



Review Article

Mutant Bacteria in Bioremediation: Harnessing Modified Microbes for Cleanup of Environmental Pollutants

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Abstract

Environmental pollution threatens global ecosystems and public health, while driving global climate change. Although bioremediation is a sustainable and cost-effective alternative to traditional chemical treatments, natural bacteria are often limited by slow degradation rates and an inability to break down highly resilient materials. To overcome these limits, genetic engineering and targeted mutation are used to improve bacterial metabolism and increase the production of key degrading enzymes. This review examines recent progress in genetically altering established pollutant-degrading bacteria through various mutation methods. Specifically, we evaluate how well these engineered strains clean up synthetic plastics, heavy metals, and crude oil hydrocarbons. We also outline practical strategies for large-scale field use, including direct bioaugmentation, cell encapsulation, *ex situ* bioreactors, and root-associated plant partnerships. Finally, the paper addresses critical biosafety and ethical concerns, focusing on the risk of altered genes transferring to native microbes. To prevent these modified bacteria from persisting in nature, we discuss synthetic safety controls like genetic kill switches and metabolic dependencies. Ultimately, this review provides a clear framework to guide future research toward developing highly efficient, scalable, and environmentally safe biotechnology for ecological restoration.

Keywords

Mutation, Bacteria, Heavy Metals, Hydrocarbons, Plastics, Pollutants, Environment

1. Introduction

The rise in population, industrialisation, and urbanisation is the main cause of significant and deep pollution of soil, surface water, and groundwater. Contaminants originate from either natural or human-made sources [1]. Environmental pollution, a widespread and urgent problem, casts a looming shadow over the planet, threatening the delicate balance of

ecosystems and endangering the health of flora, fauna, and humans [2]. Although the Industrial Revolution marked a major technological and societal advancement with numerous benefits, it also led to the release of large quantities of pollutants into the environment, which are harmful to human health. These contaminants continually enter the food web and pose a major threat to the integrity and health of global ecosystems

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Received: 17 June 2026; Accepted: 9 July 2026; Published: 14 October 2026



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[3]. Additionally, poor solid waste management, especially in developing countries, can release pollutants such as methane, carbon dioxide, and dioxins into the environment [4]. Humans exposed to these pollutants have been shown to develop upper and lower respiratory illnesses, with immunocompromised individuals and those with disabilities being particularly vulnerable to their harmful effects [5]. According to an UNESCO report, about 829,000 people die annually due to polluted drinking water and inadequate sanitation, including nearly 300,000 children under the age of five, representing 5.3% of all deaths in this age group [6]. Cleaning up pollutants is vital for protecting the environment, safeguarding human health, and preserving biodiversity. Current methods, although costly, often deliver disappointing results, such as incomplete pollutant removal, soil structural damage, or significant landscape alteration. Environmental biotechnology researchers have extensively studied and managed various contaminants, including chlorinated solvents, hydrocarbons, PAHs, and heavy metals [7]. Physical, chemical, and phytoremediation techniques have been proposed as primary strategies for reducing pollutant loads in environmental compartments. Several methods—such as coagulation, filtration, precipitation, ozonation, adsorption, ion exchange, reverse osmosis, and advanced oxidation processes—have been employed to eliminate organic pollutants from contaminated water and wastewater. However, these methods are limited because they often involve high capital and operational costs. Reverse osmosis, ion exchange, and advanced oxidation processes are often not economically viable due to their relatively high investment and operational expenses [8]. The adsorption method has been widely used in wastewater treatment, especially for removing micro- and nano-plastics (MNPs) [9]. Chemical techniques tend to be inefficient and expensive, and the chemicals used in decomposition reactions may produce toxic intermediates that also require removal.

2. The Foundations of Bioremediation

Bioremediation, or biological remediation, employs microorganisms, plants, or enzymes to detoxify inorganic pollutants in soil, water, sediments, or air [10]. It relies on naturally occurring or introduced bacteria, fungi, or plants that break down hazardous compounds. When microbes are added to enhance degradation, this process is called bioaugmentation. Successful bioremediation requires microbes to enzymatically convert pollutants into harmless substances, contingent on suitable environmental conditions. Some contaminants, such as chlorinated organics and high-aromatic hydrocarbons, resist microbial attack, complicating cleanup forecasts. Bioremediation can occur naturally or be sped up with nutrients, aeration, or specific microbial strains, often proving to be more cost-effective and environmentally friendly than conventional methods like incineration. It is usually performed on-site, decreasing exposure risks and garnering public support due to its sustainability and minimal ecological footprint

[11–14].

