Petroleum Hydrocarbons-Contaminated Soils: Remediation Approaches

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Contents

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Abstract Petroleum, the backbone of today's mechanized society, now has become a threat to environment due to extraction and transportation. Accidental oil spills occur regularly at many locations throughout the world. Contamination of soil and water resources with petroleum oil and its products has become a serious problem due to carcinogenic and mutagenic compounds. Efforts are now focused on seeking potential remediation techniques for cleanup of petroleum hydrocarbons-contaminated soils in a cost effective and eco-friendly way. Various physical, chemical and biological remediation strategies have been used to restore contaminated soils. However, plant assisted bioremediation of petroleum hydrocarbons-contaminated soil is getting more attention as compared to sole use of either microorganisms or plants. The challenging task for such efforts to be successful is not only the survival of microorganisms upon their inoculation into hostile contaminated environment but also positive plant-microbe interactions. Bacteria having ACC-deaminase enzymes are considered helpful for plants in stressed environment. We have discussed that use of bacteria equipped with dual traits of bioremediation potential and ACC-deaminase activity in association with plants can be a good approach for remediation of petroleum hydrocarbons-contaminated soil.

Keywords Petroleum hydrocarbons • Bioremediation • Soil contamination • Phytoremediation • Soil pollution

1 Introduction

All over the world, petroleum oil is considered the most precious resource that is extracted from the ground (IHRDC 2010). Huge annual consumption of oil by the major countries of the world like United States of America (USA), Japan and China has indicated the heavy reliance of the world on petroleum oil (European Energy Portal 2010). However, extraction and transportation of petroleum products are posing an inevitable risk to environment. Both human as well as mechanical errors could be the main cause of terrestrial environment pollution with petroleum products. Contamination of soil and water resources with petroleum hydrocarbons has been increasing over the time in many parts of the world due to accidental oil spills. Oil spills and consequent pollution in sea and beaches has become alarming since the seaborne trade of oil begins (Hayes et al. 2010; Defeo et al. 2009). Terrestrial environment is also contaminated by accidental spills such as the Gulf war oil spill being the worst oil spill in the history caused detrimental addition of 780,000 to 1,500,000 tons of oil to the environment (Mughal 2013). In 2010, beaches and marshes of Gulf faced pollution spillage of millions of gallons of oil because of Deepwater Horizon (DP oil spill) pipe line leakage due to explosion in a well called Macondo (Jernelov 2010). Niger delta is commonly being hit by oil pollution due to poor infrastructure, spills during refining and theft of oil (Amnesty International 2009). An independent experts group estimated a total release of 9 to 13 million barrels in the last 50 years in Niger delta (Jernelov 2010). Another famous incident of oil spill that alarmed the world about petroleum pollution was Exxon-Valdez crash in March 1989 that caused release of 11 million gallons of crude oil (Downs et al. 1993). The oil pollution is not only limited with developed or industrialized countries but it also happened in developing countries. For example, crash of a Greek ship Tasman Spirit near coastal line of Karachi, Pakistan resulted in spill of 28,000 tons of crude oil in July, 2003 (The Daily Times Pakistan, August, 16, 2003). Recently, in 2014, an oil spill of 92,000 US gallons occurred in Bangladesh (UNEP/OCHA 2015).

Due to ecological and environmental hazards linked with petroleum hydrocarbons-contamination, various physical, chemical and biological approaches have been proposed and used for remediation of petroleum hydrocarbons-contaminated soil and water resources. No doubt, physical and chemical approaches are quick and more effective but these are usually environmentally destructive, costly, may produce secondary pollutants, and cleanup is limited to small area. Alternative to these physical and chemical remediation techniques, biological approaches are environment friendly, cost effective, efficient even at low concentration of pollutants, relatively easier to adopt and do not generate perilous secondary products. The biological ways to remediate contaminated sites could be microorganisms based remediation (microbial bioremediation), plant based remediation (phytoremediation) and/or combined use of microorganisms and plants (microbe-assisted phytoremediation or rhizoremediation).

2 Health Hazards Linked with Petroleum Contamination

Petroleum hydrocarbons contamination of air, water and soil is posing serious threat to all living organisms. Some serious effects of petroleum contamination on human health are carcinogenicity, genotoxicity, (Aguilera et al. 2010) rashes on skin, childhood leukemia (Gudzenko et al. 2015), miscarriage in women (Hurtig and Sebastián 2002), irritation in skin and respiratory system disorders. For example, amongst the mono-aromatic compounds present in gasoline like benzene, toluene, ethylbenzene and xylene commonly known as BTEX; benzene is carcinogenic, toluene damages central nervous system, ethylbenzene causes skin irritation and long exposure to xylene may lead to aplastic anemia in human (Singla et al. 2012; Santiago et al. 2015). Studies conducted in Ecuador by Hurtig and Sebastián (2004) revealed that the victims of the oil pollution have been struck by cancer of skin, liver, stomach, kidney, soft tissues, lymph node and hematopoietic tissues. Inhalation of gasoline may cause nausea, numbness and drowsiness. Potential toxicity of benzene may impair nerves and cause anemia while toluene may cause impairment in central nervous system of human beings. Janjua et al. (2006) surveyed the hazardous effect of oil spill on eyes, respiratory system, skin and central nervous system of the victims of Tasman Spirit shipwreck in 2003 occurred in Arabian Sea, Karachi, Pakistan. The authors found strong linear correlation between oil pollution exposure and symptoms of eye and skin irritation and lungs problems in people while such correlation was not recorded in non-exposed group and thereby concluded that the symptoms were due to oil exposure. Similarly, Khurshid et al. (2008) recorded hematological and biochemical changes in the people residing and/or working in the vicinity of Karachi coastline and the authors observed increased level of lymphocytes and eosinophiles. Diesel is one of the most persistent constituent of petroleum oil, which is mutagenic and carcinogenic in nature (CCME 2001). Lemiere et al. (2005) investigated the mutagenicity of polyaromatic hydrocarbons (PAHs) on rats by feeding them for 2 to 4 weeks with food contaminated with polyaromatic hydrocarbons. They observed mutagenic changes in DNA and bone marrow and concluded that petroleum oil-contaminated food sources were potential genotoxic for consumer. There are 16 PAHs compounds which are listed as carcinogenic to human beings by Environmental Protection Agency of United States of America (Lampi et al. 2006). Kuwait faced one of the biggest oil polluted environment in the history as all compartments of the environment were heavily polluted with petroleum. The Kuwait's crude oil is considered sour due to higher quantity of sulfur and thereby poses more toxic effects on health of people (Husain 1994). Many people suffered from infections, nutritional disorders, damaged nervous system, respiratory system, asthma and bronchial diseases (Gastañaga et al. 2002). Of the 159 respondents of Bangladesh oil spill in 2014, 72 % reported no health effect of oil spill while respondents who reported health problems, among them, 55 % were facing difficulty in breathing (UNEP/OCHA 2015). The problem of petroleum pollution is worldwide and it is increasing significantly day by day. Therefore, worldwide struggles have been increasing to find out economical, publically acceptable and self-sustained technologies to remediate petroleum contamination to mitigate health and environmental problems (Vidali 2001).

