



Enzyme Immobilization on Nanomaterials for Cutting-Edge Environmental Remediation Solutions: A Special Emphasis on Dyes and Pesticides

Jatinder Singh Randhawa 1* and Harmanpreet Meehnian 2

1 Department of Biotechnology, School of Applied & Life Sciences, Uttarakhand University, Dehradun, Uttarakhand-248007, India

2 Department of Biotechnology Engineering, UIE, Chandigarh University, Mohali-140413, Punjab, India

ABSTRACT

The prevalence of micropollutants is growing, necessitating the development of ecologically acceptable bioremediation techniques. Researchers are investigating the use of microbial enzymes as biocatalysts for novel environmental cleaning solutions, driven by their extraordinary diversity and powerful catalytic activity. Microbial enzymes are effective bioremediation agents because they are substrate-specific, biodegradable and can degrade a wide spectrum of contaminants. However, the stability and reusability of enzymes often restrict their practical utility. To address this issue, enzyme immobilization approaches have emerged as revolutionary tools. These methods immobilize enzymes on inert or active supports, increasing their stability and reusability. These developments result in more economical bioremediation procedures and more effective pollutant breakdown. This review examines immobilization techniques and their effectiveness in degradation of environmental pollutants, such as dyes and pesticides. It demonstrates the groundbreaking potential of microbially immobilized enzyme biocatalysts in reducing micropollutant pollution and achieving a cleaner, more sustainable future. The review also emphasizes how the unique properties of nanomaterials and the catalytic activity of enzymes can be combined to provide cutting-edge, environmentally friendly remediation solutions. It suggests that future technological developments in environmental cleanup may be facilitated by this research.

Received: April 04, 2026

Accepted: May 18, 2026

Published: May 20, 2026

*Corresponding author:

Dr. Jatinder Singh Randhawa

E-mail:

jatinderbiotech@gmail.com

KEYWORDS: Enzyme immobilization; Nanomaterials; Micropollutants; Pesticide degradation; Biocatalyst

1. INTRODUCTION

Expanding industrialization and urbanization have worsened environmental degradation, becoming significant global issues (Liang and Yang, 2019). Micropollutants, which include persistent contaminants such as dyes, pharmaceuticals, and insecticides, represent major concerns (Gul et al., 2022). These pollutants exhibit high persistence in the environment and pose significant risks to living organisms and marine life, even at low concentrations. This is evidenced by alterations in aquatic ecosystems and bioaccumulation within food webs (Goutte et al., 2020; Desiante et al., 2021). Pharmaceuticals with biological activity can disturb microbial ecosystems and contribute to antibiotic resistance (Pinto et al., 2022). Discharges of industrial dyes contaminate the environment, reduce light penetration in aquatic bodies, inhibit photosynthesis, and disrupt food chains (Tkaczyk et al., 2020). Although pesticides are necessary for agriculture, they can also affect non-target species, particularly those that provide food, when they contaminate soil and water (Yang et al.,

2021). Phenolic compounds, commonly found in industrial effluents and agricultural runoff, can induce oxidative stress, disrupt endocrine function, and increase the risk of cancer and long-term disorders in humans (Anku et al., 2017). Emissions of industrial dyes not only pollute the environment but also harm beneficial insects and wildlife, causing health risks. It is vital to develop innovative, sustainable micropollutant removal technology. There are numerous methods for eliminating contaminants, including physical separation, chemical transformation, and biological treatment (Saravanan et al., 2021). Established techniques have drawbacks, such as high operational costs, inadequate micropollutant removal, and the danger of secondary pollutants with greater toxicity, which may pose serious challenges (Crini et al., 2019). Advanced oxidation processes are excellent in degrading complex organic pollutants, but they can create toxic byproducts that complicate environmental rehabilitation. The limitations highlight the need for effective, long-term

environmental restoration techniques. A novel method of environmental remediation is offered by microbial enzymes, a broad family of biocatalysts generated by microorganisms (Narayanan et al., 2023).

These enzymes have a strong chemoselectivity and catalytic efficiency when it comes to mediating biological processes required for pollution degradation. Microbial diversity generates a large pool of enzymes that can degrade a variety of environmental contaminants (Karigar and Rao, 2011). Laccases and peroxidases, for example, are very good in breaking down complex chemical compounds such as dyes, pesticides, and drugs (Akkaya et al., 2016; Das et al., 2017; Spina et al., 2020; Preethi et al., 2023; Caraene et al., 2023). Similarly, hydrolases may degrade a wide range of insecticides (Ghosh and Sarkar, 2023). Similar capabilities are shown in the degradation of agricultural pesticides like atrazine and chlorpyrifos, as well as azo dyes like methyl orange and Reactive Black 5 in textile industry effluents (Saravanan et al., 2021). Solutions based on enzymes can successfully cut pollution and advance sustainability (Dave and Das, 2021). However, fundamental limitations often prevent free enzymes from being widely employed in environmental cleaning initiatives, despite the fact that they function as homogenous biocatalysts. The constraints include intrinsic instability, environmental change susceptibility, and difficulties with recovery and reuse. One potential solution to these issues is enzyme immobilization (Homaei et al., 2013). Enzymes that are attached to solid supports in a controlled manner have increased stability, activity and longevity (Federsel et al., 2021). Enzymes have a safe environment when immobilized, which decreases degradation and increases resilience to changing environmental conditions. Since immobilized enzymes may be reused, they are a more sustainable and economical option than free enzymes (Maghraby et al., 2023). There are a number of immobilization techniques, including as encapsulation, cross-linking, covalent binding, and adsorption, each having particular advantages and restrictions that may be adjusted for use in particular environmental contexts (Cavalcante et al., 2021). One significant benefit of affinity-based immobilization is that it makes it easier to properly position the enzyme on the support material (Abdelhamid et al., 2022).

