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Bioremediation and Microbial Roles in Environmental Cleanup

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Abstract

Bioremediation is an eco-friendly and sustainable approach that utilizes microorganisms to degrade, detoxify, or remove environmental pollutants such as hydrocarbons, heavy metals, pesticides, and persistent organic compounds. This study examines the fundamental principles, microbial mechanisms, and strategies involved in environmental cleanup through bioremediation. It highlights the metabolic versatility of bacteria, fungi, and extremophiles in transforming contaminants under aerobic and anaerobic conditions. The paper further discusses *in situ* and *ex situ* remediation techniques, along with approaches such as bioaugmentation and bio stimulation to enhance degradation efficiency. Key factors influencing bioremediation, including bioavailability, environmental conditions, and microbial community dynamics, are also analyzed. Additionally, the study addresses monitoring techniques, risk assessment, and real-world applications across contaminated soils and aquatic systems. Despite its advantages, challenges such as scalability, complex contaminant mixtures, and field-level implementation remain significant. Emerging tools like genomic technologies and synthetic biology offer promising advancements for improving bioremediation efficiency and sustainability.

Keywords: Bioremediation, Microorganisms, Environmental Cleanup, Biodegradation, Bioaugmentation, Bio stimulation, Hydrocarbon Degradation, Xenobiotics, Soil Remediation, Water Treatment, Microbial Metabolism, Environmental Biotechnology

Introduction

The unique metabolic abilities of microorganisms help preserve the biosphere, a complex habitat inhabited by multiple organisms that interact with each other and their environment. Biological approaches to mitigate the hazards posed by xenobiotic compounds or petroleum hydrocarbons were first applied in the mid-1900s. Bioremediation is the process whereby either natural biological activities or nutrient enhancements favour the growth of specific microorganisms that transform or mineralize hazardous compounds into less harmful products. Knowledge of microbial metabolic capabilities and the factors that govern the growth and activity of specific organisms enables the selection of biodegradation strategies best suited for specific contaminants and their environment. Biologically managed systems are therefore often more effective than classical technology because they remain effective under conditions that are incompatible with chemical treatments, such as the presence of low concentrations of contaminants, toxic substances to chemical agents, or at extreme temperatures or pH (Biswas, 2015).

Fundamentals of Bioremediation

Bioremediation refers to the use of biological agents, such as microorganisms, to remove or neutralize contaminants from the environment. The biological agents used can be microbial and can synthesize specific enzymes, or they can be in an immobilized or biosorbent form. Bioremediation technology is being broadly used to clean up contaminated sites (Biswas, 2015). Contaminated environments include those impacted by oil spills, heavy metals, persistent organic pollutants (POPs), or pesticides. Remediation usually consists of a treatment stage followed by a polishing stage, with both stages potentially employing bioremediation (S. Karigar & S. Rao, 2011).

1. Microbial Metabolic Pathways for Contaminant Transformation

A fundamental characteristic of microbes is their metabolic versatility, allowing them to utilize a variety of electron donors and acceptors to obtain energy. Over the last few decades, an increasing number of microbes able to utilize pollutants as sole sources of carbon, nitrogen, or phosphorus have been identified. Microbial metabolism can convert organic compounds to gaseous products, thus alleviating their toxicity. Biodegradation is of two types: (1) co-metabolic (where the substrate is transformed without any benefit to the microbe) and (2) a time step removal of electrons from one substrate immediately followed by the complete breakdown of another substrate (the removed electrons substrate serves as initial growth substrate) (Migiani et al., 2022).

Because microbes are capable of detoxifying numerous compounds, attention has turned toward separating pollutants that are resistant to microbial attack; not only benzene but also phenolic and chlorinated compounds; and complete mineralization where pollutants are converted to innocuous compounds (A. Key et al., 2022).

