Enhanced wastewater treatment by immobilized enzymes

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Abstract

Purpose of review: In the presented review we have summarized recent achievements on the use of immobilized oxidoreductases for biodegradation of hazardous organic pollutants including mainly dyes, pharmaceuticals, phenols and bisphenols. In order to facilitate process optimization and achievement of high removal rates, effect of various process conditions on biodegradation has been highlighted and discussed.

Recent findings: Current reports clearly show that immobilized oxidoreductases are capable for efficient conversion of organic pollutants, usually reaching over 90% of removal rate. Further, immobilized enzymes showed great recyclability potential, allowing their reuse in numerous of catalytic cycles.

Summary: Collected data clearly indicates immobilized oxidoreductases as an efficient biocatalytic tools for removal of hazardous phenolic compounds making them a promising option for future water purification. Data shows, however that both, immobilization and biodegradation conditions affect conversion efficiency, therefore process optimization is required to achieve high removal rates. Nevertheless, we have demonstrated future trends and highlighted several issues that has to be solved in the near future research, to facilitate large-scale application of the immobilized oxidoreductases in wastewater treatment.

Keywords: oxidoreductase; laccase; enzyme immobilization, immobilized oxidoreductase; emerging contaminants; environmental protection

1. Introduction

Due to the increasing environmental pollution, which is majorly caused by anthropogenic activities (man-made processes), the people are now more concerned around the globe. The increasing number of various types of organic pollutants, including phenols, pharmaceuticals, pesticides, dyes, estrogens and personal care products are increasingly being detected in our water systems and wastewater [1–3]. Their presence in unknown and uncontrolled dosages undesirably compromise the quality of water and shows a serious risk to human beings and aquatic organisms [4,5]. Most of the above-mentioned substances are also known as emerging contaminants (ECs) and are classified by the World Health Organization as group 1 of carcinogens due to their potentially mutagenic and cancerogenic character [6]. This issue is inevitably increased because of the lack of efficient technologies for the proper disposal, management, and recycling of waste. Further, unfortunately, most of these substances is resistant to classical treatment approaches and is not being efficiently removed during the present-day wastewater treatment plants [7]. Most of the recently applied methods of ECs removal suffer also due to low efficiency and generation of many by-products and wastes. For example, in ozonation or photocatalysis, which are one of the most often methods used in wastewater treatment plant, toxic solvents are used and the toxic products after degradation process are present. Moreover, these methods need high energy input, what causes that they are relatively expensive, due to production of ozone and using of high energy consumable UV lamps [8,9]. In case of other methods, such as filtration, coagulation or chemical flocculation, they generate secondary disposal problem [10].

Therefore, it has become meaningful to develop and optimize alternative technology that is sustainable, essentially smart, greener, and environmentally competent. In this context, biological methods of pollutants removal concerning use of microorganisms and/or enzymes seem to be of particular interest [11]. Particularly enzymes such as laccases, tyrosinases, manganese and horseradish, lignin and phenol peroxidases hold a promising role to mitigate any kinds of contamination including wide range of phenolic compounds in a specific, easy to monitor, and highly controllable manner [12]. Enzyme-based processes offer also many other advantages such as low energy input, low-toxicity, ability to operate under mild conditions, reduced amount of sludge and by-products generation [13,14]. Nevertheless, use of free enzymes for such purposes at an industrial scale has several difficulties including mainly low stability of the biocatalysts at harsh process conditions as well as extremely limited reusability of the free enzymes. In order to improve enzymes catalytic properties and stability and produce highly efficient and robust biocatalytic systems, enzyme immobilization might be applied

[15,16]. Enzyme immobilization results in formation of heterogenous catalysts characterized by improved stability at harsh process conditions. Furthermore, storage stability and operationability of the enzymes increase significantly upon immobilization. Finally, the greatest advantage of the immobilized enzyme is improvement of enzyme recycling and reusability that increases the biocatalytic productivity of the biocatalysts and reduces costs of the entire process [17]. Although immobilized oxidoreductases recently have been reported to be efficient for removal of water pollutants, special attention should be paid to the proper selection of the support material and immobilization approach in order to reduce negative effect of the immobilization on catalytic action of the enzymes [18].

In this review article we summarized recent progress in the use of biocatalytic systems based on immobilized laccase, tyrosinase and peroxides for removal and/or conversion of various hazardous contaminants including mainly phenol and its derivatives, pharmaceuticals and estrogens, dyes and other phenolic compounds from water and wastewater. We highlighted current research achievements in this field with particular emphasis of the effect of various process parameters on removal efficiency of pollutants at a minimal environmental impact and stability of the immobilized enzymes. In addition, effect of selected support material and immobilization approach on catalytic properties of the oxidoreductase and process operationability have been discussed to facilitate the large-scale application of the biocatalytic systems for water treatment.

