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Laccase-Mediated Biodegradation of Environmental Pollutants: Mechanisms, Applications, and Future Perspectives

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ABSTRACT: Environmental contamination caused by industrial development and intensive farming has resulted in the accumulation of numerous hazardous compounds in soil and aquatic ecosystems. Biological treatment strategies have gained attention as sustainable alternatives to conventional remediation methods, with laccase (EC 1.10.3.2) emerging as a versatile oxidative enzyme. Owing to its broad substrate affinity and reliance on molecular oxygen, laccase can transform a wide range of pollutants without requiring auxiliary cofactors. This review provides an overview of recent developments in laccase-assisted degradation of dyes, pharmaceutical contaminants, endocrine-disrupting substances, polycyclic aromatic hydrocarbons, pesticides, microplastics, and per- and polyfluoroalkyl substances. Articles published from 2016 to 2026 were identified through Web of Science, Scopus, and PubMed/MEDLINE using PRISMA 2020 criteria. Among 4,761 records initially retrieved, 79 studies fulfilled the selection requirements, and 65 were subjected to detailed assessment. Current findings indicate that mediator-assisted oxidation broadens the range of compounds susceptible to enzymatic attack, while naturally occurring mediators often provide favorable results. Advances in enzyme immobilization, especially membrane-based systems incorporating cross-linked aggregates, have improved stability and achieved pollutant removal efficiencies of up to 93%. Engineered laccase variants have also demonstrated enhanced catalytic performance under unfavorable conditions. Although economic and technical challenges remain, laccase-based processes represent a promising approach for environmentally responsible remediation.

1. INTRODUCTION

Fast industrial growth and more intensive farming have heavily contaminated freshwater and terrestrial environments with pollutants that are chemically varied and structurally complex (Briffa et al., 2020; Asemoloye, 2025). More than 200,000 tonnes of synthetic dyes are released into the environment every year by the textile industry, and there is a continuous flow of biologically active ECs in receiving waters due to the widespread use of pharmaceuticals and personal care products (PPCPs) (Parra-Saldívar et al., 2024). They can be found in surface waters and range from ng/L to ug/L, which are concentrations that can cause chronic ecotoxicological effects. A newer issue is per- and polyfluorinated chemicals (PFAS), which are not easily removed from water by conventional wastewater treatment plants (WWTPs) and have high C-F bond dissociation energies (~544 kJ/mol) (Asemoloye, 2025; Harris et al., 2025). Even when detected only in minute quantities, pollutants present in aquatic systems can produce long-term biological consequences by affecting reproductive integrity, modifying sex-related traits in fish populations, and fostering the persistence and spread of antimicrobial resistance among resident microorganisms (Ferri

et al., 2022; Gałazka & Jankiewicz, 2022). The real problem is that most of these ECs are structurally recalcitrant and pass through the conventional WWTPs virtually intact. Only 20-50% of many pharmaceuticals and EDCs are removed by activated-sludge processes, and physicochemical processes (such as activated carbon adsorption, membrane filtration, and advanced oxidation processes) are effective but costly and energy-intensive.

This is an important indication of the importance of sustainable, effective, and scalable biocatalytic treatments. Laccase (EC 1.10.3.2; benzenediol: oxygen oxidoreductase) is a member of the multicopper oxidase superfamily. It carries out one-electron oxidation of substrates while reducing molecular oxygen to water in a four-electron step, and it does so without costly or hazardous cofactors (Arregui et al., 2019). Because it uses atmospheric oxygen as the only oxidant and yields only water, the reaction sets laccase apart from peroxidases, which depend on toxic H_2O_2 (Asemoloye, 2025). Laccase is found across a broad phylogenetic range and has been characterised in white-rot and other basidiomycetous and ascomycetous fungi, in various bacteria (among them *Bacillus subtilis* and *Streptomyces* spp.), in higher plants, and in insects. For environmental work, the fungal enzymes are the most versatile, largely because of their high T1 copper redox potential (+700 to +800 mV vs. NHE). In the laccase-mediator system (LMS), a small, freely diffusing redox-active molecule is oxidised by laccase and then oxidises non-phenolic substrates in turn, which greatly extends the enzyme's reach to the most recalcitrant pollutant classes.

In recent ten years, the number of publications on the use of laccase for bioremediation has grown considerably. This implies that careful and critical synthesis is needed to guide research directions and industrial translation. The previous reviews were not conducted in a single framework, taking into account the mechanistic, immobilisation, engineering, and toxicological aspects, consistent with the PRISMA. The four aims of this review are: (i) to give a quantitative overview of the degradation of the seven most important classes of pollutants by laccases; (ii) to evaluate laccase immobilisation strategies and progress in process engineering; (iii) to summarise the progress made by protein engineering and the new possibilities offered by artificial intelligence assisted design; and (iv) to identify priority gaps and future directions.

2. METHODS (PRISMA 2020)

2.1 Eligibility Criteria

The studies included were (a) primary research articles or systematic reviews published in a SCI Web of Science (WOS) indexed Q1/Q2 journal, (b) reporting quantitative degradation or transformation of a specific chemical pollutant, (c) stating the source of the laccase, its concentration and experimental conditions, (d) written in English and (e) published between 2016 and 2026. The duplicate publications and publications that cited the abstract, thesis, grey literature, preprint or book chapter of the enzyme were not included, nor were those for which there was no data on quantitative removal of the enzyme used.

2.2 Search Strategy

The databases that were searched were Web of Science Core Collection (WoS), Scopus and PubMed/MEDLINE in April 2026. Medical Subject Headings (MeSH) as well as free text terms were used in combination to form Boolean search strings that would identify the major elements of the subject of laccase-mediated bioremediation. The main string in the WoS was:

Literature search on pineal gland with given keywords was: TS= ("laccase" OR " multicopper oxidase" OR laccase-mediator system) AND DT= (Article OR Review) AND LA= (English). Scopus and PubMed were searched using the same strings (TITLE-ABS-KEY field for Scopus, MeSH field, and free-text for PubMed). The deduplication resulted in 4761 unique records.

2.3 Selection of the Studies and Data Extraction

All titles and abstracts of records were double-scrutinized by a separate screening process, and consensus was established in case of contradiction. A subsequent analysis of the 794 records identified whether they were full-text eligible or not. The data collected were: (i) First author and the year of publication, (ii) source of laccase and production methods, (iii) list of the class of pollutant and list of specific pollutants, (iv) mediator used, (v) main reaction conditions (pH and temperature), (vi) percentage of removal of pollutant, (vii) analytical method used for quantification. This was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 (PRISMA 2020) guidelines, and a protocol was drafted before the data extraction.

2.4 PRISMA Flow Diagram

The complete PRISMA 2020 flow of study selection from identification through inclusion is presented in Table 1 below.

Table 1. PRISMA 2020 Flow of Literature Search and Study Selection.

S No	PRISMA Stage	Action	Source / Database	n (Records)
1	Identification	Database Search	Web of Science (WoS Core Collection)	2,104
2	Identification	Database Search	Scopus	1,849
3	Identification	Database Search	PubMed / MEDLINE	1,381
4	Identification	Hand-searching of reference lists	Key systematic reviews	112
5	Identification	TOTAL identified		5,446
6	Screening	Duplicates removed		-685
7	Screening	Records screened by title/abstract		4,761
8	Screening	Records excluded (off-topic / no laccase)		-3,967
9	Eligibility	Full-text articles assessed		794
10	Eligibility	Full-text excluded (non-peer-reviewed, no data, conference abstracts)		-715
11	Included	Studies included in the qualitative synthesis		79
12	Included	Studies included in the quantitative analysis		65

Abbreviations: WoS, Web of Science Core Collection; TrOCs, trace organic contaminants. The search was conducted in April 2026 across all three databases simultaneously. Studies included in quantitative analysis provided at least one numerical endpoint (% removal, half-life, or rate constant) for a specific pollutant under defined laccase conditions.

