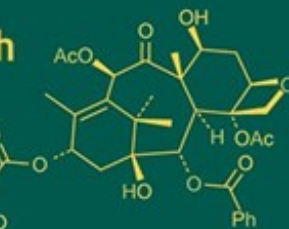
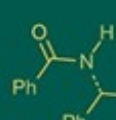


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Microbial bioremediation and enzymatic approaches: Eco-friendly strategies for aquatic pollution mitigation

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ABSTRACT

Microbial bioremediation as an eco-friendly approach to address aquatic pollution from heavy metals, PAHs, dyes, pesticides, antibiotics and plastics originating from industrial effluents, mining, agriculture and urban runoff. It explains the several microorganisms that can use enzymatic oxidation/reduction, biosorption, bioaccumulation, bioleaching, bio-stimulation and bioaugmentation to break down certain contaminants. These microbes include yeasts, protozoa, fungi like *Aspergillus*, bacteria like *Bacillus* and *Pseudomonas* and algae like *Chlorella*. Efflux pumps, mineralization to CO₂ and H₂O and metal resistance systems are important mechanisms and in-situ and ex-situ applications for aquatic ecosystems are highlighted. It highlights the use of microbial enzymes (hydrolases, proteases, cellulases, and lac-cases) as biocatalysts to degrade organophosphates, keratin and cellulose wastes, among other persistent pollutants. In addition to obstacles like microbial competition and eutrophication hazards, influencing elements including pH, temperature, nutrition and genetic engineering are examined to maximize efficiency. In general, it promotes enzyme engineering and strain optimization to improve bioremediation for sustainable aquatic resource management.

Keywords: Microbial remediation, Bioremediation, Microbial enzymes, Pollutants, Heavy metals

1. Introduction

Water pollution is a global concern, broadly classified into microbial, inorganic (heavy metals) and organic contaminants (Yadav *et al.*, 2021) [1]. Wastewater treatment is crucial for reducing pollution and ensuring sustainable aquatic resource management amid rapid population growth. Wastewater commonly contains organic contaminants such as dyes, antibiotics, pesticides, insecticides and phenols. High levels of polyaromatic hydrocarbons (PAHs) in aquatic environments stem from power generation, petroleum refining and other industrial activities (Yadav *et al.*, 2021) [1]. Heavy metal deposition from human activities and industries such as mining, textiles, leather, chemicals and electroplating poses serious threats to humans and other living organisms (Hoang *et al.*, 2021; Cui *et al.*, 2022) [2, 3]. Wastewater effluents from metallurgical, chemical, textile and leather industries are the main sources of heavy metals in water (Sinha *et al.*, 2026) [4]. Heavy metals enter the environment through natural processes (volcanic eruptions, rock weathering) and human activities (mining, industry, agriculture, mineral extraction) (Kapoor *et al.*, 2021) [5].

Excessive exposure to these metals can cause ulcers, immune deterioration, genetic disorders and liver or kidney damage. Mercury damages mitochondria, disrupts microtubules, accumulates neurotoxins and causes multiple other toxic effects. High concentrations of these metals can cause brain damage in animals (López-Berenguer *et al.*, 2020) [6]. According to (Collin *et al.*, 2022) [7], lead is highly carcinogenic, polluting the environment and causing severe harm to humans and other organisms. Ionic lead toxicity disrupts cell binding, protein folding, enzyme regulation, apoptosis and other cellular processes. This causes an imbalance between antioxidant production and free radical generation (Oladoye, 2022) [8]. Arsenic exposure occurs through ingestion, inhalation and dermal contact. Chronic arsenic exposure can lead to diabetes, hematologic disorders, cardiovascular diseases, neurological issues and other complications (Lin *et al.*, 2020) [9].

Bioremediation is a biological process where microorganisms degrade toxic compounds into simpler, less harmful forms, reducing pollutant severity (Ajona *et al.*, 2021; Pushkar *et al.*, 2021) [10, 11]. Microbes degrade pollutants using enzymes and metabolic pathways linked to their growth and metabolism. Bacteria, fungi, yeasts and algae are commonly used in bioremediation (Akerman-Sanchez *et al.*, 2021; Zhao *et al.*, 2021) [12, 13]. Microbes can degrade diverse contaminants such as dyes, hydrocarbons, polychlorinated biphenyls and heavy metals. Microbial growth and survival are influenced by genetics and environmental factors such as temperature, pH, moisture and nutrients like nitrogen and phosphorus. Thus, microorganisms can be genetically engineered to enhance their biodegradation capacity (Wu *et al.*, 2021) [14]. This review

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explores the types, mechanisms, challenges and factors affecting microbial bioremediation in aquatic environments.

2. Sources and hazards of pollution in aquatic water body

Industrial activities such as pesticide, electroplating, sugar, textile and paper industries contribute substantially to water pollution through the discharge of untreated effluents (Onyedikachi *et al.*, 2024) [15]. Domestic wastewater containing plastics, solid wastes, toxic chemicals and microorganisms further aggravate aquatic pollution (Ablankar *et al.*, 2025) [16]. Metalloids and heavy metals enter aquatic environments through industrial discharges, mining, fertilizers, ma-

nure, pesticides, sewage sludge, contaminated irrigation water, fossil-fuel combustion, lead-based products and electronic waste (Saravanan *et al.*, 2024) [17]. Natural weathering of sedimentary rocks such as dolomite, limestone, sandstone and shale, as well as igneous and ultramafic rocks, also contributes to elemental contamination. Rapid urbanization and industrialization further reduce the carrying capacity of water bodies. Mercury (Hg) contamination is associated with atmospheric deposition from waste incineration, sewage, agriculture, fossil-fuel combustion, pyrometallurgy and mining. Rainfall and snowmelt runoff can transport Hg-contaminated soils into nearby water bodies, posing risks to aquatic ecosystems. Fig 1 below represents the effect the pollutants on fish physiology.

