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Harnessing Genetic Engineering for Environmental Clean-up: A Review

Terhide Samuel Tyohemba[1,2]*, Danjuma Yakubu [2], Joy Joseph Guzol[2], Wendy Chinenye Austin-Amadi[2] ,Wilson Kitime Jonah Joyous[2] and Hajjagana Hamza[2]

[1]Department of Biological Sciences, Facu;ty of Science, Federal University, Kashere, Gombe State, Nigeria

[2]Department of Biochemistry, Faculty of Science, Gombe State University, Gombe State, Nigeria

Corresponding Author: terhide27@gmail.com

ABSTRACT

Environmental pollution caused by industrialization and urbanization poses significant risks to living organisms. Environmental pollutants, including microscopic particles, volatile organic compounds, and heavy metals, are recognized as catalysts for respiratory and cardiovascular illnesses in humans. The existing treatment methods available for addressing such environmental concerns include physical and chemical methods. However, chemical and physical remediation methods have limitations such as inefficiency and equipment, high costs, and the risk of introducing additional environmental pollution. In contrast, biological remediation is a more efficient, cost-effective, and environmentally friendly solution. Bioremediation employs plants and microorganisms to transform pollutants; however, this capacity does not give them the final advantage of cleaning pollutants completely. This paper examined the advantages of using genetically modified microorganisms as an economical and eco-friendly solutions to mitigate the negative effects of heavy metal pollution, which can have detrimental consequences on human well-being and the natural environment. This paper further explored the bioremediation mechanisms, including enzymatic oxidation, enzymatic reduction, biosorption, complexation, bioaccumulation, and precipitation. These mechanisms involve various microorganisms, such as algae, bacteria, and fungi, which aid in the mitigation of heavy metal pollution. The paper also discusses the use of metabolic and genetic engineering in bioremediation to enhance the degradation capabilities of microorganisms and improve the bioremediation process. The use of genetically modified microbes for degradation of synthetic pollutants has been highlighted. In conclusion, this paper emphasizes the need for continued research on high-throughput microbial technologies and metagenomics to advance the field of bioremediation and develop effective strategies for pollution mitigation.

Keywords: Bioremediation, Environmental pollution, Heavy metal contamination, Microorganisms, Genetic engineering, Pollution mitigation

INTRODUCTION

The development of cities and industrialization have resulted in increased exposure to harmful pollutants in the environment, which can have detrimental effects on living organisms. Manufacturing and industrial activities significantly contribute to the contamination of soil and water resources, discharging diverse heavy metals in varying quantities during their operational processes and waste disposal. For example, wastewater from dye manufacturing contains Hg, Sb, and Cr (Methneni *et al.,* 2021). Agricultural practices involving herbicides, fertilizers, and pesticides can result in the production of pollutants including copper, aluminum, zinc, lead, nickel, and

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arsenic.(Ayilara *et al.,* 2020; Prabagar *et al.,* 2021). Additionally, when untreated waste from agricultural food industries is discharged into water bodies like rivers, it can have detrimental effects on the ecosystem (Siric *et al.,* 2022a; AL-Huqail *et al.,* 2022).Environmental contamination is significantly impacted by crude oil, which can result from various sources including pipeline sabotage, transport leakages, and unintentional spillages (Ogunlaja *et al.,* 2019). Mineral extraction processes release toxic elements like lead, arsenic, cadmium, and copper into surrounding ecosystems (Liu *et al.,* 2020). Additionally, the mining industry often utilizes hazardous chemicals such as cyanide and sulfuric acid in its operations (Ayangbenro *et al.,* 2018; Orlovic-Leko *et al.,* 2022).Byproducts from industrial processes, such as those generated during cement production, contaminate the top layers of soil with harmful elements like copper, cadmium, and zinc (Jafari *et al.,* 2019). Additionally, waste from pharmaceutical production adds to environmental degradation through the discharge of lead and Chromium (Kumari and Tripathi, 2020). Furthermore, water can be contaminated by plastics that contain various heavy metals, including manganese, lead, iron, chromium, copper, silver, cadmium, mercury, and antimony (Zhou *et al.,* 2019). The coal industry also presents significant environmental hazards due to the presence of heavy metals such as arsenic, copper, mercury, lead, chromium, nickel, zinc, and cadmium (Sun *et al.,* 2019).

These heavy metals mentioned earlier present considerable threats to ecosystems in water and on land. Human exposure to mercury, cadmium, and lead can cause disruptions to the central nervous system, with infants being particularly vulnerable. Furthermore, lead exposure has been connected to impaired liver and kidney function, heart-related diseases, and compromised reproductive and immune systems (Ayangbenro and Babalola, 2019; Fashola *et al.,* 2020a; 2020b; Ayangbenro and Babalola, 2020). On the other hand, exposure to cadmium has been associated with a range of health issues, including skeletal disorders, various forms of cancer, renal impairment, neurological complications, and adverse effects on the reproductive system (Zwolak *et al.,* 2019; Fashola *et al.,* 2020a; Fashola *et al.,* 2020b; Ayangbenro and Babalola, 2020).The incorrect disposal of waste contaminated with heavy metals into soil and water bodies can result in the demise of aquatic life and the accumulation of these pollutants through the food chain, potentially leading to long-term illnesses in both humans and animals.

The pressing need for remediation techniques incorporating physical, chemical, and biological approaches is evident. Despite their long-standing use, physical and chemical methods face constraints, including the requirement for Conventional physicochemical remediation methods which are often expensive, due to limited resources, and may lead to secondary pollution, negatively impacting soil fertility and agricultural ecosystems (Zhan *et al.,* 2018; Chang *et al.,* 2018). Chemical bioremediation necessitates resources, while physical bioremediation incurs substantial expenses (Mahmood *et al.,* 2021). Consequently, exploring viable alternatives like biological remediation (bioremediation) is crucial. Bioremediation offers an efficient, eco-friendly, and economical solution for transforming pollutants (Sonune, 2021). Although both plants and microorganisms can be employed in biological remediation, microorganisms are typically favored due to their quicker growth rates and easier manipulation compared to plants (Hussain *et al.,* 2022). Additionally, microbes play a role in mitigating the impacts of heavy metals, boosting soil productivity, and stimulating plant development (Chaudhary *et al.,* 2023b).

Engineered microorganisms have emerged as a promising, cost-efficient, and eco-friendly approach to address heavy metal (HM) contamination. These modified organisms are particularly effective in this role due to their intricate degradation processes, capacity to accumulate heavy metals, and high toxicity tolerance. These characteristics make them well-suited to combat the significant risks that heavy metals pose to environmental health, human well-being, and the long-term viability of ecosystems (Barrios-Estrada *et al.,* 2018; Gong *et al.,* 2018).

Maintaining a healthy ecosystem requires the elimination and prevention of harmful pollutant spread through the use of biological materials (Jacob *et al.,* 2018). Researchers have explored various approaches to combat environmental contamination, including biological reduction and oxidation (evaporation), electrochemical methods (physicochemical treatment), innovative enzyme systems (biological molecule treatment), and reverse osmosis (landfill deposition) using microorganisms or plants (Bilal *et al.,* 2019). Phytoremediation, a technique employing green plants and beneficial soil bacteria, has demonstrated promise in breaking down and purifying pollutants in contaminated soil, water, and wastewater (Bhatt *et al.,* 2019; Bhatt *et al.,* 2021). This eco-friendly and non-invasive remediation method effectively removes metal pollutants from affected areas (Liu *et al.,* 2019; Dangi *et al.,* 2019).