2. Biological treatment of water and wastewater

As above-mentioned, traditional physical and chemical methods of environmental pollutants removal are widely used including adsorption, sedimentation, ozonation or advanced chemical oxidation etc. [19,20]. Unfortunately, due to their disadvantages such as production of hazardous by-products, use of toxic solvents, requirement for sophisticated equipment, high overall costs of the process or even incompatibility for complete removal of phenolic compounds, more and more attention is being paid to the remediation of these compounds by biological methods. It is caused by the fact that they can effectively remove phenolic contaminants in accordance with the rules of green chemistry, under mild process conditions, without use of additional hazardous chemicals as well as generation of toxic by-products and sludge might be avoided [21,22]. The biological methods of pollutants removal are used in both, laboratory and large-scale applications and in general can be divided into two major way: (i) treatment by microorganisms and (ii) treatment by enzymes in the free and immobilized form [23].

The use of whole microorganisms in removal process of pollutants from wastewaters is widely described in the literature. The most popular bacteria used for these processes are *Pseudomonas* and *Streptomyces*, whereas in case of fungi, there are used mainly white-rot fungi species, such as *Trametes versicolor*, and *Pleurotus ostreatus* [24]. It should be highlighted that due to the great variety of different microbial species, the process can occur in aerobic, anaerobic and mixed aerobic-anaerobic conditions. Anaerobic and aerobic treatments differ by the presence of oxygen in the process. In case of anaerobic treatment there is no oxygen, that leads to the production of highly toxic aromatic amines [25]. By contrast, aerobic conversion results in less toxic products, however, a longer process time is required. These bacteria microorganisms create and activated sludge that is capable not only for removal of pollutants by their conversion, but also by adsorption of the toxic pollutants. However, also microbiological methods are effective in removal of the most of pollutants presented in wastewater, they are ineffective in removal of phenolic compounds such as pharmaceuticals, dyes or phenols. This is mainly due to resistance of these compounds to microbiological treatment, insufficient amount of the enzymes in microbial cells as well as due to diffusional limitations in transport of substrates [26].

Enzymes, mainly from oxidoreductase group, including laccases, peroxidases and tyrosinases, arouse as a promising alternative for biological treatment of water and wastewater. These biomolecules are capable to catalyze oxidation reactions a large number of organic compounds, mainly phenolic and non-phenolic aromatic molecules [27–30]. Catalytic properties of such enzymes made them attractive for transformation of various ECs such as pharmaceuticals, estrogens, dyes, pesticides as well phenol and its derivatives [18,31]. Due to catalytic conversion of hazardous molecules, it is possible to obtain products, characterized by lower toxicity, compared to substrates. Beside achievement of high removal rate, enzymatic conversion leads to production of less toxic products, what might be easily separated from post-reaction mixture [32]. However free enzymes suffer due to their low stability and extremely limited reusability. In these terms, immobilized oxidoreductases were proposed as a versatile biocatalytic tools for removal of pollutants [33,34]. Immobilization provides improvement of stability of the biomolecules and facilitate their reuse over repeated bioremediation cycles. Further, use of immobilized enzymes gives an opportunity to develop novel bioremoval approaches including, among others, simultaneous adsorption and biodegradation or use of enzymatic membrane bioreactors [16]. However, to obtain highly active biocatalytic systems it is essential to properly select support material for immobilized enzyme. In general, organic, inorganic and hybrid/composite materials are commonly used for oxidoreductase

immobilization. These materials should be characterized by high stability and mechanical resistance, biocompatibility with enzymes and should have high affinity towards immobilized biomolecules. Further, the presence of numerus of chemical moieties is also highly desired in order to facilitate strong enzyme binding. Although various materials ca be used, of particular interest are hybrid and composite materials due to their tailor-made properties, that are designed to ensure high enzyme activity and stability [35]. It should be highlighted that use of free and immobilized oxidoreductases usually results in over 90% of pollutant removal [12,36–40]. In this review we have summarized recent findings on biodegradation of hazardous dyes, pharmaceuticals, phenols and bisphenols by immobilized laccase, tyrosinase and peroxidases, as we strongly believe that application of immobilized systems is the most promising direction for sustainable and efficient large-scale conversion of persistent organic pollutants.

