Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

A REVIEW ON ENZYMATIC BIODEGRADATION OF ENVIRONMENTAL POLLUTANTS: ADVANCES IN ENVIRONMENTAL BIOTECHNOLOGY

Arshul Noor Siddiqui[1], Shaista Khan[2], Sanaullah Abbasi[3], Sarfaraz[4], Javed Ahmed Abbasi[5], Dua Khowaja[6], Parwan Ali Ujjan[*7]

*[1,2,3,4,5,*7]Department of Biochemistry, Shah Abdul Latif University Khairpur*
[6]Department of Physiology, University of Sindh Jamshoro

[*7]parwanaliujjan@gmail.com

DOI: http://doi.org/10.5281/zenodo.19104965

Keywords
enzymatic bioremediation; oxidoreductases; hydrolases; directed evolution; metagenomics; enzyme immobilization; wastewater micropollutants.

Article History
Received: 19 January 2026
Accepted: 03 March 2026
Published: 19 March 2026

Copyright @Author
Corresponding Author: *
Parwan Ali Ujjan

Abstract

Escalating concentrations of organic and inorganic pollutants continue to jeopardize global ecosystems, exposing the economic and environmental limitations of conventional physicochemical remediation. Enzymatic bioremediation–leveraging the selective catalytic power of oxidoreductases, hydrolases, and oxygenases–presents a sustainable alternative capable of degrading diverse contaminants under ambient conditions. Recent breakthroughs in directed evolution and protein engineering have significantly optimized the thermostability and kinetics of plastic-degrading enzymes, enabling efficient PET depolymerization near glass transition temperatures. Furthermore, metagenomic exploration of extreme environments has expanded the biocatalytic repertoire to include recalcitrant dyes, hydrocarbons, and halogenated xenobiotics. While challenges such as enzyme deactivation in complex matrices and transformation product toxicity persist, the integration of nanobiocatalysis, AI-guided design, and hybrid systems is accelerating industrial scalability. This review synthesizes these mechanistic and engineering milestones, providing a strategic roadmap for low-footprint remediation within a circular bioeconomy framework.

1. INTRODUCTION

The fast expansion of industrial, agricultural, and urban activities has drastically increased the release of persistent pollutants ranging from hydrocarbons, synthetic dyes, pesticides, and plastics to medications and heavy metals into land, water, and air. Conventional treatments (e.g., adsorption, thermal/incineration, advanced oxidation) generally need significant energy input, generate toxic secondary waste, and fail to accomplish complete mineralization of complex compounds, particularly at trace amounts (Sudarshan *et al.*, 2023).

Enzymatic biodegradation uses the natural enzymes that microorganisms make to change or mineralize pollutants in mild environmental conditions. Oxidoreductases, which include laccases, manganese peroxidases, and dye decolorizing peroxidases, are among the most researched classes. They show extensive oxidative action on synthetic colors, aromatic pollutants, and some medications. Esterases, lipases, PETase/MHETase, and phosphotriesterases are examples of hydrolases that are essential for breaking ester bonds in plastics and pesticide residues. By catalyzing the elimination of halogen substituents from xenobiotic substances, dehalogenases facilitate subsequent degradation stages, whereas oxygenases start hydrocarbon activation through mono and dioxygenation events. (Agarwal *et al.*, 2022; Bagheri *et al.*, 2022).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Crucially, enzymatic transformation frequently combines with microbial metabolic pathways to allow for the full mineralization of contaminants; yet, cautious assessment of the toxicity of intermediates and transformation products is still necessary for environmental safety.

Recent developments in enzyme discovery, engineering, and application have revolutionized the field of enzymatic biodegradation. Metagenomic techniques have expanded the catalytic repertoire for the breakdown of plastics and dyes by identifying new enzymes from harsh environments and landfills. (Choi *et al.,* 2024; Lin *et al.,* 2025). Concurrently, protein engineering and directed evolution have produced highly efficient and thermostable PET depolymerizing enzymes, such as HotPETase and FAST PETase, enabling near complete depolymerization under industrially relevant conditions (Bell *et al.,* 2022; Lu *et al.,* 2022). Immobilization and nanobiocatalysis strategies have further enhanced enzyme stability, recyclability, and compatibility with continuous reactor systems, addressing operational challenges in wastewater and soil remediation (Zhang, Ji, *et al.,* 2023; Zhang *et al.,* 2023). Substrate scope has broadened significantly, with oxidoreductases and hydrolases now applied to plastics, microplastics, dyes, hydrocarbons, and pharmaceuticals (Thathola *et al.,* 2024). These innovations have accelerated translation from laboratory to pilot scale implementations, particularly in advanced wastewater treatment and plastic recycling, signaling a paradigm shift toward sustainable and circular bioeconomy solutions.

The primary aim of this review is to provide a comprehensive synthesis of recent advances in enzymatic biodegradation of environmental pollutants within the context of environmental biotechnology. Specifically, the review seeks to classify major pollutant types and assess their ecological impacts, while mapping key biodegradative enzymes to their respective substrates and catalytic mechanisms. It further aims to elucidate molecular and biochemical pathways, including the role of cofactors and environmental conditions influencing enzyme activity. Another critical objective is to examine technological innovations such as protein engineering, directed evolution, metagenomic discovery, enzyme immobilization, and nanobiocatalysis, highlighting their contributions to improving enzyme efficiency and stability. The review also intends to evaluate practical applications of enzymatic systems in wastewater treatment, soil remediation, air detoxification, and plastic degradation, supported by case studies and industrial scale implementations. Finally, it aims to identify existing challenges such as enzyme deactivation, transformation product toxicity, and economic constraints and to outline emerging trends, including extremophile derived enzymes, AI assisted enzyme design, biosensor integration, and strategies aligned with circular bioeconomy principles. Through this holistic approach, the review aspires to bridge the gap between mechanistic understanding and real world application, guiding future research and technology transfer toward sustainable environmental remediation.

2. Classification of Pollutants and Their Environmental Impact

2.1 Organic pollutants (hydrocarbons, pesticides, dyes, plastics)

Hydrocarbons (aliphatic and aromatic). Hydrocarbons originating from petroleum exploration, transport, refining, and accidental spills are among the most pervasive organic pollutants. Aerobic and anaerobic microbial pathways employ oxygenases (e.g., monooxygenases/dioxygenases) and peripheral enzymes to initiate activation and ring cleavage of alkanes and polycyclic aromatic hydrocarbons (PAHs); yet anaerobic degradation remains challenging, especially for high molecular weight PAHs (Ławniczak *et al.,* 2020; Agnez Lima *et al.,* 2024). Genome resolved analyses indicate extensive diversification of aerobic hydrocarbon degrading enzymes across bacterial and archaeal phyla, implying rapid adaptation via horizontal gene transfer and gene duplication (Rezaei Somee *et al.,* 2022).

Pesticides. Pesticide residues persist in soils and aquatic systems due to recalcitrant structures and

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

halogenation. Biodegradation relies on enzyme classes such as monooxygenases, dioxygenases, carboxylesterases, phosphotriesterases, haloalkane dehalogenases (e.g., LinA/LinB), and dehydrochlorinases, often organized in catabolic operons with regulatory controls (Rani *et al.*, 2022; Guerrero Ramírez *et al.*, 2023). For glyphosate, microbial pathways can utilize it as carbon, nitrogen, or phosphorus source; however, detailed enzyme gene characterization remains limited compared to fate/toxicity studies (Zhan *et al.*, 2018).

Synthetic dyes. Textile and allied industries discharge azo, anthraquinone, and triphenylmethane dyes with high chromophoric stability and toxicity. Enzymatic remediation by oxidoreductases (laccase, MnP, DyP) and azoreductases supports decolorization and transformation under varied salinities and pH, including halophilic consortia in high salt effluents (Qiu *et al.*, 2022; Adamo *et al.*, 2022). However, real wastewater matrices with salts, metals, and surfactants can inhibit enzyme activity and shift pathways toward coupling/polymerization; post treatment toxicity evaluation is therefore critical (Varga *et al.*, 2019).

Plastics and microplastics. Persistent polymers PET, PE, PP, PS, PVC fragment into micro and nanoplastics with ecological and potential health effects. Enzymatic plastic degradation is led by PETase/MHETase/cutinases for PET, with emerging reports on laccase mediated oxidation and engineered hydrolases affecting other polymers; enzyme discovery has broadened via metagenomics and high throughput platforms, and industrially relevant engineered PET hydrolases enable depolymerization near Tg with monomer recovery (Dhali *et al.*, 2024; Bell *et al.*, 2022; Tournier *et al.*, 2020). Immobilized or embedded enzymes further enhance control over degradation kinetics and compostability in designed materials (Sun, 2025).

