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A Short Review on Recent Development of Laccase Immobilization on Different Support Materials

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Abstract: Laccase is a bio catalytic agent and multi-copper enzyme containing oxidases that are potentially great for oxidizing large number of phenolic and non-phenolic compounds. However, drawbacks do arise when laccase use in large scale; low in stability, high production cost, non-reusability, sensitive towards denaturing and poor storage ability of free enzymes. These problems lead to the progress in laccase immobilization in order to facilitate the efficient recovery and re-use of the enzyme, thus enabling cost-effective in continuous processes. Apart from discussing on different methods in laccase immobilization such as entrapment, encapsulation and cross-linking in general, we have reviewed a recent development in laccase immobilization on different supports or carriers binding (natural and synthetic). Future works are recommended to focus on innovative strategies on the modified supports to improve the enzyme immobilization as well as sensible entrapment techniques for industrial applications.

Keywords: Laccase, immobilization, support materials

1. Introduction

For decades, enzymes have been a promising tool to enhance performance yield in biotechnology industry [1]. It acts as biocatalyst in biological process and is environmentally friendly. In the recent years, extensive research in application of laccases as sustainable and green biocatalyst in the textile, food, pulp and paper, cosmetic and pharmaceutical industries have been discussed [2] [3] [4].

1.1 Laccase

Laccase was initially discovered from the Japanese Rhus vernicifera lacustrine tree [5]. It is widely dispersed in bacteria, plant, fungi and shows various functions depending on their source organism, physiological and pathological conditions [5]. *S.lavendulae, S.cyaneus* and *Marinomonas mediterranea* are the example of bacteria with laccase enzyme while in plants, laccases are found in potatoes, tomatoes, apples, cabbages, pears, turnip and other vegetables [6]. Laccase plays a role in lignification whereas in fungi it has been implicated in delignification, sporulation, pigment production, fruiting body formation and plant pathogenesis. Nearly all wood decaying fungi such as *Trametes versicolor, Trametes gallica, Trametes hirsuta, Trametes ochracca, Trametes villosa, Lentinus tigrinus, Pleurotus eryngii* and *Ganoderma* are the common producers of laccase [7].

In addition, two studies reported that laccase belongs to multi-copper polyphenol oxidases with low substrate specificity which can break down a wide range of compounds including recalcitrant dyestuff and other organic contaminants [7] [8]. Due to the catalytic and eco-sustainability, this biocatalyst has wider functions in multisector industries especially those related to aromatic and non-aromatic water-based compound such as wastewater detoxification, lignin valorisation, organic synthesis and dye discoloration [9] [10].

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However, the relatively low stability due to activity loss, non-reusability, sensitive to changing conditions of the reaction environment especially in industry settings and high production costs of free laccase limit its applicability [11] [5]. Hence, modification of free laccase to enhance their properties has been discussed extensively to improve its limitation by immobilizing free laccase on various insoluble support [12].

2. Methods of Laccase Immobilization

Enzyme immobilization is defined as the attachment of soluble enzymes to a support material that results in the reduction or total loss in mobility of the attached enzyme [13]. Immobilizing laccase on various supports can protect them from denaturation, improve their stability, maintain good catalytic efficiency, facilitate efficient recovery and lead to more economical process [10] [14]. Thus, it is important to define an appropriate and suitable support for laccase immobilization [10].

Different methods of immobilization will give different results. It is based on supports, mediators and application of laccase enzyme. Theoretically, there are four methods of laccase immobilization which are entrapment and adsorption – categorized as physical immobilization; covalent binding and crosslinking method – categorized as chemical immobilization [15] [13]. The summarize methods are provided in Fig. 1.