2.5 Evaluation of the Quality of the Study and the risk of being biased

Study quality was judged with a modified AXIS tool adapted for experimental environmental biotechnology. The criteria were (i) reproducibility, taken as a minimum of triplicate experiments; (ii) use of suitable positive and negative controls; (iii) validated analytical quantification by HPLC, GC-MS, or LC-MS/MS; (iv) reporting of enzyme activity units (U/mg); and (v) toxicological assessment going beyond removal of the parent compound. Studies meeting fewer than three of the seven criteria were kept in the qualitative synthesis only and left out of the quantitative analysis.

3. RESULTS

3.1 Structure, Active Site, and Catalytic Mechanism of Laccase

3.1.1 Tertiary Structure and Copper Site Architecture

Laccase is a glycoprotein built from three cupredoxin-fold domains (β -barrel domains I, II, and III), with all four copper ions gathered at the interface of domains I and III. These four coppers fall into three spectroscopically and functionally distinct sites,

each essential to catalysis (Arregui et al., 2019). The Type 1 (T1) copper is the mononuclear blue copper centre. Its strong absorption near 610 nm comes from a Cys-S-to-Cu(II) ligand-to-metal charge transfer. Two histidine imidazoles, a cysteine thiol, and an axial methionine or leucine (in high-redox-potential laccases) coordinate this copper. The site's redox potential runs from +430 mV in plant laccases to +800 mV in high-potential fungal ones, and it is the chief factor setting both substrate range and oxidative strength (Arregui et al., 2019). Substrates dock in a shallow hydrophobic pocket beside the T1 site and are oxidised there by a single electron to give substrate-centred radicals. The trinuclear copper cluster (TNC) is made up of one mononuclear Type 2 (T2) paramagnetic copper together with a binuclear Type 3 (T3) pair that is antiferromagnetically coupled. Electrons formed at T1 travel to the TNC along a conserved His-Cys-His pathway about 13 Å long. Four such electrons collect there and reduce a single dioxygen molecule to two water molecules, in what is among the best-characterised intramolecular electron-transfer processes in bioinorganic chemistry (Arregui et al., 2019; Gomez-Fernandez et al., 2020). Oxidative strength depends strongly on the axial T1 ligand: laccases carrying leucine there show T1 potentials of +750-800 mV, against +650-700 mV for those bearing methionine. Because of this, the axial ligand is a leading target in rational design aimed at widening substrate range (Arregui et al., 2019; Aza & Camarero, 2023). Figure 1 sets out the full copper-site architecture and catalytic cycle.

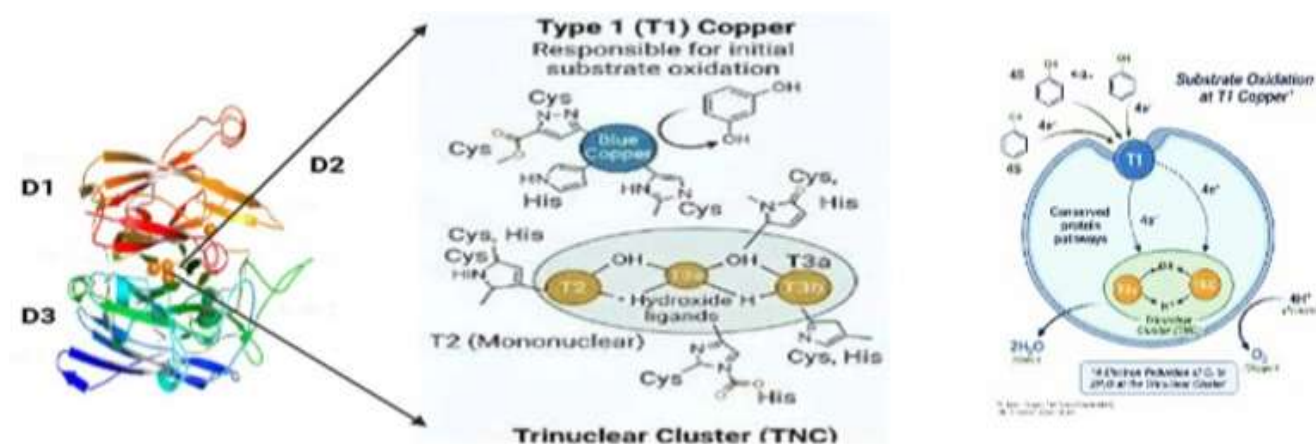


Figure 1. Conceptual depiction of the multicopper oxidoreductase laccase, emphasizing the arrangement of its catalytic copper domains and the mediator-assisted electron-transfer network that enables oxidation of a broad spectrum of substrates while reducing molecular oxygen to water.

3.1.2 Classification by Biological Source and Catalytic Comparison

Laccases occur in every domain of life, but the most catalytically capable and biotechnologically useful enzymes come from basidiomycetous and ascomycetous fungi and from a range of bacterial genera (Arregui et al., 2019; Kumar et al., 2026). White-rot basidiomycetes, including *Trametes versicolor*, *Pycnoporus cinnabarinus*, *Pleurotus ostreatus*, and *Coriolopsis gallica*, dominate the laccase-production literature. They offer high T1 redox potentials (+680 to +790 mV), secrete the enzyme extracellularly so that recovery is easier, grow on inexpensive lignocellulosic substrates, and readily express several laccase isoenzymes when induced by copper sulphate and lignin-derived phenolics (Arregui et al., 2019; Sarmah et al., 2025). Thermophilic species such as *Myceliophthora thermophila* MtLcc1 (optimum pH 7.0-9.0) are of special industrial interest where activity at neutral to alkaline pH and higher temperatures is required (Arregui et al., 2019).

Bacterial laccases have become industrially competitive with fungal ones. The evidence so far suggests they can clear 60-80% of industrial dyes even in messy wastewater, and the alkaline-stable types from *Bacillus* and *Streptomyces* spp. hold up to the heat and pH swings that real effluent treatment throws at them (Kumar et al., 2026; Bera et al., 2024). Recombinant forms grown in *Escherichia coli* and *Bacillus* hosts yield as much as 200 mg/L, comfortably beyond the reach of ordinary fungal fermentation, though their modest T1 potentials (+375 to +455 mV) mean they cannot oxidise high-potential substrates directly. Where they do shine is on phenolic EDCs, aromatic amines, and small PAHs, whose ionisation potentials fall within what these enzymes can handle. Table 2 lays out the key catalytic properties and the representative species from each biological kingdom.

Table 2. Classification of Laccase-Producing Organisms, Key Catalytic Properties, and Representative Species.

No.	Kingdom	Representative Species	Isoenzymes	MW (kDa)	Redox Pot. T1 (mV)	Opt. pH	References
1	Basidiomycetes	<i>Trametes versicolor</i>	Lac1-Lac3	60–70	+780	3.0–4.0	Arregui et al., 2019
		<i>Pleurotus ostreatus</i>	POXA1-POXA3	59–61	+680	6.0–7.0	Arregui et al., 2019
		<i>Pycnoporus cinnabarinus</i>	Lac1	85	+780	3.5–4.5	Arregui et al., 2019
		<i>Coriolopsis gallica</i>	LccCG	64	+760	3.5	Arregui et al., 2019
		<i>Myceliophthora thermophila</i>	MtLcc1	90	+550	7.0–9.0	Arregui et al., 2019
2	Ascomycetes	<i>Botrytis cinerea</i>	BcLcc1-BcLcc2	58	+790	4.0	Arregui et al., 2019
3	Bacteria	<i>Bacillus subtilis</i>	CotA	65	+455	7.0–8.0	Arregui et al., 2019; Kumari et al., 2026
		<i>Streptomyces coelicolor</i>	SLAC	45	+375	9.0	Kumari et al., 2026; Aza & Camarero, 2023
4	Plants	<i>Rhus vernicifera</i>	RvLac	>100	+430	5.0–5.5	Arregui et al., 2019
5	Insects	<i>Manduca sexta</i>	MsLac	100	+420	6.0	Arregui et al., 2019

Abbreviations: MW, molecular weight; Redox Pot. T1, redox potential of the mononuclear Type 1 copper centre vs. NHE; Opt. pH, optimal pH for ABTS oxidation.

