



Review

Plant-bacteria synergism: An innovative approach for the remediation of crude oil-contaminated soils

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Abstract

The adverse ecological and socio-economic effects of oil pollution demand that eco-friendly and proficient remediation technologies be devised as countermeasures. The synergistic use of plants and bacteria is considered as one of the efficient technologies for the remediation of crude oil-contaminated soil. In plant-bacterial synergism, plants host a large number of bacteria in its rhizosphere, root and shoot by providing nutrients and space for colonization. In return, bacterial population increases in different compartments of the plant and degrade organic pollutants. This review will highlight the issues related to soil contamination with hydrocarbons and their remediation, bioremediation and phytoremediation, plant-bacterial synergism in hydrocarbons degradation with special emphasis on the role of endo/rhizosphere bacteria for the maximum remediation of hydrocarbons contaminated soil.

Keywords: Crude oil, hydrocarbons, phytoremediation, plant-bacterial synergism, rhizobacteria, endophytic bacteria

Soil is an essential life-supporting and fundamental constituent of the biosphere which offers a number of advantages to the surroundings including primary production, control of biogenic gases, water cycling, preservation of life, and biodiversity (Alexander, 1978; Kuske *et al.*, 2002). In earlier times, it was believed that our land and its resources are in abundance and will remain available for centuries. Unfortunately, due to excessive use and now misuse, half of this natural wealth is either destroyed or is at the verge of depletion (Balba *et al.*, 1998; Andreoni *et al.*, 2004; Reda and Ashraf, 2010). The reasons behind continuous exhaustion of healthy soil ecosystem are the use of chemical fertilizers, release of other anthropogenic chemicals, and dumping of industrial/domestic wastes into the environment; all these activities are posing a significant threat to mankind itself (Kuske *et al.*, 2002; Srogi, 2007). In addition to other prevalent pollutants, petroleum hydrocarbons (PHs) are of specific concern because of their structural complexity, hydrophobicity, toxicity, and persistent nature (Tolosa *et al.*, 2004; Pinedo *et al.*, 2013; Brevik *et al.*, 2015; Fatima *et al.*, 2015; Tahseen *et al.*, 2017).

The world's energy sources depend greatly on petroleum oil and its products, and world-wide energy demand is expected to rise steeply over the next twenty years (Aqeel and Butt, 2001; Siddiqui, 2004). Due to their excessive use, there is a chance that these PHs may release in the environment and cause severe damage to the

ecosystem. Environmental contaminants enter the environment by both natural and manmade sources leading to contamination of drinking water, diminishing water and air quality, waste of non-renewable resources, and loss of soil fertility (Sebiomo *et al.*, 2010; Janbandhu and Fulekar, 2011; Prince *et al.*, 2013; Shabir *et al.*, 2013). On the other hand, continual contact with high oil concentrations may have negative effects on human health and all other life forms as well. Even at low levels of pollution, hydrocarbon causes lethal mutations in genetic material. Thus due to its mutagenic and neurotoxic effects, the United States Environmental Protection Agency (US EPA) categorizes crude oil as a significant pollutant (Aguilera *et al.*, 2010; Ordinioha and Brisbane, 2013; Robertson and Hansen, 2015).

Considering the worldwide problem of soil pollution, more suitable treatments are necessary as compared to the conventionally used more expensive and environmentally deleterious *ex situ* techniques. Conventional technologies, based on physicochemical methods (soil washing, chemical reduction or oxidation of contaminants, and incineration), are not feasible due to high cost, environmental invasion, engineering skills, labour administration, and operational management (Pandey *et al.*, 2009). Keeping in mind the limitation of conventional technologies, a much better method is needed to destroy the pollutants or to transform them into nontoxic substances. This can be achieved by the use of efficient microbes in conjunction with suitable plants i.e., microbe-assisted phytoremediation (Weyens *et al.*,

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2009; Khan *et al.*, 2013a; Ijaz *et al.*, 2015a; Fatima *et al.*, 2016). This technique provides a means of *in situ* treatment of contaminated land with high efficiency using natural biological activity. In plant-microbe synergism, plants offer nutrients and habitat to their associated bacteria and in return, microbes enhance plant growth and detoxify environmental pollutants (Chaudhry *et al.*, 2005; Compant *et al.*, 2010; Glick, 2010; Sessitsch *et al.*, 2013; Afzal *et al.*, 2014a; Ijaz *et al.*, 2015b, 2016; Ahmed *et al.*, 2017).). In this review, the contamination of environment with crude oil, remediation of soil contaminated with crude oil, plant-bacterial synergism in phytoremediation, and role of rhizo- and endophytic bacteria for the maximum remediation of the soil, are discussed.

Crude oil and environmental pollution

Crude oil primarily comprises of variable extents of carbon and hydrogen. Furthermore, it contains nitrogen, oxygen, sulfur, and a range of metal-containing compounds (Chandra *et al.*, 2013; Speight, 2014; Varjani *et al.*, 2015). It is characterized as light, moderate, or thick oil established on comparative quantities of weighty molecular components existing in it. Its configuration may differ with site, age and depth of an oil well (Bachmann *et al.*, 2014). On the basis of composition, crude oil is characterized in to four major segments: 1) aliphatics, 2) aromatics, 3) resins, and 4) asphaltthenes. Some of the constituents of crude oil are shown (Figure 1). Each fraction has a distinctive chemical and physical behaviour that influences the way it spreads and undergoes biodegradation in the environment (Abbasian *et al.*, 2015). In basic prearrangement of the aforementioned constituents of crude oil, aliphatics fraction constitutes the outmost layer while asphaltthenes, being the high molecular weight constituent, establish the intimate share of oil (Prince *et al.*, 2013; Robertson and Hansen, 2015).