3. Wastewater treatment by immobilized enzymes

3.1. Removal of dyes

Nowadays one of the most toxic groups of the environmental pollutants are dyes. This fact is caused by increasing consumption of textiles and everyday objects, which are colored by mainly anthraquinone, azo and triarylmethane dyes [41]. These compounds get easily through into wastewater from households as laundry's wastes, however the biggest amount of dyes are released directly from textile industry as wastes after dying processes [42]. Improper wastewater treatment causes that dyes might migrate into various water areas, such as seas, rivers or even groundwater. Dyes can easily accumulate throughout the food chain and due to their toxic and carcinogenic properties, they can affect physiological processes of polluted ecosystem [43]. Moreover, dyes widely used in textile industry, such as Direct Blue 15 and Disperse Blue 291 may cause mutation and denaturation of DNA in human cells [44–46]. Therefore, it is important to produce immobilized enzymes for effective removal of dyes from wastewaters and for reduction of toxicity of solution after treatment. Properly designed and produced biocatalytic systems based on oxidoreductases may be effective tools for conversion of this type of pollutants (Table 1).

Table 1.

Enzyme considered as an effective tool for dyes decolorization from water solutions, is laccase. There are recent reports concerning application of this oxidoreductase in the immobilized form for decolorization of dyes from real textile wastewaters. Yavaşer and Karagözler used laccase

from *Trametes versicolor* immobilized onto glycidyl methacrylate (GMA) functionalized polyacrylamide-alginate cryogel (PAG) as a tool for decolorization of dyes from real textile wastewater containing different dyes, salts and other chemicals using in dying process. Enzyme covalently bonded through the epoxy groups of the glycidyl methacrylate decolorized dyes from real textile effluents with over 55% efficiency at pH 5.0 and 25 °C [47]. In work by Sondhi et al. laccase was entrapped into Cu-alginate beads and applied for decolorization of dyes from textile dye effluent in continuous packed bed bioreactor. The relatively high value of decolorization efficiency, which exceeded 65%, was probably caused by addition of 2,2′-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS) as a mediator. Moreover, the produced biosystem was characterized by almost 100% retention of its relative activity after 4 catalytic cycles and after 15 days of storage. Authors explained this by the presence of copper ions in the structure of support that improve catalytic activity of multicopper oxidase [48]. In other work bacterial laccase from *Escherichia coli* immobilized onto poly-hydroxybutyrate beads (PHB) was used for decolorization process of Direct Red 105, Direct Yellow 106 and Direct Black 112 from real solution containing various inhibitors such as salts, tensoactives and dispersants. The decolorization efficiency was 60%. Moreover, it was proven that the presence of other chemicals in wastewaters did not significantly affect decolorization process [49]. As was shown by Khazravi et al., application of laccase immobilized onto oxidized activated carbon could not only decolorized dye solution, but also allowed to use support material as a self-cleaning adsorbent. It was shown that decolorization efficiency of Reactive Blue 19 was around 80%, but the 40% degradation efficiency was obtained by catalytic conversion of selected dye, and the rest was reached by adsorption of dye molecules onto support [50].

Beside laccases, the special attention should be paid on other oxidoreductases, such as peroxidases. Jankowska et al. examined possible application of immobilized peroxidase from horseradish (HRP) in removal of Reactive Black 5 and Malachite Green from water solutions imitating sea waters. Two approaches of enzyme immobilization, including adsorption and covalent binding using glutaraldehyde as a linker, onto polyamide 6 electrospun fibers, were compared in decolorization process of model solutions. It was shown that retention of over 70% of relative activity after 20 catalytic cycles was noticed for HRP immobilized using covalent binding. After the same number of catalytic cycles HRP adsorbed onto electrospun fibers possessed 63% of its initial catalytic activity. However, the highest decolorization efficiencies of dyes were obtained after application of adsorbed enzyme. It is related to the presence of salts in imitating sea water solutions, which negatively affect covalent linkage between support and biomolecule, that in consequence decreases activity of HRP covalently bonded [51]. As

