

Microbe-mediated bioremediation: Current research and future challenges

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ABSTRACT

The rise in environmental pollution over the past few decades due to rapid industrialization and unsafe agricultural practices has become a major challenge. The presence of toxic pollutants such as nuclear wastes, heavy metals, pesticides, and hydrocarbons has been languishing the environment as well as the human health. Bioremediation using microbial communities is emerging as an incredible, eco-friendly, and cost-effective approach to ameliorate the adverse effects of toxic pollutants. Microbes possess astonishing metabolic capabilities to alter most forms of organic material and can survive in extreme environmental conditions which make them attractive candidate for the bioremediation. Microbes are the treasure houses for environmental cleaning and recovering of contaminated soil and they have been reported from diverse environmental conditions including hot, cold, drought, and saline. Different groups of bioremediating microbes have reported from diverse conditions, that is, bacteria, fungi including yeast, and algae. Microbes belonging to genera *Alcaligenes*, *Aspergillus*, *Bacillus*, *Flavobacterium*, *Ganoderma*, *Methosinus*, *Nocardia*, *Phormidium*, *Pseudomonas*, *Rhizopus*, *Rhodococcus*, and *Stereum* have been reported as potential and efficient bioremediators for the degradation of different pollutants of the environment such as xenobiotics, heavy metals, hydrocarbons, and paper and pulp effluent. The present review focuses on microbial diversity in bioremediation, techniques applied in bioremediation, bioremediation of different environmental pollutants, and how bioremediation processes could be monitored.

1. INTRODUCTION

The quality of life on Earth is linked inextricably to the overall quality of the environment. The increasing civilization, urbanization, and advancements in the industrial sector have resulted in generation of wastes and their dumping in the environment. It has been estimated that about 1000 new chemicals are synthesized annually. More than 450 million kilograms of toxins are released globally into air and water in accordance to the third world network reports [1]. The pulp and paper industry is known to be the sixth largest polluter of the environment [2]. Heavy metal pollution is another significant threat to the public and environmental health for its toxicity, non-biodegradability, and bio-

accumulation [3]. Polyaromatic hydrocarbons (PAHs) are known for their mutagenic and carcinogenic properties [4]. The toxic contaminants leading to ecological imbalance are of global concern [5]. Microbial biotechnology is a rapidly growing and emerging field with diverse applications in dealing with the environmental issues. The application of the microbes for bioremediation is a versatile technology with high stability, economical, eco-friendly, lack of interference with the ecology of the ecosystem, and more public acceptance [6]. Environmental cleaning through bioremediation is a apt substitute to the physicochemical approaches, which are rather environmentally disparaging and can be the cause of the secondary pollution. Bioremediation could be utilized in cleanup of contaminated sites such as water, soils, sludge, and waste streams [7,8].

Bioremediation has been even approved by the US Environmental Protection Agency (USEPA) as an effective environmentally sound technique for revitalization of the contaminated environment and

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promotion of sustainable development [9]. Microbes belonging to genera *Alcaligenes*, *Aspergillus*, *Bacillus*, *Flavobacterium*, *Ganoderma*, *Methosinus*, *Nocardia*, *Phormidium*, *Pseudomonas*, *Rhizopus*, *Rhodococcus*, and *Stereum* have been reported to have a potential role in bioremediation [10-12]. Bioremediation is not new to human race but surely the new approaches that stem from advances in molecular biology and process engineering are emerging [13]. With the advancements of genetic engineering techniques, genetically modified organisms can be generated and utilized to reduce the burden of toxic compounds from the environment. Thus, implanting these methods and increasing their efficiency will lead to economic as well as social benefits with reduced risks of diseases and costs being spent on management of these wastes, and achievement of more ecological stability and greener environment [14].

2. BIODIVERSITY OF BIOREMEDIATING MICROBES

The enticing process, bioremediation is one of the processes that help in detoxify environmental pollutants using diverse group of microbes including fungi, yeast, and bacteria. Microorganisms are considered as to be the outstanding creatures for the detoxification of pollutants as it is cheap, simple, and eco-friendly clean-up method [15,16]. To hold the assurance for detoxification of environmental contaminants, diverse group of microbes are explored around the globe from different locations and environmental conditions [17]. In a report, phenol degrading yeasts, namely, *Candida boidinii*, *Pichia holstii*, *P. membranifaciens*, and *Saccharomyces cerevisiae*, were isolated from the olive mill wastewaters [18]. Zhang *et al.* [19] reported petroleum degrading bacterium *Bacillus* sp. from the oil contaminated soil. In another report, white rot fungi, *Trametes versicolor* was reported as a bioremediating agent of polycyclic aromatic hydrocarbons (PAH) [20]. In a report by Janbandhu, Fulekar [21], three bacterial species, namely, *Achromobacter insolitus*, *Bacillus cereus*, and *Sphingobacterium* sp., isolated from petrochemical refinery field were reported for remediating PAHs.

In a report, diversity of bioremediating bacterial isolates, that is, *Bacillus megaterium*, *B. cibi*, *B. cereus*, *Pseudomonas aeruginosa*, and *Stenotrophomonas acidaminiphila* from oily sludge contaminated soil was reported. These strains were reported for having capability of degrading aliphatic and aromatic compounds [22]. Syakti *et al.* [23] reported bioremediating potential bacterial isolated from the mangroves growing in hydrocarbons contaminated soil and they were identified as *Bacillus aquimaris*, *B. megaterium*, and *B. pumilus*, *Flexibacteraceae* bacterium, *Halobacillus trueperi*, and *Rhodobacteraceae* bacterium. In another report, crude oil degrading microbes were reported and they were reported for belonging to genera *Achromobacter*, *Alcaligenes*, *Bacillus*, *Brevibacillus*, *Delftia*, *Lysinibacillus*, *Paenibacillus*, *Pseudomonas*, and *Stenotrophomonas* [24].

In an investigation, *Pseudomonas* sp. isolated from the petroleum refinery soil and the strains were reported for degrading the hydrocarbons [25]. Godoy *et al.* [26] isolated fungal species from PAH contaminated soil which were having capability of bioremediating xenobiotics. The fungal isolates were identified as *Fomes* sp. and *Scopulariopsis brevicaulis*. In an investigation, diversity of hydrocarbons degrading bacterial strains was reported from petroleum refinery waste and they belong to genera *Bacillus*, *Burkholderia*, *Enterobacter*, *Kocuria*, *Pandoraea*, and *Pseudomonas* [27]. In another report, *Stenotrophomonas* was reported for bioremediating xenobiotics as it was having capability resist antibiotics ofloxacin, streptomycin, rifampicillin, erythromycin, ampicillin, and clindamycin. This strain

was also reported for degrading the heavy metals including arsenic, mercury, copper, nickel, and lead [28].

The microbial community belonging to genera *Shinella*, *Microbacterium*, *Micrococcus*, and *Bacillus* were reported for bioremediating heavy metal (cadmium, chromium, cobalt, nickel, and zinc) environmental pollutants [29]. In another report, *Pseudomonas nitroreducens* and *Ochrobactrum* sp. were reported as a pesticides degrading agent [30]. Ali *et al.* [31] have reported yeast cultures, namely, *Barnettozyma californica*, *Sterigmatomyces halophilus*, and *Yarrowia* sp., for having a capability of bioremediating textile Red HE3B dye.

3. BIOREMEDIATION OF DIVERSE CONTAMINANTS

3.1. Bioremediation of Paper and Pulp Effluent

Pulp and paper industry is one of the important industrial sectors simultaneously being the source of the toxic pollutants [32]. The toxic effluent released from the paper and pulp industries has adverse environmental impact as it contains high content of BOD (biological oxygen demand) and COD (chemical oxygen demand), total dissolved solids, suspended solids, color, organic acids, phosphorus, sulfur compounds, and toxic chlorophenols [33]. This environmental pollutant also affects the health of the humans working in the paper and pulp industries as it may cause headache, vomiting, nausea, diarrhea, and eye irritation. In addition, flora and fauna of land as well as aquatic are also affected by the pollutant [34]. Moreover, the effluent has also declined the quantity and quality of the water. To cope with this effluent problem, in the past decade, several projects and technologies have been implemented and upgraded, respectively. Over the many years, the wastewater generated from the industry was treated with physical methods such as adsorption, microfiltration, and photoionization; and chemical methods such as coagulation, oxidation, ozonation, and sedimentation [35]. The physical and chemical methods of the treatment of the effluent have shown to improve the quality of the effluent by enables to treat it completely.