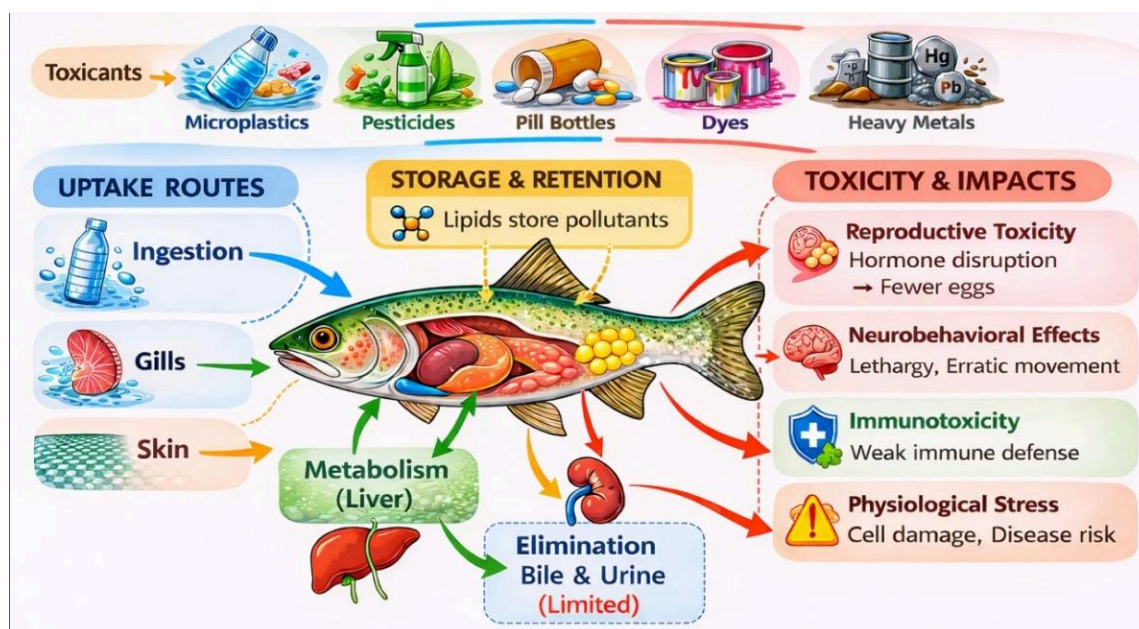


Figure 1. Effect of pollutants on Fish physiology

Table 1. Types of microorganisms used for various categories of pollutants

Microorganism	Pollutants	Citation
Bacteria		
<i>Bacillus amyloliquefaciens</i> - ASK11	Cr (VI)	[18]
<i>Sporosarcina ginsengisoli</i>	As (III)	[19]
<i>Phaeobacter</i> spp.	Phosphate triester	[20]
<i>Ruegeria mobilis</i>		
<i>Thalassospira</i>		
<i>Profundimaris</i>		
<i>Bacillus cereus</i>	Cr (VI)	[21]
<i>Acinetobacter haemolyticus</i>	Cr (VI)	[22]
<i>Kocuria flava</i>	Cu	[19]
<i>B. iodinium</i>	Pb	[23]
<i>Klebsiella pneumoniae</i>	Hg	[24]
<i>Bacillus firmus</i>	Zn	[25]
<i>Vibrio fluvialis</i>	Hg	[26]
<i>Pseudomonas veronii</i>	Cd, Zn, Cu	[27]
<i>Pseudomonas aeruginosa</i> SL-72	Crude oil	[28]
<i>Micrococcus</i> spp.	Pb	[29]
<i>Methanobacterium</i> , <i>Lactivibrio</i> , <i>Clostridium</i> , and <i>Bacillus</i>	Ciprofloxacin (85%) and sulfamethoxazole (94%)	[30]
<i>Micromonospora</i> , <i>Rhodococcus</i> , <i>Bacteroides</i> and <i>Pseudomonas</i>	DEHP (91.9%)	[31]
Fungi		
<i>Aspergillus nomius</i>	Pb	[32]
<i>Fusarium oxysporum</i>	aqueous lead and cadmium ions	[33]
<i>Candida lipolytica</i>	Cr, Cu, Ni and Zn	[34]

(continued on next page)

Microorganism	Pollutants	Citation
<i>Candida parapsilosis</i>	Hg	[35]
<i>Rhizopus oryzae</i>	Cr (VI)	[36]
<i>Aspergillus niger</i>	Ni	[37]
<i>Aspergillus versicolor</i>	Cu	[38]
<i>Aspergillus</i> spp.	chromium (Cr)	[39]
<i>Aspergillus niger</i> can	arsenic	[40]
Algae		
<i>Spirogyra</i> spp.	Cr, Pb	[41]
<i>Spirulina</i> spp.	Cu, Zn, Fe	[42]
<i>Chlorella vulgaris</i>	Pb, Cu	[43]
<i>Hydrodictylon</i> spp.	As (III)	[44]
<i>Nostoc</i> spp.	Ni, Zn	[45]
<i>Chlorella pyrenoidosa</i>	Arsenic, nickel and chromium	[46]
<i>Chlorella</i> spp.	Zinc and lead	[47]
Yeast		
<i>Saccharomyces cerevisiae</i>	Cr (VI)	[48]
<i>Saccharomyces cerevisiae</i>	lead (Pb) and cadmium (Cd)	[49]
Protozoa		
<i>Euplotes mutabilis</i>	cadmium, lead, copper and chromium	[50]
<i>Tetrahymena rostrata</i>	Hg	[35]