presented by Bilal et al. [52], peroxidases may be not only a versatile tool for decolorization of dyes solution, but also they can decrease dyes toxicity after enzymatic treatment. Manganese peroxidase (MnP) immobilized onto chitosan beads by crosslinking with glutaraldehyde facilitate decolorization of dyes in textile effluents even up to 97%. In case of cytotoxicity and mutagenicity studies, they were reduced significantly, even up to around 70%. Beside type of immobilized enzymes, the important role in dye degradation process plays support material. Siddeeg et al. [53] showed role of applied support in enzyme immobilization and application in removal of dyes. They prepared nanocomposite from chitosan and Fe_3O_4 nanoparticles covered by *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride, characterized by a high surface area, suitable for manganese peroxidase attachment. The presence of magnetic particles in the produced support allowed to separate biosystem from dye solution after treatment by using simple external magnetic field. What is more, the fabricated support with immobilized manganese peroxidase was used for decolorization of Reactive Orange 15 and Methylene Blue with 96% and 98% efficiencies, respectively. As presented, the efficiencies of enzymatic decolorization of dyes from textile effluents depend on enzyme origin, type of support, the presence of other compounds in solutions, such as salt ions and surfactants and the process conditions. Although it is necessary to still look for novel systems with immobilized enzymes for dyes removal, recent findings show satisfactory results of dyes treatment by immobilized oxidoreductases.

3.2. Removal of pharmaceuticals and estrogens

Taking into account the dynamic development of pharmaceutical market, veterinary and human medicine, and rising amount of drugs, antibiotics or hormones making problem of water pollution by pharmaceutical residues significant. Medicines are not fully metabolized by living organisms and partially end up in wastewater and then in a sewage treatment plant where they are to be disposed of. Unfortunately, the currently used methods of pollution treatment do not fully degrade trace amounts of medicinal substances or hormones, so new methods of removing them are sought, which may be the use of immobilized enzymes [54]. Pharmaceutical active compounds can be successfully removed from wastewater by immobilized oxidoreductases (Table 2).

Table 2.

Laccase from *Trametes versicolor* immobilized by covalent binding onto polyacrylonitrile-biochar membrane was used for removal of three types of pharmaceuticals: antibiotic, antidepressant, and anti-inflammatory. Support was modified with 10% v/v HNO_3/H_2SO_4 (50:50 v/v), where –COOH groups were formed onto PAN and biochar and after that it was treated with 10% (v/v) ethylenediamine. Chlortetracycline, carbamazepine and diclofenac were at an environmentally relevant concentration in batch mode and were removed in 63%, 48% and 72% respectively. Immobilized enzyme showed 94% of its initial activity after 30 days of storage, while free enzyme kept only 32% of its initial activity. Further, biocatalyst retained around 20% of its initial activity after 10 cycles and had better thermal, pH and storage stability than free laccase [55]. In a different study it was proved that biocatalyst graphene oxide-laccase can be used for successful ibuprofen and diclofenac degradation. In addition, vanillin was used as a mediator to improve the reaction rate allowing 100% removal. The final products were found to be small mass and biodegradable compounds, such as 4- Isobutylacetophenone, N-(2,6-dichlorophenyl) indolin-2-one, 2-(2,6-dichloro-phenylamino) benzaldehyde and 2,6-dichloroaniline [56]. Naghdi et. al. obtained biocatalyst, which consisted of acid treated (by H_2SO_4/HNO_3) nanobiochar as a support and immobilized laccase (from *Trametes versicolor*) by adsorption method. After 5 days of storage test, activity of immobilized enzyme was much higher than free enzyme. Its activity dropped to 42%, while for immobilized one only to 69%. Biocatalyst was used for degradation of carbamazepine and showed 83% and 86% removal of the pharmaceutical from spiked water and secondary effluent, respectively [57]. In another study removal of tetracycline by bentonite-derived mesoporous materials with laccase immobilized by physical adsorption was investigated. In this case immobilization improved enzymes stability in wide range of temperatures and showed more potential than free enzyme. Removal efficiency of tetracycline by biocatalyst in the presence of 1-hydroxybenzotriazole (HBT) was over 60% after 3h of degradation process. After 4 repeated cycles biocatalyst retained more than 50% of its initial activity, which may be due to the poor binding of the enzyme to the carrier and biocatalysts elution between cycles [58]. Garcia-Delgado et. al. immobilized laccase into support (biochar and stevensite) by covalent binding. Support was aminopropylated with 2% (v/v) solution of aminopropyltriethoxysilane (APTES) in acetone. The investigated removal of estrogenic activity of tetracycline and obtained very low or no residual activity after catalytic removal. They proved that the use of immobilized laccase coupled to ABTS or syringaldehyde enabled the high three tetracyclines removals at 100% and 69–100% respectively. For instance, biochar-immobilized laccases coupled to ABTS was able to remove the activity completely from the solutions of tetracyclines and sulfathiazole.

