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Laccase Immobilization on Magnetic Nanoparticles: Recent Techniques and Applications

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Abstract

Enzyme immobilization has emerged as a powerful strategy to enhance enzyme stability, reusability, and catalytic efficiency for diverse applications, particularly in environmental remediation, such as the removal of organic pollutants from wastewater. Among various enzymes, laccases have gained significant attention for their ability to degrade a broad spectrum of organic pollutants under mild and environmentally friendly conditions. This review focuses on the immobilization of laccases onto magnetic nanoparticles as a carrier, emphasizing their advantages as robust and reusable biocatalysts for wastewater treatment. The discussion encompasses immobilization techniques, key influencing factors, and recent advancements in optimizing catalytic efficiency, thermal stability, and operational durability. Magnetic nanoparticles, with their high surface area, ease of separation, and reusability, are highlighted as ideal carriers for enzyme immobilization. Furthermore, the review critically evaluates the latest research on laccase-magnetic nanoparticle systems and challenges in scalability, cost-effectiveness, and long-term stability. Finally, the integration of these immobilized enzyme systems with complementary treatment technologies is examined as a promising avenue for sustainable and efficient pollution control strategies.

Keywords: Enzymes; immobilization techniques; physical immobilization; chemical immobilization; biological activity; catalytic efficiency.

1. Introduction

The enormous civilization and profound growth in various industrial segments in the last century led to an increase in anthropogenic activities and produced enormous quantities of new chemicals and compounds in the aquatic environment. Consequently, the exacerbation of the problem of environmental pollution and diverse organic pollutants (such as phenols, organic dyes, pharmaceuticals, estrogens, herbicides, pesticides, and personal care products) in the water system[1]. Distinct physical and chemical treatment techniques have been utilized over the last decades to eliminate organic pollutants. Figure (1) presents the physical techniques, which comprise several methods, such as filtration, adsorption, chemical flocculation, coagulation, reverse osmosis, and filtration, etc. These physical methods present effective treatment for organic pollutants. However, they have some drawbacks. For instance, in the adsorption method was noticed that the reproduction of the adsorbents and the generation of many by-products (sludge)[2]. Furthermore, Coagulation, sedimentation, and flocculation methods are not effective towards all types of pollutants because they show selectivity to definite contaminants over others and the generation of toxic by-products [3]. According to the drawbacks and limitations of the traditional strategies of remediation more and more attention is being paid to implement environmentally competent, sustainable, essentially smart, greener, and effective strategies of pollutants decontamination whose implementation will not have an impact on the equilibrium of the entire ecosystem. Biological remediation techniques capable of effectively eliminating the organic pollutants under the mild process circumstances in accordance with the green chemistry principles, without generation of toxic by-products and the use of hazardous chemicals, and the ability to avoid sludge[4,5]. Biological techniques also involve the use of biocompatible, low-cost, and non-toxic compounds comprising fungi, yeasts, bacteria, and other microorganisms that have been demonstrated to have an exceptional capacity to eliminate organic contaminants[6]. Generally, the biological technique can be applied by two main methods: (i) treatment using microorganisms and (ii) treatment using free and immobilized enzymes [7]. In this context, biological remediation methods concerning the use of laccase enzyme seem to be of particular interest.

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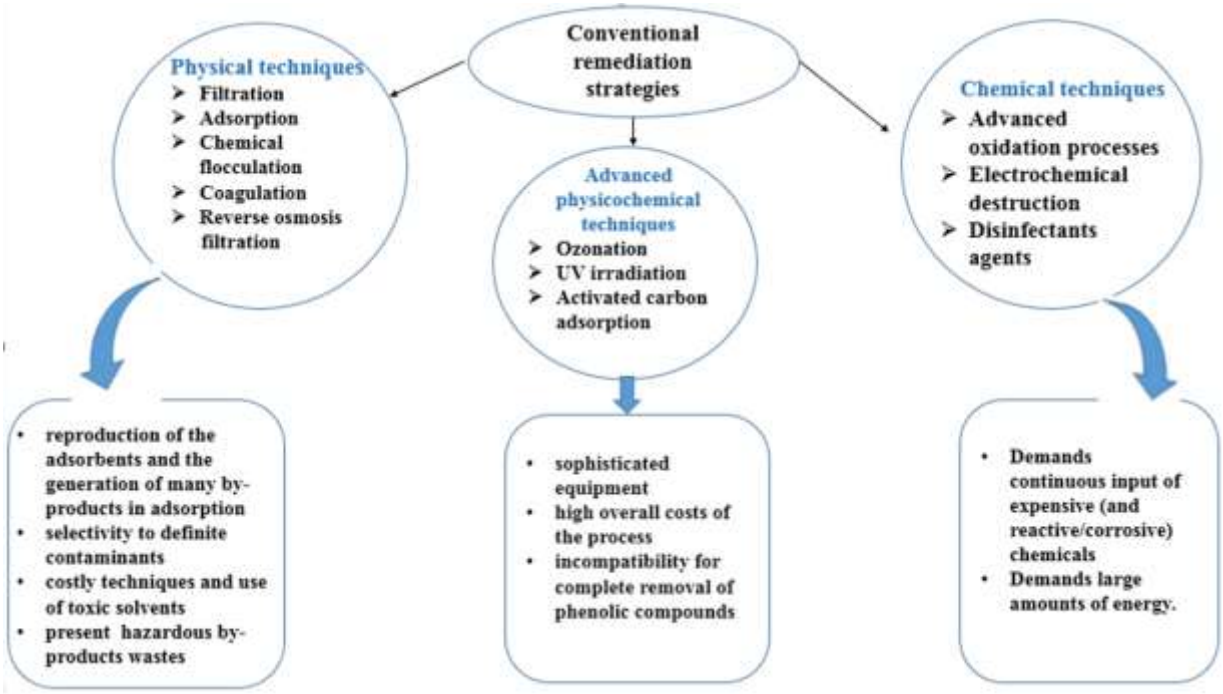

Figure 1. Physical and chemical treatment techniques with their limitations.

Although enzyme-based processes offer many benefits (the low toxicity and energy input, ability to operate under mild conditions, and reduced the amount of sludge and by-product generation)[8], using the free form of enzymes for such purposes at large industrial scale faces several drawbacks including the low thermal and operational stability of the biocatalysts as well as the extremely limited reusability of the free enzymes. As a result of that, the enzyme immobilization might be applied to enhance the catalytic activity properties as well as the stability of free enzymes and create highly effective and durable biocatalytic systems [9,10]. Finally, the major benefit behind enzyme immobilization is enhancing the biocatalytic productivity of the biocatalysts and lowering the overall operational expenses of the process through the improvement of enzyme reusability [11].

In this current review we focused on the implementation of immobilized laccases in decontamination processes of organic contaminants. Also, we presented a general overview about: immobilization (methods, affecting factors, and carriers for immobilization), laccase enzyme, and magnetic nanoparticles as a matrix for laccase immobilization. In addition, this review is crucial because it tackles a critical issue: the ecologically responsible removal of organic contaminants by application of immobilized laccase on magnetic nanoparticles and the future prospects and recommendations for optimizing immobilization techniques and integrating enzyme-based approaches with existing water treatment technologies to further improve environmental sustainability.

2. Laccase

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) have attracted the efforts of many researchers in the field of environmental purposes and biodegradation of organic pollutants from aquatic environment owing to their nontoxicity and the higher efficiency [12]. They can be widely found in nature from a lot of sources including plants, fungi, prokaryotes, bacteria, insects and lichens each species of laccase exhibit particular catalytic characteristics and sequences [13,14]. According to National Center for Biotechnology Information (NCBI), there are 191,500 items of laccases, where about 87% of them are bacterial laccases. However, fungal laccases are preferred in biotechnological applications owing to their high redox potential[15,16].