3 Approaches to Remediate Petroleum Contamination

Various physical, chemical and biological techniques have been used for remediation of petroleum hydrocarbons-contaminated soil and water resources. Physical remediation techniques may include use of different kinds of booms, inorganic absorbent, skimmers and solidifier to avoid spread of spilled oil especially in water. Thermal remediation methods include *in-situ* thermal desorption, burning and incineration. While, oxidation-reductions, encapsulation, solvent extraction and use of dispersants are listed in chemical remediation options for petroleum oil pollution. Among biological approaches that have been adopted so far are bioremediation, phytoremediation, rhizoremediation, bio-augmentation, plant assisted bioremediation, chemical oxidation coupled with bioremediation, biopiling, land farming, and infiltration galleries etc. Each strategy has its own pros and cons and is discussed in the following section.

3.1 Physical Approaches

Physical treatments to remediate petroleum contaminated water include use of booms, skimmers and adsorbents. While, physical methods adopted for treatment of petroleum oil contamination of terrestrial lands include *in-situ* and *ex-situ* techniques. *In-situ* technique includes soil aeration while *ex-situ* technique involves shifting of contaminated soil to a chemical treatment unit such as solvent/water extraction and/or bringing to a thermal treatment unit such as low temperature thermal unit or high temperature thermal unit (incineration).

3.1.1 Booms

Booms are physical barrier to the movement of spilled oil especially when spills occur in water. Booms are used as first response to oil spill to avoid spreading of oil with turbulence of water. Generally, three types of booms are used which are fence boom, curtain booms and fire-resistant booms (Potter and Morrison 2008).

3.1.2 Skimmers

Skimmers are used in connection with booms for recovering spilled oil from water surface without bringing any change in properties of oil (Hammoud 2001). Types of skimmers generally used are weir, oleophillic and suction skimmers. Choice of skimmer depends on thickness and type of oil. All types of skimmers are made from material that contains oleophillic properties.

3.1.3 Adsorbents

As a final step in clean up of oil spills after skimming, adsorbents whether natural or synthetic are used to convert liquid oil into semi-solid phase for complete removal of oil (Adebajo et al. 2003). The adsorbent may be organic, natural inorganic and synthetic.

3.1.4 Soil Washing

Soil washing is an *ex-situ* technique in which separation of fine soil particles (clay and silt) from coarse particles using water and/or solvent is done as contaminants tend to sorb on fine particles. This technique can be applied both for organic and inorganic pollutants. Selection of solvents depends on their ability to dissolve target contaminant (Madadian et al. 2014). Separated fine particles are concentrated and further treated by following other suitable approaches.

3.1.5 Thermal Treatment

Burning of oil spill on site is quick and simple way to get rid of petroleum contamination without requirement of any specialized material. However, *in-situ* burning of oil is restricted to calm conditions of wind, fresh oil spill, sufficient supply of oxygen (Buist et al. 1999) and light products of petroleum as these are burnt quickly without harming aquatic life. For remediation of oil pollution in soil environment, thermal treatments include electrical resistant heating, steam injection and extraction. In electrical resistance heating system, heat produced by moisture in soil pores in response to applied electric current is used to heat soil to vaporize the contaminant and hence steam is produced (Beyke and Fleming 2005). Steam injection and extraction also called as steam enhanced extraction is the process of injecting steam into dug well by which non-aqueous phase liquids are displaced. Upon injection, steam offers its latent heat of vaporization to the soil and when steam heat is lost it changes into hot water which moves through the soil pores. Continued process of injecting steam brings temperature of soil near to temperature required for vaporization of target compounds and produces vapors. The vapors are transported and condensed in contaminant condensate storage. The application of this technique depends on permeability of soil, depth of contaminant location and type of contamination. This technique is alternative where excavation followed by incineration is not possible such as under surface of ground and underground tanks (USEPA 1996). The mechanism involved behind extraction technique is that upon heating organic contaminant like petroleum hydrocarbons lose its density and adsorption on solid and vapor pressure and diffusion into aqueous and gaseous phase is increased (Isherwood et al. 1992). Eventually, all changes that take place due to steam temperature increase the recovery of oil from underground. Among thermal treatments, thermal desorption and incineration are *ex-situ* treatments in which contaminated soil is treated under controlled conditions. In thermal desorption technique, petroleum sludge is thermally destructed by using high temperature oxidation under controlled conditions and the waste sludge is converted into gasses such carbon dioxide, water, sulfur dioxide and oxides of nitrogen. This technique has been used as a yardstick for comparison of other remediation technologies as it serves complete destruction of contaminants (Jadidi et al. 2014). Thermal desorption is distinguished from incineration due to its ability to desorb volatile components from polluted soil without incineration of soil. As thermal desorption is characterized for the removal of volatile compounds, so the process is completely dependent upon the volatility of the contaminants. Therefore, molecules with higher molecular weight such as polycyclic aromatic hydrocarbons are difficult to remediate with this technique (Bansal and Sugiarto 1999). Incineration on the other hand is a use of high temperature (730°C–1200°C) for complete combustion of oily sludge in the presence of air and auxiliary fuels (Hu et al. 2013). Incineration process of treatment is mostly adopted in refineries for sludge disposal. The incineration process is dependent on time, temperature of combustion and rates of sludge feed. Incineration is not only remedial technology but also provides source of energy to run turbines (Naranbhai and Sanjay 1999).