2. Aerobic and Anaerobic Biodegradation

Microbial metabolism can convert a multitude of contaminants into nonhazardous substances under aerobic and anaerobic conditions. A thorough understanding of optimal growth conditions and metabolic pathways has improved bioremediation strategies tailored to specific compounds and circumstances. Many organic compounds are degraded solely under aerobic conditions, with oxygen serving as the terminal electron acceptor. Aerobic degradation typically comprises an initial oxidation step mediated by oxygenases and subsequent hydrolytic or reductive reactions, leading to the opening of aromatic rings and generation of linear intermediates like aliphatic acids or alcohols. Conditions favouring aerobic metabolism are common in surface soils, groundwater, and waters contaminated by oil spills or leakages. Aerobic bacteria continue to be the most studied microorganisms and the most widely used bioremediation agents under such conditions. By contrast, anaerobic degradation occurs in sediments, deep aquifers, and oil reservoirs, and it is important in wide-ranging anaerobic hydrocarbon-producing biodesulfurization as well as in clustering methanogenic bacteria within the genera *Methanosarcina* and *Methanosaeta*. Anaerobic techniques are being increasingly integrated into ex situ and in situ bioremediation systems. Complete mineralisation of a variety of saturated, aromatic, and chlorinated hydrocarbons has been demonstrated with synthetic or commercial microbially inert anaerobic aquifer models, representing the most significant advances in anaerobic treatment methods (D. Semrau, 2011).

3. Bioavailability and Mass Transfer Considerations

Contaminant bioavailability in bioremediation is influenced by (i) the physical and chemical nature of the contaminant and its environment at the contaminated site as well as (ii) the metabolic capability of microorganisms present at the contaminated site. Bioavailability is an indication of the degree of access microbial colonies have to a contaminant in soils. The lower the bioavailability is, the contaminant has a lower microbial access, and as a result, biodegradation is hindered. It involves the mass transfer of contaminants through porous soil elements to microorganisms (Bosma, 1994). The effective contaminant diffusion in soils is one to ten orders of magnitudes lower than that in the water phase, therefore contaminant diffusion becomes a serious rate-limiting step. Alternatively, contaminant removal is limited by the physiological or thermodynamic state of the population, ageing of contaminants bonded to the solid phase or contaminant remaining in an undissolved state. For polynuclear aromatic hydrocarbons, polyacrylamides have been used as transports to increase their bioavailability through the desorption process. For aged contaminants, microbial desorption may be employed to enhance the overall bioremediation of the contaminated site.

Microbial Taxa and Mechanisms in Contaminant Removal

Many microorganisms have been found to degrade petroleum hydrocarbons through aerobic or anaerobic biodegradation mechanisms. The rate and extent of hydrocarbon degradation depends on several factors, including the microbial community structure, carbon-source availability, chemistry of the pollutants, temperature, moisture, and pH (Kapahi & Sachdeva, 2019). Over 140 genera of bacteria are known to degrade significant quantities of hydrocarbons, including members of the genera *Acinetobacter*, *Aeromonas*, *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Gordonia*, *Hyphomicrobium*, *Methylosinus*, *Pseudomonas*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, and *Sphingomonas*. *Mycobacterium* and *Nocardia* are able to degrade long-chain n-alkanes and branched alkanes (over 30 carbon atoms), whereas *Gordonia* and *Rhodococcus* can also utilize cyclic alkanes (A. Key et al., 2022). In addition to bacterial genera fungi and yeasts have been isolated from hydrocarbon-contaminated sites that are capable of mineralizing saturated hydrocarbons (C₃-C₂₄). Some wood decaying basidiomycete fungi can degrade polychlorinated biphenyls (PCBs), diesel oil, motor oil, crude oil, and lubricants. These organisms degrade n-alkane chains of various lengths and can thus be used in bioremediation. The interaction of yeasts, fungi, bacteria, and plants has been established in residues contaminated with hydrocarbon waste.

The presence of chlorinated aliphatic and aromatic hydrocarbons from pollutants like trichloroethanes, dichloroethenes, perchloroethylene, 4-chlorophenol, and hexachloro-cyclohexane inhibits the growth of many bacteria. Yet the metabolism of these compounds is essential for biological treatment. The adaptation of an indigenous bacterium was achieved using an electrochemical continuous reactor-anaerobic suspended growth reactor culture. Dechlorination was achieved with the addition of cis-1,2-dichloroethene. The organism could also proliferate in the presence of silk effluent, an organic contaminant. Much progress has now been made in bioaugmentation in which a specialized organism or consortium is added to an indigenous population. When a neothriatoxin-degrading bacterium was introduced to a contaminated site the rate of degradation increased tenfold. Alkanes and chlorinated aliphatic hydrocarbons, as well as organophosphorus, benzoic acids, phenol, polychlorinated aromatics, and benzene, were reported to be biotransformed by Archaea. In fact, methanogenic haloalkane utilization and hydrolytic polyester degradation have also been attributed to halophilic Archaea. The potential for practical bioremediation using Archaea and other extremophiles in eluted sediments, hypersaline ponds, and in the presence of high and low concentrations of certain organic pollutants remains under evaluation.