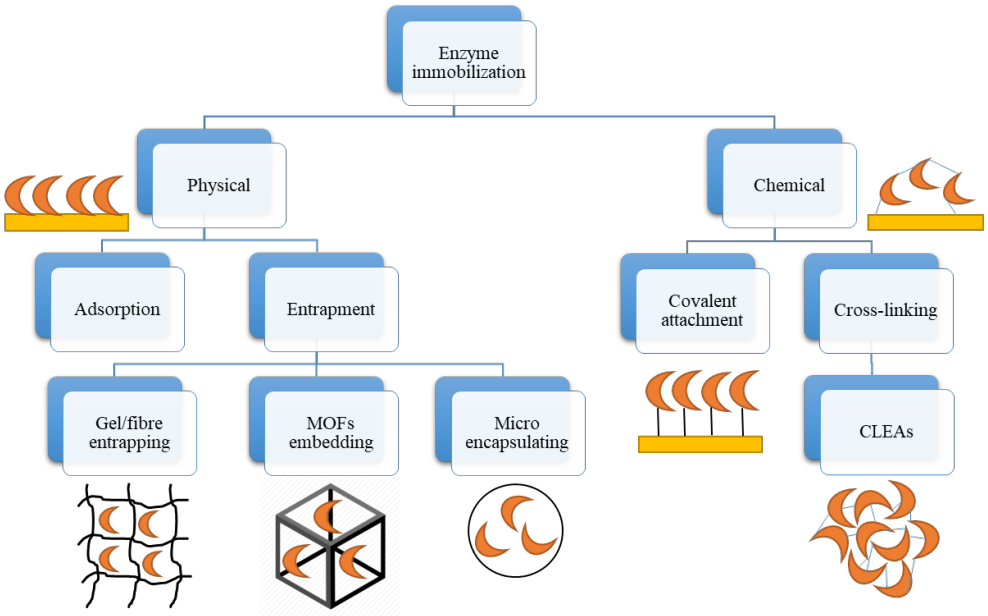

Fig. 1 - Different types of immobilization methods

2.1 Entrapment

Entrapment is defined as an immobilization technique that is physically trapped in a porous solid support which allows the path of substrates and products without changing the enzyme properties [16]. The commonly used supports for laccase immobilization are through microencapsulation, metal organic frameworks (MOF) or gel/fibre entrapping [13]. Organic supports such as polysaccharides, polymer and gelatine also are commonly used as enzyme support [17].

Entrapment known as an easy technique because there are no alterations to the enzyme as there are no covalent bonds formed between support and enzyme [15]. In addition, this technique ensures enzyme stability, reusability and retains high catalytic activities for several time of usage. However, this technique is not widely applicable in industrial scale due to the diffusion barrier that causes enzyme leakages from; large pore size distribution in support; and low binding process of substrate and active site of enzyme [18].

2.2 Adsorption

One of previous studies has reported that adsorption is the most scalable technique in the current trend of enzyme immobilization due to its high efficiency, affordability and simple mechanism [19] [20]. This is because, adsorption of enzyme has no mass transfer restriction which the easiest to perform and considers to be a low-cost technique for laccase immobilization – this might result to higher commercial potential [21]. Enzymes are physically adsorbed onto the support surface via weak forces such as ionic interaction, hydrogen bonds and Van der Waals interaction in

adsorption technique [13]. The immobilization process may be influenced by the pH and the ionic strength of the medium together with the hydrophobicity of the support [22]. However, the weak forces between the enzyme and support can be easily disrupted along the process which lead to poor operational stability and denaturation of the adsorbed enzyme [16].

2.3 Covalent Attachment

A sensible alternative to physical immobilization methods is by covalent attachment in which formation of covalent bonds between support and functional group of enzyme such as amino, carboxylic, tyrosine, and hydroxyl groups [16]. The support will be reactive when it binds with right active site of enzyme to forms strong bonds between support and enzyme. Otherwise, nonreactive carrier might be activated by another chemical called crosslinker which will be discussed next [23]. Strong bonding leads to high resistance in extreme operating conditions despite the prevention of leakage and desorption of enzyme in batch and continuous processes [24]. Unfortunately, covalent bonding may cause the alteration and destruction of enzyme active site during the reaction process thus resulting in major loss of enzymatic activity [25].

2.4 Cross-linking

Support free enzyme immobilization is another technique whereby no carrier or support involved. This technique also known as cross-linked enzyme aggregate (CLEA) involving the formation of intermolecular cross-linkage between enzyme molecules by crosslinkers. There are many types of linkers used nowadays such as glutaraldehyde, diazonium salt, diiminoesthers, diisocyanates and diamines activated by carbodiimide. In addition, the trending technology of CLEA impacts strongly in enzymatic activity and high resistance with easy recovery of enzymes [26]. The major drawback of this technique is the high amount of enzymes would be required that leads to difficulties in reaction control thus it's not a cost-effective technique [16].

3. Immobilization Support

Despite of choosing the suitable technique for enzyme immobilization, a good and suitable support plays an important role in enzymatic activity to form biocatalyst [5]. Enzymes are immobilized onto support can be used repeatedly and the reaction process will be stopped immediately by simply removing the carrier from the solution. The support should be cost-effective, easily available and eco-friendly and also conditioned by the surface, volume, porosity, shape, form and stability in given reaction condition [4]. Support materials can be divided into two main categories which are organic and inorganic carriers. Both categories have natural and synthetic supports. Natural polymers and synthetic polymers are characterized as organic support while natural minerals and synthetic minerals are characterized as inorganic support [16]. The classification or organic and inorganic supports is shown in Fig. 2.