Furthermore, the materials gathered from polluted areas can be used to extract valuable metals for commercial purposes through a method called phytomining, which can generate revenue. In addition, energy can be produced by burning plant biomass, and the land can be rehabilitated for sustainable farming and environmental preservation. Research conducted by other academics has explored the rationale, methods, and economic viability of phytoremediation (Yuan *et al.,* 2020).

This systematic review examines the connection between genetic engineering and bioremediation techniques in tackling environmental pollution. To gain a comprehensive understanding, we conducted an extensive literature review and collected insights from a diverse range of scientific journals, articles, and research studies. Our study examined the negative consequences of pollutants on the environment and living organisms, assessed various remediation techniques, elucidated the mechanisms of bioremediation, and scrutinized molecular strategies that support microbial remediation processes. Furthermore, we highlight the most recent advancements in microbial bioremediation, especially in genetic and metabolic engineering, to demonstrate their potential for sustainable pollution management.

THE NEGATIVE IMPACTS OF VARIOUS POLLUTANTS ON ORGANISMS

Research has demonstrated that human health is significantly impacted by exposure to air pollutants, resulting in a range of issues including respiratory ailments , developmental disorders, cancers, cardiovascular diseases, and early mortality (Pope *et al.,* 2019). One notable example is the connection between airborne particulate matter and an elevated risk of premature death (Pope *et al.,* 2019). Combustion processes produce nitrogen oxides, which are significant atmospheric pollutants. These compounds can irritate the respiratory tract, leading to symptoms such as breathing difficulties, coughing, and worsened asthma conditions (Zhao *et al.,* 2020). Another harmful pollutant, sulfur dioxide, a combustion by-product from fossil fuels, is known to trigger respiratory and cardiovascular conditions, including bronchoconstriction and coughing. A study conducted in China revealed a link between exposure to sulfur dioxide and higher mortality rates from respiratory illnesses (Luo *et al.,* 2015).

Sources like cleaning agents, paints, and vehicle exhaust release volatile organic compounds (VOCs), which can trigger irritation in the nose, eyes, and throat, along with symptoms such as nausea, headaches, and dizziness. Certain VOCs, including benzene, are not only irritants but also carcinogens, elevating the risk of leukemia (Bala *et al.,* 2021). Pollutants in water encompass various substances, including organic compounds, heavy metals, and pesticides, which can enter the human system through indirect ingestion or direct consumption of affected aquatic life. These pollutants present significant health hazards, such as neurological disorders, cancers, and reproductive complications. Studies have shown that exposure to heavy metals is associated with a higher risk of kidney damage and hypertension in humans (Wu *et al.,* 2018; Rai *et al.,* 2019).

Environmental pollutants have detrimental effects on animal health, causing a range of illnesses. For example, animals exposed to particulate matter (PM) may develop inflammation and damage to their respiratory systems, leading to conditions like asthma and chronic obstructive pulmonary disease (COPD) (Manisalidis *et al.,* 2020). Animals that ingest water contaminated with heavy metals, pesticides, and pharmaceuticals are at risk of developing reproductive issues, liver damage, and cancer (Hitt *et al.,* 2023). The presence of nitrogen dioxide in the environment hinders plant growth, decreases crop production, and contributes to the acidification of soil and the formation of acid rain (Manisalidis *et al.,* 2020). Similarly, sulfur dioxide plays a role in soil acidification and acid rain formation. Plants exposed to ozone may experience impaired photosynthesis and metabolic processes (Zuhara and Isaifan, 2018). In water-based ecosystems, excessive nitrogen levels can trigger eutrophication, which disrupts fish population diversity and promotes algal blooms (Zuhara and Isaifan, 2018).

TYPES OF REMEDIATION

Environmental pollution can be tackled using a range of remediation strategies, encompassing chemical, biological and physical approaches. Physical remediation techniques employ tools such as sorbent materials, skimmers, and booms to effectively contain oil spills (Vocciante *et al.,* 2019). Booms serve as physical barriers that absorb oil pollutants and prevent their spread, while skimmers and sorbents assist in the absorption and adsorption of pollutants after boom deployment (Kumari *et al.,* 2019). However, the buoyancy and roll response of booms can complicate their applications. Floating on the water's surface, buoyant booms extend their efficacy, while roll response indicates the force required to tilt the boom from its upright position. A greater roll response improves the effectiveness of cleanup efforts (Dhaka and Chattopadhyay, 2021).

The process of chemical remediation involves the introduction of substances like phosphates, biochar , clay minerals, silicocalcium materials, sulfides, and aluminum salts to neutralize and extract heavy metals from the environment (Xu *et al.,* 2022). This technique employs various mechanisms, including reduction, adsorption, oxidation, complexation, ion exchange, and precipitation

(Xu *et al.,* 2022). Although chemical remediation offers a rapid and effective solution, it carries the potential drawback of introducing additional pollutants into the environment (Xu *et al.,* 2022).

According to Kumar *et al.* (2021) and Patel *et al.* (2022), bioremediation offers an effective and environmentally friendly approach to addressing pollution issues. This technique utilizes organic entities, including plants and microorganisms, to restore polluted environments. The efficacy of bioremediation is influenced by various factors, such as the nature, site, and extent of contamination (Patel *et al.,* 2022). Microorganisms, in particular, have shown significant promise in eliminating environmental pollutants. Compared to plants, they offer numerous benefits, including simple cultivation methods, quick reproduction, and high adaptability. Consequently, expanding the application of microbial agents in bioremediation is essential for fostering environmental sustainability.

Microorganisms are essential in bioremediation, transforming hazardous materials into less harmful substances like water and carbon dioxide through a process called mineralization (Mahmoud, 2021; Kumar *et al.,* 2022). Various microorganisms, including fungi, bacteria, and algae, are found throughout nature and can absorb different pollutants by using diverse carbon sources, allowing them to exist in unusual habitats (Kour *et al.,* 2022). Their ability to adapt to specific environmental conditions further enhances their effectiveness, as demonstrated by acidophiles thriving in acidic environments, psychrophiles flourishing in cold environments, and halophiles surviving in salty conditions (Perera & Hemamali, 2022).

Bioaugmentation

Bioaugmentation is an efficient, rapid, and economical bioremediation technique that involves deliberately introducing microorganisms to contaminated areas to break down harmful pollutants (Mahmoud, 2021). This approach enhances the existing microbial community at the polluted site by adding external microbes. In certain instances, microorganisms from the contaminated area are extracted, genetically altered, and then reintroduced for remediation purposes. Genetic modification of native microbes becomes necessary when naturally occurring organisms lack the capacity to break down specific pollutants, requiring enhancements to their abilities. Another option is to introduce non-native microbes into polluted regions to assist in contaminant degradation. The effectiveness of these new strains is influenced by their capacity to thrive alongside resident microbes and adjust to the new surroundings (Ayangbenro and Babalola, 2016; Ayangbenro and Babalola, 2017; Goswami *et al.,* 2018; Babalola *et al.,* 2019). For instance, research has shown that *Burkholderia sp.* FDS-1 can degrade nitrophenolic compounds found in pesticide-contaminated soil when introduced to a polluted site, transforming them into less harmful substances at a mildly acidic pH and around 30°C (Goswami *et al.,* 2018; Ojuederie *et al.,* 2021).