3.1.6 Advantages of Physical Treatment

(a) These can be applied for all kinds of oil
(b) These are generally non destructives strategies and recovery of oil is possible
(c) These techniques are simple and easy to handle where no extensive expertise is required.
(d) Thermal treatment has advantage of time effectiveness over other technologies
(e) Efficiency of incineration and thermal desorption is approximately 99 % which is much higher than other remediation technologies

3.1.7 Disadvantages of Physical Treatment

(a) Extensive labor is required
(b) Not self-sustained
(c) Weak stability in strong wind and currents of water.
(d) Can only be used in conjunction with other techniques
(e) Prior treatment of oily sludge with high moisture content is required for incineration
(f) Thermal treatment is source of secondary pollution as thermal desorption and incineration are the sources of emission of low molecular polycyclic aromatic hydrocarbons
(g) Incomplete combustion may cause pollution in atmosphere
(h) Initial installment and running cost is very high

3.2 Chemical Approaches

Chemical approaches to remediate petroleum hydrocarbons-contaminated soil include the use of dispersants, encapsulation, and chemical oxidation.

3.2.1 Dispersants

When oil spillage occurs on water surface, it disperses into water column naturally. However, dispersion depends on the viscosity of oil. Less viscous oil disperses more due to natural energy of water currents but oil with high viscosity is less amendable with energy of sea. Dispersants are used for dispersion and dilution of highly viscous oil. These may be defined as the mixture of surface active agents dissolved in solvents and stabilizers (Dave and Ghaly 2011). Dispersants contains two parts; one is oleophilic, the other is hydrophilic; these are used for reducing surface tension and consequently increase dispersion of oil. The solvents play role of carrier for dispersant to targeted oil and water interface where it re-arranges molecules by connecting oleophilic part to oil and hydrophilic part to water molecule. Dispersant reduces the size of droplet and thereby increase dispersion of oil on water surface (Lessard and Demarco 2000).

3.2.2 Solvent Extraction

It is an *ex-situ* technique where oil contamination is separated from the media through separation followed by concentration process by using non-aqueous liquid. This method is not widely used for remediation of large area due to its expensiveness as solvents used in this technique are too costly. Soils must be dried before applying this technique and solvents themselves may be source of secondary pollution. The extraction also depends on soil type and composition, for instance, agglomeration occurs when the contaminated soil is clayey in nature and requires longer time of contact of solvent with clay particles (Berset et al. 1999). Solvent extraction, however, has advantage of being used to extract vast range of pollutants. Moreover, pollutants with higher concentration can be treated.

3.2.3 Encapsulation

In this technology, the contaminated material is physically isolated and contained by covering it with low permeable material such as textile and clay caps to avoid infiltration, leaching and resultantly migration (Robertson et al. 2003). This technology is highly dependent on the permeability of the site and saturating capacity of the covering material. This technique cannot be long lasting as sooner or later the covering material gets saturated. Also this technology is not cost effective especially in case when the contaminant depth is higher (Khan et al. 2004).

3.2.4 Chemical Oxidation

Comparing to other remedial strategies, chemical oxidation for decomposition of petroleum hydrocarbons is a short term technology which takes months or even weeks to oxidize contaminants. Chemical oxidants irreversibly reduce petroleum hydrocarbons into CO_2 and H_2O depending upon the contact time with contaminants. Mostly chemical oxidation is carried out to decontaminate sites where area is smaller and concentration of contamination is high. There are different kinds of chemical oxidants in use, however, choice of chemical oxidants depends upon the understanding of hydrogeological condition of the targeted area. The most commonly used chemical oxidants are Fenton's reagent, hydrogen peroxide, permanganate of sodium and potassium and ozone. Effectiveness of chemical oxidation can be enhanced when it is used in conjunction with ultraviolet light (Liang et al. 2003). The success of chemical oxidation technology depends on prior information about the site (soil permeability, texture of soil, soil reactivity), choice of appropriate chemical oxidant and solubility characteristics of solvents etc.

3.2.5 Advantages of Chemical Treatment

(a) It can be used as an in-situ treatment
(b) Destruction of contaminant is rapid (weeks or months)
(c) Oxidation of contaminant is complete (except Fenton's Reagent)
(d) Possesses compatibility with post treatment such as enhancement in aerobic degradation
(e) Being an *in-situ* strategy, it causes minimum disruption to other site operations

3.2.6 Disadvantages of Chemical Treatment

(a) Dispersants are of inflammable nature and can adversely affect human health during spray and also damage the sea life
(b) Higher initial and overall cost compared to other technologies
(c) In case of low permeability soils, oxidant may not get contact with contaminants
(d) Significant loss of chemical oxidant may result due to reaction with soil instead of target contaminants
(e) Clogging of capillary fringe may occur due to precipitation of minerals
(f) May be a source of secondary pollution

3.3 Biological Approaches

Remediation technologies other than biological are expensive, ecologically disruptive and demand substantial input of energy and heavy machinery. In this context, biological options for remediation are more appealing and environment friendly.