Microorganisms, especially bacteria, have versatile metabolic pathways to degrade and transform pollutants into less harmful substances or mineralize them into CO₂, water, and biomass [15]. These enzymatic processes enable the breakdown of hydrocarbons, pesticides, heavy metals, and plastics through mechanisms like oxygenation, dehalogenation, and mineralization, making bacteria essential for environmental cleanup. Bacteria also detoxify inorganic contaminants, such as metals, through processes like metal reduction, biomineralization, and biosorption. For example, dissimilatory metal-reducing bacteria can convert toxic metal ions like Cr(VI), Hg(II), or As(V) into less toxic or insoluble forms, reducing ecological and health risks [16]. Biofilms enhance bacterial resilience and interactions, improving remediation in complex environments [17]. Bacteria adapt via horizontal gene transfer and regulatory flexibility, developing more effective traits under pollutant stress. These adaptations support natural attenuation and engineered techniques such as bioaugmentation and biostimulation. Due to their efficiency, scalability, and ecological compatibility, bacteria are central to modern bioremediation efforts [11, 13]. Common biodegrading microbes include genera like *Pseudomonas*, *Bacillus*, *Burkholderia*, *Achromobacter*, *Actinomyces*, and *Alcaligenes* [18].

Biostimulation improves native microbial activity by providing nutrients or altering physical conditions, often more economical when local microbes are capable [19–21]. Its success depends on environmental factors. Bioaugmentation introduces cultivated microbes to accelerate degradation, but effectiveness depends on their survival and competitiveness [22, 23]. Combining strategies usually increases success. Natural bioremediation faces challenges like slow degradation of persistent pollutants (PAHs, chlorinated solvents, pesticides) due to limited specific pathways or gene expression in native microbes [24]. Bioavailability also limits activity, as hydrophobic pollutants either adhere to soil or dissolve into phases, making them harder to access [25]. Various environmental factors—including temperature, pH, oxygen, nutrients, and toxins—further restrict microbial activity and pollutant degradation.

Furthermore, natural microbial populations may lack the genetic diversity needed to degrade emerging contaminants such as pharmaceuticals, endocrine disruptors, and per- and polyfluoroalkyl substances (PFAS), which lack an evolutionary history and therefore do not have corresponding biodegradation pathways [26]. Even when capable microbes are present, competition with native species, predation by protozoa, and ecological disturbances can prevent effective pollutant removal. Lastly, natural bioremediation is limited by its unpredictability and lengthy timescales, making it unsuitable for situations that demand rapid or complete remediation. The limitations of natural microbial bioremediation have spurred the development of genetic engineering techniques to enhance microbial capabilities to break down or detoxify environmen-

tal pollutants. Advances in molecular biology, synthetic biology, and genome editing have enabled the strategic design of microorganisms with broader metabolic abilities, greater stress resilience, and customised regulatory systems. Initial efforts focused on inserting single catabolic genes or pathways into resilient microbial hosts, exemplified by genetically modified *Pseudomonas* strains engineered to degrade complex hydrocarbon mixtures [27]. These pioneering studies demonstrated that recombinant microbes could outperform their natural counterparts by metabolising persistent contaminants or functioning effectively in challenging environmental conditions.

This paper aims to review recent developments in the application of genetically modified organisms for the bioremediation of pollutants and to explore the opportunities and benefits of using GMOs, while examining concerns about using mutant bacteria for environmental restoration.

3. Development of Mutant Bacteria for Bioremediation

Mutations are classified as induced or spontaneous; induced mutations result from external mutagens, while spontaneous occur naturally [28]. Microorganisms create new genes through mechanisms like gene duplication, lateral transfer, de novo originations, transposable element insertions, mutations, translocations, gene fusion, and gene fission [29]. These genetic changes often improve enzymatic systems, substrate specificity, and resilience in contaminated environments [30]. Mutation in bioremediation mainly enhances catabolic pathways by overexpressing or structurally changing degradative enzymes such as oxygenases, dehydrogenases, and hydrolases, increasing pollutant breakdown efficiency. Mutations in dioxxygenase genes can boost degradation of aromatic compounds, aiding in recalcitrant pollutant removal [30, 31]. Additionally, mutations improve stress tolerance in microbes, helping them survive toxic substances, extreme pH, salinity, or heavy metals. These resistant strains enhance in situ bioremediation [32]. Techniques like chemical mutagenesis, UV irradiation, and transposon mutagenesis produce strains with better degradation abilities, while molecular biology enables targeted gene modifications. Synthetic pathways with heterologous enzymes have transformed bacteria into efficient pollutant-degrading agents [33]. Synthetic biology offers ways to sustain microbial populations, especially in degrading plastics like micro- and nanoplastics, as demonstrated by extensive research on genetically engineered microbes [9].

Recent advances in molecular biology have been applied to microorganisms to develop strains with desirable bioremediation properties, including constructing or adapting catabolic pathways, redirecting carbon flux, modifying enzyme affinity and specificity, enhancing genetic stability, increasing pollutant bioavailability, and improving process efficiency [34].

Genetic engineers create genetically engineered microorganisms (GEMs) with broad-spectrum catabolic capabilities [2]. Biotechnological tools used include enzyme engineering, genome mining, strain engineering, and metagenomics [35]. Techniques such as antisense RNA, PCR, mutagenesis, and suitable hosts are employed [36]. Mutagenesis methods include UV mutagenesis causing base conversions or deletions, and laser mutagenesis using heat, light, and electromagnetic fields to induce mutations [37]. Molecular tools for GMO construction include biolistic transformation, electroporation, conjugation, DNA transfer, cloning, and protoplast transformation [38]. Major gene editing tools are being user-friendly and highly specific and is mainly used with *Pseudomonas* and *E. coli* in bioremediation research [39–41].