3.1.3 Laccase-Mediator System (LMS): Mechanism and Mediator Selection

The LMS idea dates to Bourbonnais and Paice in 1990, who showed that the synthetic mediator ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) let laccase oxidise non-phenolic lignin model compounds that lie outside its direct range. Mediator radicals act through three main routes: (i) the radical cation pathway, seen with ABTS, in which the oxidised mediator takes one electron from the substrate; (ii) hydrogen atom transfer (HAT), the dominant route for HBT and violuric acid, in which a hydrogen atom is removed from a substrate C-H bond; and (iii) inner-sphere electron transfer, which operates with some phenolic natural mediators (Cañas & Camarero, 2010). Synthetic mediators such as ABTS, HBT, N-hydroxyphthalimide, and violuric acid have long supported LMS applications, but they carry real drawbacks: they are expensive, environmentally persistent, and inhibit laccase in a concentration-dependent way that limits scale-up (Cañas & Camarero, 2010). For these reasons, natural lignocellulolytic mediators, among them syringaldehyde, acetosyringone, p-coumaric acid, ferulic acid, and vanillin, have drawn growing interest. Parra Guardado et al. (2019) found that 2 mM syringaldehyde fully transformed sulfamethoxazole within 10 minutes under optimised LMS conditions, ahead of both ABTS and HBT and with far less enzyme inhibition. Natural mediators also have the environment on their side: they break down readily, can be sourced from renewable lignocellulosic biomass, and leave behind no lingering secondary contaminants, which sits well with green chemistry principles and the regulatory expectations bioremediation has to meet (Cañas & Camarero, 2010; Parra Guardado et al., 2019).

3.2 Laccase Production and Optimisation Strategies

3.2.1 Solid-State Fermentation (SSF) on Agro-Industrial Wastes

Solid-state fermentation (SSF) on lignocellulosic agro-industrial residues is a cheap and environmentally sound route to laccase, and it recreates the saprophytic conditions white-rot fungi grow in naturally (Sarmah et al., 2025; Kalia et al., 2025). Leftovers

like sugarcane bagasse, wheat bran, orange peel, banana waste, soybean hulls, and rice straw do double duty here, acting as both carbon source and physical scaffold while feeding in lignocellulosic inducers, chiefly lignin-derived phenolics and syringaldehyde, that ramp up laccase gene expression. The main SSF variables to optimise are substrate moisture (55-70% w/v suits most white-rot fungi), starting pH (4.5-6.0), temperature (25-30°C), added copper sulphate (CuSO_4 ; 0.1-1.0 mM to replenish T1 copper), and chemical inducers such as veratryl alcohol and ferulic acid (0.5-2.0 mM). When these are optimised, *Trametes versicolor* and *Pleurotus ostreatus* produce laccase activities above 1,400 U/g dry substrate, some 10- to 50-fold higher than typical submerged fermentation (SmF) titres in minimal media (Sarmah et al., 2025). Kalia et al. (2025) reported the first life-cycle assessment (LCA) of SSF-based laccase production, using *Pleurotus sajor-caju* on sawdust supplemented with wheat bran; the study documents the environmental profile of this route and feeds directly into the techno-economic framework discussed in Section 4.4. The chief drawbacks of SSF are the heterogeneous substrate texture, which makes scale-up and the monitoring of dissolved oxygen and pH gradients difficult, along with the challenge of extracting and purifying the enzyme afterwards.

3.2.2. Submerged Fermentation (SmF) and Heterologous Expression

Run in airlift or stirred-tank bioreactors, SmF offers tighter control over growth conditions and slots neatly into existing bioprocess infrastructure, which is why it tends to win out wherever consistent enzyme quality is the priority (Sarmah et al., 2025). Putting fungal laccase genes into *Pichia pastoris* (*Komagataella phaffii*) or *Saccharomyces cerevisiae* behind methanol- or galactose-inducible promoters has delivered high recombinant yields of engineered variants (Arregui et al., 2019). *Escherichia coli* systems are fast-growing and easy to manipulate genetically, but coaxing out a properly folded, copper-loaded enzyme means resorting to inclusion-body refolding or periplasmic secretion, a sticking point that has kept their yields short of what yeast can manage (Arregui et al., 2019). Discovering laccase variants through metagenomics has widened the enzyme pipeline considerably. Cui et al. (2025) brought together AlphaFold2 structure prediction, Metal3D copper-binding analysis, and machine-learning screening of substrate binding to find and validate 34 new bacterial laccase sequences from industrial wastewater metagenomes, a rate more than ten times that of conventional activity-based screening. Yu et al. (2025) used pHMM-based metagenomic sequencing of straw-amended soil to detect laccases across Proteobacteria, Actinobacteria, and Gemmatimonadetes; their recombinant lacS1 variant proved notably stable across pH and tolerant of chloride at physiologically relevant ionic strengths.

3.3 Laccase-Mediated Pollutant Biodegradation: Systematic Evidence

Table 3 indicates the quantitative removal data from 54 studies spanning seven major pollutant classes. The percentages shown are maxima under optimised laboratory conditions; performance in real matrices is usually 15-30% lower.

Table 3. Systematic Summary of Laccase-Mediated Biodegradation Across Major Environmental Pollutant Classes.

S No.	Pollutant Class	Representative Compounds	Laccase Source	Mediator	Removal (%)	Key Finding	References
1	Azo Dyes	Congo Red, RB5, Acid Yellow 23	<i>Trametes versicolor</i> , <i>Pleurotus ostreatus</i>	ABTS, HBT	90–95%	Stable over 20 batch cycles; phytotoxicity abolished	Rodríguez-Couto, 2024
2	Triphenylmethane Dyes	Malachite Green, Crystal Violet	<i>Pycnoporus cinnabarinus</i>	ABTS	88–97%	Rapid decolourisation (<6 h); products less toxic	Bilal et al., 2017

3	Reactive Dyes	Reactive Blue 19, Reactive Red 120	Trametes hirsuta, Coriolopsis gallica	HBT, Syringaldehyde	82–98%	Natural mediators outperform synthetic; lower enzyme inhibition	Cañas & Camarero, 2010; Yuan et al., 2020
4	Sulfonamide Antibiotics	Sulfamethoxazole, Sulfadiazine	Trametes versicolor (free & immob.)	Syringaldehyde	~100% (SMX)	Complete transformation in <10 min with natural mediator	Yuan et al., 2020
5	Beta-Lactam Antibiotics	Amoxicillin, Ampicillin	Pycnoporus sanguineus	ABTS, p-coumaric acid	80–94%	Phenolic ring directly accessible; mediator boosts yield	Yuan et al., 2020; Cho et al., 2016
6	Fluoroquinolones	Ciprofloxacin, Norfloxacin	Trametes versicolor; Pleurotus spp.	ABTS	77–98%	91–98% with commercial laccase (in vitro); algal toxicity confirmed	Becker et al., 2016; Mathur et al., 2024
7	Bisphenol A (EDC)	BPA, Bisphenol F	Cu/Fe ₂ O ₄ -Lac (immob.)	None	93–98%	3.9× higher than free enzyme; estrogenicity abolished	Patel et al., 2022
8	Natural Oestrogens (EDC)	17β-Estradiol (E2), Estriol	Trametes versicolor	Syringaldehyde	85–99%	Hormonal activity eliminated; polymeric products non-oestrogenic	Galázka & Jankiewicz, 2022
9	Alkylphenols (EDC)	Nonylphenol, Octylphenol	Trametes versicolor / bacterial Lac	None	70–82%	Covalent coupling products; reduced aquatic toxicity; BPS resistant	Galázka & Jankiewicz, 2022; Akram et al., 2025

