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REVIEW ARTICLE OPEN ACCESS

Biocatalysts for Change: Microbial Enzymes as a Sustainable Solution to Industrial Pollution

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Abstract

Industrialization and anthropogenic activity represent significant environmental hazards. Emerging pollutants in nature pose a major risk and are linked to some immediate and long-term negative effects on the ecosystem. Traditional methods of excluding pollution are futile and lead to the creation of secondary contaminants that cause diseases, cancer, mental and cardiovascular issues, allergies, and other conditions. Microbes and their enzymes are key players in reducing and removing hazardous contaminants through bioremediation by their catalytic action under ideal settings (temperature/pH/contact time/concentration). Laccases, dehalogenases, proteases, cytochrome P450s, dehydrogenases, and lipases are the primary enzymes used in bioremediation. These enzymes have demonstrated encouraging potential in the breakdown of dangerous pollutants. These enzymes use oxidation, elimination, reduction, and other numerous mechanisms to biodegrade various pollutants. Recombinant enzymes produced from genetically modified microorganisms also enhance the breakdown of pollutants. Recent developments and opportunities for microbial enzymes in the sustainable breakdown of hazardous pollutants such as dyes, polyaromatic hydrocarbons, plastics, heavy metals, pesticides, etc. in the environment due to industrial pollution are the major focus of this review.

Keywords: Bioremediation, Industrial Pollutants, Microbial Enzymes, Sustainable Solution

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INTRODUCTION

Uncontrolled release of pollutants and untreated effluents into the environment, environmental pollution is one of the biggest issues faced globally. Environmental contamination is mostly caused by population increase, industrialization, exploration, urbanization, and mining.[1] The survival of humanity is seriously threatened by the significant amount of toxins that have been dumped into the environment, ranging from untreated sewage to nuclear waste.[2] Anthropogenic activities including industrialization, farming methods, population growth, and unhealthy competition for dominance are having severely negative effects on Earth. Pollutants, mostly phenols, polyaromatic hydrocarbons, pesticides, azo dyes, polychlorinated chemicals, heavy metals, and other hazardous substances, are produced as a result of these processes. These chemicals endanger the biotic components of ecosystems because they are resistant to biodegradation.[3] These contaminants have a profound impact on every area of the planet, having carcinogenic, mutagenic, and toxic consequences on people and other living things.[4]

Clean water is essential for life, but it is often contaminated by industrial activities, leading to wastewater. This pollution, containing pesticides, pharmaceutical wastes, plastics, heavy metals, organic solvents, acids, alkali, biological wastes, and xenobiotic compounds, poses significant environmental and health risks. To address these issues, conventional methods like physical, chemical, and biological treatments are insufficient. Nanotechnology and nanofiltration are promising alternatives for industrial wastewater treatment due to their high surface area, eco-friendliness, and effectiveness in eliminating pollutants.[5]

Micropollutants (MPs) are a growing concern in wastewater due to human activities. Despite their low presence, MPs are highly toxic and non-degradable, causing global water quality and health hazards. Conventional wastewater treatment is inefficient and produces hazardous sludge. Alternative sustainable approaches are needed, and recent micro-biotechnological methods, such as bio-adsorption, membrane filtration, biocatalysts, bioreactors, and nanotechnology, have shown effectiveness in removing MPs. Bacteria, fungi, and microalgae can degrade MPs through biosorption, biotransformation, bioaccumulation, and bioconversion mechanisms. Microbial-based nanoparticles (MNPs) can be integrated into existing membrane or adsorption technologies, but systematic studies are still in its infancy.[6] Micropollutants, including pesticides, pharmaceuticals, and personal care products, have improved human quality of life but are increasingly contaminating aqueous systems due to seepage, run-off, effluent discharge, and environmental uptake. These pollutants have significant ecotoxicities and adverse effects on human health, making them a critical environmental concern. Current remediation methods are costly, consume excess chemicals, require high energy, and create harmful by-products. However, oxidative laccases and peroxidases, microalgae, and microalgae-bacteria consortia are emerging as green alternatives for remediating micropollutants in contaminated systems. These methods can be used as secondary treatment techniques for wastewater plant effluents or as tertiary treatments in conjunction with other chemical and biological technologies.[7] Pollutants have been cleaned up using a variety of physical and chemical techniques, including oxidising agents, electrochemical processes, pollutant adsorption, ion exchange, and membrane filtering. Traditional procedures were adequate for the high concentration of contaminants, but they were insufficient to reduce the pollution to allowable levels.[8] Traditional methods for cleaning up pollutants have several drawbacks, including their high cost, difficult procedures, stringent international regulations imposed on decontamination, general public rejection, non-specificity, space limitations, and potential for secondary pollution creation.[9] As a result, interest has grown in bioremediation, eco-friendly, and biological procedures. Plastic, introduced in the mid-20th century, has been crucial for modern civilization due to its convenience and cost-effectiveness. However, its extensive use has led to significant environmental issues, including waste management issues in landfills and natural ecosystems.[9] Microplastics, particularly those from polyethylene (PE), polyethylene terephthalate (PET), polyurethane (PU), polystyrene (PS),

polypropylene (PP), and polyvinyl chloride (PVC), have a profound negative impact on ecosystems and species health.[10] Enzyme technology is crucial in promoting environmental conservation by converting plastic waste into less harmful compounds. Research on *Thermobifida fusca* hydrolase has led to the discovery of various enzymes that can break down PET, with PETase enzyme from *Ideonella sakaiensis* being particularly noteworthy for its ability to effectively degrade PET into intermediates like BHET, MHET, and TPA. MHETase further processes MHET to produce terephthalic acid and ethylene glycol.[11] Significant progress has been achieved in the domain of enzymatic microplastic degradation, specifically regarding polystyrene (PS). Advancements in this domain are apparent, as numerous microbes, including bacteria and fungi, possess the capability to decompose PS. Nonetheless, the specific enzymes that initiate this breakdown process are not yet well comprehended.[12]