3. Principles and mechanisms of microbial bioremediation

3.1 Bioremediation techniques by microorganisms

Microbial removal of environmental contaminants occurs mainly through mobilization and immobilization processes (depicted in Fig. 2 and 3) (Ayilara *et al.*, 2023) [51]. Mobilization includes enzymatic reduction and oxidation, bioleaching, biostimulation and bioaugmentation, whereas immobilization involves bioaccumulation, complexation, biosorption and precipitation (Ayangbenro *et al.*, 2019) [52]. Immobilization converts contaminants into environmentally unavailable forms and can be performed in situ or ex situ (Pratish *et al.*, 2018) [53]. In situ treatment occurs at the contaminated site, while ex situ treatment involves transferring contaminated material for microbial treatment (Ayangbenro *et al.*, 2017; Cao *et al.*, 2020) [54, 55]. Microbes such as *Bacillus cereus* and *Enterobacter asburiae* can immobilize heavy metals (Fashola *et al.*, 2020) [56]. Microorganisms also resist contaminants through pollutant-degrading enzymes, metal-resistance mechanisms, and efflux systems. Organic pollutants may be mineralized into CO₂, H₂O and other metabolites, while ATPase- and proton/ion-driven pumps contribute to resistance against arsenic, cadmium and mercury (Dixit *et al.*, 2015; Ahemad, 2019) [57, 58]. Understanding these microbial responses and metabolic adaptations can improve bioremediation efficiency (Alvarez *et al.*, 2017) [59].

3.2 Mobilization techniques

3.2.1 Bioleaching

Bioleaching uses acidophilic microbes to solubilize solid heavy metals from sediments. This method is especially effective for sulphur- or iron-containing pollutants (Sun *et al.*, 2021; Bhandari *et al.*, 2023) [60, 61]. Enzymatic activity and secondary metabolites facilitate the precipitation of metals either on the cell surface or intracellularly. This process transforms poorly soluble metal compounds into more soluble forms, thereby enhancing their extractability (Kisielowska *et al.*, 2010) [62]. The majority of organisms utilized are iron- or sulfur-oxidizing microbes, such as *A. thiooxidans*, *Aspergillus*, *Mucor*, *Penicillium*, *Cladosporium* and *Rhizopus* species. These microorganisms generate an acidic environment that aids in dissolving heavy metals immobilized in water (Medfu Tarekegn *et al.*, 2020) [63].

3.2.2 Bioaugmentation

Bioaugmentation involves introducing pollutant-degrading microorganisms into contaminated environments and is considered an efficient, rapid and economical bioremediation approach (Mahmoud, 2021) [64]. Its effectiveness can be improved by using indigenous strains, optimizing inoculum and growth conditions, and integrating biostimulation or phytoremediation (Chettri *et al.*, 2024) [65]. The success of introduced microbes depends largely on their adaptability and ability to compete with native microbial communities (Ayangbenro *et al.*, 2017; Fashola *et al.*, 2016; Goswami *et al.*, 2018) [54, 66, 67]. *Burkholderia* sp. FDS-1 can degrade nitrophenolic compounds in pesticide-contaminated soil into less harmful products under mildly acidic conditions at approximately 30 °C (Goswami *et al.*, 2018; Ojuederie *et al.*, 2021) [67, 68]. However, fluctuating environmental conditions can limit bioaugmentation efficiency, emphasizing the need to consider climate-induced stressors in remediation strategies (Harsh *et al.*, 2025) [69].

3.2.3 Enzymatic oxidation

Key microbial enzymes that degrade pollutants include hydrolases, oxidoreductases, dehalogenases, oxygenases and transferases (Saravanan *et al.*, 2021) [70]. Enzymatic oxidation reduces heavy metals by transferring them from a higher to a lower oxidation state, making them less hazardous (Saravanan *et al.*, 2023) [71]. Microbes release oxidoreductase enzymes to facilitate this process. This method effectively removes hard-to-degrade pollutants like dyes and phenols. (Unuofin *et al.*) [72] report that oxidative enzymes produce radicals that form high-molecular-weight compounds. Laccase, an oxidoreductase, catalyses the oxidation of aromatic amines (Gangola *et al.*, 2018) [73]. Other examples include phenols and polyphenols reducing molecular oxygen to water (Kushwaha *et al.*, 2018; Sahay, 2021) [74, 75]. Laccase production in *Leptosphaerulina* and *Pycnoporus* has been reported to aid heavy metal breakdown (Copete-Pertuz *et al.*, 2018; Tian *et al.*, 2020) [76, 77].

3.2.4 Enzymatic reduction

Unlike enzymatic oxidation, enzymatic reduction converts pollutants into lower oxidation states, often producing less soluble and less toxic forms. Facultative and obligate anaerobic microorganisms can mediate this process and contribute to the degradation of dibenzofurans and polychlorinated dibenzo-*p*-dioxins (Zacharia, 2019) [78]. Azoreductase reduces azo compounds by cleaving azo bonds to form

colourless aromatic amines, while chromate reductase converts toxic Cr (VI) into the less toxic and less soluble Cr (III) (Saxena *et al.*, 2020) [79]. Microbial plastic degradation involves intracellular and extracellular enzymes, including depolymerases that break polymers into smaller units; *Pseudomonas* and *Bacillus* spp. have been reported to degrade brominated polystyrene through depolymerase production (Mohan *et al.*, 2016) [80]. Similarly, malathion esterase initiates malathion degradation by forming malathion monocarboxylic acid, which is subsequently converted into simpler products such as succinic acid and ethyl hydrogen formate by downstream esterases and decarboxyl-oxidoreductases (Sirajuddin *et al.*, 2020) [81]. Further research is needed to identify additional pollutant-degrading microorganisms.