The structure of laccases can exist in different forms as monomeric, homotetrameric, heterodimeric, and multimeric forms, with a molecular weight ranging from 50 to 130 kDa depending upon their origin [17]. The carbohydrate content in laccase is approximately 45% and 10-30 % of molecular weight for plant and fungal laccases, respectively [18]. Carbohydrate content in laccase enzymes is responsible for the conformational stability of the protein part in enzyme and protects them from the proteolysis and the inactivation by radicals [19]. Laccase enzymes (p-diphenol: oxidoreductase dioxide) are a family of multicopper oxidoreductase that comprise four different copper ions in their active sites. These four Copper ions are divided into three types of structures [20,21], as shown in figure (2).

According to the biological activity features of laccases, they are able to oxidize a wide range of substrates, including aromatic and non-aromatic compounds with different substituents like phenols, some inorganic ions, and several non-phenolic substrates [16,22,23]. Laccases only require oxygen molecules as reactants, and only produce water molecules as by-products for bio-catalysis process, so they can be expressed as the “green catalysts” [24] as shown in figure (3).

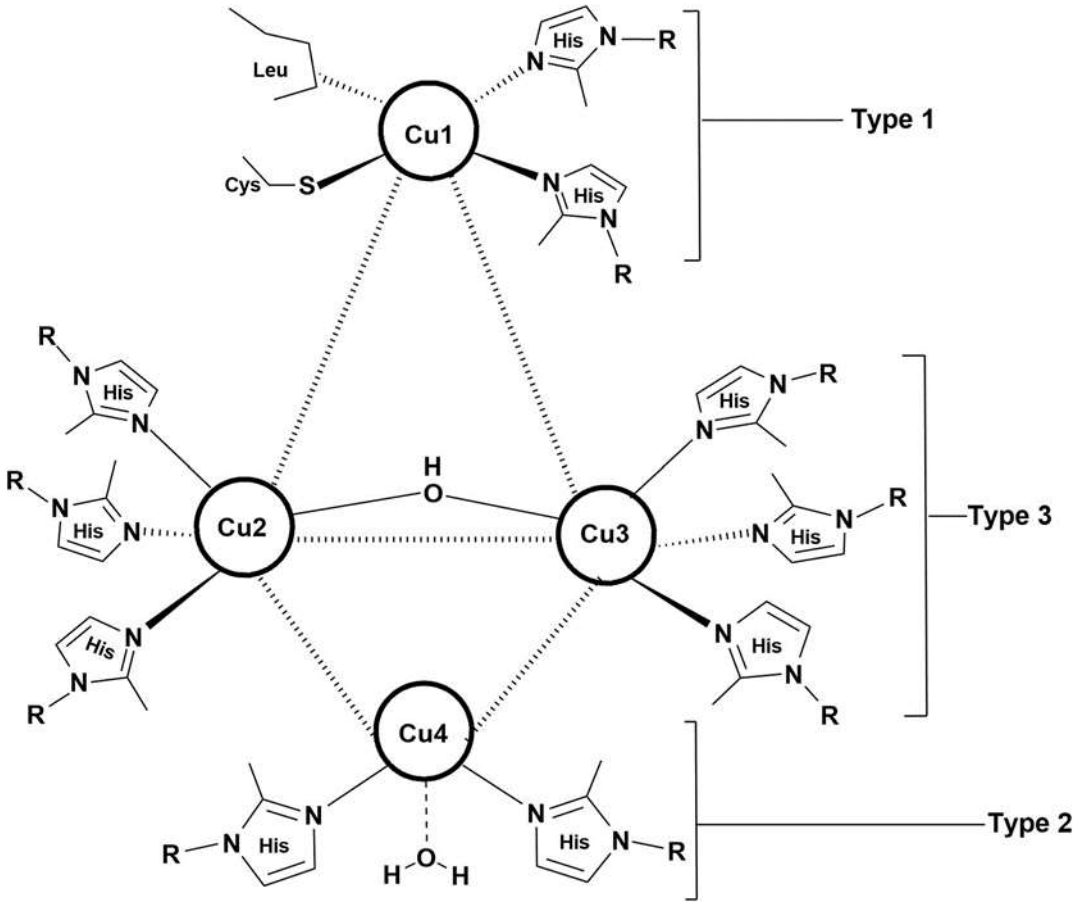

Figure 2. Copper centers of laccase enzyme: Type 1, Type 2, and Type 3 (Adapted and modified axial ligand by Garavaglia et al. (2004)).

Laccases have high catalytic efficiency, so they are widely used in technical applications in various biotechnological and industrial fields[25,26]. These fields include the removal of phenolic compounds, which cause cancer and teratogenicity when released in wastewater [27], improving the properties of fibers, environmental protection, bio-synthesis, energy exploitation, and the bio-detection and degradation of synthetic dyes[28]. Laccases can be used in the printing and dyeing industry, bio-pulping in the industry of paper, and conversion of aromatic compounds to less toxic by-products[28]. In addition, they are also used in pharmaceutical industries as anesthetics, anti-inflammatory drugs, sedatives, and antibiotics [23,29]; and in nano-biotechnology as nanoparticle-based biosensors[27].

3. Enzyme Immobilization

The immobilization process can be carried out by trapping the free enzymes within or on the surface of the insoluble support material, even if they retain their catalytic activity[30]. Enzyme immobilization aims to acquire two main merits: the enhancement of the catalytic activity and improve the enzymatic stability of enzymes against the catalytic process conditions (such as the high temperatures, the variation of pH values, the organic solvents and detergents throughout the process storage and operation). Furthermore, immobilization could lower operating expenses in several ways, like the improvement of the enzyme catalytic activity, facilitating effective recycling, and managing the conditions of the process. As well as expanding the operational domain of implementation and enabling a large-scale application of the enzymatic processes[31]. Continuing with the benefits of enzyme immobilization, sometimes the catalytic activity of immobilized enzymes becomes higher than that of their free forms. This is owing to the stability and/or the conformational changes of protein after combining with the support, which leads to an increase in the availability of the active site for biocatalysis[32].

4. Main Influencing Factors on Immobilization

Enzymes undergo chemical and physical changes in their properties as a result of immobilization. Hence, there are some fundamental factors that should be considered collectively for the success and effectiveness of enzyme immobilization: choice of the enzyme, selection of immobilization supports, methods of immobilization, and finally, cost and practical conditions of immobilization [33].

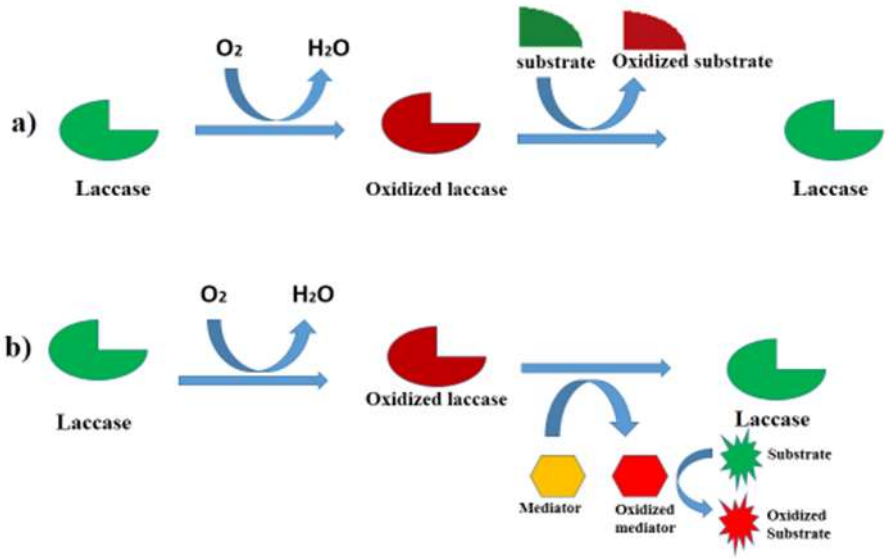

Figure 3. Schematic representation of catalyzed reaction by laccase a: direct oxidation by laccase b: in-direct oxidation in presence of ediator.