1. Bacteria Involved in Hydrocarbon Degradation

Contaminated sites associated with the release of petroleum hydrocarbons remain a prominent environmental challenge. Oil spills involving crude oil or refined petroleum products introduce a significant range of

hydrocarbons into soils and water. Hydrocarbons consist exclusively of carbon and hydrogen, making them energetically attractive to bacteria. Although all known bacteria have the ability to degrade hydrocarbons, only a limited number of genera are presently recognized as effective oil-degraders in field applications. Species within the genera *Pseudomonas*, *Bacillus*, *Alcanivorax*, *Cycloclasticus*, *Marinobacter*, *Rhodococcus*, *Mycobacterium*, and others such as *Gordonia*, *Nocardia*, *Dietzia*, and *Sphingomonas* have been identified as having hydrocarbon-utilizing capabilities. These species can thrive in the complex and stressful environment produced by oil pollution (Blaise Chikere et al., 2011). Hydrocarbon-degrading bacteria play a crucial role in bioremediation of soil and water pollutants from petroleum hydrocarbons, including n-alkanes, aliphatics, and aromatics. The biodegradability of hydrocarbons depends on their chemical structure, microbial populations, and environmental conditions. Bioremediation uses microbes to detoxify or remove hydrocarbons, relying on microbial enzymatic activities. This method is cost-effective, environmentally friendly, and results in innocuous end products like water and carbon dioxide. Monitoring the effectiveness of bioremediation involves chemical analysis to identify pollutants and microbial techniques to quantify viable populations and environmental factors influencing degradation rates.

2. Fungi and Yeasts in Xenobiotic Transformation

Fungal technology has demonstrated substantial potential for the detoxification of contaminated sites since 1985, when *Phanerochaete chrysosporium* was found to metabolize a wide range of environmental pollutants (Magan et al., 2010). Although white-rot fungi have been shown to degrade single xenobiotic contaminants under laboratory conditions, they remain largely untested on contaminant mixtures in natural soils. Moreover, biodegradation studies have focused predominantly on optimal substrate concentrations, yet white-rot fungi can grow under extreme water stress, a property that may enable bioremediation in drought-prone environments as climate change advances. To date, the influence of elevated atmospheric CO₂ concentration or temperature on fungal bioremediation has received no attention. Genome sequencing projects for white-rot fungi such as *P. chrysosporium* are commencing, providing insights into enzymes involved in xenobiotic degradation (Bosco & Mollea, 2019). Such genomic information is paving the way for a more complete “Systems” approach to harness fungi for bioremediation in terrestrial ecosystems.

Fungi possess versatile bioremediation abilities due to their longevity on Earth, adaptive evolution, and occupation of diverse ecological niches (Lúcia Leitão, 2009). Capable of degrading synthetic materials such as polyethylene, fungi contribute to the bioremediation of micropollutants and heavy metals. Species from the genus *Penicillium* colonize a variety of habitats ranging from soils to foods, beverages, and indoor air, and are predominant agents in xenobiotic and heavy-metal remediation. In fact, fungi exhibit broader substrate-utilization profiles than bacteria and can grow on more complex compounds such as polycyclic aromatic hydrocarbons (PAHs). Their rapid colonization rates and structural heterogeneity promote effective contact with solid substrates. Fungal extracellular enzymes—including hydrolases, oxidoreductases, and lyases—facilitate the degradation of complex pollutants, and the comparatively simple cultivation of fungi at large scale supports bioremediation studies. Filamentous fungi, especially *Penicillium* species, tolerate greater environmental variability than many other microorganisms, including exposure to elevated salinities, and hence are well suited for bioremediation of hypersaline wastes. Fungal treatments of water and soil wastes have been documented for centuries and continue to effectively degrade heavy metals, phenolic compounds, hydrocarbons, and other hazardous substances from substrates such as sludge, soils, and sediments.