Environmental impact. Organic pollutants reduce dissolved oxygen, impede photosynthesis, and bioaccumulate. Dye plumes cause genotoxicity and endocrine effects; pesticides affect non target organisms and soil microbiomes; microplastics act as vectors for adsorbed toxics and microbial colonizers ("plastisphere") (Dhali *et al.*, 2024; Sudarshan *et al.*, 2023).

2.2 Inorganic pollutants (heavy metals, nitrates, phosphates)

Heavy metals (e.g., Pb, Cd, Hg, Cr). Although metals are not enzymatically degraded, biocatalytic strategies influence speciation and immobilization via oxidoreductases that alter redox states, biosorption by enzyme rich biofilms, and coupling of enzymatic treatment with zero valent iron or mineralizing carriers in hybrid systems (Cheng *et al.*, 2023). Enzyme assisted processes can reduce bioavailable fractions, enabling precipitation or sequestration, but the stability of immobilized catalysts and potential metal enzyme interactions need careful assessment.

Nutrients (nitrates, phosphates). Excessive nutrient loading drives eutrophication. Enzymes contribute indirectly by degrading organic micropollutants that interfere with biological nutrient removal and by supporting biosensor detection and control strategies (Moratti *et al.*, 2022). Enzyme integrated systems may stabilize microbial communities, improving nitrification denitrification efficacy, though this remains an emerging integration area.

2.3 Emerging contaminants (pharmaceuticals, microplastics)

Pharmaceuticals and personal care products (PPCPs). Trace antibiotics, analgesics, hormones, and β blockers persist through conventional WWTPs. Laccases, peroxidases, and cytochrome P450 monooxygenases catalyze oxidative transformations; immobilization and reactor integration increase residence time and stability. Nonetheless, byproduct toxicity and mixture effects necessitate kinetic modeling and ecotoxicity assays to avoid unintended risk amplification (Barber *et al.*, 2020; de Boer *et al.*, 2023). Fungal white rot systems have shown high removal efficiencies for common

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

pharmaceuticals, providing a scalable biocatalytic polishing step (Flórez Restrepo *et al.*, 2025).

Microplastics and nanoplastics. Degradation pathways involve surface oxidation and chain scission; engineered PET hydrolases address PET, while strategies for PE/PP remain nascent. Multi omics and directed evolution are expanding target scope, and bioprocess designs emphasize coupling enzymatic depolymerization with downstream microbial assimilation to minimize microplastic persistence (Choi *et al.*, 2024; Dhali *et al.*, 2024).

3. Overview of Enzymes in Biodegradation

3.1 Classification of biodegradative enzymes

Oxidoreductases. Laccases (multi copper oxidases), MnP, lignin peroxidase, and DyP catalyze single electron or peroxidative reactions that oxidize phenolic and non phenolic substrates, often using mediators to extend substrate scope to non activated aromatics and pharmaceuticals (Barber *et al.*, 2020; Thathola *et al.*, 2024). Recent computational and activity based discovery has identified thermophilic bacterial laccases with catalytic promiscuity for dyes and even low density polyethylene surfaces (Zhang *et al.*, 2023).

Hydrolases. This category includes esterases, lipases, cutinases, and PETase/MHETase pairs that hydrolyze ester bonds in polymers and xenobiotics. Directed evolution and machine learning guided design have dramatically improved PET depolymerization rates and thermostability, enabling operation near 65–70 °C and facilitating closed loop recycling to terephthalic acid and ethylene glycol (Bell *et al.*, 2022; Lu *et al.*, 2022). Phosphotriesterases and related hydrolases degrade organophosphate pesticides via nucleophilic catalysis, sometimes enhanced through ultrahigh throughput evolution (Schnettler *et al.*, 2023).

Oxygenases. Monooxygenases and dioxygenases activate hydrocarbons by introducing oxygen into aliphatic chains or aromatic rings, producing catechols and other intermediates amenable to ring cleavage and assimilation. Genetic regulation of alkane monooxygenases underpins biosensor development for hydrocarbon detection and process control in bioremediation (Moratti *et al.*, 2022; Rezaei Somee *et al.*, 2022).

Dehalogenases. Haloalkane dehalogenases (e.g., LinA/LinB) and dehydrochlorinases catalyze hydrolytic or eliminative removal of halogens, lowering toxicity and opening pathways to further enzymatic steps. Their genes frequently reside on catabolic plasmids or islands, facilitating horizontal transfer and rapid ecological adaptation (Rani *et al.*, 2022).

3.2 Mechanisms of enzymatic degradation

Oxidative mechanisms. Laccases abstract electrons to form phenoxy radicals, initiating coupling/fragmentation; peroxidases generate high valent iron oxo species that oxidize phenols and azo linkages; DyP peroxidases exhibit unique active site architectures linked to dye decolorization. Mediator systems (e.g., ABTS, HBT) expand substrate reach but can alter effluent toxicity, underscoring the need for mediator selection and post treatment evaluation (Barber *et al.*, 2020; Varga *et al.*, 2019).

Hydrolytic depolymerization. PET hydrolases bind crystalline/amorphous PET, positioning catalytic residues and water for ester bond cleavage. Engineered variants (HotPETase, LCC mutants; ML optimized enzymes) increase Tm, reduce product inhibition, and improve surface binding; pairing PETase with MHETase accelerates conversion of intermediate MHET to TPA and EG (Bell *et al.*, 2022; Tournier *et al.*, 2020; Lu *et al.*, 2022).

Reductive cleavage and ring activation. Azoreductases catalyze reductive cleavage of azo bonds; mono/dioxygenases introduce oxygen into hydrocarbons, generating cis diols and catechols processed by intradiol/extradiol dioxygenases; dehalogenases remove halogens to yield more labile intermediates for subsequent oxidation or hydrolysis (Ławniczak *et al.*, 2020; Rani *et al.*, 2022).

3.3 Microbial sources and genetic regulation

Sources. White rot fungi (e.g., Trametes, Pleurotus) are prolific oxidoreductase producers,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

while bacteria across Proteobacteria, Actinobacteriota, Firmicutes, and Bacteroidota host diverse hydrolytic and oxygenase pathways (Barber *et al.*, 2020; Rezaei Somee *et al.*, 2022). Extremophiles and deep sea communities provide thermostable and salt tolerant enzymes useful for harsh industrial matrices (Choi *et al.*, 2024).

Regulation and genetic organization. Catabolic operons (e.g., alk operon for alkane monooxygenases) and transcriptional regulators respond to substrate induction and stress, enabling dynamic expression; mobile genetic elements accelerate dissemination of biodegradation traits. Synthetic biology repurposes these regulatory parts for biosensors and controlled expression in engineered hosts, enhancing remediation reliability (Moratti *et al.*, 2022).

I*mmobilization and nanobiocatalysis interfaces.* Genetic fusion tags and surface display facilitate immobilization on nanomaterials or porous carriers (MOFs, silica, carbon). Immobilization improves pH/temperature tolerance, reduces proteolysis, and enables continuous operation; however, diffusion limitations and potential fouling necessitate carrier optimization and reactor design (Sheldon *et al.*, 2021; Zhang *et al.*, 2023).

4. Major Classes of Biodegradative Enzymes and Their Substrates .

4.1 Oxidoreductases (laccases, peroxidases, dehydrogenases)

Scope and substrates. Oxidoreductases catalyze electron transfer reactions that initiate or accelerate the breakdown of aromatic and phenolic pollutants, synthetic dyes, and several pharmaceutical micropollutants. Laccases (multi copper oxidases; EC 1.10.3.2) oxidize phenolic substrates to radicals using O_2 as the terminal electron acceptor, enabling further coupling, cleavage, or polymer rearrangement of azo, anthraquinone, and triphenylmethane dyes, as well as phenolic pharmaceuticals (Barber *et al.*, 2020; Thathola *et al.*, 2024). Peroxidases including manganese peroxidase (MnP), lignin peroxidase (LiP), and DyP type peroxidases utilize H_2O_2 to form high valent iron oxo species that can attack recalcitrant chromophores and non phenolic aromatics; DyPs show distinctive active site features associated with dye decolorization and potential lignin modification roles (Adamo *et al.*, 2022; Pankaj & Arora, 2022). Dehydrogenases (e.g., alcohol/aldehyde dehydrogenases) often participate downstream, funneling oxidized intermediates into central metabolism (Barber *et al.*, 2020).