Microbial mutagenesis via atmospheric-pressure plasma (ARTP) involves generating reactive species that damage DNA, activating repair enzymes and generating mutations. Compared to CRISPR, ARTP offers broader pollution control potential without gene transfer risks [42, 43]. Compared with traditional mutagenesis methods like chemical mutagenesis, breeding, and X-ray irradiation, ARTP mutagenesis offers higher mutation rates, greater mutational diversity, and is faster and more stable [44]. Reporter genes are used to create genetically engineered microbial biosensors, where enzymatic activities produce colourimetric, fluorescent, or luminescent signals as indicators for contaminant detection [45]. An engineered *E. coli* strain was developed by introducing the p-Nitrophenol (PNP) pathway via the hydroquinone pathway, with a five-gene PNP degradation module from *P. putida*, granting it new PNP-biodegradation abilities [46]. Artificial microbial consortia, mimicking natural ones to perform complex biological processes, have become an important research focus in synthetic biology [47].

4. Advantages of Mutant Bacteria in Bioremediation

Biotechnologically induced microorganisms are more powerful than naturally occurring ones and may degrade contaminants faster because they can quickly adapt to new pollutants they encounter or co-metabolise [40]. Abatenh *et al.* [48] reported the advantages of GEMs in bioremediation to include speeding up the recovery of waste-polluted sites, increasing substrate degradation, displaying a high catalytic or utilisation capacity with a small amount of cell mass, and creating safe and purified environmental conditions by decontamination or neutralising any harmful substances. Ojuederie and Babalola [49] reported that the utilisation of genetic engineering ensures greater opportunities for obtaining effective pollutant-degrading microorganisms, as they could possess a higher potential for environmental cleanup than the indigenous microbes. The cyanogenic capabilities of *C. violaceum* and its promising role in the targeted application of gold bio-recovery

from e-waste make it an attractive biological chassis for synthetic biology. Han *et al.* [50] reported that even though established industrial processes for plastic recycling exist, the biological degradation of plastics could offer an ecologically

friendlier strategy. In Table 1, we present the metabolic advantages that mutant bacteria possess over wild-type strains in advancing environmental cleanup.

Table 1. Practical advantages of mutant bacteria in the biodegradation of Environmental pollutants.

Feature	Wild-Type Limitation	GM Strain Example	Key Genetic Modification	Remediation Target compounds	References
Expanded Substrate Range	Inability to degrade complex artificial chemicals.	<i>Pseudomonas putida</i> KT2440	Insertion of the BPH (biphenyl) operon.	PCBs and halogenated aromatics.	[51, 52]
Metal Sequestration	Low binding capacity; sensitive to toxicity.	<i>Escherichia coli</i>	Overexpression of metallothionein (MT2).	Cadmium (Cd) and Mercury (Hg).	[53]
Enhanced Kinetics	Slow natural enzyme turnover.	<i>Ideonella sakaiensis</i> (Mutant)	Engineered PETase (e.g., S238F/W159H).	PET Plastics (faster crystallinity breakdown)	[54]
<i>In Situ</i> Monitoring	Hard to track cleanup progress.	<i>Pseudomonas fluorescens</i> HK44	Fusion of luxCDABE to the salicylate promoter.	Naphthalene (bacteria glow during degradation).	[55]
Solvent Tolerance	Cell membrane lysis in high oil/fuel zones.	<i>Pseudomonas putida</i> S12	Upregulation of solvent efflux pumps (<i>srpABC</i>).	Toluene and organic solvents.	[56]
Biomining	Slow natural precipitation of metals.	<i>Citrobacter</i> sp.	Insertion of <i>phoN</i> (acid phosphatase).	Uranium (U) and Nickel (Ni).	[57]

4.1. Role of Genetically Modified Bacteria in Plastic Polymer Biodegradation

Plastics are long-chain carbon compounds that also include hydrogen, sulfur, and nitrogen. These materials are classified into several types, including natural, semi-synthetic, synthetic, thermoplastic, and thermosetting varieties. Due to their affordability, durability, and chemical stability, synthetic plastics have become essential to modern life. While plastics have profoundly transformed society through their versatile uses, their widespread deployment has led to a critical accumulation of waste across both human-made and ecological environments [58]. The build-up of plastic presents a significant environmental crisis, mainly because these materials do not biodegrade naturally. These persistent, insoluble polymers can endure for years before breaking down. Exacerbating this problem is the global consumption of between 500 billion and 1 trillion plastic bags annually, which destabilises ecosystems, pollutes land and air, and poses a serious threat to living organisms [59].

While numerous studies have documented bacterial biodegradation of synthetic plastics, applying this process practically faces substantial challenges. Most synthetic polymers are highly resistant to microbial breakdown, and even when biodegradation occurs, it is an exceptionally slow process that can take several years. Moreover, the absence of a standardised operational definition for plastic biodegradation complicates the development of testable hypotheses and the creation of a solid biochemical framework [60]. A key limitation in current research is reliance on controlled, culture-based laboratory methods; these static conditions do not replicate the complex and dynamic variables of natural habitats [61]. Therefore, while the biodegradability of certain synthetic plastics has been demonstrated in principle, further rigorous research is needed to bridge the gap between laboratory results and practical, large-scale waste management solutions.