3. Archaea and Extremophiles in Specialized Environments

Extremophiles are microorganisms capable of thriving in extreme environments, such as high temperatures, salinity, acidity, or radiation (Jeong & Jun Choi, 2020). These organisms evolved specialized metabolic pathways and mechanisms that enable them to endure even the harshest physical and geochemical conditions. For these reasons, extremophilic microorganisms have attracted considerable interest as potential agents for bioremediation of inorganic and organic contaminants in environments where mesophilic or moderate microorganisms are ineffective or do not survive. Recently isolated *Halobacterium* species, for example, show promise as bioaugmentation agents for petroleum-hydrocarbon-contaminated coastal waters. Extremophilic microorganisms are particularly appealing for bioremediation of metals and radionuclides, as they have natural defense mechanisms that retain activity and stability under these extreme conditions (R. Marques, 2018). Acidophilic micro-organisms resist high concentrations of hydrogen ions and heavy-metal salts by bioaccumulating metals through both metabolic and passive uptake, as well as by detoxifying intracellular metals by transforming them into less toxic or insoluble forms. Also, Archaea and Bacteria isolated from hypersaline environments, such as salterns, solar salt-works, and evaporitic salt lakes, can serve as bioremediation agents in saline environments, where only specialized microorganisms can survive.

Bioremediation Strategies and Their Applications

The selection of appropriate bioremediation strategies for practical application depends on a variety of physical, chemical, and biological parameters (Kapahi & Sachdeva, 2019). Several in situ and ex situ techniques are available for remediation of contaminated sites. In situ approaches—defined as those taking place within the affected zone—are advantageous in terms of handling minimal disturbance of the site environment, reduction of time and cost involved in excavation, and minimization of the risk of aquifer contamination due to the removal of polluted media. Also favored are methods that foster natural biodegradation or those based on selective stimulation of native microorganisms and acclimated microbial populations through inoculation. Ex situ methods, in contrast, involve the collection of contaminated media for treatment at a secondary location, although these can provide greater control

over factors affecting bioremediation. Such procedures typically entail excavation of site materials followed by treatment in lined landfills, biopiles, waste heaps, and slurry reactors.

1. In Situ Bioremediation Approaches

The in situ application of biodegradation-enhancement methods has gained traction worldwide, with a wide variety of techniques, including bio-stimulation, bioventing, air sparging, and soil vapor extraction, already being applied. Enhanced techniques prompt increasingly greater soil restoration than natural attenuation only (Simpanen et al., 2016).

Different implementation methods, including the use of monitoring wells and the application of nutrients at the surface, affect the efficiency of bioremediation. Aerobic biostimulation has been the most extensively employed means of pollution enhancement at five representative sites located within urban, industrial, and military regions (A. Key et al., 2022). By injecting oxygen or stimulating their growth, specific biogeochemical reactions involving functionalized alkyl groups can be induced even under as low as ten percent hydrocarbon saturation. Consequently, the transformation of the polycyclic aromatic hydrocarbon naphthalene to phthalate, a compound amenable to biodegradation, has been traced microbiologically. Bioremediation not only relies on native organism restoration or anaerobic/nitrate-sized organics such as within oil platforms. The sequences of hydrocarbon-affected sites at the Gama drainage basin illustrate effectively the nutrient-proof and oxygen-augment biostimulation and bio-venting enhancements. At the forward portion of the whole strata, water was collected, exhibiting that formation water can serve as a nutrient source for hydrocarbon bioremediation on the terrestrial surface.

2. Ex Situ Bioremediation Approaches

Ex situ bioremediation methods take contaminated media, such as soil or groundwater, from a contaminated site to another location for treatment. The contaminated media may be retained in place in confined structures, such as biopiles, bioreactors, or centrifuges, or they may be transported to another location for bioremediation in stockpiles or treatment units (A. Key et al., 2022). Examples of ex situ bioremediation include soil washing, landfill leaching, and ponding of landfill leachate, where the medium involved is physically separated. Ex situ bioremediation begins with the excavation and transfer of contaminated media (soil or groundwater) to a treatment facility. At the treatment facility, the contaminated media can be subjected to several operations, such as screening; particle size reduction; air drying; addition of nutrients or microbial cultures; and thermal, chemical, or biological treatment processes. The purpose of these operations is to remove contaminants or assist removal methods. The systems used in such operations can be categorized as batch, semi-continuous, or continuous.

