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REVIEW ARTICLE

Recent advancements in hydrocarbon bioremediation and future challenges: a review

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Abstract

Petrochemicals are important hydrocarbons, which are one of the major concerns when accidently escaped into the environment. On one hand, these cause soil and fresh water pollution on land due to their seepage and leakage from automobile and petrochemical industries. On the other hand, oil spills occur during the transport of crude oil or refined petroleum products in the oceans around the world. These hydrocarbon and petrochemical spills have not only posed a hazard to the environment and marine life, but also linked to numerous ailments like cancers and neural disorders. Therefore, it is very important to remove or degrade these pollutants before their hazardous effects deteriorate the environment. There are varieties of mechanical and chemical methods for removing hydrocarbons from polluted areas, but they are all ineffective and expensive. Bioremediation techniques provide an economical and eco-friendly mechanism for removing petrochemical and hydrocarbon residues from the affected sites. Bioremediation refers to the complete mineralization or transformation of complex organic pollutants into the simplest compounds by biological agents such as bacteria, fungi, etc. Many indigenous microbes present in nature are capable of detoxification of various hydrocarbons and their contaminants. This review presents an updated overview of recent advancements in various technologies used in the degradation and bioremediation of petroleum hydrocarbons, providing useful insights to manage such problems in an eco-friendly manner.

Keywords Bio-surfactants · Bioremediation · GMO · Hydrocarbons · Nanotechnology · Petrochemicals

Introduction

Slapdash exploitation of hydrocarbon fuels to meet the energy demand of the world is continuously harming our environment. Significant increase in the petroleum industry and exploitation of petroleum resources have had a negative impact on the ecosystem. Oil spillage is one such problem caused by the unintentional release of liquid hydrocarbons during oil exploration and transportation to different parts of the world (Abatenh et al. 2017; Gurav et al. 2021). Oil spillage occurs mostly due to natural disasters and anthropogenic activities such as attacks by militant organisations or accidents of ships during their transportation from one Gulf country to another. Incidents of oil spillage are of major concern because they result in consequential contamination of the sea and coastline area (Singh et al. 2015; Wang et al. 2018). Hydrocarbons present in crude oil have many adverse environmental as well as health impacts (Sayed et al. 2021). When crude oil is burned accidentally or as a spill control measure, a number of toxic gases and chemicals such as carbon dioxide, carbon monoxide, nitrogen oxides, sulphur dioxide, particulate matter, lead, polycyclic aromatic hydrocarbons, and volatile organic compounds are released, which have known health impacts (Shigenaka et al. 2015; Jaligamaet al. 2015). Because of the oil spillage, hydrocarbons, including known carcinogens like benzopyrene and polycyclic aromatic hydrocarbons pollute surface water,

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ground water, ambient air and crops (Moore 2012). Oil spills potentially affect household food security by 60% and also lower the content of ascorbic acid in vegetables and the content of cassava crude protein by 36% and 40%, respectively (Ordinioha and Brisibe 2013). The study also reported the hemotoxic and hepatotoxic nature of crude oil that leads to infertility and cancer.

Conventional physical–chemical methods are not completely effective and have adverse environmental effects. Hence, it is necessary to develop advanced and environmentally friendly technologies to treat these toxic hydrocarbons. Bioremediation is an effective method of cleaning soil and water from petroleum hydrocarbon contaminants (Bhatia et al. 2020; Bhola et al. 2021; Dange et al. 2021; Gurav et al. 2021). Microorganisms play a critical role in the biological disposal of pollutants from soils (Hamad et al. 2021). Special attention is given to the initial oxidation of hydrocarbons and enzymes involved in biodegradation and the identification of intermediates. The complete metabolic pathways and genetic control of biodegradation processes are still unknown.

Polluted sites with hydrocarbons are rehabilitated by microorganisms, which is an appealing solution. Bioremediation technology using indigenous microbial populations is an effective method to clean up contaminated sites. Hydrocarbon-degrading microorganisms include bacteria, fungi, and algae. A number of bacterial species isolated from different environments are known to have high tolerance and hydrocarbon degradation potential (Hassanshahian et al. 2012; Yuniati 2018; Bhatia 2021). Degradation of hydrocarbons can be done through lignolytic, dehydrogenase, and oxygenase enzymes (Hong et al. 2015). The microbes isolated from hydrocarbon-contaminated sites have reported high tolerance to hydrocarbons along with high catabolic activity and greater tolerance to heavy toxic metals, better degradation of pollutants, and high biodegradation ability (Thavamani et al. 2011). This review represents the current bioremediation strategies with cost-effective measures to restore the oil spill-contaminated sites.

Hydrocarbon and their types

Crude oil is a heterogeneous mixture of different classes of volatile liquid hydrocarbons such as alkanes, alkenes, cycloalkanes, and aromatic hydrocarbons (benzene and its derivatives). As hydrocarbons are the main constituents of crude oil, other compounds present are nitrogen, sulphur, and oxygen, along with a minute amount of phosphorus and heavy metals like vanadium and nickel (Yuniati 2018). Depending on the composition, each component of oil has altered physico-chemical properties, as most of these are persistent and hydrophobic in nature and vary in viscosity, solubility, and capacity to absorb. The difference in bioavailability and toxicity also decreases the ease of biodegradation in the following sequence: *n*-alkanes > methyl alkanes and alkylcyclopentanes > alkylcyclohexanes > cyclic and acyclic isoprenoids (Gros et al. 2014).

Spillage of hydrocarbon

According to the International Tanker Owners Pollution Federation (ITOPF 2017), in the last 10 years from 2007 to 2016, a total of 59,000 tonnes of persistent and non-persistent oil were lost to the environment in 79 tanker accidents worldwide, with an average of 8 oil spills per year. ITOPF is a non-profit organisation that offers information on oil spills from tankers and barges transporting oil. It is estimated that about 2.37×10^6 tonnes of petroleum enter the environment per year, either through anthropogenic or natural activities (Kennisha 1997). A significant portion of this (approximately 65.2%) comes from municipal and industrial waste or dumping in oceans, as well as atmospheric fallout, while the remaining 26.2% comes from oil spill accidents during transportation and dry docking (Fig. 1).

Various activities linked with oil exploration, installation, single point mooring (SPM) and transportation in the coastal area have the potential to create oil spills and pose a hazard to coastal ecosystems, notably those around the Indian Ocean (Kankara and Subramanian 2007). It is estimated that about 8.6% of the petroleum entering the environment comes from coastal as well as offshore production facilities and marine terminals (GESAMP 1993). As soon as the petroleum hydrocarbons enter the environment, the light fraction evaporates, while the higher molecular mass hydrocarbons with complex branched and aromatic structures pose a threat to soil habitat as they are persistent and resistant to biodegradation.