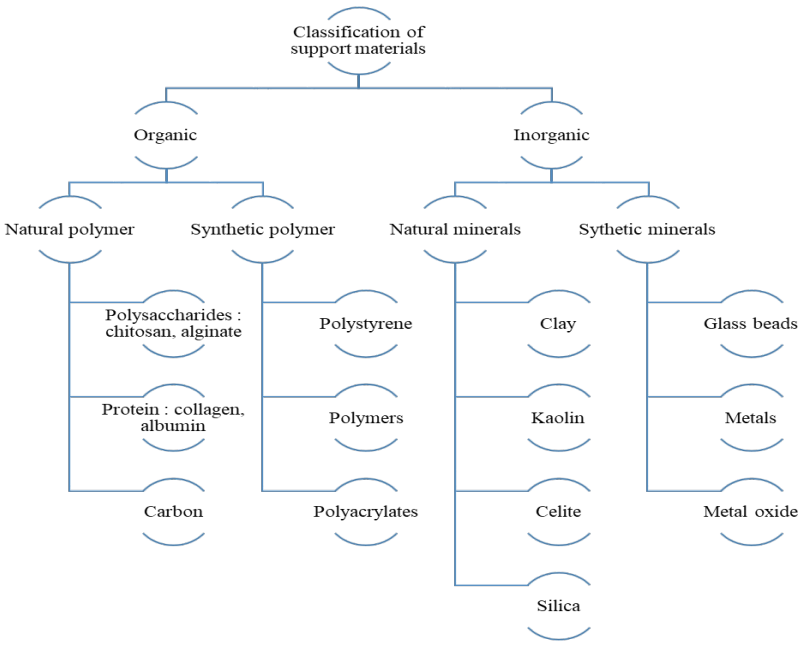

Fig. 2 - Classification of Organic and Inorganic support material

3.1 Organic Support

Organic supports are compounds of natural origin based on their chemical composition and they can be ready to be chemically modified thus fulfilling conditions of specific enzyme and its application [20]. In general, organic support can be divided into two categories which are natural polymer and synthetic polymer. The universal and common natural polymers are chitin, chitosan, alginate, collagen, cellulose and starch while the synthetic polymers comprise of polystyrene, polyacrylate, polyacrylamide, polyamides, silicons, epoxy resins and vinyl [5].

3.1.1 Natural Polymer

Natural polymers are widely use as it is biodegradable, biocompatible, non-toxic and has high affinity to proteins [5]. Numerous number of reactive functional groups mainly hydroxyl on the carrier, enhance the reaction between the support and enzyme thus lead to high catalytic activities [4]. Above all, these support materials are renewable and easy to obtain from by-products of industries which makes them inexpensive and lower the cost involved in the immobilization process.

Alginates are one of common enzymes used as support nowadays. It is made by cross linking the carboxyl group of α-L-guluronic acid from alginate with a solution of a cationic cross linker which contains enzymes such as calcium chloride, barium chloride or poly (L-lysine) [27]. Single or multiple enzymes can be immobilized in one alginate beads showing that entrapment and encapsulation methods are likely suitable. For instance, laccase is immobilized by crosslinking with glutaraldehyde prior to entrapment into Ca-alginate beads for the removal of Bisphenol A from aqueous solution [14]. The immobilization yield increased by 30% and reduced the leaking by 7-fold as compared to the immobilized laccase without crosslinking with glutaraldehyde. Moreover, a study reported that immobilized laccase into calcium alginate (Ca-AlL) and copper alginate (Cu-AlL) has higher operational stability and up to three cycles of enzyme reusability compared to free laccase for the bioremediation of Bisphenol A contaminated sites [28]. These indicate that immobilized laccase with alginate beads have greater potential in improving the current problem relating to waste water remediation especially when combine both support with crosslinker in the immobilization process.

Another natural polymer support that is quite popular – chitosan. Chitosan is the most important derivatives of chitin. It is derived by deacetylation process and composed of randomly distributed β-(1-4) linked D-glucosamine (deacetylated) and N-acetyl-D-glucosamine (acetylated) units. The interesting fact is that chitosan is likely to entrap bioactive substance such as protein and nucleic acid through chemically crosslinking method [29]. A report showed the immobilization of laccase on glutaraldehyde cross-linked chitosan beads retained the storage stability by 1.9 fold in which presenting approximately 90% of activity compared to the free enzyme after 28 days [7].

Other than chitosan, dual-functionalized cellulose beads encapsulated with ABTS are used as support material on which laccase is covalently immobilized on cellulose beads increase the degradation rate of indole up to 99.7% when it is difficult to degrade by free laccase [29]. New strategies on developing biodegradable and natural support material has come to an extent in which laccase that immobilized on amino-functionalized chicken feather has higher stability in a low-cost process [30]. It may be concluded that free laccase will have low performance in treating contaminated area unless it is immobilized to a support material. Plus, by using support alone does not produce high yield unless a crosslinker is used or embedded with mediator compare to stand alone support.

3.1.2 Synthetic Polymer

Variety types of organic synthetic polymer such as polystyrene, polyacrylate, polyacrylamide, polyamides and vinyl have been employed as support material in laccase immobilization. The massive advantage of synthetic polymer is that the polymeric chain can be selected according to the enzyme and process [4]. Moreover, laccase was encapsulated into poly ethylene glycol (PEG) hydrogel via UV assisted emulsion polymerization method preceding to crosslinking with glutaraldehyde for bisphenol A removal from aqueous solution [31]. The immobilized laccase successfully absorbed the chemical in a shorter time by 90% efficiency with eminent operational stability.

Furthermore, the removal of morphine from aqueous systems by immobilized laccase onto polypropylene beads through adsorption method showed a significant result as 70% of morphine drug successfully removed [32]. Same goes to a polymer that is abundantly carried cyclic epoxy and cyclic carbonate groups attached to laccase enzyme [3]. The polymer known as [poly(styrene-co-divinylbenzene))-graft-poly (glycidyl methacrylate)] "PS-co-DVB-g-P (GMA)" microsphere can store enormous enzyme loading due to high-catalytic activity and thus leads to significant result in degradation of Bisphenol A and Congo Red dye. In addition, it is stated that polypropylene chloride (PP) film acts as support that helps laccase enzyme to degrade three types of textile dye which are Procion Green H4G, Brilliant Blue G and crystal violet [12]. The positive outcome from the degradation process has proven that the immobilization of laccase on polypropylene chloride (PP) film escalates the stability of the enzyme in terms of thermal, storage and operational stability.