Microorganisms that break down many contaminants enzymatically and convert them into less harmful compounds or metabolites that could be beneficial products are the mainstay of the bioremediation process.[13] Reducing environmental pollution through the use of biological agents is an affordable process and reduces the risks they impose on people's health and threats to the environment. To break down and detoxify pollutants, it mostly employs intracellular accumulation or enzymatic transformation.[9] Microbial enzymes are regarded as innovative, economical, and promising when used in the bioremediation of persistent pollutants (Figure 1). Microbes are an abundant source of biocatalytic enzymes, essential in industries like textiles, agriculture, and pharmaceuticals. They reduce environmental pollution through biodegradation and bioremediation. The global enzyme market is growing due to their eco-friendliness, stability, and easy modification. Alternatives like recombinant DNA technology and protein engineering are used to create novel products.[14] Because of their metabolic activity and their ability to thrive in various environmental conditions, bacteria, fungi, etc. are present throughout the biosphere and produce enzymes. Among the various microbial enzymes, such as those from *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, *Rhodococcus, Mycobacterium* has already been shown to break down pesticides and hydrocarbons. Cytochrome P450s, dehydrogenases, laccases (Lac), proteases, hydrolases, lipases, and dehalogenases are the most prevalent microbial enzymes involved in bioremediation.[15] The bioremediation technique has some constraints. Only a few biological agents have the aptness to produce specialized enzymes with sufficient power to break down contaminants, and the process is exceedingly slow and constant. Genetically engineered microbes produce required enzymes in huge quantities under ideal conditions; we choose them for bioremediation. Industrial microbial enzymes currently have a very competitive global market. Worldwide recognized companies in the enzyme production sector are DSM, Novozymes, Amano Enzymes Inc., and DuPont. By employing enzymes, we can safeguard our planet against the accumulation of hazardous and poisonous waste in the environment.[16] Numerous novel microbial enzymes are been developed for various bioprocesses using rDNA technology, metagenomics, and protein engineering. To increase the production and efficacy of various microbial enzymes, a range of molecular approaches are used in the food, paper, pharmaceutical, biotechnological, textile, leather, and other industries.[17,18] The major focus of this review are the function and mechanism of microbial enzymes in the sustainable bioremediation of industrial pollutants. Additionally, there is a gap in suitable approaches considering the bioremediation of toxic multi-pollutants using enzymatic applications. Most of the research remains lab-scale, with few studies on pilot or field applications. Enzyme stability and activity under real environmental conditions (e.g., temperature fluctuations, mixed contaminants, pH changes) are underexplored. The mechanisms of enzymatic degradation for complex pollutants (e.g., microplastics, PFAS, pharmaceutical residues) are not fully understood. The mechanisms of enzymatic degradation for complex pollutants (e.g., microplastics, PFAS, pharmaceutical residues) are not fully understood. Few sustainable, low-cost carriers (e.g., biochar, waste-derived supports) should have been optimized for enzyme immobilization. Focus is often on well-known microbes; the microbiomes of extreme

environments (e.g., deep-sea vents, deserts, Antarctic soils) remain untapped for novel enzymes.

Production, optimization, and application of microbial enzymes for industrial pollution

Enzymes are efficient biocatalysts for various biochemical reactions, producing few by-products compared to chemical catalysts. Industrial enzymes, such as proteases, amylases, cellulases, and lipases, can be obtained from microorganisms through optimization and enzyme engineering. These enzymes are essential for industrial conditions, as wild microbial strains produce less enzyme than engineered microorganisms. Most industrial enzymes are derived from plants, animals, and microorganisms due to better yields, cost reduction, and reduced labor. Industrial enzymes are essential for various industries, used for commercial purposes (Figure 2). They are produced by microorganisms, which involve various steps such as isolation, screening, optimization of process parameters, fermentation, purification, characterization, formulation for sale, customer liaison, and working with regulatory authorities. Most bacteria and fungi used to produce industrial enzymes are genetically modified to overproduce them. Solid state and submerged fermentation are commonly used, but submerged fermentation is preferred due to the extracellular nature of the enzyme. Criteria such as pH and temperature stability, specificity, influence of activators and inhibitors, and reaction velocity are used in selecting industrial enzymes. Industrial enzymes are typically produced under controlled conditions using microorganisms, mainly bacteria and fungi being the main producers (Table 1 and 2). Industrial enzymes are frequently produced by submerged and solid-state fermentation. However, due to the extracellular character of the industrial enzyme that is released into the production medium, submerged fermentation has been consistently described as the preferred technique for industrial enzyme secretion from microorganisms. According to Sarrouh et al.,[19] some of the factors taken into consideration when choosing which industrial enzymes to manufacture from microbes include pH and temperature stability, specificity, the impact of activators and inhibitors, and reaction velocity. Figure 2 demonstrates the comparative study between various microbial enzymes.

Optimization of parameters for enzyme production by microorganisms

A number of factors, including incubation duration, agitation/shaking, pH, inoculum concentration, incubation temperature, carbon supply, metal ions, and nitrogen source, primarily affect the industrial enzymes produced by microorganisms.

Improving the industrial enzyme yield is largely dependent on optimizing these parameters.[20]

Application of microbial enzymes for the bioremediation of industrial pollutants

In the present scenario, microbial enzyme-mediated bioremediation is a viable approach for reduction and removal of hazardous industrial pollutants. Enzymes are present in almost every naturally existing organism i.e., from prokaryotes to eukaryotes.[15] Many microbial enzymes act as catalysts for producing a variety of products from a variety of substrates under controlled circumstances. Additionally, through bioconversion or biodegradation processes, several microbial enzymes can quickly convert hazardous substances into valuable by-products (Figure 1).

Because they lower the reaction activation energy without having any long-term effects, enzymes speed up a variety of biological processes that are essential to maintaining human life. The production of enzymes from microorganisms offers a number of benefits, such as simple gene manipulation, quick growth under regulated conditions, ease of handling, increased manufacturing yield, etc.[16]

Microbial enzymes are also being employed more frequently in industrial settings as a result of their catalytic activity, non-toxicity, specificity, eco-friendliness, stability, cost-effectiveness, and ease of manufacturing.[9] The sources, mechanisms involved, and uses of microbial enzymes isolated from different bacterial and fungal species for bioremediation are described in Table 1 and 2, respectively.