Environmental and economic impacts of oil spillage

Oil spillage is a quantitatively major source of aquatic forms of accidental pollution caused by the maritime sector's transportation. Oil spillage is associated with various environmental, social, and health impacts. In 2015, an oil tanker carrying 1.1 million barrels of oil collapsed near Yemen's coast, which caused huge damage to the environment (Huynh et al. 2021). Oil spills have severe acute and chronic effects on aquatic ecosystems, as oil-affected birds and mammals lose their thermal insulation and buoyancy (Lee et al. 2015). Crude oil components and dispersants used during oil spill clean-up modify the structure and dynamics of planktonic communities in the sea by disrupting the energy transmission between the trophic levels (Almeda et al. 2014). Oil pollution also damages the reproductive system of coral reefs and decreases colony viability, lowering the number of ovarian per polyp on

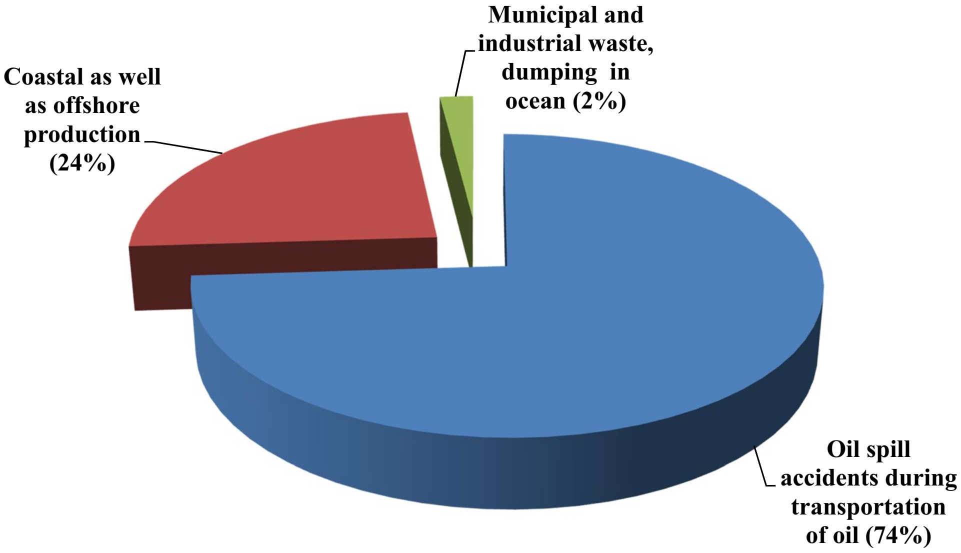


Fig. 1 Activities contributing to oil spillage

reef corals and resulting in premature planulae shedding (Loya and Rinkevich 1980). Mangrove vegetation is also adversely affected by oil pollution. Duke has reported that about 5.5 million tonnes of oil have killed at least 126,000 ha of mangrove vegetation by entering mangrove-lined coastal waters since 1958 (Duke 2016). Because the accumulation of pollutants in animal and plant tissues can result in offspring mortality or mutation, hydrocarbon-contaminated soil causes significant harm to local ecosystems (Alvarez et al. 1991). These compounds also penetrate and bind to macro and micro components of soil and limit the transport of water and air, which are crucial for remediation by microorganisms (Caravaca and Roldan 2003).

Many oil spills result in the contamination of coastal areas with high amenity values. In addition to the cost of clean-up, enterprises and individuals that rely on coastal resources may suffer significant financial losses. Typically, the tourism and fishing industries endure the most of the consequences. Fisheries and mariculture resources can be severely harmed by oil spills. By fouling gear or obstructing access to fishing grounds, physical contamination can have an impact on stocks and impede commercial operations. Recreational activities such as swimming, boating, angling, and diving are normally disrupted very briefly by oil-contaminated coastlines. Normal trade and such-like activities are likely to resume only after the shorelines are cleaned. However, when the public perception of long-term and widespread contamination persists long after the oil is gone, greater long-term and harmful economic effects can ensue. On the other hand, many other companies' operations and industries could be disrupted, resulting in a loss of earnings (ITOPF 2017).

Remediation of oil spillage

The various clean-up techniques, including in-situ burning, mechanical and chemical remediation, are used (Figs. 2, 3). The use of various cleaning agents, such as organo-clays synthesised by ion exchange, dispersants, chemical absorbents, synthetic surfactants, and additives, has several drawbacks, including higher cost, non-biodegradability, non-recyclability, as well as acute and chronic toxicity to aquatic life (Carmody et al. 2007; Nyman et al. 2007; Lee et al. 2015). The conventional techniques do not lead to the degradation of environmental pollutants and focus unduly on the separation of contaminants. Bioremediation technology has an edge over conventional physico-chemical methods as bioremediation using microbial populations is eco-friendly, cost-effective, and results in total contaminant degradation (Tyagi et al. 2011; Haritash 2020; Sadhana et al. 2021). Although, the bioremediation process also has disadvantages, it has many more benefits with high sustainability and economic feasibility (Kumari et al. 2019; Dutta et al. 2021). The major disadvantages associated with bioremediation are the longer treatment time as that of other remediation technologies, the range of contaminants (which should be biodegradable), and some time it leaves high residual levels which are persistent and toxic. However, the evaluation of performance is difficult because bioremediation has no defined level of a "clean" site, which makes the performance criteria regulations uncertain.

