Different strategies and bio-removal mechanisms of petroleum hydrocarbons from contaminated sites

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Abstract

Purpose – Petroleum hydrocarbons are naturally occurring flammable fossil fuels used as conventional energy sources. It has carcinogenic, mutagenic properties and is considered a hazardous pollutant. Soil contaminated with petroleum hydrocarbons adversely affects the properties of soil. This paper aim to remove pollutants from the environment is an urgent need of the hour to maintain the proper functioning of soil ecosystems.

Design/methodology/approach – The ability of micro-organisms to degrade petroleum hydrocarbons makes it possible to use these microorganisms to clean the environment from petroleum pollution. For preparing this review, research papers and review articles related to petroleum hydrocarbons degradation by micro-organisms were collected from journals and various search engines.

Findings – Various physical and chemical methods are used for remediation of petroleum hydrocarbons contaminants. However, these methods have several disadvantages. This paper will discuss a novel understanding of petroleum hydrocarbons degradation and how micro-organisms help in petroleum-contaminated soil restoration. Bioremediation is recognized as the most environment-friendly technique for remediation. The research studies demonstrated that bacterial consortium have high biodegradation rate of petroleum hydrocarbons ranging from 83% to 89%.

Social implications – Proper management of petroleum hydrocarbons pollutants from the environment is necessary because of their toxicity effects on human and environmental health.

Originality/value – This paper discussed novel mechanisms adopted by bacteria for biodegradation of petroleum hydrocarbons, aerobic and anaerobic biodegradation pathways, genes and enzymes involved in petroleum hydrocarbons biodegradation.

Keywords Bioremediation, Consortium, Contaminated soil, Oil spills, Hazardous pollutant

Paper type Literature review

Introduction

Soil is the uppermost surface of the earth's crust, where various biogeochemical processes aid in its development and support key ecosystem functions. It is a dynamic living ecosystem that forms a habitat for millions of living organisms, including plants, animals and microorganisms. Soil acts as a reservoir for organic and inorganic nutrients required for the growth and development of organisms and provides essential needs like food, fuel and fibers. Microorganisms found in soil plays an important role in carrying out various biochemical processes such as nitrogen fixation, phosphate solubilization, mineralization, nitrification,

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denitrification and ammonification and leads to help in plant growth. Soil functioning gets altered due to addition of harmful chemical compounds and that affects the existing living organisms and consequently decreases plant productivity. The rapid development in industrialization and urbanization has brought not only social and economic growth but also increased environmental pollution due to various anthropogenic activities.

Petroleum or crude oil is a naturally occurring flammable fossil fuel found beneath the earth's crust. It consists of mixtures of alkanes, cycloalkanes, aromatic hydrocarbons, asphaltenes and resins (Chandra, Sharma, Singh, & Sharma, 2013). Petroleum hydrocarbons are an indispensable energy resource on which a country's economic and social development depends. The daily life of human beings is dependent on petroleum hydrocarbons. Petroleum hydrocarbons are used for fuels, petrochemicals, polymers and precursors to chemical synthesis and to meet other daily needs (Adipah, 2019). Excessive use of petroleum hydrocarbons leads to soil, air and water pollution and causes environmental toxicity, ultimately leading to a serious threat to human health and other animals (McFarlin and Prince, 2021). It has various degrees of toxicity such as mutagenic, hemotoxic, genotoxic, carcinogenic, cardiotoxic, hepatotoxic, nephrotoxic, immunotoxic, neurotoxic and cytotoxic. They can damage our vital organ systems, such as nervous, circulatory, immune and endocrine systems and cause metabolic and hormonal disorders (Ossai, Ahmed, Hassan, & Hamid, 2020).

The releasing of petroleum hydrocarbons into environment through various activities such as municipal wastes, sewage release, dumping off oil and fuel, offshore and onshore petroleum industrial activities including drilling, exploration, storage, transportation, processing, refining, unplugging of oil wells as well as accidental oil spills, cause petroleum hydrocarbons pollution and contaminate soil, groundwater as well as ocean in large scale. During crude oil processing, oily sludge is generated, which is of special environmental concern because many of the constituents of this sludge are of hazardous nature. In Gulf country (Kuwait) more than 60 million gallons of crude oil were released by the destruction of oil wells by Iraqi forces in 1991 (Al-Gounaim, Diab, Al-Hilali, & Abu-Shady, 2005). According to International Tanker Owners Pollution Federation (ITOPF) report, approximately 10,000 tons of oil spilled into the marine environment from tanker spills in the year 2021 (ITOPF, 2022). Ambaye *et al.* (2022) reported that 1.7–8.8 million metric tons of petroleum hydrocarbons spill into the environment every year. Human activities are responsible for about 90% of the spillages.