Cytochrome P450 (EC 1.14.14.1)

A member of the heme enzyme superfamily, cytochrome P450 is found extensively throughout the three domains of life-bacteria, archaea, and eukaryote.[21] It carries out a variety of tasks, such as the biotransformation of harmful compounds in our ecosystem and synthesizing intricate natural products in living systems. They combine molecular oxygen with NADH or NADPH to produce oxidized products and a carbon substrate. They function as a cofactor.[22] P450s can degrade xenobiotics naturally.[23] For catalytic activity, they also require ferredoxin and ferredoxin reductase as an electron source. Research on microbial P450s for the bioremediation of hydrocarbons and organic pollutants has been done utilizing both non-engineering and protein engineering approaches.[24]

One of the recognized microbial P450s is the *Bacillus megaterium* CYP102A1 (P450BM3) model, which has been proven through protein engineering research to have the ability to oxidize PAHs.[22] Many microorganisms, mainly bacteria such as *Rhodococcus, Gordonia, Mycobacterium, Pseudomonas,* etc., have been found to include some catabolic genes and plasmids that express P450s for the reduction of POPs (persistent organic pollutants) and their elimination from our environment.[25]

Laccases (EC 1.10.3.2)

Laccases are extracellular enzymes composed of several glycoprotein subunits and contain multiple copper ions. Laccases oxidize certain phenolic and aromatic chemicals in bacteria, fungi, and plants, as well as certain amines, ethers, and esters, via a one-electron process.[26] The possibility of laccases being used

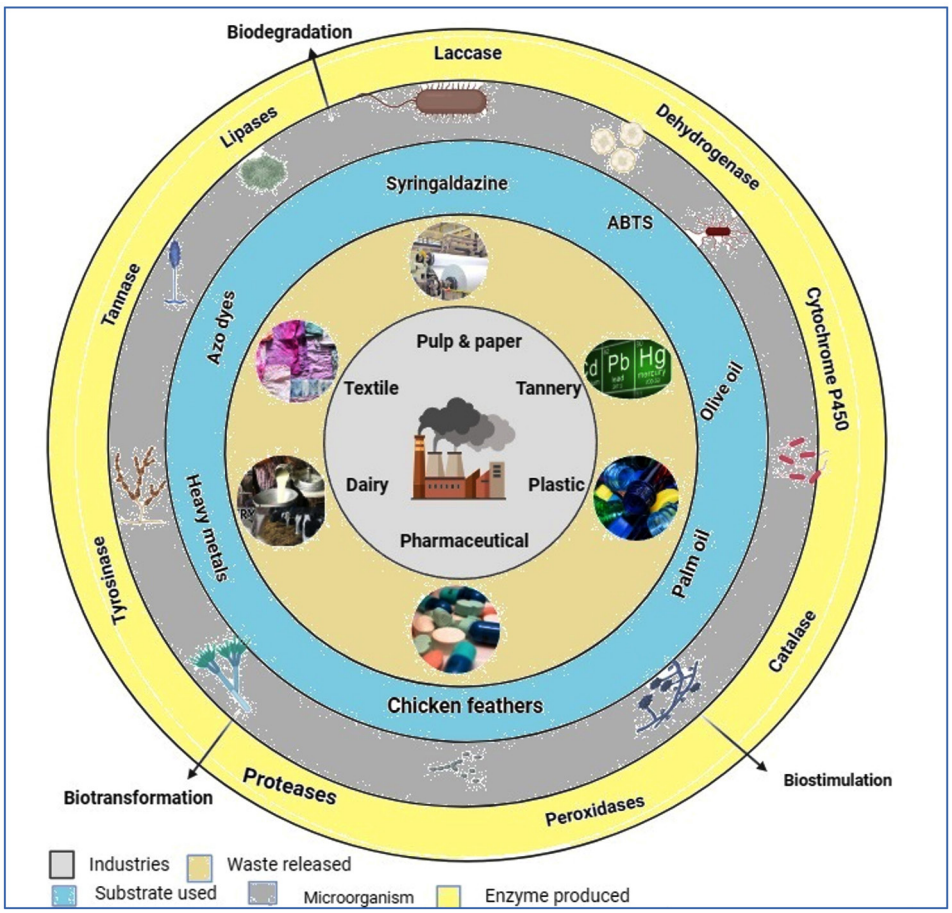

Figure 1. Microbial enzymes' use in the bioremediation of hazardous industrial wastes into non-toxic substances

Table 1. Bioremediation of industrial pollutants through various bacterial enzymes

No.	Name of enzyme	Name of bacteria	Substrate used	Mechanism Involved	Industry	Application	Ref.
1	Laccase (EC 1.10.3.2.)	*Streptomyces maltophilia*	Mainly Synthetic dyes e.g. Congo red, Methylene blue, Toluidine blue, Methyl pink, green, and methyl orange	Ring cleavage in aromatic compounds and reduce one molecule of oxygen in the water and produce free radicals	Textile	Degradation as well as decolorization of synthetic dyes in Textile effluents	78
2	Laccase (EC 1.10.3.2.)	*Streptomyces cyaneus*	2,2′-Azino-bis-(3-ethylbenzothiazoline -6-sulfonic acid (ABTS)		Plastic	Oxidation of Micro-pollutants like BPA (Bisphenol A), DFC (Diclofenac), and MFA (Mefenamic acid)	79
3	Laccase (EC 1.10.3.2.)	*Geobacillus thermocatenulatus*	ABTS		Textile	Degradation and Decolorization of Textile dyes, especially Congo red and bromophenol blue	80
4	Laccase (EC 1.10.3.2.)	*Anoxybacillus gonensis*	ABTS		Tannery	Bioremediation of tannery effluents	15,80
5	Cytochrome P450 (EC 1.14.14.1)	*Rhodococcus rhodochrous*	Hexahydro-1,3, 5-trinitro-1,3, 5-triazine (RDX)	Performs electron transfer reactions and catalysis by reduction or oxidation of heme iron	Pharmaceutical	Degradation of RDX	24
6	Lipase (EC 3.1.1.3)	*Bacillus subtilis*	Olive oil	Catalyzes the hydrolysis of mono-, di-, and triglycerides into fatty acids and glycerol as well as catalyse the esterification reactions	Food	Bioremediation of Wastewater, Cleaning detergent of tough oil or grease stains	15,81,52
7	Lipase (EC 3.1.1.3)	*Bacillus pumilus*	Palm oil		Detergent, food, cosmetic	Degradation of palm oil containing Industrial wastewater	82