Optimizing enzyme orientation during immobilization is critical for preserving effective substrate access to the active site, thereby enhancing catalytic efficiency (Abdelhamid et al., 2020). The selection of an appropriate immobilization strategy is governed by multiple factors, including the nature of the enzyme, the physicochemical properties of the target contaminant, and the operational conditions such as pH, temperature, and ionic strength. This review critically examines the potential of microbial enzymes as efficient and sustainable tools for environmental remediation. Particular emphasis is placed on their intrinsic advantages in the degradation of micropollutants, including high specificity, catalytic efficiency, and eco-friendly operation. Furthermore, advanced immobilization techniques such as adsorption, covalent binding, entrapment, and encapsulation are discussed in the context of improving enzyme stability, reusability, and resistance to environmental stressors. The review specifically focuses on the application of immobilized microbial enzymes in the removal of environmentally persistent pollutants, notably pesticides and synthetic dyes. In addition, it evaluates the broader role of enzyme-based

technologies in promoting environmental sustainability, highlighting their potential integration into green remediation strategies.

2. MULTIPOLLUTANT REMEDIATION USING MICROBIAL ENZYMES

A wide range of micropollutants can be effectively mitigated using the diverse catalytic repertoire of microbial enzymes. In particular, oxidoreductases including peroxidases, laccases, oxygenases, and tyrosinases along with hydrolases such as cutinases, lipases, dehalogenases, esterases, and PETases, exhibit strong biocatalytic potential for the degradation of structurally diverse environmental contaminants (Bhandari et al., 2021). These enzyme classes are capable of transforming or mineralizing a broad spectrum of pollutants, including pesticides, synthetic dyes, pharmaceuticals, and other recalcitrant organic compounds.

2.1. Microbial enzymes in micropollutants degradation

Microbial oxidoreductases are a broad class of enzymes that are well-known for their ability to catalyze oxidation-reduction processes. These enzymes are particularly effective in the transformation and detoxification of a wide range of chemical pollutants, including phenolics, dyes, pesticides, and pharmaceutical residues (Varga et al. 2019). Due to their robust biocatalytic capabilities, extensively studied oxidoreductases such as peroxidases, laccases, oxygenases, and tyrosinases have garnered significant scientific interest for their potential applications in environmental remediation and industrial biotechnology.

According to Janusz et al. (2020), laccases (EC 1.10.3.2) are a type of multi-copper oxidoreductases that are present in a wide range of taxa, including bacteria, fungi, and in some plants. The exceptional ability of these enzymes to oxidize a wide spectrum of both aromatic and non-aromatic compounds has established them as key biocatalysts in environmental remediation (Agrawal et al., 2018; Xu et al., 2020). Their distinctive multicopper active-site architecture is closely associated with their high catalytic efficiency and broad substrate specificity. Gianfreda et al. (1999) reported that laccases had three distinct copper cores, known as type 1 (T₁), type 2 (T₂), and type 3 (T₃). T₁ copper center, a mononuclear site, is the main electron acceptor from the targeted substrate undergoing oxidation (Malhotra et al., 2021). However, molecular oxygen binding and reduction to water depend on the trinuclear cluster made up of the T₂ and T₃ copper centers. The difference in redox potential between the substrate and T₁ copper dictates how well laccase mediates oxidation. According to Malhotra et al. (2021), laccases have the ability to selectively oxidize molecules that have a lower ionization potential, hence facilitating electron transfer. Additionally, it has been demonstrated that naturally occurring mediators like syringaldazine, which are created when lignin breaks down, are highly effective against some pollutants (Asadi et al., 2020). The selection of an optimal mediator is governed by the complex interplay between the chemical characteristics of the target micropollutant, the intrinsic properties of the laccase (e.g., redox potential and substrate affinity), and environmental parameters such as pH and temperature.

Microbial laccases, derived from both bacterial and fungal sources, are emerging as potent biocatalysts for environmental remediation (Agrawal et al., 2018; Agrawal et

al., 2022). In particular, fungal laccases especially those from the genera *Trametes* and *Pleurotus* have attracted considerable attention due to their remarkable ability to oxidatively degrade a wide spectrum of pollutants, including structurally complex dyes and pharmaceutical compounds (More et al., 2011; Loi et al., 2021). According to studies (Guo et al., 2008; Hachi et al., 2017, Singh et al., 2020), laccase extracted from *Trametes versicolor* is effective in degrading acetaminophen, carbamazepine, azophloxine and Remazol Brilliant Blue R dye. In contrast, laccase derived from *Pleurotus ostreatus* demonstrates broad substrate specificity, effectively catalyzing the oxidation of structurally diverse pollutants such as Congo Red dye and chlorophenolic compounds (Isanapong et al., 2021; Isanapong et al., 2024).

Several environmental pollutants can be effectively reduced by fungi laccases due to their inherent flexibility. As an interesting alternative to fungal laccases for laccase-based bioremediation, bacteria provide a lot of interest. According to Givaudan et al. (1993), *Azospirillum lipoferum* provided the first indication of bacterial laccase activity. Since then, it has been shown that laccases are present in the following microorganisms: *Streptomyces* (Machczynski et al., 2004), *Proteobacterium* (Narayanan et al., 2015), *Pseudomonas* (Neifar et al., 2016), *Yersinia* (Singh et al., 2016), *Klebsiella* (Liu et al., 2017), *Bacillus* (Wang et al., 2020), and *Marinomonas* (Chang et al., 2022). These monomeric extracellular or intracellular proteins, averaging 50–70 kDa in size, are known to participate in various bacterial processes, such as pigmentation, morphogenesis, toxin oxidation, and defence against UV light and oxidising agents (Martins et al., 2015; Akram et al., 2022). Notably, the CotA protein from *Bacillus subtilis* represents a distinctive copper-dependent laccase localized in the spore coat, underscoring the functional diversity and physiological significance of laccase-like enzymes in bacterial systems (Hullo et al., 2001).