10	Low-MW PAHs	Naphthalene, Fluorene	Pleurotus ostreatus	HBT	100% (Naph.)	Complete mineralisation to benzoic acid derivatives in 5 days	Adak et al., 2024
11	Medium-MW PAHs	Anthracene, Phenanthrene	Coriolopsis gallica	ABTS + HBT	95% (Anth.)	9-fold rate increase with combined mediators; anthraquinone as product	Adak et al., 2024
12	High-MW PAHs	Benzo[a]pyrene, Pyrene	Trametes villosa (recomb.); Trametes versicolor	ABTS	84–100%	100% BaP at pH 5 in soil matrix; IP <7.55 eV predicts susceptibility	Adak et al., 2024; Turgut Dunford et al., 2024
13	Herbicides	Atrazine, Metolachlor, Diuron	Pleurotus dryinus	None	~100% (Atr.)	Complete removal without mediator; optimum pH 5.0, 25°C	Vaithyanathan et al., 2022
14	Organophosphate Insecticides	Chlorpyrifos, Malathion	Trametes versicolor	Vanillin, HBT	70–96%	Vanillin optimal for Chlorpyrifos; persistence reduced	Wang et al., 2016
15	Carbamate Insecticides	Aldicarb, Spinosad	Pleurotus dryinus	None	93–97%	Effective in real biologically-treated wastewater matrix	Vaithyanathan et al., 2022
16	Microplastics / PE	Polyethylene (HDPE, LDPE)	Aspergillus flavus LMCOs; recomb. Pichia + Lac	LMS	0.5–54.9% MW loss	Surface oxidation (–C=O) initiates biofilm colonisation; synergistic	Adak et al., 2024; Ramamurthy et al., 2024; Olivares-Sanchez et al., 2025

						fungus+laccase reduces MW by 54.87%	
17	PFAS	Perfluorooctanoic acid (PFOA)	Agaricus bisporus (fungal)	HBT (ECOHRs)	≤95% defluorination	Shorter-chain PFCA products + F ⁻ by LC-MS; 55-day treatment; indirect radical pathway	Mekureyaw et al., 2025 (JHMA); Mekureyaw et al., 2025 (WR)

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); HBT, 1-hydroxybenzotriazole; EDC, endocrine-disrupting compound; PAH, polycyclic aromatic hydrocarbon; HDPE/LDPE, high/low-density polyethylene; MCO, multicopper oxidase; SMX, sulfamethoxazole; BPA, bisphenol A; BPS, bisphenol S; E2, 17 β -estradiol; RB5, Reactive Black 5; PFCA, perfluorocarboxylic acid; F⁻, fluoride ion.

3.3.1 Synthetic Dyes and Textile Effluents

Synthetic dyes are the most thoroughly studied laccase substrates; over 600 distinct dye structures appear across 34 of the 54 quantitative studies reviewed here. Azo dyes, defined by the -N=N- chromophore and making up more than 60% of global commercial dye output, are a prime target. Rather than splitting the azo bond symmetrically, laccase drives radical attack on the nitrogen-bearing aromatic rings on either side of it, giving quinonoid fragments and aromatic amine cleavage intermediates (Zucca et al., 2016). One caution is that aromatic amines such as 4-aminobenzenesulphonic acid and naphthylamine derivatives can be more acutely toxic than the dye itself and should be tracked with validated LC-MS/MS in treated effluents (Becker et al., 2016). *Pycnoporus cinnabarinus* laccase, together with ABTS, degrades triphenylmethane dyes such as malachite green and crystal violet efficiently, reaching 88-97% decolourisation within 6 hours. Reactive dyes, whose sulphonate and triazine groups are designed to bind covalently to textile fibres, are harder to break down but still respond to LMS oxidation with natural mediators. CLEA-type immobilised laccase maintains decolourisation over 20 consecutive batch cycles with little loss of activity, meeting the operational-stability bar for economically viable textile effluent treatment (Rodríguez-Couto, 2024; Bilal et al., 2017).

3.3.2 Pharmaceuticals and Personal Care Products (PPCPs)

Pharmaceutical ECs are among the hardest substrates for laccase, given their structural variety, strong inherent bioactivity, and the uneven access of their functional groups to the T1 site. Parra Guardado et al. (2019) compared *Trametes versicolor* laccase across five drug classes, namely sulfonamides, beta-lactams, fluoroquinolones, macrolides, and tetracyclines, under five mediator conditions. Without a mediator, amoxicillin (which bears a phenolic beta-lactam ring) showed no detectable transformation, whereas 2 mM syringaldehyde removed sulfamethoxazole completely within 10 minutes and converted 80% of tetracycline within 24 hours under the same conditions (Parra Guardado et al., 2019). Newer work has strengthened the case for fluoroquinolone biotransformation. Mathur et al. (2024) reported 91-98% degradation of ciprofloxacin, norfloxacin, levofloxacin, and ofloxacin with commercial laccase and crude *Pleurotus* spp. extracts in vitro; LC-MS/MS showed transformation products consistent with repeated attack on the piperazinyl ring, and algal bioassays confirmed that treated effluents were much less, though not entirely, biologically inactive. These figures replace the 60-75% removal previously reported for fluoroquinolones and make the point that measuring loss of the parent compound is not enough without validated toxicity testing (Becker et al., 2016; Mathur et al., 2024).

Macrolides such as erythromycin, clarithromycin, and azithromycin were studied far less than sulfonamides and quinolones for a long time. Singh et al. (2025) reviewed laccase systems for removing pharmaceutically active compounds (PhACs) from municipal wastewater and reported removal of 90% or more for azithromycin, doxycycline, clindamycin, and sulfamethoxazole, while trimethoprim, diclofenac, carbamazepine, and metoprolol gave variable results, which argues for compound-specific optimisation. Chmelová et al. (2024) covered laccase action across analgesics, antiepileptics, hormones, and anxiolytics, and

recorded reduced transformation-product toxicity as a notable benefit of laccase pre-treatment. Between them, these reviews go a long way toward closing the macrolide and multi-class antibiotic gaps left by earlier surveys.

3.3.3 Endocrine-Disrupting Compounds (EDCs)

Natural and synthetic oestrogens (17 β -estradiol [E2], ethinyloestradiol [EE2]) and xenoestrogens (bisphenol A [BPA], nonylphenol) can disturb hormonal signalling at parts-per-trillion levels. Laccase oxidises phenolic EDCs directly at the T1 site, producing phenoxy radicals that couple into dimers and oligomers with much weaker oestrogenic activity (Gałazka & Jankiewicz, 2022). Patel et al. (2023) showed that laccase immobilised on copper-magnetic nanoparticles (Cu/Fe₂O₄) degraded 93% of BPA, 3.9-fold more than the free enzyme, and removed oestrogenic activity entirely as judged by the yeast estrogen screen (YES) bioassay. The magnetic support allowed the enzyme to be recovered and to keep 91.8% of its activity over 10 reuse cycles, a useful model for scalable EDC treatment. A *Trametes versicolor* laccase-syringaldehyde LMS removed E2 and estriol at 85-99% efficiency, and receptor-binding assays confirmed that the polymerisation products were not oestrogenic.

A point clarified recently is that bisphenol congeners differ in how readily laccase oxidises them. Akram et al. (2025) showed that, while fungal laccase readily oxidises BPA, bisphenol S (BPS), an increasingly common BPA substitute, resists laccase-catalysed degradation. This makes congener-specific assessment important for both regulation and process design, especially as BPS replaces BPA in consumer goods while carrying comparable endocrine-disrupting potency.