As stated above, synthetic polymer support has great stability at wide range of pH which efficiently removed contaminates from aqueous solution. However, the process is time-consuming and costly as the polymer itself needs to be synthesized according to the specific enzyme and process desired.

3.2 Inorganic Carrier

Inorganic support materials establish stable performance compared to organic supports due to their higher resistance in extreme operating conditions, good thermal and chemical stability as well as great mechanical resistance. Hence it becomes a promising support than organic support in real life application especially in wastewater treatment [16]. Inorganic support can be categorized into two main categories which are natural minerals and synthetic minerals. Kaolin, silica, clay and celite are the common natural mineral support applied in laccase immobilization while carbon-based material, ceramic material, graphene and graphene oxide, magnetic particle and nano-structured material are allocated in synthetic mineral category [4].

3.2.1 Natural Mineral

Silica is one of the popular natural mineral support used in enzyme immobilization. Silica has hydrophilic characteristic on the surface and a number of hydroxyl groups. This is one of the advantages of silica as support material as the hydroxyl groups are desirable in developing covalent bonds with enzyme despite through adsorption as well as encapsulation [4]. For instance, the adsorption of laccase enzyme into mesoporous silica prior to crosslinking each enzyme inside the silica using glutaraldehyde resulting in the removal of Bisphenol A in wastewater treatment [33]. The product gave a promising result whereby it can be used more than three times without decreasing the catalytic activity and shown better reusability. In another study, they used mesostructured cellular foam (MCF) silica and covalently bind to laccase enzyme from *Trametes versicolor* [34]. The biocatalyst produced were applied for the removal of 95% tetracycline from aqueous solution with higher catalytic activity compared to the free enzyme.

Another laccase derived from the same fungal as mentioned earlier, was immobilized onto kaolinite through physical adsorption method. The highest activity obtained was 839.01 U/g with better pH stability and operational stability while the catalytic activity retained above 50% with nearly 80% decolorization of malachite green dyes effluent after 5 cycles [11]. Moreover, another natural mineral support called bentonite was successfully immobilized with laccase enzyme through adsorption between ions on the surface layer of bentonite and laccase from *Trametes versicolor* fungal. The aim for the immobilization was to remove tetracycline contaminants that accumulated in waterways from pharmaceutical usage. The bentonite-Lac support has exhibit about 60% tetracycline removal within three hours with the presence of 1-hydroxybenzotriazol (HBT) [35].

In summary, laccase enzyme has been immobilized in various natural mineral support and applied for biodegradation of dyes, pharmaceutical-based contaminants and also endocrine disrupting compound. All problems mentioned are dangerous to human health hence, serious actions need to be taken by using biocatalyst to treat those problems in safe and promising result.

3.2.2 Synthetic Mineral

Inorganic synthetic materials such as graphene and graphene oxides (GOs), nano-structured materials such as nanoparticles (NPs), nanocomposite and carbon nanotubes (CNTs) have attracted high attention for their stability as support materials for enzyme immobilization [16]. Graphene and graphene oxide (GO) has unique characteristics such as biodegradable, two-dimensional structure, high surface area and pore volume as well as excellent thermal stability and chemically stable [4]. In addition, laccase enzyme from genetically modified *Aspergillus* was covalently immobilized onto nanobiocatalyst, graphene oxide [36]. The immobilized enzyme showed good operational stability and reusability when more than 75% of Direct Red 23 dye and Acid Blue 92 dye decolorized effectively after 6 cycles. Moreover, a report stated that immobilized laccase with a mixture of iron oxide nanoparticles, gallic acid and polyacrylic acid showed more than 8 cycles used has retained catalytic activity more than 57% [37]. An appealing study reported that laccase was immobilized onto nanoporous Zeolite-X (ZX) has retained 100% activity after 7 successive decolorization process of AB 225 dye [38].

Other than nanoparticles, many findings have been carried out on combining organic and inorganic support for laccase immobilization. The electrostatic force between magnetic nanoparticles and laccase-inorganic hybrid nanoflowers improved that 100% Bisphenol A degradation could achieve in 5 minutes with 92% retained activity after 60 days and only 5% loss of activity after 5 cycles used [39]. Besides that, Zirconia-Silica doped with Cu2+ were used as laccase support resulting in 90% of Remazol Brilliant Blue R (RBBR) dye decolorization while organic-inorganic nanocomposite between mesoporous silica, chitosan and carboxyl-functionalized ionic liquid as bridging agent can remove 2,4-dichlorophenol up to 90% in 35 hours [22] [40].

In short, nanostructured materials and hybrid materials showed significant output whereby the rate of degradation of dye effluents such as Direct Red 23, Acid Blue 92, AB 225 and degradation of endocrine disrupting compound such as Bisphenol A increased with good operational stability and reusability.