3.3.1 Bioremediation

Remediation of petroleum-contaminated soil and water resources by the use of microorganisms is known as bioremediation and is a promising option for complete conversion of petroleum hydrocarbons into carbon dioxide, water and inorganic compound (Kuiper et al. 2004; Barea and Pozo 2005). It is environment friendly and cost efficient compared to traditional physico-chemical remediation strategies (Gallego et al. 2001; Bundy et al. 2002; Mulligan and Yong 2004; Bento et al. 2005; Joo et al. 2008; Gargouri et al. 2014; Fuentes et al. 2014; Pizarro et al. 2014). Numerous bacterial genera reported for biodegradation abilities include *Sphingomonos*, *Cycloclasticus* (Ho et al. 2002), *Burkholderia* (Caballero-Mellado et al. 2007), *Bacillus*, *Bravibacterium*, (Xiao et al. 2012) and *Pseudomonos* (Liu et al. 2013). Microbes have been manipulated for bioremediation of petroleum hydrocarbons in different ways which include biostimulation, biopiling, land farming, bioventing and bioaugmentation (Zhou and Hua 2004).

Stimulation of indigenous microflora by provision of inorganic nutrients like nitrogen and phosphorus to expedite the degradation of pollutants is referred to as biostimulation (Perfumo et al. 2007). Degradation of organic pollutants has been reported by many scientists because of increased microbial growth in response to addition of inorganic nutrients (Sarkar et al. 2005; Ron and Rosenberg 2014). However, blending of inorganic nutrients may also inactivate microbial population and could result in decreased bioremediation process (Mani and Kumar 2014).

Biopile is an *ex-situ* process of remediating organic contaminants in which contaminated soil is piled up where air, fertilizer and other amendments are injected in the contaminated pile to accentuate microbial oxidation of hydrocarbons (Hazen 1997). Contaminated soils are blended and mounded while aeration and moisturizing system is installed prior to piling. The amendments added are bulking agent, chemicals to adjust pH of biopile and periodical addition of nutrients and moisture. To avoid leaching of contaminants, biopile is constructed on impermeable layer of soil (USEPA 2012). Biopiles, also called biocells are constructed where growth conditions for aerobic bacteria are optimized for biodegradation of contaminants. The volatile fractions of petroleum hydrocarbons are evaporated during aeration process while heavier fraction of petroleum hydrocarbon is broken down by biodegradation process.

Land farming is *in-situ* remediation method adopted particularly for remote areas where minimum equipment are required and passive aeration is carried out by tilling periodically (Paudyn et al. 2008). Like biopiling, land farming also requires addition of amendments like bulking agent, chemicals for adjusting pH and inorganic nutrients to speed up bioremediation process (McCarthy et al. 2004). Land farming is adversely affected by environmental conditions such as rainfall and low ambient temperature that consequently affect the rate of biodegradation (Gan et al. 2009).

In bioventing oxygen is provided in contaminated soil and/or water to increase the redox potential for enhancing the bioremediation process. Bioventing is not cost effective technique and to make it cost effective biosparging have been innovated (Mani and Kumar 2014).

Bioaugmentation is addition of microorganisms which have ability to degrade petroleum oil in polluted soil or water resources to enhance the process of bioremediation. Microorganisms used for bioaugmentation could be pre-acclimated and/or genetically engineered. In this process, genes relevant to biodegradation could also be conjugated in the indigenous microbial population (El-Fantroussi and Agathos 2005). It is relatively simple and easy process of bioremediation. Alisi et al. (2009) reported that due to bioaugmentation, about 75 % of diesel contents were decreased in 42 days. However, success of bioaugmentation is limited due to inoculum failure which could be due to competition with indigenous microflora (Vidali 2001). Other possible reasons for inoculum failure could be low redox potential, unavailability of inorganic nutrients, bioavailability of target compounds and improper soil conditions like moisture, temperature and pH etc. (Suja et al. 2014).

3.3.1.1 Degradation of Petroleum Hydrocarbons

Crude oil is complex mixture of aliphatic and cyclic hydrocarbons. On refining, crude oil yields different compounds such as gasoline, kerosene, diesel and lubricating oil. Straight chain alkane and alkene are called aliphatic hydrocarbons while cyclic hydrocarbons are of two kinds; cycloalkanes (saturated hydrocarbons) and aromatic hydrocarbons (unsaturated hydrocarbons). Alkane compounds are of three types; linear alkane (n-alkanes), branched alkanes and cycloalkane. One or more rings of carbon atoms are present in cycloalkanes; however, these rings are not benzene rings because the hydrocarbon molecules characterized by the presence of one or more benzene or aromatic rings comprise separate class which is called aromatic hydrocarbons. These compounds are further categorized into mono, di and polyaromatic hydrocarbons. In crude oil the major portion is linear alkane or n-alkanes, if biodegradation has not been happened earlier (Ollivier and Magot 2005). Generally, it is considered that alkanes are degraded more rapidly and wide range of microorganisms is capable of biodegrading alkanes both short and long chain. The alkanes are degraded mono-terminally by addition of oxygen and converted into alcohols, aldehyde and fatty acids. The resistance to degradation increases with increasing length of chain (Atlas and Bartha 1981). Three mechanisms of alkane degradation are proposed by Huguenot et al. (2015) given as Fig. 1.