4.1. Choice of enzyme

The physical and chemical properties of enzymes can impact their catalytic activity. For instance, the enzyme characterization: enzyme characterization factors such as enzymatic activity, kinetics, substrate affinity, and inhibition of the immobilized enzyme should be assessed to ensure the effectiveness of the immobilization process. The size and shape of enzymes can influence the selection of immobilization technique and the choice of an appropriate carrier. Large enzymes may require matrices with a larger surface area or porous carriers to accommodate their large size, while the smaller enzymes can be a good choice for smaller support materials [34,35]. Also, the concentration of the immobilized enzyme can affect the overall activity and efficiency of the immobilization process because the loading of immobilized enzyme on the support material depends on the concentration of the enzyme. Higher enzyme concentrations may lead to higher loading on the support material, but excessive concentrations can result in enzyme crowding, decreased accessibility to the substrate, and reduced catalytic activity[36]. Some enzymes require co-factors or specific prosthetic groups to improve their catalytic activity. So, with such enzymes, it is important to ensure that the immobilization process preserves the functionality of these co-factors or prosthetic groups [37].

4.2. Selection of support material

One of the key components of the immobilization process is the selection of appropriate immobilization supports. However, the selection of the better support material can be a complicated process. Because it depends on the type of enzyme,

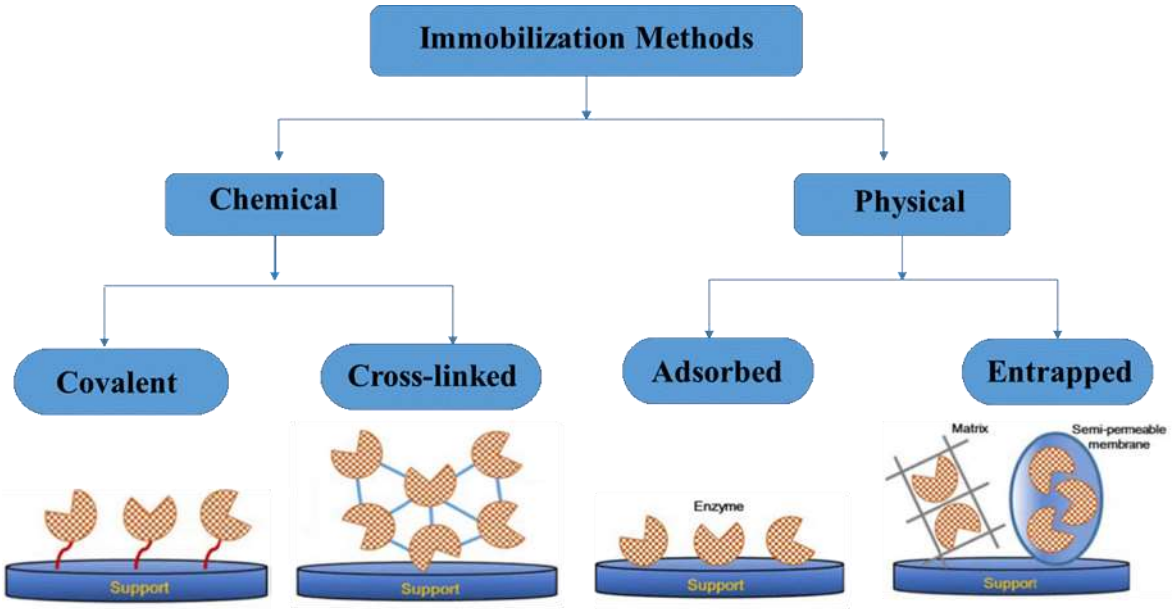

Figure 4. Various strategies of immobilization

reaction media, safety guidelines in the area of hydrodynamic conditions, and reaction conditions [38,39,40,41]. As a result, the supporting material needs to possess the following attributes [41]:

- Support should be biocompatible and possess a large internal surface area to facilitate good enzyme binding. Porosity may, therefore, be a useful property, such as vesicular silica or vesicle-like mesoporous material. However, the pores diameter must remain within a certain range, because the enzyme and the support will rarely interact intensely when the last is smaller than the protein, also too large support will result in a significant drop in the surface area [42,43,44].
- For the covalent immobilization, a high surface density of function groups should be presented in the supporting material. An intense multipoint covalent attachment can only be achieved, when the protein surface is exposed many reactive groups inside the support material[45,46]. In the reaction medium, the reactive groups in the protein and the support material should exhibit lower steric hindrances, because the multipoint covalent attachment requires contact between groups bonded to stiff structures after the initial immobilization [41].
- The active sites on the surface of the enzyme ought to interact with the reactive groups in the support material [41,46].
- To enable an extended enzyme-support reaction, the reactive function groups that participated in the immobilization must be sufficiently stable chemically and physically [41,47].
- After the immobilization process, it should be simple to obtain a final inert surface in the support matrices by obstructing the remaining function groups without changing the structure of the enzyme [48].

Support materials can be categorized into organic and inorganic supports based on their chemical composition; in addition, they can be subdivided into natural and synthetic polymers. The most widely utilized support materials include porous matrices (e.g., agarose, cellulose, or silica), magnetic particles, and solid surfaces (e.g., glass or polymers) [42,49,50,51].

4.3. Enzyme immobilization methods

In accordance with how the enzyme and support interact, the immobilization processes are based on distinct physical and chemical methods, physical methods such as physical adsorption and entrapment/encapsulation. Chemical methods like covalent bonding and cross-linking. Figure (4) presents an overview of several techniques that have been applied to laccase immobilization. Each technique has benefits and drawbacks, as summarized in table (1).

4.3.1. Adsorption

Enzymes can be immobilized using the adsorption method by attaching them to the outside surface of an insoluble substrate through several types of interactions, including hydrophobic, hydrogen bonds, ionic bonds, electrostatic, van der Waals or dipole interactions, and even chelation [9]. The simplicity of the operation of the immobilization process is the main characteristic of adsorption. While the interaction's reversibility is the main limitation, which could result in enzyme leakage into the reaction medium, they might guarantee that the enzymes original structure is preserved throughout the biocatalysis process. This is because of the mild forces interaction between support and the enzyme[9]. Also, there are some factors that limit the process, like the reduced flexibility in operating circumstances (e.g., variations in pH and ionic strength), in addition to abrasion on the enzyme surface from mixing conditions [52]. Although electrostatic interactions are a common method of adsorption immobilization, they necessitate the existence of charges on the surface of the support and the enzyme, which range from low resistance to large

ionic forces in the reaction medium [53]. Electrostatic interactions may become ionic interactions depending on the intensity of the previous ones. In terms of the chelation adsorption, the amino acid residues and the chelated metal ions on the support surface retain a very stable coordination [54,55].

4.3.2. Entrapment

In this type of immobilization, the support's porous nature allows the enzyme to be three-dimensionally confined, preserving its mobility, permitting unimpeded passage of substrate and products without causing structural alterations to the enzyme, and reducing the amount of enzyme lost into the solution, so it is the most favored option in industries [56]. The most extensively utilized supports for entrapment immobilization are polymers like alginate, alginate-chitosan microcapsules, gelatin, carrageenan, metal-linked enzyme beads, and hydrogel structures [57].

4.3.3. Covalent binding

The principle behind the covalent binding technique is the interaction between the reactive function group on the support and the enzyme's nucleophilic group and the formation of strong covalent connections that provide the immobilization with increased stability [58]. Several function groups on the carriers (like amino, hydroxyl, amino, thiol, imidazole, and guanidyl groups) are covalently attached to the functional group like the α-carboxyl group at C-terminus of the enzyme, phenol ring of tyrosine, indole ring of tryptophan, and α-amino group at N-terminal of the enzyme [59]. A wide variety of supports can be covalently bonded to the enzyme like cellulose, silica, inorganic carriers such as glass, agarose, collagen, metallic silver, and gold nanoparticles, as well as cross-linkers like glutaraldehyde [60].