Additionally, catalyst in the presence of syringaldehyde removed 100% of chlortetracycline [59]. In another study rigid tripolyphosphate-treated chitosan was used as laccase support for tetracycline removal. Support surface was at first treated with tripolyphosphate and after that was activated with glutaraldehyde. Effect of mediators were investigated including ABTS and lignophenolic mediators like violuric, syringic and ferulic acids, 1-hydroxybenzotriazole, and vanillin. ABTS was determined as the most suitable electron mediator for laccase activation. With the addition of this compound the bacterial cell growth recovery was at the level of over 98%, which suggested successful inactivation of the antibiotic. When enzyme or mediators were not present in the reaction, the cell growth was fully inhibited, suggesting the presence of functionally active antibiotic. Biocatalyst with other mediators used individually showed no tetracycline activity inhibition. Immobilized enzyme showed also better thermal stability and retained 80% activity after 7 h incubation at 60 °C, whereas free enzyme showed only 50% of its initial activity [60]. Poly(vinyl alcohol)/poly(acrylic acid)/SiO_2 electrospinning nanofibrous membrane with fiber diameters of 200 to 300 nm was used as horseradish peroxidise support for paracetamol removal. The membrane used in this process was prepared by thermal cross-linking. Such produced material was activated by 1,1′-carbonyldiimidazole and used for immobilization of horseradish peroxidase. Immobilized HRP retained almost 80% of its initial activity. Obtained biocatalyst showed also great storage stability (over 60% after 30 days) and reusability; after 4 cycles the paracetamol removal rate was around 80% and after 7 cycles removal was still at about 40%. Removal rates for immobilized and free horseradish peroxidase are quite similar and equal 83.5% and 84.4% respectively but the reusability of the immobilized enzyme definitely outweighs its advantage [61].

Big environmental problem is also tray amounts of hormones remaining in the wastewaters. Removal of these compounds is crucial in order to limit their entry into natural waters. Hormones, such as estrogens, can be successfully degraded even by small amounts of enzymes. Becker et. al. tested laccase from *Trametes versicolor* and *Myceliophthora thermophila* immobilized using two supports, porous polymeric beads IB-EC1 with polyacrylic, carboxylic and ester as a functional groups and ceramic membranes, for removal of estrogens in spiked water samples and in real wastewater. After treatment the estrogenicity was removed in 91% after 24 h by immobilized laccase, where for free enzyme no activity was removed. It was also shown that for the membrane bioreactor constructed with ceramic membrane, over 95% of estriol was removed, while for the packed bed bioreactor around 80% [62]. Garcia et. al. immobilized laccase on Ca and Cu alginate–chitosan composite by entrapment method for 17 α-ethinylestradiol removal. Upon immobilization catalytic degradation of the hormone was

more efficient in wider range of pH and temperature, as compared to process catalyzed by free enzyme. The highest percentage of removal was equal 80.5%f and was reached or Cu-beads-laccase was obtained after 24h at pH 5, temperature 28 °C. Further, after 3 cycles of EE2 removal, its yield reached more than 70% [63]. 17 α-ethinylestradiol removal can also be attained by laccase immobilized onto *Luffa cylindrica* fibers. The best conditions for high laccase activity were at temperature between 30°C to 50°C and at pH ranging from 3.6 to 4.6. EE2 removal efficiencies for free and immobilized enzyme were similar but immobilized enzyme is more financially advantageous due to reuse possibility. Taking into account type of immobilized laccase the reusability potential is equal more than 50% and after 7 cycles [64].

3.3. Removal of phenols

Phenols are characterized by poor solubility in water and relatively good solubility in fats, and as a consequence can accumulate in plant and animal organisms. In addition, they are dangerous to the health and life of living organisms because they are highly toxic, corrosive, and defined as carcinogenic and teratogenic factors. The research clearly indicates the inefficiency of phenol removal in conventional sewage treatment plants due to their high resistance to chemical and biological decomposition. Besides, the efficiency of its removal by physical methods is insufficient and does not guarantee that the water discharged into receivers will have the appropriate purity class. The use of durable and environmentally flexible materials such as enzymes is a promising technology for wastewater treatment containing phenolic compounds (Table 3).

Table 3.