Mechanistic features and process levers. Laccase activity can be extended to non activated substrates by laccase–mediator systems (LMS) (e.g., ABTS, HBT), but mediator choice and concentration influence effluent toxicity necessitating post treatment ecotoxicity assessment (Barber *et al.*, 2020; Varga *et al.*, 2019). Immobilization on nanocomposites, MOFs, or carbon materials increases operational stability, reusability, and resistance to salts/surfactants typical of industrial matrices (Zhang *et al.*, 2023; Sheldon *et al.*, 2021). Recent computationally aided discovery has delivered thermophilic bacterial laccases with catalytic promiscuity that decolorize dyes and act on low density polyethylene surfaces, hinting at broader polymer oxidation capability (Zhang *et al.*, 2023). For peroxidases, pH and H_2O_2 dosing are critical: excess peroxide can inactivate enzymes, while optimized dosing sustains catalytic cycles and dye mineralization (Pankaj & Arora, 2022; Varga *et al.*, 2019).

Representative applications. Oxidoreductases are widely studied for wastewater polishing to remove color and micropollutants that escape conventional biological steps (Barber *et al.*, 2020), and for textile effluent remediation where immobilized laccase achieves high decolorization with improved durability (Zhang *et al.*, 2023). DyPs are increasingly explored for industrial dye streams and lignocellulosic bioprocessing, expanding the enzyme toolbox for aromatic pollutants (Adamo *et al.*, 2022).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

4.2 Hydrolases (lipases, esterases, proteases, PETase/MHETase, phosphotriesterases)

Scope and substrates. Hydrolases cleave ester, amide, or phosphoester bonds in xenobiotics and polymers. Esterases and lipases act on aliphatic/aromatic esters in pesticides and plasticizers; proteases assist in proteinacious effluent treatment and in multi enzyme cascades. The most transformative advances involve polyester hydrolases PETase, MHETase, and cutinases which depolymerize poly(ethylene terephthalate) (PET) into terephthalic acid (TPA) and ethylene glycol (EG) (Tournier *et al.*, 2020; Bell *et al.*, 2022). For organophosphate pesticides, phosphotriesterases hydrolyze P–O bonds to detoxify compounds such as parathion and paraoxon; recent ultrahigh throughput evolution engineered a metal free α/β hydrolase into an efficient phosphotriesterase, showing generalizable strategies for upgrading catalytic scaffolds (Schnettler *et al.*, 2023; Rani *et al.*, 2022).

Mechanistic features and process levers. PET hydrolases bind to amorphous/crystalline PET surfaces via hydrophobic/aromatic residues that position the ester linkage near catalytic serine–histidine–aspartate triads; directed evolution (e.g., HotPETase) and machine learning guided design (FAST PETase like variants) improved thermostability (Tm > 80 °C) and surface activity, allowing depolymerization near the glass transition temperature (Tg ≈ 60–70 °C) where PET chains are more accessible (Bell *et al.*, 2022; Lu *et al.*, 2022). Co administration of PETase with MHETase mitigates product inhibition and accelerates conversion of MHET to final monomers (Tournier *et al.*, 2020). Immobilization of hydrolases onto nanocarriers or in CLEAs/MOFs improves reuse and tolerance to inhibitors; carrier selection must balance mass transfer with enzyme orientation and stability (Sheldon *et al.*, 2021).

Representative applications. Engineered PET hydrolases demonstrate fast depolymerization of post consumer PET and selective deconstruction of PET from laminated multi materials, enabling closed loop recycling of monomers into bottle grade PET (Bell *et al.*, 2022; Tournier *et al.*, 2020). Phosphotriesterases are being advanced for soil/water pesticide detoxification and biosensing, aided by evolution platforms and synthetic biology (Schnettler *et al.*, 2023; Rani *et al.*, 2022).

4.3 Oxygenases (monooxygenases, dioxygenases)

Scope and substrates. Monooxygenases introduce one atom of oxygen into hydrocarbons (e.g., alkanes, alkenes), producing alcohols and epoxides; dioxygenases insert O_2 across aromatic rings to form cis diols/catechols, which are processed by intradiol/extradiol ring cleavage enzymes (Ławniczak *et al.*, 2020; Rezaei Somee *et al.*, 2022). These enzymes constitute the activation step for hydrocarbon biodegradation and are central to microbial pathways for PAHs and mixed petroleum fractions.

Mechanistic features and regulation. Many oxygenases are multi component systems (e.g., alkane monooxygenase AlkB with rubredoxin and reductase) requiring electron donors (NADH/NADPH). Their genetic regulation responds to substrate presence via dedicated transcription factors and catabolic operons, enabling inducible expression tailored to pollutant profiles (Moratti *et al.*, 2022; Rezaei Somee *et al.*, 2022). Biosensors based on monooxygenase promoters/regulators provide rapid detection of hydrocarbons and real time control of engineered bioremediation systems (Moratti *et al.*, 2022).

Representative applications. Oxygenases are integral to soil/groundwater hydrocarbon remediation, both aerobic and, through community interactions, microaerobic niches; genome resolved surveys reveal enzyme diversity across numerous phyla, supporting bioprospecting for robust catalysts in varied environments (Rezaei Somee *et al.*, 2022; Ławniczak *et al.*, 2020).

4.4 Dehalogenases and xenobiotic degradation

Scope and substrates. Dehalogenases remove halogen substituents (Cl, F, Br) from haloalkanes, chlorinated aromatics, and lindane derivatives, converting them to less toxic and more labile intermediates. Canonical examples include LinA

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

(dehydrochlorinase) and LinB (haloalkane dehalogenase) in hexachlorocyclohexane catabolism, along with AtzA/TrzN in atrazine dechlorination (Rani *et al.*, 2022). These enzymes often operate alongside oxygenases/hydrolases in multi step pathways that culminate in ring cleavage and assimilation.

Mechanistic features and genetics. Many dehalogenases leverage SN1/SN2 type nucleophilic mechanisms (haloalkane dehalogenases) or elimination reactions (dehydrochlorinases). Genes are frequently located on plasmids/transposons, facilitating horizontal gene transfer and rapid spread of degradation capacity across environmental communities an important factor in adaptive responses to pesticide use (Rani *et al.*, 2022). Immobilization or expression in robust hosts can improve operational stability in high salinity or mixed pollutant matrices (Sheldon *et al.*, 2021).

Representative applications. Dehalogenases contribute to pesticide residue cleanup in agricultural soils and to treatment trains for chlorinated solvents and halogenated aromatics, often in consortia with oxidoreductases and oxygenases to complete mineralization (Rani *et al.*, 2022; Ławniczak *et al.*, 2020).

4.5 Plastic degrading enzymes (PETase, MHETase, cutinases, and emerging variants)

Scope and substrates. PETase/MHETase and cutinase variants target PET's aromatic polyester backbone; leaf branch compost cutinase (LCC) mutants and HotPETase/FAST PETase exemplify engineered catalysts with superior thermostability and productivity (Bell *et al.*, 2022; Lu *et al.*, 2022). Beyond PET, laccases and oxidative systems can functionalize polyethylene (PE) surfaces, increasing susceptibility to abiotic/biotic chain scission; enzyme embedded PLA and other biodegradable plastics integrate tailored hydrolases to enable home compost disintegration without compromising shelf stability (Zhang *et al.*, 2023; Sun, 2025).

Mechanistic features and process integration. Advances in structure guided engineering optimize active site geometry, surface binding loops, and thermostability; reactor design (slurry and packed bed with immobilized enzymes) addresses mass transfer limitations, while process coupling with downstream purification/repolymerization supports circular PET economy (Tournier *et al.*, 2020; Bell *et al.*, 2022). For biodegradable plastics, thermo stable embedded enzymes are encapsulated to remain latent during product life and activate under composting/methanization conditions, reconciling performance with end of life degradability (Sun, 2025).

Representative applications. Demonstrations include near complete depolymerization of post consumer PET, selective deconstruction of PET in multi material laminates, and enzyme embedded PLA that fully degrades under home compost standards illustrating the trajectory from laboratory innovation to industrial implementation and product redesign for circular bioeconomy outcomes (Bell *et al.*, 2022; Sun, 2025).

