

Review Article

Microbial Enzymes Used in Bioremediation

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Emerging pollutants in nature are linked to various acute and chronic detriments in biotic components and subsequently deteriorate the ecosystem with serious hazards. Conventional methods for removing pollutants are not efficient; instead, they end up with the formation of secondary pollutants. Significant destructive impacts of pollutants are perinatal disorders, mortality, respiratory disorders, allergy, cancer, cardiovascular and mental disorders, and other harmful effects. The pollutant substrate can recognize different microbial enzymes at optimum conditions (temperature/pH/contact time/concentration) to efficiently transform them into other rather unharmed products. The most representative enzymes involved in bioremediation include cytochrome P450s, laccases, hydrolases, dehalogenases, dehydrogenases, proteases, and lipases, which have shown promising potential degradation of polymers, aromatic hydrocarbons, halogenated compounds, dyes, detergents, agrochemical compounds, etc. Such bioremediation is favored by various mechanisms such as oxidation, reduction, elimination, and ring-opening. The significant degradation of pollutants can be upgraded utilizing genetically engineered microorganisms that produce many recombinant enzymes through eco-friendly new technology. So far, few microbial enzymes have been exploited, and vast microbial diversity is still unexplored. This review would also be useful for further research to enhance the efficiency of degradation of xenobiotic pollutants, including agrochemical, microplastic, polyhalogenated compounds, and other hydrocarbons.

1. Introduction

Earth is facing tremendously severe effects due to anthropogenic activities like modern agricultural practices, industrialization, overpopulation, and unhealthy competition for supremacy. These activities contribute to unprecedented levels of the generation of pollutants like phenols, azo dyes, polyaromatic hydrocarbons (PAHs), polychlorinated compounds, pesticides, heavy metals, and the rest. These chemicals resist biodegradation and endure in the environment for an extended period and cause acute and chronic detriment that threatens ecosystems' biotic components [1]. These pollutants have created teratogenic, carcinogenic, mutagenic, and toxic effects on humans or organisms and entirely affected every part of the earth [2]. Therefore, concerns about the removal of

organic waste from the surroundings are of great importance. The traditional approaches for eliminating xenobiotic contaminants include disposing of the garbage in a pit and pouring it in, high-temperature incineration, and chemical and ultraviolet (UV) decomposition. However, these physical and chemical approaches are losing ground due to lack of room, high expense, complicated procedures, rigorous regulatory requirements placed on decontamination by different countries, and the global public unacceptance. These techniques inherit several constraints, as they produce large amounts of sludge, which requires safe disposal, and end up with the formation of toxic secondary pollutants [3]. Therefore, there should be an immediate and rapid switch from these unfriendly physical and chemical techniques to biologically friendly techniques.

Bioremediation, a biotechnological process, is a biologically mediated change in pollutants concerned with restoring the recalculated environment and managing pollutants by detoxification and mineralization. The remediation of persistent organic pollutants (POPs) using microbial enzymes is considered environment-friendly, cost-effective, innovative, and promising [4]. However, the bioremediation process has got some limitations. It is a slow process, and so far, only a few species of bacteria capable of producing specific enzymes have displayed their potent ability to degrade pollutants. So, we prefer the genetically engineered microorganisms for bioremediation due to the large production of desired enzymes at optimized conditions. In this process, we exploit the typical characteristic of microorganisms or genetically engineered microorganisms capable of producing specific enzymes to catalyze or metabolize the xenobiotic pollutant to obtain energy and biomass, including agrochemical, microplastic, polyhalogenated compounds, and hydrocarbons. This process transforms toxic form to nontoxic form and sometimes novel products [5].

Bacteria are distributed in the biosphere due to their metabolic ability and are easily grown under a wide range of environmental conditions and produce enzymes. Various enzymes from aerobic bacteria such as *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium* have often degraded pesticides and hydrocarbons. In contrast, enzymes from anaerobic bacteria have been used for bioremediation of polychlorinated biphenyls (PCBs), dechlorination of trichloroethylene (TCE), and chloroform [6]. The most representative enzymes from microorganisms and genetically engineered microorganisms responsible for the degradation of the different classes of pollutants used in bioremediation processes include cytochrome P450, laccases, hydrolases, dehalogenases, dehydrogenases, proteases, and lipases (Figure 1). In this review, we have focused on enzymes isolated from microbial sources involved in the bioremediation of pollutants, exploring the diversity and ability of catalysis as shown in Table 1.

2. Some Microbial Enzymes Used in Bioremediation

2.1. Cytochrome P450. Cytochrome P450 (EC 1.14.14.1) is a superfamily of ubiquitous heme enzymes found across the three biological domains: Eukaryota, Bacteria, and Archaea [33], responsible for a wide range of functions ranging from the synthesis of complex natural products and drug metabolism in the living systems to biotransformation of toxic chemicals in our ecosystem [34]. P450s have an intrinsic capacity to degrade xenobiotics [35] through chemical transformations like aliphatic hydroxylations and epoxidations, dealkylations, dehalogenation, and various mechanism-based inactivations that are central to bioremediation chemistry. CYP101, CYP102, CYP1A1, CYP1A2, and CYP1B1 were known to metabolize PAHs, of which CYP1A1 shows high activity towards dibenzo-p-dioxin (DD) and mono-, di-, and trichloro-DDs whereas CYP1A1 mutant, F240A, exhibits activity toward 2,3,7,8-tetrachloro-

DD [36]. They involve using molecular oxygen and utilizing NADH or NADPH as a cofactor producing carbon substrate and oxidized products [37]. They also use ferredoxin and ferredoxin reductase as a source of electrons for catalytic function.

Various protein engineering and nonengineering studies on microbial P450s have been carried out to bioremediate organic pollutants and hydrocarbons. Early reviews on engineered P450s [38] and the application of microbial P450s for biodegradation/hydroxylation of environmentally relevant substrates were made [36].

Among the known microbial P450s, protein engineering studies on the model P450 from *Bacillus megaterium*, CYP102A1 (P450BM3), demonstrated its potential to oxidize PAHs such as phenanthrene, fluoranthene, and pyrene to a mixture of phenols and quinones. Some microbial P450s showed potential for bioremediation of polyhalogenated aromatics [39]. Lamb et al. investigated genetically modified *Acinetobacter calcoaceticus* strain BD413 to express P450 xenobiotic-metabolizing enzyme CYP105D1 from *Streptomyces griseus* ATCC 13273 to encompass persistent pollutants, herbicides, and agrochemicals, which allowed microorganisms to grow on these compounds [40]. For the efficient oxidation of hydrocarbons, Kumar et al. studied engineered CYP102A1, which showed enhanced activity towards PAHs, polychlorinated biphenyls (PCBs), and linear alkanes often used in the bioremediation of toxic compounds, detoxification of gaseous alkanes, and terpenes [41]. P450s have the ability, particularly for the degradation of recalcitrant halogenated pollutants, which are resistant to dioxygenases that the mutants F87W, F98W, Y96F, and V247L of heme monooxygenase CYP101A1 (P450_{cam}) from *Pseudomonas putida* showed activity with insoluble pentachlorobenzene, without the need of surfactants or organic solvents. So, the rational re-engineering of wild-type CYP101A1 provides active site mutants with a vastly improved ability to oxidize polychlorinated benzenes into chlorophenol products, further degraded by various microorganisms. Hence, the CYP101A1 mutants could form the basis of novel bioremediation systems for polychlorinated benzenes [42].