3. Bioaugmentation, Biostimulation, and Nutrient Considerations

Bioremediation can be classified into different operational strategies, including bioaugmentation and biostimulation. Bioaugmentation, the addition of previously enriched microbial communities, has been practiced since the first release of oil spills. Bioaugmentation or the addition of specialized microbial strains under laboratory condition enhances the degradation of petroleum products both in soil and water (Atashgahi et al., 2018). Biostimulation, the addition of organic or inorganic substrates to stimulate indigenous microbial activity for bioremediation, has been used extensively (Brzeszcz et al., 2020). A variety of organic and inorganic compounds have been successfully used during bioremediation of petrochemicals, pesticides, organo-phosphates, dyes, heavy metals and phenols. Nutrient amendments increase bioremediation of crude oil, hydrocarbons, phenols, organo-phosphates, heavy metals and other contaminants. Addition of nitrogen enhances the bioremediation of crude oil, phenols and organo-phosphates. Bioaugmentation of crude oil contaminated water with hydrocarbon degrading microorganisms, *Pseudomonad*, *Flavobacterium* and *Micrococcus* in combination with urea and superphosphate has been reported to enhance degradation. Addition of Potassium Hydroxide and Calcium Hydroxide as inhibitors increase the bioremediation of organo-phosphates by the added *Pseudomonas aeruginosa* and *Alcaligenes*. Heavy metal contaminated soil treated by saturated with 10% nutrients and potato starch as carbon source showed enhancement in the degradation of herbicides. Bioaugmentation of Organophosphate hydrolysing bacterial isolates such as *Brevundimonas*, *Pseudomonas* and *Pseudoxanthomonas* has been reported to enhance bioremediation of soils contaminated with organophosphates.

Monitoring, Evaluation, and Risk Assessment

Recovery from contamination requires ongoing evaluation of natural or engineered remediation systems. The ideal extent of monitoring depends on project size and complexity, but general recommended indicators include physical-chemical parameters (e.g., contaminant concentration, water levels, phase distribution); microbial activities, community structure and diversity; and chemical analyses of contaminant-derived end products (A. Key et al., 2022). Several specific metrics assist in bioremediation assessment. The metabolic potential or activity of target microorganisms indicates particle-transfer kinetics and bioavailability (Blaise Chikere et al., 2011). Most aqueous contaminants undergo microbial mineralization to CO₂ under aerobic conditions, yielding a value closely tied to metabolic-activity rates. Hydrocarbon bioavailability can be tracked by monitoring dissolved concentrations or extractable-phase composition, while other reaction products point to pathways and bioavailability. Determining whether degradation involves biotic or abiotic processes also provides useful insight. Prioritizing risks to health and ecosystems is crucial in gauging remediation necessity. Scores serving as life cycle assessment raw indicators of exposure, risk and hazard offer additional information. Mass-balance comparisons of biological, physical and physicochemical treatment pathways between solution inflow and outflow likewise assess/or determine reduction efficiency.

1. Molecular and Microbial Community Indicators

Pseudomonas aeruginosa, a bacterium found in soil and water, is an opportunistic human pathogen and a model organism for studies of bioremediation and biotechnology. It degrades hydrocarbons via multiple pathways, transforming petroleum alkanes and aromatics into several partially oxidized metabolites, some of which, such as 2-hydroxy-3-oxooctanoate and several other branched-chain fatty acids, are required for growth and act as siderophores. *P. aeruginosa* produces biosurfactants such as rhamnolipids that enhance hydrocarbon solubility and thus cellular bioavailability. Monitoring microbial hydrocarbon remediation in soil involves chemical analyses for pollutant identification and microbiological techniques for quantifying viable populations of microorganisms. Routine, inexpensive, and rapid methods detect hydrocarbons and hydrocarbon-utilizing microbes, and enrichment cultures reveal degrading microbes. Molecular microbial ecology techniques that do not rely on cultivation are used to monitor bioremediation progress (Blaise Chikere et al., 2011).