Further, the effluent was treated with the biological process in which the wastewater is treated with microbes which have a capability to produce ligninolytic enzymes which helps in the bioremediation without causing any harmful effect on the environment. These methods have adventurous over the physical and chemical method as it is cost effective, and appropriately reduced the BOD and COD in the wastewater. In the detoxification of the paper and pulp effluent, several types of the microbes are being recognized including bacteria, fungi, and algae [36]. A study on the secondary sludge of pulp and paper mill sample, pentachlorophenol (PCP) mineralizing bacterium, *Pseudomonas stutzeri* was reported for the having a capability of degrading the stoichiometric compounds which removes 66.8% of PCP from paper and pulp effluent and bioremediate the effluent [37]. In another report, a bacterium, *Enterobacter* sp., was isolated from the tannic acid enriched soil was reported for the bioremediation of pulp and paper mill effluents by reducing the color up to 82% and lignin content up to 73%. Moreover, the effluent has also reported for the reducing of the BOD and COD content in their 16 h of retention time in batch culture [38].

In another investigation, *Pseudomonas putida* MTCC 10510 was reported for the decolorization of up to 39.72–48.2% and chloride ions by 80.1–83.5% in 36 h in a paper and pulp mill effluent [39]. Chandra and Singh [40] have reported three ligninolytic enzyme activity exhibiting bacterial strains, namely, *Pseudochrobactrum glaciale*,

Providencia rettgeri, and *Pantoea* sp., which help in the reduction of color, COD, and BOD in the paper and pulp mill effluent by 96.02%, 91%, and 92.59%, respectively, in 219 h of the incubation period. In a report, a laccase enzyme-producing bacterium, *Pseudomonas putida* was reported for decolorizing the industrial effluent by 16–86% within 24 h of incubation [41]. Similarly, laccase enzyme-producing bacterium, *Paenibacillus* sp., was reported effectively reducing color, lignin, phenol, BOD, and COD by 68%, 54%, 86%, 83%, and 78%, respectively, in 144 h of incubation [42]. In another report, ligninolytic bacterium, *Brevibacillus agri*, isolated from the paper and pulp mill sludge was reported for bioremediating 69%, 47%, and 37% of COD, color, and lignin, respectively, in the mill effluent [43].

In a report, ligninolytic bacterium, namely, *Serratia liquefaciens*, was reported for detoxifying the color, lignin, COD, and phenols by 72%, 58%, 85%, and 95% within the 144 h of the inoculation at temperature, pH, and speed of 30°C, 7.6, and 120 rpm, respectively [44]. Abhishek et al. [45] isolated *Citrobacter freundii* and *Serratia marcescens* from wastewater sludge which were found to decolorize color by 64% and 60%, respectively, and, in combination, color was 87% decolorized in paper pulp wastewater. Bacterial isolates *C. freundii* and *S. marcescens* was also reported for removing 76% and 61% of total organic carbon (TOC), 80% and 67% of COD, and 87% and 65% of lignin, respectively. In another report, Hooda et al. [46] have reported *Brevibacillus parabrevis* for detoxification of color, chemical oxygen demand, lignin content from kraft paper mill effluent up to 59%, 62%, and 53.8%, respectively, at 37°C after 5 days of inoculation.

In a report, bacterial isolate, *Planococcus* sp. isolated from wastewater was reported for the bioremediating paper mill effluent in fluidized bed reactor. The bacterial isolates were reported for reducing the concentration of phenol, lignin, color, and COD from the effluent up to 96%, 74%, 81%, and 85%, respectively, with the 60 h of incubation [47]. Sonkar et al. [48] reported *Bacillus* sp. for degradation of decolorization of the 100% filter sterilized effluent in a batch treatment. This strain was reported for degrading 82.22, 89.50, 93.33, and 73.01% of TOC, COD, BOD, and color, respectively, after 72 h of treatment. In an investigation, paper mill sludge was reported to be detoxified by bacterial consortium of *Bacillus* sp., *Pseudomonas* sp., and *Pseudomonas stutzeri* at the 37°C temperature, 150 rpm speed, and 7.0 ± 0.2 pH. The bacterial consortium was reported for degrading the TOC, COD, BOD, lignin, total phenol, nitrogen, phosphate, absorbable organic halides, and color [49]. In another report, thermophilic ligninolytic *Serratia* sp. was reported for the degradation of papermaking black liquor. This strain was reported for the degrading the color, lignin, phenol, BOD, and COD up to 80%, 60%, 95%, 80%, and 80%, respectively [50] [Table 1].

3.2. Bioremediation of Heavy Metals

Heavy metals, the elements having greater density, are widespread environmental pollution which has generated hype in recent years due to associated health risks. The major cause of this widespread pollutant is industries including iron and steel, electroplating, electrolysis, energy and fuel, fertilizer, and pesticides producing industries. These industries release of heavy metals such as uranium, mercury, lead, chromium, cadmium, and arsenic that are poisonous to land as well as aquatic flora and fauna. Heavy metals are also known to threaten the life of humans by causing skin irritation, breathing problem, weakness, abdominal cramps, headache, diarrhea, anemia, and permanent damage of kidney and brain. The metal cadmium toxicity could also induce DNA breakage. To remove the heavy metals from the environment, conventional methods such as chemical precipitation,

electrochemical treatment, and ion exchange were being used but these methods expensive and even remove the heavy metal at very low concentration [51]. Microbial mediated bioremediation is one of the appropriates the method for the removal of heavy metal accumulated into the environment [52,53].

In the literature, numerous microbial species residing in diverse conditions have been reported for remediating the heavy metals polluting the environment. In a report, endophytic bacteria of *Solanum nigrum* L., namely, *Bacillus* sp., were reported for the bioremediating heavy metals such as copper, cadmium, and lead up to 75.78%, 80.48%, and 21.25% within 24 h if incubation [3]. Joshi et al. [54] have reported four different fungi, namely, *Aspergillus awamori*, *A. flavus*, *Phanerochaete chrysosporium*, and *Trichoderma viride* for bioremediation of lead, cadmium, chromium, and nickel. In another investigation, uranium biomineralization was reported by the bacterium *Pseudomonas aeruginosa* isolated from contaminated mine waste [55]. In a similar report, bacterium *Pseudomonas aeruginosa* and fungi *Penicillium corylophilum* isolated soil and phylloplane samples from traffic and non-traffic site of Sohag city, Egypt, were reported for bioremediating cadmium, zinc, and lead ion [56].

In a report, Gram-negative bacterial species, namely, *Enterobacter* sp., was reported for detoxification of copper metal, *Stenotrophomonas* sp. and *Providencia* sp. were reported for cadmium bioremediation, whereas *Chryseobacterium* sp., and *Comamonas* sp. were reported for the removal of cobalt and *Ochrobactrum* sp. reported for the bioremediation of chromium. These strains were reported for resist 275 mg Cu/l, 320 mg Cd/l, 140 mg Co/l, and 29 mg Cr/l in a wastewater [57]. Bhakta et al. [58] reported diverse bacterium species for remediating cadmium and arsenic and they were identified as *Acinetobacter brisouii*, *Pseudomonas abietaniphila*, *Exiguobacterium aestuarii*, and *Planococcus rifietoensis*. Kang et al. [59] have reported lead-resistant bacterium *Enterobacter cloacae* for the removal of lead up to 60% within the 48 h of incubation. In another report, endophytic bacterium, *Paenibacillus* sp., associated with plant *Tridax procumbens* was reported for bioremediating copper, zinc, lead, and arsenic up to 750 mg/L, 500 mg/L, 450 mg/L, and 400 mg/L, respectively [60].

In an investigation, the carcinogenic heavy metals lead, chromium, and cadmium were detoxified by the microbes, namely, *Gemella* sp., *Hafnia* sp., and *Micrococcus* sp. [61]. In a report by Raman et al. [62], bacterium, *Stenotrophomonas maltophilia*, isolated from the tannery effluent was reported for remediating hexavalent chromium. In a similar report, from an electroplating treatment, plant bacteria belonging to genera *Bacillus*, *Shewanella*, *Lysinibacillus*, and *Acinetobacter* genera were isolated which were reported for the detoxifying copper, nickel, manganese, cobalt, and chromium metals [63]. In another report, urease-producing bacteria *Sporosarcina pasteurii*, *Stenotrophomonas rhizophila*, and *Variovorax boronicumulans* isolated from the Iranian mine calcareous soils were reported for the biomineralizing various heavy metals [64]. Aibeche et al. [65] have reported yeast strains including *Rhodotorula mucilaginosa*, *Clavispora lusitaniae*, and *Wickerhamomyces anomalus* for the remediation of heavy metals mercury, chromium, cadmium, lead, copper, zinc, and iron. These yeast strains were isolated from the lead and cadmium highly polluted area of Dayet Oum Ghellaz Lake water [Table 2].

3.3. Bioremediation of Xenobiotics

Xenobiotics are chemicals which are considered as foreign substances in the atmosphere. These compounds are synthesized by human beings such as agro chemicals used in agriculture, and toxic waste generation

Table 1: Microbes mediated remediation of toxic effluents from pulp and paper industries.