5. Molecular and Biochemical Mechanisms of Enzymatic Degradation .

5.1 Catalytic Pathways

Enzymatic biodegradation operates through highly specific catalytic pathways that transform complex pollutants into simpler, less toxic intermediates or mineralized products. Oxidative pathways dominate for aromatic and phenolic pollutants, where enzymes such as laccases and peroxidases initiate electron abstraction, generating phenoxy radicals that undergo coupling, cleavage, or polymerization (Barber *et al.*, 2020; Thathola *et al.*, 2024). Peroxidases, including manganese peroxidase (MnP) and lignin peroxidase (LiP), utilize hydrogen peroxide to form high valent iron oxo species, enabling oxidation of non phenolic substrates and azo dye chromophores (Adamo *et al.*, 2022; Pankaj & Arora, 2022). DyP type peroxidases exhibit unique heme architectures that broaden substrate specificity to synthetic dyes and lignin derivatives (Adamo *et al.*, 2022).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Hydrolytic pathways are central to plastic and pesticide degradation. PET hydrolases (PETase, MHETase, and cutinases) catalyze ester bond cleavage in poly(ethylene terephthalate), producing terephthalic acid (TPA) and ethylene glycol (EG). The catalytic triad (Ser–His–Asp) activates water molecules for nucleophilic attack on ester linkages, while engineered variants improve thermostability and surface binding to crystalline PET (Bell *et al.*, 2022; Lu *et al.*, 2022). Similarly, phosphotriesterases hydrolyze organophosphate pesticides via nucleophilic substitution, often requiring metal cofactors for stabilization of transition states (Schnettler *et al.*, 2023; Rani *et al.*, 2022).

Reductive and oxygenation pathways govern hydrocarbon and dye degradation. Azoreductases catalyze reductive cleavage of azo bonds using NAD(P)H as electron donors, while monooxygenases and dioxygenases introduce oxygen into hydrocarbons, forming catechols that undergo ring cleavage by intradiol or extradiol dioxygenases (Ławniczak *et al.*, 2020; Rezaei Somee *et al.*, 2022). These reactions are often coupled with downstream dehydrogenases and hydrolases to complete mineralization.

5.2 Enzyme–Substrate Interactions

Substrate binding and orientation within the active site dictate catalytic efficiency. For laccases, the T1 copper site mediates electron transfer from phenolic substrates, while T2/T3 sites reduce molecular oxygen to water (Thathola *et al.*, 2024). Structural studies reveal that hydrophobic pockets and flexible loops in PETase enhance binding to PET surfaces, whereas engineered variants introduce stabilizing mutations that improve activity at elevated temperatures (Bell *et al.*, 2022; Lu *et al.*, 2022). Immobilization strategies further influence enzyme–substrate interactions by altering enzyme conformation and accessibility; nanocarrier based immobilization often enhances substrate affinity and catalytic turnover (Sheldon *et al.*, 2021; Zhang *et al.*, 2023).

5.3 Role of Cofactors and Environmental Conditions

Cofactors such as metal ions (Cu^{2+} in laccases, Mn^{2+} in MnP, Fe in peroxidases) and heme groups are essential for electron transfer and catalytic cycles. Peroxidases require controlled H_2O_2 dosing to avoid oxidative inactivation, while oxygenases depend on NAD(P)H and flavin cofactors for electron delivery (Pankaj & Arora, 2022; Moratti *et al.*, 2022). Environmental factors pH, temperature, ionic strength, and presence of inhibitors strongly modulate enzyme activity. For PET hydrolases, activity peaks near PET's glass transition temperature (60–70 °C), where polymer chains become more accessible (Bell *et al.*, 2022; Tournier *et al.*, 2020). Immobilization and nanobiocatalysis mitigate pH and thermal instability, enabling operation in harsh industrial matrices (Sheldon *et al.*, 2021; Zhang *et al.*, 2023). The interconnection between pollutant classes, catalytic mechanisms, and representative enzyme families is illustrated in **Figure 1.** Collectively, these mechanistic insights underscore the versatility and catalytic precision of enzymes in targeting structurally diverse environmental contaminants.

Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

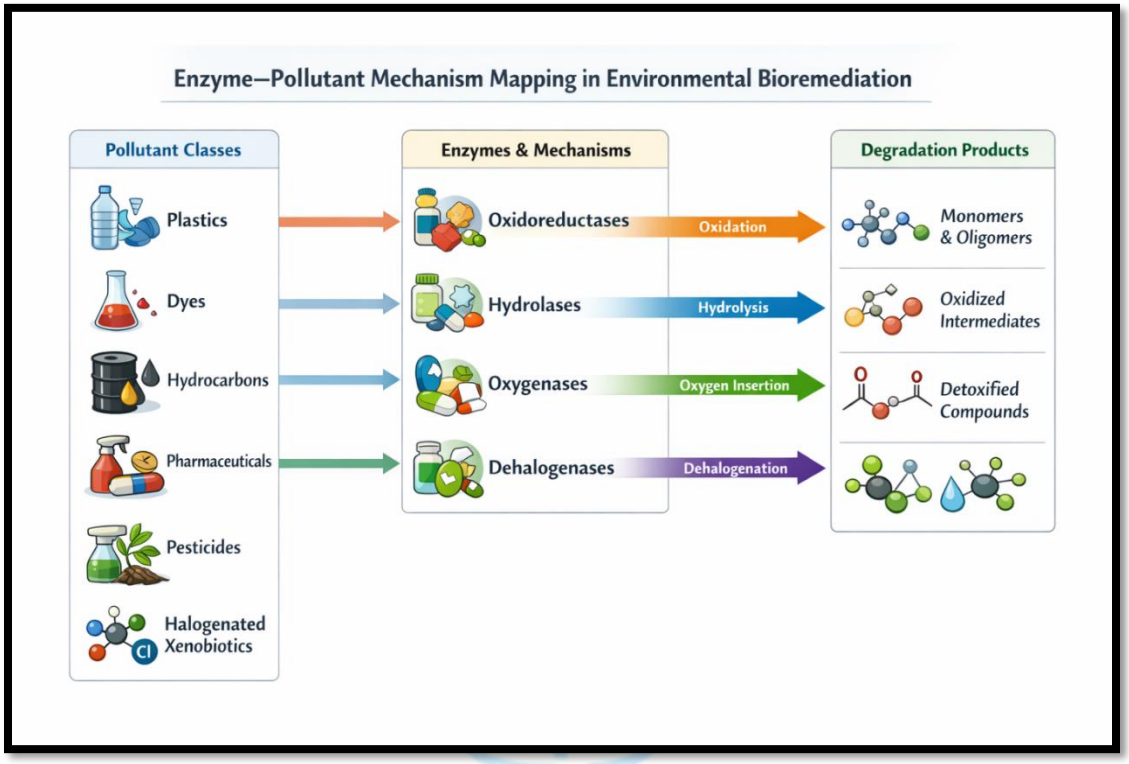


Figure 1. Enzyme pollutant mechanistic mapping in environmental bioremediation.

Figure 01 shows schematic representation of major pollutant classes (plastics, dyes, hydrocarbons, pharmaceuticals, pesticides, and halogenated xenobiotics) and their corresponding enzymatic degradation pathways. Oxidoreductases (laccases, peroxidases), hydrolases (PETases, cutinases, esterases, lipases), oxygenases, and dehalogenases are shown mediating substrate recognition, catalytic bond cleavage, oxidation–reduction reactions, and detoxification processes. The figure highlights enzyme substrate specificity and the transformation of complex contaminants into smaller, less toxic, or recyclable intermediates.

6. Advances in Enzyme Engineering and Environmental Biotechnology

6.1 Protein Engineering and Directed Evolution

Over the past decade, protein engineering combining rational, structure guided design with directed evolution has delivered substantial gains in activity, thermostability, and substrate scope for key biodegradative enzymes. In polyester depolymerization, the most visible advances are engineered PET hydrolases. Automated evolution platforms produced HotPETase with $T_m \approx 82.5$ °C, enabling depolymerization of semi crystalline PET at or above the polymer's glass transition temperature and selective deconstruction from complex laminates (Bell *et al.*, 2022). Complementarily, machine learning aided design accelerated sequence navigation to identify stabilizing and activity enhancing mutations in PET hydrolases, yielding variants capable of efficient PET depolymerization near ambient to moderate temperatures and facilitating closed loop monomer recovery (Lu *et al.*, 2022). These advances build on earlier structure guided LCC mutants that demonstrated near complete depolymerization and bottle grade PET resynthesis, validating the techno economic potential of enzymatic recycling (Tournier *et al.*, 2020).