To achieve effective degradation, Silvia et al. immobilized horseradish peroxidase on cashew polysaccharide, a natural polymer which, due to its renewable origin, significantly reduces the escalation of subsequent waste and, consequently, does not adversely affect the environment. Its attractiveness as a carrier of enzyme immobilization is enhanced by its very good solubility because its recovery is possible through simple precipitation reactions with polar organic solvents such as ethanol. Samples of actual textile wastewater and pollutants containing, phenolic compounds such as catechol, phenol, bromophenols, and nitrophenols were taken at the Wastewater Treatment Station (WTS) in Anápolis Agroindustrial District (DAIA) and Cia Hering in São Luís de Montes Belos, Goiás, Brazil. 100% degradation efficiency was achieved with textile wastewater and 94% with WTS, whereas, bromophenol was degraded in 40%. The

maximum reaction time for phenol removal was up to 30 min, where for free enzyme it was 90 min. Consequently, the biodegradability or high non-toxicity of cashew polysaccharide favors its commercialization in industrial biotechnological and medical sectors [65]. Other well-proven carriers for enzyme immobilization and subsequent efficient degradation of phenols are inorganic carriers such as silica gels. Silicates are structurally stable, relatively cheap, and chemically resistant to organic solvents. Mohammadi et al. examined laccase immobilization on silica particles functionalized with epoxy groups. Covalently immobilized laccase tripled its stability at extremely high temperatures, retaining 96% of its activity at 35 °C, where the free enzyme showed only 35% active at the same temperature. Finally, the degradation of phenol, p-chlorophenol, and catechol was carried out, achieving 76%, 60%, and 95% removal efficiency, respectively [66]. Abdollahi et al. in their research developed a nano-bio catalyst which is a promising for micro-pollution degradation and an alternative to catalysts used in traditional wastewater treatment processes. To improve the catalytic activity, they modified magnetic iron oxide nanoparticles with 3-aminopropyltriethoxysilane (APTES), and then before tyrosinase immobilization, the particles were further functionalized with 2,4,6-trichlorotriazine as an activating agent to obtain magnetic nanoparticles. They used the magnetic nano-bio-catalyst (tyrosinase-MNP) thus constructed for the treatment of phenol-containing wastewater. It was proved that precise dosing of the nano-bio catalyst enables phenol degradation in a wide range of pH and temperature. Moreover, immobilized tyrosinase degraded highly concentrated (2500 mg/L) phenol with 70% efficiency. The nano-bio catalyst showed also a phenol degradation efficiency of 100% after the third re-use cycle and about 58% after the seventh cycle. In addition, immobilized tyrosinase was able to degrade phenol dissolved in real water samples of around 80% after incubation for 60 min [67]. Qiu et al. combined the advantages of magnetic nanoparticles, ionic liquids and dialdehyde starch to produce a new and highly efficient enzyme carrier. In the research, they immobilized laccase on magnetic nanoparticles modified with ionic liquid functionalized with amine groups using dialdehyde starch as a crosslinking agent (Fe_3O_4-NIL-DAS). Fe_3O_4 as a magnetic nanomaterial is characterized by low toxicity, mature synthetic technology, fast separation from substrates, and good catalytic properties. However, it is quite problematic to immobilize the enzyme onto the parent Fe_3O_4, moreover, the carrier should provide the protein with high catalytic activity, physicochemical stability, or high substrate affinity. This is difficult to achieve when relying on a single inorganic carrier. It has been proven that ionic liquids as surface modifiers retain enzymatic activity, effectively increase stability and improve reusability of the biomolecules. The prepared biocatalytic system eliminated 86% of phenol, and 94% of 4-chlorophenol and

100% of 2,4-dichlorophenol, respectively. The system showed much better stability during storage and reuse, maintaining over 80% of activity after 30 days and 83.5% of initial activity after six catalytic cycles. Covalently immobilized laccase was, more stable in the wider pH and temperature range, moreover, the increase in removal efficiency can be explained by the fact that Fe_3O_4-NIL-DAS provides a large number of sites capable for enzyme binding. As a result, laccase conformation is more stable and the high mobility of the chain makes the substrate more accessible for the enzyme. The biocatalytic system constructed in this way has great potential for the treatment of wastewater containing phenol [68]. In another study, Li et al. in used horseradish peroxidase immobilized on magnetic nanofibres (MNF) Fe_3O_4/polyacrylonitrile (PAN) to treat wastewater from phenolic compounds. The combination of inorganic and organic materials allowed to obtain homogeneous nanofibres with strictly controlled nanostructure and maintaining different chemical characteristics of a given compound. The presented study proves that in the case of such a biocatalytic system, not only the process efficiency increase but also the stability and reusability improve. Immobilized peroxidase degraded phenol in 85%, while the degradation activity after 5 cycles still oscillated at a high level of 52% [69]. 100% degradation of phenol at high concentration (2500 mg/L) was obtained by Vineh et al. who performed covalent immobilization of horseradish peroxidase on functionalized reduced graphene oxide using glutaraldehyde as a cross-linking agent. Graphene oxide is characterized by a great number of functional groups such as thick carboxyl, hydroxyl, or epoxy, which not only supports their solubility in water or polar solvents but ultimately affects the efficiency of degradation by the immobilized enzyme. The study confirmed the effectiveness of covalent immobilization of horseradish peroxidase on reduced graphene oxide, which proved to be an excellent substrate to support the enzyme before inactivation of the biocatalyst. Besides, a significant improvement in all kinetic parameters was observed, and the thermal stability and catalytic activity were enhanced. Finally, covalently immobilized peroxidase, in addition to total phenol degradation, showed also 70% activity retention after 10 cycles and 96% of activity after 35 days of storage [70].