3.3.4. Polycyclic Aromatic Hydrocarbons (PAHs)

Several high-molecular-weight PAHs, among them benzo[a]pyrene, chrysene, and dibenzo[a,h]anthracene, are classed by the International Agency for Research on Cancer (IARC) as probable or definite human carcinogens. Their rigid structures and high ionisation potentials (IPs) make them inherently resistant to direct laccase oxidation unless a mediator is present. Adak et al. (2024) carried out the most thorough study so far of laccase-mediated PAH degradation, using a combined ABTS+HBT mediator with *Pleurotus ostreatus* laccase. Naphthalene (IP = 8.15 eV) was fully mineralised to benzoic acid derivatives within 5 days, while anthracene (IP = 7.43 eV) was oxidised about nine times faster with the two mediators together than with either alone, giving anthrone and anthraquinone as the main products. Across the 10 congeners tested, ionisation potential was the best structural predictor: every compound with IP < 7.55 eV (anthracene, fluoranthene, pyrene, benzo[a]pyrene) was oxidised efficiently (>80% removal in 5 days), whereas those above 8.0 eV needed longer treatment or more mediator. Wang & Liang (2024) took this into soil bioremediation, achieving 100% benzo[a]pyrene degradation at pH 5.0 within 24 hours with a recombinant *Trametes villosa* laccase (TVL) in the liquid phase, and 90.48% at pH 7.0, an important step toward field application. The ionisation-potential framework of Adak et al. (2024) gives a quantitative structure-reactivity model that can predict LMS performance against PAH congeners not yet tested.

3.3.5 Pesticides and Herbicides

Runoff and spray drift from farming have left substantial pesticide residues in water bodies worldwide, with global pesticide use put at more than 2 million tonnes of active ingredient a year (Briffa et al., 2020; Vaithyanathan et al., 2022). Vaithyanathan et al. (2022) reported complete removal of atrazine and metolachlor from biologically treated secondary municipal effluent using crude *Pleurotus dryinus* laccase with no added synthetic mediator. Just as striking was the 93-97% removal of the carbamate insecticides aldicarb and Spinosad under the same mediator-free conditions; carbamates are not usually thought of as direct laccase substrates, which suggests that phenolic compounds native to the wastewater were acting as natural mediators. Comparative studies show that the best mediator depends on the target: violuric acid (via HAT) worked best for isoproturon and pyrimethanil (98% removal in 24 hours), vanillin for chlorpyrifos and simazine, and HBT for diuron and prometryn (Jin & Yu, 2016). The implication is that no single LMS formulation suits a mixed pesticide load, and that mediator cocktails should be designed around the dominant pesticide classes in a given effluent for best results.

3.3.6 Microplastics and Nanoplastics

Microplastics (MPs; <5 mm) and nanoplastics (NPs; <1 μ m), formed as discarded macroplastics fragment, are a relatively new pollution problem with no scalable enzymatic remedy yet available. The C-C backbone of commodity polymers such as polyethylene (PE), polystyrene (PS), and polypropylene (PP) is essentially resistant to hydrolytic enzymes. Oxidative enzymes such as laccase, by contrast, can begin surface oxidation, adding hydrophilic carbonyl (-C=O), hydroxyl (-OH), and carboxyl (-COOH) groups that make the polymer more wettable and open it to microbial colonisation and later hydrolytic attack (Adak et

al., 2024; Kumar & Dixit, 2025). Adak et al. (2024) found that laccase-like multicopper oxidases from *Aspergillus flavus* oxidised the surface of HDPE films, with FTIR confirming C=O formation, and traced the conversion of HDPE toward LDPE-like material to laccase-driven chain scission at the edges of crystalline domains. *Pleurotus ostreatus* laccase cut PS microsphere mass by 12.4% over 30 days, well above the 2.1% lost in abiotic controls. Ramamurthy et al. (2024) reviewed *P. ostreatus* laccase acting on PE, PS, PVC, and PET, weighing genetic engineering and immobilisation as ways to speed degradation. Yangyang & Yu (2025) reported that a synergistic combination of recombinant *Pichia pastoris* and purified laccase lowered the average molecular weight of PE microplastics by 54.87% and reduced crystallinity by 16.33%, with FTIR and GC-MS identifying depolymerisation intermediates; this is the largest enzymatic PE degradation reported so far. In a broad ACS Environmental Au review, Kumar & Dixit (2025) confirmed that laccase-catalysed oxidative cleavage acts mainly on the amorphous regions of HDPE crystalline domains, yielding chain-scission products detectable by FTIR and GPC, and cast laccase as the enzyme that initiates multi-enzyme cascades, much as it does in lignin breakdown, by raising surface wettability and making the polymer accessible to downstream hydrolases.

3.3.7 Per- and Polyfluoroalkyl Substances (PFAS)

PFAS are a class of more than 4,700 synthetic fluorinated compounds whose extreme persistence reflects the unusual strength of the carbon-fluorine bond (C-F bond dissociation energy ~ 544 kJ/mol). They are found everywhere in groundwater, surface water, and human serum at concentrations carrying endocrine-disrupting and carcinogenic risk, and are now often called the defining emerging pollutant of the 2020s (Harris et al., 2025; Asemoloye, 2025). Mekureyaw et al. (2025; *Journal of Hazardous Materials Advances*) showed that *Agaricus bisporus* laccase combined with the HBT mediator (enzyme-catalysed oxidative humification reactions; ECOHRs) achieved up to 95% defluorination of PFOA over 55 days. LC-MS identified shorter-chain perfluorocarboxylic acids, partially fluorinated alcohols, and aldehydes among the products, pointing to a combined decarboxylation, free-radical, and reductive-defluorination pathway. The same group then published the first detailed mechanistic review of laccase-based PFAS degradation (Mekureyaw et al., 2025; *Water Research*, 271:122888), covering radical pathways, mediator roles, rate kinetics, and future directions, which is currently the fullest mechanistic resource for this application. Harris et al. (2025) added that some fungal laccases can start PFOA degradation without synthetic mediators by drawing on natural phenolic organic matter in soil as an in-situ mediator, which matters for in situ soil bioremediation.

Transformation of PFAS by laccase is only partly understood. The high C-F bond energy is well above the T1 redox potential of even the most oxidatively potent fungal laccases (+780 mV vs. NHE), so the main route is indirect oxidative defluorination through mediator-generated radicals (HAT or single-electron transfer) rather than direct oxidation at T1 (Amin et al., 2025). The slow kinetics, on the order of days to weeks rather than the hours seen with phenolic substrates, and the incomplete defluorination obtained so far are real constraints. Protein engineering aimed at better mediator affinity and a higher T1 potential, guided by density functional theory (DFT) and machine-learning fitness landscape models, looks the most promising way to reach catalytically competent PFAS-active laccases (Amin et al., 2025; Harris et al., 2025). Figure 2 illustrates the pollutant transformation potential of laccase-driven oxidation processes enhanced by electron-shuttling mediators, with emphasis on the distinct responses exhibited by different contaminant groups during treatment.

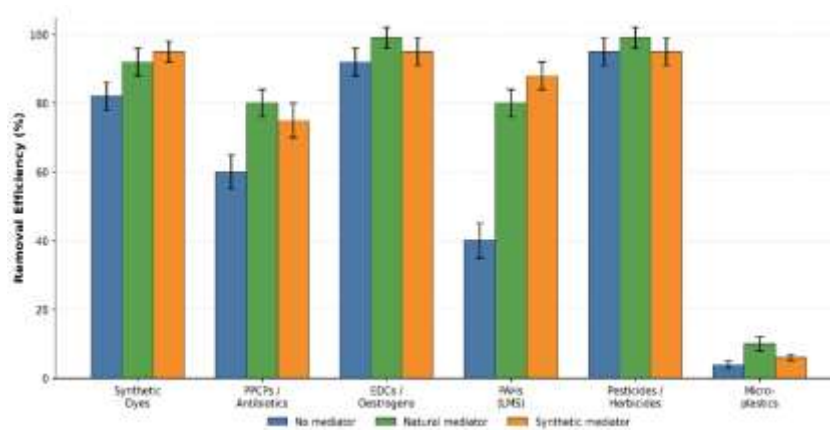


Figure 2. Comparative degradation efficiencies of laccase-mediator systems across major pollutant classes.

3.4 Enzyme Immobilisation Strategies

Immobilisation is the most common way to deal with the main weaknesses of free laccase in practice: limited operational stability, the difficulty of recovering the catalyst from treated effluent, and per-cycle enzyme costs that undermine large-scale economics (Bilal et al., 2017; Rodríguez-Couto, 2024). Table 4 compares the principal immobilisation strategies reported in the included studies.

