MICROBIAL BIOREMEDIATION: ADVANCEMENTS, CHALLENGES, AND FUTURE PROSPECTS

Abstract

Bioremediation is an eco-friendly technique that utilizes microorganisms to detoxify environmental contaminants. It emphasizes microbe-mediated bioremediation's significance and diversity, showcasing its potential in remediating diverse pollutants. The study covers hydrocarbons, heavy metals, organic pollutants, and emerging contaminants, exploring various in-situ and ex-situ techniques. Monitoring methods for assessing microbial activity, environmental parameters, and ecological impacts are discussed. Biotechnological applications in agriculture, forestry, wastewater treatment, and industrial settings are explored, along with the potential use of bioremediation in space exploration. Future prospects encompass advances in microbial genomics, synthetic biology, and the integration of bioremediation with sustainable technologies like renewable energy, along with the role of artificial intelligence and machine learning. Overall, this review emphasizes the importance of current research and highlights bioremediation as a promising solution for environmental restoration and a healthier planet.

Keywords: Bioremediation, Microorganisms, Contaminants, Monitoring, Biotechnological Applications, Future Prospects

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I. INTRODUCTION

The well-being of life on Earth is intricately tied to the condition of the environment. With the progression of civilization, urbanization, and industrialization, waste generation and improper disposal have escalated. An alarming estimate of 1000 new chemicals is synthesized each year, contributing to environmental complexities. Disturbingly, over 450 million kilograms of toxic substances are released into the air and water worldwide, as reported by the third world network (Singh et al.,2014). Heavy metal pollution poses a substantial risk to public and environmental health due to its toxicity, inability to biodegrade, and tendency to accumulate in the food chain (Guo et al.,2010). Similarly, polyaromatic hydrocarbons (PAHs) are concerning due to their mutagenic and carcinogenic characteristics (Balaji et al.,2014). Toxic pollutants causing ecological imbalances are a matter of worldwide apprehension (Kour et al.,2021). Microbial biotechnology is a swiftly expanding and emerging domain with various applications in addressing environmental problems. The utilization of microbes for bioremediation is a flexible technology with great stability, cost-effectiveness, environmental friendliness, minimal interference with ecosystem ecology, and high public acceptance (Singh et al.,2020). Environmental cleaning through bioremediation serves as a suitable alternative to physicochemical methods, which can be environmentally harmful and lead to secondary pollution. Bioremediation is applicable for cleaning up polluted sites like water, soils, sludge, and waste streams (Kaur et al.,2021). It has even gained approval from the US Environmental Protection Agency (USEPA) as an effective and environmentally friendly technique for revitalizing contaminated environments and promoting sustainable development (Bharagaya et al.,2020). Microbes from Alcaligenes, Aspergillus, Bacillus, Flavobacterium, Ganoderma, Methosinus, Nocardia, Phormidium, Pseudomonas, Rhizopus, Rhodococcus, and Stereum genera have demonstrated potential for bioremediation (Kumar et al.,2021). While bioremediation is not a novel concept, advances in molecular biology and process engineering have led to new approaches (Nduka et al.,2012). Genetic engineering techniques now allow the creation and application of genetically modified organisms to mitigate the impact of toxic compounds in the environment. Implementing these methods and enhancing their efficiency can yield economic and social benefits, reducing disease risks and waste management costs, while achieving greater ecological stability and a greener environment (Aziz et al.,2018).

II. BIODIVERSITY OF BIOREMEDIATION MICROBES

The captivating process of bioremediation involves utilizing a diverse array of microorganisms, including fungi, yeast, and bacteria, to detoxify environmental pollutants. These remarkable creatures are highly effective at cleansing pollutants from the environment, making bioremediation a cost-effective, straightforward, and environmentally friendly cleanup method (Kour et al., 2020). To ensure the efficacy of environmental contaminant detoxification, a wide range of microbes are extensively studied worldwide, originating from various locations and adapting to diverse environmental conditions (Chandran et al., 2020). In a study, yeasts capable of degrading phenol, including Candida boidinii, Pichia holstii, P. membranifaciens, and Saccharomyces cerevisiae, were isolated from olive mill wastewaters (Sinigaglia et al., 2010). According to Zhang et al. (Zhang et al., 2010), a bacterium capable of degrading petroleum, Bacillus sp., was identified from soil contaminated with oil. In another study, Trametes versicolor, a white rot fungus, was reported as an effective bioremediation agent for polycyclic aromatic hydrocarbons (PAH) (Sayara et al., 2011). In a study conducted by Janbandhu and Fulekar (Janbandhu & Fulekar, 2011), three bacterial