3.2.5 Bio-stimulation

Bio-stimulation enhances microbial activity by adding nutrients (N, P, K), metabolites, electron donors/acceptors, enzymes and biosurfactants to the soil. (Ojuederie *et al.*, 2017; Ayangbenro *et al.*, 2018) [82,

83]. (Goswami *et al.*) [67] reported bio-stimulation as an economical, eco-friendly and effective approach preferable to bioaugmentation since native microbes outcompete introduced ones. It helps preserve the natural balance of microbial diversity in the environment (Sayed *et al.*, 2021) [84]. Several bacterial genera, including *Rhodococcus* spp., *Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Citrobacter* spp., have been documented as effective in the bioremediation of heavy metals through bio-stimulation, demonstrating significant metal tolerance and removal capabilities (Nivetha *et al.*, 2022) [85]. Bio-stimulation enhances microbial activity to accelerate crude oil biodegradation (Mohammadi *et al.*, 2024) [86]. Despite its effectiveness, excess nutrients can trigger eutrophication and other issues, while synthetic nutrient sources may further pollute the environment, undermining the purpose of bioremediation (Saravanan *et al.*, 2023) [71].

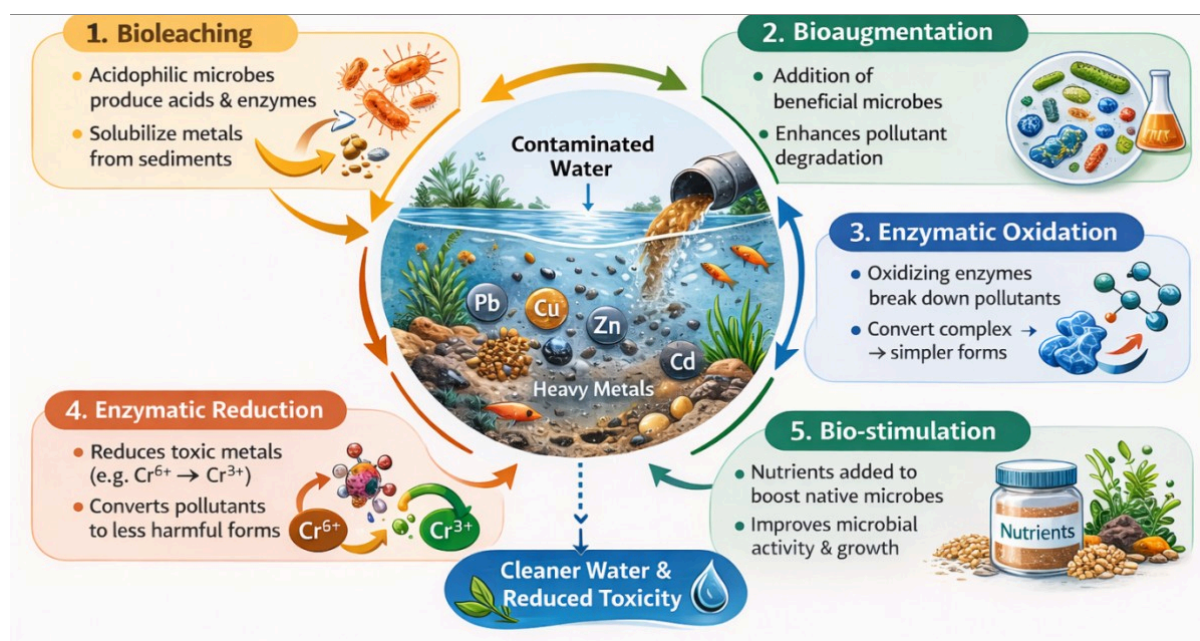


Figure 2. Microbial Mobilization techniques for aquatic pollution remediation

3.3 Immobilization techniques

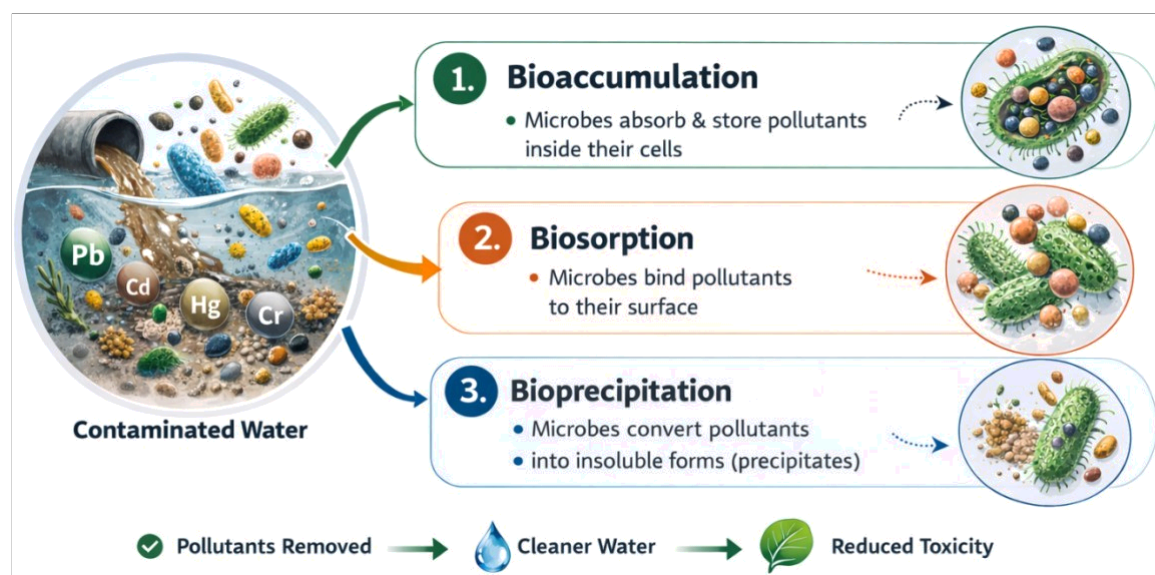


Figure 3. Microbial immobilization techniques for aquatic pollution remediation

3.3.1 Bioaccumulation

Bioaccumulation occurs when a compound's absorption rate exceeds its elimination rate. Compounds accumulate intracellularly in microbes, leading to potential toxicity (Sharma *et al.*, 2022) [87]. In microorganisms, absorbed heavy metals generally localize within the cell wall or cytoplasmic regions (Mir-Tutusa *et al.*, 2018) [88]. According to (Jain *et al.*) [89], bioaccumulation alters adsorption, pH and redox reactions of pollutants, thereby reducing their environmental solubility. Accordingly, microorganism selection depends on their chemical sensitivity, which varies by species and compound (Mishra *et al.*, 2013) [90]. Selected microorganisms require strong biotransformation capacity and high contaminant tolerance. Numerous microorganisms have been reported to exhibit high activity in the bioaccumulation of heavy metals. Notable examples include *Pseudomonas putida*, which demonstrates significant capacity for cadmium accumulation, *Aspergillus niger* known for its ability to accumulate thorium and *Rhizopus arrhizus*, which effectively bioaccumulates mercury (Sharma *et al.*, 2022) [91]. Bacteria and fungi are most frequently employed for bioremediation, while yeast and algae are also used for this purpose (Coelho *et al.*, 2015) [19].

3.3.2 Biosorption

Biosorption is a passive physicochemical process in which living or dead biological materials bind and accumulate pollutants, particularly heavy metals, from aqueous environments. Metal ions interact with microbial cell-wall functional groups such as carbonyl, hydroxyl, carboxyl, phosphate and sulphate through ion exchange, complexation, chelation and electrostatic interactions (Coelho *et al.*, 2015; Mahmoud, 2021) [19, 64]. Bacteria, fungi and algae are effective biosorbents because of their adaptability, high removal efficiency and rapid kinetics (Ayangbenro *et al.*, 2017; Francis *et al.*, 2015) [54, 92]. Reported biosorbents include *Bacillus anthracis*, *Streptomyces* sp. K11 and *Rhodococcus erythropolis* (Mathew *et al.*, 2018; Baltazar *et al.*, 2019; Sedlakova-Kadukova *et al.*, 2019) [93, 94, 95]. Biosorption