Hydrocarbons are one of the most recalcitrant organic contaminants in the environment. Because of their toxic nature, they cause wide-ranging and permanent damage to human as well as all other life forms. Although microbes eradicate soil pollution, whereas the quantity of pollutants surpasses the buffering capability of soil, it leads to enduring undesirable effects on its value and ecology (Gan *et al.*, 2009).

Petroleum hydrocarbons pollution of soil is an anxiety for a number of causes (Figure 2). At the outset, when discharged into soil, the volatility of hydrocarbons can lead to fire or even hazardous outbursts, particularly when fumes enter restrained places (Souza *et al.*, 2014). Secondly, contaminants can adsorb on soil particles and be retained in soil for years thus leading to land degradation. Though these chemicals can profit the soil microflora as an energy

basis, they still have toxic and mutagenic effects on microorganisms even at low concentrations (Walker *et al.*, 2012). PHs also destroy the aesthetics of land by inducing unpleasant odour, taste in associated groundwater, or appearance to surroundings. Persistent seepage and continuous runoffs occur due to their mobile nature which extends their impact to adjacent areas (Albaigés, 2014).

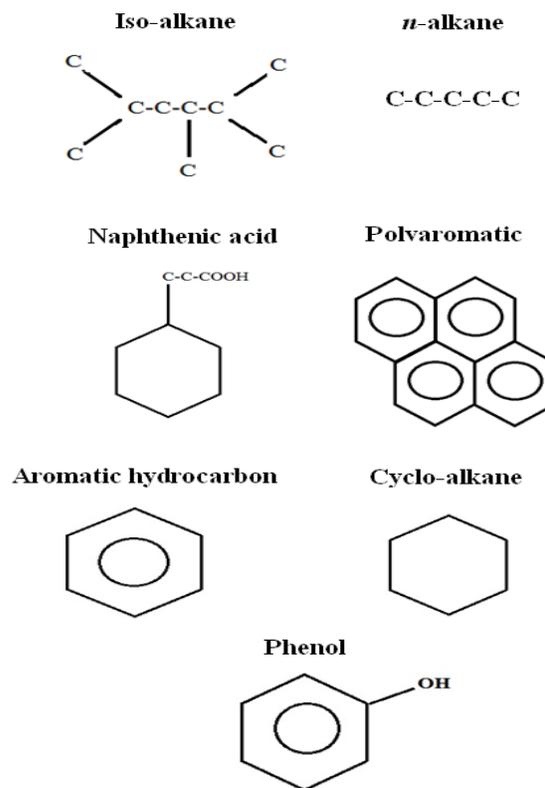


Figure 1: Chemical structure of some crude oil components

Fate of PHs in soil environment

It is essential to advance understanding about the fortune of PHs within the surroundings in order to regulate and remove pollution. The soil rhizosphere helps in structural rearrangement of hydrocarbons arriving from several sources (Varjani and Upasani, 2016). As soon as go in to the soil, the multifaceted compounds of PHs can detach into distinct amalgams dependent on their physicochemical possessions. The environmental fate of these pollutants may be dissimilar from that of distinct petroleum compounds due to the structural arrangements and interactions among hydrocarbons, soil, and microflora (Atlas, 1981; Salanitro, 2001). Resistance of hydrocarbons to soil microflora in soil/water lean towards to rise with the nature and molecular weight of hydrocarbons. Petroleum



crude oil undergoes a number of weathering courses, such as sorption, degradation, vaporisation, photodestruction, emulsification, or biodegradation, which naturally degrade its constituents (Al-Majed *et al.*, 2012; Wu and Coulon, 2016). Amalgams of lower molecular weight, e.g. benzene, xylene and toluene can easily move in the atmosphere and are possibly more prone to volatilize in to the air or penetrate towards the groundwater as compared to hydrocarbons of higher molecular weight (Huang *et al.*, 2013; Li *et al.*, 2013). Commonly, hydrocarbon compounds with straight and limited chains are degraded more easily than those having five or six rings. Compounds with high molecular weight, such as polyaromatic hydrocarbons (PAHs), have a high tendency to adsorb on soil particles and persist relatively fixed at the site where they are dropped till they are dispersed into minor segments and are mineralized by microbes (Atlas, 1981; Salanitro, 2001; Glick, 2010; Sessitsch *et al.*, 2013; Fatima *et al.*, 2017).