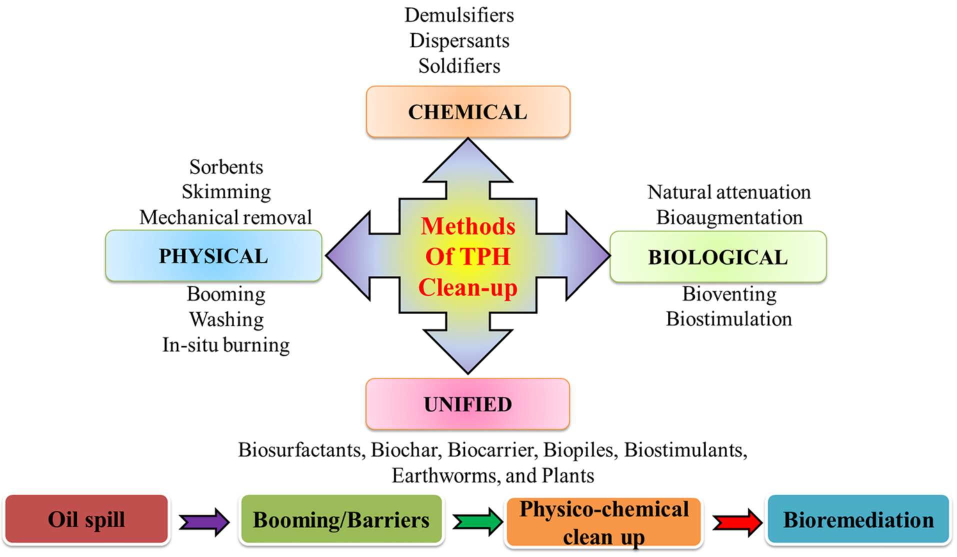


Fig. 2 Proposed methods of clean up spill

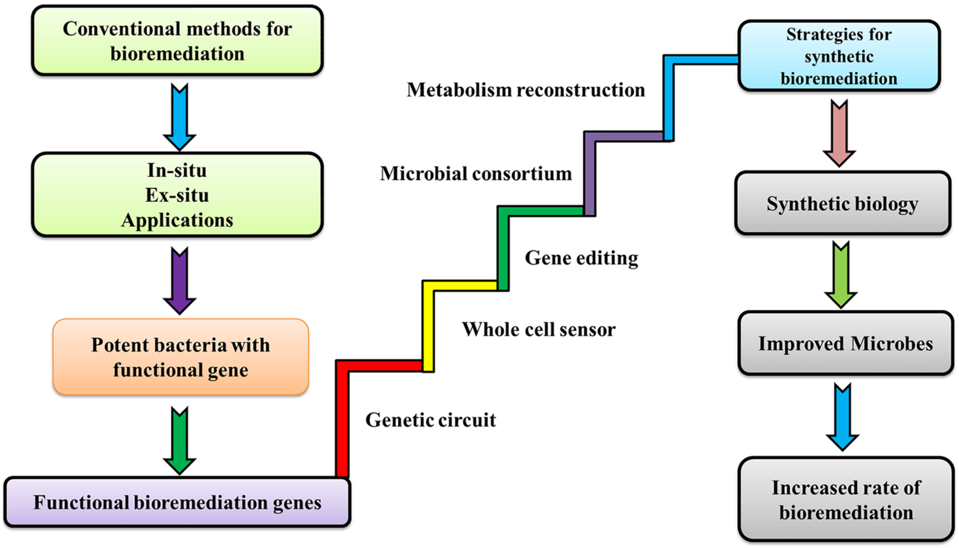


Fig. 3 Improvement of microbes for hydrocarbon bioremediation

Hydrocarbon bioremediation

Microorganisms in both the terrestrial and aquatic environments serve as primary biodegradation agents for environmental significance, majorly against petroleum hydrocarbons. Bacteria, algae, yeast, and fungus that degrade hydrocarbons are found in abundance in marine, freshwater, and soil ecosystems (Fig. 3). In aquatic ecosystems, yeast and bacteria are dominating degraders, whereas in soil, fungi and bacteria appear to be the primary degraders (Hossain et al. 2022). Algae-based bioremediation has a significant capacity for producing biomass and has a greater ability for collection, detoxification, and degradation of xenobiotics and contaminants. Many algal enzymes have been discovered to be involved in the biodegradation of a variety of organic contaminants (Baghour 2019). The intermediate length, *n*-alkanes (C10–C20), is the favourite substrate, which can be easily degradable to the various petroleum fractions, but shorter chain molecules are more hazardous to bacteria. However, *n*-alkanes of short length have great aqueous solubility, but they are toxic to microbes or cells, which directly inhibit microbial growth. The toxicity mechanisms are associated with their uptake and dissolution in the cell membrane and have shown more bioavailability of short-chain n-alkanes than midsized or long-chain n-alkanes. Waxes (C20–C40) are hydrophobic solids with long-chain alkanes that are difficult to decompose due to their low solubility and bioavailability in water; alkanes with branched chains also decay more slowly than conventional alkanes (Balba et al. 1998). For creating an artificial microbial-bacterial consortium, a microalgae, i.e., *Scenedesmus obliquus* GH2, is utilised for crude-oil degradation (Tang et al.

2010b). According to a study on the *Rhodococcus* genus, it is involved in the degradation of n-alkanes (up to C36) and heavy alkanes in Prestige fuel (Jimenez et al. 2007). Microorganisms' ability to degrade polyaromatic hydrocarbons (PAH) is largely determined by their molecular weights (Sakhuja et al. 2021).

Various microorganisms have the ability to degrade aromatic compounds, mainly low-molecular weight PAH compounds, i.e., phenanthrene, anthracene, and naphthalene. The degradation rate of cycloalkanes is variable, but it is as slow as that of alkanes, which also involves several microbial species. Bitumen, tars, and asphaltic materials having high-condensed aromatic and cycloparaffinic structures with a high boiling point greatly resist biodegradation (Balba et al. 1998). Hong et al. (2008) investigated the accumulation and biodegradation of two common PAHs, phenanthrene (PHE) and fluoranthene (FLA) by diatoms utilising two algal species, *Skeletonema costatum* and *Nitzschia* sp. The scientists discovered that *Nitzschia* sp. had better accumulation and degradation abilities than *S. costatum*. FLA degradation was slower in both algal species, showing that FLA was a more resistant PAH molecule. Some organic xenobiotics, such as poly-aromatic hydrocarbons (PAHs) and some fractions of crude oil and coal, are resistant to degradation. Microorganisms, on the other hand, are thought to be capable of decomposing practically all the complex and resistant xenobiotics found on the planet (Hossain et al. 2022). Phytoplankton is high in lipid content and serves as the foundation for benthic and pelagic food chains, which have a role in determining persistent organic pollutants (POPs), i.e., polychlorinated biphenyls (PCBs) in the water column (Lynn et al. 2007).

The most common genera known to be responsible for oil degradation comprise mainly *Nocardia*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Micrococcus*, *Arthrobacter*, *Corynebacterium*, *Achromobacter*, *Scenedesmus, Nitzschia*, *Rhodococcus*, *Alcaligenes*, *Mycobacterium*, *Bacillus*, *Aspergillus*, *Mucor*, *Fusarium*, *Penicillium*, *Rhodotorula*, and *Sporobolomyces* (Atlas 1981; Bossert and Bartha 1984; Hassanshahian et al. 2012). The different microorganisms and their hydrocarbon degradation potential are given in Table 1.