Similarly, Chakraborty and Das have reported several microorganisms such as *Rhodococcus*, *Gordonia*, *Mycobacterium*, and *Pseudomonas* harbor catabolic genes, plasmids, and genomes expressing P450s for the degradation and removal of POPs from the environment [43]. Awad and Mohamed found that P450 BM3 (CYP102A1) from *Bacillus megaterium*, engineered from *E. coli* BL21, is useful in the degradation of various organic gases pollutants by immobilizing hollow nanosphere particles of Pt/TiO₂-Cu under solar radiation where the degradation of isopropanol was found high (95%) at a pH of 7.0, ambient temperature, and concentration of 20 mg/L with the continuous supply of electrons via nanoparticles [44]. Bisphenol A (BPA), plasticizer, was degraded by strain YC-JY1 isolated from *Sphingobium* sp. This strain could utilize 4-hydroxybenzaldehyde and 4'-hydroxyacetophenone as a sole carbon source. Strain YC-JY1 Δ *bisdB* was constructed in *E. coli* to explore the role of the P450 [45]. Also, Kan et al. isolated

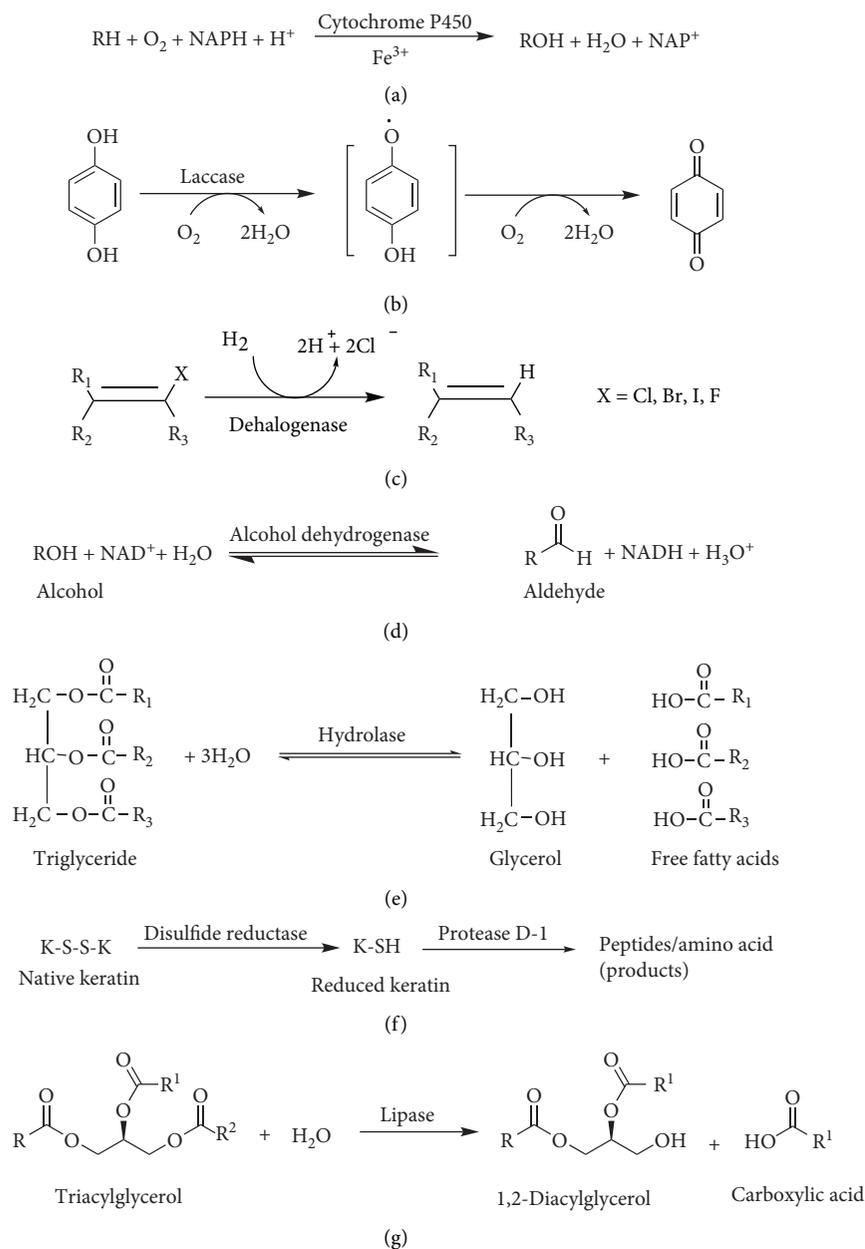


FIGURE 1: Some microbial enzymes used in bioremediation; general enzymatic reactions catalyzed by (a) cytochrome P450, (b) laccase, (c) dehalogenase, (d) dehydrogenase, (e) hydrolase, (f) protease, and (g) lipase.

Rhodococcus sp. P14 from crude oil-contaminated sediments where the regulatory expression of CYP108J1 resulted in PAHs' degradation, which can be used as the sole source of carbon and energy.

Further mutational analysis showed that NarL (nitrate-dependent two-component regulatory factor) acts as a novel repressor for the expression of CYP108J1 during PAHs degradation [46]. The list of other microbial P450s, their optimum catalytic conditions, along with their application, is mentioned in Table 1. Likewise, Table 2 illustrates the enzymes involved in bioremediation along with their functions. Further, other genetically engineered bacteria, with their role in bioremediation, are listed in Table 3.

2.2. Laccases. Laccases (benzenediol oxygen oxidoreductases, EC 1.10.3.2) are multi-copper-containing extracellular enzymes that consist of monomeric, dimeric, and tetrameric glycoproteins characterized by plants, bacteria, and fungi [47]. Microbial laccase from different microorganisms, especially the *Streptomyces* laccase from actinomycetes, are mostly identified, characterized, and studied. Such species include *S. cyaneus*, *S. coelicolor*, *S. bikiniensis*, and *S. ipomoea*, of which *S. coelicolor* is most extensively characterized [73]. Lignin and phenolic compounds present in agricultural wastes such as sawdust, banana peel, and rice bran enhanced laccase production [74]. Laccase has the catalytic capacity to affect the oxidation of phenolic compounds (ortho- and paradiphenol), aromatic amine, and their substituted

TABLE 1: Properties and applications of various microbial enzymes from different microorganisms.