Table 1 - Enzyme immobilization on various support materials, methods of immobilization and applications

Support	Method	Application	Reference
Calcium alginate beads	Entrapment	Removal Bisphenol A from aqueous solution	(14)
Bentonite(BDMMs)	Adsorption	Removal of tetracycline	(35)
Porous polyvinyl alcohol(PVA) / halloysite hybrid beads(HNT)	Covalent	Dye removal	(41)
Glutaraldehyde-crosslinked (NH4)2SO4, glutaraldehyde-crosslinked MNPs, alginate beads, glutaraldehyde-crosslinked chitosan beads	CLEAs, M-CLEAs , Entrapment, Covalent	Malachite green decolorization	(8)
Magnetic metal organic framework (MOF), Fe3O4-NH2@MIL-101(Cr)	Adsorption and Covalent	Phenolic compound	(42)
Nonwoven polyethylene/polypropylene fibers	Glutaraldehyde cross-linking	Dye decolorization	(43)
Superparamagnetic iron oxide nanoparticles	Covalent	Dye decolorization	(37)
Uniform polyurea (PU) microsphere	Crosslinking using glutaraldehyde	Degradation of Remazol Brilliant Blue	(40)
Mesoporous silica SBA-15 and chitosan was combined using carboxyl-functionalized ionic liquid as the bridging agent (SBA-CIL-CS)	Physical adsorption	Chlorophenol removal	(22)
Glutaraldehyde cross-linked chitosan beads	Covalent	Degrade bisphenol A	(7)
Nanoporous zeolite-X	-	Dyes decolorization	(38)
Meso-MIL-53(Al)	Physical adsorption	Catalytic conversion of triclosan	(44)
Amino-functionalized magnetic metal organic framework	-	Phenolic compound removal	(42)
Poly(glycidyl methacrylate) microspheres	Covalent	Degradation of azinphos-methyl	(45)
Novel dual-functionalized cellulose beads	Covalent	Biodegradation of indole	(29)
Chicken Feather Derived Novel Support Material	Covalent	Oxidation of Veratryl Alcohol	(30)
Hollow mesoporous carbon nanospheres	Adsorption and Covalent	Antibiotic contaminants removal	(46)
Kaolinite	Adsorption	Treatment of malachite green effluent with the coexistence of Cd (Π)	(11)
E-CLEA	Entrapped cross-linked laccase aggregates	Catalytic phenol removal	(47)

Laccase-loaded magnetic nanoflowers	-	Degradation of bisphenol A	(39)
Citric acid functionalized micro-biochars derived from different feedstock	Covalent	Removal of diclofenac	(48)
Hollow fiber membranes	Covalent cross-linkage of the adsorbed laccase	Novel biodegradation system for bisphenol A	(49)
Multi-channel ceramic membrane with glutaraldehyde	Crosslinked	Bisphenol A degradation	(2)
Stabilized laccase in porous silica	-	High efficiency biotransformation of bisphenol A	(33)

4. Conclusion

Immobilized laccase emerge as green approach in effective degradation of various severe environmental and health problems caused by endocrine disrupting compound, dye effluent from textile industries and also from pharmaceutical waste. For example, a study immobilized laccase onto porous polyvinyl alcohol/halloysite hybrid beads for reactive blue dye removal [41]. Other than that, another study removed Bisphenol A from aqueous solution by immobilizing laccase by glutaraldehyde crosslinking prior to entrapment into Ca-alginate beads [14]. Contaminants from pharmaceutical waste were reported by [35] where the usage of bentonite as support in tetracycline removal and known as economical and eco-friendly biocatalyst which leads to wide opportunities for the elimination of micropollutants from wastewater.

Hence, the usage of green technology to solve environmental issues should be our main concern. Furthermore, as compared to free enzyme, immobilized enzyme has significantly excellent catalytic activity, longer storage and operational stability as well as enable reusability. The catalytic activity seems to be influenced by method of immobilization, support used, operating conditions and also different applications. Recent developments of laccase immobilization have demonstrated the incorporation of hybrid materials can enable a vast impact in wastewater treatment.

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References

[1] Karam, J., & Nicell, J. A. (1997). Potential applications of enzymes in waste treatment. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental AND Clean Technology, 69(2), 141-153. https://onlinelibrary.wiley.com/doi/abs/10.1002/(SICI)1097-4660(199706)69:2%3C141::AID-JCTB694%3E3.0.CO;2-U

[2] Barrios-Estrada, C., de Jesús Rostro-Alanis, M., Muñoz-Gutiérrez, B. D., Iqbal, H. M., Kannan, S., & Parra-Saldívar, R. (2018). Emergent contaminants: endocrine disruptors and their laccase-assisted degradation–a review. Science of the total environment, 612, 1516-1531. https://www.sciencedirect.com/science/article/pii/S0048969717323550

[3] Bayramoglu, G., Karagoz, B., & Arica, M. Y. (2018). Cyclic-carbonate functionalized polymer brushes on polymeric microspheres: Immobilized laccase for degradation of endocrine disturbing compounds. Journal of industrial and engineering chemistry, 60, 407-417. https://www.sciencedirect.com/science/article/pii/S1226086X17306330

[4] Zdarta, J., Meyer, A. S., Jesionowski, T., & Pinelo, M. (2018). Developments in support materials for immobilization of oxidoreductases: A comprehensive review. Advances in colloid and interface science, 258, 1-20. https://www.sciencedirect.com/science/article/pii/S0001868618300848

[5] Deska, M., & Kończak, B. (2019). Immobilized fungal laccase as" green catalyst" for the decolourization process–State of the art. Process Biochemistry, 84, 112-123. https://www.sciencedirect.com/science/article/pii/S1359511319300789