3. IMMOBILIZED ENZYMES IN MICROPOLLUTANT DEGRADATION

The widespread use of dyes and pigments, particularly in the textile industry, poses a significant environmental challenge due to their toxicity, persistence, and resistance to degradation. Azo and synthetic dyes commonly found in industrial effluents can adversely affect aquatic ecosystems and water quality. Conventional treatment methods are often inadequate for their complete removal. In this context, immobilized enzymes offer a sustainable and efficient alternative for dye degradation. Enzymes such as laccases and peroxidases, when immobilized, exhibit enhanced stability and reusability, enabling effective breakdown of complex dye molecules. Thus, enzyme immobilization represents a promising approach for mitigating dye pollution in wastewater systems.

3.1 Dyes degradation by immobilized enzyme

Immobilized manganese peroxidase (MnP) has demonstrated considerable potential in cleaning wastewater. In one investigation, iron oxide nanoparticles were used to immobilize MnP from the fungus *Aspergillus flavus* (Kalsoom et al., 2022). The immobilized MnP demonstrated improved thermal stability and performed well across a larger pH and temperature range (ideal pH 5.0, 50°C). Additionally, compared to the free enzyme, it demonstrated enhanced catalytic activity, completely decolorizing Direct Red 31 and greatly improving (92%) decolorization of Acid Black 234.

Additionally, the incorporation of magnetic nanoparticles facilitated efficient separation and recovery of the biocatalyst using an external magnetic field. This property enables repeated reuse of the immobilized enzyme, thereby improving process economics and contributing to a more sustainable and environmentally friendly wastewater treatment strategy. A key limitation of manganese peroxidase (MnP) is its strict dependence on Mn²⁺ ions as mediators for catalytic activity, which can restrict its practical application (Kalsoom et al., 2022). To address this constraint, Jiménez et al. (2023) developed an innovative co-immobilization strategy in which MnP and Mn²⁺ ions were simultaneously entrapped within silica gel matrices. This approach resulted in a substantial enhancement of enzymatic activity, with a 4–5-fold increase compared to MnP immobilized alone. The sustained presence of Mn²⁺ within the gel matrix confirmed the effective and continuous redox cycling facilitated by immobilized Mn²⁺, a critical step in MnP-mediated catalysis. Consequently, dye degradation efficiency improved significantly, with removal rates increasing by 2–4 times. This strategy enables effective MnP application even in Mn²⁺-limited environments, thereby broadening its potential for environmental remediation.

Laccase is a widely studied oxidoreductase known for its ability to degrade a broad spectrum of synthetic dyes, making it a valuable biocatalyst for environmental remediation, particularly in textile wastewater treatment. Recent advances in immobilization strategies have significantly enhanced its operational stability and catalytic efficiency. For instance, laccase derived from *Aspergillus* sp. has been co-immobilized with the redox mediator ABTS onto a metal–organic framework (MOF) grown on polyethylene terephthalate (PET) fibers (Lou et al., 2023). This approach improves enzyme stability under acidic conditions and enables the degradation of recalcitrant dyes such as malachite green and crystal violet. The co-immobilized laccase–mediator system achieved 58.8% removal of crystal violet within 24 hours, approximately sevenfold higher than free laccase, demonstrating significantly enhanced performance. Additionally, the recyclability of both the enzyme and mediator contributes to reduced operational costs and improved sustainability.

Zhu et al. (2020) have proposed a unique approach to tackle the problem of eliminating harmful azo dyes. Their method involves creating a biocatalytic membrane with a strong polyvinylidene fluoride base. Using a multi-step procedure, researchers coated the PVDF membrane with polydopamine, attached iron oxide cubes that were specifically designed and modified with silica (Fe₂O₃@SiO₂) and a coupling agent (APTES), and then immobilized laccase onto this structure. This resulted in the creation of a hybrid bio-inorganic structure on the membrane's surface. Lac-FS@cubes-PDA@PVDF membrane demonstrated excellent operational performance, characterized by high storage stability and notable reusability, retaining over 75% removal efficiency after five successive cycles. Moreover, it exhibited outstanding catalytic efficiency, achieving up to 97.1% decolorization of the model dye Congo Red, thereby highlighting its potential for practical wastewater treatment applications.

Tyrosinase is a well-characterized oxidoreductase that exhibits high efficiency in the removal of toxic dyes and phenolic compounds, both of which are significant environmental pollutants. A recent work used EDC/NHS chemistry to covalently link *Agaricus bisporus* tyrosinase onto

silver-coated Fe₃O₄ nanoparticles, thereby immobilizing the enzyme. According to Yava et al. (2023), this designed biocatalyst showed excellent efficiency, usefulness, and reusability in pollution removal applications. It was determined that each gram of nanoparticles contained 216.6 ± 1.250 mg of immobilized tyrosinase. 48.9% of the enzyme's initial activity was retained after six reuses, and immobilization enhanced the substrate affinity by 1.4 times. The immobilized enzyme's residual activity after 84 days of storage was 68.3%, whereas the free enzyme's residual activity was 45.8%. High effectiveness was demonstrated by the immobilized tyrosinase in eliminating phenolic chemicals and azo dyes from aqueous solutions. For Congo Red and Reactive Green 19, it obtained decolourization rates of 95.0% and 36.9%, respectively. Additionally, a variety of phenolic compounds were effectively removed by the biocatalyst, including 87.8% of 4-chlorophenol and 92.3% of phenyl acetate. Furthermore, cyclic voltammetry was used to evaluate the electrochemical characteristics of immobilized tyrosinase in order to assess its suitability for use as a catechol biosensor. Overall, this approach highlights the effectiveness of enzyme-based, environmentally benign strategies for the remediation of phenolic and dye contaminants in wastewater.