Hydrocarbon-contaminated soils suffer from salinity due to industrial activities (Gao et al. 2015). Soil

Table 1 Hydrocarbon degradation potential of indigenous microbial population

Hydrocarbon substrate	Microorganism	Degradation (% age)	Isolated from	References
Crude oil	*Acinetobacter calcoaceticus*	98	Persian Gulf and Caspian Sea	Hassanshahian et al. (2012)
	Alcanivorax dieselolei	98		
	Alcanivorax dieselolei strain PM07	71		
	Yarrowia lipolytica PG-20	68		
	Yarrowia lipolytica PG-32	58		
	C2 strain (99% *Citrobacter sedlakii)*	69	Petroleum polluted soils, Iran	Ghoreishi et al. (2017)
	C4 and SI1 (99% *Enterobacter hormeachei)*	48		
	SI2 (99% *Entrobacter cloacae*)	42		
TPH	*B. subtilis* DM-04	53.57	North east India	Das and Mukherjee (2007)
	P. aeruginosa M	75		
Mixture of petroleum hydrocarbons	*Bacillus* sp. SV9	59	Ankleshwar, India	Verma et al. (2006)
	Acinetobacter sp. SV4	37		
	Pseudomonas sp. SV17	35		
Alkanes	*Pseudomonas* strain PS-1	70.69	Lingala oil field project India	Mittal and Singh (2010), Brown et al. (2016), Imperato et al. (2019)
Aromatic hydrocarbons	*Gordonia sihwensis*	45.37		
Anthracene	*Mycobacterium* sp. strain PYR-1	92	Contaminated estuarine sediment	Moody et al. (2001)
Phenanthrene		90		
Phenanthrene	*Staphylococcus* sp. strain PN/Y	Unknown	Noonmati refinery site, India	Mallick et al. (2007)
Acinetobacter baumannii S30 pJES	Hydrocarbon degradation	Unknown	Petro contaminate site	Mishra et al. (2004)
Streptomyces coelicolor M145	n-hexadecane degradation	Unknown	Crude oil contaminate site	Gallo et al. (2012)
Acinetobacter sp. BS3	Aromatic hydrocarbons	Unknown	Crude oil contaminate site	Xie et al. (2014)

salinity suppresses microbial growth and reduces the ability of microbes to degrade petroleum pollutants (Hua et al. 2010). Therefore, in such conditions, it is necessary for the microbes to be resistant to salinity. Bacterial communities ("consortia") are more flexible and are efficient degraders of a wider range of pollutants than individual microorganisms (Jobson et al. 1972; Shankar et al. 2014; Li et al. 2016). Recently, Ghoreishi and his co-workers isolated the bacteria from petroleum and kerosene-contaminated soils for the treatment of kerosene and investigated their degradation potential (Ghoreishi et al. 2017). These bacteria were capable of thriving in a medium containing 20% v/v kerosene and decomposing sulphur and carbon compounds in 7 days to 5% v/v kerosene. According to the results of GC analysis, the isolate C2 (97 percent comparable to *C. sedlakii*) had carbon degradation capacity in kerosene. SI2 (99 percent linked to *E. cloacae*) also demonstrated a significant proclivity for sulphur degradation in kerosene. These two strains in a microbial consortium could aid in the biodegradation of petroleum-contaminated soil more effectively (Ghoreishi et al. 2017). This finding paves the way for more focused and advanced research in the field of biodegradation, mainly in petroleum biodegradation.

Enzymes involved in hydrocarbon biodegradation

Pollutants in the environment have acute and chronic effects on biotic components, degrading the ecosystem (Bhandari et al. 2021). Oxidase, dehydrogenase, and lignolytic enzymes are involved in the breakdown of hydrocarbons. Aromatic ring hydroxylation is carried out by monooxygenases and dioxygenases; monooxygenases insert one oxygen atom while dioxygenases insert two oxygen atoms into their substrates. Through a series of processes, the ring is then cleaved and transformed into 2-ketoadipate or another chemical that the organism can use (Fan and Krishnamurthy 1995; Kora 2018).

Lignin peroxidase, manganese peroxidase, and laccase are fungal lignolytic extra-cellular enzymes that catalyse the formation of radicals through oxidation for bond disruption in a molecule (Punnapayak et al. 2009; Yadav et al. 2011; Lombard et al. 2014). Temperature, pH, salinity, oxygen availability, nutrition availability, and light conditions all have an impact on these enzymes. The majority of enzymes are most active at mesophilic temperatures, but others have been found to be active even at high temperatures. According to a study by Farnet and colleagues, the laccase activity of the fungus *Marasmius quercophilus* is best around 80 °C (Farnet et al. 2000). Although enzymes have substrate specificity, lignolytic enzymes are non-specific, creating cation radicals after one electron oxidation on both phenolic and non-phenolic organic compounds (Juhasz et al. 1997; Sadhana et al. 2021). *Pleurotus laccase* generates hydroxyl radicals, whereas *Nematoloma forwardii* and *Aspergillus oryzae* Mn-dependent peroxidase degrades a wide range of polyaromatic and aliphatic compounds to carbon dioxide and polar fission products (Asemoloye et al. 2020).

In a cell free reaction mixture enriched with manganese (II) ions (Mn^{2+}) at a concentration of 200 M, *Stropharia coronilla*, a litter-decomposing basidiomycete, has also been found to metabolise and mineralize benzo(a) pyrene (BaP) (Steffen et al. 2003; Sharma et al. 2018). This degrading action could be attributed to the lignolytic enzyme manganese peroxidase (MnP), which is activated by the presence of Mn^{2+} and accelerated by the addition of Tween 80. Tween 80 is a surfactant that stimulates or enhances the release of enzymes into the culture medium. In a cell-free reaction mixture, crude and purified MnP from *S. coronilla* efficiently oxidized BaP.

Cytochrome P450 is also identified as being used in the microbial decomposition of petroleum hydrocarbons. An alkane hydroxylase enzyme belongs to the ubiquitous heme-thiolate Monooxygenases superfamily (Van-Beilen and Funhoff 2007; Punetha et al. 2022). Eukaryotes have a number of P450 families, each of which contains a huge number of unique P450 forms that can help in the metabolic transformation of a specific substrate. Zimmer et al. (1996) found that only a few kinds of microorganisms possess P450 multiplicity. *Candida maltosa, Candida tropicalis*, and *Candida apicola* have all been found to have numerous microsomal Cytochrome P450 types of enzyme (Scheller et al. 1998; Punetha et al. 2022). They are capable of obtaining carbon and energy solely from *n*-alkanes and other aliphatic hydrocarbons. Some of the enzymes used in hydrocarbon bioremediation are given in Table 2.