For xenobiotic detoxification, ultrahigh throughput microfluidic screening has evolved a metal free α/β hydrolase into an efficient phosphotriesterase, an important proof of concept

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

that organophosphate degrading activity can be installed in alternative scaffolds and improved orders of magnitude for remediation or biosensing (Schnettler *et al.*, 2023). In oxidoreductases, computational discovery and protein design recently identified thermophilic bacterial laccases with catalytic promiscuity, enabling robust dye decolorization and even surface oxidation on low density polyethylene, expanding oxidative pre treatment options for recalcitrant polymers (Zhang *et al.*, 2023). Directed evolution and AI aided engineering have transitioned several enzymes from lab curiosities to process relevant biocatalysts with the kinetic and thermal properties needed for industrial effluents, plastic waste streams, and mixed matrices (Bell *et al.*, 2022; Lu *et al.*, 2022).

6.2 Metagenomics and Synthetic Biology Approaches

Metagenomic mining of underexplored and extreme habitats continues to expand the biocatalyst repertoire. Genome resolved surveys show that hydrocarbon degrading oxygenases are far more widely distributed than previously appreciated across bacterial and archaeal phyla, reflecting extensive gene duplication and horizontal gene transfer drivers of rapid adaptation to anthropogenic compounds (Rezaei Somee *et al.*, 2022). In plastics, deep environment and landfill meta omics have uncovered novel PETases/BHETases with distinct stability profiles, highlighting the value of niche targeted sampling to discover enzymes suited to harsh conditions (Choi *et al.*, 2024).

Synthetic biology enables translation of these discoveries into deployable systems. Regulatory parts associated with monooxygenases are being repurposed as transcription factor based biosensors that report on hydrocarbons and other pollutants, offering low cost, field deployable monitoring and control layers for engineered bioprocesses (Moratti *et al.*, 2022). Moreover, modular cloning, promoter tuning, and chassis optimization (e.g., *Pseudomonas*, *Bacillus*, yeast) support high level expression of multi enzyme pathways, while pathway balancing mitigates intermediate toxicity and product inhibition critical for sustained operation in real effluents (Moratti *et al.*, 2022; Rezaei Somee *et al.*, 2022). Metagenomics broadens the search space for robust enzymes, while synthetic biology converts these parts into sensors and high expression biocatalysts aligned with process needs (Choi *et al.*, 2024; Moratti *et al.*, 2022).

6.3 Enzyme Immobilization and Nanobiotechnology

Immobilization transforms soluble enzymes into recyclable heterogeneous catalysts, improving operational stability, resistance to proteolysis, and tolerance to pH/temperature excursions. Contemporary strategies include covalent grafting on porous supports, carrier free aggregates (CLEAs), and incorporation into metal–organic frameworks (MOFs) or nanocomposites that tune microenvironments around the active site (Sheldon *et al.*, 2021). For laccases, immobilization on nanocomposites markedly increases reusability and activity retention during dye removal in challenging matrices rich in salts and surfactants (Zhang *et al.*, 2023). These designs reduce the cost per treated volume by extending enzyme lifetime and enabling continuous flow reactors.

At the interface of materials science and biocatalysis, nanobiotechnology contributes magnetic nanoparticles for easy recovery, conductive carbons for redox mediation, and MOFs providing high surface area and protective microenvironments. While immobilization can introduce mass transfer limitations, careful control of pore size, hydrophilicity, and enzyme orientation mitigates diffusion barriers and preserves turnover (Sheldon *et al.*, 2021; Zhang *et al.*, 2023). Beyond wastewater, enzyme embedded materials are emerging: encapsulating thermally robust hydrolases directly into polymer matrices yields self degrading biodegradable plastics that remain inert in use yet activate under composting or methanization, shortening end of life persistence without sacrificing performance (Sun, 2025). Immobilization and nano enabled supports convert fragile proteins into process tolerant, reusable biocatalysts, and enzyme embedded materials align product design with

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

desired degradation pathways (Sheldon *et al.*, 2021; Sun, 2025). Technological advancements enabling enzyme optimization and large scale deployment are summarized in Figure 02.

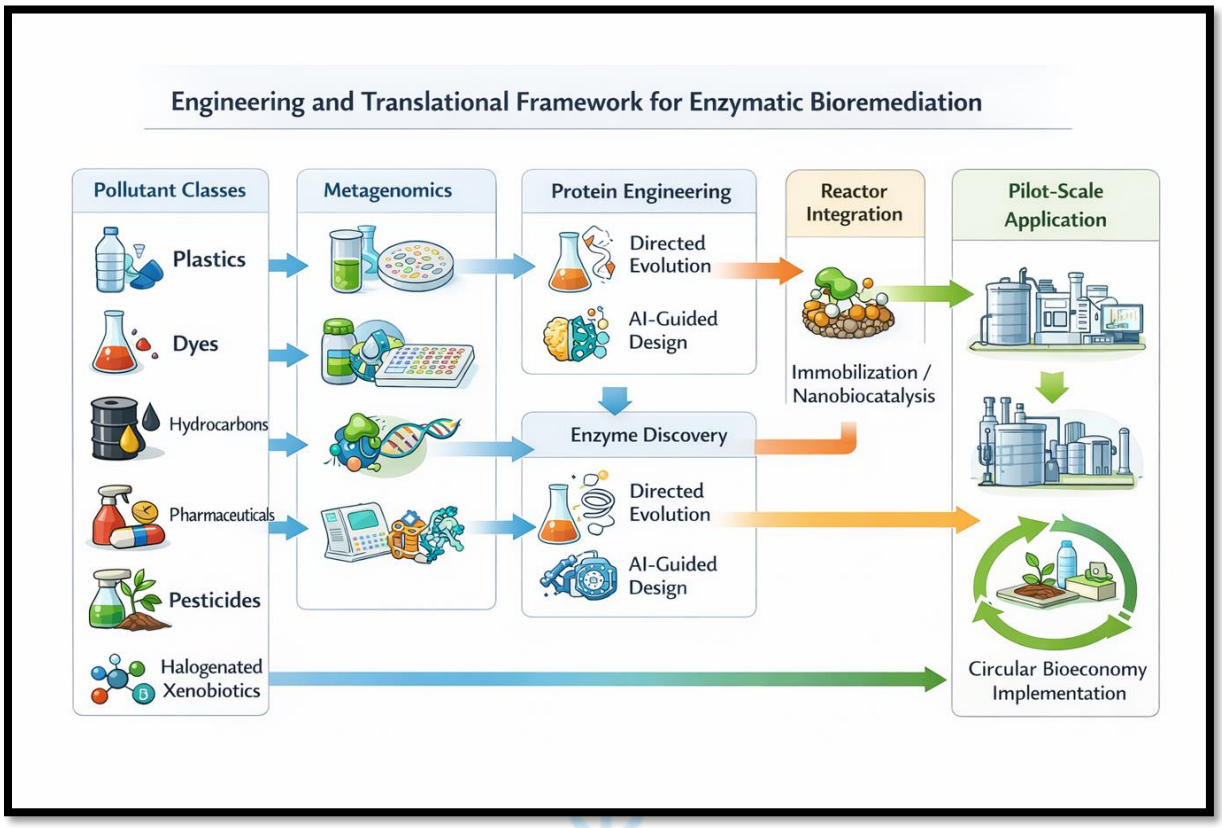


Figure 2 Engineering and translational framework for scalable enzymatic bioremediation.