also allows recovery of valuable metals such as Cu, Zn and Au through desorption using mineral solutions or chelating agents (Medfu Tarekegn *et al.*, 2020) [63].

Siderophore-mediated complexation is another microbial mechanism in which siderophores produced under iron-deficient conditions chelate metals through hydroxamate, catecholate and phenolate groups, enhancing their solubility and mobilization (Khan *et al.*, 2018) [96]. Cyanobacteria produce siderophores such as schizokinen, synechobactin and anachelin and have potential for bioremediation of complex pollutants (Albelda-Berenguer *et al.*, 2019; Arstol *et al.*, 2019; Sarmah *et al.*, 2020) [97, 98, 99]. Complexation involves binding of heavy metals by microbial ligands, including low- and high-molecular-weight organic compounds, siderophores and extracellular polymeric substances (Pratish *et al.*, 2018; Xie *et al.*, 2020) [53, 100]. Biochar can also remove Cu (II) and Cr (VI) from wastewater through complexation, while *Bacillus licheniformis* and *Rhodobacter blasticus* have been associated with this mechanism (Xiao *et al.*, 2019; Wang *et al.*, 2019; Bai *et al.*, 2019) [101, 102, 103].

3.3.3 Bioprecipitation

Microbial precipitation of heavy metals is the process by which metals in water react with microbial metabolites to form solids (Kumari *et al.*, 2016) [104]. Enzyme activity and secondary metabolites, such as galactoside, can facilitate the deposition of metals including iron and manganese, as well as mineralized manganese, silver and microfossils during biogeochemical cycling. This process converts heavy metals or pollutants into precipitates or crystals, reducing their toxicity (Sharma *et al.*, 2022) [105]. At alkaline pH (> 7), sulphate-reducing bacteria can convert organophosphates (Pratish *et al.*, 2018) [53]. *Bacillus subtilis* and *Oceanobacillus indicireducens* are also linked to the precipitation of heavy metals in environmental settings, facilitating metal removal through bioprecipitation mechanisms (Maity *et al.*, 2019) [106]. Further, table 2 represents various microbes used for bioremediation and mechanisms involved.

Table 2. Various types of microbial-based bioremediation mechanism

Microorganism Group	Examples	Pollutants	Mechanism	References
Bacteria	<i>Bacillus subtilis</i> X3	Lead	Bio-immobilization	[107]
	<i>Pseudomonas aeruginosa</i>	Cadmium	Biosorption	[108]
	<i>Pseudomonas aeruginosa</i> and <i>Bacillus cereus</i>	Cadmium and lead	Bioaugmentation	[109]
	<i>Bacillus</i> spp.	Nickel	Biosorption	[110]
	<i>Lysinibacillus sphaericus</i>	Azo dyes	Enzymatic reduction	[111]
	<i>Cupriavidus</i> spp. strain <i>Cd²⁺</i>	Cadmium	Bioprecipitation	[112]
	<i>Desulfovibrio desulfuricans</i>	Copper, zinc and cadmium,	Extracellular sequestration	[113, 114]
	<i>Synechococcus</i> spp.	zinc and cadmium,	Intracellular sequestration	[115]
	<i>Escherichia coli</i>	Zinc, mercury and cadmium	Active export	[116]
	<i>White rot fungi</i>	PAHs, pesticides, synthetic dyes, industrial effluents	Ligninolytic enzymes (laccase, peroxidases)	[117]
Fungi	<i>Phanerochaete chrysosporium</i> , <i>Trametes versicolor</i> , <i>Pleurotus ostreatus</i>			
	<i>Fusarium solani</i>	Crude oil derivatives, diesel,	Breakdown by extracellular enzymes	[118, 119]
	<i>Aspergillus flavus</i> , <i>Trichoderma</i> spp.			
	<i>Trametes versicolor</i> with bacteria	persistent organic pollutants (POPs), pesticides	Synergistic enzymatic pathways	[120]
				(continued on next page)