The development of methods that can effectively enhance the biodegradation of these polymers has become a fundamental strategy for reducing the environmental impact of plastic persistence, as most developing nations lack advanced technological facilities and proper rules and regulations concern-

ing plastic production, use, and waste management [62]. Biotechnological advancements such as bioinformatics, genetic engineering, and the cultivation of synthetic microbial consortia have been adopted to address issues associated with traditional disposal methods such as burning and landfilling. Genetic engineering tools have been instrumental in altering the genetic makeup of microorganisms to boost their capacity to degrade plastic contaminants.

Progress in genetic engineering, especially through recombinant DNA technology and gene cloning, has notably improved the ability of microorganisms to remediate environmental pollutants like plastics. These techniques involve precise modifications of enzyme active sites to optimise substrate interaction, such as increasing the hydrophobicity of the binding groove and neutralising surface electrostatic charges. This reduces repulsion and enhances the affinity between the enzyme and the plastic substrate. Further structural modifications refine these interactions by improving hydrophobic packing and catalytic flexibility. In addition to molecular im-

provements, selecting suitable microbial hosts is vital for diverse environmental applications. Halophilic bacteria are increasingly targeted for marine plastic degradation because of their high salt tolerance, while fungi are being investigated for their unique spore dispersal mechanisms. *Escherichia coli* continues to be a central organism in this field, serving as a reliable platform for expressing high levels of plastic-degrading enzymes such as laccase and manganese-dependent peroxidase. Notable breakthroughs include the development of BIND-PETase in engineered *E. coli*, which has shown the ability to depolymerise 9.1% of highly crystalline post-consumer PET waste within seven days under ambient conditions, offering a promising foundation for sustainable biocatalytic recycling strategies [63].

Several studies have investigated the use of genetically modified bacteria in enhanced plastic polymer bioremediation. Table 2 below shows the application of genetic manipulation of bacteria for enhanced breakdown of plastic polymers, improving degradation potential compared to wild-type strains.

Table 2. Applications of mutation and genetic modification of bacteria in the enhancement of plastic biodegradation.

Microorganism / Enzyme	Modification Method	Plastic Type	Key Results / Improvements over wild-type strains	References
<i>Pseudomonas aeruginosa</i> D5	UV Irradiation	LDPE	9% better weight reduction than wild type (28 days).	[64]
<i>Ideonella sakaiensis</i> (PETase)	Active site mutation	PET	2.5x higher activity; 22.5 mg per $\mu\text{mol L}^{-1}$ per day.	[65]
<i>Pseudomonas putida</i>	UV Mutation	LDPE	34% weight loss vs. 11% in wild type (60 days).	[66]
<i>E. coli</i> PHL628	BIND Strategy (Genetic Fusion)	PET (Microplastics)	Degraded microplastics from bottles and water plants.	[67]
<i>Thermobifida fusca</i> (TfCut2)	Single-site substitution	PET	2.7-fold increase in weight loss.	[68]
<i>E. coli</i> (from <i>P. aestuans</i> gri)	Gene Cloning	Amorphous PET	2.5x higher degradation than parental strain.	[69]
<i>Stenotrophomonas paucimotus</i>	Overexpression (DuraPETase)	PET (BHET)	100% degradation of BHET within 4 hours at 30 °C.	[70]
PES-H1 (Variant L92F/Q94Y)	Protein Engineering	PET Waste	3.4-fold improved activity against real-world waste.	[71]
Bacterium XZ-A	ARTP Mutagenesis	PE (Microplastics)	53.65% increase in degradation efficiency.	[37]
<i>P. putida</i>	Genomic Integration	PET / PBAT	Successfully metabolised terephthalic acid (TA).	[72]
Cutinase-CBMCenA	Site-directed mutagenesis	PET Fiber	1.4–15fold improvement in catalytic efficiency.	[73]
Modified Cutinase	Genetic Modification	PET	12 mg/h/mg vs 0.05 mg/h/mg for native enzyme.	[74]
BS-10L	Plasma Treatment	LDPE	27.78% mass loss vs. 13.40% for untreated.	[75]

Microorganism / Enzyme	Modification Method	Plastic Type	Key Results / Improvements over wild-type strains	References
<i>Bacillus velezensis</i> (BCAv-PEase)	Pathway Engineering	PE (Microplastics)	Enhanced MP degradation and microbial abundance.	[76]
Amidases (PA_PBM fusion)	Enzyme Absorption Improvement	Polyurethane	4x more active than the native enzyme.	[77]
Cutinases	DM Strategy	PBAT	Achieved complete biodegradation to monomers.	[78]