8	Dehydrogenase (EC 1.1.1.1)	*Pseudomonas putida*	4-Hydroxybenzaldehyde and 4-hydroxy-3-methylbenzaldehyde	Oxidizing organic compounds and generating energy	Tannery	Breakdown of 2,4- xylenol	83,84
9	Dehydrogenase (EC 1.1.1.1)	*Stenotrophomonas rhizophila*	Vinyl alcohol oligomer and polyvinyl alcohol		Textile, paper, food	Polyvinyl alcohol degradation	83,84
10	Protease (E C 3.4.21.12)	*Bacillus subtilis*	Feather culture medium	Assist in the breaking of protein peptide bonds	Poultry, tannery	Deterioration of casein as well as feathers and degradation of proteins like keratin, casein, etc., leather dehairing, and wastewater treatment	85
11	Protease (E C 3.4.21.12)	*Chryseobacterium* sp. strain kr6, *Bacillus pumilus*	Chicken feathers		Poultry	Deterioration of feathers	85
12	Protease (E C 3.4.21.12)	*Streptomyces thermoviolaceus*	Hair, collagen, Muscle, nail, feathers		Poultry	Hydrolyze the fibrin, collagen, muscle, nail, and hair	85
13	Protease (E C 3.4.21.12)	*Thermoanaerobacter keratinophilus*	Complex medium without oxygen having merino wool, human hairs, chicken feathers		Poultry	Breakdown of keratin fibers	85
14	Dehalogenase (EC 3.8.1.5)	*Bacillus* sp.	2,4,6-Trinitrobromophenol (TBP)	Cleaves the carbon-halogen bond and eliminates the halogens	Pesticides	Degradation of TBP	21

Table 2. Bioremediation of industrial pollutants through various fungal enzymes

No.	Name of enzyme	Name of fungus	Substrate used	Mechanism involved	Industry	Application	Ref.
1	Laccase (EC 1.10.3.2)	*Coniophora puteana*	Syringaldazine (SGZ)	Cleavage of ring in aromatic compounds as well as reduction of one molecule of oxygen in the water molecule and production of free radicals	Textile	Deterioration of artificial dye	80
2	Laccase (EC 1.10.3.2)	*T. versicolour*	Pellets of *T. versicolour*		Textile	Detoxifying and reducing the colour, aromatic compounds, and chemical oxygen demand (COD) were reduced up to 70–80% and COD was reduced up to 60%.	42,80
3	Laccase (EC 1.10.3.2)	*Aspergillus flavus*	Dyes		Textile	Removing surfactants and dyes	86
4	Laccase (EC 1.10.3.2)	*Cerrena unicolor*	Sugarcane bagasse		Paper & pulp	Reduce lignin	87
5	Laccase (EC 1.10.3.2)	*Flavodon flavus, Pycnoporus sanguineus, Trichosporon beigelii* NCIM-3326	Bromophenol blue, malachite green		Textile	Dye Decolorization (Azure B, Brilliant Blue R, bromophenol blue and malachite green)	88,89
6	Laccase (EC 1.10.3.2)	*Phanerochaete chrysosporium*	Azo black reactive dye		Tannery	Degradation of Azo Black Reactive 5 Dye	24
7	Laccase (EC 1.10.3.2)	*Phanerochaete chrysosporium* URM 6181 and *Curvularia lunata* URM 6179	Indigo dye		Textile	Decolourize effluent containing textile indigo dye by approximately 95%	26,27
8	Laccase (EC 1.10.3.2)	*Flavodon flavus, Coriolopsis gallica*	Sugar cane bagasse		Pulp and paper	Decolourize the effluent from a Kraft paper mill bleach plant	25

9	Laccase (EC 1.10.3.2)	*Phanerochaete chrysosporium*	Polyaromatic hydrocarbon (PAHs)		Pharmaceutical	Catabolize PAHs including anthracene and the endocrine disrupting alkylphenols, a variety of pharmaceutical compounds, antibiotics, anti-inflammatories, and β-blockers are detoxified	90
10	Lipases (EC 3.1.1.3)	*Aspergillus terreus*	Disposed engine oil	Catalyses the hydrolysis of mono-, di-, and triglycerides into fatty acids and glycerol as well as catalyse the esterification reactions	Petroleum	Remediate oily polluted soils	51
11	Lipases (EC 3.1.1.3)	*Aspergillus terreus*	Dairy effluent	Oxidizing organic compounds and generating energy	Dairy	Bioremediation of dairy effluent to reduce over 90% oil and grease	45
12	Dehydrogenase (EC 1.1.1.1)	*Phanerochaete chrysosporium* (acidic conditions) and *Humicola insolens* (alkaline conditions)	Pulp mill effluent	Catalyses the hydrolysis of mono-, di-, and triglycerides into fatty acids and glycerol as well as catalyse the esterification reactions	Pulp and paper	Used to treat the acid effluent stream discharged from a pulp plant and remove colour	91
13	Dehydrogenase (EC1.1.1.1) Laccase (EC 1.10.3.2)	*Funalia trogii*	Textile Dyes	Oxidizing organic compounds and generating energy	Textile	Applicable to the effluent released from the caustic sewer of bleach plant and for the decolorization of textile dyes	92
14	Catalase (EC 1.11.1.6)	*Neurospora crassa*	Hydrogen Peroxide	Convert hydrogen peroxide to water and oxygen	Tannery	Bioremediation of heavy metals from tannery effluent	93
15	Peroxidases.	*Thanatephorus* sp.,	Phenols,	Catalyze the oxidation	Textile	Degradation of	41

	(EC. 1.11.1.7)	*Auricularia* sp., *Penicillium geastrovirus*, *Candida tropicalis*	hydroquinone, dyes, amines, aromatic alcohols and xenobiotic	of various organic and inorganic substrates by reacting with hydrogen peroxide and similar molecules			synthetic dyes such as azo, remazol blue, Cibacron red, remazol brilliant blue, anthraquinone
16	Proteases (EC 3.4.21.12) Keratinase (EC 3.4.99.11)	*Aspergillus flavus*, *A. oryzae*, *A. niger* , *Cladosporium herbarum*, *Fusarium solani*, *Penicillium chrysogenum*	Hides and skins	Catalyze hydrolytic reactions that help in the degradation of protein molecules firstly into peptides and finally to free amino acids	Tannery		Widely used in the leather processing industries, waste management and wastewater treatment
17	Proteases (EC 3.4.21.12)	*Aspergillus niger*, *A. oryzae*, *A. japonicus*, *A. gallonyces*, *A. awamori*	Tannin	Catalyzes the hydrolysis of ester bonds	Tannery	55	Used in the degradation of tanneries effluents containing tannins

in many biotechnological processes has been explored because oxygen is the final electron acceptor. These qualities have piqued the researcher's interest in using laccases.