Contamination of soil with oil spills is a major global concern nowadays. The toxicity of petroleum hydrocarbons is well established, affecting our ecosystem, agricultural practice and human health. Soil contaminated with crude oil has a serious hazard and causes organic pollution of ground water which limits the agricultural productivity of the soil. The persistent nature of petroleum hydrocarbons negatively impacts on soil and water ecosystems. It alters soil biological properties by affecting microbial diversity and enzymatic activities (Wang, Liang, Wang, & Gao, 2018). They also have phytotoxic impact on seed germination and plant growth due to inhibiting uptake of minerals and water, primarily reducing the root length and leaf area of plants (Haider *et al.*, 2021). It adversely affects the physicochemical characteristics such as pH, moisture, soil texture nutrient contents (Czarny *et al.*, 2020; Sui, Wang, Li, & Ji, 2021). Hence the removal of petroleum hydrocarbons pollutants from the environment is an urgent need of hour to maintain the proper functioning of soil ecosystems. This paper deals with the various physical, chemical and biological approaches for remediation of petroleum hydrocarbons contaminated soil. This paper briefly explains the mechanisms adopted by bacteria for biodegradation of petroleum hydrocarbons, aerobic and anaerobic biodegradation pathways, genes and enzymes involved in petroleum hydrocarbons biodegradation.

Different strategies for petroleum hydrocarbons degradation

Physical method

Flotation. Flotation is used to separate petroleum from contaminated soil via a gas-liquid-solid system. This process can separate very low-weight particles with low settling velocities by forming bubbles. The gas bubbles are attached to the hydrophobic pollutants and form bubble-oil pollutants. Gas bubbles-oil contaminants are aggregated on the surface where they are skimmed off (Tao, 2005). Lim, Lau, and Poh (2018), studied the interaction of micro and macro bubbles in floatation techniques for recovering oil from contaminated sandy soil in laboratory conditions. The oil recovery efficacy rate was approximately 70% from sandy soil. In this technique a large amount of wastewater is produced and the efficiency rate of contaminated soil is significantly low (Wang, Yin, Ge, & Zheng, 2010).

Incineration. Incineration is the burning of petroleum contaminants at high temperatures up to 1000°C. The oil contaminants and other organic pollutants are burned and decomposed into nontoxic molecules. This process can reduce up to 95% of petroleum sludge (Hao, Chen, van Loosdrecht, Li, & Jiang, 2020). Harmful gas and residual waste produced during incineration leads to air pollution by raising the carbon dioxide, nitrogen oxide and sulfur oxide levels in the atmosphere (Ali, Dashti, Khanafer, Al-Awadhi, & Radwan, 2020). Establishing a high-temperature-resistant incinerator is very expensive, increasing the overall remediation cost.

Ultrasonication. Ultrasonication involves using ultrasonic waves to separate solid/liquid from highly concentrated suspension. This technique leads to desorption of hydrocarbon contaminants from the soil and water (Hu, Li, Thring, & Arocena, 2014). Wulandari and Effendi (2018) demonstrated that stainless steel tube reactor is used for ultrasonic remediation of petroleum-contaminated soil using 28 kHz and 48 kHz ultrasound frequencies. The results showed 67% total petroleum hydrocarbon (TPH) removal at an optimum frequency of 48 kHz. The disadvantage of this process is high energy requirement and cost for setting up this process, which is only useful in laboratory scale.

Electrokinetics remediation. Electrokinetic technique separates organic pollutants and heavy metals from contaminated soil. In this technique, low-intensity direct current is used for treatment with low hydraulic permeability (Cameselle & Gouveia, 2018). The remediation of contaminated soil through electrokinetics uses various mechanisms such as electrolysis, electrophoresis, electromigration and electro-osmosis (Hassan, 2016). When an electric current is applied through the electrode pairs fixed in an impregnated soil grid, the charged ions, soil particles and water molecules move toward a particular direction. Electrokinetic remediation is an *in situ* remediation technique. However, it is not suitable for large-scale remediation of petroleum hydrocarbons contaminated soil.

Thermal desorption. In the thermal desorption method, a high temperature is used to increase the vapor pressure of the organic pollutants and subsequently pollutant is removed from contaminated soil (Kastanek *et al.*, 2016). The volatile contaminants are passed through sweep gas to carry away the contaminants for further treatment. This method is appropriate for inflammable and semiinflammable pollutants such as polycyclic aromatic hydrocarbons (PAHs) and TPH (Liu, Zhang, Yao, Li, & Tang, 2019). In the presence of oxygen, petroleum hydrocarbon degradation efficiency reached up to 99% at 100°C to 300°C, but the treatment time may vary due to the nature of the contaminants (Zhao, Dong, Feng, Li, & Dong, 2019). The major drawback of this method is the use of high energy for increasing high temperature, which is expensive and not useful in large-scale contaminated fields.

Microwave frequency heating. This technique converts microwave energy into thermal energy to remove oil contaminants via heating and volatilization (Rushton, Ghaly, & Martinell, 2007). In this method, high frequency of infrared or radio waves (300 MHz (Megahertz) to 3000 GHz (Gigahertz)) is used to generate high thermal energy for the remediation of petroleum hydrocarbon-contaminated soil (Aguilar-Reynosa *et al.*, 2017). Sivagami, Padmanabhan, Joy, and Nambi (2019) demonstrated that microwave heating using spent graphite in treatment of petroleum hydrocarbon-contaminated soil at frequency of 2.45 GHz removed 91% TPH from the soil. This method is limited to laboratory conditions for small-scale remediation and is not suitable in the field or at large scale. Few amounts of residual waste are also persistent in the environment.