Microbes	Role	References
<i>Pseudomonas stutzeri</i>	Degrades pentachlorophenol	Karn <i>et al.</i> [37]
<i>Pseudomonas putida</i>	Degrades color and lignin	Garg <i>et al.</i> [39]
<i>Pseudochrobactrum glaciale</i>	Decolorization	Chandra, Singh [40]
<i>Providencia rettgeri</i>	Decolorization	Chandra, Singh [40]
<i>Brevibacillus agri</i>	Reduces COD, color, and lignin	Hooda <i>et al.</i> [43]
<i>Serratia liquefaciens</i>	Detoxifying color, lignin, COD, and phenols	Haq <i>et al.</i> [44]
<i>Brevibacillus parabravis</i>	Reduces COD and lignin	Hooda <i>et al.</i> [46]
<i>Citrobacter freundii</i>	Decolorization	Abhishek <i>et al.</i> [45]
<i>Serratia marcescens</i>	Decolorization	Abhishek <i>et al.</i> [45]
<i>Planococcus</i> sp.	Reduces of phenol, lignin, color, and COD	Majumdar <i>et al.</i> [47]
<i>Bacillus</i> sp.	Reduces color, COD, BOD, and TOC	Sonkar <i>et al.</i> [48]
<i>Pseudomonas stutzeri</i>	Reduces absorbable organic halides	Sonkar <i>et al.</i> [49]
<i>Serratia</i> sp.	Reduces color, lignin, phenol, BOD, and COD	An <i>et al.</i> [50]
<i>Pseudomonas aeruginosa</i>	Reduces BOD, COD, color, and lignin	Tiku <i>et al.</i> [214]
<i>Bacillus megaterium</i>	Reduces BOD, COD, color, and lignin	Tiku <i>et al.</i> [214]
<i>Bacillus megaterium</i>	Degrades pentachlorophenol	Karn <i>et al.</i> [215]
<i>Bacillus pumilus</i>	Degrades pentachlorophenol	Karn <i>et al.</i> [215]
<i>Bacillus thuringiensis</i>	Degrades pentachlorophenol	Karn <i>et al.</i> [215]
<i>Bacillus cereus</i>	Degrades pentachlorophenol	Tripathi <i>et al.</i> [216]
<i>Klebsiella pneumoniae</i>	Reduce of COD, BOD, and color	Chandra <i>et al.</i> [217]
<i>Citrobacter</i> sp.	Reduce of COD, BOD, and color	Chandra <i>et al.</i> [217]
<i>Pseudomonas</i> sp.	Removes of chlorinated compounds	Das <i>et al.</i> [218]
<i>Aspergillus oryzae</i>	Reduces COD and color	Chavan <i>et al.</i> [219]
<i>Paenibacillus</i> sp.	Reduces color, lignin, phenol, BOD, and COD	Raj <i>et al.</i> [42]
<i>Paenibacillus glucanolyticus</i>	Degrade black liquor and lignin	Mathews <i>et al.</i> [220]
<i>Pseudomonas plecoglossicida</i>	Degrade black liquor	Paliwal <i>et al.</i> [221]
<i>Bacillus megaterium</i>	Degrade black liquor	Paliwal <i>et al.</i> [221]
<i>Phlebia brevispora</i>	Decolorization	Fonseca <i>et al.</i> [222]
<i>Bacillus subtilis</i>	Degrades kraft lignin	Yadav, Chandra [223]
<i>Klebsiella pneumoniae</i>	Degrades kraft lignin	Yadav, Chandra [223]
<i>Achromobacter xylosoxidans</i>	Biodegradation of catechol	Bramhachari <i>et al.</i> [224]
<i>Aspergillus flavus</i>	Degrades color and lignin	Barapatre, Jha [225]
<i>Kocuria turfanesis</i>	Reduces color, COD, and BOD	Ahmadi <i>et al.</i> [226]
<i>Halomonas alkaliphila</i>	Reduces color, COD, and BOD	Ahmadi <i>et al.</i> [226]
<i>Pseudomonas balearica</i>	Reduces color, COD, and BOD	Ahmadi <i>et al.</i> [226]
<i>Rhodospiridium kratochvilovae</i>	Reduces color, lignin, phenol, and COD	Patel <i>et al.</i> [227]
<i>Pleurotus ostreatus</i>	Reduces COD and BOD	Rivera-Hoyos <i>et al.</i> [228]
<i>Bacillus aryabhatai</i>	Degrades color and lignin	Zainith <i>et al.</i> [229]
<i>Rhodococcus pyridinivorans</i>	Degrades phenol	Barik <i>et al.</i> [230]

from paint, plastics, and textile industries [66,67]. Xenobiotics including DDT and halogenated aromatic compounds pose harmful impact on the environment [68]. The biota is negatively affected by xenobiotics in the environment. These toxic substances can cause skin problem in human and can possibly cause cancer if exposed for lengthy periods of time. Bioaccumulation of xenobiotics can lead to their entry into food chain in turn increasing the tropical level of the ecosystem [69]. The degradation of such compounds is not easy due to their recalcitrant nature [70]. Chemical processes do not break down compounds containing group such as halogen, nitro, or sulfonyl into simple inorganic materials. As

a result, microbial bioremediation is an effective method for removing or breaking down specific pollutants in the environment. The finest tool for bioremediation is the utilization of minute organism that cover half of our planet's biomass since they can quickly grow and proliferate on a large scale in a short amount of time and are also cost effective. Microbes can breakdown such harmful compounds using certain enzymes that breakdown xenobiotics compound into harmless end products.

Azo dyes have been found to be mostly used in the textile industries and are used for coloring of various materials including cosmetics,

Table 2: Microbes mediated remediation of heavy metals.

Microbes	Heavy metals	References
<i>Aspergillus awamori</i>	Pb, Cd, Cr, and Ni	Joshi <i>et al.</i> [54]
<i>Aspergillus flavus</i>	Pb, Cd, Cr, and Ni	Joshi <i>et al.</i> [54]
<i>Phanerochaete chrysosporium</i>	Pb, Cd, Cr, and Ni	Joshi <i>et al.</i> [54]
<i>Trichoderma viride</i>	Pb, Cd, Cr, and Ni	Joshi <i>et al.</i> [54]
<i>Pseudomonas aeruginosa</i>	Uranium	Choudhary, Sar [55]
<i>Pseudomonas aeruginosa</i>	Cd, Zn, and Pb	Mohamed, Abo-Amer [56]
<i>Penicillium corylophilum</i>	Cd, Zn, and Pb	Mohamed, Abo-Amer [56].
<i>Acinetobacter brisouii</i>	As and Cd	Bhakta <i>et al.</i> [58]
<i>Pseudomonas abietaniphila</i>	As and Cd	Bhakta <i>et al.</i> [58]
<i>Exiguobacterium aestuarii</i>	As and Cd	Bhakta <i>et al.</i> [58]
<i>Planococcus riftetoensis</i>	As and Cd	Bhakta <i>et al.</i> [58]
<i>Enterobacter cloacae</i>	Pb	Kang <i>et al.</i> [59]
<i>Stenotrophomonas maltophilia</i>	Cr	Raman <i>et al.</i> [62]
<i>Sporosarcina pasteurii</i>	Zn, Pb, and Cd	Jalilvand <i>et al.</i> [64]
<i>Stenotrophomonas rhizophila</i>	Zn, Pb, and Cd	Jalilvand <i>et al.</i> [64]
<i>Variovorax boronicumulans</i>	Zn, Pb, and Cd	Jalilvand <i>et al.</i> [64]
<i>Ralstonia pickettii</i>	Cu, Pb, and Ni	Xie <i>et al.</i> [230]
<i>Pseudomonas lubricans</i>	Cu, Cr, Ni, and Hg	Rehman <i>et al.</i> [232]
<i>Clostridium subterminale</i>	Fe, Zn, and Cu	Alexandrino <i>et al.</i> [233]
<i>Clostridium pascui</i>	Fe, Zn, and Cu	Alexandrino <i>et al.</i> [233]
<i>Clostridium mesophilum</i>	Fe, Zn, and Cu	Alexandrino <i>et al.</i> [233]
<i>Clostridium peptidovorans</i>	Fe, Zn, and Cu	Alexandrino <i>et al.</i> [233]
<i>Desulfovibrio desulfuricans</i>	Fe, Zn, and Cu	Alexandrino <i>et al.</i> [233]
<i>Aspergillus niger</i>	Cu and Pb	Iskandar <i>et al.</i> [234]
<i>Aspergillus fumigatus</i>	Cu and Pb	Iskandar <i>et al.</i> [234]
<i>Trichoderma asperellum</i>	Cu and Pb	Iskandar <i>et al.</i> [234]
<i>Penicillium simplicissimum</i>	Cu and Pb	Iskandar <i>et al.</i> [234]
<i>Penicillium janthinellum</i>	Cu and Pb	Iskandar <i>et al.</i> [234]
<i>Graphium putredinis</i>	Cd, Ce, Ni, Pb, and Zn	Vargas-García <i>et al.</i> [235]
<i>Fusarium solani</i>	Cd, Ce, Ni, Pb, and Zn	Vargas-García <i>et al.</i> [235]
<i>Penicillium chrysogenum</i>	Cd, Ce, Ni, Pb, and Zn	Vargas-García <i>et al.</i> [235]
<i>Exiguobacterium aestuarii</i>	Ni and Cr	Gupta <i>et al.</i> [236]
<i>Bacillus weihenstephanensis</i>	Ni and Cr	Gupta <i>et al.</i> [236]
<i>Bacillus firmus</i>	As and Cr	Bachate <i>et al.</i> [237]
<i>Azotobacter chroococcum</i>	Cd, Cu, Cr, Co, Hg, Ni, Zn, and Pb	Abo-amer <i>et al.</i> [238]
<i>Rhizobium leguminosarum</i>	Cu, Cd, Ni, and Zn	Chiboub <i>et al.</i> [239]
<i>Microbacterium oxydans</i>	Uranium	Sánchez-Castro <i>et al.</i> [240]
<i>Cronobacter muytjensii</i>	Cd, Cr, Cu, and Zn	Saranya <i>et al.</i> [241]
<i>Pseudomonas putida</i>	Ag, Cr, Co, Mg, and Pb	Nokman <i>et al.</i> [242]
<i>Pseudomonas monteilii</i>	As, Cr, Co, Cd, Hg, Pb, Se, and Zn	Sher <i>et al.</i> [243]