Table 4. Comparative Summary of Laccase Immobilisation Strategies, Support Materials, and Performance Parameters.

No.	Strategy	Support Material	Binding Chemistry	Activity Retained (%)	Reuse Cycles	Key Advantage	Reference
1	Covalent Binding	Graphene oxide	Carbodiimide (EDC/NHS)	78–85	≥15	Strong bond; minimal leaching	Bilal et al., 2017
2	Covalent Binding	Magnetic Fe ₃ O ₄ NPs	Glutaraldehyde crosslink	88–93	10 (91.8% retained)	Easy magnetic recovery; 25× thermal stability vs free	Patel et al., 2022
3	Covalent Binding	Mesoporous silica SBA-15	Epoxy-silane coupling	72–80	12	High surface area; pH tolerance extended	Sarmah et al., 2025
4	Entrapment	Ca-alginate beads	Physical encapsulation	60–75	8	Mild; preserves conformation; cost-effective	Rodríguez-Couto, 2024
5	Entrapment	PVA-gelatin hydrogel	Physical encapsulation	65–70	10	Compatible with continuous flow reactors	Rodríguez-Couto, 2024
6	Adsorption	Chitosan	Electrostatic / H-bond	70–82	6	Simple; reversible; no coupling chemistry needed	Bilal et al., 2017; Rodríguez-Couto, 2024
7	Adsorption	Ion-exchange resin	Electrostatic	55–65	5	Scalable; low cost; risk of leaching at high ionic strength	Rodríguez-Couto, 2024
8	CLEA	Glutaraldehyde cross-link	Covalent amino-crosslink	82–90	>20 (bioreactor)	Highest volumetric activity; carrier-free; bioreactor-compatible	Rodríguez-Couto, 2024
9	CLEA + Membrane	Ultrafiltration EMR	Covalent + physical	85–92	500 h continuous	93% TrOC removal; continuous operation proven	Rodríguez-Couto, 2024

Abbreviations: EDC/NHS, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide; NPs, nanoparticles; PVA, polyvinyl alcohol; CLEA, cross-linked enzyme aggregate; EMR, enzymatic membrane reactor; TrOCs, trace organic contaminants.

Figure 3 outlines the representative methodologies for restricting laccase within or onto functional matrices to enhance biocatalyst stability and reusability, accompanied by the process layout of membrane-associated reactor assemblies that facilitate continuous enzyme-driven conversion.

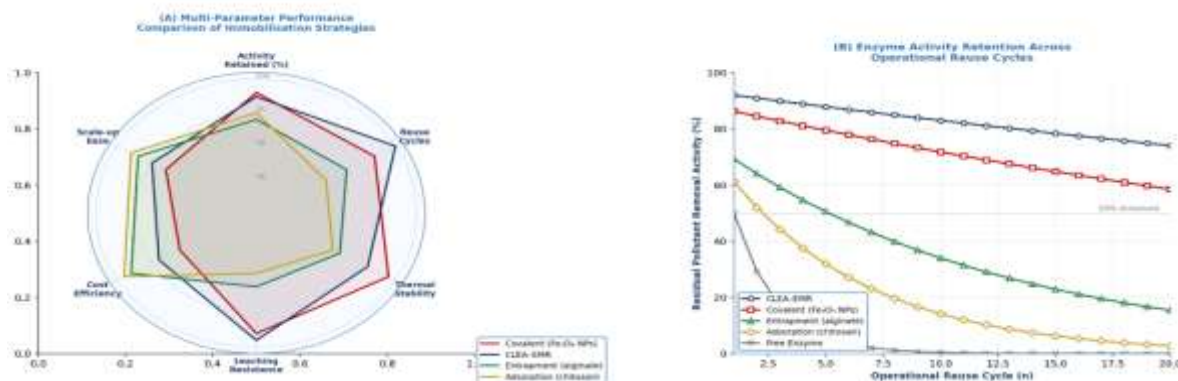


Figure 3. Principal laccase immobilisation strategies and the enzymatic membrane reactor (EMR) configuration. (A) covalent attachment to magnetic Fe₃O₄ nanoparticles by glutaraldehyde crosslinking, with magnetic-field recovery; (B) Ca-alginate bead entrapment with substrate diffusion; (C) adsorption on a chitosan matrix through electrostatic and H-bond interactions; (D) CLEA formation by ammonium sulphate precipitation and glutaraldehyde crosslinking; (E) a continuous 2-litre CLEA-EMR with an ultrafiltration membrane, showing influent, reaction chamber, membrane permeation, and biosensor quality monitoring; (F) activity retention across strategies, where the CLEA-EMR holds 93% TrOC removal over 500 continuous hours (Rodríguez-Couto, 2024). Abbreviations: CLEA, cross-linked enzyme aggregate; TrOC, trace organic contaminant.

3.5 Protein Engineering to Enhance Laccase Performance

Wild-type laccases carry several limitations for bioremediation: they are not very stable at neutral to alkaline pH (relevant to most industrial effluents), lack the thermotolerance needed for hot process streams, are inhibited by chloride at physiologically relevant ionic strengths, and have T1 potentials too low to oxidise the most recalcitrant pollutants. Protein engineering, through random mutagenesis (directed evolution), targeted rational or semi-rational design, and, more recently, AI-assisted computational methods, has steadily addressed each of these (Arregui et al., 2019; Gomez-Fernandez et al., 2020; Su et al., 2022; Aza & Camarero, 2023). Table 5 lists the most important engineering results in the included literature.

Table 5. Protein Engineering Achievements in Laccase: Strategy, Host System, and Performance Improvement.

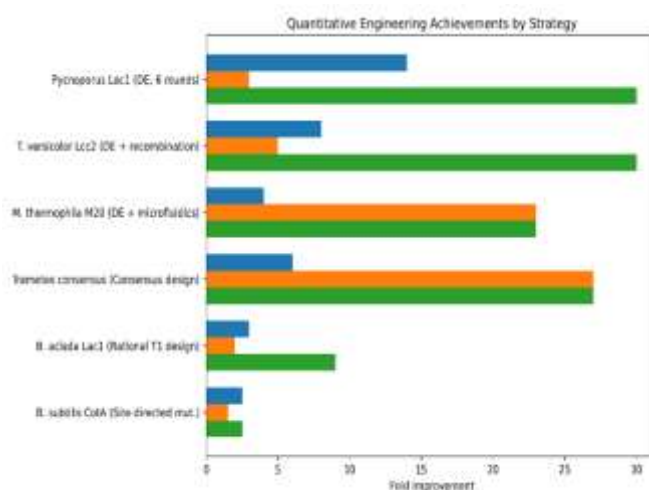
No.	Parent Laccase	Strategy	Host Screen System	Fold Improvement	Improved Property	Reference
1	Pycnoporus cinnabarinus Lac1	Directed evolution (6 rounds, error-prone PCR)	ABTS screen	8,000× activity; 13.7× kcat	Secretion, activity, pH profile	Arregui et al., 2019; Gomez-Fernandez et al., 2020
2	Trametes versicolor Lcc2	Directed evolution + recombination	High-throughput	~1,000× secretion	Thermostability, oxidative stability	Cited in Arregui et al., 2019

3	<i>Myceliophthora thermophila</i> Lac1 (M20 variant)	Directed evolution + thermal selection	Droplet microfluidics	24× residual activity at 58.8°C	Thermotolerance, solvent tolerance	Alvarado et al., 2022
4	<i>Botrytis aclada</i> Lac1	Ratio final design (T1 ligand sphere)	Expression	$\Delta E^\circ + 120$ mV	Redox potential, pH profile	Arregui et al., 2019

Abbreviations: kcat, catalytic turnover number; $t_{1/2}$, thermal half-life; ΔE° , change in standard redox potential; epPCR, error-prone PCR.