Laccase is typically a highly stable, industrially applicable, heat-resistant enzyme. This enzyme can eliminate xenobiotics and generate polymeric compounds employed in bioremediation procedures. Textile industry-produced phenols and dyes can be eliminated and detoxified using laccase.[27] Laccase is a promising method for treating organic micropollutants in wastewater. It effectively degrades and detoxifies various compounds, including pharmaceuticals, steroid hormones, personal care products, pesticides, and industrial chemicals. Laccases have been successfully utilized to remove pharmaceuticals, personal care products, biocides, endocrine-disrupting agents, steroid hormones, and microplastics. Although no commercially available laccase-based wastewater treatment preparations exist, studies demonstrate its effectiveness.[28]

Agricultural wastes, including sawdust, banana peels, and rice bran, contain lignin and phenolic substances that increase laccase production.[29] There are numerous biotechnological uses for laccases in food, textile, paper, cosmetics, and other industries. This is because of their high oxidative capability. Laccases are also employed to break down agricultural items like pesticides and herbicides, protecting the environment from dangerous chemicals. Laccases remediate soil contaminated by oil hydrocarbons, treat wastewater containing textile industry dyes, and perform bio-bleaching procedures.[30,31] Laccases, known to have a huge substrate range, can oxidize a wide variety of aromatic chemicals, especially phenolic substrates.[32] According to Guan et al.[33] the majority of laccases from different microorganisms are identified, described, and studied, especially the *Streptomyces laccase* from *actinomycetes*. Simulated textile effluents (STE) can be discolored by *E. coli* recombinant CotA laccase. Pure and unpurified CotA laccase decolorized dyes more rapidly when simulated textile effluents were buffered at pH 7.[34] In roughly four hours, the pure recombinant laccase eliminates over 93% of the tested colors at a basic pH of 9.0. Laccase-mediated dye degradation is an environmentally

Table 3. Recombinant DNA technology produced microbial enzymes for enhanced bioremediation

No.	Recombinant enzyme	Host	Origin	Expression vector	Application	Ref.
1.	Laccase cotA	*Escherichia coli* DH5α	*Bacillus subtilis* 168	pMD18-T	Degradation of artificially produced dye	33
2.	Laccase CueO	*Pichia pastoris* GS115	*Escherichia coli* K12	pHBM905BDM	Decolorization of effluent from the textile industry	105
3.	Cellobiohydrolase (CBH1)	*Pichia pastoris* KM71H	*Aspergillus niger*-NL-1	pPICZαC	Degradation of pulp, cellulose etc.	106
4.	Endoglucanase (ReEG I)	*Pichia pastoris* GS115, X-33 and KM71H	*Trichoderma reesei* Rut C-30	pPICZαA	Degradation of pulp, cellulose, oat xylan, birch xylan, corn straw	107
5.	Laccase (Fmb- rL 103)	*E. coli* BL 21	*Bacillus vallismortis* fmb-103	pMD19-Tlac103	Degradation of triphenyl methane dye	36
6.	Cytochrome P450 (CYP105D1)	*Acinetobacter calcoaceticus* strain BD413	*Streptomyces griseus*	pSP19g10L	Deterioration of pollutants herbicides	108
7.	Dehydrogenase (17β-HSDx)	*E. coli*	*Rhodococcus* sp. P14	pET-32a	Bioremediation of steroids contaminated environment	109
8.	Dehalogenase	*E. coli* BL21 (DE3)	*Ochrobactrum* sp. T.	pET30a-a6	Degradation of tetra bromo bisphenol A (TBBPA)	110

acceptable substitute for conventional dye degradation methods, which are sometimes costly and have negative effects.[35] Recombinant laccase, which is utilized in aquaculture wastewater bioremediation, is produced by *B. vallismortis* strain fmb103.[36] Five of the seven dyes that were tested-Evans Blue, Brom Cresol Purple, Remazol Brilliant Blue, Reactive Black 5, and Amido Black 10B-were destroyed by it.[37] Titanium dioxide, iron oxides, aluminum, mica flakes, cadmium, mercury, and lead are among the heavy metals known to be present in the many colored pigments used in the paint industry. According to reports, the laccase enzyme can break down heavy metals utilized in several industries. The laccase enzyme belongs to a broad class of enzymes known as polyphenol oxidases, which are helpful in the printing and tannery, baking, wine and beverage, textile, and pharmaceutical sectors. Laccase is one of the enzymes produced by microorganisms for bioremediation.

The purpose of this study is to separate bacteria from paint industry wastewater, screen for possible laccase-producing bacteria, and produce the laccase enzyme.[38]

Laccases can be produced from filamentous fungi such as white rot fungi.[39] Strong Laccase producers include *Pleurotus florida*, *P. ostreatus*, *P. pulmonarius*,[40] *P. tailandia*[41] and *Trichoderma*.[42] The fungi *Trametes* sp., *Coriolopsis* sp., *Grifola* sp., and many others also create laccases. The *Rhus vernicifera* tree served as the first source of laccase isolation.[22] Bacteria such as *E. coli*, insects' example *Bombyx*, *Drosophila, Papilio*, *Schistocerca,* etc., and plants that produce laccases include mango, peaches, and pines.[39]

Dehydrogenase (EC1.1.1.1)

Dehydrogenases belong to the oxidoreductase family and are found in bacteria, yeast, plants, animals, & human beings. The bacteria's cell-free extracts that break down xenobiotics manufactured industrially showed signs of polyethylene glycol dehydrogenase activity. Xenobiotic polyvinyl alcohol which is water soluble is broken down by recombinant polyvinyl alcohol dehydrogenase.[40,43] It has been discovered that aldehyde dehydrogenase is involved in the anabolic and catabolic processes of aromatic compounds and according to a protein expression analysis of *Amycolatopsis tucumanensis* DSM 45259 used in the biodegradation of phenanthrene, it is extensively and specifically expressed.[44] Table 1 lists a few dehydrogenases

together with information about each one's characteristics and function in bioremediation.