6.4 Hybrid Bio catalytic Systems and Reactor Design

Real effluents are complex: competing substrates, salts, surfactants, metals, and variable pH/temperature can inhibit enzymes and microbes. Hybrid systems combining enzymes with microbial consortia, adsorbents, membranes, or advanced oxidation leverage complementary strengths. In wastewater polishing, oxidoreductases (laccase/MnP/DyP) target micropollutants that evade conventional secondary treatment, while immobilization allows operation in packed bed or membrane reactors with online regeneration (Barber *et al.*, 2020; de Boer *et al.*, 2023). For plastics, slurry and packed bed reactors maximize enzyme–polymer contact while controlling residence time and product removal to limit feedback inhibition; integration with downstream purification and repolymerization closes the loop to bottle grade PET (Bell *et al.*, 2022; Tournier *et al.*, 2020). Process intensification increasingly uses sensing and control. Transcription factor based biosensors and electrochemical probes inform feed forward dosing of H_2O_2 for peroxidases and oxygen supply perturbations to upregulate oxidoreductases in microbial cultures (Moratti *et al.*, 2022; de Boer *et al.*, 2023). AI assisted frameworks help select immobilization supports, predict stability under matrix conditions, and optimize reactor set points. Crucially, modern development shifts from percent "removal" metrics to kinetic modeling and ecotoxicity informed assessment of transformation products to ensure that treatment reduces risk, not merely parent compound concentration (de Boer *et al.*, 2023). Combining engineered enzymes, smart immobilization, and sensor guided reactors enables robust treatment of complex streams and

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

supports scale up to pilot and industrial settings (Barber *et al.*, 2020; de Boer *et al.*, 2023). These technological innovations significantly enhance enzyme performance and represent a decisive step toward industrial scale environmental biotechnology.

7. Applications of Enzymes in Pollution Control

7.1 Wastewater Treatment

Enzymatic polishing has emerged as a practical complement to conventional biological treatment, particularly for micropollutants (pharmaceuticals, personal care products, dyes) that escape secondary processes. Oxidoreductases notably laccase, manganese peroxidase (MnP), lignin peroxidase (LiP), and DyP type peroxidases oxidize a wide spectrum of aromatic compounds and dye chromophores, reducing color and toxicity in complex effluents (Barber, Liu, & Smith, 2020; de Boer, Schäffer, & Moreira, 2023). Immobilized laccase systems on nanocomposites enhance enzyme lifetime and reusability under high salt, surfactant rich matrices typical of textile and industrial streams (Zhang *et al.*, 2023). Importantly, mediator selection and dosing must be tuned to minimize the formation of toxic transformation products; recent reviews emphasize coupling removal metrics with ecotoxicity assays to ensure net risk reduction (Barber *et al.*, 2020; de Boer *et al.*, 2023). In high salinity scenarios, halophilic/halotolerant consortia using azoreductase and peroxidases have achieved rapid decolorization with verified detoxification, offering enzyme compatible microbial co treatments for challenging dye laden wastewaters (Qiu *et al.*, 2022; Varga *et al.*, 2019).

From a process perspective, packed bed or membrane reactors hosting immobilized oxidoreductases allow continuous operation and straightforward regeneration, while biosensor guided control leveraging transcription factor–based reporters can optimize dosing of cofactors (e.g., H_2O_2 for peroxidases) and aeration for redox balance (Moratti, Scott, & Coleman, 2022; de Boer *et al.*, 2023). These integrations help stabilize performance across diurnal fluctuations in influent quality. Co immobilization of laccase with adsorption media can simultaneously remove color and residual organics; however, routine toxicity profiling (e.g., Daphnia/Algae assays) remains critical to validate environmental safety (Barber *et al.*, 2020; de Boer *et al.*, 2023).

7.2 Soil Bioremediation

In soils contaminated by pesticides and hydrocarbons, enzymatic approaches are deployed within microbial consortia or as exogenous enzyme additions to accelerate detoxification. Phosphotriesterases and hydrolases degrade organophosphate pesticides; advances in ultrahigh throughput directed evolution have produced metal free α/β hydrolase variants with dramatically enhanced phosphotriesterase activity, improving practical feasibility for field applications and biosensing (Schnettler *et al.*, 2023; Rani, Kumar, & Gupta, 2022). For hydrocarbon contamination, monooxygenases/dioxygenases catalyze initial activation and ring cleavage, and genome resolved surveys reveal broad phylogenetic distribution of these pathways supporting bioprospecting for robust catalysts adapted to local soil conditions (Ławniczak *et al.*, 2020; Rezaei Somee *et al.*, 2022).

Operational strategies include bioaugmentation with enzyme rich strains, biostimulation via nutrient amendments to strengthen endogenous enzyme expression, and immobilization of enzymes on soil friendly carriers to prolong activity under variable moisture and pH (Sheldon, Basso, & Brady, 2021; Rani *et al.*, 2022). Given the likelihood of halogenated xenobiotics in agricultural contexts, dehalogenases (e.g., LinA/LinB) reduce toxicity by removing halogens prior to oxygenase driven ring cleavage (Rani *et al.*, 2022; Ławniczak *et al.*, 2020). Pair enzyme enhanced treatments with monitoring of metabolites (e.g., LC–MS) to verify detoxification and prevent accumulation of persistent intermediates (Rani *et al.*, 2022; Varga *et al.*, 2019).

7.3 Air and Industrial Effluent Detoxification

Although enzymatic remediation is most mature in aqueous matrices, enzymes can mitigate

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

industrial off gas and volatile emissions by integrating biofilters/biotrickling filters that exploit oxygenase and oxidoreductase activities in hydrated catalytic beds. Laccase and peroxidase modified surfaces can catalyze oxidative removal of phenolic VOCs and colorants in spray or trickle phase reactors, while oxygenase driven biofilters metabolize alkanes/alkenes, aided by synthetic biology biosensors to tune airflow and nutrient supply (Moratti *et al.*, 2022; Barber *et al.*, 2020). For industrial liquid effluents, hybrid trains that combine enzymatic steps with adsorption/membranes often outperform single unit processes, especially when effluents contain recalcitrant aromatics and mixed pollutant loads; immobilization reduces fouling and simplifies catalyst recovery (Sheldon *et al.*, 2021; de Boer *et al.*, 2023).

A persistent challenge is enzyme stability in high temperature or solvent rich streams. Here, nanobiocatalyst supports (MOFs, magnetic nanoparticles) and carrier free aggregates (CLEAs) improve tolerance to shear, temperature, and organic content, extending operational windows and reducing enzyme replacement costs (Sheldon *et al.*, 2021; Zhang *et al.*, 2023).

7.4 Plastic and Microplastic Degradation

Enzyme enabled plastic control spans end of pipe handling of microplastics and closed loop recycling of bulk polymers. Engineered PET hydrolases (e.g., HotPETase, ML optimized PETases) achieve rapid depolymerization near PET's glass transition temperature, enabling recovery of monomers (TPA, EG) for re polymerization to bottle grade PET demonstrated at pilot relevant scales (Bell *et al.*, 2022; Tournier *et al.*, 2020). For microplastics in water, coupling oxidoreductase pre oxidation (e.g., laccase mediated surface functionalization) with hydrolase action improves fragmentation and downstream capture, while immobilized enzymatic beds can be integrated into tertiary treatment (Choi *et al.*, 2024; Zhang *et al.*, 2023).

Beyond remediation, enzyme embedded biodegradable plastics (e.g., PLA with stabilized hydrolases) are being designed to remain inert in use yet activate in home compost/methanization, achieving full disintegration and reducing microplastic release (Sun, 2025; Bell *et al.*, 2022). Reviews highlight complementary strategies pretreatments, additives, and consortia to address non PET polymers and broaden substrate scope (Choi *et al.*, 2024; Dhali, Parida, Kumar, & Bala, 2024). For PET rich streams, slurry or packed bed enzyme reactors with online product removal minimize feedback inhibition; for microplastics, coagulation/filtration post enzymatic treatment enhances solid capture before discharge (Bell *et al.*, 2022; Choi *et al.*, 2024). Real world implementations demonstrate the growing feasibility of enzymatic systems in both wastewater treatment and solid waste valorization pathways.

8. Industrial Implementations

8.1 PET Recycling at Industrial Scale

One of the most prominent demonstrations of enzymatic bioremediation at scale is PET recycling using engineered hydrolases. The French biotech company Carbios successfully piloted a process employing engineered PET depolymerases (derived from *Ideonella sakaiensis* PETase and optimized via structure guided mutagenesis) to depolymerize post consumer PET into terephthalic acid (TPA) and ethylene glycol (EG) under mild conditions (Tournier *et al.*, 2020). This process achieved >90% depolymerization within 10–16 hours at 65–72 °C, enabling closed loop recycling of PET bottles into virgin grade resin. Techno economic assessments indicate competitive costs compared to chemical recycling, especially when integrated with monomer purification and repolymerization units (Bell *et al.*, 2022). Carbios has since scaled up to a demonstration plant and announced plans for full industrial deployment by 2025, marking a milestone for enzymatic recycling in the circular economy.