food, textile, and leather. Textile industry is the second largest industry in India. It makes use of natural and synthetic dyes, heavy metals, and mordants for manufacturing of textile fabrics. Recently, more than 2000 azo dyes are being used in various industries, in which textile coloration industries are the largest users. There is estimation that only 10% of these dyes bind when applied to the material and remaining is released into the water bodies and affects the aquatic life [71]. The treatment of these pollutants through microbes is

emerging as a potential tool and a number of microbial species have been reported to degrade these dyes including *Zobellella* sp., *Sphingomonas paucimobilis*, *Shewanella indica*, *Rheinheimera* sp., *Pichia occidentalis*, *Paenibacillus polymyxa*, *Oceanimonas smirnovii*, *Micrococcus luteus*, *Marinobacterium* sp., *Halomonas* sp., *Brevibacillus* sp., *Bacillus fusiformis*, and *Acinetobacter junii* [72].

The use of chemical fertilizers and pesticides to increase grain output has led to several environmental issues such as reduction in

soil fertility and biodiversity as well as increase of soil acidification and weed species resistance. These substances contaminated the air, groundwater, and bodies of water [73]. The persistence of these chemicals in the environment for a long time can affect the entire ecosystem. The increasing challenges to remove these pollutants from the environment shifted the paradigm toward the utilization of the microbes. Many microbes have been reported for their ability of degrading alachlor, atrazine, chlorpyrifos, DDT, endosulfan, fenprothrin, paichongding, profenofos, phenylurea, parathion, mefenacet, and methyl parathion [74]. Degradation of endosulfan by *Staphylococcus* sp., *Bacillus circulans*-I, and *Bacillus circulans*-II has been reported [75]. *Acremonium* sp., *Alcaligenes faecalis*, *Bacillus licheniformis*, and *Bacillus thuringiensis* [76-79] have been reported for degrading chlorpyrifos. *Pseudomonas aeruginosa*, *P. nitroreducens* and *P. putida* [80], *Providencia stuartii* [81], *Botryosphaeria laricina* [82], *Cupriavidus taiwanensis* [83] have been reported for degradation of chlorpyrifos bacteria.

DDT was the most widely used pesticides in the 1940s. Several studies have been conducted that it has negative impact on the ecosystem as well as non-target creatures such as fishes and birds. It's upregulation in adipose tissue and its estrogenic properties raised concerns about its potential long-term adverse effects. It is further known to be carcinogenic in nature, affects neurobehavioral functions and associated with premature birth. Sweden was the first country to prohibit its use in 1970, citing environmental concerns. Its production was banned by the USSR in 1981. Further, in 1989, it was banned for medical-disinfecting purposes. By 1972, most of the countries banned its use due to negative impact on the wildlife. It takes 3 to 30 years for the degradation thereby remaining in the atmosphere for an extended period and effect the surrounding environment [84]. Many microbes such as *Alcaligenes* sp., *Ochrobactrum* sp., *Sphingobacterium* sp., and *Stenotrophomonas* sp. have been reported for degrading this hazardous pesticide [85-88]. Endosulfan widely used worldwide for regulatory beetle, cabbage worms, Colorado potato, and peach tree borer has been reported to be degraded by *Bacillus subtilis* and *Mycobacterium* sp. [89,90]. Furthermore, *Alcaligenes xylooxidans*, *Arthrobacter globiformis*, *Nocardioides* sp., *Providencia rustigianii*, *Pseudomonas marginalis*, *Pseudomonas putida*, *Rhodococcus rhodochrous*, and *Stenotrophomonas* sp. have been reported for degrading different herbicides [91-93].

Nitro-aromatic compounds, another harmful xenobiotics produced by incomplete combustion of fossil fuels are released into the atmosphere, largely from anthropogenic sources. Second, nitration is an essential chemical reaction for the commercial synthesis and uses of numerous amino aromatic intermediates as a feedstock for the production of explosive, pesticides, herbicides, polymers, dyes, medicine, and other products. Various microbes such as *Arthrobacter ureafaciens*, *Pseudomonas* sp., *Rhodococcus wratislaviensis*, *Shewanella oneidensis*, and *Streptomyces mirabilis* have been reported for the degradation of nitro-aromatic compounds [94-98]. Another microbes such as *Pseudomonas putida*, *P. putida*, *P. mendocina*, *Burkholderia cepacia*, *B. cepacia* and *Ralstonia pickettii* [99], *Arthrobacter* sp., *Enterobacter agglomerans*, *Escherichia coli*, *Pseudomonas cepacia*, *Pseudomonas* sp., *Rhizobium* sp., *Staphylococcus aureus*, and *Xanthomonas* sp. have been reported for degrading chlorinated hydrocarbons [100,101].

Di-(2-ethylhexyl) phthalate (DEHP) widely used in the production of polyvinylchloride is another major contributor of environmental pollution. DEHP is known to be an endocrine disrupting substance.

In a study Li, Gu [102] reported that *Klebsiella oxytoca* Sc and *Methylobacterium mesophilicum* Sr have degradation of PAEs. In one of the investigations, Baek *et al.* [103] proposed *Micrococcus luteus* for the degradation of DEHP. Yuan *et al.* [104] reported degradation of DEHP by *Bacillus* sp. Furthermore, *Rhodococcus* sp. has been shown to have a strong ability to degrade DEHP [105,106]. Biodegradation through microbes is one of the most promising, relatively efficient, and cost-effective technologies. In particular, this technique is economically viable and enhances the quality of life for farmers and society as a whole.

3.4. Bioremediation of Polyaromatic Hydrocarbons

PAHs, a class of toxic-fused ring aromatic compounds, are widespread organic pollutants accumulated in the environment either due to anthropogenic or natural activities. These compounds have a high molecular weight and can persist for years [107]. They are mostly produced from incomplete combustion of fossil fuels, petroleum products, and industrial activities. The natural calamities such as forest fires and volcanic eruptions also contribute to their accumulation in the environment [108]. PAHs exist as a complex mixture in many different petroleum-based products. Soils and waters surrounding gas plants, soil refineries, air bases, petrol stations, and chemical industrial sites are common sources of contamination. PAHs have been shown to be carcinogenic and mutagenic to human and animal health, and as a result, the US EPA has classified them as priority pollutants [109]. PAHs can enter the human body through a variety of pathways, including air, food, soil, water, and occupational exposure. PAH can also enter the water supply through a variety of sources, including industrial and home waste, as well as urban runoff and automobile emissions. The removal of these specific contaminants of the atmosphere has extended attention because it caused damage ranging from human to the environment, marine and land animals, and agricultural soil. PAHs are challenging to remove from soil due to their insolubility in water and degrade slowly [110].

The clean-up of soil through bioremediation is one of the most efficient means to restore original ecosystem conditions [111]. The PAH-degrading microorganisms include algae, bacteria, and fungi. The use of microorganisms for bioremediation of PAH-contaminated environments seems to be an attractive technology for restoration of polluted sites. Microbes play a major role in the removal of PAHs from the environment. Different pathways used by diverse microbes for the biodegradation of different PAHs are shown. A wide variety of microbes have been observed to be capable of PAH degradation using metabolic pathways and substrate ranges. In a study, Krivobok *et al.* [112] reported anthracene degrading *Cryphonectria parasitica*, *Ceriporiopsis subvermispora*, *Oxysporum* sp., *Cladosporium herbarum*, *Rhizopus arrhizus*, *Phanerochaete chrysosporium*, *Irpex lacteus*, and *Pleurotus ostreatus* were isolated from soil and help to degradation of. Another investigation Annweiler *et al.* [113] reported bacteria *Bacillus thermoleovorans* isolated from contaminated compost, have ability to degradation of naphthalene compounds. Similarly, Chauhan *et al.* [114] reported *Comamonas testosteroni*, *Pseudomonas stutzeri*, *Sphingomonas paucimobilis*, *Mycobacterium* sp., *Nocardioides* sp., and *Alcaligenes faecalis* have capability to degrade naphthalene, anthracene, benzo[b] fluoranthene, pyrene, benzo[a]pyrene, 1-nitropyrene, and phenanthrene. Another report Chaudhary *et al.* [115] reported *Haemophilus* sp., *Mycobacterium* sp., *Pseudomonas* sp., and *Rhodococcus* sp. separated from soil for their ability to degrade to phenanthrene, naphthalene, anthracene, pyrene, and benzo[a]pyrene. Mangwani *et al.* [116] reported

Stenotrophomonas acidaminiphila and *Alcaligenes faecalis* isolated from Chilika lagoon, *Pseudomonas mendocina* from Rushikulya estuary, *Pseudomonas aeruginosa* from Paradeep port; all microbes have ability to degradation of PAHs compounds such as phenanthrene and pyrene [Table 3].