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species, namely, Achromobacter insolitus, Bacillus cereus, and Sphingobacterium sp., which were isolated from a petrochemical refinery field, were found to be effective in remediating polycyclic aromatic hydrocarbons (PAHs). In another report, a diverse group of bioremediating bacterial isolates, including Bacillus megaterium, B. cibi, B. cereus, Pseudomonas aeruginosa, and Stenotrophomonas acidaminiphila, were identified in soil contaminated with oily sludge. These strains were reported to possess the capability of degrading both aliphatic and aromatic compounds (Cerqueira et al., 2012). In a study conducted by Syakti et al. (Syakti et al., 2013), bacterial isolates with the potential for bioremediation were found in mangroves growing in soil contaminated with hydrocarbons. The identified bacterial species included Bacillus aquimaris, B. megaterium, B. pumilus, a Flexibacteraceae bacterium, a Halobacillus trueperi, and Rhodobacteraceae bacterium. In a different study, microbes capable of degrading crude oil were identified, and they were found to belong to the genera Achromobacter, Alcaligenes, Bacillus, Brevibacillus, Delftia, Lysinibacillus, Paenibacillus, Pseudomonas, and Stenotrophomonas (Roy et al., 2014). In a study, Pseudomonas sp. was isolated from soil at a petroleum refinery, and the strains were found to be capable of degrading hydrocarbons (Goudarztalejerdi et al., 2015). (Godoy et al.,2016), in their research, isolated fungal species from soil contaminated with polycyclic aromatic hydrocarbons (PAHs), which showed the ability to bioremediate xenobiotics. The identified fungal isolates were Fomes sp. and Scopulariopsis brevicaulis. In a research study, a diverse array of bacterial strains capable of degrading hydrocarbons was reported from petroleum refinery waste. These strains were found to belong to the genera Bacillus, Burkholderia, Enterobacter, Kocuria, Pandoraea, and Pseudomonas (Sarkar et al., 2017). In a separate study, Stenotrophomonas was identified as a bacterium with the capability of bioremediating xenobiotics. Notably, this strain exhibited resistance to antibiotics such as ofloxacin, streptomycin, rifampicillin, erythromycin, ampicillin, and clindamycin. Additionally, it was reported to effectively degrade heavy metals, including arsenic, mercury, copper, nickel, and lead (Aslam et al., 2018). The microbial community consisting of the genera Shinella, Microbacterium, Micrococcus, and Bacillus was found to be effective in bioremediating heavy metal environmental pollutants, including cadmium, chromium, cobalt, nickel, and zinc (Bhakat et al., 2019).

Table 1: Bioremediating Microorganisms and their Capabilities

SI NO.	Microorganisms	Bioremediation Capability	Reference
1	Candida boidinii, Pichia holstii, P. membranifaciens, Saccharomyces cerevisiae	Degrading phenol in olive mill wastewaters	Sinigaglia et al.,2010
2	Bacillus sp.	Degrading petroleum in oil-contaminated soil	Zhang et al.,2010
3	Trametes versicolor	Bioremediation agent for polycyclic aromatic hydrocarbons (PAH)	Sayara et al.,2011
4	Achromobacter insolitus, Bacillus cereus, Sphingobacterium sp.	Remediating PAHs from petrochemical refinery soil	Janbandhu et al.,2011
5	Bacillus megaterium, B. cibi, B. cereus, Pseudomonas aeruginosa,	Degrading aliphatic and aromatic compounds in oily	Cerqueira et al.,2012

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	Stenotrophomonas acidaminiphila	sludge contaminated soil	
6	Achromobacter, Alcaligenes, Bacillus, Brevibacillus, Delftia, Lysinibacillus, Paenibacillus, Pseudomonas, Stenotrophomonas	Degrading crude oil	Roy et al.,2014
7	Fomes sp., Scopulariopsis brevicaulis	Bioremediating xenobiotics from PAH-contaminated soil	Godoy et al.,2016
8	Bacillus, Burkholderia, Enterobacter, Kocuria, Pandoraea, Pseudomonas	Degrading hydrocarbons from petroleum refinery waste	Sankar et al.,2017
9	Stenotrophomonas	Bioremediating xenobiotics and degrading heavy metals	Aslam et al.,2018
10	Shinella, Microbacterium, Micrococcus, Bacillus	Bioremediating heavy metal pollutants (cadmium, chromium, cobalt, nickel, zinc)	Bhakat et al.,2019