[6] Shekher, R., Sehgal, S., Kamthania, M., & Kumar, A. (2011). Laccase: microbial sources, production, purification, and potential biotechnological applications. Enzyme research, 2011. https://downloads.hindawi.com/archive/2011/217861.pdf

[7] Bilal, M., Iqbal, H. M., & Barceló, D. (2019). Mitigation of bisphenol A using an array of laccase-based robust bio-catalytic cues–a review. Science of The Total Environment, 689, 160-177. https://www.sciencedirect.com/science/article/pii/S0048969719329766

[8] Yang, J., Lin, Y., Yang, X., Ng, T. B., Ye, X., & Lin, J. (2017). Degradation of tetracycline by immobilized laccase and the proposed transformation pathway. Journal of hazardous materials, 322, 525-531. https://www.sciencedirect.com/science/article/pii/S0304389416309165

[9] Bilal, M., Asgher, M., Parra-Saldivar, R., Hu, H., Wang, W., Zhang, X., & Iqbal, H. M. (2017). Immobilized ligninolytic enzymes: an innovative and environmental responsive technology to tackle dye-based industrial pollutants–a review. Science of the Total Environment, 576, 646-659. https://www.sciencedirect.com/science/article/pii/S0048969716323269

[10] Zhang, C., Gong, L., Mao, Q., Han, P., Lu, X., & Qu, J. (2018). Laccase immobilization and surface modification of activated carbon fibers by bio-inspired poly-dopamine. RSC advances, 8(26), 14414-14421. https://pubs.rsc.org/ko/content/articlehtml/2018/ra/c8ra01265b

[11] Wen, X., Du, C., Wan, J., Zeng, G., Huang, D., Yin, L., ... & Zhang, J. (2019). Immobilizing laccase on kaolinite and its application in treatment of malachite green effluent with the coexistence of Cd (Π). Chemosphere, 217, 843-850. https://www.sciencedirect.com/science/article/pii/S0045653518321805

[12] Arica, M. Y., Salih, B., Celikbicak, O., & Bayramoglu, G. (2017). Immobilization of laccase on the fibrous polymer-grafted film and study of textile dye degradation by MALDI–ToF-MS. Chemical Engineering Research and Design, 128, 107-119. https://www.sciencedirect.com/science/article/pii/S0263876217305415

[13] Wong, J. K. H., Tan, H. K., Lau, S. Y., Yap, P. S., & Danquah, M. K. (2019). Potential and challenges of enzyme incorporated nanotechnology in dye wastewater treatment: A review. Journal of Environmental Chemical Engineering, 7(4), 103261. https://www.sciencedirect.com/science/article/pii/S2213343719303847

[14] Lassouane, F., Aït-Amar, H., Amrani, S., & Rodriguez-Couto, S. (2019). A promising laccase immobilization approach for Bisphenol A removal from aqueous solutions. Bioresource technology, 271, 360-367. https://www.sciencedirect.com/science/article/pii/S0960852418313828

[15] Fernandez-Fernandez, M., Sanromán, M. Á., & Moldes, D. (2013). Recent developments and applications of immobilized laccase. Biotechnology Advances, 31(8), 1808-1825. https://www.sciencedirect.com/science/article/pii/S073497501200064X

[16] Jun, L. Y., Yon, L. S., Mubarak, N. M., Bing, C. H., Pan, S., Danquah, M. K., ... & Khalid, M. (2019). An overview of immobilized enzyme technologies for dye and phenolic removal from wastewater. Journal of Environmental Chemical Engineering, 7(2), 102961. https://www.sciencedirect.com/science/article/pii/S2213343719300843

[17] Datta, S., Christena, L. R., & Rajaram, Y. R. S. (2013). Enzyme immobilization: an overview on techniques and support materials. 3 Biotech, 3(1), 1-9. https://link.springer.com/article/10.1007/s13205-012-0071-7

[18] Górecka, E., & Jastrzębska, M. (2011). Immobilization techniques and biopolymer carriers. Biotechnology and Food Science, 75(1), 65-86. https://eczasopisma.p.lodz.pl/BFS/article/view/11

[19] Homaei, A. A., Sariri, R., Vianello, F., & Stevanato, R. (2013). Enzyme immobilization: an update. Journal of chemical biology, 6(4), 185-205. https://link.springer.com/content/pdf/10.1007/s12154-013-0102-9.pdf

[20] Jesionowski, T., Zdarta, J., & Krajewska, B. (2014). Enzyme immobilization by adsorption: A review. Adsorption, 20(5-6), 801-821. https://link.springer.com/content/pdf/10.1007/s10450-014-9623-y.pdf

[21] Wang, F., Guo, C., Liu, H. Z., & Liu, C. Z. (2008). Immobilization of Pycnoporus sanguineus laccase by metal affinity adsorption on magnetic chelator particles. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 83(1), 97-104. https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.1793

[22] Qiu, X., Qin, J., Xu, M., Kang, L., & Hu, Y. (2019). Organic-inorganic nanocomposites fabricated via functional ionic liquid as the bridging agent for Laccase immobilization and its application in 2, 4-dichlorophenol removal. Colloids and Surfaces B: Biointerfaces, 179, 260-269. https://www.sciencedirect.com/science/article/pii/S0927776519302255