4. TECHNIQUES FOR BIOREMEDIATION

The development of eco-friendly, cost-effective, and reliable clean-up technology is a priority to decontaminate the environment. Microbes are the readily available and omnipresent bioresources which can utilize these noxious elements as their source of nutrition. They possess amazing capabilities to survive in varying environment and produce metabolites that can transform environmental pollutants thus making it possible to revive contaminated sites naturally. Different remediation techniques can be utilized, but due to the number of advantages offered by microbes and rise in the costs of the physical and the chemical treatments, microbe-mediated bioremediation is the most preferred approach to tackle the worldwide contamination. The US Environmental Protection Agency has described two methods

of bioremediation, that is, *in situ* and *ex situ* [117]. Microbes can be applied in both in situ and ex situ conditions.

4.1. In situ Bioremediation

The technique involves the application of a biological treatment for cleaning up of hazardous compounds and has been commonly applied for degradation of contaminants in saturated soils and groundwater [118-120]. It relies on the microbial activities for destruction and detoxification of contaminants present in a place. On the contrary, the capability of the microbes to convert the toxic contaminants into less toxic or non-toxic forms is completely dependent on availability of the nutrients and electron acceptors and donors. Bioremediation through *in situ* approach is sustainable as the requirements of transport, deposition of contaminated soil, groundwater pumping, treatment, and discharge to recipients are removed [121]. Moreover, it offers many advantages such cost-effectiveness, utilization of native harmless microbial species, and large volume of contaminated soil or water could be treated with less release of toxic contaminants. *In situ* bioremediation approach

Table 3: Microbe mediated bioremediation of different toxic compounds.

Microbes	Compound	Source	References
<i>Aeromonas hydrophila</i>	Acenaphthene and fluorene	Water	Alegbeleye <i>et al.</i> [244]
<i>Alcaligenes faecalis</i>	Pyrene	ChilikaLagoon	Mangwani <i>et al.</i> [116]
<i>Alcaligenes faecalis</i>	Naphthalene, phenanthrene and chrysene degradability	Soil	John <i>et al.</i> [245]
<i>Anthracytophyllum discolor</i>	Phenanthrene, Anthracene, Fluoranthene and Pyrene	Soil	Acevedo <i>et al.</i> [246]
<i>Bacillus megaterium</i>	Acenaphthene and fluorene	Water	Alegbeleye <i>et al.</i> [244]
<i>Bacillus thermoleovorans</i>	Naphthalene	Contaminatedcompost	Annweiler <i>et al.</i> [113]
<i>Burkholderia cepacia</i>	Acenaphthylene, anthracene, benzo(b) fluoranthene	Claysoil	Reda [247]
<i>Burkholderia sp.</i>	Fluorene, naphthalene, and phenanthrene	Oilrefinerywastewaterdrainage	Andreolli <i>et al.</i> [248]
<i>Ceriporiopsis subvermispora</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Cladosporium herbarum</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Cryphonectria parasitica</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Irpex lacteus</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Micrococcus varians</i>	Naphthalene, phenanthrene, and chrysene degradability	Soil	John <i>et al.</i> [245]
<i>Oxysporum sp.</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Paracoccus sp.</i>	Anthracene	PollutedGreeksoil	Zhang <i>et al.</i> [249]
<i>Phanerochaete chrysosporium</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Pleurotus ostreatus</i>	Benzo(a)pyrene and benzo(a)anthracene	Contaminatedsoil	Li <i>et al.</i> [250]
<i>Pleurotus ostreatus,</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Pseudomonas aeruginosa</i>	Phenanthrene	PAH-contaminatedsoil	Wong <i>et al.</i> [251]
<i>Pseudomonas citronellolis</i>	Anthracene	Petrochemicalsludgelandfarmingsite	Jacques <i>et al.</i> [252]
<i>Pseudomonas mendocina</i>	Phenanthrene, Pyrene	Rushikulyaestuary	Mangwani <i>et al.</i> [116]
<i>Pseudomonas mendocina</i>	Phenanthrene	Soils	Chaudhary <i>et al.</i> [115]
<i>Pseudomonas pseudoalcaligenes</i>	Phenanthrene, pyrene	Paradeeport	Mangwani <i>et al.</i> [116]
<i>Pseudomonas putida</i>	Naphthalene, phenanthrene, and chrysene degradability	Soil	John <i>et al.</i> [245]
<i>Pseudomonas sp.</i>	Naphthalene	Soils	Chaudhary <i>et al.</i> [115]
<i>Raoultella ornithinolytica</i>	Acenaphthene and fluorene	Water	Alegbeleye <i>et al.</i> [244]
<i>Rhizobium tropici</i>	Phenanthrene (PHE) or benzo[a]pyrene (BaP)	Phaseolusvulgaris	Yessica <i>et al.</i> [253]
<i>Rhizopus arrhizus</i>	Anthracene	Soil	Krivobok <i>et al.</i> [112]
<i>Rhodococcus erythropolis</i>	Phenanthrene, anthracene, and fluoranthene	Mangroveecosystem	Lang <i>et al.</i> [254]
<i>Rhodococcus sp.</i>	Phenanthrene (Phe), pyrene (Pyr), and benzo[a]pyrene (BaP)	Crudeoil	Song <i>et al.</i> [255]

has been mostly used for the degradation of anilines, chlorinated hydrocarbons, nitrobenzenes, nitriles, and plasticizers in soil and groundwater [122-128]. *In situ* bioremediation includes

4.1.1. Biosparging

This approach involves the injection of the air under pressure below the water table. This, in turn, increases oxygen concentrations of groundwater and rate of biodegradation by naturally occurring bacterial species [129]. Biosparging finds major applications in the treatment of aquifers polluted with kerosene and diesel, which have good biodegradation of the BTEX group and naphthalene [130]. The effectiveness of biosparging depends on soil permeability as well as the pollutant biodegradability [131].

4.1.2. Bioventing

Bioventing is a potential technology that stimulates the natural *in situ* biodegradation of compounds that can be degraded aerobically by existing soil microbial communities [132]. The technique involves controlled stimulation of the air flow, providing oxygen in sufficient levels to sustain activities of the microbes thereby enhancing the process of bioremediation [133]. The levels of nutrients and humidity are maintained to achieve transformation of pollutants. This technique has been successfully used in the remediation of soils polluted by oil products [134].

4.1.3. Bioaugmentation and biostimulation

In bioaugmentation, the autochthonous microflora of the polluted site is enriched by adding previously selected indigenous or genetically modified species of microbes to enhance the process of remediation. Bioaugmentation is used for the soils and groundwater contaminated with tetrachloroethylene and trichloroethylene where the approach ensures that the *in situ* microbes degrade these contaminants to non-toxic compounds such as ethylene and chlorides [135]. Biostimulation involves the use of native microorganisms which are stimulated to grow with the addition of nutrients including phosphorus and nitrogen, O₂, or other oxidizing agents. Stimulating agents are usually applied underground by means of injection wells [136]. The involvement of well-adaptive autochthonous microorganisms is the major advantage of using this approach. Recently, it has been suggested that both these techniques can be also applied *ex situ* though classified into *in situ* bioremediation approach [137].

4.1.4. Biopiling

In biopiling, excavated soils are mixed with soil amendments, placed on a treatment area, and bioremediated using forced aeration. The contaminants are reduced to carbon dioxide and water. The conditions such as levels of moisture and nutrients, heat, oxygen, and pH are controlled to enhance the process of biodegradation [132].