2. Chemical and Physical Metrics of Remediation

Bioremediation approaches employ a number of indicators and monitoring strategies to detect treatment progress and assess overall efficacy (Blaise Chikere et al., 2011). Depending on the contaminant, it may be more suitable to study biological, chemical, isotopic, or toxicological indicators. For example, microbial population shifts can indicate changes in contaminant concentrations. Chemical indicators directly measure target analytes or their degradation products, thus confirming the remediation process. Site characterization can be complemented with bioassay approaches that measure the bioavailability and ecotoxicology of persistent contaminants before, during, and after remediation treatments. Physical and chemical measurements on soil amendments and aqueous and gaseous phases are important for monitoring bioremediation processes. Soil texture, bulk density, moisture content, pH, and organic carbon must be determined before and after treatment to evaluate bioremediation feasibility and progress. Measurement of hydraulic or gaseous conductivity aids in the design of in situ bioremediation systems and assessment of permeable treatment materials. Dissolved oxygen and other key parameters are monitored to evaluate aerobic biodegradation and the performance of bioreactors and land treatment systems, and soil temperature measurements can indicate metabolic rates.

3. Ecological and Human Health Risk Considerations

Bioremediation is a widely accepted and environmentally friendly technology suitable for cleaning up sites contaminated with organic or inorganic pollutants. The process essentially relies on naturally occurring microorganisms and/or plants to complete the remediation task. The underlying premise of many bioremediation approaches is that modification of environmental conditions or the introduction of additional substrates could stimulate the metabolism of indigenous microorganisms toward the biodegradation of specific contaminants. However, sites undergoing bioremediation could also remain potentially hazardous to human health and ecosystems until significant contaminant reduction has been achieved. Contaminated sites undergoing bioremediation should be monitored for indicators of a decrease in the pollutant concentration and appropriate risk assessment should be performed alongside. Many of the monitoring metrics are already covered in the previous sections. For example, an increase in bacteria capable of degrading specific organic pollutants, an increase in relevant metabolites, or a drop in other biogeochemical indicators would suggest significant loss of the targeted contaminant even if it has not disappeared entirely. Likewise, attention to chemicals (e.g., volatile fatty acids or phenolic compounds) generated during microbial metabolism could reveal the overall metabolic pathways being path through and whether mammalian toxic intermediates are being produced.

Different organic classes of pollutants would carry different types of indicators and nature of the toxicologic assessment during bioremediation (Hellal et al., 2023).

Case Studies Across Contaminant Classes

Biostimulation and bioaugmentation strategies for reducing contaminant concentrations were adopted in additional field demonstrations in Southern California. A passive biobarrier constructed in an aquifer contaminated with petroleum hydrocarbons utilized naturally occurring microbes and the introduction of nutrients to stimulate the growth of indigenous bacteria capable of degrading the contaminants. Bioaugmentation, the introduction of exogenous microorganisms capable of degrading specific contaminants, was employed in several remedial systems. The indigenous microbial community was stimulated to degrade chlorinated solvents within a contaminated aquifer in Southern California. Molecular biological tools were combined with biogeochemical monitoring to evaluate the bioremediation process and assess the bioaugmentation approach (A. Key et al., 2022). Hydraulic fracturing at a natural gas production field in Northern Mississippi engaged water re-injection systems to maintain water in the target production zone. Tracing existing and injected water in an aquifer contaminated with PCE and TCE required a methodology that would not interfere with the bioremediation process. A migration study monitored the bacterial processes involved in the degradation of chlorinated solvents, and the microbial community structure demonstrated the activity across fracture and porous media regions (M. Hlihor et al., 2017).

1. Petroleum Hydrocarbons in Soils and Groundwater

Petroleum hydrocarbons, including n-alkanes and aromatic compounds, are among the world's most common organic pollutants. They result from the refining, storage, and distribution of crude oil and its derivatives. Their widespread use in industrial applications presents a challenge for the remediation of polluted sites. For a variety of reasons, including the presence of hydrocarbons in deep geological formations and the unintentional release of hydrocarbons through accidents, industry and government agencies have designated numerous sites for cleaning. Effective remediation relies on an understanding of the mechanisms of transport, concentration, and degradation, as well as the use of techniques that exploit these mechanisms to promote efficient removal. Petrochemical hydrocarbons have the ability to sorb onto both mineral and organic matter, thereby reducing their

free concentration and bioavailability and greatly influencing the efficiency of remedial actions (Blaise Chikere et al., 2011). Bioremediation shows significant promise, but further research is needed to address location-specific implementation and to assess success based on hydrocarbon fraction removals (Koshlaf & S Ball, 2017). Consequently, laboratory, field, and pilot-scale trials remain important for evaluation of process efficiency and establishment of key characteristic parameters. Remediation techniques should be selected and designed with consideration of site-specific factors.