4.3.4. Cross linking / self-immobilization

Bifunctional cross-linkers allow for the immobilization enzymes without the need for carriers, in cross-linking method enzymes can be immobilized by two different procedures. The crosslinking enzyme aggregates (CLEA) technique precipitate laccase, integrating immobilization and purification into a single process. In this method, the application of the crosslinking agent encourages the crosslinking between the groups that are not involved in the catalytic process of the enzyme [61]. Diisocyanates, dialdehydes, diiminoesthers, and diamines activated by carbodiimide are some of these crosslinking agents [62]. The enzymatic immobilization is conferred by the enzymes crossing each other, hence this approach does not require any matrix to act as a support [63]. Cross-linked enzyme crystals (CLECs) are the second way for cross-linking immobilization. Although CLECs exhibit high activity and operational stability; there are drawbacks like the high purity

demand for the crystalization of the enzyme, the high laccase activity loss, difficulty controlling enzyme orientation, and the harsh reaction conditions[59] [62].

Table 1. Advantages and limitations of the most common immobilization techniques.

Immobilization technique	Advantages	Limitations
Adsorption	• operational simplicity • The enzyme has not been chemically modified	• reversibility of interaction may result in enzyme leakage • The reaction's low specificity (because adsorption and ion-exchange may overlap) • Decreased operational condition flexibility
Entrapment/ encapsulation	• The enzyme has not been chemically modified • Enzymes should remain catalytically active during the support transitions or polymerizes	• Possibility of mass transfer • Possibility of enzyme leakage
Covalent bonding	• Durability of the binding • Reducing the enzyme leaching • Enhance the enzyme stability	• Usually the irreversibility of the connection limiting the reusability of enzyme • Support needs the chemical modification • Possibility of decreasing of enzyme catalytic activity • Possibility of enzyme's sterical modifications
Enzyme cross-linking	• Support is not required • Enhancement of activity and operational stability of the enzyme • Reducing the enzyme's leakage	• Possibility of mass transfer • harsh reaction conditions • pore blockage issues • Potentially significant chemical modification of enzyme

4.4. Selection of suitable immobilization conditions

The operational conditions of the immobilization process, such as temperature, pH, and presence of redox mediators, can affect the efficiency and stability of the immobilized enzyme.

4.4.1. Temperature

The temperature of the immobilization process is one of the most effectual parameters on the enzymatic reaction because it improves the enzymatic degradation process by providing the reaction with the demand activation energy[64]. Nevertheless, the enzymatic reaction may retarded and the enzyme's catalytic activity may decrease after the optimal temperature [65,66,67].

4.4.2. pH

Enzymes are extremely sensitive to pH changes, because of the variations in the buffer solution can alter the ionization states of amino acids at the active sites, which can impact substrate binding and catalysis [68]. The enzymatic activity shows an increasing tendency before its optimal pH value, but it then declines with raising the pH [69]. Hereby, the influence of pH level and temperature on the enzymatic elimination of contaminants tend to be similar. In strongly acidic or alkaline solution the immobilized laccase activity is inhibited, so the enzymatic elimination rate is significantly reduced [70].

4.4.3. Reaction time

The non-complementary enzyme's multipoint interaction with the support surface is a sluggish and time-dependent reaction and the removal efficiency increases over time until leveling off [71]. This could be due to a number of factors, including the inactivation of enzymes during the degradation process, the inhibition of catalytic degradation brought on by the accumulation of bi-products, and the saturation of support material by adsorption [72,73,74]. So, this reaction requires the correct alignment of the enzyme active sites and the rigid surface of the support [75].

4.4.4. Presence of redox mediators

As previously reviewed, enzymatic degradation of some organic pollutants needed to be activated by using redox mediators[76]. The efficacy of redox mediators relies on their redox potentials. In which there is a direct relationship between the degradation efficiency of the redox mediators and their redox potentials[76].

Table 2. Some recent studies of the optimized immobilization conditions.

Enzyme	**Immob. Carrier**	**enzyme conc.**	**pH**	**Temp. (°C)**	**Incubation time**	**Ref.**
Trichoderma asperellum laccase	polymer coated $Fe_3O_4@SiO_2$ nanoparticles	24.11 µg	5.95	NA/NG	5.3 h	[88]
laccase	Cu^{2+} modified recyclable magnetite nanoparticles, Fe_3O_4-NH_2	2.5 mg mL^{-1}	4.5	65	72 h	[87]
laccase	magnetic nanoparticles (MNPs)	NA/NG	5	50	9 h	[90]
laccase	nanoparticles of magnetic iron oxide (Fe_3O_4) and copper ferrite ($CuFe_2O_4$)	0.1 mL/mL	4	25	4 h	[91]
laccase	magnetic amino-functionalized metal–organic framework Fe_3O_4 -NH_2@MIL-101(Cr)	6 mg·ml^{-1}	3	20-30	5 h	[92]
laccase	Copper ferrite magnetic nanoparticles (CuMNPs)	1:5 enzyme:nanoparticles (NPs) ratio	5	50	9 h	[89]

4.5. Other factors

Great efforts are being made to remove organic pollutants because of their hazardous effect on the ecosystem and the living organisms present in it because of their toxicity. So, the target of remediation techniques (like enzymatic degradation) is the elimination of the toxicity of the organic pollutants[77,78]. Also, the structure of organic pollutants can affect the efficiency of catalytic removal of pollutants. As in previous studies, Enzymes degrade the organic pollutants through the binding and orientation of these substances within the enzyme's active sites. Consequently, the degradation potential of the enzymes directly related to the organic pollutants structures. [79,80,81]. Cost and practical considerations such as ease of production, handling, and downstream processing are essential factors that must be taken into account in the selection of the immobilization strategy. Because they can influence the feasibility and scalability of the immobilization process.

When taking the immobilization efficiency into account, it was observed that immobilization circumstances play a significant role. So the immobilization circumstances should be optimized, for instance, the impact of protein weight ratio to linker and immobilization carrier (e.g., GTA), the concentration of protein and support, and time of immobilization are vital characteristics that specify the residual enzyme activity. GTA concentration is typically less than 3%, and the duration of incubation varies depending on the specific enzyme. The optimization process is crucial for the immobilization efficiency, enzyme stability, catalytic activity, and reusability of immobilized enzyme. Studies that have previously optimized the immobilization conditions have often improved the immobilization yield and efficiencies[82,83,84,85,86,87,88,89], table (2) presents some recent studies of the optimized immobilization conditions.

5. Magnetic Nanoparticles as a Precise Matrix for Laccase Immobilization

Recently, nanoparticles have been extensively utilized as carrier agents for the immobilized enzymes in biocatalytic systems; this trend is owing to their high surface area, which leads to a high capacity of loading, in addition to enhancing the biocatalytic activity of enzymes [93]. To immobilize enzymes, a variety of materials have been created as nanoparticles, these materials included silica, chitosan, nanotubes of carbon, nanofibers of cellulose, nanocomposites through the combination of certain nanomaterials and iron oxide nanometals [94]. Surface modifications of these nanomaterials, such as silanization, activation of carbodiimide, and crosslinking with glutaraldehyde, among others, can contribute in the conjunction of the enzyme and the support by creating single or multi-point systems through covalent interactions [95].

Table 3. Catalytic properties of free and immobilized laccase with magnetic carrier of recently reported.