III. BIOREMEDIATION OF VARIOUS POLLUTANTS

1. **Microbe-Mediated Degradation of Hydrocarbons:** Polycyclic aromatic hydrocarbons (PAHs) are hazardous fused-ring aromatic compounds that can be found as organic pollutants in the environment. These compounds have a high molecular weight and can persist for years (Wei et al., 2022). They accumulate due to human and natural activities, including fossil fuel combustion and industrial processes, as well as natural disasters like forest fires and volcanic eruptions (Wattiau P., 2002). These compounds are present in various petroleum-based products and are commonly found in areas near gas plants, refineries, petrol stations, and chemical industrial sites, leading to contamination in soil and water. PAHs have been identified as carcinogenic and mutagenic, earning them priority pollutant status by the US EPA (Quinn et al., 2009). Human exposure to PAHs can occur through multiple pathways, such as inhalation, ingestion of contaminated food and water, and occupational contact. Additionally, these pollutants can enter the water supply from various sources like industrial and household waste, urban runoff, and vehicle emissions. The removal of PAHs from the atmosphere is critical due to the extensive harm they cause to human health, the environment, marine life, land animals, and agricultural soil. However, their insolubility in water and slow degradation make their removal from soil particularly challenging (Bossert et al., 2003).

   Bioremediation is a highly effective method for restoring ecosystems by cleaning up soil contamination (Haritash et al., 2009). Microorganisms, including algae, bacteria, and fungi, are crucial in degrading PAHs (polycyclic aromatic hydrocarbons) found in polluted environments. These microorganisms use various metabolic pathways to break down different PAH compounds. Studies have identified numerous microbes capable of degrading specific PAHs. For instance, Krivobok et al. discovered several isolates, including Cryphonectria parasitica, Ceriporiopsis subvermispora, Oxysporum sp., Cladosporium herbarum, Rhizopus arrhizus, Phanerochaete chrysosporium, Irpex lacteus, and Pleurotus ostreatus aiding in anthracene degradation (Krivobok et al., 1998). Another investigation Annweiler et al. reported bacteria Bacillus thermoleovorans from

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contaminated compost demonstrated the ability to degrade naphthalene compounds (Annweiler et al., 2000). Additionally, Chauhan et al. reported Comamonas testosterone, Pseudomonas stutzeri, and other microbes capable of breaking down various PAHs like anthracene and benzo[b] fluoranthene (Chauhan et al., 2008). Another report Chaudhary et al. reported different microbial species like Haemophilus sp., Mycobacterium sp., Pseudomonas sp., and Rhodococcus sp. separated from soil for their potential to degrade to phenanthrene, naphthalene, anthracene, pyrene, and benzo[a]pyrene (Chaudhary et al., 2015). Mangwani et al. reported Pseudomonas mendocina isolated from Rushikulya estuary, Pseudomonas aeruginosa from Paradeep port, Stenotrophomonas acidaminiphila and Alcaligenes faecalis from Chilika lagoon; all microbes have potential to degradation of PAHs compounds such as pyrene phenanthrene (Mangwani et al., 2017).

2. **Microbial Detoxification of Heavy Metals:** Heavy metals possess a greater atomic mass and higher density compared to other elements. The environment contains over 20 heavy metals, but some, like lead, nickel, zinc, chromium, cadmium, copper, argon, silver, mercury, arsenic, and uranium, are particularly worrisome due to their high toxicity. Globally, soil and groundwater contamination by heavy metals has emerged as a significant environmental issue, necessitating their removal from contaminated areas. This is crucial because these metals can accumulate in the food chain and impact the health of organisms. Research by Mesa et al. (2015) demonstrated that Spartina maritima, with the assistance of indigenous rhizobacteria, exhibited enhanced heavy metal removal from metal-contaminated estuaries, leading to increased plant biomass and heavy metal uptake. Similarly, Tiecher et al. (2016) utilized Brachiaria mutica and Zea mays to treat a heavy metal-contaminated site through phytoremediation. Several studies on heavy metal bioremediation have reported removal rates of 19%-65% for As, Cu, Pb, and Zn, 99.3% for heavy metals (Fe, Zn, Cd, Cu, B, and Cr), 50%-100% for Pb, 25%-60% for Ni, and 20%-70% for silver nanoparticles using bioaugmented rhizoaccumulation, rhizofiltration, and phytoaccumulation techniques of phytoremediation (Elias et al., 2014; Mesa et al., 2015).

3. **Biodegradation of Organic Pollutants:** Various human activities, including industrial agriculture, oil spills, and petroleum industries, have led to the release of a wide range of persistent organic pollutants into the environment. Nevertheless, scientific research has showcased the effective degradation of these hazardous organic pollutants through microbial interventions. For instance, Almansoory et al. (2015) documented a substantial 93.5% reduction in total petroleum hydrocarbon (TPH) levels in soil contaminated with gasoline. This reduction was achieved by applying a biosurfactant produced by a combination of Serratia marcescens and the plantation of Ludwigia octavalvis. Similarly, Gomez and Sartaj (2013) accomplished a noteworthy 90.17% removal of TPH in a field-scale system where bioaugmentation and biostimulation were employed in biopiles, even when faced with low-temperature conditions. Furthermore, Dias et al. (2015) observed a 71% decrease in TPH during a 50-day experimental period through biopile treatment following soil pretreatment.