Biostimulation

Biostimulation, a technique for soil remediation, enhances the natural cleanup process by introducing substances such as nutrients, metabolites, electron donors and acceptors, enzymes, and biosurfactants to activate native microorganisms (Ojuederie and Babalola, 2017; Ayangbenro and Babalola, 2018). This approach is recognized for its cost-effectiveness and eco-friendly nature, demonstrating considerable success in bioremediation efforts (Goswami *et al.,* 2018). Unlike bioaugmentation, biostimulation is typically favored as it gives an edge to

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indigenous microbes over introduced species (Sayed *et al.,* 2021), thus helping to preserve the ecosystem's natural microbial diversity balance. Research by Nivetha *et al.* (2022) highlighted the efficacy of diverse microbial organisms, such as *Rhodococcus sp*., *Bacillus spp*., *Staphylococcus sp*., *Pseudomonas sp*., *Klebsiella sp*., and *Citrobacter sp*., in bioremediation processes utilizing biostimulation techniques. Nevertheless, it is essential to address potential environmental concerns, including the risk of eutrophication due to excessive nutrient addition and contamination from artificial chemical origins.

Bioleaching

Bioleaching is a remediation method that employs microorganisms thriving in acidic conditions to extract heavy metals in solid form from sediment-based materials. This approach is especially useful for treating pollutants like sulfur and iron (Sun *et al.,* 2021; Bhandari *et al.,* 2023). Commonly used bacteria that oxidize iron or sulfur, including *Aspergillus sp., Acidothiobacillus thiooxidans.*, *Mucor sp*., *Cladosporium sp*., *Penicillium sp*., and *Rhizopus sp*., create an acidic environment that promotes the dissolution of heavy metals, effectively immobilizing them in a watery solution (Medfu Tarekegn *et al.,* 2020).

MECHANISMS OF BIOREMEDIATION

Microbes utilize diverse methods to remove pollutants from their surroundings, which can be classified into two primary categories: immobilization and mobilization (Ndeddy Aka and Babalola, 2016; Verma and Kuila, 2019). Mobilization approaches include enzymatic oxidation and reduction, bioleaching, biostimulation, and bioaugmentation. In contrast, immobilization techniques involve complexation, biosorption, bioaccumulation, and precipitation (solidification) (Tak *et al.,* 2012; Ayangbenro *et al.,* 2019).). During mineralization, microbes facilitate the conversion of pollutants into final products such as carbon dioxide, water, or intermediate metabolic compounds. In contrast, immobilization involves transforming substances into forms that are less accessible in the environment, such as converting nitrate-nitrogen to organic nitrogen (Pratush *et al.,* 2018). Immobilization methods can be implemented through in situ or ex situ approaches. In situ treatment addresses pollution directly at the contaminated site (Cao *et al.,* 2020), whereas ex situ methods involve relocating contaminated soils for microbial processing to immobilize the metal ions causing pollution (Ayangbenro and Babalola, 2017). Notably, microorganisms like E. asburiae and B. cereus have been linked to the immobilization of heavy metals in environmental pollution scenarios (Fashola *et al.,* 2020a). Microbes protect themselves from toxic substances by developing hydrophobic or solvent efflux pumps that shield their outer cell membranes (Verma and Kuila, 2019).

Enzymatic Oxidation

The conversion of pollutant compounds from higher to lower oxidation states through enzymatic oxidation is facilitated by oxidoreductases, enzymes produced by microorganisms. This technique is particularly useful for treating dyes, phenols, and other pollutants that resist bacterial breakdown (Unuofin *et al.,* 2019). Oxidative enzymes create radicals that can be further broken down into various components, ultimately forming high molecular weight compounds (Unuofin *et al.,* 2019). Laccase, an oxidoreductase enzyme, facilitates the oxidation of aromatic amines (Gangola *et al.,* 2018). This process also involves the reduction of molecular oxygen to water, with polyphenols and phenols participating (Kushwaha *et al.,* 2018; Sahay, 2021).

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Organisms such as *Pycnoporus* and *Leptosphaerulina* have been recognized as laccase producers, contributing to the degradation of heavy metals (Copete-Pertuz *et al.,* 2018; Tian *et al.,* 2020).

Enzymatic Reduction

Unlike enzymatic oxidation, this method transforms pollutants into a reduced, oxidized state, rendering them insoluble. Both facultative and obligate anaerobes perform this process, which is an efficient technique for bioremediation of substances such as dibenzo-p-dioxins ,dibenzofurans, and polychlorines (Zacharia, 2019). The enzyme chrome reductase is responsible for converting hexavalent chromium to trivalent chromium, while azoreductase splits azo compounds by severing azo bonds (Saxena *et al.,* 2020). Additional studies are required to identify other microorganisms capable of bioremediating environmental pollutants.

Biosorption

Heavy metal removal from pollutants through biosorption involves various mechanisms, including chelation, ion and proton displacement, complexation, and electrostatic interactions (Mahmoud, 2021). This technique utilizes the external cellular structures of microorganisms such as bacteria, fungi, and algae as bioremediation agents to extract pollutants from solutions. The binding of metals occurs through active groups on microbial cell surfaces, enabling ion transfer between metal cations and the negatively charged active sites on the microorganisms' outer structures (Mathew and Krishnamurthy, 2018; Baltazar *et al.,* 2019; Sedlakova-Kadukova *et al.,* 2019). Research has demonstrated that specific microorganisms, including *Rhodococcus erythropolis*, *Streptomyces sp.* K11, and *Bacillus anthracis*, can effectively remediate heavy metals via biosorption (Baltazar *et al.,* 2018; Baltazar *et al.,* 2019; Sedlakova-Kadukova *et al.,* 2019). Additionally, the reversible nature of biosorption allows for the extraction of valuable metals through desorption using mild mineral solutions or chelating agents (Tarekegn *et al.,* 2020).

Complexation

The formation of complexes, a process known as complexation, occurs when ligands bind to inorganic metals. These complexes are frequently encountered in solid waste and are considered environmental pollutants (Ayangbenro and Babalola, 2017). Various factors influence complex formation, including high-molecular-weight ligands like siderophores and toxic metal-binding compounds, as well as low-molecular-weight organic acids such as alcohols, tricarboxylic acids, and citric acids (Pratush *et al.,* 2018). This phenomenon takes place when extracellular polymeric substances on microbial surfaces interact with environmental heavy metals (Xie *et al.,* 2020). Certain bacteria, including *Rhodobacter blasticus* and *Bacillus licheniformis*, have been recognized as important contributors to this process (Xiao *et al.,* 2019).

Microorganisms produce iron-binding compounds called siderophores in environments with limited iron availability. These molecules play a vital role in the process of complexation. These compounds possess binding groups such as phenolate, hydroxamate, and catecholate, which form complexes with heavy metals and increase their solubility (Khan *et al.,* 2018). Additionally, siderophores can produce reactive oxygen species, enhancing their efficacy as bioremediation agents for organic pollutants (Albelda-Berenguer *et al.,* 2019). For example, cyanobacteria have demonstrated the ability to effectively remediate complex compounds like polythene,

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owing to their capacity to produce siderophores such as *schizokinen* ,*synechobactin*, and *anachelin* (Arstol and Hohmann-Marriott, 2019; Sarmah and Rout, 2020).