Laccase source	**Magnetic Carrier**	**Kinetic parameters**		**Thermal Stability**	**Operational stability**	**Ref.**
		Km (µM)	**Vmax (µM/min)**			
Cerrena sp. HYB07	Modified silicon dioxide coated ferroferric oxide magnetic nanoparticles	14.8 81.4 22.5 28.4 59.7	4.5 x 10^3 22.8 x 10^3 13.32x 10^3 11.6 x 10^3 111.0x 10^3	At 50 ◦C, t $(_{1/2})$(h) 1.6 1.7 2.3 1.6 Fold of that for free lac.	7 cycles: 53 % 7 cycles: 52 % 8 cycles: 61 % 8 cycles: 51 % ----------	[90]
Trametes versicolor	Laccase@$CuFe_2O_4$ Laccase@Fe_3O_4 free laccase	(mg/mL) 3.64 3.76 3.65	(U/mL) 34.80 30.93 33.60	Laccase@$CuFe_2O_4$ and Laccase@Fe_3O_4 retained 71% and 61% of their activity at 70.0 ◦C	Residual activity was 70 % for both the nanobiocatalysts after 6 cycles	[91]
Trametes versicolor	Magnetic amino-modified metal–organic framework Fe_3O_4-NH_2@MIL-101(Cr)	Free lac. 0.1 mM Immob. 0.7 mM	Free lac. 1.9 Immob. 1.2	Free and immob. laccase retained 34% and 87% of their initial activity at 45 ◦C	Residual activity was 92 % for the nanobiocatalyst after 5 cycles	[92]
Trametes versicolor	Amino-functionalized magnetic nanoparticles (MNPs)	NA/NG	NA/NG	At 40 °C, Free laccase loses 50% of its initial activity and at 60 °C almost completely inactivates. At 20–30 °C, immobilized laccase retained relative activity > 90%	After four cycles, immobilized laccase preserved 70% of its initial activity, while after eight cycles, it retained 30%.	[100]
Aspergillus oryzae	Amino-functionalized ionic Liquid-modified magnetic chitosan nanoparticles	NA/NG	NA/NG	Free and immobilized laccase retained 7 % and 70 % of their initial activity at 60°C for 7 h, respectively.	Immobilized laccase retained 93.2% of its initial activity after 6 operational cycles.	[101]
Trametes versicolor	Magnetic iron nanoparticles	0.58 mM	0.25 mM min^{-1}	The lowest activity of free and immob. enzyme was recorded at 60 °C	95% of residual activity was retained after 5 cycles	[102]

Aspergillus oryzae	Magnetic nanoparticles	NA/NG	NA/NG	The immobilization enhanced the activity of the enzyme	83.5% of residual activity was preserved after 6 cycles	[103]
Commercial laccase	Magnetic nanoparticles	0.0062 mM	0.062 mM min^{-1}	Half life time of immobilized enzyme at 60 °C was 1.5 h, while for free enzyme was 4.1 h	80%of residual activity, after 5 operational cycles	[104]
Trametes pubescens MB89	Glutaraldehyde cross-linking prior to entrapment in Ca-alginate beads	0.272 mM	0.869 mM min^{-1}	above 50ºC, the immobilized laccases retained 50% of their initial activities	Greater than 70% of residual activity was preserved after 10 cycles	[105]
Trametes versicolor IBL-04	Calcium alginate beads	77.5µ M	876.4 µM min^{-1}	After the incubation at 60 °C for 6 h, free and immobilized laccase retained about 3.7 % and 33.2% relative activity, respectively.	retained 68% of residual activity after 3 cycles	[106]
Laccase Novozymes 513003	Granular activated carbon (GAC)	NA/NG	37 µM/min	More than 85% of activity of immobilized laccase was retained, while 43% of free laccase activity was lost.	55% of residual activity was retained after 20 cycles	[107]
Alternia tenuissima	Calcium alginate with chitosan beads	4.375 U /mL	1250 U/ml/min	After 60 min at 60 ˚C the immobilized laccase superior the free enzyme by 10% relative activity	Residual activity was 58% after 19 cycles	[108]

Specifically, Fe3O4 magnetic nanoparticles have attracted considerable attention as support agents and carrier matrices for the immobilized enzymes in the immobilization technologies[96], as shown in table (3). This is owing to their unique properties, including a strong magnetic response, which enhances the reusability of immobilized enzymes by saving easy separation from the biocatalytic system by applying an external magnetic field. Furthermore, magnetic nanoparticles have low toxicity, easy surface modification and biocompatibility with biological materials, and high mass-transference [97,98]. The magnetic materials always tend to aggregate owing to their substantial surface area-to-volume ratio and magnetic dipole-dipole attractions [99].

6. Conclusions and Future Perspectives

Laccase immobilization onto magnetic nanoparticles as carriers have been shown to be a successful method for improving the catalytic properties of enzymes, such as the stability, reusability, and catalytic activity of enzymes for the bioremediation of organic pollutants. Magnetic nanoparticles present distinct benefits as immobilization matrices, owing to their high surface area, ease of recovery, and reusability by applying an external magnetic field. This was proved by the recent studies that demonstrated the ability of various laccase-magnetic nanoparticles biocatalytic systems in the removal and degradation of a wide range of contaminants, like organic dyes, phenols, and pharmaceutical residues. Nevertheless, a number of obstacles need to be resolved before the practical application of these bio-catalytic systems. So, further studies are required to optimize

the immobilization process, considering enzyme loading and operational conditions (temperature, pH, presence of mediators, and reaction time). Furthermore, the long-term stability and reusability of the immobilized enzymes should be assessed under actual environmental circumstances.

Developing scalable and affordable immobilization strategies ought to be another priority, moreover to the investigation of alternative magnetic nanoparticles or nanocomposites with improved qualities. More effective pollutants removal biocatalytic systems, can be obtained by the integration of immobilized laccase system with other treatment technologies, such as membrane filtration or advanced oxidation processes.

In general, immobilization of laccase enzyme on the surface of modified magnetic nanoparticles offers a promising avenue for the creation of sustainable and environmentally friendly bioremediation techniques. Researchers should make more efforts in this area to develop a more sustainable future and circular economy.

7. Conflicts of interest

There are no conflicts to declare.