S. no.	Microorganisms and enzymes	Substrate	Optimum temperature (°C)	Optimum pH	Mol. wt (kDa)	Application	Reference
1	<i>Pseudomonas putida</i> F6 (cell-free extracts laccase)	Syringaldazine (SGZ)	30	7.0	59	Degradation of synthetic dyes	[7]
2	<i>Streptomyces cyaneus</i> (cell-free extracts laccase)	2,2'-Azino-bis-(3-ethylbenzothiazoline -6-sulfonic acid (ABTS))	60	4.5	—	Oxidation of micropollutants such as BPA, DFC, and MFA	[8]
3	<i>Geobacillus thermocatenulatus</i> (cell-free extracts laccase)	ABTS	60	4.5	42.5 65	Decolorization of textile dyes, especially Congo red and bromophenol blue	[9]
4	<i>Bacillus safensis</i> (cell-free extracts laccase)	—	37	6.2	—	Decolorization of commercially available dyes	[10]
5	<i>A. gonensis</i> (cell-free extracts laccase)*	ABTS	60	5.0	160	Bioremediation of wastewater	[11]
6	<i>Rhodococcus rhodochrous</i> (cell-free extracts P450)*	Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	30	7.2	—	Degradation of RDX	[12]
7	<i>Bacillus megaterium</i> (purified P450)	1-Monochloro-dibenzo-p-dioxin (1-MCDD), 2,3-dichlorodibenzo-p-dioxin (2,3-DCCD), 2,3,7-trichloro-dibenzo-p-dioxin (2,3,7-triCDD)	30	7.5	120	Hydroxylation of PCCDs	[13]
8	<i>Mycobacterium</i> sp. (cell-free extracts P450)*	—	30	6.6	—	Biodegradation of morpholine	[14]
9	<i>Bacillus</i> sp; <i>Geobacillus</i> (purified amylase)	Diethylethanolamine (DEAE)	90	8.0	—	Starch liquefaction	[15]
10	<i>Bacillus subtilis</i> (cell-free extracts lipase)	Olive oil	45	7.0	—	Bioremediation of wastewater	[16]
11	<i>Bacillus pumilus</i> (cell-free extracts lipase)	Palm oil	50	7.0	—	Degradation of palm oil containing industrial wastewater	[17]
12	<i>Chromobacterium viscosum</i> (cell-free extracts lipase)*	—	37	7.0	—	Degradation of PBSA, PCL, PBS	[18]
13	<i>Bacillus subtilis</i> (cell-free extracts lipase)	Olive oil	37	8.0	—	Removal of tough oil or grease stains from detergent	[19]
14	<i>Sphingobacterium</i> sp. strain S2 (cell-free extracts lipase)	Chromogenic PNPL	37	7.0	40	Degradation of PLA	[20]
15	<i>Pseudomonas putida</i> (cell-free extracts dehydrogenase)*	4-Hydroxybenzaldehyde and 4-hydroxy-3-methylbenzaldehyde	30	8	—	Catabolism of 2,4-xylenol	[21]
16	<i>S. rhizophila</i> (cell-free extracts dehydrogenase)*	Polyvinyl alcohol and vinyl alcohol oligomer	30	7.2	—	Polyvinyl alcohol degradation	[22]
17	<i>Bacillus subtilis</i> (cell-free extracts serine protease)	Feather culture medium	25	5–8	25.4	Degradation of casein and feather	[23]
18	<i>Chryseobacterium</i> sp. strain kr6 (cell-free extracts protease)	Chicken feathers	30	8	—	Complete degradation of feathers	[24]
19	<i>Bacillus pumilus</i> keratinase (cell-free extracts protease)	Chicken feathers	37	8	—	Complete degradation of feathers	[25]

TABLE 1: Continued.

S. no.	Microorganisms and enzymes	Substrate	Optimum temperature (°C)	Optimum pH	Mol. wt (kDa)	Application	Reference
20	<i>Streptomyces thermoviolaceus</i> (cell-free extracts protease)*	Muscle, collagen, hair, nail, feathers	55	8	40	Hydrolyze fibrin, muscle, collagen, nail, and hair	[26]
21	<i>Thermoanaerobacter keratinophilus</i> (purified extracellular protease)	Anaerobic complex medium with chicken feathers, merino wool, or human hair	85	8	135	Degradation of keratin fibers	[27]
22	<i>B. subtilis</i> (purified protease)*	Casein	50	8	44	Deproteinization of crustacean wastes	[28]
23	<i>Ancylobacter aquaticus</i> (purified dehalogenase)	Monochloroacetate	37	9	25	Degradation of halogen acid ester	[29]
24	<i>Bacillus</i> sp. (cell-free extracts dehalogenase)	2,4,6-Trinitrobromophenol (TBP)	37	7.5	—	Degradation of TBP	[30]
25	<i>Ochrobactrum</i> (purified dehalogenase)*	TBBPA	35		117	Degradation of TBBPA	[31]
26	<i>Pseudomonas</i> sp. TL (purified dehalogenase)	2-Chloropropionate	65	9.5	28	Degradation of halogen acid	[32]

Note: *denotes incubation temperature and pH in enzyme-catalyzed reactions are not optimum.

TABLE 2: Microbial enzymes involved in bioremediation and their function.

S. no.	Enzymes	Mechanism	Function	Reference
1	Cytochrome P450	Performs electron transfer reactions and catalysis by reduction or oxidation of heme iron. Utilizes pyridine nucleotides as electron donors producing carbon substrates and oxidized products. $\text{NAD(P)H} + \text{O}_2 + \text{R} \longrightarrow \text{NAD(P)}^+ + \text{RO} + \text{H}_2\text{O}$	Synthesis and metabolism of various molecules and chemicals within cells oxidize steroids, fatty acids, and xenobiotics	[37]
2	Laccase	Reduction of the O_2 molecule, including the oxidation of one electron with a wide range of aromatic compounds.	Ring cleavage in aromatic compounds and reduce one molecule of oxygen in the water and produce free radicals	[47]
3	Dehalogenase	Mainly occurred through three mechanisms: (1) Hydrolytic mechanism: water molecule serves as a cofactor; halogen substituent is replaced in S_N reaction by the hydroxyl group (2) Oxygenlytic mechanism: catalyzed by mono/dioxygenase incorporating one/two atoms of molecular oxygen into the substrate (3) Reductive mechanism: it is related to the carbamide family; in this course, halogen is substituted by hydrogen under aerobic conditions, where organohalides are used as the terminal electron acceptors	Cleaves the carbon-halogen bond and eliminates the halogens	[48, 49]
4	Dehydrogenase	Catalyze the reactions with coenzymes such as NAD^+ / NADP^+ or flavin such as FAD and FMN as an electron acceptor. It transfers two hydrogen atoms from organic compounds to electron acceptors.	Oxidizing organic compounds and generating energy	[5, 50]
5	Hydrolase	In triglyceride hydrolysis, one-mole triglyceride (T) reacts with three moles of water (W) to produce one-mole glycerol (G), and three-mole fatty acids (P) peptide bond of protein is broken down by hydrolyzing.	Degradation of fats and proteins	[3]
6	Protease	Catalyze the breakdown of peptide bonds of proteins	Degradation of proteins like keratin, casein, etc., leather dehairing, and wastewater treatment	[51]
7	Lipase	The transfer of a proton between the aspartate, the histidine, and the serine residues of the lipase followed by hydroxyl residue of the serine attacks the carbonyl group of the substrate. In the deacylation step, nucleophile attacks the enzyme regenerating the enzyme and releasing the product.	Catalyzes the hydrolysis of mono-, di-, and triglycerides into fatty acids and glycerol. Also, catalyze the esterification and transesterification reactions.	[52]

TABLE 3: Microbial enzyme produced by recombinant DNA technology for enhanced bioremediation.