Bioaccumulation

The process of bioaccumulation occurs when microorganisms absorb substances at a rate faster than they can eliminate them, leading to an accumulation of toxic compounds within their cells (Sharma *et al.,* 2022a). Heavy metals can penetrate microbial cell membranes through various pathways, including transport proteins, ion channels, and active pumps (Mir-Tutusaus *et al.,* 2018). Specific microorganisms have been identified as efficient accumulators of particular heavy metals, with examples such as *Rhizopus arrhizus* for mercury, *Aspergillus niger* for thorium, and *Pseudomonas putida* for cadmium (Sharma *et al.,* 2022a).

Precipitation

The conversion of toxic heavy metals or pollutants into less harmful precipitates or crystals is known as precipitation. This transformation can take place during biogeochemical cycles, leading to the deposition of metals such as iron and manganese, along with mineralized forms of manganese and silver, and the creation of microfossils. According to a recent study by Sharma *et al.* (2022a), these changes are facilitated by enzyme activity and the galactosis of secondary metabolites. For example, under alkaline conditions, sulfate-reducing bacteria can transform organophosphates into orthophosphates (Pratush *et al.,* 2018). Additionally, research has shown that *Oceanobacillus indicireducens* and *Bacillus subtilis* play a role in heavy metal precipitation across various environmental settings (Maity *et al.,* 2019).

Phytoremediation

Phytoremediation relies on various processes, including phytodegradation , phytoextraction, ,phytostabilization, and rhizodegradation. During phytoextraction, plants uptake pollutants from the soil through their root systems using mechanisms such as diffusion, active transport, facilitated diffusion, or concentration gradient. These pollutants are then stored in the plants' aboveground parts, which can be harvested and disposed of later. The process of phytodegradation involves plants breaking down pollutants within their tissues using enzymes, leading to detoxification. Phytostabilization focuses on limiting contaminant movement in the soil, reducing their accessibility and potential for spreading or leaching. Rhizodegradation occurs when plant roots interact with microorganisms in the rhizosphere, enhancing contaminant breakdown through microbial action (Maity *et al.,*2019).

MOLECULAR STRATEGIES FOR ESTABLISHING MICROBIAL REMEDIATION

Understanding the metabolism, genetic makeup, diversity, and dynamics of microorganisms involved in microbial remediation relies heavily on molecular techniques. Scientists have utilized various molecular methods to investigate microbes employed in bioremediation, including analyses of metabolic profiles and proteins, genetic sequencing, and sophisticated bioinformatics approaches (Sharma *et al.,* 2022b).

Beyond sophisticated techniques, traditional and culture-reliant molecular approaches have been employed to track microbial populations during the bioremediation process. These methodologies encompass a range of analytical tools, including terminal-restriction

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fragment (T-RF) length polymorphism ,amplified ribosomal DNA restriction analysis, randomly amplified polymorphic DNA analysis, temperature gradient gel electrophoresis, length heterogeneity polymerase chain reaction, denaturing gradient gel electrophoresis ,amplified fragment length polymorphisms, single-strand conformational polymorphism , and automated ribosomal intergenic spacer analysis (Bhargava *et al.,* 2019).

Additionally, various omics approaches, such as proteomics, metagenomics, and transcriptomics, have made significant contributions to this field. Metagenomics involves extracting genomic DNA from all organisms in a sample, followed by fragmentation, cloning, transformation, and screening using a metagenome library (Bhargava *et al.,* 2019). Metagenomic methodologies encompass fluxomics, metabolomics, and metatranscriptomics. Microbial function and expression within a sample can be understood through metatranscriptomics, which utilizes metagenomic mRNA (Mukherjee and Reddy, 2020). The analysis of all protein samples from environmental sources is conducted through metaproteomics (Bargiela *et al.,* 2015). In contrast, metabolomics focuses on the detection and measurement of all metabolites released into the environment (Liu *et al.,* 2022).Fluxomics comprises various techniques aimed at investigating metabolic activity rates in biological samples (Kumar *et al.,* 2022). In recent times, next-generation sequencing has become a formidable method for analyzing genes (Eisenhofer *et al.,* 2019).

ADVANCEMENTS IN MICROBIAL BIOREMEDIATION

Advancements have been made in utilizing microorganisms for bioremediation purposes. Microbial glycoconjugates are crucial in reducing surface tension, improving the accessibility of organic pollutants, and establishing solvent interfaces that aid in pollutant elimination (Bhatt *et al.,* 2021b). Research by Atakpa *et al.* (2022) demonstrated effective biodegradation of petroleum hydrocarbons using glycoconjugates obtained from *Scedosporium sp.* and Acinetobacter sp. Furthermore, microbial biofilms consisting of extracellular DNA, polysaccharides, and proteins have shown promise as efficient tools for organic pollutant bioremediation (Sonawane *et al.,* 2022). These biofilms are particularly effective in addressing persistent pollutants. Current research efforts are directed towards enhancing environmental conditions, quorum sensing, and surface adhesion to boost the effectiveness of this technology (Sonawane *et al.,* 2022). A study by Andreasen *et al.* (2018) revealed that *Exiguobacterium profundum* substantially decreased arsenic levels in synthetic wastewater within 48 hours of incubation.

The integration of electrochemical and biological techniques in bioelectrochemical systems has shown great potential for managing pollutants (Ambaye *et al.,* 2023). These systems excel in addressing petroleum hydrocarbon pollution through the collaborative and mutually beneficial interactions among various microbial communities (Ambaye *et al.,* 2023). Research by Sharma *et al.* (2020) has identified several bacterial species, including *Pseudomonas sp., Ralstonia sp.*,*Thauera sp.*, and *Rhodococcus sp.* , as efficient organisms for eliminating phenanthrene in soil contaminated with petroleum hydrocarbons.

Nanotechnology is widely acknowledged as a promising approach to address pollution, with nanomaterials from various sources showing potential for bioremediation (Shanmuganathan *et al.,* 2019). The efficacy of nanoparticles in bioremediation is influenced by multiple factors, including their dimensions, surface modifications, chemical makeup, and geometry (Tan *et al.,* 2018). Additionally, the nature of the pollutants, ambient temperature, medium type, and environmental acidity significantly impact the performance of nanoparticles in bioremediation processes (Tan *et al.,* 2018). In recent years, carbon dots nanoparticles have garnered interest due to their high efficiency in treating environmental pollutants, attributed to their minimal toxicity, widespread availability, and distinctive optical characteristics (Long *et al.,* 2021). Additional studies are necessary to explore the techniques and mechanisms that can boost the effectiveness of bioremediation methods.