3.2 Pesticides degradation by immobilized enzyme

The extensive use of pesticides, including fungicides, herbicides and insecticides has unquestionably increased agricultural output. On the other hand, their ability to remain in soil and water presents a serious environmental risk. The need for alternate remediation solutions arises from bioaccumulation throughout ecosystems and possible damage to non-target animals, including people. One emerging green technology that shows promise is immobilized enzymes. A number of enzyme biocatalysts that have been immobilized are particularly engineered to break down pesticide molecules into less hazardous chemicals, hence lowering their toxicity and environmental persistence. With this strategy, pesticide contamination may be reduced in a targeted and sustained manner. A different research used immobilized *Bacillus* sp. laccase on magnetic iron nanoparticles to address the problem of chlorpyrifos breakdown (Srinivasan et al., 2020). In order to obtain almost full recovery of enzyme activity, they improved the procedure. The resulting immobilized laccase claimed numerous improvements: better stability (lasting 100 h), resistance to alkaline pH, higher temperatures, and critically, the capacity to successfully breakdown the chlorpyrifos. Researchers have created a very efficient approach that uses immobilized phosphotriesterase (PTE) enzyme from *Sulfolobus solfataricus* within a customized biocatalytic membrane in order to tackle the difficulty of decomposing the harmful pesticide paraoxon (Vitola et al., 2023). The key innovation lies in the integration of immobilized phosphotriesterase (PTE) with either cationic or anionic surfactants. This approach significantly enhances enzymatic performance, with activity reaching up to 90% compared to the free enzyme. The improvement is likely attributable to surfactant-induced conformational stabilization of the enzyme, particularly in the presence of CTAB as well as increased substrate accessibility and affinity facilitated by SDS. Because of their decreased flexibility, the immobilized enzyme activity improved less, but the resultant biocatalytic membranes had significant benefits. These membranes achieve an impressive 96% conversion rate of paraoxon in a biocatalytic reactor, taking only one-third of the time reported in previous methods. The surfactant-

assisted immobilized system exhibited approximately a twofold increase in specific activity compared to surfactant-free counterparts. Moreover, it retained catalytic efficiency over multiple reaction cycles, particularly when surfactants were replenished, indicating enhanced operational stability and reusability. This work demonstrates how extremely active and stable biocatalytic membranes may be made using immobilized PTE enzymes and surfactants. This method overcomes obstacles that previously prevented the widespread use of this technology and marks a substantial advancement in the degradation of organophosphate insecticides.

Pest management can be greatly aided by pyrethroid insecticides, such as bifenthrin, permethrin, cypermethrin, and fenpropathrin. Their strong neurotoxic effects and environmental durability, however, raise questions about how they may affect human health and non-target creatures. By attacking sodium channels in the neurological system of insects, pyrethroids cause paralysis and, in the end, insect death. Immobilized esterase enzymes are a unique technique for pyrethroid breakdown that has been studied recently. Zong et al. (2022) identified a novel gene, *est882*, encoding a highly efficient esterase (*Est882*) capable of rapidly degrading pyrethroid pesticides such as permethrin, cypermethrin, and fenpropathrin. The enzyme exhibited remarkable catalytic performance, achieving over 80% degradation of these compounds within 30 minutes. To enhance its stability and broaden its practical applicability, *Est882* was subsequently immobilized. This modification significantly improved its tolerance to diverse environmental conditions—such as variations in pH, temperature, and the presence of inhibitors, thereby enhancing its catalytic efficiency and suitability for real-world remediation applications.

A study conducted by Yang et al. (2020) investigated *Ix-EstM160K*, an immobilised esterase produced from *Geobacillus uzonensis*, and provided evidence of the potential of these esterases for pyrethroid remediation. In a reactor system with high concentrations of bifenthrin (500 mg/L), *Ix-EstM160K* exhibited a degradation efficiency of 90.4%. Additionally, after 10 consecutive cycles of bifenthrin degradation, *Ix-EstM160K* demonstrated operational stability, retaining 72% of its original activity. These findings highlight the efficacy of immobilised esterases for bioremediation in pyrethroid-contaminated environments.

Carbamates, a class of pesticides that includes fungicides, insecticides, and herbicides, are generally considered to pose lower acute toxicity to humans compared to organophosphate pesticides (Hernández et al., 2013). Bayramoğlu et al. (2019) investigated an advanced wastewater treatment strategy targeting carbamate contaminants through the application of immobilized laccase enzymes, demonstrating the potential of this biocatalytic approach for efficient and sustainable pollutant removal. Using this approach, laccase enzymes isolated from the fungus *T. versicolor* were immobilized onto specially engineered microbeads functionalized with carbonate or epoxy groups, enabling efficient enzyme attachment and enhanced operational stability. The procedure of immobilization greatly improved the performance of the enzymes. In contrast to the free enzyme, the immobilized laccase nearly completely biodegraded the model carbamate pesticide, carbaryl, when a mediator was present.

Furthermore, the immobilized enzyme demonstrated enhanced stability during storage and a wider tolerance for pH and temperature fluctuations. After a day of testing in a fluidized bed reactor, the immobilised enzyme showed minimal activity loss for carbaryl, highlighting its excellent reusability. This study underscores the potential of using immobilised laccase on microbeads as an effective approach for removing carbamate micropollutants from wastewater treatment. The three key advantages of this technique are improved degradation efficiency, enhanced enzyme stability, and superior reusability.