S.no.	Recombinant enzyme	Bacteria host	Origin	Expression vector	Gene size/ enzyme	Application	Reference
1	Laccase CotA	<i>E. coli</i> DH5	<i>B. subtilis</i>	pMD18-T	1542 bp	Degradation of synthetic dyes	[53]
2	Laccase CueO	<i>P. pastoris</i> GS115	<i>E. coli</i> K12	pHBM905BDM	1470 bp/ 55 kDa	Decolorization of wastewater from the textile printing factory	[54]
3	Laccase (Fmb-rL103)	<i>E. coli</i> BL21 (DE3)	<i>B. vallismortis</i> fmb-103	pMD19-T-lac103	1542 bp/ 70 kDa	Degradation of MG (a triphenylmethane dye)	[55]
4	CYP105D1	<i>Acinetobacter calcoaceticus</i>	<i>Streptomyces griseus</i>	pSP19g10L	1500 bp	Degradation of pollutant herbicides	[40]
5	CYP153A6	<i>Pseudomonas putida</i> GPo12	<i>Mycobacterium</i> sp. HXN-1500	pGEc47B	—	Hydroxylation of alkanes to 1-alkanols	[56]
6	CYP153	<i>E. coli</i>	<i>Acinetobacter</i> sp. EB104	pUC18	1494 bp/ 56 kDa	Hydroxylation of unsubstituted alkanes	[57]
7	CYP154H1	<i>E. coli</i> C43 (DE3)	<i>Thermobifida fusca</i>	pIT2-MCS	NR/ 48kDa	Hydroxylation of small aliphatic and aromatic compounds to alcohol and epoxide	[58]
8.	CYP151A2	<i>E. coli</i> SG13009	<i>Mycobacterium</i> sp. strain RP1	pQE30	3900 bp/ 45 kDa	Degradation of secondary amines by ring cleavage	[59]
9	Dehydrogenase	<i>E. coli</i>	<i>Pseudomonas</i> sp. Strain WBC-3	pET29b	38 kDa	Catabolism of para-nitro phenol (PNP) to Krebs cycle intermediates	[60]
10	Aldehyde dehydrogenase	<i>E. coli</i> ER2566	<i>Bacillus cereus</i>	pRSFDuet-1	54 kDa	Regulate harmful metabolic intermediate aldehydes	[61]
11	Dehydrogenase	<i>E. coli</i>	<i>Pseudomonas putida</i> S12	pET28	30 kDa	Catalyzes the NAD ⁺ -dependent oxidation of phenylacetaldehyde to phenylacetic acid in the styrene catabolic and detoxification pathway	[62]
12	Dehydrogenase	<i>E. coli</i>	<i>Azoarcus evansii</i> KB740	pMal-c2x	54 kDa	Oxidizes ring cleavage product 3,4-dehydroadipyl-CoA semialdehyde	[63]
13	Dehydrogenase	<i>E. coli</i>	<i>Rhodococcus</i> sp. P14	pET-32a	45 kDa	Bioremediation of steroids	[64]
14	Dehalogenase	<i>E. coli</i> BL21 (DE3)	<i>Ochrobactrum</i> sp. T.	pET30a-a6	117 kDa	Degradation of TBBPA	[65]
15	Dehydrogenase	<i>E. coli</i> BL21	<i>Pseudomonas umsongensis</i> YCIT1612	pET28a(-)	729 bp/ 25.6 kDa	Epoxide assimilating opening reaction	[66]
16	Dehydrogenase	<i>E. coli</i> BL21	<i>Bacillus</i> sp. GZT	pET30a(+)	565 bp/ 63.4 kDa	Degradation of TBP (tribromophenol)	[67]
17	Dehydrogenase	<i>E. coli</i> K-12 NM522	<i>Rhizobium</i> sp.	pUC19	6500 bp	Degradation of halo alkanoate	[68]
18	Hydrolase CPD	<i>E. coli</i> BL21 (DE3)	<i>Paracoccus</i> sp. TRP	pET-32a (+)	51.7 kDa	Degradation of chlorpyrifos	[69]
19	Hydrolase OPH	<i>E. coli</i> DH5 α	<i>Pseudomonas diminuta</i>	pQE30	35kDa	Bioremediation of organophosphorus pesticides	[70]
20	Opd A Opd E	<i>E. coli</i> BL21	<i>Leuconostoc mesenteroides</i> WCP307	pET32a(+)	930 bp/ 35 kDa 894 bp/ 33 kDa	Biodegradation of organophosphorus insecticides	[71]
21	6-OCH-CoA hydrolase	<i>E. coli</i>	<i>Geobacter metallireducens</i>	pCR [®] T7/CT-TOPO [®]	41.9 kDa	Degradation of aromatic compounds	[72]

compounds having various functional groups by the formation of two water molecules with concomitant electron loss of a single oxygen molecule [75] as well as nonphenolic compounds that are less soluble and more stable, which helps to control groundwater and underwater pollution [76].

The most attentive biochemical properties of laccases are their stability under various conditions of pH, temperature,

organic solvents, and salt concentrations [73]. Usually, laccase is a highly stable, industrially relevant thermostable enzyme, as seen in CotA from *B. subtilis* at 75°, whereas recombinant CotA from *E. coli* has a half-life of 2 hours [75]. Laccase can remove xenobiotic substances and produce polymeric products used for bioremediation processes. The major known pollutant, PAHs, are distributed evenly in a

natural environment, consisting of a benzene ring arranged linearly [77]; [78]. Such pollutants, along with their intermediate, are of great environmental concern due to their toxicity, persistence, mutagenicity, and carcinogenicity in nature, produced due to incomplete combustion of fossil fuels and various industrial wastes. Due to their low water solubility and poor degradation rate, these aromatic hydrocarbons are xenobiotic [79]. Laccase thereby converts PAHs to their quinone form and subsequently degrade further to carbon dioxide, whereas on employed with mediator 1-hydroxy benzene triazole (HBT), the most effective laccase mediator [80], it converts acenaphthylene to 1,2-acenaphthalenedione and 1,8-naphthalic acid [81]. Laccase can be used to remove and detoxify textile dyes and phenols produced by the textile industry [82]. The microbial laccase has also been reported for the degradation and decolorization of amino carbonyl complex containing distillery effluent, including the complex mixture of phenolic metals and detoxification of postmethanated distillery effluent and chlorolignin containing pulp paper mill waste. A study has shown that the recombinant CotA laccase from *E. coli* can decolorize simulated textile effluents (STE). Both purified and crude recombinant CotA laccase efficiently decolorized seven structurally different dyes. When STE was buffered at neutral pH, the decolorization rates of purified and crude CotA laccase were higher [83].