Chemical method

Chemical oxidation. This method uses different chemical oxidants such as hydrogen peroxide (H_2O_2), Fenton's reagent, persulfate, peroxymonosulfate, permanganate and ozone for the treatment of oil from contaminated soil (Usman, Faure, Hanna, Abdelmoula, & Ruby, 2012). Petroleum pollutants are rapidly degraded into nonhazardous or less toxic compounds. These compounds are more stable, less mobile or inert (Lim, Von Lau, & Poh, 2016). In this method, oxidants used for remediation are expensive and some are persistent in the environment after remediation leading to toxicity effects.

Soil washing/soil flushing method. In this method, the petroleum hydrocarbons are separated from soil particles using washing solvents. The efficacy of separation depends on the degree of association between soil and solvent. Organic or nonaqueous solvents are used to remove oil pollutants by extraction and concentration process. Different types of organic solvents ethyl acetate, acetone, hexane, ether, rhamnolipid and supercritical acetone (CO_2 in presence of acetone) have been used to remove petroleum oil from contaminated soil (Tran, Lin, Hoang, Bui, & Vu, 2022). Complete removal of petroleum hydrocarbons from contaminated soil is quite difficult. In addition to this, large amount of costly solvents are used. After treatment, these solvents enter nearby water bodies and cause water pollution.

Synthetic surfactants or detergents. Synthetic surfactants are amphiphilic molecules composed of hydrophilic head and hydrophobic tail, used to remove oil from contaminated soil. When surfactant is added to an aqueous medium, the surface tension of the oil-contaminates mixture is reduced, micelles are formed and then oil-contaminates are separated (Rocha e Silva *et al.*, 2019; Liu *et al.*, 2021). The commercial surfactants used for the removal of oil from petroleum-contaminated soil are alkylbenzene sulfonate, sodium dodecyl sulfate, Triton X-100, polysorbate 80 (Tween 80) and Tween 20. The surfactants or detergents used in this method are expensive and cause water pollution.

Photocatalytic degradation. Photocatalysis is an advanced oxidation process in which ultraviolet radiation (UV), ozone (O_3), hydrogen peroxide (H_2O_2) and other metal oxides such as titanium oxide and zinc oxide act as a catalyst and are used to degrade petroleum hydrocarbons pollutants (Koe, Lee, Chong, Pang, & Sim, 2020). The UV light reacts with H_2O_2 and produces more oxidizable hydroxyl radicals ($OH^{\bullet}$), which react with the contaminant soils and degrade contaminated compounds into simple compounds (Ossai *et al.*, 2020). This method is limited to small scale, and it is difficult to remove petroleum contaminants on large scale.

Bioremediation

Bioremediation refers to the use of living organisms for the removal of pollutants, or unwanted substances from soil or water. It is the process by which living organisms degrade complex organic pollutants or transform hazardous organic contaminates to inorganic components such as CO_2, H_2O, NO^{3-} (Fernndez-Luqueno *et al.*, 2011). There are various processes used for the removal of organic contaminants.

Biostimulation. Biostimulation is the addition of stimulatory ingredients such as nutrients, bio-surfactants, vitamins, minerals and cofactors to enhance and support the microbial growth already present in the polluted soil for bioremediation processes (Adams, Fufeyin, Okoro, & Ehinomen, 2015; Lim *et al.*, 2016). The limiting factors such as nitrogen, phosphorus, potassium and oxygen are added to media, which are available in the contaminated soil at low concentrations to increase microbial growth. It is very successful and effective bioremediation technique compared to other techniques in soil contaminated with petroleum hydrocarbons (Simpanen *et al.*, 2016).

Bioaugmentation. This method involves the addition of exogenous microbial culture, microbial consortium; genetically engineered microbes and biodegradation-relevant genes packaged in vectors to be transferred into indigenous microorganisms. These microbes have specific catabolic activity, which has proved and recognized to degrade petroleum contaminants or enhance the rate of degradation (Poi, Aburto-Medina, Mok, Ball, & Shahsavari, 2017).

Phytoremediation. In phytoremediation, plants remediate contaminants from the soil and water. Plants can breakdown or bioaccumulate the contaminants through various processes such as phytoextraction, phytoaccumulation, phytodegradation, phytostabilisation, phytotransformation and phytovolatilsation. In these processes, the pollutants are transformed into harmless or less toxic materials in the soil or stabilized in the plant cells (Cristaldi *et al.*, 2017 ;Hussain *et al.*, 2018).

Removal of petroleum hydrocarbons from contaminated site

Removal of pollutants from the contaminated sites is a great challenge among researchers for sustainable environmental management. Various physical and chemical methods are used for the remediation of petroleum hydrocarbons. These methods have significant drawbacks such as cost expensive; limited efficiency; high energy consumption; production of secondary pollutants such as dioxins, polychlorinated biphenyls, heavy metals and less eco-friendly (Wang *et al.*, 2018; Sui *et al.*, 2021). Due to the drawback of these methods, it encourages the development of alternative methods, i.e. bioremediation techniques for the cleanup of pollutants. Bioremediation technology is focused on low energy consumption, lower operating costs and eco-friendly potentiality of plants and microorganisms.