Organochlorine pesticides, including dichlorophen and DDT (dichlorodiphenyltrichloroethane), are well known for their prolonged environmental persistence and strong tendency to bioaccumulate. These properties lead to long-term contamination of soil and aquatic systems and pose significant risks to ecosystems and human health. Researchers have created laccase-MSU-F, a unique biocatalyst that particularly targets the breakdown of dichlorophen, as a potential remedy for environmental cleanup (Vidal-Limon et al., 2018). The laccase enzyme, which is sourced from the fungus *Corioliopsis gallica*, is combined with mesoporous synthetic silica foam (MSU-F) to create this novel hybrid nanomaterial. Laccase-MSU-F demonstrated effective degradation of dichlorophen, likely through mechanisms such as dechlorination and oxidative polymerization into higher-molecular-weight products. This transformation significantly reduced the genotoxic and apoptosis-inducing effects of the parent compound in cellular assays, indicating decreased cytotoxicity. Importantly, the degradation products were also suggested to exhibit a lower affinity for steroid hormone receptors, thereby potentially reducing the risk of endocrine disruption. Collectively, these findings highlight the strong potential of laccase-MSU-F as an efficient biocatalyst for environmental remediation, with implications extending to reducing toxicological impacts beyond environmental systems.

Salem et al. (2024) reported the development of an efficient biocatalytic system based on covalently immobilized ligninolytic enzymes for the remediation of DDT (dichlorodiphenyltrichloroethane). In this approach, a multi-enzyme consortium was extracted from the fungus *Pleurotus ostreatus*, comprising key ligninolytic enzymes such as aryl alcohol oxidase, laccase, lignin peroxidase, and manganese peroxidase. The enzyme mixture was partially purified and subsequently covalently immobilized onto nano-silica particles using glutaraldehyde as a crosslinking agent. This immobilization strategy markedly enhanced both the stability and reusability of the biocatalyst. The resulting system exhibited robust catalytic performance across a broad operational range (pH 4–9 and 20–55 °C). Notably, it achieved complete degradation of p,p'-DDT within 12 hours under optimized conditions (pH 5 and 30 °C), highlighting its high efficiency and potential for practical environmental remediation applications.

To address the growing challenge of persistent organic pollutants, considerable research is focused on developing microbial enzyme-based biocatalysts for efficient degradation. One promising strategy involves co-immobilized enzyme–membrane composites (Jia et al., 2024). In this context, a novel system, Lac-HBT-Pd/BC, integrates laccase, the redox mediator 1-hydroxybenzotriazole (HBT), and palladium (Pd),

all co-immobilized on a functionalized bacterial cellulose support. This synergistic design enhances electron transfer and catalytic efficiency. The biocatalyst demonstrated remarkable performance in atrazine degradation, achieving complete removal within 5 hours under mild conditions. Additionally, it facilitated “deep degradation,” with ~85% removal of toxic intermediate products, indicating effective detoxification and improved environmental safety. The incorporation of palladium (Pd) played a critical role in enhancing the stability of mediator-derived radicals and improving the overall catalytic efficiency of the Lac-HBT-Pd/BC system. Furthermore, this biocatalyst exhibited excellent reusability and adaptability across varying water quality conditions, underscoring its suitability for practical water treatment applications. These findings highlight the considerable potential of co-immobilized enzyme–membrane composites as efficient and sustainable platforms for the removal of persistent contaminants such as atrazine. Given the well-documented environmental persistence and toxicological impacts of such pesticides, the application of immobilized microbial enzymes offers a viable remediation strategy. By leveraging the high specificity and catalytic efficiency of enzymatic systems, it is possible to reduce both the toxicity and longevity of pesticide residues, thereby contributing to the protection of aquatic ecosystems and human health.

4. CONCLUSION

In conclusion, the immobilisation of enzymes represents a transformative approach to enhancing bioremediation efforts, offering significant advantages in terms of stability, reusability, and efficiency. By enabling the breakdown of a diverse array of environmental pollutants, from dyes to complex industrial chemicals, immobilised enzymes provide a sustainable solution to pressing environmental challenges. The ongoing exploration of innovative immobilisation methods and new enzyme designs holds great promise for expanding the scope of enzymatic bioremediation, particularly in addressing emerging contaminants. Furthermore, integrating enzyme-based solutions with complementary technologies can create more effective and holistic remediation strategies. To ensure the widespread adoption of these techniques in large-scale industrial applications, it is crucial to assess their economic viability, optimize production processes, and conduct life cycle assessments to understand their environmental impacts comprehensively. By addressing these factors, researchers can foster the long-term implementation of enzyme-based bioremediation as a key strategy for environmental restoration, paving the way for a cleaner and more sustainable future.