Bioengineering offers a method for addressing pollution and waste by employing genetically altered microbial strains to enhance the breakdown process (Peng *et al.,* 2020; Mishra *et al.,* 2020a, 2020b; Lawniczak *et al.,* 2020). This approach involves altering the genetic makeup of domesticated plants and animals to produce specific results, such as hybridization, substitution, and induced mutation (Saxena *et al.,* 2020). The genetic modification of plants and animals is typically carried out with particular goals in mind (Perera and Hemamali, 2022). The term "genetically engineered organisms" (GEMs) specifically refers to microorganisms, including bacteria, fungi, and yeast, that have undergone transformation through in vitro molecular biology techniques (Jafari *et al.,* 2013; Liu *et al.,* 2019; Hou *et al.,* 2020; Sanghvi *et al.,* 2020). Through genetic modification, these microorganisms can be altered by introducing specific genes, creating various strains capable of breaking down and eliminating pollutants from the environment, a process known as bioremediation (Jafari *et al.,* 2013; Liu *et al.,* 2019; Hou *et al.,* 2020; Sanghvi *et al.,* 2020).

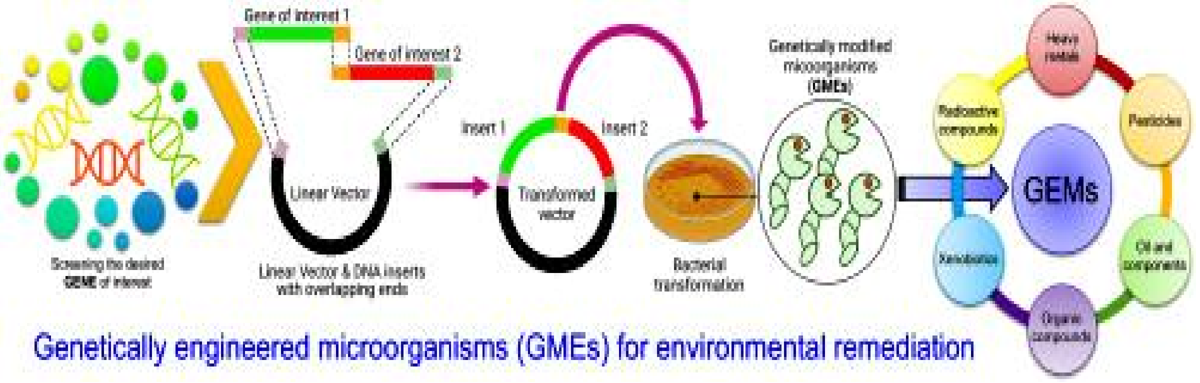

Figure 1: Genetically engineered microorganisms (GMEs) for environmental remediation (Liu *et al.,*2019).

Microorganisms, particularly bacteria, exhibit exceptional abilities to break down environmental pollutants. Numerous bacterial strains have been recognized for their capacity to metabolize various pollutants, including nitro-aromatics, chloro-aromatics, and polycyclic aromatic compounds, making them ideal candidates for cleaning up polluted areas (Gupta and Prakash, 2020). Nevertheless, certain highly persistent and dangerous

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synthetic chemicals, such as halogenated aromatic compounds or heavily nitrated insecticides and explosives, present difficulties due to their chemical stability under natural conditions, which impedes their effective breakdown by most microorganisms (Pande *et al.,* 2020). The intricacy resulting from pollutant mixtures and the toxicity of some of these substances to existing microbial communities further complicates successful biodegradation (Rylott and Bruce, 2020). To overcome these obstacles, scientists have developed genetically modified bacterial strains with enhanced metabolic pathways to improve bioremediation potential beyond that of naturally occurring microbes (French *et al.,*2020). Techniques such as bio-stimulation and bioaugmentation, including composting, electro-bioremediation, and microbe-assisted phyto-bioremediation, are crucial components of microbial bioremediation (French *et al.,* 2020). Recent scientific progress has sparked increased interest in using genetic engineering to enhance the natural remediation capabilities of microorganisms (Jaiswal and Shukla, 2020 ; Sharma, 2020). Effective in situ bioremediation using genetically modified organisms (GMOs) necessitates a thorough understanding of biotechnology, ecology, field biochemical processes, and genetic engineering (Kumar *et al.,* 2018).

Engineered Microbes for Mitigating Environmental Pollutants

Environmental biotechnology has been significantly impacted by progress in synthetic biology. Genetically Engineered Microbes (GEMs) are increasingly utilized for eliminating harmful chemicals, xenobiotics, and pesticides from the environment (Pant *et al.,* 2021). Understanding natural microbial communities is essential for creating synthetic ones, but pinpointing specific species involved in bioremediation within natural settings remains a significant obstacle (Nwankwegu *et al.,* 2022). One approach to address this issue involves establishing synthetic microbial communities by growing two microorganism species with specialized functions under controlled conditions, based on their interactions. This approach serves as a model for investigating the structural, functional, and ecological aspects of these communities (Anjum *et al.,* 2022). Various factors influence the structure and dynamics of these communities (Patel *et al.,* 2022a). The dynamics of these communities are affected by metabolic interactions between microbial populations (Saleh *et al.,* 2022). Different outcomes can arise from interactions between two microbial populations, such as competition , mutualism, and collaboration, resulting in synergistic (+/+) and antagonistic (+/-) relationships (Yue *et al.,* 2022).

Synthetic communities, which emerge from planned partnerships, play a role in shaping the impact of collaboration on community development. Scientists can employ various methods, including gene removal and insertion, to alter environmental conditions and promote cooperation between two microorganisms (De Melo and Solleder, 2022). Additionally, studies have examined interactions among engineered microbial species within synthetic communities to gain insights into additional interaction patterns, particularly in bioremediation systems (Huang, 2022). The use of synthetic microbial communities as bioremediation strategies for various contaminated environments, such as those affected by petroleum oil spills, pesticides, and acid drainage, offers a promising approach that can improve the sustainability of microbial populations (Abdi *et al.,* 2022).

Table 1: Advantages and disadvantages genetic engineering in bioremediation

S/N	Advantages	Disadvantages
1	Genetic engineering can create microorganisms with enhanced capabilities to degrade specific pollutants more efficiently than natural strains.	The use of genetically modified organisms (GMOs) raises ethical questions and public concerns about safety and environmental impact.
2	Engineered organisms can be designed to target specific pollutants, leading to more effective and focused remediation efforts	There may be unintended effects on non-target organisms and ecosystems, potentially disrupting local biodiversity.
3	Genetically modified organisms can be engineered to survive in harsh environmental conditions, increasing their effectiveness in contaminated sites	The regulatory framework for GMOs can be complex and may delay the implementation of bioremediation projects.
4	Genetic engineering allows for the rapid development and testing of new microbial strains tailored for specific remediation tasks.	Public skepticism and opposition to GMOs can hinder the acceptance and deployment of genetically engineered bioremediation solutions.
5	Engineered microorganisms can be designed to degrade multiple pollutants simultaneously, increasing the versatility of bioremediation efforts.	The development and application of genetically engineered organisms can be costly and resource-intensive, requiring specialized knowledge and facilities. (Rafeeq *et al.,* 2023).

Bacterial communication through quorum sensing is a concentration-dependent process that allows for cell-to-cell interaction and group-level synchronization. Microorganisms within a population interact by emitting and detecting chemical cues, enabling the formation of engineered communities with distinct capabilities by leveraging this collective behavior (Yin *et al.,* 2022). Researchers can develop artificial models to create designs that maintain functionality while vying with other organisms in real-world settings (Chavan *et al.,* 2022). Nevertheless, genetic circuits may face the risk of disappearance over time due to alterations in genomic elements and gene exchange. The competition between different species significantly influences the durability of microbial populations, which can subsequently impact the stability of engineered communities. Enhancing these processes is crucial for the advancement of synthetic communities engineered for particular bioremediation purposes (Fan *et al.,* 2022).