A group tried the degradation of petrol in an aqueous system with immobilized cells and cell free suspension of *Pseudomonas* sp. The increase in rhamnolipid production was also observed in this experiment (Sarkar et al. 2005). The amphipathic characteristics of rhamnolipids diminish oil–water interfacial tension systems, allowing better diffusion of water-insoluble n-alkanes in the aqueous phase. The study found that immobilizing cells boosted cell-to-cell interaction with solubilized hydrocarbon droplets and resulted in fast hydrocarbon uptake. A study by Diaz and colleagues found that immobilizing cells of bacteria enhance crude oil biodegradation rate as that of free-living cells (Dıaz et al. 2002; Shahzad et al. 2020).

Immobilized enzymes have higher efficiency, thermostability, half-life and resistance to protease degradation (Sarkar et al. 1989; Solanki et al. 2021). The activity of the immobilised enzyme lasts substantially longer than that of the free enzyme. Furthermore, the immobilised enzyme can be extracted from the reaction fluid and used to convert substrate with minimal activity loss (Guleria et al. 2016; Ruggiero et al. 1989). For the reasons described above, using

Table 2 Enzymes involved in hydrocarbon degradation

Enzyme	Substrate	Strain	References
Mono-oxygenases Methane, propane and butane mono-oxygenases, CYP153 mono-oxygenases, AlkB non-heme iron mono-oxigenase, flavin-mono-oxigenase AlmA, flavin-dependent mono-oxygenase LadA	Alkanes, Alkenes, cycloalkenes	*Methylococcus, Methylosinus, Methylocystis, Methylomonas, Methylocella,* *Pseudomonas putida* GPo1 *Burkholderia cepacia* G4, *Ralstonia pickettii* PKO1, *Pseudomonas mendocina* KR1, *Pseudomonas stutzeri* OX1	McDonald et al. (2006), Rojo (2010), Imperato et al. (2019)
Di-oxygenases (copper flavin-dependent Di-oxygenase)	Alkanes	*Bacillus* sp. *Pseudomonas* sp.	Kora (2018)
Cytochrome P450 enzymes (Eukaryotic and bacterial)	C10–C16 alkanes, fatty acids, cycloalkanes	*Candida maltosa,* *Candida tropicalis, Candida apicola,* *Acinetobacter, Caulobacter, Mycobacterium*	Scheller et al. (1998), van Beilen and Funhoff (2007), Punetha et al. (2022)
Dehydrogenases	Petroleum hydrocarbon	*Pseudomonas aeruginosa*	Ebadi et al. (2017)
Laccase	Polycyclic Aromatic Hydrocarbon	*Marasmius quercophilus,* *Coriolopsis gallica* UAMH 8260	Lombard et al. (2014), Sharma et al. (2018), Farnet et al. (2000)
Manganese peroxidase	Bezo(a)pyrenes (BaP)	*Nematoloma forwardii,* *Stropharia coronilla* *Aspergillus oryzae*	Steffen et al. (2003), Asemoloye et al. (2020)
Aldehyde dehydrogenase	Total Petroleum Hydrocarbon	*Acinetobacter baumannii* S30 pJES	Mishra et al. (2004)
Alkane monoxygenase	n-hexadacane	*Streptomyces coelicolor* M145	Gallo et al. (2012)
Catechol 2,3-dioxygenas	Aromatic Hydrocarbon	*Acinetobacter* sp. BS3, *Pseudomonas putida*	Xie et al. (2014), Haritash (2020)

cell-free enzymes (ideally immobilized) to speed the cleanup of petroleum-contaminated soils holds a lot of potential.

Advances in petroleum hydrocarbon bioremediation

Conventional methods used for oil spill treatment are now replaced by advanced and sustainable techniques such as genetically engineered microorganisms, nanotechnology-based remediation materials, and metabolic engineering to treat toxic bioremediation intermediates (Naeem and Qazi 2020; Dutta et al. 2021; El-Sheshtawy et al. 2022). Use of indigenous microbial communities for effective bioremediation is a current approach as they are resistant to toxic pollutants and can grow in harsh conditions (Karunanithi et al. 2017; Shakya et al. 2021).

For the remediation of highly hydrocarbon-contaminated sites, the application of xenobiotic-resistant genetically modified microorganisms is more suitable as the microbial population is not able to dissipate them completely (Fernandez-Luqueno et al. 2011). Sarkar et al. (2005) and Taccari et al. (2012) describe the efficient techniques involved in hydrocarbon biodegradation in oil spills. Bioaugmentation can increase the degradation efficiency by enhancing the metabolic activity and survival rate of a microbial population by increasing the expression of the alkB gene. Suja and co-workers have studied the combined effects of bioaugmentation and biostimulation and reported the highest TPH degradation of 79% with the fastest rate constant of 0.0390 day1 (78) (Suja et al. 2014; Sui et al. 2021).

Role of biosurfactants in hydrocarbon biodegradation

The main disadvantage of the soil biodegradation process is the restriction of mass transfer and bioavailability of pollutants, which directly hinders microbe access to petroleum components and is responsible for the decrease in biodegradation rate (Onwurah et al. 2007; Karlapudi et al. 2018). Some microorganisms can produce amphipathic molecules called biosurfactants. These surfactants pseudo-solubilize hydrocarbons and desorbed them from the soil matrix into the aqueous phase, which led to better availability of oil for microbial cell uptake. Such microbes have been considered as a source of crude oil contaminate bioremediation in soil (Cameotra and Makkar 1998; Whang et al. 2008; Ganesh and Lin 2009; Rocha et al. 2011; Hua and Wang 2012). Thavasi and his co-workers have reported that biosurfactants are capable of enhancing biodegradation rates by up to 4–5% without adding fertilizers, which also helps in reducing the biodegradation cost (Thavasi et al. 2011).

The application of two biosurfactants was tested in a series of bench-scale experiments, a lipoprotein type biosurfactant surfactin (SF) and a glycolipid biosurfactant rhamnolipid (RL), for increased soil and water biodegradation for removing diesel-contamination has also been described. *Bacillus subtilis* ATCC 21332 created the surfactin, while *Pseudomonas aeruginosa* J4 produced the rhamnolipid; both biosurfactants reduced surface tension to < 30 dynes/cm, down from 72 dynes/cm, and increased diesel solubility with increased biosurfactant (Whang et al. 2008; Rikalovic et al. 2015). A consortium of hydrocarbon degrading bacteria sown in crude oil-contaminated soil and added with different combinations of rhamnolipids and nutrients produced a maximum of 77.6% biodegradation (Tahseen et al. 2016).