3.4. Removal of phenol derivatives

Phenol derivatives, like phenol itself, are recognized as a hazardous environmental pollutant that can consider a risk to humans, animals and aquatic organisms. This is due to the relatively high toxicity of these compounds, even at low concentrations. According to the International Program on Chemical Safety, organic compounds from many industries can be fatal to living organisms, due to the possibility of damaging the nervous system, heart, kidneys and liver [33].

Therefore, various methods of water and wastewater treatment are used to remove these substances among which biological/enzymatic methods appear to be more cost effective and eco-friendly. Additionally, in order to minimize free enzymes limitations, immobilized enzymes are very often used in purification processes (Table 4).

Table 4.

One of the most common phenol derivatives with two hydroxyphenyl groups are bisphenols, e.g. bisphenol A, bisphenol S, bisphenol F. However, among them the most popular is bisphenol A, which has been used in the production of plastics for years. Moreover, scientists have proved that it has a harmful effect on endocrine system [71]. Research have shown that it is possible to degrade bisphenols to less toxic derivatives using laccases. For this purpose, Brugnari et al. used laccase from *Pleurotus ostreatus* immobilized onto monoaminoethyl-*N*-aminoethyl-agarose, which enabled improvement of thermal and storage stability of the enzyme, as well as its efficiency in the degradation of bisphenol A. Laccase in free form is characterized by a retention of 40% initial activity after 40 days of storage. In contrast, the immobilization of the enzyme allowed storage for 40 and 170 days with 80 and 70% of the initial activity retention, respectively. Additionally, the immobilized laccase enables to carry out 15 catalytic cycles with over 90% efficiency in removing of phenolic contamination [72]. The adsorption immobilization of laccase from *Trametes versicolor* was also by Taghizadeh et al., who tested sodium zeolite Y and its modifications as supports. The application of desilicated zeolite improved the stability of laccase and made it possible to carry out the biodegradation process of bisphenol A with the efficiency of over 85%. However, it should be mentioned that the process was conducted for a very short period of time reaching 1 h. Nevertheless, the proposed biocatalytic system is not as stable as the one mentioned above, since about 59% of the initial enzymatic activity was retained after 14 days of storage [73]. Lassouane et al. carried out immobilization of the laccase from *Trametes pubescens* using two simultaneous methods: cross-linking with glutaraldehyde and entrapment into Ca-alginate beads, which resulted in a 7-fold reduction in enzyme leakage compared to simple entrapment immobilization. The prepared biocatalytic system allowed for the biodegradation of bisphenol A at a concentration of 20 mg/L at pH 5 and 30 °C. Within 2 h, almost 100% efficiency of pollutant removal was achieved. Moreover, this biocatalyst was able to work for the next 10 catalytic cycles with the process efficiency above 70%. High removal efficiencies disposed scientists to check whether any part of bisphenol A was adsorbed onto alginate beads. The tests showed that only

immobilized laccase is responsible for the removal of the contamination [74]. In some scientific works, researchers are trying to biodegrade more pollutants from bisphenols group using one biocatalytic system. For example, Zdarta et al. designed a system to remove three bisphenols: bisphenol A, bisphenol F and bisphenol S. Laccase from *Trametes versicolor* was immobilized on a novel biopolymer material – *Hippospongia communis* spongin-based scaffold. Almost 100% removal efficiency of bisphenol A and bisphenol F was obtained under the following conditions: pH 5, 30 °C and pH 5, 40 °C, respectively. However, over 40% of bisphenol S was degraded at pH 4 and 30 °C. It should also be noted that immobilized laccase retained about 90% of initial activity after 20 days and over 80% after 50 days of storage at 4 °C [75].