Bioremediation, a well-established and environmentally friendly method for cleaning contaminated areas, is known for its safety, cost-effectiveness, and sustainability (Dai *et al.,* 2020; Singh *et al.,* 2021). However, the process of identifying and developing suitable microbial strains can be lengthy. When designing and cultivating bacteria for bioremediation, it is crucial to consider various factors, including the presence of genes responsible for metal absorption, chelator production, and those that provide survival advantages in both natural and human-altered environments. The use of recombinant DNA technology enables the transformation of organisms into desired forms (Haritash, 2020; Landa-Acuña *et al.,* 2020) by incorporating the target gene into another organism's genome using vectors such as phages, plasmids, or viruses, thus improving the host organism's bioremediation capabilities (Patel *et al.,* 2022b). This process requires several tools, including DNA ligase, restriction enzymes, T4 polynucleotide kinase, reverse transcriptase, alkaline phosphatase, and appropriate host organisms (Pal *et al.,* 2020).

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ADVANCEMENTS IN GENE EDITING TECHNOLOGIES FOR ENHANCED BIOREMEDIATION APPLICATIONS

Recent advancements in scientific methodologies have enabled researchers to manipulate DNA molecules with exceptional precision, facilitating targeted genetic modifications at both the genomic and gene levels (Soutullo *et al.,* 2016). Gene editing techniques such as transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated nucleases are widely employed (Sharma and Shukla, 2022). Notably, the CRISPR-Cas system has gained prominence as a user-friendly and efficient gene-editing tool with considerable potential in bioremediation applications. TALENs incorporate a DNA-binding module that specifically recognizes the host genome, generating double-stranded breaks (DSBs) with sticky ends to enhance stability. Another technique, zinc finger nucleases (ZFNs), features a 30-amino acid DNA-binding domain that creates DSBs at specific sites within the host genome (Priya *et al.,* 2022).

In response to the complex issues related to genetic engineering, researchers have developed hybrid nucleases like TALENs and ZFNs, which paved the way for the CRISPR-Cas system. This advanced technology enables precise modification of multiple genes concurrently (Gao *et al.,* 2022). The CRISPR-Cas system employs guide RNA (gRNA) to merge crisper-derived RNA (crRNA) and trans-acting antisense RNA (trcRNA), offering a defense mechanism against viral infections (Sarma *et al.,* 2021). Guided by gRNA, the Cas9 enzyme creates double-stranded breaks (DSBs) at specific DNA sequences by identifying the matching gRNA sequence. These gene-editing techniques show potential for bioremediation studies, facilitating the creation of knock-in and knock-out organisms (Liu *et al.,* 2021). Current research has largely concentrated on applying the CRISPR-Cas system in model organisms such as *Pseudomonas* and *E. coli* (Okoli *et al.,* 2021). Furthermore, non-model organisms like *Comamonas testosteroni* and *Achromobacter sp*. HZ01 and can be utilized for bioremediation using CRISPR tools and for expressing function-specific genes relevant to remediation (Zhang *et al.,* 2021). Bacteria adapted to pollutants are particularly well-suited for metabolic engineering and genome editing due to their capacity to survive in various toxic environments (Patel *et al.,* 2022b).

Understanding the metabolic processes involved in microbial bioremediation is essential. For example, certain microorganisms produce haloalkane dehalogenases that can effectively treat toxic pollutants, while the biodegradation mechanism of fenpropathrin in *Bacillus sp*. DG-02 aids in removing pyrethroids from soil (Kurade *et al.,* 2021). The bioremediation process can be significantly enhanced through metabolic engineering by modifying existing metabolic pathways. Key enzymes in this process include oxidases, esterases, monooxygenases, oxidoreductases, and phenol oxidases (Mehta *et al.,* 2021). As a sustainable method, enzyme-based bioremediation offers several benefits, and genetic engineering techniques can be used to produce recombinant enzymes. Many studies have highlighted the importance of extracellular enzymes in bioremediation. Enzymes produced by white-rot fungi can degrade polycyclic aromatic hydrocarbons (PAHs), 2,4,6-trinitrotoluene (TNT), and polychlorinated biphenyls (PCBs). Esterase D has been shown to break down endosulfan into simpler components, while hemoproteins encoded by *Phanerochaete chrysosporium* assist in the degradation of PAHs (Gallo *et al.,* 2021).

Contaminant breakdown can yield energy or function as intermediates in biological processes. For example, benzopyrene can be transformed into three quinone compounds by lignin peroxidase (LiP). In the presence of Mn(II), manganese peroxidase (MnP) oxidizes organic molecules, while other enzymes such as laccase, glutathione S-transferase, mixed-function oxidases, and cytochrome P450 contribute to the biodegradation of stubborn substances. Furthermore, catechol 1,2-dioxygenase derived from Pseudomonas NP-6 converts catechol into muconate molecules. The immobilization of enzymes can substantially improve their half-life, stability, and activity, facilitating effective and eco-friendly enzymatic bioremediation of persistent xenobiotic compounds (Wiener and LeFevre, 2022). Nevertheless, the identification and characterization of microorganisms that produce these enzymes can be difficult due to their low production rates. Organophosphates (OP) and organochlorines (OC), which are frequently used in insecticides, can contaminate agricultural soils and runoff, presenting considerable risks to water resources.

Research has shown that genetically modified microbes can effectively eliminate oil pollution and its byproducts, as exemplified by the application of *Pseudomonas putida* KT2440 (Sazanova *et al.,* 2022). These organisms, through metabolic engineering, are capable of breaking down various pollutants. For example, *Sphingobium japonicum* and *Pseudomonas sp.* WBC-3 have demonstrated the ability to degrade methyl parathion and hexachlorocyclohexane. Additionally, heterologous catabolism in *E. coli* can aid in dispersing the fumigant 1,2,3-trichloropropane into the environment by utilizing enzymes from diverse microbial origins. Scientists have engineered *P. putida* to express pnp operon enzymes and organophosphorus hydrolase (OPD) , enabling the transformation of p-nitrophenol into adipate (Hussain *et al.,* 2022). In *Rhodococcus opacus* R7, the gene clusters pobA and chcpca facilitate the bioremediation of naphthenic acids, while the aliA1 gene encodes an enzyme that degrades long-chain naphthenic acids. The use of microorganisms allows for the partial or complete breakdown of persistent chemicals, thereby reducing their environmental accumulation (Kharitonov *et al.,* 2022).

The CRISPR-Cas system, renowned for its exceptional gene-editing capabilities, can be employed to alter bacteria for organic contaminant elimination. Various CRISPR-Cas systems, including types I, II, and III, enable genome modification for bioremediation purposes. The CRISPR-associated Cas9 protein acts as a molecular scissor, precisely cutting DNA at specific sites. Within the CRISPR framework, crRNA, a complementary copy of foreign DNA, is generated by the direct repeat sequence, while gRNA can also be produced. The combination of crRNA and Cas protein forms cr ribonucleoproteins (crRNPs), which trigger the breakdown of both DNA and RNA in the target organism. The precision of the CRISPR system is primarily due to gRNA. CRISPR-Cas9 can be utilized to introduce or remove target genes in microbial strains, thereby enhancing their degradation efficiency. For instance, eliminating the yvmC gene through CRISPR-Cas9 led to a 100% increase in biotransformation efficiency in *B. licheniformis*. In the future, CRISPR-Cas9 technology may be used to improve microbial strain capabilities, potentially resulting in the development of strains specifically engineered

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for environmental bioremediation of targeted pollutants (Qian *et al.,* 2022a).