According to a recent study by Ebadi and colleagues, inoculating contaminated soil with a combination of four biosurfactant-producing *Pseudomonas aeruginosa* strains improved the adverse effects of salinity on biodegradation and enhanced the rate of degradation of petroleum hydrocarbons by about 30% when compared to non-treated soil. Inoculation of polluted soil with the consortium considerably increases the dehydrogenase enzyme activity (about twofold) in saline conditions (Ebadi et al. 2017; Mandalenaki et al. 2021). Table 3 lists some of the biosurfactant-producing strains used in hydrocarbon bioremediation.

Role of nanotechnology in bioremediation

Instead of sustainable and economic methods, research is also focused on the use of advanced and inexpensive bio-based materials for the clean-up of petroleum hydrocarbons (Pete et al. 2021). Various nanomaterials and nanostructures have been successfully deployed by various research groups for the treatment of hydrocarbon spills, as shown in Fig. 4. Wu and colleagues created a new form of carbon nanofiber (CNF) aerogel that has a high absorption capacity, more recyclability, and working temperature versatility (up to 400 °C) (Wu et al. 2014). Generally, macroporous materials are used for the absorption of oil spills. Pan and co-workers synthesised new macro-porous and hydrophobic polyvinyl formaldehyde (PVF-H) sponges with improved reusability and oil recovery of 90% (Pan et al. 2014). Nano-TiO2-Induced photocatalysis is a recent technique used for the treatment of water contamination through oil and gas production, transportation, and storage (Liu et al. 2017). For the stabilisation of oil in water emulsion, naturally occurring halloysite clay nanotubes have been reported by Owoseni and colleagues as effective interfacially active carriers for delivering oil spill treatment agents (Owoseni et al. 2014). Therefore, nanotechnology has the ability to provide immense potential in cleaning up pesticides, metal-contaminated sites, and petroleum hydrocarbons (Benjamin and Lima 2019; Pete et al. 2021; Kapoor et al. 2021).

Phytoremediation of hydrocarbon

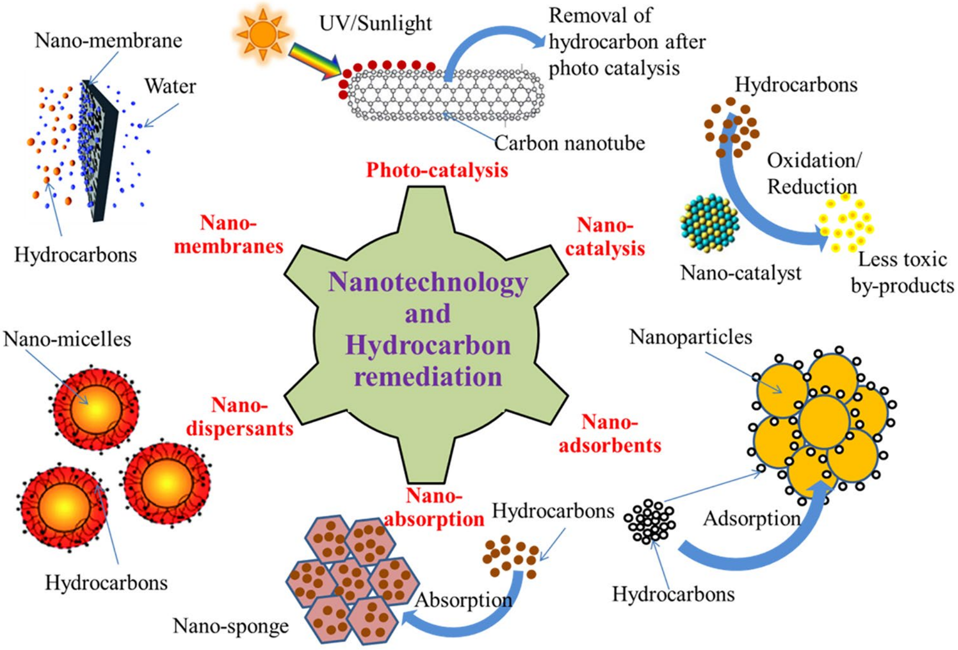
Phytoremediation is an emerging technology that involves the use of plant life for the clean-up of hydrocarbon-contaminated soil and groundwater. Phytoremediation is a cost-effective technique that provides aesthetic value to the site and has long-term applicability. In addition, phytoremediation reduces disturbance on land and removes the liability and transportation expenses involved with off-site treatment and disposal (Das and Chandran 2011; Nedjimi 2021).

The Alabama Department of Environmental Management has approved a location for field-scale total petroleum hydrocarbon degradation. The study used around 1500 cubic yards of soil, with 70% of baseline samples containing more

Table 3 Biosurfactant producing strains used for degrading hydrocarbons

Biosurfactants	Micro-organisms	References
Surfactin	*Bacillus subtilis* ATCC 21332 *Bacillus subtilis* *Bacillus licheniformis*	Whang et al. (2008), Karlapudi et al. (2018)
Rhamnolipid	*Pseudomonas aeruginosa* J4 *Pseudomonas fluorescens* *Pseudomonas aeruginosa* MM1011	Whang et al. (2008), Amani et al. (2013), Rikalovic et al. (2015)
Glycolipid	*Aeromonas* sp., *Bacillus* sp., *Pseudomonas aeruginosa*	Tabatabaee et al. (2005), Rikalovic et al. (2015)
Sophorolipids	*Candida tropicalis*	Imura et al. (2014), Karlapudi et al. (2018)
Lipopeptide biosurfactant	*Pseudomonas aeruginosa*	Thavasi et al. (2011), Ebadi et al. (2017)
NA	*Bacillus* sp., *Paenibacillus* sp.	Najafi et al. (2015)
di-rhamnolipid	*Pseudomonas* sp.	Varjani and Upasani (2016)
NA	*Agrobacterium* sp.	Ohadi et al. (2017)
Lipopeptide biosurfactants	*Aeribacillus* sp.	Mehetre et al. (2019)
Lipopeptide	*Acinetobacter* sp.	Sharma et al. (2019)

Fig. 4 Role of nanotechnology in hydrocarbon remediation



than 100 parts per million of total petroleum hydrocarbons (TPH). Nedunuri et al. (2000) found that when field sites contaminated with total petroleum hydrocarbons (TPH) at 1700–16,000 mg/kg were bioremediated, around 83 percent of the TPH was eliminated after one year. Three coastal trees, milo (*Thespesia populnea*), kiawe (*Prosopis pallida*), and kou (*Cordia subcordata*), as well as the native shrub *Scaevola serica*, a beach naupaka, survived under diesel fuel soil conditions for clean-up among diverse plants in tropical conditions (USACE 2003). At some locations, organic pollutants are removed by planting grass in a row in between the trees. The thin roots of grasses at the surface of soil are capable of binding effectively with hydrophobic contaminants (TPH, PAHs, and BTEX) and converting them into non-hazardous form. Following harvest, the grass can be composted or burned. The most commonly used legumes to replace the nitrogen requirements of low soils are alfalfa (*Medicago sativa*) and peas (*Pisum* sp.). In numerous areas contaminated by petrochemical wastes, reed canary grass (*Phalaris arundinacea*), fescue (*Vulpia myuros*), rye (*Elymus* sp.) and clover (*Trifolium* sp.) have successfully been utilised (Ahmad 2021; Nero 2021).