Similarly, Lu et al. reported the complete degradation of synthetic dyes such as carmine and reactive black within 1 hour by alkaline laccase produced from a recombinant strain of *Bacillus licheniformis*. The recombinant laccase was purified that decolorizes more than 93% of the tested dyes within 4 hours at pH 9.0 [84]. The recombinant laccase produced from *B. vallismortis* strain fmb103 shows its applications in wastewater bioremediation in aquaculture [55]. In contrast, the recombinant CueO from *E. coli* K12, expressed in *Pichia pastoris* was applied to decolorize wastewater from the textile printing industry [54]. This showed an ability of recombinant laccase in the bioremediation process, as shown in Table 3.

The current study has shown that two laccases were isolated from cell-free extracts of the soil bacterium *Pseudomonas putida* F6, of which one CopA degraded five out of seven tested dyes, namely, Amido Black 10B, Brom Cresol Purple, Evans Blue, Reactive Black 5, and Remazol Brilliant Blue [85]. The purified laccase from *Bacillus* sp. converts 92% bisphenol A (BPA) into 4-ethyl-2-methoxy phenol as an end product [86]. Similarly, A laccase, purified Lac15 from a marine microbial metagenome, showed decolorization of 50 mM reactive azo dyes, reactive brilliant orange K-7R, and reactive deep blue M-2GE under alkaline conditions within 1 hour [53]. The biobleaching of eucalyptus kraft pulps using ABTS and HOBT as mediators have been evaluated from microbial laccases obtained from *Streptomyces cyaneus* CECT 3335 [87] and *Pseudomonas stutzeri* [88]. Hence, laccase holds tremendous potential for the economic treatment of wastewater containing phenolic as well as nonphenolic compounds, PHAs, synthetic dyes, and various emerging pollutants. The list of bacteria producing microbial laccase along with its application is mentioned in Table 1.

2.3. Dehalogenase. Microbial dehalogenase (EC 3.8.1.5) has attracted a great deal of attention because of its significant application in halogenated organic compounds' bioremediation. A wide range of halogenated compounds is degraded using dehalogenase enzyme, which cleaves C-X bonds [89, 90] through three mechanisms, including hydrolytic, reductive, and oxygenolytic ones, which could perform dehalogenation by the replacement of the halogen atom by hydroxyl group from water and a hydrogen atom from H₂, respectively [48].

Zu et al. have isolated a pure strain of *Bacillus* sp. GZT from the sludge of waste recycling site which has an excellent capacity to simultaneously debrominate and mineralize the TBP. The debromination step has occurred in two pathways, of which reductive bromination is the major path and methyl bromination is the minor one. The reductive debromination proceeds with the debromination at both ortho and para positions to give 2,4-DBP and 2,6-DBP, respectively. The mineralization product is CO₂, and the highest mineralization efficiency of 29.3% was observed from 3 mg/L TBP solution at 148 hours [30]. Liang et al. have grown the same species under favorable conditions and then lysed the cells and obtained three fractions: extracellular enzyme, intracellular crude extract, and membrane protein. These three fractions were assayed for dehalogenase activity, of which only intracellular crude extract displayed the debromination activity confirming that the dehalogenase is an intracellular enzyme. The open reading frame, namely, ORF05005, coding peptide ABC transporter substrate-binding protein, was predicted to be involved in the degradation pathway of TBP. The TBP dehalogenase gene sequence from *Bacillus* sp. GZT was transformed into competent *E. coli* BL21 (DE3) to confirm TBP dehalogenase activity. The purified enzyme has degraded TBP within 120 minutes at 35°C at 200 rpm, where 80% efficiency was achieved. H₂O₂, NADPH, Mn²⁺, and Mg²⁺ enhanced the dehalogenase activities while EDTA, methyl viologen, Ni²⁺, Cu²⁺, Ca²⁺, and Fe²⁺ were strongly inhibited [31]. Similarly, the novel bacterial strain *Ochrobactrum* sp. when cloned with *tbbpaA* produced recombinant strain *E. coli* BL21 (DE3). Thus, purified dehalogenase enzyme from recombinant strain exhibited high TBBPA degrading ability (78%) [31]. The gene of *Rhizobium* sp. encoding haloalkane dehydrogenase [68] and haloalcohol dehydrogenase (HHDH) producing strain *Pseudomonas umsongensis* YCIT1612 [66], both expressed in *E. coli*, also help in bioremediation by process of degradation of haloalkane and performing epoxide assimilating reaction, respectively, as shown in Table 3.

Many other bacterial species such as *Pseudomonas* sp. [32], *Ancylobacter aquaticus* strain UV5 [29], *Pseudomonas umsongensis* YCIT1612 [66], and *Rhizobium* sp. [68] released enzymes capable of degrading various halogenated substrates. Fricker et al. used *Dehalococcoides mccartyi* strain JNA in pure culture to dechlorinate highly toxic ubiquitous environmental pollutants pentachlorophenol, and other chlorinated phenols into 3, 5-dichlorophenol (DCP) in three possible ways [91]. Similarly, Nelson et al. used the enrichment culture of three *Dehalobacter* sp. strains (12DCB1,

13DCB1, and 14DCB1) to dehalogenate chlorobenzenes, dichlorotoluenes, and tetrachloroethene, the common environmental pollutants, of which strain 12DCB1 preferentially dehalogenated singly flanked chlorines. In contrast, 13DCB1 dehalogenated the recalcitrant 1, 3, 5-trichlorobenzene to monochlorobenzene (MCB), and both these strains (pure cultures of *Dehalobacter* sp.) dehalogenated polychloroethene to cis-dichlorethene. Strain 14DCB1 was the only strain to dehalogenate para-substituted chlorines, and all strains dehalogenate 3, 4-DCT to mono chlorotoluene. These results showed that *Dehalobacter* sp. are versatile dehalogenators [92]. Zhang et al. reported 2-haloacid dehalogenases (EC 3.8.1.2) from the marine bacterium *Pseudomonas stutzeri* DEH130; dehalogenase 1 and dehalogenase 2. The dehalogenase activity assay was carried out for both enzymes, and dehalogenase 2 was preferentially active towards substrate L-2-CPA while they could not purify dehalogenase properly. Dehalogenase 2 was more stereospecific towards halogenated substrate than dehalogenase 1 [93]. Boyer et al. isolated 2,4,6-trichlorophenol reductive dehalogenase from *Desulfitobacterium frappieri* PCP-1, which dechlorinated pentachlorophenol (PCP) into 3-chlorophenol at the ortho, meta, and para positions. These halogenated compounds, which are toxic to the environment, are transformed by reductive dehalogenation to less toxic and readily biodegradable forms [94].

2.4. Dehydrogenase. Dehydrogenases belong to the family of oxidoreductase and are found in organisms ranging from bacteria, yeast, plants to animals, including humans. The microbial alcohol dehydrogenase (ADH; EC1.1.1.1) catalyzes the reversible conversion of alcohol to aldehyde or ketone and can be categorized as (a) NAD⁺ or NAD(P)⁺-dependent dehydrogenases and (b) NAD⁺ or NAD(P)⁺-independent enzymes that use pyrroloquinoline quinone, heme, or cofactor F420 as a cofactor [95, 96]. Similarly, aldehyde dehydrogenase (ALDHs, EC 1.2.1.3) catalyzes the NAD(P)⁺-dependent oxidation of the aldehyde to a carboxylic acid [97].