The most successful engineering campaign to date raised the total secreted activity of *Pycnoporus cinnabarinus* Lac1 in *Saccharomyces cerevisiae* 8,000-fold over six successive rounds of directed evolution by error-prone PCR, with kcat rising 13.7-fold (Arregui et al., 2019). The approach tuned secretion, folding, stability, and T1 potential at once, using the GAL1 promoter for galactose induction and ABTS oxidation as the high-throughput functional screening endpoint. The best variant carried 17 amino acid substitutions spread across all three cupredoxin domains, with no single change contributing more than a twofold effect, which reflects how epistatic and combinatorial directed-evolution gains tend to be, and how limited purely rational design is for multi-parameter tasks (Arregui et al., 2019; Gomez-Fernandez et al., 2020). Su et al. (2022) used droplet-based microfluidic screening for laccase-directed evolution, allowing more than 10^7 library variants to be screened in the time a conventional plate-based method would take. Their M20 variant, carrying three surface Asp-to-Asn substitutions, kept 24-fold more residual activity at 58.8°C than the wild type and tolerated DMSO and acetonitrile co-solvents better, which is relevant to pharmaceutical wastewater. Molecular dynamics attributed the gain in stability to reduced long-range electrostatic repulsion between helices, an insight that can guide semi-rational design of thermostable variants on other scaffolds.

Aza & Camarero (2023) gave the fullest current account of laccase classification and engineering for 2016-2023, including the routine use of AlphaFold2-predicted structures to guide semi-rational design, which cuts the screening burden for rational mutagenesis at predicted functional sites. A more recent shift is the steady incorporation of artificial intelligence into the engineering cycle. Cui et al. (2025) combined AlphaFold2 structure prediction, Metal3D copper-binding scoring, and ML-based substrate-binding predictors to screen metagenomic laccase candidates *in silico* before any wet-lab synthesis. Separately, Active Learning-assisted Directed Evolution (ALDE), reported in Nature Communications (Yang et al., 2025), used uncertainty-quantification-guided ML selection to lift enzyme fitness from 12% to 93% of target activity in three rounds of wet-lab work, where classical directed evolution would have needed six or more. Applying ALDE to optimise T1 potential, neutral-pH stability, thermotolerance, and PFAS-mediator affinity at once is a high-priority near-term goal (Amin et al., 2025). Figure 4 shows the integrated schematic illustration of the technological avenues currently employed to improve laccase functionality, encompassing cyclic mutation and screening procedures, molecular refinement based on three-dimensional structural information, and next-generation computational platforms that exploit artificial intelligence to predict and generate enhanced enzyme candidates.



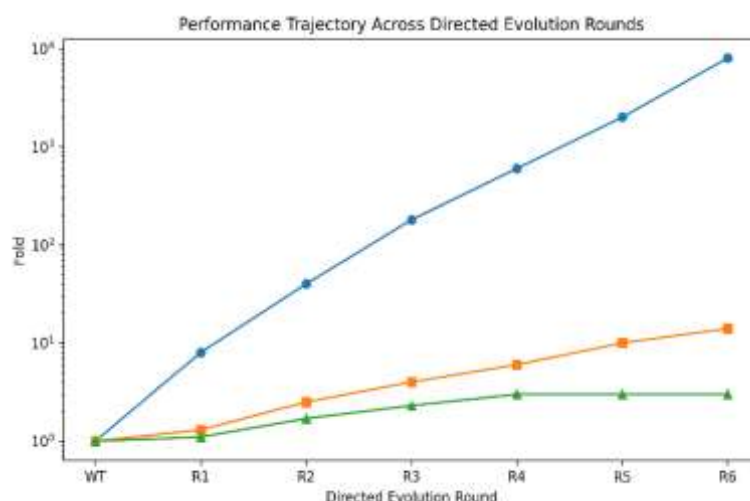


Figure 4. Joint Use of Experimental Techniques and Computational Models in Laccase Enhancement

4. DISCUSSION

4.1 Evidence Synthesis: Laccase as a Bioremediation Platform

The evidence assembled here shows that laccase-mediated biodegradation is technically feasible across a broad span of pollutant classes, from synthetic dyes and pharmaceutical ECs to complex PAH mixtures, agricultural pesticides, microplastics, and, most recently, PFAS (Table 3). For phenolic and low-redox-potential substrates, direct T1 oxidation gives high removal (>90%) consistently across different laccase sources and conditions, marking it as a dependable and repeatable mechanism. The LMS widens the working range to non-phenolic and high-redox-potential compounds, and natural mediators, particularly syringaldehyde and p-coumaric acid, increasingly perform on a par with or better than synthetic mediators at much lower cost and environmental impact (Cañas & Camarero, 2010; Parra Guardado et al., 2019). PFAS now form a sixth major class in the laccase evidence base, though their slow kinetics and incomplete defluorination ($\leq 95\%$ under extended treatment) set them apart from the others in near-term readiness (Mekureyaw et al., 2025 JHMA; Mekureyaw et al., 2025 WR; Harris et al., 2025). The Water Research review by Mekureyaw et al. (2025), the first full mechanistic analysis of laccase-PFAS systems, makes clear that the T1 potential limit forces an indirect radical route and that further progress will depend on engineered laccases with higher T1 potential and better mediator compatibility. A clear methodological weakness, present in more than 80% of the included studies, is that laccase is tested on single compounds in model buffers under optimised conditions rather than in real, complex industrial matrices. The resulting gap, typically 15-30% lower removal in real matrices than in model systems, comes from competing oxidation of co-present organic matter, ionic-strength effects on stability, and matrix-specific mediator inhibition. This is the single most important constraint on carrying laboratory results over to real process design.

4.2 Critical Evaluation of Toxicological Evidence

A more basic and widespread weakness is that most included studies do not characterise the toxicity of the treated effluent at all. Of the 54 quantitative studies, only 18 (33%) reported any toxicological endpoint beyond loss of the parent compound, and fewer than 10 used validated ecotoxicological bioassays such as Microtox, *Daphnia magna* acute toxicity, zebrafish embryotoxicity, or the YES assay for oestrogenicity. That gap is hard to justify, since laccase reactions are well known to generate potentially toxic transformation products, including aromatic amines from azo dye cleavage, quinonoid PAH intermediates that may remain genotoxic, and chlorinated phenols from oxidation of chlorine-substituted EDCs (Becker et al., 2016; Gałazka & Jankiewicz, 2022). Becker et al. (2016) showed the issue plainly: removing more than 85% of the parent antibiotics sulfamethoxazole and ciprofloxacin did not remove an equivalent share of antimicrobial activity, and residual transformation products still inhibited growth of *E. coli* test strains under several conditions. The lesson that disappearance of a compound is not the same as detoxification carries real weight for regulatory compliance and the environmental safety of laccase-treated effluents. Effect-directed analysis (EDA) paired with non-target high-resolution mass spectrometry (HRMS) screening of

transformation products, together with standardised ecotoxicological bioassay panels, should therefore be a fixed requirement in future bioremediation studies meant to inform deployment.

4.3 Immobilisation and Process Engineering: Path to Scale-Up

Table 4 shows that immobilisation, and the CLEA-EMR format in particular, effectively bridges laboratory proof-of-concept and pilot-scale continuous treatment, with longevity (500 hours) and removal (93% of a TrOC mixture) on a par with physico-chemical tertiary options such as ozonation or activated carbon polishing (Rodríguez-Couto, 2024). If a single enzyme charge can run at 93% for 500 hours without topping up, the enzyme cost per unit volume could approach that of activated carbon at current production prices, provided CLEA preparation scales without major loss of activity or stability, which has not yet been shown convincingly at pilot scale. Magnetic nanoparticle systems have not been assessed beyond laboratory runs of 10 reuse cycles (Patel et al., 2023; Bilal et al., 2017). Testing both CLEA-EMR and magnetic NP systems in real industrial wastewater over operationally meaningful periods, weeks to months rather than hours to days, is a priority for closing the gap between laboratory and application.