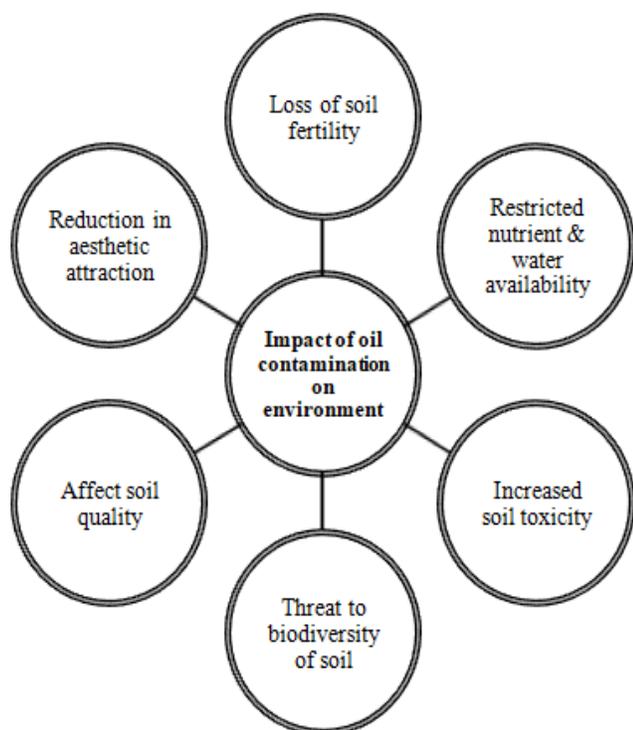


Figure 2: Concerns due to crude oil contamination of soil

Soil remediation: Preserving a precious resource

Physicochemical vs. biological methods

Many conventional physical decontamination methods, e.g., soil washing, incineration, and solvent extraction, are expensive due to diggings and moving of huge amount of

contaminated material for *ex-situ* treatment (Afzal *et al.*, 2014a). Other physicochemical techniques are the use of dispersants, cleaners, emulsifiers, surfactants, soil oxidizers, abiotic transformations, and chemical inactivation (potassium permanganate/hydrogen peroxide are used as chemical oxidants to mineralize non-aqueous hydrocarbons) (Kuppusamy *et al.*, 2016). Though, there is growing discussion about the practice of these techniques as they involve the relocation of contaminants away from the original site or produce secondary pollution (Bao *et al.*, 2012; Ferradji *et al.*, 2014). Therefore, the increasing cost and limiting efficacy of these out-of-date methods have impelled the progress of innovative and substitute expertise for *in situ* remediation of contaminated lands, particularly based on biological approaches. On-site operation of biological technology is less expensive and causes minimal site disruption, therefore, it has greater public acceptance (Afzal *et al.*, 2011, 2012; Chen *et al.*, 2012; Burghal *et al.*, 2015; Athar *et al.*, 2016).

Biological methods are efficient, versatile, cost-effective, and environmentally safe (Abhilash and Yunus, 2011; Abhilash *et al.*, 2012; Shabir *et al.*, 2016; Ahsan *et al.*, 2017; Arslan *et al.*, 2017). Different biotic apparatuses that can be applied for soil clean-up are: 1) use of microbes (fungi/bacteria) to degrade carbon-based chemicals, 2) usage of plants, particularly fast-growing plants with huge biomass and 3) soil fauna (e.g., earthworms) to store or stabilize the non-degradable pollutants in their body or in the soil; and 4) the combined use of plants and bacteria i.e., microbe-assisted phytoremediation (Singh, 2009; Hodson, 2010; Banwart, 2011; Yousaf *et al.*, 2011; Ahmad *et al.*, 2012; Shehzadi *et al.*, 2014; Tara *et al.*, 2014).

Bioremediation: A natural method for the restoration of polluted sites

Bioremediation utilizes biological agents (green plants and microorganisms) or their metabolic capabilities to degrade or transform many environmental pollutants in both terrestrial and aquatic ecosystems (Ron and Rosenberg, 2014). Due to the abundance of microorganisms, their capacity to grow even in anaerobic conditions, and large biomass relative to different residing organisms inside the Earth, they make a suitable means for bioremediation. In biodegradation, microbes utilize chemical contaminants within soil as sole carbon source and degrade the desired contaminant into carbon through redox reactions (Singh, 2009; Yousaf *et al.*, 2010; Abhilash *et al.*, 2012; Hassanshahian *et al.*, 2012; Ron and Rosenberg, 2014). Byproducts are released again into the environment usually in a lesser toxic form. Microorganisms present in contaminated areas adjust themselves in keeping with the situation accordingly of which genetic



transformations are activated in next generations organising them to emerge them as hydrocarbon degraders (Beškoski *et al.*, 2017). It is a well-known fact that hydrocarbon-degrading microbes in uncontaminated ecosystem constitute less than 0.1% of the total microscopic microbes. This quantity may rise up to 1-10% of the whole inhabitants in PHs contaminated site. However, overall microbial diversity in a polluted environment is declined (Nazina *et al.*, 2005; Ferradji *et al.*, 2014; Mnif *et al.*, 2014; Varjani *et al.*, 2015). Aerobic environment and suitable microorganisms are necessary for an optimal rate of bioremediation of soils contaminated with PHs. Therefore, hydrocarbon-degrading bacteria are the best candidate to be used in bioremediation of soil contaminated with crude oil because they can adapt rapidly to the contaminated environment and release variety of enzymes to detoxify pollutants (Khan *et al.*, 2013a).

Biodegradative bacteria

Hydrocarbons might be degraded completely within couple of hours, days, or months by action of microbes. A number of investigation reports indicated that low molecular weight alkanes are degraded more quickly by soil microorganisms than high molecular weight alkanes (Fuentes *et al.*, 2011; Hamzah *et al.*, 2013). Petroleum is a combination of various compounds and no individual bacterium can utilize all components present inside petroleum because single bacterium can degrade only a narrow range of hydrocarbons (Mukred *et al.*, 2008; Alkhatib *et al.*, 2011). Bioremediation requires the dynamic synergy of different microbes to treat a wide ranging environmental contaminants such as pesticides and complex hydrocarbons (Mittal and Singh, 2009; Varjani and Upasani, 2013; Sajna *et al.*, 2015). It has been suggested that certain microorganisms may make PHs more bioavailable. This could happen through the development of a bacterial biofilm specifically on PHs (Norman *et al.*, 2002; Flemming and Wingender, 2010). Several microbes have the tendency to form multi-cellular aggregates joined together to form biofilms (Dasgupta *et al.*, 2013). Biofilms can be formed by single type of bacteria or even by different species of bacteria. The potential of microbial aggregates in the biofilm communities for bioremediation is always a safer method than free-living microorganisms as the biofilm protects them during stress giving the bacterial cells a better chance of adaptation to harsh environments (Decho, 2000; Klein *et al.*, 2010).