4.2. Application of Genetically Modified Bacteria for Petroleum Hydrocarbons Biodegradation

Environmental contamination from oil hydrocarbons mainly results from crude oil spills and the discharge of industrial wastewater from petroleum refineries [79]. Large oil spills can cause extensive harm to marine life, as exposure to these substances often results in immediate toxicity or death among aquatic organisms [80]. Microbes are known to aid in the biodegradation of hydrocarbons by breaking down pollutants into energy, carbon, and nitrogen for their own use, thanks to specialised metabolic processes that convert hazardous substances into fuel for essential cell functions [81, 82]. For cleaning highly hydrocarbon-contaminated sites, the use of xenobiotic-resistant genetically modified microorganisms is more appropriate, as natural microbial populations cannot generally fully break down these substances [83, 84]. Genetically modified bacteria have transformed hydrocarbon bioremediation by combining metabolic efficiency with real-time monitoring. An early example is *Pseudomonas fluorescens* HK44, engineered with the luxCDABE gene cassette to both degrade naphthalene and emit bioluminescent signals, enabling researchers to track its activity in contaminated soil [85]. Similarly, strains such as *P. putida* KT2442 and different *Escherichia coli* derivatives have been developed to target specific aromatic compounds, utilising genes like bphA2cA1c and nahH to break down salicylate, phenanthrene, and pyrene [86, 87].

Beyond simple degradation, these engineered strains can transfer catabolic plasmids—such as pNF142 or the petroleum-degrading pWW0—to native microorganisms via horizontal gene transfer [88]. This process effectively "teaches" existing soil microbes how to process pollutants. Research into strains like *Mycobacterium* sp. 6PY1 and *Ralstonia eutropha* further demonstrates that introducing these tailored genes greatly enhances the breakdown of complex toxins like 2,4-dichlorophenoxyacetic acid and multi-ringed pyrenes, providing a sophisticated solution for restoring contaminated environments [80, 89]. In a comparative study of hydrocarbon remediation, Ogujiofor *et al.* [90] examined the degradative

effectiveness of wild-type and mutant strains of *Pseudomonas putida* and *Bacillus subtilis* isolated from refinery effluents. Over 15 days, they monitored Hydrocarbon Utilizing Bacterial (HUB) counts and reductions in oil and grease concentrations at three-day intervals. They found that UV-mutant strains showed superior metabolic activity, with higher HUB counts than their wild-type equivalents. Conversely, mutagenesis caused by nitrous acid resulted in the lowest HUB counts among all tested groups. They also observed that the oil and grease degradation efficiency mirrored these microbial growth trends. The UV-irradiated mutants achieved the highest performance, with degradation rates of 96.23% for *P. putida*, 92.60% for *B. subtilis*, and an almost complete 99.38% for the co-culture. These results outperformed the wild strains, which peaked at 88.89% in co-culture, and were significantly better than the nitrous acid mutants, which recorded the lowest degradation levels. Ultimately, the study concludes that although indigenous strains of *B. subtilis* and *P. putida* are naturally effective at breaking down hydrocarbons, UV irradiation markedly improves their efficiency, and the combined action of microbial co-cultures consistently yields the most robust remediation outcomes.

In another study, Zhang *et al.* [91] isolated, identified, and optimised a high-efficiency petroleum-degrading bacterium, *Enterobacter* sp. strain MX1, from petrochemical wastewater sludge. Under these optimal conditions, the wild-type strain managed a crude oil degradation rate of 37.41% over seven days, reaching 61.08% after 21 days. To enhance this natural capability, researchers used a composite mutagenesis approach, combining 30 seconds of microwave exposure with four minutes of ultraviolet (UV) irradiation. This produced the mutant strain MXM3U2, which showed a significantly improved petroleum hydrocarbon breakdown efficiency of 56.74% within just seven days—a 51.66% increase over the wild type. Shahi *et al.* [92] assessed microbial populations and functional genes during bioremediation of petroleum-contaminated soil as an effective monitoring method. Their results indicated that gene expression analyses identified gram-negative bacteria and alkB as crucial to successful bioremediation.

Baoune *et al.* [93] inoculated *Zea mays* with *Streptomyces* sp. H1W to remove crude oil pollution; they recorded 70% degradation of hydrocarbons (C-8 to C-30). Their bacterial

consortium, comprising two salt-tolerant, biosurfactant-producing bacteria—*Dietzia* sp. CN-3 and *Acinetobacter* sp. HC8-3S—degraded 95.8% of crude oil (including n-alkanes, cycloalkanes, and aromatic hydrocarbons) in 10 days, outperforming individual isolates [94]. Liu *et al.* [95] applied targeted mutagenesis through genetic deletion of *alkMa* and *alkMb* genes to study specific alkane hydroxylases in *Acinetobacter venetianus* RAG-1. These engineered strains achieved higher degradation rates than the wild type, reaching up to 90.1% for different crude oil chain lengths (C10–C38), with excellent emulsification activity and cell surface hydrophobicity, along with broad environmental tolerance.