Microorganism Group	Examples	Pollutants	Mechanism	References
Algae	<i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> , <i>Chlamydomonas reinhardtii</i> , <i>Nannochloris oculata</i> , <i>Spirogyra</i> spp.	Heavy metals, hydrocarbons, organic pesticides, dyes, nutrients (N, P)	Biosorption Bioaccumulation Enzymatic degradation	[121, 122]
	<i>Asparagopsis armata</i>	zinc, Cadmium, lead and nickel	Biosorption	[123]

4. Microbial diversity and enzymatic systems in aquatic bioremediation

4.1 Microbial enzymes as biocatalysts

Enzymes are biological catalysts that accelerate specific biochemical and metabolic reactions by converting substrates into products without being consumed (Chanda *et al.*, 2025; Farhan *et al.*, 2025) [124, 125]. Although most enzymes are proteins, some RNA molecules also exhibit catalytic activity as ribozymes. Microbial enzymes produced by bacteria, fungi and yeasts are widely used in industrial and biotechnological applications due to their high specificity and efficiency. Enzyme research has progressed from laboratory-scale production to industrial applications, supported by advances in enzyme extraction, characterization and engineering (Jain *et al.*, 2025) [126]. Modern approaches such as protein engineering, metagenomics and biochemical engineering enable the development of tailored enzymes with improved properties and applications (Chirumamilla *et al.*, 2001) [127]. Microorganisms including *Bacillus*, *Pseudomonas*, *Aspergillus*, *Penicillium* and *Clostridium* produce industrially important enzymes such as amylases, proteases, asparaginase, catalase, laccase and cellulases (Anguraj *et al.*, 2024) [128].

4.2 Classification of microbial enzymes

4.3 Different types of microbial enzymes produced by microorganisms

4.3.1 Hydrolases

Hydrolases catalyse the hydrolytic cleavage of chemical bonds, converting complex molecules into simpler, less toxic compounds. Their advantages include low cost, eco-friendliness, wide availability, co-factor independence and stereoselectivity (Karigar *et al.*, 2011) [129]. Microbial hydrolases, such as lipases, esterases, proteases, amylases and cutinases, are applied in biofilm and plastic degradation, pesticide detoxification, food waste treatment and oil-contaminated soil remediation (Kumar *et al.*, 2019) [130]. Species of *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Nocardia* and *Bacillus cereus* degrade pesticides such as carbofuran, carbaryl, parathion, diazinon and coumaphos. Organophosphates (OPs) pose environmental risks due to their neurotoxicity and persistence, requiring phosphodiester bond hydrolysis, whereas pyrethroids and malathion can be detoxified through carboxyl ester hydrolysis (Haque *et al.*, 2020) [131]. Malathion-degrading microbes include *Brevibacillus* spp., *Bacillus cereus*, *B. licheniformis* and *Alicyclobacillus tengchongensis* (Littlechild, 2015) [132]. Furthermore, *Rhodococcus* and *Nocardia* spp. can degrade highly toxic cyanide-containing compounds (Rao *et al.*, 2010) [133].

4.3.2 Proteases

Protease (EC 3.4.21.12) is a hydrolase enzyme that catalyses the breakdown of peptide bonds in proteins. It can be derived from microorganisms like *Amycolatopsis* spp., *Bacillus* spp. and *Aspergillus* spp. Microbial proteases are widely used due to their high yield, low cost and efficient activity, with applications in wastewater treatment, the food industry and leather processing. Keratin, found in animal horns, nails, poultry waste and shed appendages, is highly resistant to degradation and contributes to environmental pollution and

unpleasant odours (Dwivedi *et al.*, 2023) [134]. Keratinase (EC 3.4.21/24/99.11), a type of protease, can degrade keratin and is used in bioremediation by converting keratinous waste into valuable byproducts. These enzymes are hydrolytic and belong to one of the most commercially viable enzyme groups. Microbial proteases can be produced by neutral, acidophilic, or alkaliphilic organisms (Lai *et al.*, 2023) [135]. Alkaliphilic proteases are particularly useful as they remain active under high pH, high temperatures and in the presence of inhibitory substances. In marine waste management, proteases are used for chitin extraction from crustaceans through deproteinization. For instance, 75% of shrimp waste can be deproteinized using alkaline protease from *Bacillus licheniformis* MP1133. By breaking down keratinous and marine waste, proteases reduce pollution and replace harmful chemicals, making them essential for environmental bioremediation (Canpulat *et al.*, 2024) [136].

4.3.3 Cellulases

This enzyme is mainly produced by some bacteria, fungi and protozoan species, which can catalyse the reaction of cellulose decomposition and also some other related polysaccharides (Datta, 2024) [137]. Cellulase enzymes break down cellulose-containing materials into simpler sugars such as monosaccharides like β -glucose and oligosaccharides. Globally, cellulase is regarded as the third most important industrial enzyme due to its widespread use in sectors including bio-fuels, textiles, food processing, animal feed and pulp and paper (Borthakur *et al.*, 2024) [138]. In the field of research, focus is given on techniques to enhance the cellulases enzyme production to increase the glucose commercial production from residues of plants containing cellulose. There are three different forms of cellulase enzyme which works synergistically because of its crystalline and amorphous structure. These three forms are endoglucanase, exoglucanase and β -glucosidase carried out the process of cellulose saccharification. The endoglucanase acts on the fibres of amorphous cellulose and releases reducing and non-reducing ends by randomly hydrolysing the chain of glucose polymer. The 2nd form exoglucanase hydrolyses the free ends and produce cellobiose and further the 3rd most form β -glucosidase hydrolyses the cellobiose and convert into glucose which is a final end product of the saccharification. The thermal stability of the cellulases enzyme is essential to ensure the completion of the cellulose saccharification process which is performed at higher temperatures (Wu *et al.*, 2023) [139].