2. Chlorinated Solvents in Aquifers

Chlorinated solvents are frequently disposed into the environment due to their widespread application in diverse industrial practices. The extensive use of chlorinated solvents renders them one of the most prevalent organic groundwater pollutants, highlighting the need for efficient and sustainable remediation strategies (Blázquez Pallí et al., 2019). Several dehalorespiring microorganisms that can carry out the reductive dechlorination of chlorinated ethylenes have been isolated. Species of *Dehalococcoides*, an obligate organohalide-respiring genus, are capable of reducing tetrachloroethene (PCE) and trichloroethene (TCE) to the non-toxic ethylene compound in aquifers contaminated with chlorinated solvents. Microorganisms that reside in the competent microbial consortia considerably limit the chlorinated ethenes in the aquifer system via anaerobic bioremediation under in situ conditions (Herrero et al., 2022). The active microbial community of the aquifer containing chlorinated solvents consists of *Geobacter*, *Desulfovibrio*, and *Desulfotobacterium* genera, indicating that these key microbial species and propagation processes would largely assist in the assessment of bioremediation efficiency and the elucidation of bioremediation dynamics.

3. Pesticides and Persistent Organic Pollutants

Contaminated sites may contain various persistent organic pollutants (POPs), including pesticides. Pesticide use continues to increase, and the high toxicity of many pesticides often leads to groundwater contamination, posing a significant threat to human health (Maria Raffa & Chiampo, 2021). Bioremediation has emerged as a promising option for decontaminating agricultural soils contaminated with pesticides. Bioremediation involves the use of living organisms, particularly microorganisms, to remove contaminants, whereby microorganisms utilize pesticides as nutrients to promote cellular growth and mineralization to carbon dioxide, water, and other harmless end products. Microbial pesticide degradation is carried out mainly by bacteria and fungi, which transform pesticides into less toxic products or completely mineralize them, the action being catalyzed by enzymes such as hydrolases, peroxidases, and oxygenases. The time required for pesticide degradation depends on several factors, including pesticide structure, bioavailability, environmental conditions, and microbial activity. Microbial degradation of pesticides is influenced by soil characteristics that affect pesticide biopersistence, such as soil pH, organic matter content, temperature, soil moisture, clay content, and microbial biomass. Measuring these characteristics provides a numeric indication of the extent of bioremediation. The main advantages of the bioremediation process are its low cost and that it is an environmentally friendly method. Nevertheless, when dealing with large-persistent and slowly biodegradable POPs prevalent in contaminated sites, the treatment process requires extra attention.

Challenges, Limitations, and Future Directions

Modern bioremediation is characterized by its diverse array of techniques in soil or water systems. Beyond the challenges associated with scaling bacterial science from laboratory settings to field trials, the degradation of complex mixtures of chemical contaminants remains a major hurdle (Xiang et al., 2021). Efforts continue to design model pathways for the removal of municipal contaminants while optimizing the degradation of mixed chemical wastes. Synthetic biology, which permits the rational engineering of microbial metabolic pathways and regulatory circuits to create microbial cell factories, offers key advantages in fields such as green production and bioremediation. Genomic sequencing provides insight into metabolic capabilities and degradation genes, along with model editing to construct and design further pathways and functions. Boxed information files with in-depth information on relevant biological sensors, degradation pathways, and existing bioremediation systems can be linked to assist this process for tailored cell-factory design. Long-term evolutionary engineering facilitates the design of degradation pathways and substrate mixtures capable of creating adaptive evolvable microbial consortia to maintain and govern biodegradation processes.