Principles of microbial bioremediation of petroleum hydrocarbons. Bioremediation is a technique in which microorganisms mitigate, degrade or reduce the toxicity of complex hazardous organic contaminants to simple harmless compounds without adversely affecting the environment. Biodegradation is the principal mechanism in which hydrocarbonoclastic microbes are used for the degradation of hydrocarbon pollutants, as hydrocarbons are used as natural source of energy and carbon. Indigenous microorganisms or consortium can mitigate petroleum hydrocarbon due to their catalytic activities, which enhance the rate of pollutant degradation (Varjani, 2017).

Mechanism of bioremediation. Microbial bioremediation is a widely used technique for degrading petroleum hydrocarbons in terrestrial and aquatic ecosystems (Varjani & Upasani, 2012; Abbasian, Lockington, Mallavarapu, & Naidu, 2015). Numerous studies have been done on the biodegradation of petroleum hydrocarbons pollutants (Sajna, Sukumaran, Gottumukkala, & Pandey, 2015; Cai *et al.*, 2021; Rodriguez-Uribe, Pena-Cabriales, del Carmen Rivera-Cruz, & Delano-Frier, 2021). Some bacteria can degrade aliphatic, some are aromatic, and some are degrading resins (Rahman *et al.*, 2003). Different petroleum hydrocarbons degrading bacteria and the type of petroleum hydrocarbons constituents they degrade are shown in Table 1. Microorganisms catabolize petroleum hydrocarbons to obtain energy or assimilate them into the cell biomass. Microorganisms catabolize petroleum hydrocarbons through aerobic or anaerobic processes (Abbasian *et al.*, 2015; Meckenstock *et al.*, 2016). Chemical reactions such as oxidation, reduction, hydroxylation and dehydration are frequent in the metabolic pathway of petroleum hydrocarbon degradation. Petroleum hydrocarbons are hydrophobic in nature and microorganisms release different biosurfactants which adhere to the hydrophobic pollutants and enhance the dissolution or emulsification of petroleum hydrocarbons (Varjani & Upasani, 2017). At the initial stage bacteria intake petroleum

Table 1. Petroleum hydrocarbon degrading Bacteria

Petroleum hydrocarbon components	Bacterial species	References
Aliphatics	*Acinetobacter* sp.	Foght (2008)
	Alcanivorax sp.	Brooijmans, Pastink, and Siezen (2009)
	Azoarcus sp.	Widdel and Rabus (2001)
	Bacillus sp.	Das and Mukherjee (2007)
	Dietzia sp.	Wang *et al.* (2011)
	Desulfosarcina sp. and *Desulfococcus* sp.	Jaekel *et al.* (2013)
	Geobacillus thermodenitrifican	Abbasian *et al.* (2015)
	Gordonia sihwensis	Brown, Gunasekera, Striebich, and Ruiz (2016)
	Marinobacter sp.	Yakimov, Timmis, and Golyshin (2007)
	Micrococcus sp.	Roy, Hens, Biswas, Biswas, and Kumar (2002)
	Ochrobactrum sp.	Varjani *et al.* (2015)
	Oleispira sp.	Brooijmans *et al.* (2009)
	Oleispira antarctica	Yakimov *et al.* (2007)
	Pseudomonas sp.	Sajna *et al.* (2015), Varjani *et al.* (2015)
	Rhodococcus sp.	Abbasian *et al.* (2015)
	Rhodococcus ruber	Zhukov, Murygina, and Kalyuzhnyi (2007)
	Stenotrophomonas sp.	Varjani *et al.* (2015)
Aromatics	*Achromobacter insolitus*	Janbandhu and Fulekar (2011)
	Achromobacter xylosoxidans	Li, Tian, Hao, and Ma (2020)
	Acinetobacter sp.	Batista, Mounteer, Amorim, and Totola (2006)
	Aeribacillus pallidus	Mnif, Sayadi, and Chamkha (2014)
	Archaeoglobus fulgidus and *Aromatoleum aromaticum*	Wilkes *et al.* (2016)
	Bacillus sp.	Janbandhu and Fulekar (2011)
	Bacillus licheniformis and *Bacillus mojavensis*	Eskandari *et al.* (2017)
	Cycloclasticus sp.	Kasai, Kishira, and Harayama (2002)
	Halomonas sp.	Widdel and Rabus (2001)
	Mycobacterium cosmeticum	Zhang, Zhang, Cheng, Yao, and Chen (2013)
	Neptunomonas naphthovoran	Hedlund, Geiselbrecht, Bair, and Staley (1999)
	Novosphingobium sp.	Ghosal, Ghosh, Dutta, and Ahn (2016)
	Phanaerochaete chrysporium	Salleh *et al.* (2003)
	Pseudomonas aeruginosa	Das and Mukherjee (2007)
	Pseudomonas sp.	Widdel and Rabus (2001)
	Rhodococcus sp.	Salleh *et al.* (2003)
	Sphingomonas sp. and *Sphingobium* sp.	Ghosal *et al.* (2016)
Resins	*Moraxella sp.*	Chandra *et al.* (2013)
	Pseudomonas aeruginosa	Ma *et al.* (2020)
Asphaltenes	*Bacillus sp.* and *Citrobacter sp.* and *Enterobacter sp.*	Jahromi, Fazaelipoor, Ayatollahi, and Niazi (2014)
	Staphylococcus sp.	Jahromi *et al.* (2014)

Source(s): Authors work

hydrocarbons which undergo an oxidative process, forming various intermediate compounds. These intermediate compounds undergo central intermediate metabolic pathways and are transformed into simple compounds or accumulated as cell biomass.