4.3. Application of Genetically Modified Bacteria for Heavy Metals Remediation

Heavy metal pollutants present a serious environmental threat due to their high toxicity, environmental persistence, and significant potential for bioaccumulation. These contaminants endanger both aquatic and terrestrial ecosystems while posing serious risks to human health. Resulting from a combination of natural phenomena and human activities, heavy metal levels often surpass safe thresholds, disrupting global biogeochemical cycles and contaminating various food chain levels. Unlike organic pollutants, these inorganic contaminants are not broken down by metabolism, leading to their gradual build-up within the food chain. Once inside the body, heavy metals interfere with cellular balance, impair enzymatic functions, and cause oxidative stress, which can lead to multi-organ failure and cancer [96]. The following summary highlights the specific pathological impacts and toxicological profiles linked to key heavy metal species.

Heavy metals can have damaging effects on different organs and systems. Exposure to cadmium (Cd^{2+}) reduces cell vitality and causes apoptosis, leading primarily to kidney failure, liver injury, and bone weakening. Long-term intake is linked to fractures and reproductive problems [96, 97]. Mercury (Hg) is a toxicant affecting multiple systems, damaging the nervous, digestive, and immune systems, and presents as cognitive issues, high blood pressure, and severe dysfunction in the lungs, kidneys, and eyes [98]. Lead (Pb) continues to be a major neurotoxin, especially harming cardiovascular and nervous health. In children, it impairs neurodevelopment, disrupts the gastrointestinal system, and can cross the placental barrier, harming fetal brain development and circulation [99]. Arsenic (As) mainly causes skin problems like hyperkeratosis and pigmentation changes, which may develop into skin cancer [100].

Genetic engineering combined with bioremediation has been shown to aid in the manipulation of bacterial genomes, which can improve the detoxification of toxic metals—a process not typically performed by ordinary bacteria. Microorganisms employ a variety of sophisticated strategies to survive and interact in metal-contaminated environments, including active extrusion, biotransformation, enzymatic degradation,

and the production of exopolysaccharides and metallothioneins. Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins that bind metals. Modifying the genes of microbial membrane transporters can make the removal of heavy metals from soils more effective, as transporters and binding mechanisms are essential for this process [101]. In addition to enzymatic transformation, mercury can be bioremediated through the use of metallothioneins and polyphosphates, which sequester mercury ions in a biologically inactive form [102].

Bacteria exhibit substantial detoxification capabilities through physicochemical processes such as ion exchange, surface complexation, precipitation, redox reactions, and electrostatic interactions [102]. They employ key resistance mechanisms, including modifying metal valency via methylation or demethylation, synthesizing metal chelators like bio-surfactants, and utilizing specialized efflux pumps to expel toxins. Additionally, bacteria cope with metal stress by sequestering metals intracellularly or extracellularly, establishing permeability barriers to prevent entry, destroying metal ligands, and facilitating metal-organic complexation or oxidation [103]. Genetically modified (GM) and mutant bacteria act as high-efficiency "bio-sponges" and catalytic agents in remediating heavy metal-contaminated environments. While natural isolates often possess inherent resistance, GM bacteria are engineered with precision—such as the addition of poly-cysteine tags or optimized metallothioneins (e.g., ShMT3)—to significantly increase intracellular binding sites and tolerance. Conversely, mutant bacteria created through chemical or physical induction (e.g., acridine orange or UV radiation) display enhanced metabolic resilience and altered surface chemistry, enabling near-quantitative removal efficiencies (up to 99.4%) across diverse pH ranges. Together, these advanced microbial strains surpass the limitations of wild-type organisms by accelerating the biotransformation of toxic ions into stable forms and maximizing bioaccumulation, providing a scalable and cost-effective approach for restoring industrial and terrestrial ecosystems.

Rojas *et al.* [104] generated a broad-spectrum mercury-resistant strain, MSR33, by incorporating plasmid pTP6, which was directly isolated from the environment, into *C. metallidurans* CH34. The strain MSR33 was found capable of removing mercury from polluted waters. *Bacillus subtilis* as a chassis organism successfully engineered the bacterium to sense and remove Pb^{2+} , Cu^{2+} , and Hg^{2+} , achieving metal removal efficiencies above

Dash and Das [105] in their investigation reported a significant advancement in molecular bioremediation through the development of the transgenic strain *Bacillus cereus* BW-03(pPW-05). By mobilizing a mer operon-bearing plasmid from the marine isolate *B. thuringiensis* PW-05 into the mercury-resistant host *B. cereus* BW-03, the researchers engineered a robust microbial platform capable of dual-mode mercury detoxification. This transformant achieved a mercury removal efficiency exceeding 99% *in vitro*, utilizing a synergis-

tic pathway of enzymatic volatilization (>53%) and cell-surface biosorption (~40%), the latter of which was facilitated by the interaction of mercury ions with sulfhydryl (-SH) and carboxyl (-COOH) functional groups.

The engineered strain demonstrated profound physiological plasticity, maintaining its remediative efficacy across diverse environmental gradients, including fluctuations in salinity (5–30 ppt), varying pH levels, and acute mercury concentrations up to 50 ppm. Notably, the study established that microbial encapsulation could optimize sequestration potential to near-quantitative levels (~100%). Furthermore, microcosm simulations indicated that the transgenic strain could persist for seven days and operate synergistically with indigenous microbiota resulting in a 96.4% reduction in inorganic mercury, underscoring its viability for the bioaugmentation of mercury-contaminated marine and industrial effluents.