4.2. *Ex situ* Bioremediation

This technique involves digging pollutants from polluted sites and successively transporting them to another site for treatment. Certain factors are taken into consideration for applying *ex situ* bioremediation techniques such as depth of pollution, type of pollutant, treatment cost, and geographical location of the polluted site [138]. The technique is further categorized into solid-phase and slurry-phase systems depending on the state of the pollutant to be removed. The solid-phase system involves treatments of agricultural, domestic, industrial, organic, and municipal solid wastes. Solid-phase treatment processes further include land farming, composting, and soil biopile techniques. Land farming, also known as land treatment, involves the excavation of the contaminated soil and spreading it on a thin surface [139]. The

target of applying this technique is to stimulate indigenous microbes with biodegrading potential and facilitate degradation of contaminants under aerobic conditions [120]. Soil biopiles, also known as biocells, are used for the remediation of excavated soil contaminated chiefly with petroleum contents. Biopiles provide a favorable environment for indigenous aerobic as well as anaerobic microbes. Compositing involves the combining of contaminated soil with non-hazardous organic amendments including agricultural wastes, corncobs, hay, manure, and straw so as to maintain optimum levels of air and water to the microbes. The types of amendments used depend on the soil porosity and the carbon and nitrogen balance of needed to encourage microbial activity. Slurry-phase bioremediation, also known as bioreactors, is a controlled treatment that involves the excavation of the contaminated soil, mixing it with water and placing it in a bioreactor.

5. MONITORING OF BIOREMEDIATION PROCESSES

As a result of population explosion and rapid industrialization, different contaminants have been generated and dumped into the environment. These harmful materials have a negative impact on human health as well as on the environment. However, microbial-mediated bioremediation seems to have great promise in restoring contaminated surroundings in an environmentally friendly manner. It is necessary to prove that there is an enough microorganism population able of fighting the specific pollutants before bioremediation can be deemed a remediation approach [140]. Monitoring begins with the use of standard microbiological techniques for quantifying viable populations of microbes and basic chemical analysis for the identification of pollutant. Although when specific microbial populations are difficult to grow, the enrichment culture may disclose the existence of essential degrading bacteria and prove that they have the inherent inclination to decompose the contaminant at a satisfactory rate [141]. There are new molecular microbial ecology tools which do not depend on culturing due to functional and non-culturable phenomenon and have been proven to be highly beneficial for monitoring bioremediation progress [142].

Microbial population changes can be explored during bioremediation, as well as more detailed analytical work, such as gas chromatography [flame ionization detector (FID) or: electron capture detector (ECD)], tests on the fate of ¹⁴C-radiolabeled substrates to determine whether mineralization or biodegradation of the substrate has taken place or a simple transformation to a more stable state, and high-performance liquid chromatography (HPLC) [141]. These strategies have been used in a variety of field scale and laboratory bioremediation investigations, and they have proven to be effective in tracking the development of bioremediation in various environmental media [140,143,144].

The intake of molecular oxygen or the generation of CO₂ can be used to measure microbial activity and aerobic metabolism using respirometry [145]. Furthermore, respirometric studies can be used to examine the possible decomposition in soil of petroleum hydrocarbons, nutritional limits, the heavy metal ability to, toxic chemicals, clayey acidic soil, and the impact of pH on soil microbial activity [146-149]. Respirometry studies could also be used to assess various biological treatment procedures, the impact of culture bioaugmentation, and nutrient supplementation, and to demonstrate active hydrocarbon breakdown during a full-scale bioremediation.

Soil microcosm experiments can help to determine the biodegradation capability of hydrocarbon-contaminated soils and developing models to predict their destiny. The pollutant concentrations and their metabolic by products could be evaluated during the test to acquire meaningful

biodegradation kinetics data and to determine the best bioremediation method for a large-scale application. Slurry bioreactors of various sizes can also be used to test biodegradation capability. These bioreactors have numerous benefits, such as effective aeration, mixing and better substrate supply, as well as can drastically shorten treatment time [145,149]. Fourier-transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), mass spectrometry (MS), gas chromatography, infrared (IR) absorption, and thin-layer chromatography (TLC) among other techniques are used to evaluate the rates of contaminant degradation and product creation [148]. The biodegradation of semi volatile hydrocarbons in diesel fuel-contaminated soil and water, as well as volatile hydrocarbons during growth of bacteria on crude oil, has been monitored using solid-phase micro extraction (SPME) [150,151]. In petroleum biodegradation systems, solid-phase microextraction has been shown to be a quick and precise approach for evaluating semi-volatile and volatile hydrocarbons.

Microbial interactions in the atmosphere and how they utilize hydrocarbons as a substrate can be learned using traditional culture techniques. Specific hydrocarbon-degrading microbial counts and total heterotrophic microbial counts in polluted soil give helpful info on how well the native microbial community has adapted to the polluted environmental conditions and whether it is able to maintain bioremediation. Microbial counts are often assessed in representative soil composite samples, and there has been evidence of a substantial link between microbial numbers and hydrocarbon breakdown [152]. Non-hydrocarbon-degrading bacteria can grow on agar plates with volatile, liquid, or solid hydrocarbons; hence, caution should be used when reporting counts of hydrocarbon-degrading organisms [153,154]. In a study of mineral agar plates either with or without toluene-xylene fumes, it was discovered that few choices were made over bacteria that degrade xylene and non-toluene. For non-volatile hydrocarbons depending on emulsion formation, a fast MPN test (sheen screen) utilizing tissue culture plates can be used [155]. The microbial biomass is one of the other potential indications of soil contamination evaluation. Muramic acid can be used to identify bacterial biomass, while ergosterol has been proposed as a fungus indication [156,157].

Physiological or biochemical method includes phospholipid fatty acid analysis (PLFA). Microbial cell membranes contain phospholipid fatty acid (PLFA), which are important components. PLFA isolated from soils can be analyzed to learn more about the general organization of terrestrial microbial populations. PLFA profiling has been widely employed as a biological indicator of overall soil quality and a quantitative indication of soil response to land management and other environmental stresses in a variety of habitats [158]. Soil enzymes play a key role in major degradation processes such as xenobiotic detoxification and organic matter decomposition. Because of their key function in the soil environment, soil enzymes such as lipases, ureases, dehydrogenases, alkaline and acid phosphatases, catalases, and arylsulfatase can be considered useful markers for monitoring the effects of contaminated soils. Despite the fact that soil enzymatic activity has been utilized as bioindicators of pollution with herbicides, heavy metals, and organic pollutants, little is known about their potential as bioindicators of hydrocarbon biodegradation [159,160]. There is mounting evidence that soil bioactivity is vulnerable to environmental pressures and, as a result, can be utilized as a rapid approach to test soil decontamination in association with other appropriate methods. Enzymatic assays, on the other hand, will need more research and data before they can be utilized as the primary tool for assessing bioremediation.

BIOLOG microtiter plate assay can be used as a quick way to track changes in microbial communities metabolic fingerprints [161]. This approach was developed to classify bacteria on the basis of their ability to oxidize 95 distinct carbon sources, but it was later refined to assess functional aspects of microbial communities that produce habitat-specific and reproducible patterns of carbon substrate oxidation [162]. The density and composition of the inoculum utilized have been found to affect substrate utilization patterns. Although growth of bacteria happens in the microtiter plate wells during the experiment, the patterns of substrate usage seen are shown to reflect only those microorganisms that can thrive under the standard assay conditions. Despite its drawbacks, this quick approach is nevertheless a helpful tool for microbial community study.

The interaction of an antibody (detector) and an antigen is the basis of immunochemical procedures (pollutant) [163]. To prevent the groundwater pollution caused by pesticide, best management practices for agricultural (BMP) have been recommended by pesticides and groundwater strategy (the US Environmental Protection Agency). Hydrocarbon-degrading microbes can be quantified in near real time using ELISA and direct immunofluorescence [164]. For speedy analysis of complex sample matrices in the field, immunodetection has shown to work quite well. Antibody mixtures could be created to tackle certain bacterial groups, although measuring the expression of individual genes involved in hydrocarbon metabolism would be more useful in most cases. Immunoassay test kits are now accessible on the market, and immunoassay procedures are widely used in a wide range of applications. Monitoring of underground storage tanks for volatile organic compounds leakage, agricultural runoff for pesticides, chemical and biological testing of poultry, dairy, and meat products for safety, and monitoring the characterization and bioremediation of hazardous waste sites are just a few of the environmental remediation.

Molecular techniques for evaluation of microbial community profiles include 16S rRNA sequencing, reverse sample genome probing (RSGP), the polymerase chain reaction (PCR) along with denaturing and temperature gradient gel electrophoresis (DGGE and TGGE), ribosomal intergenic spacer analysis (RISA), single-strand conformation polymorphism (SSCP), 16S pyrotags, ITS-restriction fragment length polymorphism (ITS-RFLP), terminal restriction fragment length polymorphism (T-RFLP), automated ribosomal intergenic spacer analysis (ARISA), random amplified polymorphic DNA (RAPD), 16S-23S internally transcribed spacer (ITS) typing, amplified ribosomal DNA restriction analysis (ARDRA), single-strand conformation polymorphism (SSCP), and fluorescent *in situ* hybridization (FISH) [165-168].