Aerobic biodegradation pathways. Petroleum hydrocarbon degradation by aerobic microbes goes after several steps of oxidation such as terminal, subterminal, ω-oxidation and *β*-oxidation (Abbasian *et al.*, 2015). The schematic pathway of aerobic petroleum hydrocarbon degradation is shown in Figure 1. Alkanes having short chain lengths of about C_{10}-C_{24} are easily degraded by bacteria. The pathways for biodegradation of n-alkanes are initiated by the oxidation of the terminal methyl group which consequences the incorporation of alcohol. The alcohol is first dehydrogenated to aldehyde and next to carboxylic acid. Carboxylic acid is further metabolized by *β*-oxidation pathway to form fatty acids. The consequences of *β*-oxidation leads to the removal of acetyl coenzyme-A by which fatty acid is degraded to two carbon compounds. In di-terminal pathway, oxidation of alkane occurs through ω-hydroxylation at both the terminal ends of alkane and transformed into di-carboxylic acid. In subterminal oxidation alkanes are oxidized to form secondary alcohol and then to corresponding ketone and ester, then ester is hydrolyzed to generate an alcohol and fatty acid (Rojo, 2009). Salleh, Ghazali, Rahman, and Basri (2003), considered aromatic hydrocarbons as priority pollutants due to their less biodegradable properties and the presence of benzene ring. Microbial degradation of aromatic hydrocarbons involves the cleavage of benzene ring, then the formation of a diol and di-carboxylic acid (Zhang *et al.*, 2011). The cleavage of benzene ring achieved by microbial enzymes through ortho or meta-cleavage pathways which form unstable central intermediates which are further transformed into tricarboxylic acid (Li & Liu, 2002).

Enzyme responsible for aerobic petroleum hydrocarbons degradation. Bacteria release some specific oxygenase and dehydrogenase enzymes such as phthalate dioxygenase, naphthalene dioxygenases, catechol dioxygenase and biphenyl dioxygenase that have ability to degrade petroleum hydrocarbons and transform them into CO_2 and H_2O (Haritash, 2020). Enzyme alkane 1-monooxygenase, alcohol dehydrogenase, cyclohexanol dehydrogenase, methane monooxygenase and cyclohexanone 1,2 monooxygenase are employed in degradation of alkanes. Naphthalene 1,2-dioxygenase ferredoxin reductase, cis-2,3-dihydrobipheny l-2,3-diol

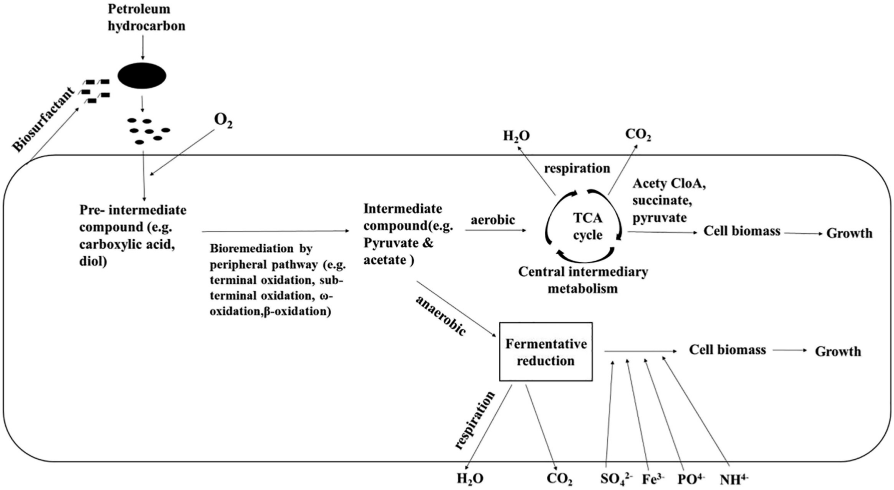

Figure 1.
Schematic pathway of aerobic and anaerobic petroleum hydrocarbon degradation of microorganisms

Source(s): Authors work

dehydrogenase and salicylaldehyde dehydrogenase are accountable for naphthalene degradation (Bacosa *et al.*, 2018). Enzyme secreted by genetically modified *Pseudomonas putida* F1 has the ability to degrade benzene, toluene and phenol (Abuhamed, Bayraktar, Mehmetoglu, & Mehmetoglu, 2004). Ortho and meta-cleavage of benzene is catalyzed by an enzyme intradiol and extradiol dioxygenases (Li & Liu, 2002).