Several research studies have highlighted significant reductions achieved through bioremediation using the bioreactor technique, including 82% to 97% in TPH, 51% to 68% in BTEX (benzene, toluene, ethylbenzene, xylene), 97% to 100% in 2,4-dichlorophenoxyacetic acid, and 88% to 97% in carbofuran from contaminated soil (Plangklang and Reungsang, 2010; Firmino et al., 2015; Mustafa et al., 2015; Chikere et

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al., 2016). Additionally, Kao et al. (2008) accomplished a reduction exceeding 70% in BTEX contaminants present in contaminated groundwater using the biosparging bioremediation technique.

4. **Microbial Strategies for Remediating Emerging Contaminants:** Microorganisms have a significant impact on breaking down xenobiotics, and it has been discovered that pharmaceuticals can also be degraded by these microbes. Certain microorganisms even use these contaminants as an energy source through complete mineralization. Biodegradation offers a viable approach for eliminating pollutants, as conventional methods like advanced oxidation, activated carbon, and physical treatments face limitations due to high energy consumption and the generation of toxic by-products (Homem and Santos, 2011; Schwarzenbach et al., 2006).

   A significant pharmaceutical contaminant, Paracetamol (PAM), is commonly used as an antipyretic over-the-counter drug. Researchers have developed a method combining microbial fuel cell and Fenton oxidation to degrade PAM without an external power supply (Zhang et al., 2015). Microbial fuel cells consist of anode and cathode, where microorganisms called electricigens facilitate electron transfer to reduce oxidized pollutants on the cathode (Logan, 2009). Another area of interest is nootropic drugs as environmental contaminants, which are poorly metabolized and mostly excreted through urine (Mache et al., 2012). An instance is Piracetam, fully transformed by two strains of Ochrobactrum bacteria, breaking down the heterocyclic ring at the C-N linkage (Wo´zniak-Karczewska et al., 2018). Although more comprehension is required, a thermophilic microorganism known as Thermus thermophilus C419, was discovered with the capability to break down fluoroquinolones. This implies that microorganisms can also be harnessed to remediate challenging environments that are also polluted (Pan et al., 2018). Corynebacterum sp. D5, which degrades acrylonitrile, utilized nitrile hydratase and amidase for partial transformation (Sunarko and Sulistinah, 2019). Furthermore, fungi such as Gymnopilus luteofolius and Stropharia rugosoannulata, which have self-immobilized in pellet-like structures, have demonstrated potential in breaking down 90% of Iopromide and 70% of Carbamazepine respectively.

IV. METHODS FOR BIOREMEDIATION

Developing eco-friendly, cost-efficient, and dependable cleanup technology is crucial for environmental decontamination. Microorganisms, abundant and widely available, can utilize harmful substances as their food source. They possess remarkable adaptability to diverse environments and produce metabolites that naturally transform environmental pollutants, enabling the restoration of contaminated sites. While various remediation methods exist, microbe-mediated bioremediation is preferred due to its numerous benefits and the rising costs of physical and chemical treatments. The US Environmental Protection Agency has outlined two bioremediation methods: in situ and ex situ. Microbes can be effectively applied in both in situ and ex situ conditions.

1. **In Situ Bioremediation:** The technique involves utilizing a biological treatment method to clean up hazardous compounds. It is commonly used to degrade contaminants in saturated soils and groundwater (Girma G., 2015; Evan et al., 2016; Vidali et al.,2001). This process relies on microbial activities to destroy and detoxify contaminants. The effectiveness of the microbes in converting toxic substances into less harmful forms

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depends on the availability of nutrients and electron acceptors and donors. In situ bioremediation is a sustainable approach as it eliminates the need for transporting, depositing, pumping, treating, and discharging contaminated soil and groundwater (Jørgensen K., 2011). This method has several advantages, such as cost-effectiveness, the use of harmless native microbial species, and the ability to treat large volumes of contaminated soil or water while minimizing the release of toxic substances. In situ bioremediation has proven successful in degrading various pollutants in soil and groundwater, including anilines, chlorinated hydrocarbons, nitrobenzenes, nitriles, and plasticizers . In situ bioremediation include.

- **Bioaugmentation:** Bioaugmentation involves enhancing the native microorganisms at the polluted location by introducing specifically chosen local or genetically altered microbes. This improves the remediation process and is particularly effective in treating soils and groundwater polluted with tetrachloroethylene and trichloroethylene. By using this approach, the on-site microbes can break down these contaminants into harmless substances like ethylene and chlorides (Niu et al., 2009).
- **Biostimulation:** Biostimulation utilizes indigenous microorganisms, which are encouraged to thrive by introducing nourishing elements like phosphorus, nitrogen, O2, or other oxidizing substances. These stimulants are commonly administered below the surface through injection wells (Zeneli et al., 2019). The significant benefit of this method lies in the utilization of well-adapted local microorganisms. Furthermore, it has been proposed that both of these methods can also be employed ex situ, although they are categorized as in situ bioremediation techniques (Bodor et al., 2020).