Another valuable tool for evaluating microorganisms and their activity in environmental materials is DNA microarray technology because it allows conducting high number of hybridizations concurrently [169]. Many existing analytical techniques for pollution monitoring necessitate costly equipment and substantial processing of environmental samples. Traditional analytical methods are unable to distinguish between chemicals that are inaccessible and those that are bioavailable. Analytical methods used in the past only provided data on concentrations in polluted stages. The shortcomings of traditional analytical methods have sparked interest in developing new approaches, such as innovative bacterial biosensors. A biosensor is a type of analytical device that combines a biological sensing element (such as an antibody or an enzyme) with a physical transducer (such as mass, an optical, or electrochemical) to convert the interaction between bio-recognition molecules and the target into a measurable electrical

signal [170]. *Escherichia coli*, *Pseudomonas putida*, *Burkholderia* sp., and *Rhodococcus eutropha* are some examples of biosensors developed for monitoring different contaminants [171-174].

Bioremediation also utilizes the engineered microbial biosensors developed from bacterial two-component regulatory systems (TCRSs) [175]. Our understanding of microbial population diversity and communities present in the environment is evolving because of the rapid improvements in molecular technologies. The ability of inherent unpredictability of microbial populations with time, on the other hand, remains a significant challenge. To eliminate background variability, rapid automated methods will be necessary to process and assess huge amounts of data. Even so, it is important to remember that, while molecular approaches are strong and appealing, a community's genetic composition cannot be used to correctly extrapolate the function of ecosystem.

Thus, over the past two decades, monitoring of the microbial processes during bioremediation of contaminated sites has always been a challenge and a key research focus for the development of quick and reliable approaches. Various advanced molecular and biochemical approaches can be used to assess the existence of dangerous substances and the ecological risk. Molecular methods are an essential interdisciplinary endeavor that involves both biological and computational knowledge. Various advanced molecular and biochemical approaches can be used to assess the existence of dangerous substances and the ecological risk. Molecular methods are an essential interdisciplinary endeavor that involves both biological and computational knowledge.

6. BIOTECHNOLOGICAL APPLICATIONS

Microbes, including bacteria, archaea, fungi, and algae through their enzymatic activity, can degrade, transform, or neutralize toxic and hazardous pollutants [176]. The identification of enriched microbes at polluted sites has been accelerated by the growth and accessibility of DNA sequencing technology. The polluted sites are often allowed to naturally remediate in a process called natural attenuation which is largely dependent on microbial activity [177]. The growth and activity of the indigenous microbes could be enhanced by supplementing the soil with nutrients such as plant or animal waste, a process known as biostimulation. Alternatively, pollutant degradation could be accelerated by bioaugmentation, which involves introduction of new wild type or genetically engineered microbes that specialize in the degradation of toxic compounds into the contaminated site [176]. Bioremediation through microbes has found practical applications as an economical tool in the treatment of pollution in agricultural settings as well as in other environments.

6.1. Environmental Applications

The environment could be polluted by various sources ranging from industrial emissions and effluents to incineration, fossil fuel combustion and automobile exhausts, chemical spills, and landfills. Gaseous pollutants could be removed by passing industrial gases through a microbiological filter surface in a process called biofiltration before being released into the environment [178,179]. Sewage treatment conducted worldwide to remediate water is a largely microbe-driven process. The decontamination of land with hazardous pollutants may be undertaken *in situ* often through biostimulation, but the natural process of bioremediation and the microbes involved are frequently investigated through the study and testing of polluted samples and relevant microbes in laboratory settings.

A common pollutant, petroleum products such as gasoline or petrol contain alkanes, cycloalkanes, aromatic, and heterocyclic compounds, all of which may be subjected to enzymatic degradation by microbes. Studies on gas station environments reveal the presence of microbe's actively metabolizing petrochemicals and decommissioned gas stations are frequently remediated through microbial bioremediation. In fact, one of the earliest examples of bioremediation, as reported in the 1970s, was implemented to remove toxic petroleum products by promoting microbial activity through the addition of nutrients [180]. Since then, bioremediation has been actively employed worldwide to address environmental pollution. One of the most notable applications of *in situ* bioremediation was the reclamation of an area that was heavily polluted by a chemical storage facility, converting it into a safe site called Olympic Park where the 2012 London Olympics were held [181]. In this case, toxic ammonia was converted into safe nitrogen gas, through the activity of archaea making the land area reusable for public activity.

Toxic pollutants such PAHs and polychlorinated biphenyls (PCBs) can persist in the soil long term, posing risks to human and environmental health [182]. Semi-volatile polycyclic aromatic hydrocarbons (PAHs) are among the most common industrial pollutants which are not only present in petroleum products but also industrial wastes, wood preservatives, and numerous other sources and can contaminate soil and water aquifers. Many PAH compounds, including naphthalene, phenanthrene, chrysene, and benzanthracene, are carcinogenic, making the removal of these compounds from contaminated environments close to human activity imperative. Environmental PAH can be degraded or transformed into less toxic forms through the activity of bacteria, archaea, and fungi. The mechanisms of naphthalene degradation by *Pseudomonas* and *Rhodococcus* and the decomposition of phenanthrene by *Ochrobactrum* have been elucidated [183]. Some industrial effluents are inhospitable to normal microbial life and under these conditions, for example in hypersaline environments; extremophiles like halophilic archaea can thrive and undertake the process of bioremediation. The white rot fungus, *Trametes versicolor*, can decompose wood and has also evolved to disintegrate toxic pollutants such as creosote, a wood preservative which is largely made up of PAHs [184]. A study focusing on the bioremediation of industrially polluted soil revealed competition between PAH-degrading bacteria and the fungi like *Trametes* and each could be enriched through specific amendments that discouraged the other species. This indicates that careful selection and balance of amendments are necessary for optimal biodegradation by members within a microbial community.

Heavy metals from industrial and automobile waste also threaten the environment and animal health. One application of bioremediation involved alleviation of chromium toxicity in industrial wastewater using genetically engineered *Alcaligenes eutrophus* ae104 which employed metallothionein and heavy metal transport proteins to sequester toxic metal [10]. Toxic heavy metals are also commonly found in landfills. Waste material from human civilization is dumped into landfills where the disintegration of the biodegradable material is accomplished primarily by microbes. As cities grow, landfills become scarce or overloaded or start leaching pollutants into waterways or may be decommissioned, in which cases bioremediation is an ideal resort. The greenhouse gas methane and toxic ammonia are common airborne pollutants emanating from landfills which could be reduced through bioremediation. Accidental oil spills from man-made oil lines as well as seepage of crude oil from natural resources both stimulate blooms of hydrocarbon degrading bacteria that accelerate the recovery

of the site. One practical example of bioremediation was treatment of the Exxon Valdez oil spill from a crude oil tanker in Alaska in the 1990s. Follow-up studies revealed that the microbial degradation under anaerobic conditions alleviated the oil contamination in the beaches and that biostimulation with inorganic fertilizer enhanced oil biodegradation [185,186].

It is well known how detrimental non-degradable plastic waste can be to our environment, especially as it ends up in the oceans, threatening marine life [187]. With the promise and success of bioremediation, efforts are underway to explore microbial decomposition of plastic. For example, the most commonly used plastic polyethylene (PE) can be partially broken down by the bacterium *Acinetobacter* sp., using the enzymes alkane hydroxylase and laccase [188]. Over 20 genera of bacteria including *Pseudomonas* and *Bacillus* sp. as well as cyanobacteria like *Oscillatoria* are found to be capable of plastic degradation using it as a carbon source; many of them function through the formation of biofilms on plastic surfaces [189]. These microbes have been isolated from a variety of sources including plastic dumps, ocean water, sewage sludge, and intestines of plastic-eating worms [190]. Another commonly used plastic made with polylactic acid can be degraded using Actinobacteria [191]. Another bacterium, *Ideonella sakaiensis*, can enzymatically degrade the plastic polyethylene terephthalate (PET)-producing ethylene glycol and terephthalic acid as byproducts. Interestingly, wax worms consuming polyethylene excrete large amounts of glycol which are presumably produced by the plastic degradation activity of the worm's gut bacteria [192]. Thus, microbes are instrumental in a bioremediation in a wide range of environmental pollution scenarios.

6.2. Agricultural Applications

Due to the increase in modern agriculture worldwide, there has been dramatic contamination of produce, soil, groundwater, and the surrounding environment by agricultural pollutants and toxins from fertilizers and heavy metals to pesticides. By exposure to agricultural fields and through consumption of agricultural products, this pollution also impacts the safety of the consumer [15].

A key aspect of agricultural pollution stems from the usage of polluting inorganic fertilizers, especially those containing nitrogen, phosphorus, and potassium (N, P, and K). Over-fertilized fields and those applying animal waste can be bioremediated using microbes. Ammonia and nitrogen oxides emanating from nitrogen based fertilizers could be released into the atmosphere aggravating the greenhouse effect, create ground-level smog, could contaminate waterways and affect aquatic organisms, as well as pose harm to livestock and humans. Nitrogenous compounds such as nitrates and nitrites released in agriculture could result in respiratory distress, heart, or kidney diseases [193,194]. Bacterial bioremediation can remove compounds like nitrate in runoff through assimilatory nitrate reduction [195]. Bioremediation is not only limited to naturally occurring microbes but can also implement genetically modified microbes (GMMs) to specifically attack certain toxins or pollutants. Aerobic GMMs, such as *Pseudomonas*, *Mycobacterium*, and *Rhodococcus*, use contaminants for carbon and energy sources and thus will degrade them into less toxic products [196]. Studies have shown success with using indigenous *Rhodobacter sphaeroides* in wastewater to remove both nitrogen and phosphorus species, indicating a potential remediation for agriculture as well [197].