Gene responsible for aerobic petroleum hydrocarbons degradation. Microbial plasmids encode enzymes related to biodegradation. Plasmids such as Q15 (*Rhodococcus* sp. Strain Q15), OCT (Octane degrading plasmid, *Pseudomonas* sp.), TOL (Toluene degrading plasmid, *Pseudomonas* sp.), NAH7 (Naphthalene-catabolic plasmid, *Pseudomonas* sp.), pND140 (Naphthalene-catabolic plasmid, *Pseudomonas* sp.) and pND160 (Naphthalene-catabolic plasmid, *Pseudomonas* sp.) with the existence of genes *alkA* (alkane degrading gene), *alkB* (alkane degrading gene), *alkM* (alkane degrading gene), *thcA* (aldehyde dehydrogenase gene), *ladA* (long-chain alkane monooxygenase encoding gene), *assA1*(alkyl succinate synthase (ASS) encoding gene), *assA2* (ASS encoding gene) and *nahA-M* (naphthalene degrading gene) are engaged in PAHs degradation (Abbasian *et al.*, 2015). Wang *et al.* (2011) reported that *Dietzia* sp. DQ12-45-1b has gene *alkB* (alkane degrading gene) and *CYP153* (cytochrome CYP153 gene), which encodes enzymes alkane mono-oxygenase and cytochrome P450 alkane hydroxylase. They suggested that these genes are responsible for the degradation of small and medium-length n-alkanes ranging upto C40. The strains of *Geobacillus toebii B-1024*, *Geobacillus sp. 1017 and Aeribacillus pallidus 8m3* consinting gene *alkB* and *ladA*, which encodes enzymes rubredoxin-dependent alkanemonooxygenase and flavin-dependent alkanemonooxygenase and are capable for degrade C10–C30 n-alkanes (Nzila, 2018). The alkane hydroxylase gene (*alkJ*) found in *G. thermoleovorans*, *G. stearothermophilus*, *G. anatolicus* and *Bacillus aeolius* can degrade n-alkanes (Meintanis, Chalkou, Kormas, & Karagouni, 2006). Zhang, Zhao, Liang, Li, and Zhou (2013) detected 7,714 microbial functional genes responsible for PAHs degradation from PAH-contaminated sites. These genes belongs to 6 types genes, including *bph* (2,3-dihydroxybiphenyl 1,2-dioxygenase encoding gene), *nah* (naphthalene 1,2-dioxygenase encoding gene), *nidA* (PAH dioxygenase encoding gene), *phd* (hydratase–aldolase encoding gene), *dfb* (angular dioxygenase encoding gene) and *qor* (putative quinoline 2-oxidoreductas encoding gene). Their results showed that the gene *nidA* was dominant among all other genes and encodes enzyme dioxygenase which acts as a promoter to initiate degradation of aromatic hydrocarbons (Pagnout *et al.*, 2007). Rest of the genes *bph, nah, phd, dfb and qor* are encoded for the synthesis of enzyme 2,3-dihydroxybiphenyl 1,2-dioxygenase, naphthalene 1,2-dioxygenase, hydratase–aldolase, angular dioxygenase and putative quinoline 2-oxidoreductas respectively which play a significant role in petroleum hydrocarbons degradation (Taguchi, Motoyama, & Kudo, 2004; Pohlmann *et al.*, 2006). The gene cluster *pah* (polycyclic hydrocarbon degrading gene), *dox* (dibenzothiophene degrading gene), *phn* (phenanthrene and naphthalene degrading gene), *nag* (naphthalene through gentisate), *fln* (Fluorene degrading gene) are also involved in hydrocarbon degradation (Haritash, 2020).

Anaerobic biodegradation pathways. The anaerobic microorganisms such as nitrate-reducing, sulfate-reducing and methanogenic bacteria can degrade n-alkane (Callaghan, Tierney, Phelps, & Young, 2009; Jaekel *et al.*, 2013). General pathways for anaerobic petroleum hydrocarbon degradation is shown in Figure 1. In the initial steps n-alkanes are activated by carbon-carbon addition of an alkyl radical to fumarate, which results the formation of 1-methylalkyl-succinates. 1-methylalkyl-succinate is employed by nitrate and sulfate-reducing bacteria and then converted into fatty acid 4-methylalkanoyl-CoA. This fatty acid is formed via the activation of 1-methylalkyl-succinate to a CoA ester. The 4-methylalkanoyl-CoA is further degraded by β-oxidation (Callaghan *et al.*, 2009; Wilkes, Buckel, Golding, & Rabus, 2016). These compounds are finally undergoing fermentative reduction (Abbasian *et al.*, 2015).

Aromatic compounds such as benzene, toluene, ethyl benzene and xylene are first oxidized and then transformed into long-chain fatty acids, which are finally metabolized to CH_4 and

CO_2 under anaerobic pathways (Wilkes *et al.*, 2016). The benzene degradation pathway is activated by hydroxylation, alkylation or carboxylation and formed phenol, toluene and benzoic acid respectively. Phenol, toluene followed by fumarate addition and benzoic acid is then converted to benzoyl-CoA as a common intermediate in the anaerobic catabolism of aromatic compounds. Alkyl benzene, ethyl benzene and toluene are also oxidized to benzoyl-CoA. Benzoyl-CoA is eventually oxidized to acetyl-CoA and CO_2 (Foght, 2008; Atashgahi *et al.*, 2018).