In a study by Li *et al.* [106], the primary structure of metallothionein from *Sinopotamon henanense* (ShMT) was engineered using site-directed mutagenesis. The results demonstrated that these structural modifications significantly bolstered the proteins' ability to bind heavy metals. When expressed in *Escherichia coli*, the mutant proteins improved cellular tolerance and the bioaccumulation of Cd²⁺, Cu²⁺ and Zn²⁺. Crucially, this enhanced performance followed a clear dose-response relationship correlated with the density of point mutations, where the most heavily modified variant (SUMO-ShMT3) outperformed the intermediate versions and the wild-type control.

Yetunde Mutiat *et al.* [107] demonstrated the efficacy of chemical mutagenesis as a strategic approach for optimizing the bioremediation potential of *Klebsiella variicola* against cadmium (Cd) contamination. By exposing wild-type isolates to the mutagenic intercalating agents; acridine orange and ethidium bromide, the researchers induced physiological enhancements that significantly bolstered the bacterium's sequestration efficiency. The experimental data revealed that while the wild-type strain exhibited a substantial Cd removal rate of 97.9% at pH 7, the chemically induced mutants displayed superior performance and adaptive resilience to varying environmental conditions. Specifically, the acridine orange mutant (Mut Ac) achieved a near-quantitative removal efficiency of 99.4% at neutral pH. In contrast, the ethidium bromide mutant (Mut Eb) demonstrated peak performance in more alkaline conditions, maintaining a 97.7% efficiency at pH 8. These findings underscore the utility of random mutagenesis in refining microbial metabolic responses to heavy metal stress, providing highly efficient biological candidates for the treatment of industrial effluents across a broader pH spectrum.

5. Deployment Approaches for Engineered Bacteria in Bioremediation

The successful application of engineered bacteria in environmental cleanup depends not only on their design but also

on the strategies used to deploy them effectively in contaminated ecosystems. Deployment approaches must consider the nature of the pollutant, the environmental matrix (soil, water, sediment), microbial survival, and containment to prevent unintended ecological impacts. Several deployment strategies have been developed, ranging from direct inoculation to encapsulation and controlled bioreactor systems.

5.1. Direct Inoculation (Bioaugmentation)

Direct inoculation, or bioaugmentation, involves introducing engineered bacteria directly into contaminated soils, sediments, or water bodies. This approach is widely used for hydrocarbons, heavy metals, and xenobiotic compounds. For example, modified *Pseudomonas putida* strains can be directly applied to petroleum-contaminated soils to accelerate the degradation of toluene and benzene. Successful bioaugmentation requires optimization of bacterial inoculum size, compatibility with native microbial communities, and environmental conditions such as pH, temperature, oxygen availability, and nutrient supply. While effective in controlled settings, direct inoculation faces challenges related to microbial survival and competition with indigenous microbiota [108].

5.2. Immobilization and Encapsulation

To improve survival and stability, engineered bacteria can be immobilized or encapsulated in materials such as alginate beads, biochar, or polymer matrices. Immobilization protects microbes from environmental stress, prevents washout in flowing water, and can provide a sustained release of bacterial activity over time. For instance, encapsulated PETase-producing bacteria have been tested for plastic degradation in aqueous systems, where direct application would otherwise result in rapid loss or inactivation of the cells. Encapsulation also enables safer deployment by containing genetically modified organisms and minimizing ecological risks [109].

5.3. Bioreactors and Ex Situ Systems

Ex situ bioreactors offer controlled environments for efficient pollutant removal before releasing treated water or soil back into the environment. Engineered bacteria are particularly effective in bioreactors designed for hydrocarbon-contaminated wastewater, pharmaceutical residues, or dye-laden industrial effluents. Bioreactors allow precise control over temperature, pH, oxygen levels, and nutrient supply, maximizing microbial activity and pollutant degradation rates. Additionally, bioreactor deployment reduces the risk of horizontal gene transfer of engineered traits to native microbes [110].

5.4. Rhizosphere and Plant-Associated Deployment

For soil remediation, engineered bacteria can be applied in

the rhizosphere, where they associate with plant roots to enhance pollutant degradation while promoting plant growth. Plants can facilitate pollutant bioavailability through root exudates, while bacteria degrade contaminants in situ. Engineered strains with heavy metal sequestration capabilities or hydrocarbon degradation pathways have been used in conjunction with phytoremediation strategies, providing a synergistic approach to soil cleanup [15].

5.5. Biosensor-Assisted Deployment

Biosensor strains can be deployed alongside remediation efforts to monitor pollutant concentrations in real time. Engineered bacteria with reporter genes under pollutant-responsive promoters allow continuous assessment of contaminant levels, enabling dynamic adjustments to remediation strategies and optimization of bacterial activity. This approach is particularly valuable in complex environmental matrices, where pollutant distribution and bioavailability may vary spatially and temporally [111].

5.6. Safety and Containment Considerations

Deployment strategies must also address biosafety. Engineered bacteria are often equipped with kill switches, auxotrophy, or other containment mechanisms to prevent uncontrolled proliferation. Regulatory frameworks and field containment protocols guide the application of genetically modified microbes to minimize ecological risks while maximizing remediation efficiency [112].