2. **Ex Situ Bioremediation:** In this strategy, contaminants are extracted from polluted sites and then relocated to an alternate location for remediation. To implement ex situ bioremediation techniques, multiple factors are taken into account, including the degree of pollution, the nature of pollutants present, treatment costs, and the geographic setting of the polluted area (Sharma et al., 2020). The method is categorized into two primary groups: solid-phase and slurry-phase systems, depending on the state of the targeted pollutants for elimination. Solid-phase systems address diverse waste types, including agricultural, household, industrial, organic, and municipal solid wastes. Within solid-phase treatment processes, there are methods such as land farming, composting, and soil biopile approaches. Land farming, also recognized as land treatment, involves excavating contaminated soil and spreading it thinly on the ground (LV et al., 2008). The goal of this approach is to activate local microorganisms with biodegradation capabilities and facilitate the breakdown of contaminants under aerobic conditions (Vidali et al., 2001). Soil biopiles, also referred to as biocells, are employed to remediate excavated soil primarily polluted with petroleum-based substances. Biopiles establish a conducive environment for both native aerobic and anaerobic microorganisms. Composting entails mixing contaminated soil with non-hazardous organic additives, like agricultural residues, corncobs, hay, manure, and straw. The aim is to maintain optimal levels of air and water for the microorganisms. The selection of additives used depends on soil permeability and achieving the necessary carbon and nitrogen equilibrium to promote microbial activity. Conversely, slurry-phase bioremediation, or bioreactors, is a regulated treatment approach. It involves excavating polluted soil, blending it with water, and depositing the blend into a bioreactor.

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3. **Microorganisms Assisted Nanotechnology:** Biomaterial fabrication of nanomaterials, combined with the utilization of microorganisms, presents a more sustainable and environmentally friendly approach to nanotechnology. In contrast to chemically produced nanoparticles, which might have limitations associated with chemical utilization and self-agglomeration in aqueous solutions, the eco-friendly synthesis of nanoparticles from botanical extracts, fungal, and bacterial enzymes provides a promising substitute. These natural agents act as reducing agents for metal complex salts, facilitating the creation of metallic nanoparticles. As a result, the resultant nanoparticles display increased stability in aqueous environments via co-precipitation or the incorporation of proteinaceous and bioactive components onto their surfaces. A noteworthy investigation conducted by Mahanty et al. (2020) illustrates the biofabrication of iron oxide nanoparticles using Aspergillus tubingensis (STSP 25) isolated from the rhizosphere of Avicennia officinalis in Sundarbans, India. Impressively, these produced nanoparticles displayed a remarkable ability to eliminate over 90% of heavy metals (Pb [II], Ni [II], Cu [II], and Zn [II]) from wastewater and could be regenerated for up to five cycles. The chemical adsorption of metal ions onto the nanoparticle surface took place via endothermic reactions (Mahanty et al., 2020). Similarly, another investigation focused on employing exopolysaccharides (EPS) derived from Chlorella vulgaris to co-precipitate with iron oxide nanoparticles. Fourier-transform infrared spectroscopy (FT-IR) analysis verified the successful alteration of the nanoparticles by the functional groups of EPS. Furthermore, this nanocomposite exhibited a remarkable capacity to eliminate 91% of PO43– and 85% of NH4+ from its environment (Govarthanan et al., 2020). The application of microorganisms in nanoparticle synthesis has surfaced as an economical and eco-friendly approach. An illustrative instance involves the production of copper nanoparticles using copper-resistant Escherichia sp. SINT7. These biogenic nanoparticles exhibited notable efficacy in breaking down azo dye and textile effluent. When used at a concentration of 25 mg/L, the reduction percentages for reactive black-5, congo red, direct blue-1, and malachite green were 83.61, 97.07, 88.42, and 90.55%, respectively. At a higher concentration of 100 mg/L, these values slightly decreased to 76.84, 83.90, 62.32, and 31.08%, respectively. Significantly, the treatment of industrial effluent with these nanoparticles led to significant reductions in suspended solids, chloride, and phosphate ions in the treated samples, highlighting the potential of this strategy for economical and sustainable utilization in industries (Noman et al., 2020).