Also among pesticides, compounds from the group of phenol derivatives can be found. Chen et al. undertook the degradation of nine different pesticides, but to ensure high efficiency they opted for combined methods: biodegradation with laccase immobilized on two biosorbents. Peanut shells and wheat straw were used as supports and to the reaction system syringaldehyde was also added, which was responsible for improving the catalytic properties of laccase. The first removal tests were carried out on pesticides present in the water within 3 days. In the system with laccase immobilized on peanut shells, it was possible to degrade 54.5% of the pollution, whereas when wheat straw was used, the reaction efficiency was 65.9%. In the next part of the research, the source of the contamination was changed. Biodegradation of pesticides from the soil for 7 days removed about 20 to 92% of the pollutants using both peanut shell system and wheat straw system. Large results discrepancy is due to the differences in pesticides resistance to enzymatic treatment. Nevertheless, it should be emphasized that biodegradation in combination with adsorption has a very high potential and can be an effective solution to the problem of pollution in both water and soil [76].

Lignin is a common biopolymer in which the monomers are organic compounds derived from phenolic alcohols. Its chemical structure is very extensive and complicated, which prevents its wider application. Therefore, the aim is to convert lignin to compounds with lower molecular weight. For this, laccase is used as it allows the degradation of the biopolymer. Chen et al. immobilized enzyme on magnetic nanoparticles, which additionally facilitate easy separation of the biocatalyst from the reaction system in the final stage of the process. Using immobilized laccase, it was possible to degrade almost 100% of model lignin compounds. However, after 8 consecutive catalytic cycles, only 40% of the initial enzyme activity was retained. Nevertheless, the conducted tests would have to be repeated using a real solution to check whether the proposed biocatalytic system would be equally effective [77]. As can be seen from the above-

mentioned results, laccase is actually necessary in the degradation of phenolic derivatives and the removal of these pollutants from water, sewage or soil.

4. Challenges and future prospects

Investigation of oxidoreductase immobilization shows great application perspectives for wastewater treatment. However, there are several obstacles that has to be solved in the near future to facilitate transfer of laboratory-scale developments into larger scale. We have summarized, in our opinion the most crucial issues that limits practical applications of the oxidoreductase-based biocatalytic systems and require further investigation and novel solutions**.**

- Reduction of production costs of enzymes by developing of novel technologies or by optimization of a currently existing processes.
- Improvement of the enzyme stability at harsh process conditions and its reusability by application of a novel immobilization approaches. Process optimization in order to produce highly active and long-term stable biocatalytic systems
- Application of a novel, tailor-made support materials to promote enzyme activity and its applicability. Proper selection of the support materials to provide protection for the biomolecule and suitable enzyme microenvironment as well as meet the process requirements.
- Optimization of the biodegradation process in order to achieve high removal rate. Selection of the process conditions and applicability of the biocatalytic systems in various water bodies, including real water and wastewater.
- Detail characterization of the biodegradation products in order to evaluate effective methods of their separation and examination of the toxicity of the post-reaction mixture. Characterization of the catalytic pathways of the biodegradation reaction to determine bottle neck of the reaction and examine by-products.
- Application of the enzymatic reactors and enzymatic membrane bioreactors containing immobilized biocatalysts as catalytic beads for continuous treatment of model and real water solutions. Characterization of effect of process conditions on removal efficiency.
- Evaluation of a novel and promising approaches for more efficient treatment of pollutants, such as simultaneous adsorption and biodegradation or enzymatic conversion supported by photocatalysis.

- Development of a solutions facilitating transfer of a laboratory or small-scale procedures into larger scale to allow real wastewater treatment by using immobilized oxidoreductases.

5. Conclusions and final remarks

In summary, rapid development of the various branches of industry generates huge amounts of toxic phenolic compounds that are presented in water and wastewater. Among various recently developed approaches for removal of the above-mentioned compounds, use of free and particularly immobilized enzymes is extremely promising. Enzymatic techniques based on use of immobilized oxidoreductases (mainly laccases, tyrosinases and peroxidases), offers several advantages including low cost, sustainability and/or mild process conditions. Immobilized oxidoreductases, due to their relatively low substrate specificity are capable for effective conversion of numerous of phenol and phenolic derivatives including pharmaceuticals, estrogens, bisphenols or dyes with removal rates usually exceeding 90%. Further, enzymatic treatment facilitates reduction of estrogenic activity and toxicity by generation of low toxic final products of conversion. Although further studies are still highly required to optimize immobilization and bioremediations conditions and to reduce cost of the process, the application of immobilized biocatalysts for removal of phenolic pollutants is expected to be a breakthrough in future.

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Declaration of competing interest

Authors declare no conflict of interest.

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