Use of genetically modified strains in hydrocarbon bioremediation

In situ bioremediation is the combination of traditional microbiology, ecology, genetic engineering, and biochemistry, a promising solution to hydrocarbon bioremediation. A genetically modified microorganism, especially for enzyme specificity, metabolic pathway and its regulation, can be used for bioremediation purposes as well as a biosensor for detection of specific chemical hazardous compounds in the environment (Sheth et al. 2016; Wasilkowski et al. 2012; Mandeep 2020; French et al. 2020).

The first genetically modified strain, *Pseudomonas fluorescens* HK44, was utilised for long-term naphthalene contaminated soil bioremediation along with bioluminescence imaging of inoculation cells. The plasmid pUTK21, containing the *P. fluorescens* HK44 strain, was developed from the *P. fluorescens* 5R NAH7 plasmid by inserting the Tn4431 transposon element into it. *Vibrio fischeri* was the source of this transposon, which also carried the luxCDABE gene cassette. As a result, this gene is both responsible for naphthalene breakdown as well as for the production of luminous signals (Sayler and Ripp 2000). Filonov and colleagues created the other *P. putida* KT2442 (pNF142: TnMod-OTc), a genetically modified strain capable of degrading soil naphthalene (Filonov et al. 2005). Another naphthalene degrading strain *Escheriachia coli* having bphA2cA1c gene encoding for salicylate oxygenase from *S. yanoikuyae* B1 (Liu et al. 2019). This strain contains three bacteria:

(1) pTnMod-OTc plasmid conatining *E.coli* S17-1 with tetracycline resistance gene.

(2) *Pseudomonas* sp. 142NF (pNF142) capable of depredating naphthalene.

(3) *P. putida* KT2442 with the green fluorescent protein gene.

These findings demonstrate that genetically modified bacteria are capable of digesting naphthalene and transferring the plasmid pNF142: TnMod-OTc to native microorganisms. The possibility of plasmid pWW0 being transferred into rhizosphere bacteria from the PaW85 strain of *Pseudomonas putida* is capable of digesting petroleum hydrocarbons (Jussila et al. 2007). In petroleum-contaminated soil, they also confirmed horizontal gene transfer events between PaW85 and *Pseudomonas oryzihabitans* 29. Lipthay and colleagues looked at how *Escherichia coli* HB101 and *Ralstonia eutropha* harbouring the pRO103 plasmid degraded the aromatic hydrocarbon 2,4-dichlorofenoxyacetic acid (2,4-D) (Lipthay et al. 2001). The gene for 2,4-dichlorophenoxyacetic acid/2-oksoglutaric dioxygenase was found on the plasmid. It was proven that the derived transconjugant *R. eutropha* (pRO103) considerably boosted 2,4-D decomposition in soil. *Pseudomonas putida*and *Pseudomonas* sp. CGMCC2953 having nahH gene cloned in pUC18 and C230 gene cloned in plasmid pK4 responsible for Phenanthrene and Pyrene degradation (Haritash 2020). *Mycobacterium* sp. strain 6PY1 having pdo gene undertaking the degradation of Pyrene (Sadhana et al. 2021).

Toxic substances in the soil not only impair soil fertility and the number of beneficial microbes, but they also hinder plant growth. As a result, genetically modified microbes can be utilised to both degrade hazardous substances and promote plant development at the same time (Cases and Lorenzo 2005; Pimentel et al. 2011). Yang and his colleagues created genetically modified bacteria that could stimulate maize plant development while also degrading phenol (Yang et al. 2011). The phenol-degrading *Pseudomonas aeruginosa* SZH16 (unable to boost plant development) and PGPB *Pseudomonas fluorescens* strains were utilised to create modified bacteria (without the ability to degrade phenol). They discovered horizontal gene transfer, which led to the emergence of recombinant strain P13. This strain was able to increase maize plant growth while also degrading phenol.

Barac et al. (2004) reported the transfer of pTOD, a toluene-degradation plasmid from *Burkholderia cepacia* G4 (donor) to *B. cepacia* L.S.2.4 (natural endophyte strain). The findings revealed that genetically modified bacteria had the capacity to degrade toluene and reduce transpiration through the leaves by 50–75% (Taghavi et al. 2005). In a similar study, Taghavi and Barac demonstrated the development of another strain, *B. cepacia* VM1468, for toluene degradation. This endophyte strain was created by conjugating *B. cepacia* BU0072 with the pTOM-Bu61 plasmid from *B. cepacia* BU61. The results revealed five-times lower toluene transpiration than that of control plants. Furthermore, the root and leaf mass were increased by 30%. According to these findings, the pTOMBu61 plasmid might spontaneously transfer to other natural endophytes in plants and accelerate toluene breakdown. Germaine and his colleagues created *Pseudomonas putida* VM1441, a naphthalene-degrading endophytic bacteria (pNAH7) (Germaine et al. 2009). In comparison to un-inoculated control plants, this strain inoculation in the soil enables a reduction of naphthalene's toxic effects on pea plants. The use of genetic engineering to remove heavy metals has attracted people's interest. *Alcaligenes eutrophus* AE104 (pEBZ141) was used to remove chromium from industrial wastewater (Srivastava et al. 2010), and *Rhodopseudomonas palustris* was engineered for Hg^{2+} removal from heavy metal wastewater (Srivastava et al. 2010; Xu and Pei 2011). Table 4 contains a list of genetically modified bacteria.