4.3.4 Laccase

Laccases (benzenediol oxygen oxidoreductases, EC 1.10.3.2) are extracellular enzymes containing multiple copper ions. They exist as monomeric, dimeric, or tetrameric glycoproteins and are present in bacteria, fungi and plants (Shraddha *et al.*, 2011) [140]. The majority of microbial laccases have been identified, described and studied across various microorganisms, with particular attention given to *Streptomyces laccases* from actinomycetes. Among the species studied like *S. cyaneus*, *S. coelicolor*, *S. bikiniensis* and *S. ipomoea*, *S. coelicolor* is the most extensively characterized (Guan *et al.*, 2018) [141]. Laccase production was enhanced by lignin and phenolic compounds found in agricultural wastes like sawdust, banana peels and rice bran etc. (Muthukumarasamy *et al.*, 2015) [142]. To help control groundwater and underwater pollution, laccase exhibits catalytic activity that enables the oxidation of phenolic compounds (such as *ortho*- and *para*-diphenols), aromatic amines and their substituted deriv

atives containing various functional groups. This process involves the reduction of a single oxygen molecule into two water molecules through the simultaneous loss of electrons and it can also act on non-phenolic compounds, converting them into more stable and less soluble forms (Chandra *et al.*, 2015) [143]. Laccases most notable biochemical characteristics include their stability in a range of pH, temperature, organic solvent and salt concentration conditions (Guan *et al.*, 2018) [141]. Laccase has the ability to eliminate xenobiotics and creates polymeric products that are utilized in bioremediation procedures. The well-known pollutant PAHs, which are made up of linearly arranged benzene ring are evenly distributed in the natural environment (Zeng *et al.*, 2011; Li *et al.*, 2010) [144, 145]. Because of their toxicity, persistence, mutagenicity and carcinogenicity, these pollutants as well as their intermediates are extremely dangerous to the environment. They are created when fossil fuels and other industrial wastes are not completely burned. These aromatic hydrocarbons are xenobiotics because of their poor degradation rate and low water solubility (Ihssen *et al.*, 2015) [146].

4.3.5 Lipases

Lipases (EC 3.1.1.3) are well-known biocatalysts that hydrolyse the ester bonds in triglycerides, producing fatty acids and glycerol. These enzymes belong to the serine hydrolase family. They help reduce hydrocarbon levels in contaminated soils by breaking down lipids from various microorganisms, animals and plants. Lipolytic reactions occur at the lipid-water interface, where lipase substrates exist in an equilibrium between monomeric, micellar and emulsified forms. Microbial lipases are widely used in the bioremediation of oil residues, petroleum pollutants, industrial effluents and in soil restoration, due to their high substrate specificity, low energy requirements, strong stability, rapid processing and cost-effective production from diverse

raw materials. Lipases produced by species such as *Acinetobacter*, *Mycobacterium* and *Rhodococcus* have been effective in degrading oil spills containing n-alkanes, aromatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) (Casas-Godoy *et al.*, 2012) [147]. Furthermore, *P. aeruginosa* is verified in the bioremediation of crude oil-contaminated wastewater by a progressive drop in oil concentrations and a reduction of roughly 80% of waste toxicity over a week (Verma *et al.*, 2012) [28]. Further the table 3 below depict the several enzymes synthesized by different microbes.

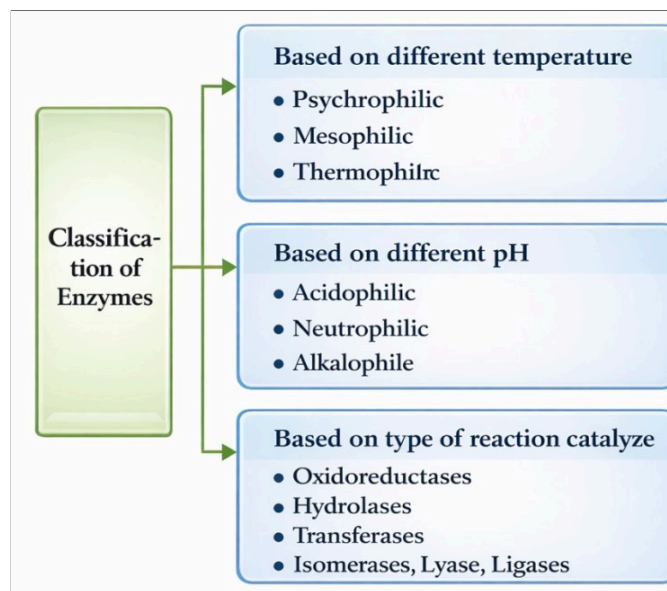


Figure 4. Classification of microbial enzymes

Table 3. Categories of enzymes used for microbial bioremediation

Enzymes	Pollutant remediated	Microbial sources	References
Oxidoreductase	Xenobiotics	<i>Bacillus safenis</i>	[148]
Hydrolases	Polyester plastics Hydrocarbons	<i>Pseudomonas</i> spp., <i>T. fusca</i> <i>Burkholderia</i> spp., <i>Ralstonia</i> spp., <i>Achromobacter</i> spp., <i>Comamonas</i> spp., <i>Bacillus pumilus</i>	[149, 150] [151]
Lipase	Oil containing industrial wastewater	<i>Brevundimonas diminuta</i>	[152]
Phosphodiesterase	Pesticides	<i>Pseudomonas putida</i>	[153]
Laccase	Synthetic dyes	<i>S. rhizophila</i>	[154]
Dehydrogenase	Polyvinyl alcohol	<i>E. coli</i>	[155]
Amylase	Steroids	<i>Bacillus cereus</i>	[148]
Oxygenase	Waste water pollutants	<i>Pseudomonas</i> spp.	[148]
	Pesticides		