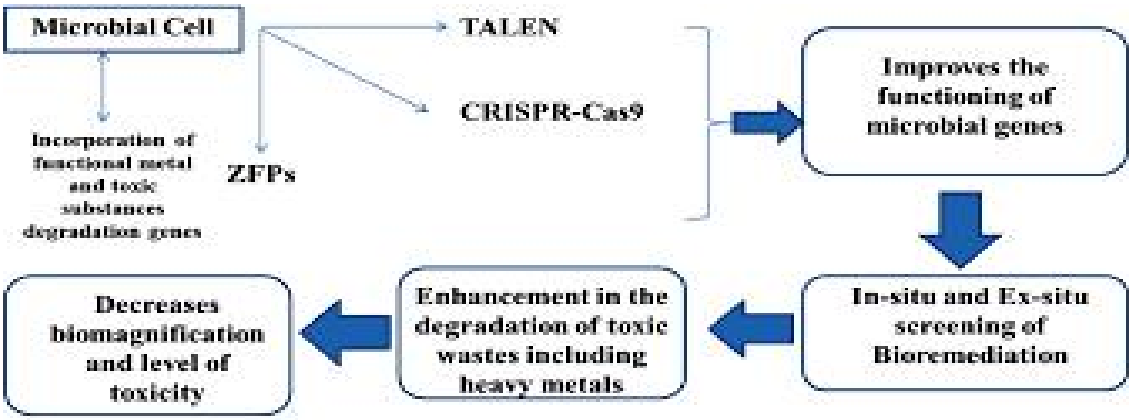

Figure 2: Different gene editing tools for bioremediation (ZFPs- Zinc Finger Proteins ,TALEN: Transcription Activator Like Effector Nuclease) (Paul *et al.,*2021).

Overview on the Application of Genetically Enhanced Microbial Strains for Effective Pollutant Degradation

In their natural environments, microorganisms possess the capability to degrade unfamiliar compounds as a survival mechanism. Nevertheless, there is a demand for remediation approaches that utilize microbes able to thrive in diverse environmental settings and effectively break down pollutants, thereby swiftly eliminating dangerous substances from the surroundings (Siwal *et al.,* 2022).Achieving this requires developing genetically stable microbial strains with broad substrate specificity that are easily manipulated. Researchers can engineer strains with desired degradation capabilities by using insights from the biochemistry, physiology, and genetics of microorganisms, making them effective tools for remediation efforts (Tahir *et al.,* 2022).

Mutation

Mutation is a widely recognized strategy used to alter the genetic traits of organisms, resulting in the creation of new strains (Patel *et al.,* 2022b). This process can significantly impact an organism's phenotype. Mutations can occur spontaneously or be intentionally induced, with both detrimental and advantageous effects, and natural selection tends to favor the survival of more suitable populations (Patel *et al.,* 2022b). Specifically, plasmids, integrons, and transposons contain genes linked to degradation, allowing bacteria to better cope with polluted environments through genetic changes. Targeted site-specific mutations can be employed to break down persistent pollutants in polluted soil and water. Furthermore, engineered enzymes created through mutagenesis techniques have demonstrated the ability to improve bioremediation processes by enhancing the efficiency of degradation pathways (Mutanda *et al.,* 2022).

Spontaneous and Induced Mutation

Genetically modified organisms (GMOs) are microbes that have undergone genetic alterations to survive in contaminated environments. These microorganisms can adjust their genetic makeup when exposed to polluted conditions, enabling them to adapt to new environmental challenges (Chakdar *et al.,* 2022). One method by which microbial populations evolve is through mutations, which can be triggered by physical or chemical mutagens. Physical mutagens include X-rays, while chemical mutagens encompass base analogs and intercalating agents (Xue *et al.,* 2022). In industrial applications, mutagenesis is frequently employed to enhance the production of desired metabolites and can also be utilized in bioremediation to improve the pollutant-degrading abilities of microorganisms. The development of mutant strains capable of efficiently breaking down specific pollutants has been achieved through random mutations. Common mutagenic techniques include UV irradiation, ethyl methyl sulfate, and ethidium bromide (EtBr) (Téllez *et al.,* 2022). For example, Gopinath *et al.* utilized random mutations to expedite the biodegradation of Congo Red, while Joshi and Thagard (2013) created a UV-irradiated mutant of *Pseudomonas sp.* 1F to biodegrade the sulfonated azo dye Green HE4B (GHE4B). The mutant strain exhibited a more rapid degradation rate of the resistant dye GHE4B compared to the wild-type strain, with UV irradiation resulting in enhanced enzymatic activity (Téllez *et al.,* 2022).

Site-Directed Mutagenesis

A commonly employed technique for altering specific DNA regions is site-directed mutagenesis, which enables the development of optimized strains for particular applications. This method has been successfully applied to enhance the catalytic efficiency and heat resistance of phytase in *Aspergillus niger* N25, resulting in a 20% improvement in thermostability compared to the original strain, as noted by Liao *et al.* (2013). Furthermore, site-directed mutagenesis has been used to boost the thermal resistance of a protease derived from *Bacillus stearothermophilus*, elevating its stability from 86.1 °C to 100.1 °C. Researchers have also utilized this approach to enhance the performance of proteases and lipases in cold environments, as demonstrated in the work of Saravanan *et al.* (2022).

Recombination by Natural Methods

Microorganisms can gain novel genetic characteristics through natural mechanisms such as conjugation, transformation, and transduction. These processes of genetic exchange are commonly employed in laboratory environments to introduce desired genes into bacterial organisms, creating modified strains with specific advantages. Conjugation necessitates direct physical contact between donor and recipient strains to enable genetic material transfer. This technique can be employed to transfer degradation genes to indigenous bacteria in contaminated environments, leading to the development of specialized degradation abilities. For example, a *Pseudomonas putida* strain capable of breaking down camphor, octane, salicylate, and naphthalene was engineered through conjugation. This process involved transferring XYL and NAH genes via a hybrid plasmid formed by the recombination of OCT and CAM genes (Glick and Patten, 2022).

Bacterial transformation is a process where bacteria actively absorb free DNA and incorporate it into their genetic material, representing a type of horizontal gene transfer that can occur naturally in populations of competent bacteria (Deckers *et al.,* 2022). This technique was employed to explore the molecular mechanisms involved in breaking down pollutants like polychlorinated biphenyls (PCBs) and trichloroethylene (TCE). Additionally, the combination of transduction with conjugation and transformation can create highly enhanced bacterial strains with diverse genetic makeup.

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In this method, fragments of the host's genome are encapsulated in capsids, which serve as vehicles to deliver specific genes to target recipient cells (Sendhil *et al.,* 2022).