Specific microbes have the capability to degrade aliphatics, certain can degrade monoaromatics or polyaromatics, whereas others degrade resins (Khan *et al.*, 2013a). Hydrocarbons degrading microbes and the nature of hydrocarbons degraded by them is enlisted in Figure 3.

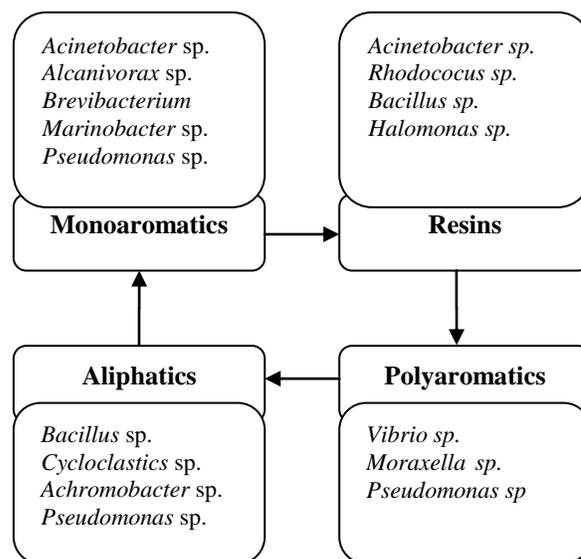


Figure 3: Bacterial species involved in the degradation of different types of PHs

Concerns associated with bioremediation

Bioremediation of PHs is thought to be a complicated phenomenon due to the lethal and hydrophobic nature of the contaminants, changes in microbial surroundings, and certain biotic and abiotic factors of soil including temperature, pH, composition, and moisture (Boopathy, 2000; Klein *et al.*, 2010). Certain factors modify the rate of microbial uptake and alter the rate of movement of contaminant to the bacteria (bioavailability) (Shukla *et al.*, 2010; Adams *et al.*, 2014; Afzal *et al.*, 2014a; Adams *et al.*, 2015). Important factors that have a significant impact on the effectiveness of bioremediation process are shown in Figure 4.

Contaminant concentration and characteristics

The type and concentration of environmental contaminants have a direct influence on microbial growth and activity. When the concentration is too high, it may have a toxic effect on bacteria. On the other hand, low concentration may prevent induction of pollutant-degrading genes present in bacteria (Bhatnagar and Kumari, 2013; Nandal *et al.*, 2015).

Bioavailability of contaminants

Bioremediation efficiency to a great extent relies upon the degree of the bioavailability of the contaminant and consequent metabolism by the microorganisms. It is generally believed that bioavailability of hydrocarbons decreases with increasing molecular mass. Moreover, the rate of



bioremediation in soil decreases with increase in residence time of PHs. Aging hinders the movement of pollutants into soil leading to the alteration and/or absorption of pollutants on soil particles. This practice restrains the release of PHs into the liquid phase making them unavailable to microorganisms, thus lowering their biodegradation rate (Tang *et al.*, 1998).

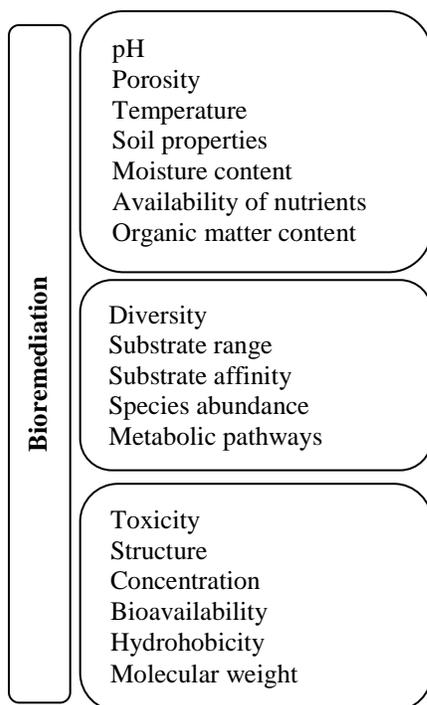


Figure 4: Factors influencing the process of bioremediation of soil contaminated with PHs

Soil properties

Another significant aspect that affects the rate of biodegradation is the chemical/physical/biological properties of soil. Due to the hydrophobic nature of PHs, they become hypothetically inaccessible for microbial degradation. Their degradation occurs when they come in contact with aqueous material as only small fraction of these mixtures become in water-dissolved condition. Additionally, the rate of biodegradation largely depends upon the soil type. Low fractions of clay and slit in soil have been associated with higher availability of hydrocarbon (Mohan *et al.*, 2006).

Temperature

It also influences the biodegradation of hydrocarbons. Despite the fact that hydrocarbon biodegradation can occur over an extensive range of

temperatures, the rate of biodegradation usually declines with the diminishing temperature. It also has a major effect on microbial metabolism and consequently on microbial activity in the environment (Srivastava *et al.*, 2014).