   In a distinct study, Cheng et al. (2019) effectively fabricated iron-sulfur nanoparticles without the need for additional sulfur. These nanoparticles demonstrated the capability to break down Napthol Green B dye through extracellular electron transfer. Utilizing Pseudoalteromonas sp. CF10-13 in the nanoparticle creation offered an environmentally friendly method for biodegradation. The innate nanoparticle production effectively mitigated the release of harmful gases and metal complexes. The utilization of biogenic particles emerges as a superior technology for remediating industrial effluents. Beyond direct nanoparticle generation from microorganisms, these adaptable microorganisms can also supply catalytic enzymes, working in conjunction with nanoparticles to expedite effluent remediation and further enhance nanotechnology's applications. The following table provides succinct details concerning the implementation of nanotechnology in wastewater remediation. Additionally, microorganisms play a role in yielding beneficial products from industrial waste, a topic that will be further explored.

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Table 2: Bioremediation of Different Industrial Effluents Using Advanced Nanotechnology Processes.

SI NO.	Nanotechnology applied	Associated Microorganisms	Modification	Specific Feature	Advantage /Mechanism	Removal or Adsorption Capacity	References
1.	Electrospunnanofibrous webs	*Pseudomonas aeruginosa*	Bacterial encapsulation	Genetic engineering or more potent bacterial cell could prove more promising	Biological removal of dye	55–70% removal of methylene blueat different concentrations	Sarioglu et al., 2017
2.	NiO and MgO nanoparticles	–	Silica-embedded	Regeneration and reusability proved sustainability	Spontaneous, endothermic, and physical adsorption of Cu^{2+} and Cr^{3+} and exothermic and chemical of Zn^{2+}	Maximum uptake of 41.36, 13.76, 7.23 (ions per nm^2) for Cr^{3+}, Cu^{2+}, and Zn^{2+}	Abuhatab et al., 2020
3.	Cobalt and cobalt oxide nanoparticles	–	Microwave and reductive chemical heating	Greener, easy, and faster to make, cost-effective and photocatalytic degradation efficiency	Irradiation and large surface area	43.6 and 39.4% degradation of murexide dye by Cobalt and cobalt oxide nanoparticles, respectively	Adekunle et al., 2020
4.	Mesoporous organosilica nanoparticles (MONs)	–	Incorporation of ferrocene	Novel organic-inorganic hybrid nanomaterial	More surface area and πnjugation derived from non-covalent	High removal rate of dyes by MONs-50% and metals by	Yang et al., 2019

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SI NO.	Nanotechnology applied	Associated Microorganisms	Modification	Specific Feature	Advantage /Mechanism	Removal or Adsorption Capacity	References
					interaction facilitated by ferrocene	MONs-25%	
5.	Zirconia nanoparticles	*Pseudomonas aeruginosa*	Synthesis from microbial cell free culture supernatant	Green synthesis of nanoparticles and sustainable bioremediation	Chemisorptions and strong electrostatic interaction among zwitter ions	Tetracycline adsorption of 526.32 mg/g	Debnath et al., 2020
6.	Electrospuncyclodextrinfibers	*Lysinibacillus* sp. NOSK	Bacterial encapsulation	Cyclodextrin provides extra carbon source for growth of bacteria	Bacterial bioremediation	Removal efficiency of Ni(II) = 70 ± 0.2%, Cr(VI) = 58 ± 1.4% and Reactive black 5 = 82 ± 0.8	San Keskin et al., 2018
7.	Enzyme immobilized nanoparticles	*P. ostreatus*	Laccase immobilization	Reusable enzyme and cost-effective	Oxidation by immobilized laccase	Degradation of bisphenol-A = 90% and carbamazepine = 10%	Ji et al., 2017
8.	Silica nanoparticles	Actinomycetes	Synthesized from actinomycetes	Cost-effective and sustainable	Photocatalytic degradation	80% decolorization of industrial effluent	Mohanraj et al., 2020
9.	Graphene oxide and	–	Nano-sized	Superior	Hydrophobic	Methylene blue	Ahsan et

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SI NO.	Nanotechnology applied	Associated Microorganisms	Modification	Specific Feature	Advantage /Mechanism	Removal or Adsorption Capacity	References
	carbon nanotubes		nickel metal organic framework	interaction of nanocomposite	and/or π-π interactions, high surface area, occurrence of the pores among the MOFs and the platforms and diverse morphological features of mixed nanocomposites	adsorption of 222 mg/g	al., 2020

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V. Microbial-Mediated Bioremediation Techniques and Monitoring Methods

Due to rapid population growth and extensive industrial activities, diverse contaminants have been generated and released into the environment. These hazardous substances exert detrimental effects on both human health and the ecosystem. Nonetheless, the application of microbial-mediated bioremediation shows tremendous potential in restoring contaminated environments in an eco-friendly manner. However, before considering bioremediation as a viable approach, it is imperative to demonstrate the presence of a sufficient population of microorganisms capable of effectively combatting the specific pollutants (Wolickaet al.,2009).