Lipases (EC 3.1.1.3)

Lipases are triacylglycerol ester hydrolyses that facilitate the conversion of triglycerides into glycerol and fatty acids. Lipases catalyze the reaction in which bonds are broken due to acid (acidolysis), alcohol (alcoholysis), and amino acids (aminolysis) in addition to hydrolysis. Lipases are the most adaptable biocatalysts. Plants, animals, and microbes all have lipases in varying concentrations. The most common source of lipases are microorganisms. *Bacillus* sp. is one of the most common bacteria that produces lipase. *Bacillus alcalophilus, Staphylococcus caseolyticus*, *B. licheniformis, Serratia rubidaea, B. stearothermophilus, Pseudomonas aeruginosa,* and *Acinetobacter radioresistens* are the most common producers of lipase.[45]

Microbial lipases are used in industry to create biosensors, which are diagnostic instruments used to identify a range of illnesses. In addition to the pharmaceutical, polymerization, pulp and paper, and cosmetic industries, microbial lipases are commercially useful in the bioremediation of oil residues, petroleum pollutants, and effluents.[46,47] Lipases can accelerate the bioremediation of oily effluents that are generated from a range of sources that contain fats, proteins, and oils. Lipase from the bacteria *Acinetobacter* sp*., Rhodococcus* sp., and *Mycobacterium* sp*.,* has been used to manage oil spills containing n-alkanes, aromatic hydrocarbons, and PAHs.[48,49]

Pseudomonas aeruginosa lipase has been observed to degrade castor oil[50] and to remediate soil having waste oil released from industries. Bacteria that are isolated from soil polluted with motor oil produce lipase that helps in the remediation of hydrocarbon.[51] In household laundry, lipases are used to reduce environmental contaminants and improve the effectiveness of detergent to get rid of stubborn grease or oil stains. Crude lipase isolated from *Bacillus subtilis* strain is used to lessen the impact of phosphate byproducts in laundry detergents.[52] It was discovered that *L. plantarum* displayed the highest polycaprolactone (PCL) degradation efficiency in comparison with other lipases when it came to the breakdown of artificial polyester polycaprolactone (PCL) by co-cultures of *L. brevis* and *L. plantarum* lipases.[53] In fungus, lipase makers are *Rhizopus* (*Rhizopus arrhizus, R. niveus*)*, Penicillium, Aspergillus* sp*.,* etc. Lipases are expected to expand at the fastest rate among enzymes due to their extensive use in numerous industrial domains. Table 1 and 2 lists a few microbial lipases together with information about each one's characteristics and bioremediation function.

Protease (E.C 3.4.21.12)

It is an enzyme that catalyzes the formation of peptide bonds in proteins and is a member of the hydrolase family. Proteases are mainly secluded from *Bacillus* and *Aspergillus* species. Microorganism-derived proteases are extremely important because of their low cost, high yield, and practical usage. They are widely used in industries like the food, leather, and wastewater treatment sectors.[54] Protease is used in the removal of polymers because it aids in the breakdown of -ester bonds created by polyhydroxyl butyrate (PHB) and c-linkages.[55] Because of the presence of keratin protein, which is insoluble in nature, animal horns, nails, poultry feces, and the shedding and molting of appendages are resistant to breakdown. Aside from their disagreeable smell, they are the cause of environmental pollution. By dissolving and recycling keratinous wastes into useful by-products, the protease enzyme keratinase aids in the breakdown of keratin proteins and can be applied to the bioremediation of bird excrement. *Stenotrophomonas maltophilia* KB13's keratinase enzyme has shown its ability to use resistant keratinous waste in the biological deprivation of chicken feathers.[56] Protease producing bacteria has been discovered and identified from soil of tanneries.[57] These are used for silk degumming, leather, waste management, and in silver recovery.[58] *Bacillus* sp. FPF-1 has shown its ability to use resistant keratinous waste biomass from the agricultural industry (accession number MG214993) by degrading chicken feathers at an 82% rate.[59] By-products of feather degradation can be employed as ferric ion reducers, fertilisers for plant development, feed additives, free radical scavengers,[60] and hazardous hexavalent chromium-reducing agents. Keratinase is utilized in

place of the conventional chemicals CaO and Na_2S in environmentally friendly enzymatic de-hairing operations in tanneries, which reduce pollution by preventing the release of harmful waste into water bodies.[61] The bioremediation of marine crustacean wastes employed in the de-proteinization phase of chitin extraction uses protease enzymes. By producing alkaline protease, *Bacillus licheniformis* strain MP1 reduces the protein content of shrimp waste by 75%.[62] At a temperature of 30 °C and a predominant pH of 7 to 8, *Pseudomonas chlororaphis* broke down the Impranil substrate while displaying protease activity (beta clearing zone) and esterase activity (alpha clearing zone).[63] By breaking down and transforming marine crustacean debris and keratinous waste products into beneficial molecules, proteases lessen environmental pollution.

Microorganisms are a natural source of commercial enzyme preparations as they be cultured in simple conditions, easy cell manipulation etc. About 40%-60% of all enzyme sales from microbial sources is of proteases.[64]

Peroxidases (E.C. 1.11.1.7)

They are oxidoreductases that utilizes free radical mechanism for transforming a variety of chemical substances into oxidized or polymerized products.[65] Peroxidase activity transforms ferricyanides and ascorbic acid into innocuous components. They lessen water pollution by helping in the bioremediation of wastewater contaminated with chlorinated phenolic chemicals, cresol, and phenol. Peroxidases are suitable enzymes that help in the development of enzyme-linked immunosorbent assay (ELISA) kits, as they can produce chromogenic products at very low concentrations.[66] These enzymes are used in several industrial and analytical bioprocesses because of their strong reduction potential. They degrade artificial dyes such as brilliant blue, azo dyes, Cibacron red, remazol blue etc.[67,68]