Cycloalkanes are recalcitrant constituent of petroleum hydrocarbons and are found abundantly in petroleum products. These are mostly degraded through co-metabolism by alkane degraders. This process of co-metabolism is initiated by conversion of cycloalkanes into alcohol or ketone by monooxygenase (Sayyed and Patel 2011).

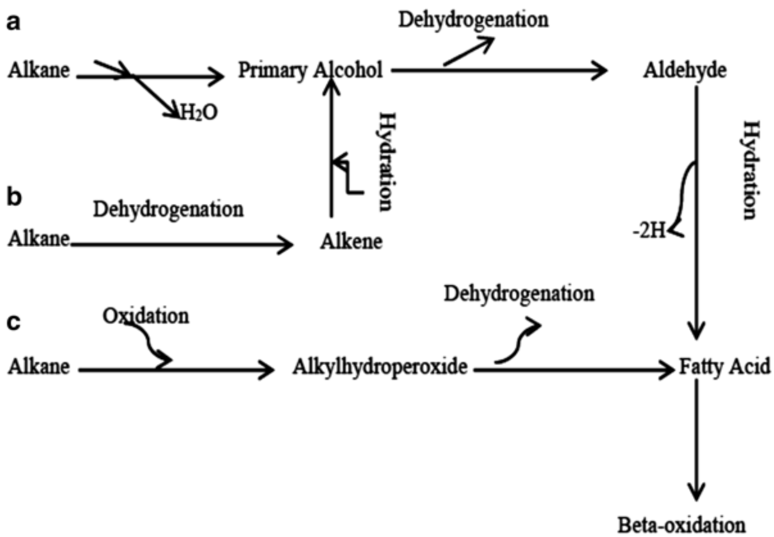

Fig. 1 Terminal methyl oxidation pathways adapted by alkane degrading microorganisms (Extracted from Atlas and Bartha 1981; Huguenot et al. 2015)

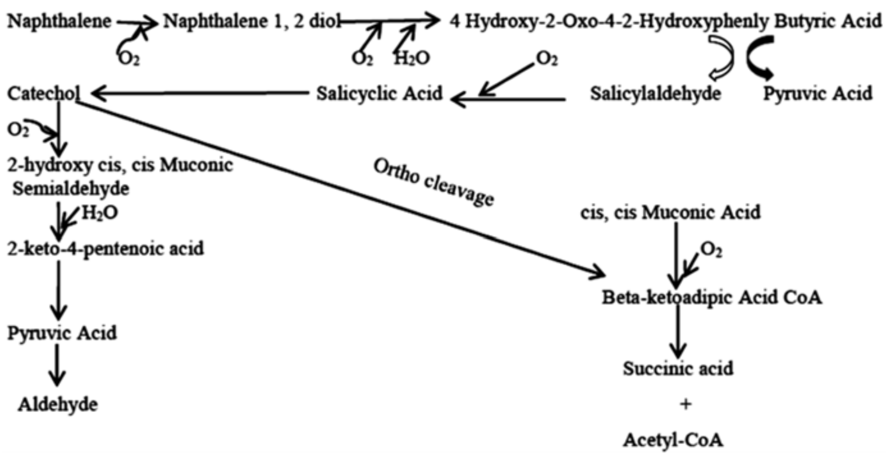

Fig. 2 Degradation of aromatic ring by microorganism showing ortho- and meta- cleavage of catechol (Extracted from Atlas and Bartha 1981; Huguenot et al. 2015)

Degradation of aromatic hydrocarbons especially of polycyclic aromatic hydrocarbons is slow as compared to alkane because one or more oxidation steps are required to convert into catechol which is further opened by oxidation on ortho or meta points of the ring (Atlas and Bartha 1981). The initial oxidative step is mediated by monooxygenase or hydroxylating dioxygenase and this dioxygenase mediated opening of catechol results into the production of cis,cis muconate and unsaturated dicarboxylic acid (Fig. 2). Finally acetyl-CoAs are produced from this product through beta-oxidation.

3.3.1.2 Constraints in Bioremediation

Bioremediation process faces a number of serious constraints including petroleum contamination in more than one medium like soil, water and gaseous phase and complex matrix of inorganic and organic contaminants with variety of toxicological behavior. Moreover, heterogenic subsurface conditions, uncontrollable sub-optimal environmental conditions, costly analytical procedure and difficulty in monitoring process could cause limitation in bioremediation (Pollard et al. 1994). Some of these important constraints have been discussed below.

3.3.1.2.1 Composition of Petroleum Waste

Petroleum hydrocarbons are a complex mixture of thousands of molecules with unique chemical structure and behavior that make the bioremediation process uncertain as molecules with different chemistry make them either easily biodegradable or very difficult to break down (American Academy of Microbiology 2011). Microbial degradation order of petroleum hydrocarbons is alkane > monoaromatic hydrocarbons > cycloalkane > polycyclic aromatic hydrocarbons > asphalthene (van Hamme et al. 2003).

3.3.1.2.2 Weathering of Petroleum Waste

Weathering process limits the susceptibility of petroleum hydrocarbons to biodegradation as easily biodegradable fractions of petroleum hydrocarbons are weathered due to evaporation, reaction of sunlight leaving behind the recalcitrant portion of contamination (Bossert and Bartha 1984). An aqueous phase is required for microorganism to grow on petroleum hydrocarbons which is limited due to weathering as hydrophobicity/octanol water portioning co-efficient increases during weathering. In other words, the bioavailability of compounds is restricted and consequently the process of bioremediation is constrained (Leahy and Colwell 1990; Cerniglia 1993).

3.3.1.2.3 Climatic Condition

Climatic conditions controlling biodegradation are temperature, soil moisture, redox potential, oxygen, pH and nutrient status.