Nutrients

Nutrients (nitrogen, phosphorus and/or iron) play an essential role in biodegradation of PHs. Appropriate amounts of these nutrients are already present in the soil but with high concentrations of pollutants there, they become limiting factors thus affecting the process of biodegradation. To overcome this limitation, nutrients can be added in useable form or with organic amendments (Foght *et al.*, 1996).

Moisture content

For efficient microbial activity, optimum amount of water in soil environment is essential. For optimal growth and development, microorganisms require approximately 25% of moisture contents in the soil (Das and Chandran, 2010).

Redox potential

It is influenced by the presence of electron acceptors including manganese and iron oxides in soil. It is suggested that redox potential increases the degradation of PHs by expanding their bioavailability thus increasing microbial metabolism (Varjani and Upasani, 2016).

Phytoremediation: Using green technology to restore contaminated environment

In general, it was believed that plants can only supply fibre, energy, and food. However, their promising role in eliminating contaminants from environment have been documented in the past two eras (Khan *et al.*, 2013a). Phytoremediation is a promising phenomenon where green plants are used to diminish, eliminate, detoxify, and immobilize toxins with the purpose of restoration of a site to a condition that can be used for private or public applications (Ijaz *et al.*, 2015). It provides a solution to the problem of sites contaminated with organic and inorganic contaminants which includes metals, insecticides, solvents, explosives, and PHs. Growth of plants and their capacity to tolerate high concentrations of pollutants are the factors responsible for their efficiency in phytoremediation (Chen *et al.*, 2015). The benefits and limitations of phytoremediation are listed in Table 1.

Plants use various mechanisms to remove and/or uptake organic and inorganic contaminants that forms the basis of phytoremediation technology. For removal of



environmental contaminants, they utilize dynamic processes including rhizofiltration, phytovolatilization, phytostabilization, phytodegradation, and rhizodegradation (Meagher, 2000; Liang *et al.*, 2017). The initial step of efficient phytoremediation is plant uptake of hydrocarbons. Contaminant uptake and transport takes place in the two-vessel system of xylem and phloem for subsequent accumulation and degradation within the plant (Chen *et al.*, 2015; Patowary *et al.*, 2017; Wei *et al.*, 2017). The pathway by which pollutants enter the plants depends upon their physicochemical properties including hydrophobicity, water solubility, and vapour pressure. Hydrophobicity is usually expressed as coefficient of octanol/water partition (K_{ow}), wherein a log of K_{ow} value (0.5-3.5) make sure take-up of pollutants by plants whereas higher values mainly result in sorption to roots and insignificant translocation in aerial parts of plants (Gerhardt *et al.*, 2017; Kuppusamy *et al.*, 2017; Thomas *et al.*, 2017).

Table 1: Pros and cons of phytoremediation

Pros	Cons
<i>In situ</i> , proficient and environment friendly technology	Technology is limited to shallow ground water and soils. Highly dependent on soil properties and environmental conditions
Applicable on moderate and low levels of contamination	Not applicable in high concentrations of contaminants
Fast and beneficial for breaking down diverse organic pollutants	Slower than physico-chemical treatments and often in need of supplementary treatments such as nutrient supply
High public acceptance	Toxicity and nature of biodegradation products are not known
Aesthetically pleasant	Results are variable
Reduces landfill wastes	Effects to food web might be unknown
Harvestable plant material	Contaminant fates might be anonymous

Plant selection for phytoremediation

Selection of appropriate plant species is critical consideration for implementing phytoremediation strategies. Common factors for selection of trees or grasses generally include: 1) resistance to contaminants, 2) tolerance to environmental conditions, 3) high productivity, 4) low bioaccumulation, 5) suitability for various soil types, and 6) native to avoid the introduction of invasive species. Several reports indicated that shrubs,

grasses, herbs and trees are suitable candidates that can be utilized for phytoremediation (Farraji *et al.*, 2017; Patel and Patra, 2017; Singha and Pandey, 2017; Shahsavari *et al.*, 2016). Legumes (e.g. alfalfa, clover, and peas), grasses (e.g. ryegrass, kallar grass, and para grass), and trees (e.g. *Populus* sp., *Conocarpus erectus* and *Acacia nilotica*) have been proven to be tolerant to hydrocarbon pollutants (Yousaf *et al.*, 2010).

Benefits of grasses, legumes, and trees in phytoremediation

Grass species are excellent contenders for phytoremediation because of their widespread fibrous root structure that result in increased rhizosphere and ultimately abundant area for microbial activity and growth (Shahsavari *et al.*, 2016). Additionally, grasses can proficiently eradicate hydrocarbons from polluted soil, without any nutrient requirement, and show broad usage to the harms associated with hydrocarbon pollution (Yi and Crowley, 2007).

The other important factor to be considered in phytoremediation is the level of nutrients in polluted soils. Soils contaminated with elevated amounts of crude oil are often deficient in nitrogen. Legumes might be utilized in phytoremediation because of their symbiotic-association with nitrogen fixers (bacteria and fungi) and arbuscularmycorrhizal fungi (Farraji *et al.*, 2017; McIntosh *et al.*, 2017). The root system of leguminous plants generally is not as flourished as grasses to reach deeper soil layers (Safronova *et al.*, 2011). Legumes have preference over non-leguminous plants on account of their inherent capacity to fix nitrogen. Moreover, legumes do not need to contend with microorganisms and different plants for accessible soil nitrogen at oil-contaminated sites; they additionally stimulate attached microorganisms by discharging nutrients into the rhizosphere (Brígido and Glick, 2015). Legumes, such as *Medicago sativa*, *Vulpamyuros*, *Elymus* sp., *Trifolium* sp. and *Phalarisarundinacea* have been effectively applied to clean polluted places, particularly petrochemical waste polluted sites (Minoui *et al.*, 2015).