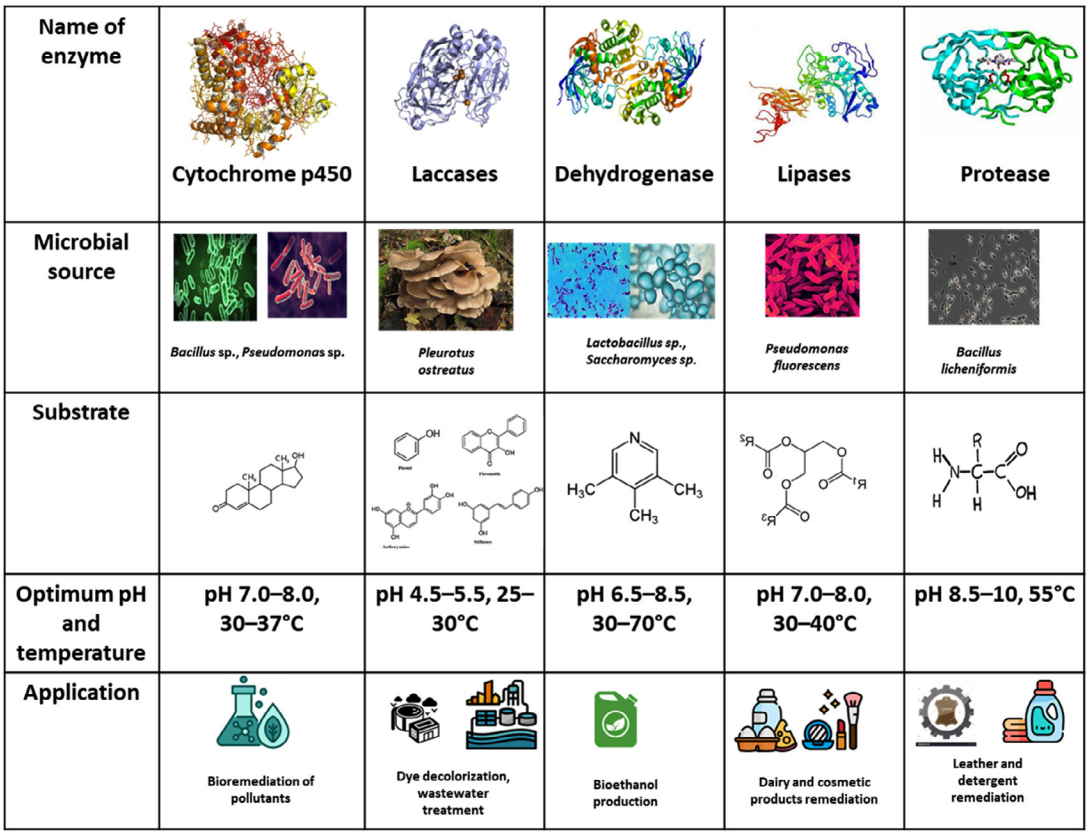

Figure 2. Comparative studies of microbial enzymes and their applications

Peroxidases are also used in the paper industry to hydrolyze cellulose and lignin into carbon dioxide and water, respectively, which breaks down wood components.

Microbes, plants, and animals are the main sources of peroxidases. There have been reports of peroxidase production from a variety of plant sources, including papaya (*Carica papaya*), banana (*Musa paradisiaca*), horseradish (*Armoracia rusticana*),[69] and so on. *Pseudomonas* sp., *Escherichia coli*, and *Bacillus* sp. are the main bacterial strains for production of peroxidase. However, *Pleurotus ostreatus., Umbelopsis isabelline, Auricularia* sp., and, *Thanatephorus* sp. have been reported to be efficient peroxidase producers in the case of fungal strains.[70,71]

Cellulases (E.C. 3.2.1.4)

The hydrolysis of cellulosic substrates into monomeric products is facilitated by the cellulase enzymes. They are created when microbial strains hydrolyze the α-1,4-glycosidic linkages of cellulose while growing on cellulosic substrates. Several strains of bacteria, yeast, and fungi have been found to contain cellulases. Because they can use additional pathways to develop higher cellulase activity, fungi are the primary microbiological species that manufacture cellulase enzymes. *Aspergillus* and *Trichoderma* are well-known fungal genera that produce cellulase. Strong cellulase production has been reported for *A. niger, T. asperellum*,[72] *Trichoderma viride*,[73] *A. fumigatus,*[74] *A. ellipticus*,[75] and *Aspergillus protuberus. Myceliophthora thermophile, Penicillium echinulatum,* and *Rhizopus oryzae* are among the other fungal strains that may have cellulase activity. *Thermomonospora* sp., *Cellulomonas* sp., *Microbispora* sp., *Clostridium* sp., *Cellvibrio* sp., and *Ruminococcus* sp. are among the bacteria that are known to be powerful cellulose-producing genera.[76]

Cellulase enzyme is mostly utilized in textile industry for bio-polishing, to modify the structure of fibres and are also used in the last phase of textile manufacturing to soften and lessen the tendency of fabric to pill as well as to remove the starch sizing.[77]

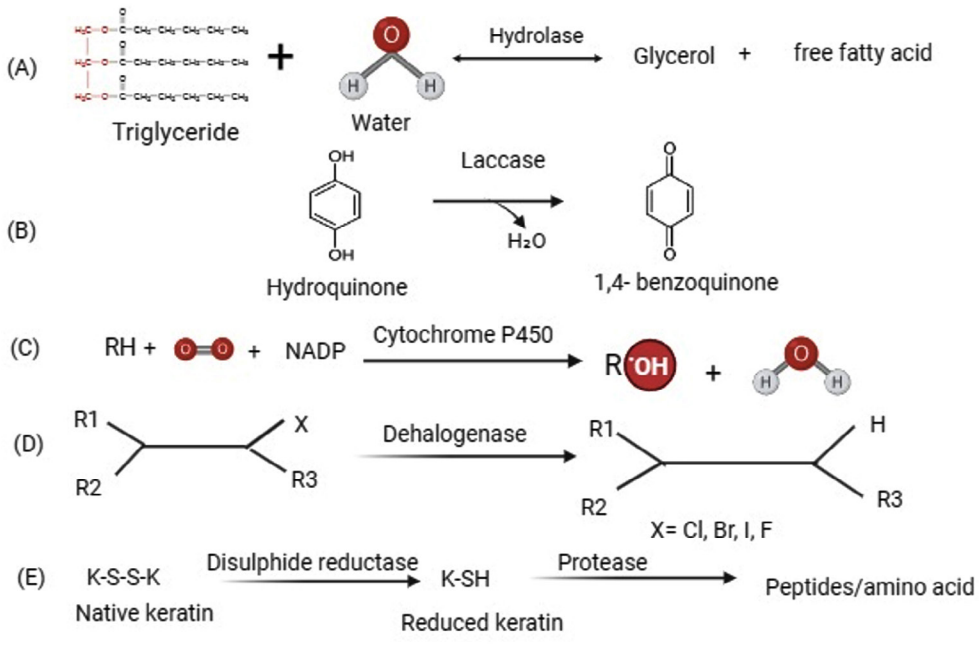

Figure 3. Enzymatic reactions of microbial enzymes employed in bioremediation
(a) Hydrolase, (b) Laccase, (c) Cytochrome P450, (d) Dehalogenase, (e) Protease

Microbial enzymes mechanism for bioremediation

In nature, several genes are known to express different types of enzymes and that regulate how these enzymes function and what kind of structure they should adopt to perform a certain function. The numerous protein folds of the enzyme are regulated by highly distinct sets of genes, which not only adapt the structure of the enzyme for a variety of uses but also determine the catalytic mechanism, which establishes the function of enzymes at a given site. Figure 3 shows the mechanism of action for some of the most important microbial enzymes utilized in bioremediation.