[23] Alshabib, M., & Onaizi, S. A. (2019). A review on phenolic wastewater remediation using homogeneous and heterogeneous enzymatic processes: Current status and potential challenges. Separation and Purification Technology, 219, 186-207. https://www.sciencedirect.com/science/article/pii/S1383586618337912

[24] Singh, R. K., Zhang, Y. W., Jeya, M., & Lee, J. K. (2011). Covalent immobilization of β-1, 4-glucosidase from Agaricus arvensis onto functionalized silicon oxide nanoparticles. Applied Microbiology and Biotechnology, 89(2), 337-344. https://link.springer.com/article/10.1007/s00253-010-2768-z

[25] Secundo, F. (2013). Conformational changes of enzymes upon immobilisation. Chemical Society Reviews, 42(15), 6250-6261. https://pubs.rsc.org/en/content/articlehtml/2013/cs/c3cs35495d

[26] Ba, S., & Vinoth Kumar, V. (2017). Recent developments in the use of tyrosinase and laccase in environmental applications. Critical reviews in biotechnology, 37(7), 819-832. https://www.tandfonline.com/doi/abs/10.1080/07388551.2016.1261081

[27] Draget, K. I., Skjåk-Bræk, G., & Smidsrød, O. (1997). Alginate based new materials. International journal of biological macromolecules, 21(1-2), 47-55. https://www.sciencedirect.com/science/article/pii/S0141813097000408

[28] Olajuyigbe, F. M., Adetuyi, O. Y., & Fatokun, C. O. (2019). Characterization of free and immobilized laccase from Cyberlindnera fabianii and application in degradation of bisphenol A. International journal of biological macromolecules, 125, 856-864. https://www.sciencedirect.com/science/article/pii/S0141813018360720

[29] Yaohua, G., Ping, X., Feng, J., & Keren, S. (2018). Co-immobilization of laccase and ABTS onto novel dual-functionalized cellulose beads for highly improved biodegradation of indole. Journal of hazardous materials, 365, 118-124. https://europepmc.org/article/med/30412808

[30] Suman, S. K., Patnam, P. L., Ghosh, S., & Jain, S. L. (2018). Chicken Feather Derived Novel Support Material for Immobilization of Laccase and Its Application in Oxidation of Veratryl Alcohol. ACS Sustainable Chemistry & Engineering, 7(3), 3464-3474. https://pubs.acs.org/doi/abs/10.1021/acssuschemeng.8b05679

[31] Piao, M., Zou, D., Yang, Y., Ren, X., Qin, C., & Piao, Y. (2019). Multi-Functional Laccase Immobilized Hydrogel Microparticles for Efficient Removal of Bisphenol A. Materials, 12(5), 704. https://www.mdpi.com/1996-1944/12/5/704

[32] Huber, D., Bleymaier, K., Pellis, A., Vielnascher, R., Daxbacher, A., Greimel, K. J., & Guebitz, G. M. (2018). Laccase catalyzed elimination of morphine from aqueous systems. New biotechnology, 42, 19-25. https://www.sciencedirect.com/science/article/pii/S1871678417300808

[33] Piao, M., Zou, D., Ren, X., Gao, S., Qin, C., & Piao, Y. (2019). High efficiency biotransformation of bisphenol A in a fluidized bed reactor using stabilized laccase in porous silica. Enzyme and microbial technology, 126, 1-8.https://www.sciencedirect.com/science/article/pii/S0141022918302345

[34] Zdarta, J., Feliczak-Guzik, A., Siwińska-Ciesielczyk, K., Nowak, I., & Jesionowski, T. (2020). Mesostructured cellular foam silica materials for laccase immobilization and tetracycline removal: A comprehensive study. Microporous and Mesoporous Materials, 291, 109688. https://www.sciencedirect.com/science/article/pii/S1387181119305451

[35] Wen, X., Zeng, Z., Du, C., Huang, D., Zeng, G., Xiao, R., ... & Hu, L. (2019). Immobilized laccase on bentonite-derived mesoporous materials for removal of tetracycline. Chemosphere, 222, 865-871. https://www.sciencedirect.com/science/article/pii/S004565351930236X

[36] Kashefi, S., Borghei, S. M., & Mahmoodi, N. M. (2019). Covalently immobilized laccase onto graphene oxide nanosheets: Preparation, characterization, and biodegradation of azo dyes in colored wastewater. Journal of Molecular Liquids, 276, 153-162. https://www.sciencedirect.com/science/article/pii/S0167732218353479

[37] Iriarte-Mesa, C., Díaz-Castañón, S., & Abradelo, D. G. (2019). Facile immobilization of Trametes versicolor laccase on highly monodisperse superparamagnetic iron oxide nanoparticles. Colloids and Surfaces B: Biointerfaces, 181, 470-479. https://www.sciencedirect.com/science/article/pii/S092777651930311X

[38] Wehaidy, H. R., Abdel-Naby, M. A., El-Hennawi, H. M., & Youssef, H. F. (2019). Nanoporous Zeolite-X as a new carrier for laccase immobilization and its application in dyes decolorization. Biocatalysis and agricultural biotechnology, 19, 101135. https://www.sciencedirect.com/science/article/pii/S1878818119301239