Enzyme responsible for anaerobic petroleum hydrocarbons degradation. Various enzymes released by bacteria are involved in anaerobic degradation of petroleum hydrocarbons. In aliphatic hydrocarbon degradation, the activation of n-alkane by adding fumarate is catalyzed by glycyl radical enzyme family. The genomic studies of *Aromatoleum* sp. strain HxN1 revealed that the addition of carbon-carbon atom of (C3–C16) n -alkanes to fumarate is catalyzed by a glycyl radical enzyme, i.e. 1-methylalkyl succinate synthase (MAS) or ASS (Wilkes *et al.*, 2016). In aromatic hydrocarbons, the formation of phenol, toluene and benzoic acid from benzene is catalyzed by the enzyme benzene hydroxylase, benzene methylase and benzene carboxylase. The formation of benzyl succinate from toluene and fumarate is catalyzed by benzyl succinate synthase (BSS) (Foght, 2008). Various nitrate and sulfate reducing bacteria transformed toluene into (R)-benzylsuccinate via fumarate addition (Abdel-Shafy & Mansour, 2018). Then, (R)-benzylsuccinate is substituted by succinyl-CoA using enzyme CoA transferase and formed benzyl-CoA as an intermediate. The benzyl-CoA is further breakdown into aliphatic compounds and is oxidizes by enzyme Benzyl-CoA reductase. These aliphatic compounds are then converted into CO_2 and CH_4 (Hazaimeh & Ahmed, 2021).

Gene responsible for anaerobic petroleum hydrocarbons degradation. The nitrate-reducing, sulfate-reducing and methanogenic bacterial strains contain genes in their chromosome and plasmids for petroleum hydrocarbons degradation in anaerobic conditions. Grundmann *et al.* (2008) reported that n-alkene degradation by the enzyme MAS is encoded by MAS-encoding genes (*mas*) present in *Aromatoleum* sp. strain HxN1. Sulfate-reducing bacterium *Desulfatibacillum alkenivorans* strain AK-01 has n-alkane metabolic gene *assA1* (ASS) and *assA2* gene, which encoded for catalytic subunits of glycyl radical-type enzymes. The gene *bssA* encoded the α-subunit of the enzyme BSS, which helps in the addition of fumarate to methyl group of toluene in all anaerobic toluene degrading pathways (Winderl, Schaefer, & Lueders, 2007). The gene *bssA* is also involved in fumarate addition to benzene and ethylbenzene. The gene *abcA* (anaerobic benzene carboxylase) encoded benzene carboxylase enzyme which involved carboxylation of benzene to benzoic acid (Bouchard *et al.*, 2018). Genomic analysis revealed that dissimilatory sulfate reductase gene *dsrA and dsrB* abundant in sulfate reducing bacteria involved in dissimilatory sulfite reduction and mineralized the organic contaminants under anaerobic condition (Roy *et al.*, 2018).

Factors affecting bioremediation

Several environmental factors, including biotic and abiotic, affect various biological processes occurring in a microbial community and thereby influence the growth of microbial cells.

Abiotic factors

Temperature. Temperature influences petroleum hydrocarbon degradation by changing the physical and chemical properties of petroleum hydrocarbons. Bisht *et al.* (2015) observed that biodegradation rate decreases at low temperatures. When the temperature in biodegradation process increases, the rate of hydrocarbon metabolism is also increasing, and it reaches maximum level at temperature ranges from 30°C to 40°C (Srivastava, Naraian, Kalra, & Chandra, 2014).

pH. The pH affects the degradation process by changing the cell membrane transport and catalytic reaction. Thavasi, Jayalakshmi, Balasubramanian, and Banat (2007) observed that the maximum biodegradation of petroleum crude oil in water was at pH 8.0 by bacterial strain *Pseudomonas aeruginosa*. The optimum pH range for the maximum degradation of petroleum hydrocarbons is 6.5-7.5 observed by Al-Hawash *et al.* (2018). Pawar (2015) observed that the degradation of petroleum hydrocarbons was most convenient at pH 7.5 in soil ecosystem.

Salinity. Qin, Tang, Li, and Zhang (2012) reported that soil salinity also influences the biodegradation process by affecting the growth and diversity of microbes. It adversely influences the activity of enzymes in the process of hydrocarbon degradation (Ebadi, Sima, Olamaee, Hashemi, & Nasrabadi, 2017). Hydrocarbon metabolism rates were greatly reduced with the increase in salinity. The optimum salinity range for significant petroleum hydrocarbon degradation is 0.1-2M NaCl concentration (Kebede, Tafese, Abda, Kamaraj, & Assefa, 2021).