Trees additionally play an essential role in the process of phytoremediation. Proper selection of tree species and variety/genotype is important criteria to predict the phytoremediation efficacy (Hussain *et al.*, 2017; Luo *et al.*, 2017). Trees typically have greater root biomass and deeper root systems than grasses, thereby occupying a greater soil volume than grasses. Common reasons behind the excessive use of trees in phytoremediation are their easy propagation, fast



growth, deep root systems that stretch out to the water table, high water take-up rates, maximum absorption surface areas, perennial growth, and/or tolerance to contaminants (Iori *et al.*, 2017). For instance, poplar and willows have been carefully chosen as potential contestants in phytoremediation of both organic and inorganic contaminants (Mathur *et al.*, 2010).

Microbe-assisted phytoremediation: An optimal approach to revitalize ecosystem

The efficacy of plant-based remediation is often restricted by two factors: (1) the toxic nature of environmental contaminants, and (2) loss of soil fertility in the form of unavailability of nutrients and modification of soil texture. In contrast, microbial degradation often faces difficulty due to the inability of existing microflora to degrade the contaminants, insufficient nutrients in contaminated soil, and low bioavailability of pollutants (Noroozi *et al.*, 2017; Patel and Patra, 2017). Therefore, an optimal system is obligatory in order to overcome these constraints. Dynamic synergy between plant roots and soil microorganisms has received great attention due to the possible role of bacteria in plant development and degradation of PHs (Kamath *et al.*, 2004; Vaziri *et al.*, 2013). The inoculation of specific bacteria increases plant resistance to pollutant stress and enhances plant biomass. In response, vegetation, through its rhizospheric effects, supports the proliferation of hydrocarbon degrading microbes, which results in the mineralization of recalcitrant organic pollutants (Kuiper *et al.*, 2004; Glick, 2010). The combined use of phytoremediation and microbial augmentation techniques develop a more effective strategy for the restoration of recalcitrant pollutants, predominately polyaromatic hydrocarbons (Afzal *et al.*, 2013; Khan *et al.*, 2013b; Arslan *et al.*, 2014).

While it is broadly accepted that bacteria and fungi are chief mediators in hydrocarbon degradation, bacteria have been revealed to be more versatile than fungi (Weyens *et al.*, 2009; Khan *et al.*, 2013a; Sessitsch *et al.*, 2013). Bacteria are ubiquitous -living in the rhizosphere, rhizoplane/ phyllosphere, and plant interior, thus can be considered active players in the cleanup strategy for hydrocarbon remediation (Truu *et al.*, 2015; Pandey *et al.*, 2016a). Microbes having both hydrocarbon-degrading and plant growth promoting (PGP) abilities more actively reduce stress symptoms in plants and detoxify soil pollutants as compared to microorganisms having just contaminant degrading/PGP capabilities (Ma *et al.*, 2011). Plant growth promoting bacteria actively stimulate the

growth via different mechanisms, such as fixation of N₂, P-solubilization, siderophores, and production of 1-amino cyclopropane 1-carboxylate (ACC) deaminase, thus assisting plants to overcome stress, enhance plant defense towards pathogens, and stimulate biodegradation process (Figure 5) (Compant *et al.*, 2010). Ethylene (plant hormone) assumes a vital part in root initiation, extension, fruit ripening, and in stress signaling too. The inhibition of growth that occurs as a result of environmental stress is the outcome of the plant reaction to increased amounts of ethylene (Belimov *et al.*, 2001). However, bacteria producing ACC deaminase can bring down levels of ethylene by cleaving the ethylene precursor ACC and mitigate stress in developing plant (Saleem *et al.*, 2007; Rai *et al.*, 2016). Numerous bacteria produce indole 3-acetic acid (IAA) which plays an important role in the development of extensive root system and prompting enhanced uptake of nutrient that, thus, stimulates bacterial propagation in root zone (Saleem *et al.*, 2007). It has been recommended that bacterium producing IAA might prevent the deleterious impacts of environmental stresses on plant development. Thus, the collective application of vegetation and such microorganisms may be an important alternative for remediation of oil-polluted soils (Benson *et al.*, 2017; Salam *et al.*, 2017; Spada *et al.*, 2017).

The adequacy of plant-bacteria partnership relies to a great extent upon the persistence and metabolic capability of bacteria harboring catabolic genes necessary for the enzymatic degradation of PHs (McGenity *et al.*, 2012; Shankar *et al.*, 2014). It is additionally essential to screen the abundance and expression of specific genes during remediation of oil-polluted soil to get proof of the survival and metabolic activity of the inoculated microbes (Afzal *et al.*, 2011, 2012, 2013). Culture-dependent techniques are classical means of determining microbial population changes. Though, less than 1% of environmental bacteria are cultivable (Andria *et al.*, 2009). Culture-independent procedures have opened gateways for a deeper understanding of microbial communities enlightening information about gene abundance and expression (Yan *et al.*, 2016). Culture-independent approaches give molecular insights of the bacteria present in a particular site at a definite time (Gandolfi *et al.*, 2017; Wang *et al.*, 2017). Nucleic acids are also analyzed by way of fingerprinting of functional genes (e.g., *alkB* gene) or a quantitative PCR (*qPCR*) to reveal the presence of specific bacteria in an environment (Blain *et al.*, 2017; Li *et al.*, 2017).