Polyethylene glycol dehydrogenase activity was observed in the cell-free extracts from the bacteria degrading, industrially produced xenobiotic, polyethylene glycol of various molecular weights [98]. Several *Sphingomonas* sp. use polyethylene glycol as a source of energy and carbon by oxidizing terminal alcohol groups of the polymer chain; both crude and purified enzymes oxidize the corresponding aldehyde though the process is slow [99]. The NAD⁺-dependent polypropylene glycol dehydrogenase (PPG-DH) from *Stenotrophomonas maltophilia* preferentially oxidizes hydrophobic polymermedium-chain secondary alcohols, di- and tri-propylene glycols, and polypropylene glycols, including those with secondary alcohol groups in their molecular structure [100]. Similarly, in *Stenotrophomonas maltophilia*, dye-linked PPG dehydrogenase located in periplasm or membrane is active in degrading high-molecular-weight PPG; however, the enzyme present in the cytoplasm is active in metabolizing low-molecular-weight

PPG [101]. Cell-free extracts of *Sphingobium* sp. strain PW-1 grown on 0.5% PPG show that the PPG dehydrogenase activity confirms the PPG's oxidative metabolic pathway [102]. Moreover, recombinant polyvinyl alcohol dehydrogenase degrades water-soluble xenobiotic polyvinyl alcohol, which is found to oxidize glycols such as polypropylene glycols and 1,3-butane/cyclohexanediol and 2,4-pentane-diol, but neither primary nor secondary alcohols [103].

Aldehyde dehydrogenase is found to be active in the metabolism of aromatic compounds. The gene (*orf9*) from *Azoarcus evansii* responsible for producing 3,4-dehydroadipyl-CoA semialdehyde dehydrogenase, which is used in oxidation 3,4-dehydroadipyl-CoA semialdehyde, has been expressed effectively [63]. *Rhodococcus* sp. NCIMB12038 utilizes naphthalene as a sole source of carbon; heterologous expression of the NCIMB12038 *cis*-naphthalene dihydrodiol dehydrogenase demonstrated 39% amino acids identity with the *cis*-naphthalene dihydrodiol dehydrogenase from *Pseudomonas putida* G7 [104]. The enzyme NahB (*cis*-dihydrodiol naphthalene dehydrogenase), which catalyzes the second reaction of naphthalene degradation pathway, binds to various substrates such as *cis*-1,2-dihydro-1,2-dihydroxy-naphthalene (1,2-DDN), *cis*-2,3-dihydro-2,3-dihydroxybiphenyl (2,3-DDB), and 3,4-dihydro-3,4-dihydroxy-2,2',5,5'-tetrachlorobiphenyl (3,4-DD-2,2',5,5'-TCB) [105]; flexible substrate binding loops permit NahB to fit in with various substrates. The aldehyde dehydrogenase (NidD) is found to catalyze degradation of 1-hydroxy-2-naphthaldehyde to 1-hydroxy-2-naphthoic found in the analysis of the metabolic intermediate [106]. Analysis of protein expressed in the biodegradation of phenanthrene by *Amycolatopsis tucumanensis* DSM 45259 shows that aldehyde dehydrogenase is expressed abundantly and uniquely [107]. Ethylbenzene dehydrogenase (EBDH), an enantioselective enzyme from the denitrifying bacterium *Azoarcus* sp. strain EbN1 (to be renamed *Aromatoleum aromaticum*) catalyzes the oxygen-independent, stereospecific hydroxylation of ethylbenzene to 1-(S)-phenylethanol, the direct anaerobic oxidation of a non-activated hydrocarbon, which helps in the biomineralization of ethylbenzene which is a product from crude oil [108, 109]. The fate of the ring cleavage product 3,4-dehydroadipyl-CoA semialdehyde was studied in the β -proteobacterium *Azoarcus evansii*. Cell extracts contained a benzoate-induced, NADP⁺-specific aldehyde dehydrogenase, which oxidized this intermediate [63]. The list of a few dehydrogenases with their properties and their role in bioremediation is given in Table 1, along with a genetically engineered bacteria enlisted with their role in bioremediation in Table 3.

2.5. Hydrolase. Hydrolases break a chemical bond utilizing water and convert larger molecules to smaller ones to reduce pollutants' toxicity. They facilitate the cleavage of C-C, C-O, C-N, S-S, S-N, S-P, C-P, and other bonds by water and catalyze several related reactions, including condensations and alcoholics. This enzyme class's main advantages are ready availability, economical, eco-friendly, lack of cofactor stereoselectivity, and tolerance of the addition of water-miscible solvents [3].

Microbial hydrolytic enzymes (lipases, esterases, amylases, proteases, cellulases, nitrilases, peroxidases, cutinase, etc.) can be pressed into service for the management of waste produced during food processing, degradation of plastics and insecticides, treatment of biofilm deposits, and oil-contaminated soils, etc. Hydrolytic enzymes possess quite diverse potential usages in different areas such as feed additive, biomedical sciences, and chemical industries [110]. The breakdown of ester, amide, and peptide bonds by esterases, amidases, and protease may lead to products with little or no toxicity. Microbial hydrolase such as carbamate or parathion hydrolase from *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Nocardia*, and *Bacillus cereus* has been successfully used in the conversion of pollutants such as carbofuran, carbaryl or parathion, diazinon, and coumaphos by the process of hydrolysis [111].

Organophosphate (OP) compounds are highly lethal neurotoxins. They have widely used pesticides in agriculture, representing a threat in the biotic environment where pyrethroids and malathion are unsafe for living environments. Organophosphate pesticides can be detoxified by the hydrolysis of phosphodiester bonds, whereas pyrethroids and malathion by the hydrolysis of carboxyl ester bonds. The OP pesticides usually consist of phosphorus either as a phosphate ester or a phosphonate, and being ester, the principal reactions involved are hydrolysis, oxidation, alkylation, and dealkylation. Thus, microbial degradation through hydrolysis of P-O-alkyl and P-O-aryl bond is considered as the most significant step in detoxification [112]. A high level of OP contamination was found in grounds, drinking water, grains, and fruit, leading to OP poisoning [70]. *Alicyclobacillus tengchogenesis*, *Brevibacillus* sp., *Bacillus licheniformis*, and *Bacillus cereus* are known microorganisms for the degradation of malathion [113]. For many bacteria like *B. licheniformis*, malathion was observed to be a carbon source, and hence, hydrolytic enzymes of *B. licheniformis* help in the bioremediation of malathion-containing soil [114]. Therefore, microbial enzymes OP hydrolases, OP acid anhydrolases, or methyl parathion hydrolases (MPH) have been used as potent agents for OP decontamination, thereby gaining attention as a clean bioremediation strategy [115]. Similarly, the gene for organophosphorus hydrolase (GenBank accession no. M20392) from *Pseudomonas diminuta* expressed in *E. coli* showed its detoxification ability and methyl parathion ranging between 10–80% and 3.6–45% [70]. In another study, Su et al. have shown that the OP hydrolase enzyme was tethered onto outer membrane vesicles (OMVs) of Gram-negative bacteria, resulting in high activity degradation of parathion chemical compounds [116]. Similarly, the chlorinated herbicides (s-triazine), used as pesticides in agriculture, are carcinogenic and cause several disorders to human health. Amidohydrolase of bacteria coded by *atZ* genes and product of these genes degrade the s-triazine to carbon dioxide and ammonia through dechlorination, deamination, and degradation of cyanuric acid. *Pseudomonas* sp. ADP is a model organism involved in the degradation of s-triazine as it degrades the s-triazine ring of chlorinated herbicides [117].