5. REFERENCES

- Abdelhamid MA, Meligy AM, Yeo KB, Lee C-S and Pack SP (2020) Silaffin-3-derived pentyllysine cluster as a new fusion tag for one-step immobilization and purification of recombinant *Bacillus subtilis* catalase on bare silica particles. *Int. J. Biol. Macromol.* 159:1103–1112.
- Abdelhamid MA, Son RG, Park KS and Pack SP (2022) Oriented multivalent silaffin-affinity immobilization of recombinant lipase on diatom surface: Reliable loading and high performance of biocatalyst. *Colloids Surf. B Biointerfaces* 219:112830.
- Agarwal N, Solanki VS, Gacem A, Hasan MA, Pare B, Srivastava

- A, Singh A, Yadav VK, Yadav KK, Lee C et al. (2022) Bacterial laccases as biocatalysts for the remediation of environmental toxic pollutants: A green and eco-friendly approach—A review. *Water* 14:4068.
- Agrawal K, Chaturvedi V and Verma P (2018) Fungal laccase discovered but yet undiscovered. *Bioresour. Bioprocess.* 5:4.
- Akkaya A, Erdogan Ozseker E and Akdogan HA (2016) Degradation of dyes by laccase. *Anal. Lett.* 49:790–798.
- Akram F, Ashraf S, Haq I, Shah FI and Aqeel A (2022) Eminent industrial and biotechnological applications of laccases from bacterial source: A current overview. *Appl. Biochem. Biotechnol.* 195:2336–2356.
- Ali SS, Elsamahy T, Koutra E, Kornaros M, El-Sheekh M, Abdelkarim EA, Zhu D and Sun J (2021) Degradation of conventional plastic wastes in the environment: A review on current status of knowledge and future perspectives of disposal. *Sci. Total Environ.* 771:144719
- Anku WW, Mamo MA and Govender PP (2017) Phenolic compounds in water: Sources, reactivity, toxicity and treatment methods. In *Phenolic Compounds-Natural Sources, Importance and Applications*; InTechOpen: London, UK, pp.419–443.
- Asadi E, Makhdoumi A and Asoodeh A (2020) Laccase mediator system obtained from a marine spore exhibits decolorization potential in harsh environmental conditions. *Ecotoxicol. Environ. Saf.* 191:110184.
- Bayramoglu G and Arica MY (2019) Biodegradation of methylene blue and carbaryl by *Trametes versicolor* laccase preparations in the presence of a mediator compound. *J. Macromol. Sci. Part A.* 56:277–285
- Bhandari S, Poudel DK, Marahatha R, Dawadi S, Khadayat K, Phuyal S, Shrestha S, Gaire S, Basnet K and Khadka U (2021) Microbial enzymes used in bioremediation. *J. Chem.* 2021:8849512.
- Caraene ID, Gruchlik Y, Busetti F, Linge KL and Joll CA (2023) Degradation of selected pharmaceuticals detected in wastewater systems using an enzyme-mediator system and identification of resulting transformation products. *Biocatal. Biotransform.* 41:133–144.
- Cavalcante FT, Cavalcante AL, de Sousa IG, Neto FS and dos Santos JC (2021) Current status and future perspectives of supports and protocols for enzyme immobilization. *Catalysts* 11:1222.
- Chang F, Wu L, Xiong Z, Yang Y, Xia X, Wu Q, Ge C and Chen H (2022) Light-induced expression of a novel marine laccase in *Escherichia coli* from *Marinomonas profundimaris* and its application in synthetic dye decolorization. *Protein Expr. Purif.* 197:106108.
- Crini G, Lichtfouse E, Wilson LD and Morin-Crini N (2019) Conventional and non-conventional adsorbents for wastewater treatment. *Environ. Chem. Lett.* 17:195–213.
- Das A, Singh J and Yogalakshmi KN (2017) Laccase immobilized magnetic iron nanoparticles: fabrication and its performance evaluation in chlorpyrifos degradation. *International Biodeterioration & Biodegradation*, 117, pp.183–189.
- Dave S and Das J (2021) Role of microbial enzymes for biodegradation and bioremediation of environmental pollutants: Challenges and future prospects. In *Bioremediation for Environmental Sustainability*; Elsevier: Amsterdam, The Netherlands, pp.325–346.
- Desiante WL, Minas NS and Fenner K (2021) Micropollutant biotransformation and bioaccumulation in natural stream biofilms. *Water Res.* 193:116846.
- Federsel H-J, Moody TS and Taylor SJ (2021) Recent trends in enzyme immobilization—Concepts for expanding the biocatalysis toolbox. *Molecules* 26:2822.
- Ghosh S and Sarkar B (2023) Microbial enzymes for biodegradation and detoxification of pesticides. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands pp.321–356.
- Gianfreda L, Xu F and Bollag J-M (1999) Laccases: A useful group of oxidoreductive enzymes. *Bioremediat. J.* 3:1–26
- Givaudan A, Effosse A, Faure D, Potier P, Bouillant M-L and Bally R (1993) Polyphenol oxidase in *Azospirillum lipoferum* isolated from rice rhizosphere: Evidence for laccase activity in non-motile strains of *Azospirillum lipoferum*. *FEMS Microbiol. Lett.* 108:205–210.
- Goutte A, Alliot F, Budzinski H, Simonnet-Laprade C, Santos R, Lachaux V, Maciejewski K, Le Menach K and Labadie P (2020) Trophic transfer of micropollutants and their metabolites in an urban riverine food web. *Environ. Sci. Technol.* 54:8043–8050.
- Gu Y, Yuan L, Jia L, Xue P and Yao H (2021) Recent developments of a co-immobilized laccase–mediator system: A review. *RSC Adv.* 11:29498–29506.
- Gul B, Naseem MK, Malik W-N, Gurmani AR, Mehmood A and Rafique M (2022) Environmental micropollutants and their impact on human health with special focus on agriculture. In *Hazardous Environmental Micro-Pollutants, Health Impacts and Allied Treatment*. Springer, Cham, pp.1–19.
- Guo M, Lu FP, Liu MY, Li TP, Pu J, Wang N, Liang P and Zhang CY (2008) Purification of recombinant laccase from *Trametes versicolor* in *Pichia methanolica* and its use for the decolorization of anthraquinone dye. *Biotechnol. Lett.* 30:2091–2096.
- Hachi M, Chergui A, Yeddou AR, Selatnia A and Cabana H (2017) Removal of acetaminophen and carbamazepine in single and binary systems with immobilized laccase from *Trametes hirsuta*. *Biocatal. Biotransform.* 35:51–62.
- Hernández AF, Parrón T, Tsatsakis AM, Requena M, Alarcón R and López-Guarnido O (2013) Toxic effects of pesticide mixtures at a molecular level: Their relevance to human health. *Toxicology* 307:136–145.
- Homaei AA, Sariri R, Vianello F and Stevanato R (2013) Enzyme immobilization: An update. *J. Chem. Biol.* 6:185–205.
- Hullo M-F, Moszer I, Danchin A and Martin-Verstraete I (2001) CotA of *Bacillus subtilis* is a copper-dependent laccase. *J. Bacteriol.* 183:5426–5430.
- Isanapong J, Suwannoi K, Lertlattanapong S and Panchal S (2024) Purification, characterization of laccase from *Pleurotus ostreatus* HK35, and optimization for congo red biodecolorization using Box-Behnken design. *3 Biotech* 14:73.
- Janusz G, Pawlik A, Świdzka-Burek U, Polak J, Sulej J, Jarosz-Wilkolazka A and Paszczyński A (2020) Laccase