8. References

[1] L. Aleya, M.S. Uddin, Environmental pollutants and the risk of neurological disorders, Environmental Science and Pollution Research 27 (2020) 44657-44658.
[2] I. Ali, New generation adsorbents for water treatment, Chemical reviews 112 (2012) 5073-5091.
[3] Z. Daud, H. Awang, N. Nasir, M.B. Ridzuan, Z. Ahmad, Suspended solid, color, COD and oil and grease removal from biodiesel wastewater by coagulation and flocculation processes, Procedia-Social and Behavioral Sciences 195 (2015) 2407-2411.
[4] O.M. Ontanon, P.S. González, G.G. Barros, E. Agostini, Improvement of simultaneous Cr (VI) and phenol removal by an immobilised bacterial consortium and characterisation of biodegradation products, New biotechnology 37 (2017) 172-179.
[5] D. Bhatia, N.R. Sharma, J. Singh, R.S. Kanwar, Biological methods for textile dye removal from wastewater: A review, Critical Reviews in Environmental Science and Technology 47 (2017) 1836-1876.
[6] R.S. Monisha, R.L. Mani, B. Sivaprakash, N. Rajamohan, D.-V.N. Vo, Green remediation of pharmaceutical wastes using biochar: a review, Environmental Chemistry Letters (2022) 1-24.
[7] M. Meena, P. Sonigra, G. Yadav, Biological-based methods for the removal of volatile organic compounds (VOCs) and heavy metals, Environmental Science and Pollution Research 28 (2021) 2485-2508.
[8] V. Ivanov, V. Stabnikov, O. Stabnikova, S. Kawasaki, Environmental safety and biosafety in construction biotechnology, World Journal of Microbiology and Biotechnology 35 (2019) 1-11.
[9] T. Jesionowski, J. Zdarta, B. Krajewska, Enzyme immobilization by adsorption: a review, Adsorption 20 (2014) 801-821.
[10] J. Zdarta, A.S. Meyer, T. Jesionowski, M. Pinelo, Multi-faceted strategy based on enzyme immobilization with reactant adsorption and membrane technology for biocatalytic removal of pollutants: a critical review, Biotechnology advances 37 (2019) 107401.
[11] L.-J. Li, W.-J. Xia, G.-P. Ma, Y.-L. Chen, Y.-Y. Ma, A study on the enzymatic properties and reuse of cellulase immobilized with carbon nanotubes and sodium alginate, Amb Express 9 (2019) 1-8.
[12] M.D. Cannatelli, A.J. Ragauskas, Two decades of laccases: advancing sustainability in the chemical industry, The Chemical Record 17 (2017) 122-140.
[13] H. Claus, H. Decker, Bacterial tyrosinases, Systematic and applied microbiology 29 (2006) 3-14.
[14] N. Santhanam, J.M. Vivanco, S.R. Decker, K.F. Reardon, Expression of industrially relevant laccases: prokaryotic style, Trends in biotechnology 29 (2011) 480-489.
[15] C.M. Rivera-Hoyos, E.D. Morales-Álvarez, R.A. Poutou-Piñales, A.M. Pedroza-Rodríguez, R. RodrÍguez-Vázquez, J.M. Delgado-Boada, Fungal laccases, Fungal Biology Reviews 27 (2013) 67-82.
[16] G. Songulashvili, S. Flahaut, M. Demarez, C. Tricot, C. Bauvois, F. Debaste, M.J. Penninckx, High yield production in seven days of Coriolopsis gallica 1184 laccase at 50 L scale; enzyme purification and molecular characterization, Fungal biology 120 (2016) 481-488.
[17] N. Jaiswal, V.P. Pandey, U.N. Dwivedi, Purification of a thermostable alkaline laccase from papaya (Carica papaya) using affinity chromatography, International journal of biological macromolecules 72 (2015) 326-332.
[18] P. Baldrian, Fungal laccases–occurrence and properties, FEMS microbiology reviews 30 (2006) 215-242.
[19] O. Morozova, G. Shumakovich, S. Shleev, Y.I. Yaropolov, Laccase-mediator systems and their applications: a review, Applied Biochemistry and Microbiology 43 (2007) 523-535.
[20] F.J. Enguita, L.O. Martins, A.O. Henriques, M.A. Carrondo, Crystal structure of a bacterial endospore coat component: a laccase with enhanced thermostability properties, Journal of Biological Chemistry 278 (2003) 19416-19425.
[21] S. Garavaglia, M.T. Cambria, M. Miglio, S. Ragusa, V. Iacobazzi, F. Palmieri, C. D'Ambrosio, A. Scaloni, M. Rizzi, The structure of Rigidoporus lignosus laccase containing a full complement of copper ions, reveals an asymmetrical arrangement for the T3 copper pair, Journal of Molecular Biology 342 (2004) 1519-1531.
[22] I. Matera, A. Gullotto, S. Tilli, M. Ferraroni, A. Scozzafava, F. Briganti, Crystal structure of the blue multicopper oxidase from the white-rot fungus Trametes trogii complexed with p-toluate, Inorganica Chimica Acta 361 (2008) 4129-4137.

[23] S.V. Surwase, S.A. Patil, S. Srinivas, J.P. Jadhav, Interaction of small molecules with fungal laccase: a surface plasmon resonance based study, Enzyme and Microbial Technology 82 (2016) 110-114.
[24] A. Kunamneni, I. Ghazi, S. Camarero, A. Ballesteros, F.J. Plou, M. Alcalde, Decolorization of synthetic dyes by laccase immobilized on epoxy-activated carriers, Process Biochemistry 43 (2008) 169-178.
[25] A. Xenakis, M. Zoumpanioti, H. Stamatis, Enzymatic reactions in structured surfactant-free microemulsions, Current opinion in colloid & interface science 22 (2016) 41-45.
[26] S. Datta, R. Veena, M.S. Samuel, E. Selvarajan, Immobilization of laccases and applications for the detection and remediation of pollutants: a review, Environmental Chemistry Letters 19 (2021) 521-538.
[27] S. Pang, Y. Wu, X. Zhang, B. Li, J. Ouyang, M. Ding, Immobilization of laccase via adsorption onto bimodal mesoporous Zr-MOF, Process Biochemistry 51 (2016) 229-239.
[28] F. Zheng, B.-K. Cui, X.-J. Wu, G. Meng, H.-X. Liu, J. Si, Immobilization of laccase onto chitosan beads to enhance its capability to degrade synthetic dyes, International Biodeterioration & Biodegradation 110 (2016) 69-78.
[29] S. Nicotra, M.R. Cramarossa, A. Mucci, U.M. Pagnoni, S. Riva, L. Forti, Biotransformation of resveratrol: synthesis of trans-dehydrodimers catalyzed by laccases from Myceliophtora thermophyla and from Trametes pubescens, Tetrahedron 60 (2004) 595-600.
[30] R.A. Sheldon, S. van Pelt, Enzyme immobilisation in biocatalysis: why, what and how, Chemical Society Reviews 42 (2013) 6223-6235.
[31] M.A. Rao, R. Scelza, F. Acevedo, M. Diez, L. Gianfreda, Enzymes as useful tools for environmental purposes, Chemosphere 107 (2014) 145-162.
[32] J. Mehta, N. Bhardwaj, S.K. Bhardwaj, K.-H. Kim, A. Deep, Recent advances in enzyme immobilization techniques: Metal-organic frameworks as novel substrates, Coordination Chemistry Reviews 322 (2016) 30-40.
[33] D. NOUROUZIAN, Enzyme immobilization: the state of art in biotechnology, (2003).
[34] J. Bié, B. Sepodes, P.C. Fernandes, M.H. Ribeiro, Enzyme immobilization and co-immobilization: main framework, advances and some applications, Processes 10 (2022) 494.
[35] J. Kim, J.W. Grate, P. Wang, Nanostructures for enzyme stabilization, Chemical engineering science 61 (2006) 1017-1026.
[36] H. Zaak, E.-H. Siar, J.F. Kornecki, L. Fernandez-Lopez, S.G. Pedrero, J.J. Virgen-Ortíz, R. Fernandez-Lafuente, Effect of immobilization rate and enzyme crowding on enzyme stability under different conditions. The case of lipase from Thermomyces lanuginosus immobilized on octyl agarose beads, Process Biochemistry 56 (2017) 117-123.
[37] C. Rodriguez, I. Lavandera, V. Gotor, Recent advances in cofactor regeneration systems applied to biocatalyzed oxidative processes, Current Organic Chemistry 16 (2012) 2525-2541.
[38] W. Tischer, F. Wedekind, Immobilized enzymes: methods and applications, Biocatalysis-from discovery to application (1999) 95-126.
[39] C. Spahn, S.D. Minteer, Enzyme immobilization in biotechnology, Recent patents on engineering 2 (2008) 195-200.
[40] A.A. Khan, M.A. Alzohairy, Recent advances and applications of immobilized enzyme technologies: a review, Res J Biol Sci 5 (2010) 565-575.
[41] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente, Improvement of enzyme activity, stability and selectivity via immobilization techniques, Enzyme and microbial technology 40 (2007) 1451-1463.
[42] C.-H. Lee, T.-S. Lin, C.-Y. Mou, Mesoporous materials for encapsulating enzymes, Nano today 4 (2009) 165-179.
[43] E. Magner, Immobilisation of enzymes on mesoporous silicate materials, Chemical Society Reviews 42 (2013) 6213-6222.
[44] D.N. Tran, K.J. Balkus Jr, Perspective of recent progress in immobilization of enzymes, Acs Catalysis 1 (2011) 956-968.
[45] P. Zucca, E. Sanjust, Inorganic materials as supports for covalent enzyme immobilization: methods and mechanisms, Molecules 19 (2014) 14139-14194.
[46] C. Garcia-Galan, Á. Berenguer-Murcia, R. Fernandez-Lafuente, R.C. Rodrigues, Potential of different enzyme immobilization strategies to improve enzyme performance, Advanced Synthesis & Catalysis 353 (2011) 2885-2904.
[47] H.H. Weetall, Preparation of immobilized proteins covalently coupled through silane coupling agents to inorganic supports, Applied biochemistry and biotechnology 41 (1993) 157-188.
[48] R.M. Blanco, J.J. Calvete, J. Guisán, Immobilization-stabilization of enzymes; variables that control the intensity of the trypsin (amine)-agarose (aldehyde) multipoint attachment, Enzyme and microbial technology 11 (1989) 353-359.
[49] J.M. Guisan, Immobilization of enzymes and cells, Springer, 2006.
[50] C. Wu, G. Zhou, X. Jiang, J. Ma, H. Zhang, H. Song, Active biocatalysts based on Candida rugosa lipase immobilized in vesicular silica, Process Biochemistry 47 (2012) 953-959.
[51] P. Gemeiner, Materials for enzyme engineering, Ellis Horwood, New York, NY, 1992, pp. 13-119.
[52] H. Fang, J. Huang, L. Ding, M. Li, Z. Chen, Preparation of magnetic chitosan nanoparticles and immobilization of laccase, Journal of Wuhan University of Technology-Mater. Sci. Ed. 24 (2009) 42-47.
[53] M.F. Vieira, A.M.S. Vieira, G.M. Zanin, P.W. Tardioli, C. Mateo, J.M. Guisán, β-Glucosidase immobilized and stabilized on agarose matrix functionalized with distinct reactive groups, Journal of Molecular Catalysis B: Enzymatic 69 (2011) 47-53.
[54] T. Bala, B. Prasad, M. Sastry, M.U. Kahaly, U.V. Waghmare, Interaction of different metal ions with carboxylic acid group: a quantitative study, The Journal of Physical Chemistry A 111 (2007) 6183-6190.
[55] C.S. Farinas, P.C. Reis, H.C. Ferraz, V.M. Salim, T.L. Alves, Adsorption of myoglobin onto hydroxyapatite modified with metal ions, Adsorption Science & Technology 25 (2007) 717-727.