Free radicals are created when laccase oxidizes the substrate. Laccase-mediated catalysis can be expanded by utilizing mediators. Laccase oxidizes mediators, which are organic molecules with a low molecular weight. The glycerol backbone of a lipid substrate serves as the typical site of lipase activity in the case of the lipase enzyme.

Electrons are transferred from the substrate to an electron carrier by dehydrogenase enzyme. NAD^+, FAD, and $NADP^+$ are common electron acceptors utilized by this subclass. In this process, electron carriers are reduced and are regarded as oxidizers of the substrate. Co-enzymes such as flavin groups, nicotinamide adenine dinucleotide (NAD), flavin mononucleotide (FMN), or nicotinamide adenine dinucleotide phosphate (NADP) catalyze the reaction with dehydrogenase enzymes.[83] The dehydrogenase enzyme, which is present in all living organisms, transports hydrogen atoms from organic transporters to electron-acceptor substances.[84] Hydroxylase enzymes have active sites containing metal that can catalyze the reactions.

Dioxygen molecules can serve as an electron acceptor for oxidases to use in catalyzing processes.[83] Oxidases transfer electrons using a variety of substances, including metals and cofactors. These substances consist of amine oxidases, metals based on alcohol or flavin, or both.[85] Utilizing molecular oxygen and NADH or NADPH as a cofactor, cytochrome P450 produces oxidized products and carbon substrate.[24] As a source of electrons, cytochrome P450 uses ferredoxin reductase and ferredoxin for catalytic function.

Enzymatic biodegradation potential for sustainability

The enzymatic activity of microorganisms that convert harmful substances into less toxic or non-hazardous compounds is the primary focus of biological remediation of toxic contaminants.[96,97] Using microbial enzymes over microbial cell majorly depends on the Standardizable activity, ease of handling and storage, more selectivity, improved mobility because of their smaller size, ability to function in the face of high concentrations of harmful substances, and biodegradability.[98] Microbial enzymes enhance the remediation process by yielding non-flammable, non-corrosive byproducts that can be easily disposed of Perpetuini et al.[99] It has been seen that immobilization increases the stability and lifetime of enzymes by reducing enzymatic activity.[100,101] Recombinant DNA technology has helped to generate more effective enzymes in significant amounts.[102] Because enzymes are sustainable, naturally occurring catalysts derived from renewable resources, microbially produced enzyme remediation is a safe and cost-effective bioremediation method. They use as many natural products as possible and are biodegradable.[103] Enzyme-mediated degradation of toxic pollutants provides an environmentally friendly technique by reducing post-treatment environmental hazards, making it more socially acceptable.[54]

Recombinant microbial enzymes produced by different expression systems

Studies have demonstrated effective methods to create various microbial enzymes by diverse microorganisms (Table 3) using a variety of study approaches that have been improved in recent years. Recombinant laccase has been investigated for the treatment of dyes, phenolic compounds, insecticides, and polycyclic aromatic hydrocarbons due to its capacity to oxidize a variety of substrates.[104] Laccase-mediated degradation has been investigated for the treatment of a number of pollutants, including dyes, phenolic compounds, insecticides, and polycyclic aromatic hydrocarbons. Endo-1,4-glucanase-encoding *Aspergillus fumigatus* gene (Afu6g01800) was cloned in the pET-28a (+) vector and was expressed in the Rosetta TM (DE3) strain of *E. coli.* The findings of this study demonstrated

that the afegl7 enzyme belongs to the GH7 family. The afegl7 gene encodes a protein of 460 amino acids, a CBM1 domain at residues 424-460, and a molecular weight of 52kDa.[105]

Laccase cotA produced from *Bacillus subtilis*, was expressed in *E. coli* DH5 using the pMD18-T vector, is well known for its synthetic dye efficiency.[30] Another laccase, CueO, derived from *E. coli* K12, was expressed in *Pichia pastoris* GS115 using the pHBM905BDM vector, demonstrating efficacy in the textile effluents dye decolorization. Laccase Fmb-rL103, from similarly *Bacillus vallismortis* fmb-103 and expressed in *E. coli* BL21 using the pMD19-Tlac103 vector, is effective against triphenyl methane dyes. Cellobiohydrolase CBH1 from *Aspergillus niger*, expressed in *Pichia pastoris*, is effective in the degradation of pulp and cellulose. Similarly, endoglucanase ReEG I, derived from *Trichoderma reesei* and expressed in *Pichia shepherds*, can degrade various substrates including pulp, cellulose, oat xylan, birch xylan, and corn straw.

Cytochrome P105D1, sourced from *Streptomyces griseus* and expressed in *Acinetobacter calcoaceticus* using the pSP19g10L vector, plays a key role in the breakdown of various pollutants and herbicides.[108] It has been observed that the Dehydrogenase from *Rhodococcus* sp. P14, expressed in *E. coli* with pET-32a, significantly contributes to the bioremediation of steroids.[106] A study suggests that dehalogenase produced by *Ochrobactrum* species, and expressed in *E. coli* BL21 (DE3) using the pET30a-a6 vector, can be used for the degradation of the environmental contaminant Tetra Bromo Bis Phenol A (TBBPA).[110] Thus, Several recombinant enzymes have been developed and expressed in various host systems for applications in biodegradation and bioremediation.

CONCLUSION

Rising contaminants harm biotic components through chronic exposure, even at low concentrations. When optimal conditions of temperature, pH, and concentration are met, microbial enzymes transform pollutant substrates into harmless products. Polyhalogenated aromatics and PAHs interact with cytochrome P450's active site to produce non-toxic substances. Laccase's halotolerant capabilities convert antibiotics, synthetic colours, PAHs, and phenolic contaminants through oxidation. Dehalogenase enzymes cleave carbon-halogen bonds, converting halogenated contaminants into substrates that reduce chlorinated environment. Dehydrogenase converts hydroxyl groups in synthetic polymers and alcohols into aldehydes. Protease enzyme breaks down keratinous waste, colours, marine crustacean waste, and biodegradable plastics through hydrolysis. Microbial hydrolases degrade plasticizers, cyanides, and nitrile chemicals into less hazardous byproducts. Microbial lipase decomposes copolymers, synthetic polyester, and parabens into biodegradation products. Therefore, bioremediation by microbial enzymes provides a safe, sustainable method for biodegrading dangerous organic and inorganic materials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SY, GKA and NJ conceptualized the study. SY collected resources. GKA and NJ performed supervision. SY and NJ performed data curation. NJ performed data validation. SY wrote the original draft. SY, NK, GKA, NJ and AB wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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