6. Risk, Safety, and Ethical Frameworks in Engineered Bacterial Bioremediation

The deployment of genetically modified microorganisms (GMMs) for environmental restoration requires comprehensive biosafety frameworks to reduce potential ecological and public health risks. Main concerns include horizontal gene transfer (HGT), which may lead to the unintended spread of engineered traits to native populations, and disruption of microbial communities through competitive displacement [113, 114]. Health risks associated with accidental acquisition of virulence factors or antibiotic resistance must be managed through strict Biosafety Level (BSL) protocols.

To ensure biosafety, researchers apply advanced biocontainment methods, such as metabolic auxotrophy and genetic kill switches that induce programmed cell death without synthetic substrates [113]. Physical containment using encapsulation further limits environmental dispersal. These measures are supported by strict regulatory oversight from agencies like the EPA and EFSA, which require detailed assessments of environmental persistence and gene stability before field deployment. Incorporating real-time monitoring and adaptive risk

management provides a strategic balance between biotechnological advantages and ecological integrity [111].

The interest in transgenic microorganisms for various environmental applications, which surged during the 1980s, has sparked ethical debates about their use in open environments [115]. Skepticism remains regarding the field applications of GMOs for pollutant cleanup, mainly due to concerns over horizontal gene transfer and displacement of native organisms. Transfer of genetic material between bacteria is a significant obstacle in developing engineered microbial systems. However, employing suitable design techniques during bacterial system development can greatly reduce these risks. Such transfer, especially in response to environmental changes, allows organisms—particularly prokaryotes—to access genes beyond those inherited naturally [116]. When GMOs are introduced into ecosystems, they may cause unintended environmental consequences, including increased replication rates that lead to dominance and long-term effects on native systems. Jafari *et al.* [117] noted that engineered strains might not survive long in soil once their specific substrate is depleted. To address these issues, technologies like suicidal GEMs or biocontainment systems—using toxin/antitoxin gene systems—are considered promising, efficient, and environmentally friendly. Genetic engineering offers solutions such as equipping bacteria in plastic-polluted areas with enzymes for plastic degradation. However, careful monitoring and strategies for programmed cell death are essential once bioremediation is complete or if gene transfer occurs [118].

Furthermore, the safety and biohazard concerns associated with potentially released GM bacteria demand thorough assessment [119]. While gene-editing tools like CRISPR offer significant potential, their misuse raises substantial ethical and religious issues due to the risks of illegal germline alterations. Off-target effects of CRISPR can result in lethal mutations or genetic disorders, emphasizing the importance of careful use to avoid unintended outcomes [120].

7. Future Prospects and Research Directions

This review has covered recent developments in the application of genetically modified organisms for the bioremediation of pollutants, as well as the ethical concerns. We have been able to establish that GMOs have the ability to clean up a variety of pollutants, particularly plastics, crude oil hydrocarbons and heavy metals. Moreover, when GMOs are used in conjunction with other physical and chemical methods, bioremediation can provide a comprehensive approach toward removing pollution from the environment. However, strict regulations on the applicability and field trials of genetically modified microbes are yet another factor that should be addressed to evaluate the success rates of further research in this direction. The use of enzymes to treat the problem of this polymer has the advantage of being harmless to living beings and the environment, so further

research should be carried out. Atmospheric and room temperature plasma (ARTP) is emerging as a powerful mutagenesis tool due to its rapid, non-thermal generation of diverse mutations via reactive chemical species, electromagnetic energy, and cell membrane disruption. Unlike UV or chemical mutagens, ARTP operates under mild, non-toxic conditions, making it especially adaptable to a range of microbial taxa, including bacteria, fungi, and microalgae. While ARTP has not yet been widely applied to microbes engineered for specific pollutant degradation (e.g. lindane, TCP), its success in enhancing metabolic and stress-tolerance traits with its well-developed protocols, including helium flow rates, energy input, and exposure durations that can be adapted to pollutant-degrading strains, makes it well-suited for future studies.

Abbreviations

PAHs	Polycyclic Aromatic Hydrocarbons
MNPs	Micro- and Nano-plastics
PFAS	Per- and Polyfluoroalkyl Substances
UV	Ultraviolet
PNP	p-Nitrophenol
PCB	Polychlorinated Biphenyl
PET	Polyethylene Terephthalate
LDPE	Low-Density Polyethylene
BHET	Bis(2-Hydroxyethyl) Terephthalate
PBAT	Polybutylene Adipate Terephthalate
GMOs	Genetically Modified Organisms
GEMs	Genetically Engineered Microorganisms
ARTP	Atmospheric and Room Temperature Plasma
DNA	Deoxyribonucleic Acid
HGT	Horizontal Gene Transfer
GMM	Genetically Modified Microorganism
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
UNESCO	United Nations Educational, Scientific and Cultural Organization
EPA	Environmental Protection Agency
EFSA	European Food Safety Authority
BSL	Biosafety Level

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Conflicts of Interest

The authors declare no conflicts of interest.

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