Temperature is probably the most crucial factor that affects bioremediation process as the solubility and bioavailability of hydrophobic compounds directly depends upon temperature. The temperature controls the viscosity of petroleum hydrocarbons and thereby increases or decreases the distribution and diffusion of petroleum hydrocarbons (Gibb et al. 2001). Also, the microbial metabolism is directly affected by the temperature and consequently the activity of microorganisms in the environment is affected.

Bioremediation of various component of oil may be influenced due to inorganic nutrients. Soil, contaminated with hydrocarbons, suffers from deficiency of inorganic nutrients due to the higher concentration of carbon in contaminants. Generally, nitrogen (N) and phosphorus (P) are limiting in hydrocarbon-contaminated soils and affect the rate of bioremediation by suppressing the proliferation of microorganism because N and P are major cellular components of bacteria (Alamri 2009; King et al. 1998). Most of the petroleum hydrocarbons are degraded aerobically with the exception of heavier molecules like asphalthene and polycyclic aromatic hydrocarbons. For aerobic degradation, proper provision of oxygen is of immense importance. Parallel to oxygen supply for bioremediation process, soil moisture is of same importance for growth of microbial population and bioavailability of low molecular weight compounds. Hence the delicate balance between soil oxygen and soil moisture is necessary for successful bioremediation process.

3.3.1.2.4 Bioavailability

In bioremediation context, bioavailability is called the portion of petroleum hydrocarbons available for microbial degradation (Pollard et al. 1994). Bioavailability of different fractions of petroleum hydrocarbons is very important regulating factor for acceptable rate of bioremediation. Main reason of decreased bioavailability of contaminants is the ageing process which is attributed to sequestration of contaminants into solid phase either on clay minerals or on organic matter (Stewart et al. 2003). Break down of petroleum hydrocarbon compounds is either by direct attachment of microorganism to non-aqueous phase, liquid-water interface or by mass transfer of non-aqueous phase liquid to aqueous phase (Mohanty and Mukerji 2008). Problem of bioavailability may be overcome by the addition of surface active agent or the bacteria with ability to secrete bio-surfactants.

Although, bioremediation is very appealing strategy for remediation of petroleum hydrocarbons-contaminated soils. However, its efficiency is limited because of limited growth of microbes in oil contaminated soil environment due to large molecules of hydrocarbons, improper moisture and anaerobic conditions etc. If, microbes are used in association with plants, the remediation processes could be expedited many times than their separate application.

3.3.2 Phytoremediation

Phytoremediation is use of plants for remediation and/or restoration of contaminated soil or ground water resources. There are various mechanisms involved in phytoremediation like phytoextraction, rhizofiltration, phytotransformation, phytostabilization, phytodegradation, rhizoremediation, and phytovolatilization for the remediation of contaminated soil and ground water (Glick 2010; Hakeem et al. 2014). Degradation of contaminants by plants could be either intracellular metabolic process after uptake of contaminants in their cells or by releasing extracellular enzymes that degrade contaminants (Mougin 2002; Liu et al. 2013; Sabir et al. 2014). However, there are some limitations for metabolic degradation of organic pollutants like polycyclic aromatic hydrocarbons and other components of petroleum hydrocarbons due to limited uptake by plants of such type of contaminants (Mougin 2002; Newman and Reynolds 2005; Martin et al. 2014). The hydrophobic nature of organic pollutants is the main hindrance for plant uptake and it has been reported that plants can only uptake, translocate and metabolize the organic compounds with octanol-water partition coefficient (Kow) log Kow $\leq$ 1 (Limmer and Burken 2014). The petroleum hydrocarbons with octanol water partitioning coefficient (Kow) greater than 3 are not soluble in water thus cannot be taken up by plants (Rojo 2009). Different types/mechanisms of phytoremediation are elaborated precisely in following section:

3.3.2.1 Phytodegradation/Phytotransformation

Breakdown of organic contaminants by plants either internally by their metabolic process or externally by releasing enzymes is called phytodegradation or phytotransformation (Vaziri et al. 2013). Plants possess several enzymes such as cytochrome P450s and glutathione-s-transferase by which plants are able to transform toxic chemicals (Mougin 2002). There are very few reports of direct degradation of petroleum hydrocarbons by plants (Mougin 2002; Newman and Reynolds 2005). Soybeans (*Glycine max*) has been reported for uptake and degradation of 14C-anthracene (Edwards et al. 1982).

3.3.2.2 Phytostabilization

Containment or immobilization of contaminant in soil or ground water by plant roots is called phytostabilization. It involves the use of plants to reduce the bioavailability and migration of contaminants in soil (Germida et al. 2002). Stabilization of contaminants by plants occurs due to adsorption on the root surface, accumulation by the roots, or isolation within the root zone using plants as organic pumps (Adam and Duncan 1999; Pilon-Smits 2005). Schnoor (2002) suggests that organic chemicals with log Kow values greater than 3.0 are strongly sorbed to plant roots. Schwab et al. (1998) reported up to 30 % of naphthalene adsorption to the roots of alfalfa, while 15 % on roots of tall fescue. Conclusion drawn by the authors was that adsorption of lipophilic compounds onto the surface of roots may be an important sink for PAHs in soils and an initial step in phytoremediation. Binet et al. (2000) reported 0.006 and 0.11 % of extractable PAHs by ryegrass (Lolium perenne) as determined by GC-MS. In case of petroleum hydrocarbons, phytostabilization may simply involve the establishment of a vegetative cover to minimize potential migration of the contaminant through soil erosion or leaching (Germida et al. 2002).