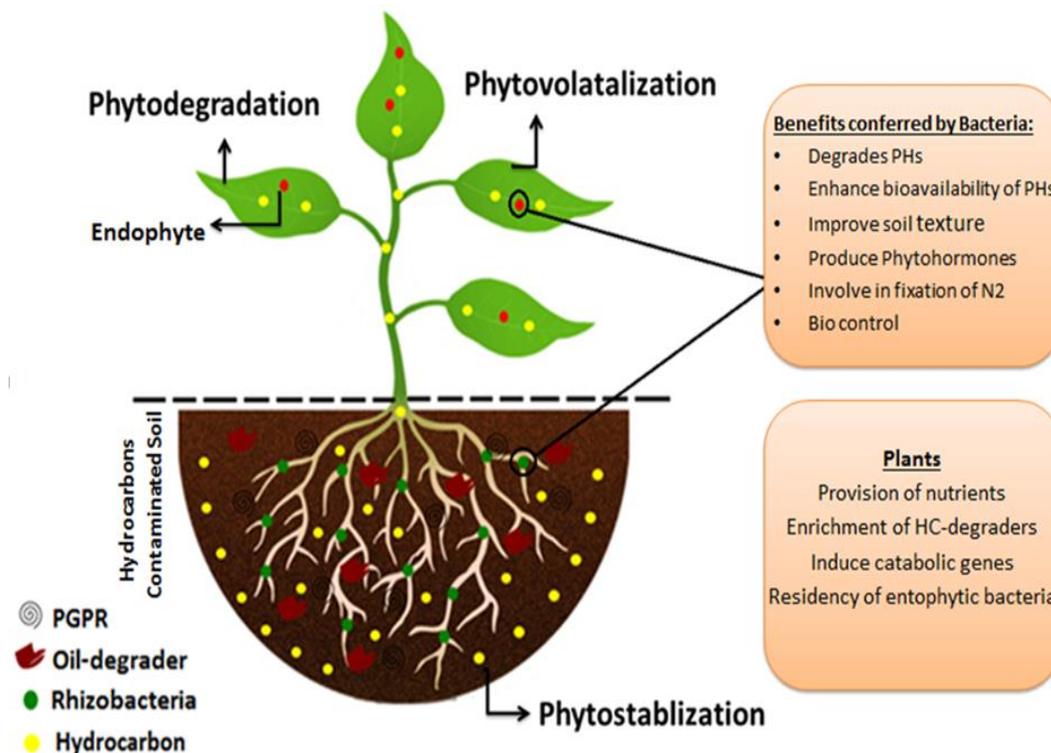


Figure 5: Plant-microbe interactions that lead to remediation of soils contaminated with PHs

Rhizoremediation: Use of rhizobacteria to enhance hydrocarbon phytoremediation

Rhizoremediation is described as the use of plants and their root-associated microorganisms to decontaminate oil-polluted soils. This beneficial association relies on the fact that bacteria increase the bioavailability and degradation of organic pollutants, in turn, plants provide residency and food to the bacteria (Muratova *et al.*, 2003; Weyens *et al.*, 2009). Despite the fact that rhizoremediation happens naturally, but through deliberate manipulation (inoculating the soil with contaminant degrading and/or PGP bacteria) in rhizosphere it can be enhanced (Hernández-Vega *et al.*, 2017; Vergani *et al.*, 2017).

The rhizosphere is a densely-populated zone wherein enhanced microbial activities are witnessed and plant roots have interaction with soil-borne microorganisms by exchange of essential supplements, growth factors and so forth (Abioye *et al.*, 2017; Kong and Glick, 2017). Increased removal of persistent contaminants in the rhizosphere is perhaps the consequence of greater microbial populations and functional actions around the roots of plants. Growing plants release a diversity of chemicals in root exudates. Following root exudation, the proliferation of specific group of bacteria is 10-1000 folds more prominent in the rhizosphere than in the bulk soil; this occurs due to excessive level of nutrients found in exudates (Adam, 2016; Maddela *et al.*, 2016; Wu *et al.*, 2017). The properties, amount and timing of root

exudation are critical for rhizoremediation process (Kang, 2014). In soil, plants may react to chemical stress with the aid of changing the composition of nutrients that, thus, adjust the metabolic potential of microorganisms. The microbial activity in the close vicinity of the root seems to offer a promising environment for degradation of obstinate chemicals (Hassanshahian *et al.*, 2012; Hassanshahian *et al.*, 2014). These activities also increase the bioavailability of soil-bound nutrients and degradation of phytotoxic soil contaminants in the rhizosphere (Gkorezis *et al.*, 2016).

Rhizobacteria with plant growth-promoting capability have been conventionally used in agricultural science to improve crop yields. Nevertheless, prospective role in the remediation of environmental pollutants have been sightseen in recent times (Dong *et al.*, 2014; Ajuzieogu *et al.*, 2015; Hou *et al.*, 2015). Organic compounds including PHs, pesticides, chlorinated compounds (polychlorinated biphenyl), explosives, organophosphate insecticides (diazinon and parathion), and surfactants (detergents) are more rapidly degraded by rhizospheric bacteria (PizarroTobías *et al.*, 2015; Thijs and Vangronsveld, 2015).

The successful application of rhizoremediation largely depends upon survival and establishment of bacteria in the rhizosphere. This phenomenon has been widely studied, but the complete mechanism is as yet not clear; it has been suggested that it may be because of the secretion of certain compounds (e.g. polysaccharides) and other phenomenon such as chemotaxis (Afzal *et al.*, 2013; Fester *et al.*, 2014).