Unified methods of microbial remediation

The three basic categories of microbial integrated techniques for remediation are physical, chemical, and biological treatments, but due to hydrophobicity and fluidity of petroleum, none of these strategies is effective independently. Hence, integrated approaches are preferred as they are able to remediate hydrocarbon-contaminated land or water systems in a much better way and can boost microbial activity. Various research groups have conducted a number of studies for the breakdown of petroleum-contaminated soil using microbial integrated or combination techniques. To boost the system's degradation rate in petroleum-contaminated areas, several technologies such as chemical oxidation, electric fields, fertilizers, biosurfactants, biochar, bio carrier, bio-piles, biostimulants, earthworms, and plants were used (Mukome et al. 2020; Shahzad et al. 2020). The efficacy of microbial degradation of petroleum pollutants can be increased by combining these physical or chemical approaches with the use of specific microorganisms. The addition of several components to highly polluted petroleum-contaminated soil (10,000 mg/kg) such as biochar, electric field, nutrients, and biosurfactants can increase the clearance rate of petroleum pollutants by more than 60% (Tang et al. 2010a). With a 58% breakdown rate within 162 days (the original oil content was 6.19%), the combination of ryegrass and mixed microbial strains had the best degrading impact (Tang et al. 2010a). Within 60 days, a combination of alfalfa and microorganisms may degrade 63% of petroleum hydrocarbons (the starting oil concentration is 12%) (Shahzad et al. 2020). It has been also found that degradation of PAH from the contaminated sites can also be enhanced by ozone, hydrogen peroxide oxidation which later on helps the microorganisms in rapid degradation of less complex hydrocarbon compounds. These findings proved that the combined approaches are much better than the individual approaches for the treatment of hydrocarbon contaminated soil and water sources.

Challenges and future prospects of hydrocarbon bioremediation

Oil pollution is of great environmental and public concern worldwide due to its long-term effects on ecosystems and health. Despite the fact that bioremediation is a

Table 4 Genetically modified microorganisms known for degrading hydrocarbons

GMMs	Introduced gene	Substrate	References
Alcaligenes eutrophus AE104	pEBZ141	Chromium	Srivastava et al. (2010)
Rhodopseudomonas palustris	NA	Hg^{2+}	Xu and Pei (2011)
Pseudomonas fluorescens HK44	transposon Tn4431 insertion into NAH7 plasmid	Naphthalene	Sayler and Ripp (2000)
Mycobacterium sp. strain 6PY1	pdo	Pyrene	Sadhana et al. (2021)
Escheriachia coli	bphA2cA1c gene encoding for salicylate oxygenase from *S.yanoikuyae* B1	Naphthalene	Liu et al. (2019)
P. putida KT2442 (pNF142:TnMod-OTc)	pNF142 plasmid and gfp gene	Naphthalene	Filonov et al. (2005)
Pseudomonas putida	nahH gene cloned in pUC18 plasmid	Phenanthrene and Pyrene	Haritash (2020)
P. putida PaW85	plasmid pWW0	Petroleum	Jussila et al. (2007)
Pseudomonas sp. CGMCC2953	C230 gene cloned in plasmid pK4	Phenanthrene	Liu et al. (2019)
Ralstonia eutropha (pRO103)	pRO103 plasmid	2,4-D	Lipthay et al. (2001)
Recombinant strain P13	Combination of *Pseudomonas aeruginosa* SZH16 and PGPB *Pseudomonas fluorescens*	Phenol	Yang et al. (2011)
Bacillus cepacia L.S.2.4	pTOD plasmid	Toluene	Barac et al. (2004)
Pseudomonas sp.	alkB	Alkanes	Imperato et al. (2019)
B. cepacia VM1468	pTOM-Bu61	Toluene	Taghavi et al. (2005)
Sphingomonas sp. GY2B		Pyrene	Zhao et al. (2017)
Streptomyces coelicolor M145	alkB	n-hexadacane	Gallo et al. (2012)
Acinetobacter sp. BS3	Xyl E gene	Aromatic hydrocarbon	Xie et al. (2014)
Pseudomonas putida strain BNF1	NA	Aromatic hydrocarbon	
Pseudoalteromonas haloplanktis TAC125	NA	Toluene and xylene	Parrilli et al. (2010)

promising approach for cleaning up oil-contaminated sites, a variety of factors influence the degradation process, i.e., type of hydrocarbons, physico-chemical properties of contaminated sites, suitability of the technique used, type of microbes, and other intrinsic as well as extrinsic factors involved. Organic pollutant biodegradation metabolic routes are poorly understood, and inefficient transformation results in the accumulation of increasingly complex intermediate pollutants that resist further degradation by existing pathways (Jones and Voogt 1999; Sadhana et al. 2021). The selection of the inoculum is critical for the complete and effective bioremediation of organic pollutants. Isolation of indigenous microorganisms from polluted sites is resistant to toxic pollutants due to evolved catabolic genes, and they are able to degrade a wide range of pollutants (Korda et al. 1997; Liu et al. 2019). Slower bioremediation rates of microbial populations in contaminated sites are because of harsh environmental conditions where new engineering tools are of prime importance in research to boost the bioremediation process by improving catabolic pathways. Co-contamination with heavy metals is another constraint as heavy metals could adversely affect the microbial population by extending acclimatisation phases and reducing biodegradation rates (Arjoon et al. 2013). The evolution of more heavy metal resistant xenobiotic degrading microbes is underway to eliminate the effect of heavy metal toxicity on bioremediation.

A number of genetic engineering approaches have been introduced in the field of microbial bioremediation in recent years to evolve the desired genetic manipulation of native soil microorganisms and metabolic pathways (Imperato et al. 2019). Rayu et al. (2012) describe metagenomics as an advanced field that enhances the microbial bioremediation potential by obtaining novel pollutant-degrading genes and enzymes involved in the bioremediation process. Although a number of approaches have been developed in the field of microbial remediation of hydrocarbon bioremediation, much is yet to be explored. Better understanding of the metabolic pathways, complete degradation of organic pollutants, use of bio-based treating agents, and flexible legislation for the proper use of genetically engineered microbes can make the bioremediation process more sustainable and economic.

Conclusion

Biodegradation is a method for converting substances to less hazardous forms with minimal chemical and energy input in an environmentally friendly manner. Microorganisms (bacteria, fungus, and algae) can be used singly or in combination to repair oil-contaminated environments. Environmental circumstances, the quantity and kind of microorganisms present, and the chemical structure and nature of the molecule being degraded determine the rate of bioremediation of a contaminant. Exposure to greater levels of pollutants can lead to the acclimatisation of hydrocarbon-degrading microbial species and genetic alterations that can lead to improved degradation and the induction of degradation capacity. An integrated method of physical, chemical, and biological degradation should be applied to rehabilitate contaminated places in an environmentally sustainable manner.

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Declarations

Conflict of interest There is no conflict of interest between the authors.

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