Cyanide-containing compounds are highly toxic and deadly poisonous; there are two different enzymatic pathways known for the degradation of nitrile compounds; one is a two-step degradation involving nitrile hydratase and amidase via an amide as an intermediate, whereas the other is the direct hydrolysis of nitriles to the corresponding acids and ammonia, catalyzed by nitrilases (EC 3.5.5.1). Such enzyme-producing bacteria are *Nocardia* sp. and *Rhodococcus* sp. [118].

Phthalates are frequently used as a plasticizer and are ubiquitous environmental pollutants. Phthalate esters (PEs) and short-chain (C1–C4) alkyl esters of phthalates are a category of toxic organic compounds that are widely used as additives or plasticizers (softeners) in the manufacture of plastics. These have become great environmental concerns globally because of their suspected carcinogenic, estrogenic, and endocrine-disrupting properties. Dialkyl PEs hydrolases catalyze the microbial degradation of PEs from *Acinetobacter* sp.M673 and *Micrococcus* sp.YGJ1 to form monoalkyl PEs [119]. Cutinase is a biocatalyst isolated from bacteria *Fusarium solani* f. *pisi* used for the degradation of plastics and polycaprolactone [120]. Dang et al. isolated a thermophilic bacteria *Bacillus* sp. BCBT21 from composting agricultural residual, which secretes hydrolases including chitinase, CMCase, protease, xylanase, and lipase, acts in high temperature, and plays a pivotal role to degrade biodegradable and oxo-biodegradable plastics from various resources [121]. Singh et al. isolated three bacterial strains *Pseudomonas* sp. PKDM2, *Pseudomonas* sp. PKDE1, and *Pseudomonas* sp. PKDE2, which is capable of degrading di-(2-ethylhexyl phthalate) (DEHP) into phthalic acid mediated by mono-2-hexyl ethyl phthalate (MEHP) hydrolase via reduction [122]. Poly(ethylene terephthalate) (PET) is commonly used in plastic goods around the world, and its concentration in the atmosphere has become a problem [123]. A recent study has shown the potential of polyester hydrolases on the enzymatic degradation of synthetic polyesters obtained from thermophilic actinomycetes (*Thermobifida fusca* KW3) at a high rate as the comparison to other hydrolases such as lipase [124]. Hydrolase may further be classified according to the type of bond hydrolyzed. The extracellular hydrolytic enzymes such as amylases, lipases, proteases, etc. have diverse potential in bioremediation. The two enzymes, protease and lipase, have been described below.

2.5.1. Protease. Protease (E.C 3.4.21.12) belongs to the hydrolase family of enzymes that catalyze proteins' peptide bonds. They can also be isolated from microorganisms such as *Bacillus* sp., *Aspergillus* sp., and *Amycolatopsis* sp. Microbial proteases are of immense importance because of their low cost, high production, and efficient activity. They are widely used in industries such as the leather industry, the food industry, and wastewater treatment [110]. Microbial protease accounts for two-thirds of commercial protease production, and these enzymes are extensively used and account for more than 60% sale of total enzyme sales. Protease is capable of degrading α -ester bonds,

poly(hydroxybutyrate) (PHB) depolymerase β -ester bonds, and lipase γ - ω bonds and thus can be used in bioremediation for the degradation of polymers [125].

Shedding and molting of appendages, death of animals, poultry wastes, horns, and nails of animals are resistant to degradation due to the presence of insoluble keratin protein. They are responsible for causing environmental pollution along with their foul-smelling. The protease enzyme, keratinase (E.C. 3.4.21/24/99.11), can degrade keratin proteins and apply in poultry wastes' bioremediation by degrading and recycling keratinous wastes into useful byproducts. The protease enzyme, keratinase produced from *Stenotrophomonas maltophilia* KB13, has shown significant activity on the biodegradation of chicken feathers [126]. The keratinase enzyme produced by *Bacillus* sp. FPF-1 (accession number MG214993) showed a degradation rate of $82.0 \pm 1.41\%$ of chicken feathers showing its potential to valorize recalcitrant keratinous waste biomass from the agro sector [127]. The cooperation of two enzymes effectively carried out the keratin degradation: serine protease and disulfide reductase from the bacterium *Stenotrophomonas* sp., increasing the keratinolytic activity 50-folds compared to that of protease only. The disulfide reductase catalyzes the breakdown of disulfide bonds of keratin protein [128].

Similarly, the keratinase enzymes produced from *Bacillus* sp. and *Pseudomonas* sp. have shown significant hydrolysis of keratinous wastes from poultry [129]. The products released from the degradation of feathers are rich in amino acids and nutrients and thus can be used as feed additives, fertilizers for plant growth [130], free radical scavengers, ferric ion reducing agent [131], and toxic hexavalent chromium reducing agent [126]. Keratinase is used in enzymatic environment-friendly dehairing processes in the leather industry to replace the traditional chemicals CaO and Na₂S, which prevent toxic waste release in water bodies to avoid pollution [132].

The protease enzymes are also used in the bioremediation of marine crustacean wastes used in chitin extraction after deproteinization. The alkaline protease produced by *Bacillus licheniformis* MP1 leads to 75% deproteinization of shrimp wastes [133]. The microorganism *S. marcescens* FS-3 deproteinized up to 84% of the crab shell proteins on 7-day fermentation [134], and *P. aeruginosa* K-187 removes 72% of protein from shrimp and crab shell powder (SCSP) while it removes 78% of protein from natural shrimp shells (NSS) and 45% from acid-treated SCSP [135]. The chitinase enzyme BsChi, produced by *Bacillus subtilis* expressed in *E. coli*, degraded crystalline chitin effectively to N-acetyl-D-glucosamine which can be considered as an environment-friendly strategy for the degradation of crab shell wastes [89]. The commercial neutral protease enzyme from *Bacillus subtilis* degraded chitosan by its chitosanolytic activity to low-molecular-weight chitosan and chitosan oligosaccharides by endo action [136] that can be used in the valorization of marine crustacean wastes [137].

Pseudomonas fluorescens degrade 3 g/L of Impranil, water-dispersible polyurethane within 4-5 days by protease activity of enzyme exhibiting beta action. The crude enzyme was purified as protein and reported as protease, active at

optimum pH 5.0 [138]. Similarly, *Pseudomonas chlororaphis* hydrolyzed the Impranil substrate displaying protease activity (beta clearing zone) along with esterase activity (alpha clearing zone) at an ambient temperature of 30°C and prominent pH of 7 and 8 [139]. Proteases reduce environmental pollution by degrading and converting the keratinous wastes and marine crustacean wastes into useful products. Similarly, the use of toxic chemicals has been replaced by the enzyme's action, reducing its release into the environment. Thus, protease has a significant role in the bioremediation of a polluted environment. The list of microbial proteases with their properties and role in bioremediation is given in Table 1, along with genetically engineered bacteria producing protease enlisted with their role in bioremediation in Table 3.