3.3.2.3 Phytovolatilization

Phytovolatilization is a process in which contaminants are taken up by plants and subsequently moved into atmosphere by the process of evapotranspiration through stomata into the atmosphere (Pilon-Smits 2005). Watkins et al. (1994) assessed phytovolatilization of radiolabelled [7-14C] naphthalene with the Rhodes grass (*Chloris gayana*). The authors concluded that the pollutant was taken up by the grass roots, translocated within the plant, and volatilized through the above ground biomass. Overall, plant uptake and accumulation of hydrocarbons from contaminated soil is quite small and limited to low molecular weight compounds. Many petroleum hydrocarbons are large, high molecular weight molecules which are also lipophilic, thus excluding them from the plant root (Qui et al. 1997). Phytodegradation and phytovolatilization are therefore considered minor pathways of hydrocarbon removal from soil systems.

3.3.2.4 Advantages of Phytoremediation

(a) Minimum disturbance to the environment
(b) On-site remediation technology
(c) Cost effective

(d) No excavation and transportation is required
(e) Can be applied to remediate different kinds of hazardous materials
(f) Large area can be covered
(g) It is aesthetic to environment and therefore has favorable public perception.

3.3.2.5 Disadvantages of Phytoremediation

(a) The contaminated area must be large enough to grow the plants.
(b) Phytoremediation is limited to low concentration of contamination
(c) Phytoremediation is limited to root zone depth
(d) Problem to handle contaminated plants

No doubt, phytoremediation is a promising strategy to remediate polluted soil and ground water with xenobiotic compounds, but it has limitations due to toxicity of pollutants and it has been observed in several studies that under petroleum hydrocarbon stress plants fail to attain sufficient biomass for meaningful remediation and reduced plant growth with decreased root and shoot lengths has been observed (Merkl et al. 2005; Germaine et al. 2009).

3.3.3 Plant Assisted Bioremediation/Microbial Assisted Phytoremediation

Surely, both plants and microbes have potential to biodegrade petroleum hydrocarbons but their individual success is limited. Plants cannot absorb and degrade the hydrophobic compounds with high molecular weight and microbes may face problem in biodegradation due to deficiency of nutrients and oxygen (Pilon-Smits 2005). However, partnership between plants and microbes expedites the biodegradation of hydrophobic compounds (Kuiper et al. 2004). Remediation of petroleum hydrocarbons in rhizosphere of plants by the assistance of microbes is known as rhizoremediation. However, endophytic microbial degradation of petroleum hydrocarbons has also been observed which refers to the symbiotic relationship between plants and endophytic microorganism (Newman and Reynolds 2005). Work by van Aken et al. (2004) revealed that symbiotic relationship between *Methylobacterium* sp. strain BJ001 and hybrid poplar approximately degraded 60 % of explosives such as TNT (2,4,6-trinitrotoluene) and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) into carbon dioxide in 2 months. However, in case of PAHs and petroleum compounds, rhizosphere degradation (rhizoremediation) is more prevalent than direct uptake and metabolism by the plant or its associated symbionts (Hutchinson et al. 2003). Plant assisted bioremediation is more pleasing and publically accepted because of its low cost and meaningful remediation of organic compounds (Gurska et al. 2009). Both plants and microbes must have ability to tolerate stress imposed by petroleum hydrocarbons for the success of this strategy (Germida et al. 2002 and Bona et al. 2011). Bioremediation assisted by plants is useful to overcome constraints that limit alone application of plants or microorganisms and thereby enhances degradation of recalcitrant soil contaminant like polyaromatic hydrocarbons (Huang et al. 2004). Roots of higher plants play important role in assisting microbial population to degrade pollutants in many ways such as provision of molecular oxygen, inorganic nutrients through sloughed off cells, soluble exudates and lysates (Phillips 2008; Martin et al. 2014). Plants improve soil aeration by directly giving off oxygen to the root zone as well as allowing improved entry of oxygen into the soil by diffusion along old root channels (Marschner 2012). If microbes do not have degradative pathways for petroleum hydrocarbons and cannot use them as carbon source, even then petroleum hydrocarbons can be mineralized through the process of co-metabolism (Gojgic-Cvijovic et al. 2012). Co-metabolism is mechanism by which plants assist microbes to co-metabolize a contaminant in the soil using the root exudates as an energy source. Plant enzymes degrade the compounds, and then further degradation is carried out by microbes ultimately into carbon dioxide and water. Some structural analogues of polyaromatic hydrocarbons such as phenols, terpenes and flavonoids are released by some plant roots as exudates and thus promote the growth of petroleum hydrocarbons degrading bacteria (Rentz et al. 2005; Martin et al. 2014) and can act as trigger of PAHs degradation-pathway (Singer et al. 2003). Although cooperation of plant with microorganisms is true yet organic pollutants present in the system cause impairment in plant growth and limit the benefits of adding vegetation to a contaminated soil (Gartler et al. 2014).