5. Recent advances in enzyme engineering and immobilization for enhanced stability and activity

5.1 Genetic engineering approach

Genetically modified microorganisms (GMOs) can enhance bioremediation through bioaugmentation and engineered metabolic pathways (Gupta *et al.*, 2017) [156]. Advances in sequencing and bioinformatics enable identification of remediation-related genes and enzymes, while genetic engineering improves enzyme production and efficiency. Engineered laccases from *Fusarium oxysporum*, *Bacillus subtilis* and *Ganoderma* spp. expressed in *Pichia pastoris* have been used to degrade dyes such as malachite green, indigo carmine and Congo red (Huy *et al.*, 2021) [157]. Similarly, genetically engineered PET hydrolase from *Ideonella sakaiensis* expressed in *E. coli* BL21 enhanced

plastic degradation (Janatunaim *et al.*, 2020) [158]. However, environmental release of GMOs requires stringent containment, risk assessment and regulatory monitoring.

5.2 Enzyme immobilization

Immobilized microbial enzymes offer advantages such as enhanced stability, easy separation, reusability and tolerance to varying pH and temperature. Horseradish peroxidase (HRP) immobilized on calcium-alginate beads using glutaraldehyde achieved 70–80% decolourization of reactive dyes and retained 40% activity after seven cycles (Saravanan *et al.*, 2021; Priyadarshane *et al.*, 2022) [70, 159]. Laccase from *Pleurotus nebrodensis* immobilized on chitosan beads achieved 84–90% dye decolourization, with 84% and 54% activity retained for dispersive and reactive dyes, respectively, after repeated cycles (Aslam *et al.*, 2017) [18]. Similarly, immobilized organophosphate-de

grading enzyme OpdA on non-woven polyester enabled reusable methyl parathion degradation (Bilal *et al.*, 2016) [160]. Laccase immobilized on magnetic metal-organic structures achieved 90–95% degradation of methylene blue and crystal violet in both batch and continuous systems (Ladole *et al.*, 2020) [161].

5.3 Molecular and systems biology tools in microbial bioremediation

Microbial bioremediation exploits the natural metabolic potential of microorganisms to degrade pollutants, but nearly 99% of environmental microbes remain unculturable under standard laboratory conditions (Suneja *et al.*, 2020; Malla *et al.*, 2018) [162, 163]. Advanced approaches such as next-generation sequencing (NGS), omics and bioinformatics enable characterization of these microbial communities and their bioremediation functions (Sharma *et al.*, 2022; Rawat *et al.*, 2019) [91, 164]. Genomics identifies catabolic genes, operons and degradation pathways, while transcriptomics reveals gene-expression responses to pollutants (Huang *et al.*, 2022) [165]. Proteomics identifies stress-response and functional proteins involved in contaminant degradation (Algahmadi *et al.*, 2024; Chandran *et al.*, 2020) [166, 167], whereas metabolomics profiles metabolites and detoxification pathways, providing insights into microbial physiological responses (Ayilara *et al.*, 2023) [51]. Integration of these omics approaches provides a comprehensive understanding of pollutant degradation mechanisms. Furthermore, metabolic engineering and synthetic biology enable the design and optimization of microbial catabolic pathways for persistent contaminants. For example, engineered *Escherichia coli* carrying *opd* and *pnpA-pnpE* genes achieved complete mineralization of methyl parathion and its toxic intermediates into non-toxic products (Bala *et al.*, 2022) [168].

6. Challenges and limitations

Microbial bioremediation is governed by biotic, abiotic and climatic factors, which influence microbial growth, pollutant bioavailability and degradation efficiency. Key factors include pollutant concentration and speciation, redox potential, pH, temperature, oxygen and moisture content, soil characteristics, metal solubility and microbial competition (Medfu Tareegn *et al.*, 2020) [63]. pH strongly affects metal speciation and microbial surface charge, thereby influencing heavy-metal biosorption; most bioremediation processes perform effectively around pH 6.5–8.5. Temperature regulates microbial growth, metabolism, pollutant solubility and metal bioavailability, with extreme temperatures generally reducing microbial activity and biosorption efficiency (Mahmoud, 2021) [64]. Pollutant structure, concentration, toxicity, stability and bioavailability also determine degradation rates, while the type of metal influences microbial metabolism and removal efficiency (Collin *et al.*, 2022) [7].

Climatic changes, including increased temperature and atmospheric CO₂, may alter microbial activity and organic matter decomposition. Soil amendments and chemical modification can further enhance microbe–metal interactions and heavy-metal removal. However, limitations include nutrient deficiency, pollutant toxicity and poor biodegradability, incomplete treatment of pharmaceutical pollutants, scale-up challenges and the potential formation of toxic degradation by-products. Molecular and omics-based approaches have improved understanding of microbial metabolic pathways, while bio-stimulation, through controlled nutrient supplementation, can enhance indigenous microbial activity. Nevertheless, excessive nutrient addition may inhibit microbial processes, necessitating careful optimization.

7. Conclusion and future prospectus

Future microbial bioremediation is expected to advance aquatic pollution control through synthetic biology, enzyme engineering, omics, and AI-based technologies. Genetically engineered microorganisms and designer enzymes can enhance the degradation of persistent pollutants, including pharmaceuticals, PCBs, hydrocarbons, pesticides and dyes, by improving metabolic pathways, catalytic activity and environmental stability. Integration with nanotechnology, microbial fuel

cells, biosensors and bio-electrochemical systems can further improve pollutant removal, catalyst stability, reusability and real-time monitoring while enabling simultaneous energy recovery. However, biosafety, ecological risks and regulatory challenges must be carefully addressed through containment, environmental risk assessment and updated regulatory frameworks. Future progress will rely on omics-guided microbial consortia, AI-assisted process optimization, pilot-scale validation and collaboration among researchers, industry and policymakers, supporting the development of safe, efficient and sustainable solutions for aquatic pollution management.

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