2.5.2. Lipase. Lipases (EC 3.1.1.3) belong to the serine hydrolases and are a well-known biocatalyst for the hydrolysis of the ester bond of triglycerides into fatty acids and glycerol [52]. Lipase degrades lipids derived from various microorganisms, animals, and plants, thereby reducing hydrocarbon from contaminated soil. Lipolytic reactions occur at the lipid-water interface, where lipolytic substrates usually form an equilibrium between monomeric, micellar, and emulsified states [3]. Microbial lipase has full commercial application in the bioremediation of oil residues, petroleum contaminants, effluents, and soil recovery [140] as well as applicable in various industries, including therapeutic, polymerization, pulp and paper, and cosmetic industries due to their low energy requirement, high substrate specificity, maximum stability, lesser processing time, and not costing industrial production using various available raw materials [141, 142].

Lipases can enhance the bioremediation of greasy effluents containing oils, fats, and protein discharged from various areas [143]. Lipase from *Acinetobacter* sp., *Mycobacterium* sp., and *Rhodococcus* sp. has been used to suppress oil spills (e.g., n-alkanes, aromatic hydrocarbons, and PAHs) [52]. On the other hand, the lipase from *Pseudomonas* sp. has been used in the bioremediation of soil contaminated with industrial waste oil, and the degradation of castor oil has been reported from the lipase produced from *Pseudomonas aeruginosa* [144]. In addition to this, *P. aeruginosa* is also confirmed in the bioremediation of wastewater contaminated with crude oil by a gradual decrease of oil concentrations and reduction of approximately 80% of waste toxicity over a week [145]. The mineral oil hydrocarbon derived from petroleum products causing soil pollution is one of the major environmental problems. The hydrocarbon, the main soil contaminant, can be degraded with the help of lipase, produced by bacterial isolates from the soil, being contaminated by automobile engine oil [146]. Lipases are also used in household laundry to reduce environmental pollution and enhance detergent's ability to remove tough oil or grease stains. The crude lipase from *Bacillus subtilis* strain is utilized in detergent industries to minimize phosphate-based chemicals in detergent formulations [19].

A study found that when methanol or ethanol is presented as the external carbon in the activated sludge, the transesterification process by Amano lipase from *Pseudomonas* is essential for the degradation of para hydroxybenzoic acid esters (parabens). These results demonstrated that transesterification might be an important pathway for the degradation of parabens pollutants in engineering aquatic environments [89]. Further, a study on densitometry-based microassay suggested that polyhydroxyalkanoate (PHA), a substitute for synthetic plastic, degrades the activity of lipases derived from the bacteria *Chromobacterium viscosum* [145] that offers the solution for the emerging environmental problem caused by a lack of degradability in conventional plastics. On increasing concern about environmental pollution, biodegradable polymers have been promoted as one of the approaches to solve this issue. Lipase PL isolated from *Alcaligenes* sp. catalyze the hydrolysis reaction from poly(L-lactide) (PLA) polymers to oligomers and finally to the monomers [18]. On the other hand, the copolymers (PCL/PLA) prepared by ring-opening polymerization of 3-caprolactone and DL-lactide were successfully degraded using *Pseudomonas* lipase into various soluble degradation products, including LA homo-oligomers and CL₂LA₁ co-oligomer, which suggests that *Pseudomonas* lipase can degrade not only PCL but also LA short blocks along PCL/PLA copolymer chains [146].

Similarly, the degradation of synthetic polyester, polycaprolactone (PCL), by lipases derived from *Lactobacillus brevis*, *Lactobacillus plantarum*, and their coculture revealed that the *L. plantarum* lipase exhibited the maximum PCL degradation efficiency as compared to others [147]. Hence, microbial lipase shows its significance in the bioremediation process by biodegrading polymers such as PCL and PLA. The list of a few microbial lipases with their properties and role in bioremediation is given in Table 1.

3. Conclusions

Emerging pollutants in nature are explicit and linked to the various chronic detriments in biotic components and subsequently deteriorate the ecosystem as exposure even in small concentration and large spaces of time. Specific pollutant substrate is recognized with specific microbial enzymes at optimum condition (temperature/pH/contact time/concentration) so it efficiently transforms into different innocuous products enzymatically by various enzymatic reaction mechanisms including oxidation, reduction, elimination, and ring-opening. Similarly, introducing the bioengineered microorganisms in today's research field enhances the degradation of pollutants from the recalcitrant environment more effectively and efficiently.

High-molecular-weight PAHs and polyhalogenated aromatics interact with cytochrome P450 enzyme active site and transform into innocuous products by oxidation. Low substrate specificity and higher thermostability halotolerant capacity of laccases transformed various classes of pollutant substrates such as antibiotics, synthetic dyes, PAHs, and phenolic pollutants via the oxidation process. Dehalogenase

enzymes exhibit carbon-halogen bond cleavage activity, either hydrolytic or reductive or oxygenolytic; the elimination of halides effectively transforms halogenated pollutants substrate to reduce chlorinated environment. The enzyme dehydrogenase preferentially oxidized medium-chain secondary alcohols and synthetic polymers containing various hydroxyl groups in their molecular structure into corresponding aldehydes. The protease enzyme degrades the recalcitrant keratinous waste biomass, marine crustacean wastes, dyes, protein polymers, and oxo-biodegradable plastics using the hydrolysis reaction mechanism. Microbial hydrolases act on additives or plasticizers, cyanides, and nitriles containing compounds and transform the pollutant into less harmful products by condensations and alcoholysis reactions. Substrate specificity and maximum stability of microbial lipase successfully degraded copolymers PCL, PLA, synthetic polyester, PHA, and parabens into various biodegradation products. Hence, this concludes that microbial enzymes in the biodegradation of toxic organic and inorganic pollutants in bioremediation are eco-friendly, safe, and economical for restoring the biological and physicochemical properties of degraded soil.

Abbreviations

1-MCDD:	1-Monochloro-dibenzo-p-dioxin
2,3-DCCD:	2,3-Dichlorodibenzo-p-dioxin
2,3,7-triCDD:	2,3,7-Trichloro-dibenzo-p-dioxin
ABTS:	2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)
HHDH:	Halohydrin dehydrogenase
BPA:	Bisphenol A
DCP:	3,5-Dichlorophenol
DFC:	Diclofenac
MFA:	Mefenamic acid
MPH:	Methyl parathion hydrolases
OMVs:	Outer membrane vesicles
PAHs:	Polycyclic aromatic hydrocarbons
PBS:	Polybutylene succinate
PBSA:	Polybutylene succinate-co-adipate
PCBs:	Polychlorinated biphenyls
PCCDS:	Polychlorinated di-benzo-p-dioxins
PCL:	Poly(ϵ -caprolactone)
PHA:	polyhydroxyalkanoate
PLA:	Poly(lactic acid)
PNPL:	P-Nitrophenyl laurate
POPs:	Persistent organic pollutants
SGZ:	Syringaldazine
STE:	Simulated textile effluents.

Data Availability

No data were used to support the findings of this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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