8.2 Textile Effluent Detoxification

In the textile sector, immobilized laccase reactors have been implemented for continuous decolorization and detoxification of dye laden wastewater. A notable case involves a packed bed bioreactor using laccase immobilized on magnetic nanocomposites, which achieved >85% color

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

removal and significant reduction in chemical oxygen demand (COD) across multiple cycles without major activity loss (Zhang *et al.*, 2023). Integration with membrane filtration further improved effluent quality, meeting discharge standards for azo and anthraquinone dyes. Pilot trials in India and China have demonstrated scalability, though cost optimization for enzyme production and immobilization remains a challenge (Barber *et al.*, 2020; Varga *et al.*, 2019).

8.3 Pharmaceutical Micropollutant Removal

Municipal wastewater treatment plants in Europe have piloted laccase based polishing steps for removing pharmaceuticals such as diclofenac, ibuprofen, and carbamazepine. Immobilized fungal laccases in fluidized bed reactors achieved 40–70% removal efficiencies for selected micropollutants under real effluent conditions (de Boer *et al.*, 2023). While promising, variability in removal rates due to matrix complexity and transformation product toxicity underscores the need for hybrid systems combining enzymatic oxidation with adsorption or advanced oxidation processes (AOPs). These pilots highlight enzymatic treatment as a viable tertiary option for micropollutant control in compliance with EU Water Framework Directive goals.

8.4 Biodegradable Plastics with Embedded Enzymes

Industrial packaging companies have begun incorporating enzyme embedded biodegradable plastics to accelerate end of life degradation. For example, PLA films embedded with thermostable cutinases remain inert during use but activate under composting conditions, achieving >90% weight loss within 60 days in home compost environments (Sun, 2025). This approach addresses microplastic concerns and aligns with extended producer responsibility (EPR) regulations, offering a scalable route to sustainable packaging. Representative enzymes, target pollutants, and reported applications are summarized in Table 01.

Table 1 Representative enzymes, target pollutants, mechanisms, and application contexts in environmental bioremediation.

Pollutant Class	Representative Enzymes	Mechanism Type	Key Products	Application Context
PET/Plastics	PETase, Cutinase	Ester bond hydrolysis	TPA, EG	Plastic recycling
Azo dyes	Laccase, Peroxidase	Oxidative cleavage	Aromatic amines	Textile wastewater
Hydrocarbons	Monooxygenase, Dioxygenase	Oxygen insertion	Alcohols, acids	Oil spill remediation
Pharmaceuticals	Laccase, Peroxidase	Oxidation	Detoxified metabolites	WWTP polishing
Pesticides	Dehalogenase, Hydrolase	Dehalogenation	Non toxic intermediates	Soil remediation

9. Challenges and Limitations

Despite remarkable progress in enzymatic bioremediation, several technical, economic, and ecological constraints hinder large scale deployment. These challenges span enzyme performance, process integration, cost structures, and regulatory compliance.

9.1 Enzyme Stability and Activity in Complex Matrices

Enzymes often exhibit instability under harsh environmental conditions extreme pH, salinity, temperature, and presence of inhibitors (e.g., surfactants, heavy metals). Industrial effluents and soil systems introduce mixed pollutant loads, which can denature enzymes or block active sites,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

reducing catalytic efficiency (Sheldon, Basso, & Brady, 2021; Barber *et al.*, 2020). While immobilization on nanocomposites and MOFs improves resilience, mass transfer limitations and fouling remain unresolved issues in continuous flow reactors (Zhang *et al.*, 2023). Furthermore, enzyme activity can decline due to product inhibition, particularly in PET depolymerization where MHET accumulation slows reaction rates (Bell *et al.*, 2022; Tournier *et al.*, 2020).

9.2 Transformation Product Toxicity

Enzymatic degradation does not always equate to detoxification. Partial oxidation or hydrolysis can yield intermediates with equal or greater toxicity than parent compounds (Varga *et al.*, 2019). For example, azo dye cleavage may generate aromatic amines with mutagenic potential, and pharmaceutical oxidation can produce persistent metabolites (de Boer *et al.*, 2023). This necessitates comprehensive ecotoxicity profiling and LC–MS monitoring of transformation products alongside removal metrics a step often overlooked in pilot studies.

9.3 Economic and Scalability Constraints

High costs of enzyme production, purification, and stabilization remain a barrier to industrial adoption. Although directed evolution and machine learning have improved catalytic efficiency, large scale enzyme manufacturing still relies on fermentation and downstream processing, which can be cost intensive (Bell *et al.*, 2022; Lu *et al.*, 2022). Immobilization adds material and processing costs, and carrier regeneration is not always feasible. Techno economic analyses suggest that enzymatic PET recycling approaches competitiveness only when integrated into closed loop circular economy models with monomer recovery and repolymerization (Tournier *et al.*, 2020; Sun, 2025).

9.4 Regulatory and Ecological Considerations

Deployment of engineered enzymes and genetically modified microbial hosts raises biosafety and regulatory concerns, particularly in open environments. Approval processes for synthetic biology based bioremediation remain stringent, slowing commercialization (Moratti *et al.*, 2022). Additionally, public perception of GMOs and enzyme treated effluents can influence adoption, necessitating transparent risk assessments and stakeholder engagement.

9.5 Knowledge Gaps and Standardization

Current literature lacks standardized performance metrics for enzymatic remediation. Studies often report percentage removal without kinetic modeling or toxicity endpoints, complicating cross comparison and regulatory validation (Varga *et al.*, 2019). Harmonized protocols for enzyme activity assays, immobilization stability tests, and life cycle assessments are essential to accelerate industrial translation.

10. Future Perspectives and Emerging Trends

The next decade promises transformative advances in enzymatic bioremediation, driven by synthetic biology, computational design, and circular economy imperatives. Below are key directions shaping the future landscape:

10.1 Discovery of Novel Enzymes from Extremophiles

Extreme environments deep sea vents, hypersaline lakes, and geothermal springs harbor enzymes with intrinsic thermostability, halotolerance, and solvent resistance, traits essential for industrial effluent treatment and plastic depolymerization under harsh conditions. Metagenomic mining and single cell genomics will accelerate the identification of such biocatalysts, expanding substrate scope beyond PET to polyolefins and mixed polymer blends (Choi *et al.*, 2024; Rezaei Somee *et al.*, 2022).

10.2 AI Guided Protein Engineering

Machine learning and generative AI models are revolutionizing enzyme design by predicting stabilizing mutations, optimizing active site geometry, and simulating catalytic pathways. Recent successes in ML aided PET hydrolase engineering (Lu *et al.*, 2022) demonstrate the potential for rapid iteration cycles, reducing development timelines from years to months.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Integration of physics informed neural networks and high throughput screening will enable bespoke enzymes tailored to specific pollutants and process conditions

10.3 Synthetic Biology and Modular Biocatalytic Systems

Synthetic biology offers modular platforms for multi enzyme cascades, enabling complete mineralization of complex pollutants. Engineered microbial consortia with dynamic regulatory circuits can co express oxidoreductases, hydrolases, and dehalogenases, ensuring sequential degradation of mixed contaminants. Coupling these systems with biosensors allows real time monitoring and adaptive control, enhancing reliability in variable environments (Moratti *et al.,* 2022).

10.4 Nanobiotechnology and Smart Immobilization

Future immobilization strategies will leverage stimuli responsive nanomaterials that modulate enzyme activity in response to pH, temperature, or pollutant concentration. Magnetic nanocarriers and metal–organic frameworks (MOFs) will enable easy recovery and reuse, while 3D printed bioreactors incorporating immobilized enzymes promise scalable, customizable designs for wastewater and soil remediation (Sheldon *et al.,* 2021; Zhang *et al.,* 2023).

10.5 Integration with Circular Bioeconomy

Enzymatic processes will increasingly align with circular economy principles, converting waste into value added products. PET depolymerization for monomer recovery and repolymerization exemplifies this trend (Tournier *et al.,* 2020; Bell *et al.,* 2022). Emerging strategies include enzyme enabled upcycling of plastics into specialty chemicals and biodegradable plastics embedded with latent enzymes, ensuring controlled degradation post use (Sun, 2025).

10.6 Digital Twins and Process Automation

The convergence of AI, IoT, and process modeling will enable digital twins for enzymatic bioreactors, facilitating predictive maintenance, real time optimization, and lifecycle assessment. These tools will support scale up and regulatory compliance, reducing operational risks and costs.