[39] Fu, M., Xing, J., & Ge, Z. (2019). Preparation of laccase-loaded magnetic nanoflowers and their recycling for efficient degradation of bisphenol A. Science of The Total Environment, 651, 2857-2865.https://www.sciencedirect.com/science/article/pii/S0048969718340282

[40] Jankowska, K., Ciesielczyk, F., Bachosz, K., Zdarta, J., Kaczorek, E., & Jesionowski, T. (2019). Laccase immobilized onto zirconia–silica hybrid doped with Cu2+ as an effective biocatalytic system for decolorization of dyes. Materials, 12(8), 1252. https://www.mdpi.com/1996-1944/12/8/1252

[41] Chao, C., Guan, H., Zhang, J., Liu, Y., Zhao, Y., & Zhang, B. (2018). Immobilization of laccase onto porous polyvinyl alcohol/halloysite hybrid beads for dye removal. Water Science and Technology, 77(3), 809-818. https://iwaponline.com/wst/article-abstract/77/3/809/38533

[42] Wu, E., Li, Y., Huang, Q., Yang, Z., Wei, A., & Hu, Q. (2019). Laccase immobilization on amino-functionalized magnetic metal organic framework for phenolic compound removal. Chemosphere, 233, 327-335. https://www.sciencedirect.com/science/article/pii/S0045653519310628

[43] Lee, S. H., Yeo, S. Y., Cools, P., & Morent, R. (2019). Plasma polymerization onto nonwoven polyethylene/polypropylene fibers for laccase immobilization as dye decolorization filter media. Textile Research Journal, 89(17), 3578-3590. https://journals.sagepub.com/doi/abs/10.1177/0040517518817102

[44] Jia, Y., Chen, Y., Luo, J., & Hu, Y. (2019). Immobilization of laccase onto meso-MIL-53 (Al) via physical adsorption for the catalytic conversion of triclosan. Ecotoxicology and environmental safety, 184, 109670. https://www.sciencedirect.com/science/article/pii/S0147651319310012

[45] Vera, M., Nyanhongo, G. S., Pellis, A., Rivas, B. L., & Guebitz, G. M. (2019). Immobilization of Myceliophthora thermophila laccase on poly (glycidyl methacrylate) microspheres enhances the degradation of azinphos-methyl. Journal of Applied Polymer Science, 136(16), 47417. https://onlinelibrary.wiley.com/doi/abs/10.1002/app.47417

[46] Shao, B., Liu, Z., Zeng, G., Liu, Y., Yang, X., Zhou, C., ... & Yan, M. (2019). Immobilization of laccase on hollow mesoporous carbon nanospheres: noteworthy immobilization, excellent stability and efficacious for antibiotic contaminants removal. Journal of hazardous materials, 362, 318-326. https://www.sciencedirect.com/science/article/pii/S0304389418307556

[47] Fathali, Z., Rezaei, S., Faramarzi, M. A., & Habibi-Rezaei, M. (2019). Catalytic phenol removal using entrapped cross-linked laccase aggregates. International journal of biological macromolecules, 122, 359-366. https://www.sciencedirect.com/science/article/pii/S0141813018335980

[48] Lonappan, L., Liu, Y., Rouissi, T., Pourcel, F., Brar, S. K., Verma, M., & Surampalli, R. Y. (2018). Covalent immobilization of laccase on citric acid functionalized micro-biochars derived from different feedstock and removal of diclofenac. Chemical Engineering Journal, 351, 985-994. https://www.sciencedirect.com/science/article/pii/S1385894718311951

[49] Mokhtar, A., Nishioka, T., Matsumoto, H., Kitada, S., Ryuno, N., & Okobira, T. (2019). Novel biodegradation system for bisphenol A using laccase-immobilized hollow fiber membranes. International journal of biological macromolecules, 130, 737-744. https://www.sciencedirect.com/science/article/pii/S0141813018359233

[50] Yang, J., Wang, Z., Lin, Y., Ng, T. B., Ye, X., & Lin, J. (2017). Immobilized Cerrena sp. laccase: preparation, thermal inactivation, and operational stability in malachite green decolorization. Scientific reports, 7(1), 1-9. https://www.nature.com/articles/s41598-017-16771-x

[51] Iriarte-Mesa, C., Díaz-Castañón, S., & Abradelo, D. G. (2019). Facile immobilization of Trametes versicolor laccase on highly monodisperse superparamagnetic iron oxide nanoparticles. Colloids and Surfaces B: Biointerfaces, 181, 470-479. https://www.sciencedirect.com/science/article/pii/S092777651930311X

[52] Crini, G. (2019). Historical review on chitin and chitosan biopolymers. Environmental Chemistry Letters, 1-21. https://link.springer.com/article/10.1007/s10311-019-00901-0

[53] Zhang, Y., Lv, Z., Zhou, J., Xin, F., Ma, J., Wu, H., ... & Dong, W. (2018). Application of eukaryotic and prokaryotic laccases in biosensor and biofuel cells: recent advances and electrochemical aspects. Applied microbiology and biotechnology, 102(24), 10409-10423. https://link.springer.com/article/10.1007/s00253-018-9421-7