The monitoring process commences by employing standard microbiological techniques to quantify viable microbe populations, along with basic chemical analysis for pollutant identification. In cases where cultivating specific microbial populations proves challenging, enrichment culture can reveal the presence of crucial degrading bacteria and demonstrate their inherent ability to effectively break down the contaminant at a desirable rate (Atlas & Philip, 2005).

Emerging molecular microbial ecology tools have revolutionized the field by circumventing the need for culturing, taking advantage of functional and non-culturable microorganisms. These innovative tools have been extensively validated and shown to be immensely advantageous in monitoring the progress of bioremediation processes (Zengler et al., 2008).

During bioremediation, shifts in microbial populations can be investigated using various methods, including more comprehensive analytical techniques such as gas chromatography with flame ionization detector (FID) or electron capture detector (ECD). Moreover, tests involving radiolabeled substrates like 14C can be employed to ascertain whether the substrate has undergone mineralization or biodegradation, or has been transformed into a more stable state. Additionally, high-performance liquid chromatography (HPLC) can be utilized for detailed analysis (Atlas & Philip, 2005).

These methodologies have been extensively employed in both field-scale and laboratory bioremediation studies, demonstrating their efficacy in monitoring the progress of bioremediation across various environmental media (Chikere et al., 2009).

The measurement of microbial activity and aerobic metabolism can be accomplished through respirometry, where the uptake of molecular oxygen or the production of CO2 serves as indicative parameters (Singh et al., 2004).

Additionally, respirometric investigations can be employed to explore several aspects related to soil, including the potential decomposition of petroleum hydrocarbons, nutritional limitations, the capacity of heavy metals to affect microbial activity, the impact of toxic chemicals, and the response of clayey acidic soil to varying pH levels (Bosco et al., 2019).

Respirometry studies are versatile and can extend to assessing different biological treatment procedures, evaluating the impact of culture bioaugmentation and nutrient supplementation, as well as demonstrating active hydrocarbon breakdown in full-scale bioremediation processes. Soil microcosm experiments aid in determining the biodegradation

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potential of hydrocarbon-contaminated soils and developing predictive models for their fate. During these tests, pollutant concentrations and their metabolic byproducts are analyzed to obtain valuable biodegradation kinetics data and identify the most suitable bioremediation approach for large-scale application. Slurry bioreactors of various sizes can also be utilized to assess biodegradation capabilities. These bioreactors offer several advantages, including efficient aeration, enhanced mixing, improved substrate supply, and significantly reduced treatment time (Bosco et al., 2019).

Various techniques, including Fourier-transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), mass spectrometry (MS), gas chromatography (GC), infrared (IR) absorption, and thin-layer chromatography (TLC), are utilized to evaluate the rates of contaminant degradation and the creation of degradation products. These analytical tools play a crucial role in monitoring and quantifying the progress of bioremediation processes by providing valuable information about the transformation of contaminants into less harmful substances (Singh et al., 2004).

Solid-phase microextraction (SPME) has been efficiently employed to observe the biodegradation of semi-volatile hydrocarbons in soil and water contaminated with diesel fuel, along with the decomposition of volatile hydrocarbons while bacteria metabolize crude oil. This technique allows for the extraction of target compounds from complex matrices, making it valuable in tracking the progress of bioremediation processes involving hydrocarbon-contaminated environments (Chang et al., 2010).

Solid-phase microextraction has proven to be a rapid and accurate method for assessing both semi-volatile and volatile hydrocarbons in petroleum biodegradation systems. Traditional culture techniques are used to study microbial interactions in the atmosphere and their utilization of hydrocarbons as a substrate. Specific counts of hydrocarbon-degrading microbes and total heterotrophic microbial counts in polluted soil provide valuable insights into the adaptation of the native microbial community to the contaminated environmental conditions and its capacity to sustain bioremediation efforts. Microbial enumerations are frequently determined in representative soil composite samples, and compelling evidence supports a significant correlation between microbial populations and hydrocarbon degradation (Balba et al., 1998).

Table 3: Overview of Microbial-Mediated Bioremediation Techniques and Monitoring Methods

SI NO.	Techniques	Description	References
1	Bioremediation Potential	Microbial-mediated bioremediation has potential to restore contaminated environments in an eco-friendly manner	Wolicka et al.,2009
2	Monitoring Techniques	Standard microbiological techniques, chemical analysis, and molecular microbial ecology tools are used for monitoring	Zengler et al.,2008
3	Analytical Techniques	Gas chromatography (FID/ECD), radiolabeled substrate tests (14C), HPLC,	Atlas and Philp, 2005