Heavy metal exposure, no matter the route, is potentially dangerous. Specifically metals such as cadmium, lead, chromium, and mercury,

even in miniscule amounts, tend to be hazardous to animals and humans [198]. Often, heavy metals from power plant emissions, electroplating plants and fertilizers can enter into agricultural soil directly or through irrigation channels and can be detrimental to crop quality [199,200]. The damage caused by heavy metals in agricultural soils is dependent on their bioavailability, specifically their oxidation state and chemical form [199]. Due to their non-biodegradable nature, heavy metals can accumulate in an organism's tissues causing further health implications. Bioremediation of heavy metals has been done in both *in situ* and *ex situ* conditions in a multitude of ways, including, bioventing, biostimulation, and land farming [200]. The previous studies have shown success with *Agrobacterium* species ability to absorb iron from its surroundings as were also done by microbial biofilms of *Rhodotorula species*. Other bacteria used in the bioremediation of heavy metals include *Flavobacterium*, *Pseudomonas*, and *Corynebacterium* [10]. One application of bioremediation is biotransformation, in which microbes can transform metals from a very toxic form into less toxic ones; for example, chromium VI, which is highly toxic is converted by microbial activity to a less harmful chromium III, which is also more easily removable [201]. Another implication of bioremediation is through bioleaching with fungi. Some fungi, such as *Mucor* sp. or *Cladosporium* sp., are able to resist varied environmental factors (pH, temperature, etc.) and mobilize heavy metals by producing organic acids [197]. Microbes often have optimal conditions in which they work, which allow them to bioaccumulate metalloids at their ideal pH and temperature. One study showed that heavy metal-resistant *Aspergillus* sp. were able to efficiently remove 90% of Cr (VI) and 55% of Ni (II) under a pH of 7.0, whereas in conditions 2 pH units above or below, there was a drastic decrease in bioaccumulation [202]. Thus, optimized microbial bioremediation is a promising tool to eradicate heavy metals from agricultural settings.

Pesticides and herbicides, heavily used in agriculture, are substances that are used to regulate the presence of weeds and prevent or minimize the damage to crops done by insects, rodents, and molds [203]. Many pesticides are carcinogenic and phenoxy acid herbicide, a weedicide, has been associated with the development of soft-tissue sarcoma (STS) and malignant lymphoma in humans [204]. Organochlorides like the notoriously toxic chemical dichlorodiphenyltrichloroethane (DDT) that has been banned from use and lindane are highly toxic, persistent chemicals demonstrated to be harmful to the environment. Organophosphates like diazinon are another group of pesticides designed to kill insects, but they can cause damage to nerve function in humans [205]. Exposure to organochlorides and/or organophosphates can cause cancer and convulsions. Bioremediation is an effective tool to combat pesticide pollution in agriculture. For instance, the bacterium, *Serratia*, has been observed to metabolize DDT [182]. Chlorinated compounds in pesticides are preferentially degraded under anaerobic conditions with fewer harmful byproducts by bacteria such as *Rhodococcus* and *Rhizobium* [180,206,207]. Other bacteria that are able to degrade pesticides include *Flavobacterium*, *Arthrobacter*, *Azotobacter*, *Burkholderia*, and *Pseudomonas* genera. Fungi such as *Pleurotus* can also degrade a variety of pesticide compounds [208] [Figure 1].

Many pollutants that are not agricultural products also make their way into agricultural fields and the underlying groundwater and pose a threat to agriculture and consequently human health. Substances such as gasoline, oil, and road salts can all run off into agricultural soil and subsequently affect the produce. For example, benzene can pollute groundwater through a gas line leak, landfill,

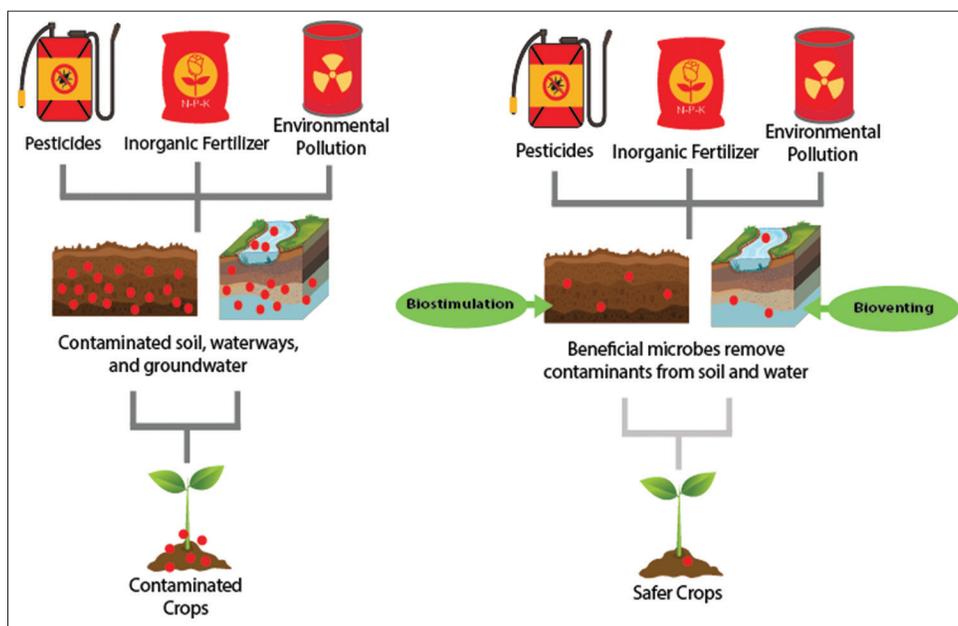


Figure 1: Application of microbes to tackle pollution of agricultural settings. Left panel, toxic chemicals from agricultural products such as pesticides, inorganic and organic fertilizers, and other environmental pollutants such as industrial compounds such as PAH and PCB can contaminate agricultural soil, groundwater, or waterways and potentially taint the crop itself. Right panel, promotion of the growth bioremediating microbes by amending the soil (biostimulation) and/or making holes in the soil to promote the release of detoxified by products (bioventing) or by introduction of bioremediation microbes (bioaugmentation) can result in safer agriculture and greater food security.

or hazardous waste runoff and exposure to benzene can lead to anemia or damage to bone marrow. *Mycobacterium vaccae* is one bacterium that can catabolize benzene and similar compounds such as acetone, trichloroethylene, ethylbenzene, and other dangerous chemicals [209]. Oil spills release petroleum hydrocarbons into the area surrounding the spillage and can also contaminate agriculture. Studies have found that there are a number of bacteria such as *Achromobacter*, *Acinetobacter*, *Kocuria*, *Mycobacterium*, *Pseudomonas*, *Staphylococcus*, *Streptobacillus*, and *Streptococcus* that degrade petroleum hydrocarbons and could be employed in an agricultural environment [210]. Petroleum by-products that enter the soil can destroy farmlands and ruin soil fertility. Petrol or gasoline, kerosene, and engine oil can be degraded by enteric bacteria *Escherichia coli*, *Proteus*, *Klebsiella*, and *Pseudomonas* sp. in soil [211-213]. Thus, microbes are promising tools for bioremediation of toxic pollutants threatening agriculture. Incidentally, many of the same microbes that are used for bioremediation such as *Pseudomonas* and *Bacillus* sp. can also act as plant growth-promoting rhizobacteria (PGPR) and contribute to plant growth promotion and disease resistance, thus potentially boosting agricultural productivity [208].

7. CONCLUSION

There is a growing public concern for removal of the toxic pollutants introduced into the environment by diverse human activities. Bioremediation through biological systems is a novel technology and receiving immense credibility in the field of the pollution management. Bioremediation is a viable and economical approach for waste disposal as compared to various physiochemical methods. The continuous search for novel bioresources is still required for successful implementation of this technology and safeguard nature and environment. Studying the effect of microbes singly or in combination on diverse range of the pollutants is the need of the hour. The application

of genetically engineered microbes with potential to degrade a wider range of pollutants could be a step forward. The enzymes involved in the process of bioremediation could be over expressed, purified, and utilized. The understanding of the mechanisms of microbial mediated bioremediation could be studied. Awareness and education among the people about the role of the microbial communities in environmental cleaning is either important. Field trials for demonstrating the efficiency of the bioremediation technology will prove important. Further, metagenomics could be a useful approach to study the microbial communities within polluted sites and genes could be identified to improve the degradation abilities of the microbial strains. Thus, there is a great potential for the development of the process for bioremediation using the microbes.

8. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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Not applicable.

12. DATA AVAILABILITY

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