[56] M.R. Javed, A. Buthe, M.H. Rashid, P. Wang, Cost-efficient entrapment of β-glucosidase in nanoscale latex and silicone polymeric thin films for use as stable biocatalysts, Food chemistry 190 (2016) 1078-1085.
[57] L. Lu, M. Zhao, B.-B. Zhang, S.-Y. Yu, X.-J. Bian, W. Wang, Y. Wang, Purification and characterization of laccase from Pycnoporus sanguineus and decolorization of an anthraquinone dye by the enzyme, Applied Microbiology and Biotechnology 74 (2007) 1232-1239.
[58] N.R. Mohamad, N.H.C. Marzuki, N.A. Buang, F. Huyop, R.A. Wahab, An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes, Biotechnology & Biotechnological Equipment 29 (2015) 205-220.
[59] D. Brady, J. Jordaan, Advances in enzyme immobilisation, Biotechnology letters 31 (2009) 1639-1650.
[60] M. Fernandez-Fernandez, M.Á. Sanromán, D. Moldes, Recent developments and applications of immobilized laccase, Biotechnology advances 31 (2013) 1808-1825.
[61] R. Sheldon, Cross-linked enzyme aggregates (CLEA® s): Stable and recyclable biocatalysts, Biochemical Society Transactions 35 (2007) 1583-1587.
[62] D.M. ARRoyo, Inmovilización de enzimas. Fundamentos, métodos y aplicaciones, Ars Pharmaceutica (Internet) 39 (1998) 23-39.
[63] K. Periyasamy, L. Santhalembi, G. Mortha, M. Aurousseau, S. Subramanian, Carrier-free co-immobilization of xylanase, cellulase and β-1, 3-glucanase as combined cross-linked enzyme aggregates (combi-CLEAs) for one-pot saccharification of sugarcane bagasse, RSC advances 6 (2016) 32849-32857.
[64] Y. Dai, J. Yao, Y. Song, X. Liu, S. Wang, Y. Yuan, Enhanced performance of immobilized laccase in electrospun fibrous membranes by carbon nanotubes modification and its application for bisphenol A removal from water, Journal of hazardous materials 317 (2016) 485-493.
[65] M.S.L.F. Kargi, M. DeLisa, Bioprocess engineering: basic concepts, Prentice Hall, 2017.
[66] R. Xu, Y. Si, X. Wu, F. Li, B. Zhang, Triclosan removal by laccase immobilized on mesoporous nanofibers: strong adsorption and efficient degradation, Chemical Engineering Journal 255 (2014) 63-70.
[67] J. Zdarta, K. Antecka, R. Frankowski, A. Zgoła-Grześkowiak, H. Ehrlich, T. Jesionowski, The effect of operational parameters on the biodegradation of bisphenols by Trametes versicolor laccase immobilized on Hippospongia communis spongin scaffolds, Science of the Total Environment 615 (2018) 784-795.
[68] R.A. Alberty, V. Bloomfield, Multiple Intermediates in Steady State Enzyme Kinetics: V. EFFECT OF pH ON THE RATE OF A SIMPLE ENZYMATIC REACTION, Journal of Biological Chemistry 238 (1963) 2804-2810.
[69] I.E. Touahar, L. Haroune, S. Ba, J.-P. Bellenger, H. Cabana, Characterization of combined cross-linked enzyme aggregates from laccase, versatile peroxidase and glucose oxidase, and their utilization for the elimination of pharmaceuticals, Science of the Total Environment 481 (2014) 90-99.
[70] R. Xu, C. Chi, F. Li, B. Zhang, Laccase–polyacrylonitrile nanofibrous membrane: highly immobilized, stable, reusable, and efficacious for 2, 4, 6-trichlorophenol removal, ACS applied materials & interfaces 5 (2013) 12554-12560.
[71] Q.-Y. Li, P.-Y. Wang, Y.-L. Zhou, Z.-R. Nie, Q. Wei, A magnetic mesoporous SiO 2/Fe 3 O 4 hollow microsphere with a novel network-like composite shell: Synthesis and application on laccase immobilization, Journal of Sol-Gel Science and Technology 78 (2016) 523-530.
[72] G. Bayramoğlu, M.Y. Arıca, Immobilization of laccase onto poly (glycidylmethacrylate) brush grafted poly (hydroxyethylmethacrylate) films: Enzymatic oxidation of phenolic compounds, Materials Science and Engineering: C 29 (2009) 1990-1997.
[73] Y. Liu, Z. Zeng, G. Zeng, L. Tang, Y. Pang, Z. Li, C. Liu, X. Lei, M. Wu, P. Ren, Immobilization of laccase on magnetic bimodal mesoporous carbon and the application in the removal of phenolic compounds, Bioresource Technology 115 (2012) 21-26.
[74] G. Bayramoglu, M. Yilmaz, M. Yakup Arica, Preparation and characterization of epoxy-functionalized magnetic chitosan beads: laccase immobilized for degradation of reactive dyes, Bioprocess and biosystems engineering 33 (2010) 439-448.
[75] Y. Tanaka, M.N. Slyadnev, K. Sato, M. Tokeshi, H.-B. Kim, T. Kitamori, Acceleration of an enzymatic reaction in a microchip, Analytical sciences 17 (2001) 809-810.
[76] L. Martins, B. Baêta, L. Gurgel, S. Aquino, L. Gil, Application of cellulose-immobilized riboflavin as a redox mediator for anaerobic degradation of a model azo dye Remazol Golden Yellow RNL, Industrial Crops and Products 65 (2015) 454-462.
[77] A. Anastasi, T. Coppola, V. Prigione, G. Varese, Pyrene Degradation and Detoxification in Soil by a Consortium of Basidiomycetes Isolated from Compost: Role of Laccases and Peroxidases, Journal of hazardous materials 165 (2008) 1229-1233.
[78] M. Bilal, M. Asgher, H. Hu, X. Zhang, Chitosan beads immobilized manganese peroxidase catalytic potential for detoxification and decolorization of textile effluent, International Journal of Biological Macromolecules 89 (2016).
[79] F. Jamal, T. Qidwai, D.P. Pandey, R. Singh, S. Singh, Azo and anthraquinone dye decolorization in relation to its molecular structure using soluble Trichosanthes dioica peroxidase supplemented with redox mediator, Catalysis Communications 12 (2011) 1218-1223.
[80] N. Veitch, Veitch, N. C. Horseradish peroxidase: a modern view of a classic enzyme. Phytochemistry 65, 249-259, Phytochemistry 65 (2004) 249-259.
[81] M. Sundaramoorthy, J. Terner, T. Poulos, Stereochemistry of the chloroperoxidase active site: Crystallographic and molecular-modeling studies, Chemistry & biology 5 (1998) 461-473.