properties, physiological functions, and evolution. *Int. J. Mol. Sci.* 21:966.

Jia J, Xue P, Ma L, Li P and Xu C (2024) Deep degradation of atrazine in water using co-immobilized laccase-1-hydroxybenzotriazole-Pd as composite biocatalyst. *J. Hazard. Mater.* 468:133779.

Jiménez Vizcarra MJ, Mahendra S and Wang M (2023) A Co-immobilized enzyme-mediator system for facilitating manganese peroxidase catalysis in solution free of divalent manganese ions. *Bioresour. Technol.* 390:129897

Kalsoom U, Ahsan Z, Bhatti HN, Amin F, Nadeem R, Aftab K and Bilal M (2022) Iron oxide nanoparticles immobilized *Aspergillus flavus* manganese peroxidase with improved biocatalytic, kinetic, thermodynamic, and dye degradation potentialities. *Process Biochem.* 117:117–133.

Karigar CS and Rao SS (2011) Role of microbial enzymes in the bioremediation of pollutants: A review. *Enzym. Res.* 2011:805187.

Liang W and Yang M (2019) Urbanization, economic growth and environmental pollution: Evidence from China. *Sustain. Comput. Inform. Syst.* 21:1–9.

Liu XP, Deng W and Yang Y (2021) Characterization of a novel laccase LAC-Yang1 from white-rot fungus *Pleurotus ostreatus* strain Yang1 with a strong ability to degrade and detoxify chlorophenols. *Molecules* 26:473.

Liu Y, Huang L, Guo W, Jia L, Fu Y, Gui S and Lu F (2017) Cloning, expression, and characterization of a thermostable and pH-stable laccase from *Klebsiella pneumoniae* and its application to dye decolorization. *Process Biochem.* 53:125–134.

Loi M, Glazunova O, Fedorova T, Logrieco AF and Mulè G (2021) Fungal laccases: The forefront of enzymes for sustainability. *J. Fungi* 7:1048.

Lou X, Zhi F, Sun X, Wang F, Hou X, Lv C and Hu Q (2023) Construction of co-immobilized laccase and mediator based on MOFs membrane for enhancing organic pollutants removal. *Chem. Eng. J.* 451:138080.

Machczynski MC, Vijgenboom E, Samyn B and Canters GW (2004) Characterization of SLAC: A small laccase from *Streptomyces coelicolor* with unprecedented activity. *Protein Sci.* 13:2388–2397.

Maghraby YR, El-Shabasy RM, Ibrahim AH and Azzazy HME-S (2023) Enzyme immobilization technologies and industrial applications. *ACS Omega* 8:5184–5196.

Malhotra M and Suman SK (2021) Laccase-mediated delignification and detoxification of lignocellulosic biomass: Removing obstacles in energy generation. *Environ. Sci. Pollut. Res.* 28:58929–58944.

Martins LO, Durão P, Brissos V and Lindley PF (2015) Laccases of prokaryotic origin: Enzymes at the interface of protein science and protein technology. *Cell. Mol. Life Sci.* 72:911–922.

More SS, Renuka PS, Pruthvi K, Swetha M, Malini S and Veena SM (2011) Isolation, purification, and characterization of fungal laccase from *Pleurotus* sp. *Enzym. Res.* 2011:248735.

Narayanan M, Ali SS and El-Sheekh M (2023) A comprehensive review on the potential of microbial enzymes in multipollutant bioremediation: Mechanisms, challenges, and future

prospects. *J. Environ. Manag.* 334:117532.

Narayanan M, Murugan S, Eva A, Devina S and Kalidass S (2015) Application of immobilized laccase from *Bacillus subtilis* MTCC 2414 on decolorization of synthetic dyes. *Res. J. Microbiol.* 10:421–432.

Neifar M, Chouchane H, Mahjoubi M, Jaouani A and Cherif A (2016) *Pseudomonas extremorientalis* BU118: A new salt-tolerant laccase-secreting bacterium with biotechnological potential in textile azo dye decolorization. *3 Biotech* 6:1–9.

Pinto I, Simões M and Gomes IB (2022) An overview of the impact of pharmaceuticals on aquatic microbial communities. *Antibiotics* 11:1700.

Preethi PS, Vickram S, Das R, Hariharan N, Rameshpathy M, Subbaiya R, Karmegam N, Kim W and Govarthanam M (2023) Bioprospecting of novel peroxidase from *Streptomyces coelicolor* strain SPR7 for carcinogenic azo dyes decolorization. *Chemosphere* 310:136836.