Plant growth impairment is a serious limitation in petroleum hydrocarbons-contaminated soils because plant species are sensitive to contaminants and fail to produce sufficient biomass and root activities (Huang et al. 2001). Successful plant-assisted microbial remediation of petroleum hydrocarbons-contaminated soil is highly dependent on elimination of root inhibition. This inhibition of root growth in contaminated soils is mainly due to stress-induced ethylene; an established stress phytohormones (Arshad et al. 2007). Synthesis of ethylene in plant at a rate where it exerts inhibitory effects on plant roots is in response to contaminant-induced stress. Thus, a preferential target is to regulate/limit the biosynthesis of ethylene so that normal or more root biomass can be achieved in stressed environment. Some plant growth promoting rhizobacteria (PGPR) are reported to be equipped with ACC-deaminase enzyme that hydrolize ACC (an immediate precursor of ethylene in plants) into α-ketobutyric acid and ammonia and thus, regulate the biosynthesis of ethylene when plants are inoculated with such bacteria (Glick 2005). This reveals the fact that just as plant can affect microbial growth, microorganism can affect and protect plant growth in contaminated soils. Thus rhizobacteria containing ACC deaminase activity regulate plant growth in both biotic and abiotic stresses and dramatically increase the biomass of plant especially roots which is a desirable parameter for plants to be used to assist the process of biodegradation of petroleum hydrocarbons-contaminated soils. But, unfortunately, the isolation and subsequent inoculation with bacteria having ACC-deaminase activity does not fulfill the job of remediating the petroleum hydrocarbons contamination unless they are acclimated to such contaminants. According to suggestions made by Glick (2010) for the enhancement of degradation of organic contaminants in soil that the bacteria inoculated for remediation of organic contaminants must possess twin nature of plant growth promotion as well as degrader of soil contaminant. In plant assisted bioremediation process, biomass production in considerable quantity is of key importance as root biomass provides multi-fold benefits to bacteria in facilitating the degradation of petroleum hydrocarbon compounds and shoot biomass provides cover to soil which not only accentuates degradation process but is also aesthetically more pleasant. To aid plants in production of higher biomass both under normal and stress conditions, bacteria which are usually called plant growth promoting bacteria (PGPB) play their role directly or indirectly (Ahemad and Kibret 2014). Plant growth promoting bacteria benefit directly by solubilizing mineral nutrients such as phosphorus (Hussain et al. 2013), fixing atmospheric nitrogen symbiotically or in rhizosphere of plants in associative manner, siderophore production, phytohormones production (Vessey 2003) or regulating stress induced ethylene (Shaharoona et al. 2006; Arshad et al. 2007) and production of volatile compounds (Ryu et al. 2003; Blom et al. 2011). Indirect mechanisms of plant growth promotion by PGPB include inducing defense mechanism of plant (Ryu et al. 2004), biocontrol by producing antibiotic against pathogenic microorganism and induced systemic resistance (Ryu et al. 2004).

Plant-assisted bioremediation occurs naturally, however it can be accentuated by exploiting suitable plant and microbe synergism especially PGPB with bioremediation potential may be beneficial as these bacteria not only degrade pollutants of the interest but also get rid of the plants from toxic effect of pollutants (Kuiper et al. 2004). In case of plant-assisted bioremediation of mixture of petroleum hydrocarbons, plants are unable to take up petroleum hydrocarbons, however, PGPB equipped with bioremediation potential break these large molecular weight compounds by their catalyzing action (Huang et al. 2004). Bacteria with ability to utilize 1-aminocyclopropane-1-carboxylate (ACC) as sole nitrogen source are of crucial importance when used in combination with plants under stress conditions (Arshad et al. 2007). Huang et al. (2004) conducted study to evaluate different processes such as bioremediation, phytoremediation, land farming and combination of plant growth promoting bacteria having ACC-deaminase activity for remediation of PAHs-polluted contaminated site. Their results revealed that most efficient process was multi process system rather than bioremediation or phytoremediation alone and the authors concluded that success of the multi process system was due to tolerance of plants to contaminants and PGPR that enhanced the tolerance of plants by reducing stress induced ethylene. ACC-deaminase containing bacteria hydrolyze the ACC exuded from germinating seed and roots. To maintain the equilibrium of ACC inside and outside of the roots or seed, ACC move outside the roots or seed as exuded ACC is being cleaved by the bacteria into ammonia and α-ketobutyrate and thus lowers ethylene production inside plant which results into better germination and root growth (Glick 2005; 2007). However, the survival and proliferation of inoculated ACC-deaminase bacteria in the polluted environment is of special consideration. Glick (2010) suggested bacteria with twin nature of plant growth promotion and biodegradation potential as better option instead of only plant growth promoting bacteria to be used in plant-assisted bioremediation process. Some examples of successful remediation of petroleum hydrocarbons by plant assisted bioremediation process are given in Table 1.

Table 1 Plant growth-promoting bacteria in plant-assisted bioremediation

Plant growth-promoting bacteria	Plant	Type of compound	Reference
Methylobacterium populi VP2	*Lycopersicon esculentum*	Phenanthrene	Ventorino et al. 2014
Indigenous Bacterial Consortia	*Trigonella foenumgraecum*	Mixture of Petrol, Diesel and Engine oil	Shanker et al. 2014
PGPR	*Avena sativa*	Petroleum oil	Xun et al. 2015
Serratia marcescens BC-3	*Avena sativa*	Petroleum hydrocarbon	Dong et al. 2014
Pseudomonas putida	Pasture Plants	Polycyclic aromatic hydrocarbons	Pizarro et al. 2014
Burkholderia sp.	*Axonopus affinis*	Diesel	Tara et al. 2014
Pseudomonas sp.	*Testuca arundinacea* L.	Petroleum hydrocarbon	Liu et al. 2013
PGPR	Indian mustard	Petroleum hydrocarbon	Graj et al. 2013

4 Conclusion

Plant assisted bioremediation could be an efficient mean for remediation of petroleum hydrocarbons-contaminated soil and water resources as compared to other physical, chemical and biological approaches. However, for successful establishment of plant-microbe partnership, it is necessary that microbes with biodegradation potential must have the ability to effectively colonize and survive in the rhizosphere of plants. Moreover, it is also of prime importance that plants must produce sufficient biomass with prolific root system. Bacteria having ability to produce ACC deaminase help the plants to tolerate contaminants stress by improving root growth in contaminated soils which ultimately results in improved uptake of water and nutrients. Better establishment of plants in contamination results in enhanced microbial survival and activities due to more availability of root-exuded nutrients. Therefore, co-existing plants and microbes could be more effective for remediation of petroleum hydrocarbons-contaminated soil as compared to their individual application.

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