Microbial ecotoxicology and the bioremediation of hazardous substances constitute only some of the activities involved in tackling globally disseminated pollutants from a biological perspective. Conserving microbial community structure and viability in native, polluted systems, along with equipping already-prescribed biodegradation consortia with new functionalities capable of degrading simultaneous mixtures of fresh and persistent products, constitutes an unparalleled challenge (Hellal et al., 2023). Strategies primarily consist of an initial screening to identify consortia involved in mineralizing target molecules, bypassing non-preferred or alternative metabolic pathways, and subsequent isolation of the most desirable strains. Real-time methods capable of monitoring contaminant degradation, availability of breakdown products, or toxicity at both the metabolic and community levels could substantially improve control over ongoing bacterial processes.

1. Scaling from Laboratory to Field

Scaling bioremediation from laboratory demonstration to full-scale field application poses significant challenges. Matrices and conditions that are amenable to laboratory experimentation are ill-suited for extrapolation to large-scale, heterogeneous conditions, which differ widely in chemical composition and physical characteristics (A. Key et al., 2022). The presence of multiple, complex contaminant mixtures, the need to respect regulatory requirements and achieve final end-state objectives, and the lack of robust and reproducible assessment

methodologies further complicate the bioremediation process. From the outset of any investigation, it is critical to assess the compatibility of the contaminant, the setting, and the proposed remediation technique (S. Madison et al., 2023). In cases where either the contaminant or the conditions are beyond the capabilities of conventional techniques, it is paramount to explicitly state this limitation: acknowledgment of the restriction allows rigorous exploration of alternate methods, rather than a potentially expensive and futile attempt at implementation.

2. Complex Contaminant Mixtures

The degradation of contaminants is complicated by the presence of multiple compounds that can influence the overall biodegradation rates. The complexity of mixtures can arise either from the nature of the contaminants (hydrophobic, hydrophilic, acidic, neutral, etc.) or from the composition of the source material itself. Clean-up of crude oil spills, for example, often involves the use of bioremediation because crude oil is composed of many compounds, including saturated hydrocarbons, branched-chain alkanes, cyclic hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes. Most synthetic mixtures consist of four types: halogenated alkanes, halogenated alkenes, halogenated aromatics, and dioxins/furans. Composition information is essential for computing the bioavailability of target compounds and for remedy design (A. Key et al., 2022). Microbial consortia evolved by engineering strategies such as community assembly can broaden the range of contaminants eliminated by consortia, improve the efficiency with which existing compounds are catabolized, and enhance general bioremediation performance (Cao et al., 2022).

3. Emerging Technologies and Genomic Tools

Natural bioremediation processes provide important physical and chemical processes; still, they do not always clean contaminated sites rapidly enough to prevent human or ecological exposure (Prakash et al., 2013). Biostimulation and bioaugmentation are among the most widely used strategies to enhance the rate of bioremediation. They are required when competent microorganisms are already present in the contaminated environment but do not remove the contaminant(s) at a rate sufficient to reduce human or ecological exposure. Bioaugmentation involves the addition of living cells. Systematic laboratory experiments can identify suitable microorganisms. The selection process becomes more complex when treating multi-contaminated sites, with the need to consider possible interactions with non-target micro-organisms and their indigenous competitors (Plewniak et al., 2018). Bioaugmentation with the addition of cells represents only one step in a broader spectrum of bioremediation processes aiming to enrich or control microbial function. Genomic tools can contribute to the understanding of these more complex processes. Incorporating genomic methods into bioaugmentation studies would significantly boost the knowledge about existing microbial communities and their behaviour during bioremediation treatments.

Conclusion

Bioremediation, which meets the need for sustainable and environment friendly methods to detoxify hazardous contaminants, is now gaining importance as an alternative to physicochemical treatment. The use of microorganisms or their metabolites, for the removal of toxic pollutants like heavy metals, organics, and pathogens from spoiled environment, becomes an attractive focus area for the researcher to deal with environmental disaster. The bioavailability of pollutants and the identification of efficient microorganisms showing specificity to pollutant degradation from complex environmental samples is the challenging task in carrying out bioremediation process. Native contaminated sites, such as landfills, mine tailing, oil spills, and metal amended site from battery or electronic industry, are selected to study the association of pollutant and microbes for possible selection of efficient strain and for its natural bioremediation capability (Kapahi & Sachdeva, 2019).

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