Conclusion

Enzymatic bioremediation offers a precise and sustainable alternative to traditional pollutant removal. By leveraging innovations in enzyme engineering and systems biology, researchers are overcoming historical limitations in stability and substrate specificity. Although challenges in scalability and cost persist, the future of the field lies in the integration of artificial intelligence and advanced materials. Moving forward, a collaborative, interdisciplinary approach is essential to translate these molecular breakthroughs into robust, large-scale environmental technologies that support a circular bio economy.

REFERENCES:

Adamo, M., Comtet Marre, S., Büttner, E., Kellner, H., Luis, P., Prego, R., & Marmeisse, R. (2022). Fungal dye decolorizing peroxidase diversity: Roles in either intra or extracellular processes. *Applied Microbiology and Biotechnology, 106*, 2993–3007. https://doi.org/10.1007/s00253 022 11923 0

Agnez Lima, L. F., Vainstein, M. H., & Zhang, X. (2024). Editorial: Microbial hydrocarbon degradation and bioremediation: From genes to pathways. *Frontiers in Microbiology, 15*, 1416516. https://doi.org/10.3389/fmicb.2024.1416 516

Bagheri, A. R., Aramesh, N., Munir, H., Ahmed, Z., Yahaya, A., & Bilal, M. (2022). Oxidoreductases for removal of environmental pollutants. In *Enzymes for Pollutant Degradation* (Vol. 30, pp. 1–17). Springer Nature. https://doi.org/10.1007/978 981 16 4574 7

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Barber, E. A., Liu, Z., & Smith, S. R. (2020). Organic contaminant biodegradation by oxidoreductase enzymes in wastewater treatment. *Microorganisms, 8*(1), 122. https://doi.org/10.3390/microorganisms8010122

Bell, E. L., Smithson, R., Kilbride, S., Foster, J., Hardy, F. J., Ramachandran, S., & Green, A. P. (2022). Directed evolution of an efficient and thermostable PET depolymerase. *Nature Catalysis, 5*(9), 754–769. https://doi.org/10.1038/s41929 022 00821 3

Cheng, X., Khan, A. R., ElKarrach, K., & Wang, F. (2023). Editorial: Environmental bioremediation: Application of enzymes and microbes. *Frontiers in Bioengineering and Biotechnology, 11*, 1327124. https://doi.org/10.3389/fbioe.2023.1327124

Choi, J., Kim, H., Ahn, Y. R., Kim, M., Yu, S., Kim, N., & Kim, H. O. (2024). Recent advances in microbial and enzymatic engineering for the biodegradation of micro and nanoplastics. *RSC Advances, 14*, 9943–9966. https://doi.org/10.1039/D4RA00844H

de Boer, S. R., Schäffer, A., & Moreira, M. T. (2023). Towards oxidoreductase based processes for the removal of antibiotics from wastewater. *Reviews in Environmental Science and Bio/Technology, 22*, 899–932. https://doi.org/10.1007/s11157 023 09676 x

Dhali, S. L., Parida, D., Kumar, B., & Bala, K. (2024). Recent trends in microbial and enzymatic plastic degradation: A solution for plastic pollution predicaments. *Biotechnology for Sustainable Materials, 1*, 11. https://doi.org/10.1186/s44316 024 00011 0

Flórez Restrepo, M. A., López Legarda, X., & Segura Sánchez, F. (2025). Bioremediation of emerging pharmaceutical pollutants acetaminophen and ibuprofen by white rot fungi A review. *Science of the Total Environment, 977*, 179379. https://doi.org/10.1016/j.scitotenv.2025.179379

Lin, X., Zhang, R., Cui, F. Q., Hong, W., Yang, S., & Ju, F. (2025). Natural selected plastics biodegradation species and enzymes in landfills. *PNAS Nexus, 4*(3), pgaf066. https://doi.org/10.1093/pnasnexus/pgaf066

Lu, H., Diaz, D. J., Czarnecki, N. J., Zhu, C., Kim, W., Shroff, R., & Beckham, G. T. (2022). Machine learning aided engineering of hydrolases for PET depolymerization. *Nature, 603*, 835–841. https://doi.org/10.1038/s41586 022 04599 z

Ławniczak, Ł., Woźniak Karczewska, M., Loibner, A. P., Heipieper, H. J., & Chrzanowski, Ł. (2020). Microbial degradation of hydrocarbons Basic principles for bioremediation: A review. *Molecules, 25*(4), 856. https://doi.org/10.3390/molecules25040856

Moratti, C. F., Scott, C., & Coleman, N. V. (2022). Synthetic biology approaches to hydrocarbon biosensors: A review. *Frontiers in Bioengineering and Biotechnology, 9*, 804234. https://doi.org/10.3389/fbioe.2021.804234

Qiu, H., Shen, F., Yin, A., Liu, J., Wu, B., Li, Y., & Xu, B. (2022). Biodegradation and detoxification of azo dyes by halophilic/halotolerant microflora. *Frontiers in Microbiology, 13*, 877151. https://doi.org/10.3389/fmicb.2022.877151

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Rani, R., Kumar, V., & Gupta, P. (2022). Role of enzymes in biodegradation of pesticides: General aspects and recent advances. In *Pesticides Bioremediation* (pp. 145–163). Springer. https://doi.org/10.1007/978 3 030 97000 0_6

Rezaei Somee, M., Amoozegar, M. A., Dastgheib, S. M. M., Shavandi, M., Ghanbari Maman, L., Bertilsson, S., & Mehrshad, M. (2022). Diversification in key aerobic hydrocarbon degrading enzymes across bacteria and archaea. *BMC Genomics, 23*, 690. https://doi.org/10.1186/s12864 022 08906 w

Sheldon, R. A., Basso, A., & Brady, D. (2021). New frontiers in enzyme immobilisation: Robust biocatalysts for a circular bio based economy. *Chemical Society Reviews, 50*, 5850–5862. https://doi.org/10.1039/D1CS00015B

Sun, S. (2025). Enzyme embedded biodegradable plastic for sustainable applications: Advances, challenges, and perspectives. *ACS Applied Bio Materials, 8*, 1785–1796. https://doi.org/10.1021/acsabm.4c01628

Sudarshan, S., Harikrishnan, S., RathiBhuvaneswari, G., Alamelu, V., Aanand, S., & Rajasekar, A. (2023). Impact of textile dyes on human health and bioremediation of textile industry effluent using microorganisms. *Journal of Applied Microbiology, 134*(2), lxac064. https://doi.org/10.1093/jambio/lxac064

Thathola, P., Melchor Martínez, E. M., Adhikari, P., Hernández Martínez, S. A., Pandey, A., & Parra Saldívar, R. (2024). Laccase mediated degradation of emerging contaminants: Unveiling a sustainable solution. *Environmental Science: Advances, 3*, 1500–1512. https://doi.org/10.1039/D4VA00173G

Tournier, V., Topham, C. M., Gilles, A., David, B., Folgoas, C., Moya Leclair, E., & Marty, A. (2020). An engineered PET depolymerase to break down and recycle plastic bottles. *Nature, 580*(7802), 216–219. https://doi.org/10.1038/s41586 020 2149 4

Varga, B., Somogyi, V., Meiczinger, M., Kováts, N., & Domokos, E. (2019). Enzymatic treatment and subsequent toxicity of organic micropollutants using oxidoreductases: A review. *Journal of Cleaner Production, 221*, 306–322. https://doi.org/10.1016/j.jclepro.2019.02 .135

Zhan, H., Feng, Y., Fan, X., & Chen, S. (2018). Recent advances in glyphosate biodegradation. *Applied Microbiology and Biotechnology, 102*, 5033–5043. https://doi.org/10.1007/s00253 018 9035 0

Zhang, W., Ji, L., Lu, Z., Liu, R., Nian, B., Liu, R., & Hu, Y. (2023). Laccase immobilized on nanocomposites for wastewater pollutants degradation: Current status and future prospects. *Bioprocess and Biosystems Engineering, 46*, 1513–1531. https://doi.org/10.1007/s00449 023 02907 z

Zhang, Y., Plesner, T. J., Ouyang, Y. C., Zheng, Y. C., Bouhier, E., Berentzen, E. I., & Guo, Z. (2023). Computer aided discovery of a novel thermophilic laccase for low density polyethylene degradation. *Journal of Hazardous Materials, 458*, 131986. https://doi.org/10.1016/j.jhazmat.2023.1 31986