Nutrients. Nutrients such as nitrogen, phosphorus, potassium and iron are essential for the successful degradation of petroleum hydrocarbons (Al-Hawash *et al.*, 2018; Kebede *et al.*, 2021). Zafra, Absalon, and Cortes-Espinosa (2015) reported that the high concentration of petroleum hydrocarbons limited the growth of microorganisms. In aquatic ecosystems, the released petroleum oil increases level of carbon and decreases nitrogen and phosphorus levels, reducing biodegradation ability. Thus, the addition of nutrients requires enhancing the biodegradation of petroleum waste (Hesnawi & Adbeib, 2013). The required amount of nutrient addition increases microbial growth, which also leads to promoting the biodegradation process.

Oxygen. The concentration of oxygen determined the degradation of petroleum hydrocarbons in aerobic conditions. The biodegradation of petroleum hydrocarbons in anaerobic conditions is not as fast as in aerobic conditions (Grishchenkov *et al.*, 2000). The catabolism of all aliphatic, cyclic and aromatic compounds by microorganisms in the presence of enzyme oxygenases is considered a key step in biodegradation. The availability of a significant amount of oxygen in the soil increases the rate of petroleum hydrocarbon biodegradation by up to 10-40% (Kebede *et al.*, 2021).

Biotic factors

Biotic factors include microbial communities such as bacteria, fungi and algae. The degradation ability of microorganisms makes them suitable for cleaning the environment from petroleum pollution. Indigenous bacteria have capability to degrade petroleum hydrocarbons because they utilize petroleum hydrocarbons for energy and carbon sources. Single microbial species take more time or cannot degrade the entire components of petroleum hydrocarbons, whereas complete degradation of petroleum hydrocarbons is achieved by microbial consortium. Interactions between the microbial communities can exchange genetic information between species conferring resistance, tolerance and faster rate of degradation ability (Srivastava *et al.*, 2014). Researchers evaluated that the microbial consortium has higher efficacy than the pure culture for bioremediation. Sathishkumar, Binupriya, Baik, and Yun (2008), demonstrated that the crude oil degradation rate by the consortium of four bacterial strains was 77%, which was higher than that of individual strains, *Pseudomonas* sp. BPS1-8 (69%), *Bacillus* sp. IOS1-7 (64%), *Pseudomonas* sp. HPS2-5 (45%), and *Corynebacterium* sp. BPS2-6 (41%). Varjani, Rana, Jain, Bateja, and Upasani (2015), reported that bacterial consortium consisting of *Ochrobactum* sp., *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* have good degrading capacity of crude oil (3% v/v) with degrading percentage as 83.49% and 86.33% by three *Pseudomonas sp.* as compare to pure individual. In the field study of diesel oil-contaminated soil, Szulc *et al.* (2014), reported that bioaugmentation with an artificial bacterial consortium consisting of *Aeromonas* *hydrophilia, Alcaligenes, Pseudomonas fluorescens, P. putida, Xylosoxidans, Gordonia* sp., *Rhodococcus equi, S. maltophilia* and *Xanthomonas* sp. have shown high degradation efficacy (89%) in 365 days.

Future prospective

Microbial remediation of petroleum hydrocarbons is considered an eco-friendly and efficient technique. But still, this technique has several problems, which have been discussed in this article that naturally occurring microbial remediation of petroleum hydrocarbons is not faster process. Hence, scientists may develop effective genetically modified bacteria to enhance the rapid degradation rate. The altered environmental conditions may be the main thrust of future studies of the bioremediation process. Identification and development of novel microbes for various organic compound resistances are required for faster and complete degradation of organic pollutants. Researchers are also required to investigate the new-fashioned functional genes that control the metabolic pathway of petroleum hydrocarbon degradation to provide new looks on microbial remediation process. For easy uptake of petroleum hydrocarbons by bacteria, required to develop novel biosurfactants which enhance the attachment between bacteria and petroleum hydrocarbons.

Conclusions

Petroleum hydrocarbons are crucial environmental pollutants due to their excessive toxicity to human and environmental health. Removal of petroleum hydrocarbons from the environment is a global problem. Several traditional physico-chemical methods have been applied for the removal of petroleum hydrocarbons but are less preferred due to their high operating cost and non–eco-friendly properties. Bioremediation is one of the most popular and advanced techniques preferred by scientists and researchers due to its low operating cost, eco-friendly as well as efficient techniques. Microbial bioremediation, especially with bacteria is widely used due to its high efficacy in petroleum hydrocarbon degradation. The degradation rate of petroleum hydrocarbons varies between 41% to 69% by bacterial strains *Corynebacterium sp., Bacillus sp.* and *Pseudomonas sp.* Bacterial consortium with petroleum hydrocarbons degrading capability have been applied for faster degradation rate and no secondary pollutant is released by bacteria. The research studies demonstrated that bacterial consortium have high biodegradation rate of petroleum hydrocarbon ranging from 83% to 89%. Several factors, including physical condition, nutrients and microbial community play an important role in the biodegradation process of petroleum hydrocarbons contaminated soil and aquatic environment. Therefore, it is necessary to understand the process of microbial bioremediation of petroleum hydrocarbons and their mechanism for further development of this technique, development of more effective bacterial consortium and genetically modified bacteria to increase their enzymatic activity, which is cheap, easy to handle and feasible to the environment.

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