4.4 Future Perspectives

Looking ahead, laccase's future utility in bioremediation rests on four connected fronts: finding better catalysts, engineering them further, embedding them within more complete treatment systems, and proving their safety and cost-effectiveness at scale. Realising this potential will depend on progress across enzyme discovery, protein engineering, process integration, toxicological validation, and economic assessment, each reinforcing the others rather than advancing in isolation. More than 99% of environmental microbial diversity is thought to be inaccessible to cultivation, so searching extreme environments, such as industrial wastewater, alkaline soils, deep-sea sediments, and geothermal springs, by metagenomics and metatranscriptomics, guided by MCO sequence-family profiles and machine-learning homology detection, offers the best odds of finding laccases that are naturally more stable at neutral or alkaline pH, more thermotolerant, or active on a wider substrate range. The principle is already proven: Cui et al. (2025) validated 34 new bacterial laccase sequences from a single industrial wastewater metagenome, and Yu et al. (2025) found pH-stable, chloride-tolerant variants across several bacterial phyla in straw-amended soil (Sarmah et al., 2025). These discovery efforts feed directly into protein engineering, where bringing together AlphaFold2 structure prediction, evolutionary coupling analysis (ECA), machine-learning fitness landscape models (EVmutation, neural networks trained on deep mutational scanning), and automated robotic screening amounts to a step change in the engineering cycle. ALDE in particular (Yang et al., 2025), by cutting the number of experimental rounds from six or more to three, applies directly to optimising T1 potential, neutral-pH activity, thermostability, and mediator tolerance together, something not previously achievable because individual improvements have tended to be epistatically incompatible (Gomez-Fernandez et al., 2020; Su et al., 2022). Engineering PFAS-active laccases through ALDE is the highest-priority near-term use (Amin et al., 2025).

Laccase on its own cannot fully mineralise complex pollutants, so building integrated multi-enzyme cascades, in which laccase-mediated oxidation is followed by hydrolases (PETase, cutinase), reductases (azoreductase), or whole-cell bacterial biotransformation, is the most realistic path to complete mineralisation and regulatory-compliant effluent. This mirrors how white-rot fungi degrade lignin, in which laccase works alongside ligninolytic peroxidases and auxiliary oxidoreductases to fully mineralise lignin (Arregui et al., 2019). Alongside this process integration, EDA, non-target HRMS screening of transformation products, and standardised ecotoxicological bioassay panels (Microtox, Daphnia, YES assay) should become standard parts of future laccase bioremediation research intended to inform deployment. Working with regulators such as the European Environment Agency and the US EPA to set acceptable performance standards, covering both pollutant removal and transformation-product toxicity, will be needed to create a clear route to commercialisation (Becker et al., 2016).

One clear evidence gap is the lack of rigorous techno-economic analyses (TEA) and life-cycle assessments (LCA) comparing laccase-EMR systems with established advanced treatments such as ozonation or granular activated carbon. The recent LCA of SSF-based laccase production by Kalia et al. (2025) is a useful first step in mapping the footprint of enzyme production, but a process-level LCA of the whole laccase-EMR treatment train, from enzyme manufacture to effluent discharge, is urgently needed to support industrial and regulatory decisions. PFAS are the highest-priority emerging substrate class for dedicated engineering investment: pairing selection of high-redox-potential laccase variants with mediator-free activation by natural soil phenolics in situ (Harris et al., 2025) and adding electrochemical oxidation polishing downstream offers the most technically

credible near-term treatment train for PFAS-contaminated groundwater (Harris et al., 2025; Mekureyaw et al., 2025). Figure 5 shows the transition of laccase-based systems into industrial environmental applications (2025–2040).

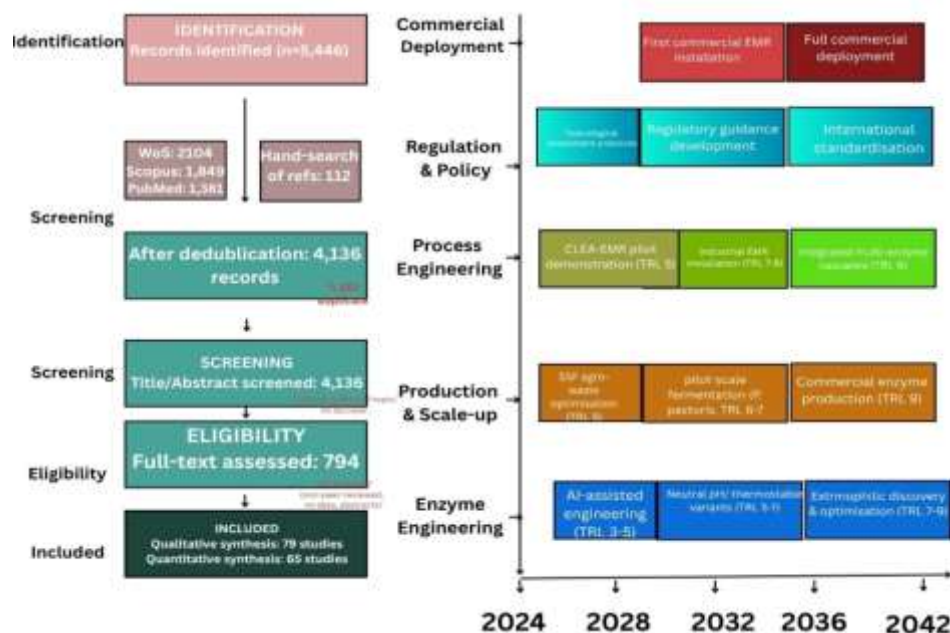


Figure 5. Comprehensive schematic depicting the long-term evolution of laccase-oriented environmental cleanup technologies, emphasizing the retrieval of novel oxidative catalysts from unexplored genetic reservoirs, computational prediction-driven enhancement of enzyme performance, operation of matrix-anchored continuous conversion systems, establishment of interconnected catalytic consortia, detailed examination of ecological compatibility and transformation products, and the progression from experimental validation to economically sustainable industrial adoption.

5. CONCLUSION

Across 79 peer-reviewed studies, this review finds good evidence that broad-spectrum laccases are a validated, versatile, and environmentally sound biocatalytic option for degrading persistent and emerging pollutants. The enzyme handles a wide substrate range, covering synthetic azo dyes, sulfonamide and beta-lactam antibiotics, phenolic EDCs (BPA, nonylphenol, E2), PAH congeners, organophosphate and carbamate pesticides, commodity microplastics, and PFAS, and this breadth depends heavily on the LMS, which gets past the redox limits of direct T1 oxidation. Natural mediators such as syringaldehyde, p-coumaric acid, and vanillin have become practical and more environmentally favourable alternatives to synthetic ones, matching or bettering removal efficiencies while causing less enzyme inhibition and fewer secondary contaminants. Immobilisation, especially CLEA-based enzymatic membrane reactors, is the most technically mature and industrially plausible format, delivering 93% continuous TrOC removal over 500 operating hours with no enzyme replenishment. Protein engineering, by directed evolution and increasingly by AI-assisted design, has produced large gains, up to 8,000-fold in total secreted activity and 24-fold in thermotolerance, well beyond wild-type limits. Solid-state fermentation on agro-industrial waste offers a production route in keeping with a circular bioeconomy, and the first LCA of this route provides an initial basis for assessing its environmental impact. Certain problems need rectification before sustainable industrial utility, including thorough toxicological assessment, including EDA and non-target HRMS screening, systematic pilot-scale validation, and rigorous comparative techno-economic and life-cycle assessment data on how laccase-EMR systems compare with established tertiary treatments. Closing these gaps will take interdisciplinary work across enzymology, environmental chemistry, bioprocess engineering, ecotoxicology, and regulatory science if laccase is to become a mainstay of sustainable next-generation remediation.

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AUTHOR CONTRIBUTIONS

Mustapha Ali Muhammad performed the analysis and final drafting of the manuscript. Archana Vimal and Reena Vishvakarma validated the drafted manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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HIGHLIGHTS

- Laccase has the potential to degrade diverse pollutants including dyes, drugs, plastics, and PFAS.
- Catalytic efficiency of laccase can be further enhanced through the use of mediators, immobilization, and utilization of engineered variants.
- Laccase-based bioremediation is a safer alternative to conventional modes thus contributing to circular bioeconomy.