Rahman MM, Sultan MB and Alam M (2023) Microplastics and adsorbed micropollutants as emerging contaminants in landfill: A mini review. *Curr. Opin. Environ. Sci. Health* 31:100420.

Salem T, Fetyan NA, Malhat F, Abdelhafez AA and Ramadan AI (2024) A novel multi-enzyme immobilized biocatalyst for Biodegradation of p, p'-DDT. *Egypt. J. Chem.* 67:21-36.

Saravanan A, Kumar PS, Jeevanantham S, Karishma S, Tajsabreen B, Yaashikaa P and Reshma B (2021) Effective water/wastewater treatment methodologies for toxic pollutants removal: Processes and applications towards sustainable development. *Chemosphere* 280:130595.

Saravanan A, Kumar PS, Vo D-VN, Jeevanantham S, Karishma S and Yaashikaa P (2021) A review on catalytic-enzyme degradation of toxic environmental pollutants: Microbial enzymes. *J. Hazard. Mater.* 419:126451.

Singh D, Rawat S, Waseem M, Gupta S, Lynn A, Nitin M, Ramchiary N and Sharma KK (2016) Molecular modeling and simulation studies of recombinant laccase from *Yersinia enterocolitica* suggests significant role in the biotransformation of non-steroidal anti-inflammatory drugs. *Biochem. Biophys. Res. Commun.* 469:306–312.

Singh J, Das A and Yogalakshmi KN (2020) Enhanced laccase expression and azo dye decolorization during co-interaction of *Trametes versicolor* and *Phanerochaete chrysosporium*. *SN Applied Sciences* 2:1-8.

Spina F, Gea M, Bicchi C, Cordero C, Schilirò T and Varese GC (2020) Ecofriendly laccases treatment to challenge micropollutants issue in municipal wastewaters. *Environ. Pollut.* 257:113579.

Srinivasan P, Selvankumar T, Paray BA, Rehman MU, Kamala-Kannan S, Govarthanam M, Kim W and Selvam K (2020) Chlorpyrifos degradation efficiency of *Bacillus* sp. laccase immobilized on iron magnetic nanoparticles. *3 Biotech* 10:366.

Tkaczyk A, Mitrowska K and Posyniak A (2020) Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: A review. *Sci. Total Environ.* 717:137222.

Varga B, Somogyi V, Meiczinger M, Kováts N and Domokos E (2019) Enzymatic treatment and subsequent toxicity of

organic micropollutants using oxidoreductases—A review. *J. Clean. Prod.* 221:306–322.

Vidal-Limon A, García Suárez PC, Arellano-García E, Contreras OE and Aguila SA (2018) Enhanced degradation of pesticide dichlorophen by laccase immobilized on nanoporous materials: A cytotoxic and molecular simulation investigation. *Bioconjug. Chem.* 29:1073–1080.

Vitola G, Mazzei R and Giorno L (2023) Biohybrid membranes for organophosphate pesticides degradation: Hyperactivation of immobilized phosphotriesterase by surfactants. *Environ. Technol. Innov.* 30:103053.

Wang H, Huang L, Li Y, Ma J, Wang S, Zhang Y, Ge X, Wang N, Lu F and Liu Y (2020) Characterization and application of a novel laccase derived from *Bacillus amyloliquefaciens*. *Int. J. Biol. Macromol.* 150, 982–990.

Xu PF, Du H, Peng X, Tang Y, Zhou YY, Chen XY, Fei J, Meng Y and Yuan L (2020) Degradation of several polycyclic aromatic hydrocarbons by laccase in reverse micelle system. *Sci. Total Environ.* 708:134970.

Maghraby YR, El-Shabasy RM, Ibrahim AH and Azzazy HME-S (2023) Enzyme immobilization technologies and industrial applications. *ACS Omega* 8:5184–5196.

Yang X, Tang X, Dong F, Lin L, Wei W and Wei D (2020) Facile one-pot immobilization of a novel thermostable carboxylesterase from *Geobacillus uzonensis* for continuous pesticide degradation in a packed-bed column reactor. *Catalysts* 10:518.

Yang Y, Zhang X, Jiang J, Han J, Li W, Li X, Yee Leung KM, Snyder SA and Alvarez PJJ (2021) Which micropollutants in water environments deserve more attention globally? *Environ. Sci. Technol.* 56:13–29.

Yavaşer R, Aktaş Uygun D and Karagözler AA (2023) Removal of selected azo dyes and phenolic compounds via tyrosinase immobilized magnetic iron oxide silver nanoparticles. *Catal. Lett.* 153:1265–1277.

Zhu Y, Qiu F, Rong J, Zhang T, Mao K and Yang D (2020) Covalent laccase immobilization on the surface of poly(vinylidene fluoride) polymer membrane for enhanced biocatalytic removal of dyes pollutants from aqueous environment. *Colloids Surf. B Biointerfaces* 191:111025.

Zong W, Su W, Xie Q, Gu Q, Deng X, Ren Y and Li H (2022) Expression, characterization, and immobilization of a novel SGNH esterase Est882 and its potential for pyrethroid degradation. *Front. Microbiol.* 13:1069754.

Author Contributions

All the authors conceived the concept, wrote and approved the manuscript.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Competing interest

The authors declare no competing interests.

Ethics approval

Not applicable.

Open Access

The authors retain the copyright of this article. It is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution, and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. Visit for more details <http://creativecommons.org/licenses/by/4.0/>.

Citation: Jatinder Singh Randhawa and Harmanpreet Meehnian (2026) Enzyme Immobilization on Nanomaterials for Cutting-Edge Environmental Remediation Solutions: A Special Emphasis on Dyes and Pesticides. *Technol TIMES* 1(1): 32-39.