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		FTIR, MS, GC, IR absorption, and TLC are employed	Chikere et al.,2011 Chang et al.,2010
4	Respirometry	Respirometry measures microbial activity and aerobic metabolism	Singh et al.,2004
5	Soil Microcosm Experiments	Soil microcosms determine biodegradation potential of hydrocarbon-contaminated soils	Bosco et al.,2019
6	Slurry Bioreactors	Slurry bioreactors assess biodegradation capabilities with improved efficiency	Singh et al.,2004 Bosco et al.,2019
7	Solid-Phase Microextraction (SPME)	SPME monitors biodegradation of semi-volatile and volatile hydrocarbons	Chang et al.,2010
8	Traditional Culture Techniques	Culture techniques study microbial interactions and specific counts of hydrocarbon-degrading microbes in polluted soil	Balba et al.,1998

VI. FUTURE PERSPECTIVES OF BIOREMEDIATION TECHNIQUES

The preceding extensive discussion on bioremediation emphasizes the widespread use of ex situ and in situ techniques for waste minimization and the remediation of contaminated soil and water. Advancements in genomic, molecular, and biotechnological methods hold potential for expanding bioremediation approaches. Employing multiple bioremediation techniques simultaneously can prove to be a highly efficient, promising, and cost-effective solution to pollution problems. Of growing interest is the application of biosurfactant-mediated bioremediation, which enhances the solubilization and bioavailability of pollutants to microbes, particularly in hydrocarbon-contaminated sites. This approach leverages the low-cost production of biosurfactants using microbes supplemented with agroindustrial waste, taking advantage of their biodegradable nature. Additionally, techniques like bioaugmentation and biostimulation can further augment the biodegradation potential of indigenous microbes. Advances in molecular techniques (metagenomics, genomics, metabolomics, transcriptomics, and proteomics) have overcome challenges related to microbial culturing, providing deeper insights into microbial diversity and their functions. This knowledge enhances the mitigation of emerging pollutants and related environmental issues. Microbial fuel cells, inoculated with specific microbes such as Shewanella sp. and Pseudomonas sp., present promising candidates for remediating polyaromatic hydrocarbons (e.g., phenanthrene)-contaminated sites. The use of genetically engineered microbes is another progressive approach in bioremediation. This technique allows for the effective degradation of recalcitrant pollutants through novel and efficient catabolic pathways, expanding the substrate range for degradation and increasing microbial degradation activity stability (Paul et al., 2005). The field of nanoscience and technology has also contributed significantly to bioremediation by developing various nanomaterials that act as biocatalysts. These nanomaterials increase the surface area and reduce the activation energy required for biodegradation processes.

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Nanotechnology has captured the attention of researchers due to its numerous benefits, including a large surface area, versatility for multiple applications, stability under harsh conditions, and easy material manipulation, among others. When combined with microorganisms and enzymes, nanotechnology offers a greener approach to managing industrial effluents (Dixit et al., 2020; Zhang et al., 2020). This integration mitigates the risks associated with chemically synthesized nanoparticles, as the residues are biocompatible or can be easily separated using simple filtration or precipitation techniques. However, a significant challenge lies in the commercialization of these nanotechnological advancements, with only 1% having been commercialized thus far (Dwevedi, 2019). Scaling up the application of microorganism-assisted nanotechnology techniques could serve as a crucial milestone for industries. To achieve this, continuous support and funding from researchers and government bodies are essential to harness the full potential of nanotechnology for sustainable and cost-effective industrial production.

VII. CONCLUSION

In conclusion, bioremediation is a powerful and promising approach for mitigating environmental pollution caused by various contaminants. Microorganisms play a central role in this process, demonstrating their ability to efficiently degrade pollutants and transform them into less harmful forms. The biodiversity of bioremediation microbes offers a diverse range of species with unique capabilities for tackling different types of contaminants. Advancements in genomic, molecular, and biotechnological methods have expanded our understanding of microbial communities and their functions, enabling the development of more effective bioremediation strategies. The combination of multiple bioremediation techniques, such as biosurfactant-mediated bioremediation, bioaugmentation, and biostimulation, has shown great potential for increasing the efficiency and cost-effectiveness of remediation processes. Nanotechnology has emerged as a valuable tool in bioremediation, with the synthesis of biocompatible nanomaterials that enhance microbial degradation and pollutant removal. The integration of nanotechnology with microorganisms offers a greener and more sustainable approach to managing industrial effluents. Monitoring plays a crucial role in assessing the progress and success of bioremediation processes, ensuring that they are effective in restoring contaminated environments. Various monitoring techniques, including standard microbiological methods and advanced molecular analyses, provide valuable insights into microbial activities and pollutant transformations. As we move forward, continuous support and funding for research and commercialization are essential to fully harness the potential of bioremediation and microorganism-assisted nanotechnology. These technologies hold great promise in addressing environmental challenges, offering eco-friendly and efficient solutions for cleaning up contaminated sites and safeguarding our ecosystems for future generations. With ongoing advancements and interdisciplinary collaborations, bioremediation is set to play a vital role in building a sustainable and cleaner environment.

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