[82] R.E. Abraham, M.L. Verma, C.J. Barrow, M. Puri, Suitability of magnetic nanoparticle immobilised cellulases in enhancing enzymatic saccharification of pretreated hemp biomass, Biotechnology for biofuels 7 (2014) 1-12.
[83] D. Sillu, S. Agnihotri, Cellulase immobilization onto magnetic halloysite nanotubes: enhanced enzyme activity and stability with high cellulose saccharification, ACS sustainable chemistry & engineering 8 (2019) 900-913.
[84] A. Bhattacharya, B.I. Pletschke, Magnetic cross-linked enzyme aggregates (CLEAs): a novel concept towards carrier free immobilization of lignocellulolytic enzymes, Enzyme and Microbial Technology 61 (2014) 17-27.
[85] K. Periyasamy, L. Santhalembi, G.r. Mortha, M. Aurousseau, A.s. Boyer, S. Subramanian, Bioconversion of lignocellulosic biomass to fermentable sugars by immobilized magnetic cellulolytic enzyme cocktails, Langmuir 34 (2018) 6546-6555.
[86] S. Ariaeenejad, E. Motamedi, G.H. Salekdeh, Immobilization of enzyme cocktails on dopamine functionalized magnetic cellulose nanocrystals to enhance sugar bioconversion: A biomass reusing loop, Carbohydrate Polymers 256 (2021) 117511.
[87] Z. Gou, N.L. Ma, W. Zhang, Z. Lei, Y. Su, C. Sun, G. Wang, H. Chen, S. Zhang, G. Chen, Innovative hydrolysis of corn stover biowaste by modified magnetite laccase immobilized nanoparticles, Environmental research 188 (2020) 109829.
[88] S. Shanmugam, S. Krishnaswamy, R. Chandrababu, U. Veerabagu, A. Pugazhendhi, T. Mathimani, Optimal immobilization of Trichoderma asperellum laccase on polymer coated Fe3O4@ SiO2 nanoparticles for enhanced biohydrogen production from delignified lignocellulosic biomass, Fuel 273 (2020) 117777.
[89] K.S. Muthuvelu, R. Rajarathinam, R.N. Selvaraj, V.B. Rajendren, A novel method for improving laccase activity by immobilization onto copper ferrite nanoparticles for lignin degradation, International journal of biological macromolecules 152 (2020) 1098-1107.
[90] X. Xu, T. Chen, L. Xu, J. Lin, Immobilization of laccase on magnetic nanoparticles for enhanced polymerization of phenols, Enzyme and Microbial Technology 172 (2024) 110331.
[91] N.S. Alsaiari, A. Amari, K.M. Katubi, F.M. Alzahrani, H.N. Harharah, F.B. Rebah, M.A. Tahoon, The biocatalytic degradation of organic dyes using laccase immobilized magnetic nanoparticles, Applied Sciences 11 (2021) 8216.
[92] A. Amari, F.M. Alzahrani, N.S. Alsaiari, K.M. Katubi, F.B. Rebah, M.A. Tahoon, Magnetic metal organic framework immobilized laccase for wastewater decolorization, Processes 9 (2021) 774.
[93] E.T. Hwang, M.B. Gu, Enzyme stabilization by nano/microsized hybrid materials, Engineering in Life Sciences 13 (2013) 49-61.
[94] M. Puri, C.J. Barrow, M.L. Verma, Enzyme immobilization on nanomaterials for biofuel production, Trends in Biotechnology 31 (2013) 215-216.
[95] E.P. Cipolatti, M.J.A. Silva, M. Klein, V. Feddern, M.M.C. Feltes, J.V. Oliveira, J.L. Ninow, D. de Oliveira, Current status and trends in enzymatic nanoimmobilization, Journal of Molecular Catalysis B: Enzymatic 99 (2014) 56-67.
[96] H. Markides, M. Rotherham, A. El Haj, Biocompatibility and toxicity of magnetic nanoparticles in regenerative medicine, Journal of Nanomaterials 2012 (2012).
[97] H. Vaghari, H. Jafarizadeh-Malmiri, M. Mohammadlou, A. Berenjian, N. Anarjan, N. Jafari, S. Nasiri, Application of magnetic nanoparticles in smart enzyme immobilization, Biotechnology letters 38 (2016) 223-233.
[98] L.M. Rossi, N.J. Costa, F.P. Silva, R. Wojcieszak, Magnetic nanomaterials in catalysis: advanced catalysts for magnetic separation and beyond, Green Chemistry 16 (2014) 2906-2933.
[99] J. Alftrén, T.J. Hobley, Covalent immobilization of β-glucosidase on magnetic particles for lignocellulose hydrolysis, Applied biochemistry and biotechnology 169 (2013) 2076-2087.
[100] X. Chen, B. He, M. Feng, D. Zhao, J. Sun, Immobilized laccase on magnetic nanoparticles for enhanced lignin model compounds degradation, Chinese Journal of Chemical Engineering 28 (2020) 2152-2159.
[101] X. Qiu, S. Wang, S. Miao, H. Suo, H. Xu, Y. Hu, Co-immobilization of laccase and ABTS onto amino-functionalized ionic liquid-modified magnetic chitosan nanoparticles for pollutants removal, Journal of Hazardous Materials 401 (2021) 123353.
[102] A. Das, J. Singh, K. Yogalakshmi, Laccase immobilized magnetic iron nanoparticles: fabrication and its performance evaluation in chlorpyrifos degradation, International Biodeterioration & Biodegradation 117 (2017) 183-189.
[103] X. Qiu, Y. Wang, Y. Xue, W. Li, Y. Hu, Laccase immobilized on magnetic nanoparticles modified by amino-functionalized ionic liquid via dialdehyde starch for phenolic compounds biodegradation, Chemical Engineering Journal 391 (2020) 123564.
[104] C.C. Fortes, A.L. Daniel-da-Silva, A.M. Xavier, A.P. Tavares, Optimization of enzyme immobilization on functionalized magnetic nanoparticles for laccase biocatalytic reactions, Chemical Engineering and Processing: Process Intensification 117 (2017) 1-8.
[105] F. Lassouane, H. Aït-Amar, S. Amrani, S. Rodriguez-Couto, A promising laccase immobilization approach for Bisphenol A removal from aqueous solutions, Bioresource technology 271 (2019) 360-367.
[106] S. Noreen, M. Asgher, F. Hussain, A. Iqbal, Performance improvement of Ca-alginate bead cross-linked laccase from Trametes versicolor IBL-04, BioResources 11 (2016) 558-572.
[107] L.N. Nguyen, F.I. Hai, A. Dosseto, C. Richardson, W.E. Price, L.D. Nghiem, Continuous adsorption and biotransformation of micropollutants by granular activated carbon-bound laccase in a packed-bed enzyme reactor, Bioresource Technology 210 (2016) 108-116.
[108] A.A. Abd El Aty, F.A. Mostafa, M.E. Hassan, E.R. Hamed, M.A. Esawy, Covalent immobilization of Alternaria tenuissima KM651985 laccase and some applied aspects, Biocatalysis and Agricultural Biotechnology 9 (2017) 74-81.