Protoplast Fusion

Protoplast fusion is a process that involves the combination of bacterial cells that have been devoid of their cell walls to produce strains with specific traits that are desired. The cell wall can be removed mechanically or by using osmotic stabilizers, such as lysozyme. Polyethylene glycol can facilitate this process either directly or indirectly. The preferred method for protoplast fusion is electrofusion, which uses low-intensity electric fields. This technique has been proven to enhance the degradation of both organic and inorganic compounds, as demonstrated in a study by Saravanan *et al.* (2022). Genetic engineering is a crucial aspect of enhancing microbial strains, and genetically engineered microorganisms (GEMs) have gained significant attention in bioremediation due to recent technological advancements. GEMs can be genetically modified to incorporate specific genes that enhance the biodegradation of xenobiotic substances. Techniques such as recombinant DNA (rDNA) methods, including PCR, electroporation, site-directed mutagenesis, and particle bombardment, can be used to improve biodegradation capabilities. The rate and extent of microbial degradation are influenced by several environmental factors, such as pH, temperature, the availability of nitrogen and phosphorus, and the genetic potential of the microorganisms and their inherent ability to metabolize pollutants (Yaashikaa *et al.,*2022).

Degradation of Synthetic Pollutants Through Gene Cloning

Amplifying specific genes through gene cloning can enhance the production of desired products in microbial strains. This method has been utilized in various bioremediation applications, including the breakdown of 4-chlorobiphenyl by inserting and expressing the gene responsible for its degradation in *Pseudomonas* using the cosmid pCP13. The complete mineralization of 4-chlorobiphenyl is facilitated by enzymes such as dioxygenases, dehydrogenases, and hydrolases. The insertion and expression of TOD genes in the chromosome of *Dinococcus radiodurans* allowed for the degradation of toluene, chlorobenzene, indole, and 3,4-dichloro-1-butene. Genes encoding carbofuran hydrolase, an enzyme involved in breaking down carbamate pesticides, show wide-ranging enzymatic activity. The hydrolase gene responsible for methyl carbamate degradation was successfully cloned and isolated from *Achromobacter sp.* WM11 (Liu *et al.,* 2022). Moreover, the expression of cloned serine alkaline protease in the *Shewanella strain* AC10 resulted in increased enzyme production. The gene encoding nitrile hydrolase (NHase) was extracted and employed for converting nitriles into valuable amides. Additionally, the genes atzA and atzB, which are involved in atrazine degradation in boreal groundwater, were cloned and expressed. Hydroquinone dioxygenase, a multifunctional enzyme, plays a role in the bioremediation of p-nitrophenol. The mpd gene and its variants have been cloned, characterized, and applied to degrade chlorpyrifos in contaminated environments. The cloning of specific bacterial and fungal genes has proven effective in exploring the potential of various bioremediation-specific genes. These genes can be introduced into model bacterial cells for use in treating widespread pollution in soil and water systems (Duque *et al.,* 2022).

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FUTURE PROSPECTS AND CONCLUSION

The key to environmentally sustainable development lies in environmental biotechnology's ability to create genetically modified microorganisms capable of effectively breaking down pollutants. Scientists are identifying and incorporating genes associated with pollutant degradation into new strains that exhibit enhanced resilience to environmental pollutants, including heavy metals. Liu *et al.* (2022) suggest that introducing these genes into organisms with robust adaptability and high reproductive rates could enhance pollutant breakdown. Nevertheless, the limitations of individual bacterial strains in pollutant removal have prompted researchers to explore micro-ecological systems. These systems comprise multiple organisms working in concert to improve the elimination of pollutants (Yaashikaa *et al.,* 2022).

Recent research has focused on developing novel methods to enhance pollutant remediation strategies. A study by Stanton *et al.* (2020) investigated multiple origins of microplastic contamination in freshwater systems, such as wastewater treatment facilities, urban areas, and runoff from roads. In another investigation, Huang *et al.* (2022) analyzed the effectiveness of iron-activated carbon systems in boosting aerobic denitrifying bacterial communities for treating reservoir water contaminated with micro-pollutants. Jaiswal and Shukla (2020) provided an overview of alternative techniques for microbial pollutant remediation using synthetic biology, emphasizing recent progress in this field. Additionally, Urso *et al.* (2023) explored the application of intelligent micro- and nanorobots in water purification, focusing on crucial design aspects that could improve remediation efficiency. Rebello *et al.* (2021) emphasized the importance of genetically modified microorganisms (GMMs) in improving soil health, highlighting their crucial role in bioremediation processes. The understanding of soil biphenyl degraders was advanced by Chen *et al.* (2021), who employed isotope probing, multi-omics approaches, and single-cell analyses to gain valuable insights into microbial biodegradation mechanisms. The sustainable nature of bioremediation as a pollution control strategy was emphasized by Singal and Kaur (2023), who demonstrated the development of genetically engineered microbes for remediating synthetic organic chemicals.

The expansion of industrial activities and the introduction of new pollutants have made the identification of genes capable of breaking down emerging pollutants a top priority. The creation of metagenomic libraries with large insert fragments can aid in discovering novel and unique genes, providing a basis for bacterial genetic modification (Glick and Patten, 2022). Although recombinant bacteria show significant potential and positive results in pollution treatment, their practical application faces several obstacles. In complex environments with diverse substrates and microbial interactions, only a few engineered bacteria are currently used for toxin treatment and removal (Duque *et al.,* 2022). Furthermore, the presence of plasmids in bacterial strains can negatively impact their development, reproduction, and effectiveness in pollutant treatment due to the metabolic burden imposed on cells. Managing multiple plasmids with desirable traits in engineered bacteria presents challenges related to compatibility and incompatibility, and the loss of plasmids during microbial reproduction can negatively affect the degradation process (Sendhil *et al.,* 2022).

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The development of engineered bacteria for biodegradation applications has shown promising results using protoplast fusion technology. However, it is important to note that the protoplast fusion process may inadvertently introduce unnecessary or detrimental genes into bacterial strains, as highlighted by Saravanan *et al.* (2022). The widespread application of genetically modified bacteria in natural environments remains a contentious issue due to concerns about their potential negative effects on human health and the delicate balance of biological ecosystems. Nonetheless, the application of microbial metabolism for bioremediation offers significant potential for mitigating soil and water pollution by reducing harmful contaminant levels in the environment, as demonstrated by Deckers *et al.* (2022). Further high-throughput microbial technology studies are necessary to advance bioremediation efforts in areas contaminated with specific pollutants, such as those associated with synthetic compounds, as emphasized by Gupta *et al.* (2022).

In conclusion, contemporary research has highlighted the vital importance of genetically engineered microorganisms in pollutant breakdown, paving the way for cutting-edge biotechnological approaches to address environmental issues. The field of environmental biotechnology shows great potential for developing genetically modified microbes capable of efficiently breaking down pollutants. Nevertheless, substantial research gaps and obstacles remain, including the need to enhance engineered bacterial strains, discover new genes involved in degradation processes, and comprehensively assess their possible effects on ecosystem health and human well-being.

Collaborative interactions among various microorganisms within micro-ecological systems provide promising strategies for improving pollutant removal, contributing to a more sustainable and healthy environment. Ongoing research on high-throughput microbial technologies and metagenomics is necessary for advancing this field and establishing effective pollution mitigation strategies.

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