It is supposed that a plant and its related bacteria establish bacterial colonization on root surface through complex chemical signals which includes hydrogen peroxide, superoxide anion and especially flavonoids (Lugtenberg *et al.*, 2001; De Weert *et al.*, 2002; Kamilova *et al.*, 2006; Nadeem *et al.*, 2016). This communication is of extreme significance for the persistence and colonization of applied bacteria in the plant rhizosphere. Numerous investigations have been executed to see persistence of inoculated bacteria, especially through labelling of inoculated bacterial strains with a marker gene, for example *gfp* encoding green fluorescent protein, *gusA* encoding β -glucuronidase and so forth (Swamy *et al.*, 2016).

Endophyte-assisted phytoremediation

In addition to rhizobacteria, plants are internally colonized by bacteria, fungi, and actinomycetes. Endophytes can be defined as pathogenic and nonpathogenic microbes living inside plant organs (root/shoot). They are ubiquitous (found in all plant species), diverse in nature, and residing in a dormant or active state in the plant tissues (Compant *et al.*, 2010). Endophytes interact more closely with the host while savoring a less competitive environment which has high amount of nutrients and is highly protective against wide-ranging fluctuations than the environment that rhizo- or phyllospheric bacteria usually face. Endophytes gain entry in plant tissue through the roots, followed by habitation in the root cortex or aerial parts of plants via plant vascular system. Additionally, cell wall-degrading enzymes favor the entrance of such microbe into plants. Endophytes have to proliferate in the rhizosphere before entering the plant (Afzal *et al.*, 2014a). Additionally, cell wall-degrading enzymes favour the entrance of such microbe into plants. Endophytes have to proliferate in the rhizosphere before entering the plant (Blain *et al.*, 2017; Oliveira *et al.*, 2017). During endophytic colonization, bacteria move to the plant roots either inertly by soil water oscillations or actively through particular stimulation of flagella. In addition, root exudates, act as indicators for chemotactic movements and provide a nutrient-rich environment for active colonization (Shehzadi *et al.*, 2016; Zhu *et al.*, 2017a).

Despite the fact that rhizoremediation seems promising, the contaminant is not accessible to the rhizospheric microflora because its residence time is very much lower in the rhizosphere (Pandey *et al.*, 2016b; Su *et al.*, 2016; Zhu *et al.*, 2017b). Here, endophytic bacteria get the chance to breakdown the contaminants with the assistance of their intracellular enzymes before than the contaminants are evapotranspired. Additionally, a most important advantage of endophytic bacteria over rhizobacteria is that they are living inside the tissues of the

host plant and consequently have lesser competition for nutrients and space (Shehzadi *et al.*, 2016; Goodwin and Gao, 2017).

Endophytes assume a key role in plant's adaptation to contaminated surroundings and furthermore improve phytoremediation by transforming contaminants, stimulating plant growth, subsiding phytotoxicity, and improving overall plant's health (Compant *et al.*, 2005; Compant *et al.*, 2008). Many endophytic bacteria exhibit PGP activities, for example nitrogen fixation, production of phytohormones (IAA and ACC deaminase) and hydrolytic enzymes (HCN and siderophores) (Weyens *et al.*, 2009). These PGP actions of endophytic bacteria improve the plant growth in contaminated soils and eventually phytoremediation efficiency. Further to modify the phytohormone intensities in plants, some endophytes can speed up plant growth via fixation of nitrogen (Naveed *et al.*, 2014). An outstanding illustration is the nitrogen-fixing endophytes of sugarcane, which provides ample nitrogen to the plant and enhance plant growth. Moreover, some endophytes enhance plant growth by enhancing mineral nutrition or increasing resilience to biotic and abiotic stresses (Compant *et al.*, 2016).

Metabolic pathways for biodegradation of PHs

Most microbial species do not contain all the appropriate enzymes so degradation is a collective function of a consortium of microorganisms belonging to different genera. Microbes either catabolize organic contaminants to get energy or integrate them into cell biomass (Rahman *et al.*, 2003).

Hydrocarbon-degrading bacteria may be categorized into two groups: 1) aerobic, and 2) anaerobic. Aerobic conditions facilitate the fastest and complete degradation of most hydrocarbons because during metabolic activities oxygen is available as an electron acceptor (Abbasian *et al.*, 2015; Pugazhendhi *et al.*, 2016). Conceivable exterior pathways for aerobic biodegradation of *n*-alkanes and aromatic hydrocarbons are described in Figure 6.

Aerobic biodegradation

In aliphatic hydrocarbons, the crucial step for aerobic degradation involves the addition of oxygen by oxygenases and peroxidases (Tolosa *et al.*, 2004). Peripheral degradation pathways (terminal/sub-terminal oxidation) convert activated molecules to intermediates in a step-by-step process followed by conversion into a fatty acid. This molecule is then conjugated to coenzyme A which forms an acyl-CoA which is then converted into acetyl-CoA (final product). Acetyl-CoA enters in the Krebs cycle and eventually completely oxidized to CO₂ (Chandra *et al.*,



2013). Other oxidation pathways include di- and sub-terminal oxidation of *n*-alkanes. In di-terminal pathway, oxidation of both ends of alkane molecule takes place through α -hydroxylation (α position represents terminal methyl group) of fatty acids. It is then further converted

bioremediation programs. For initial activation, four different enzymes are involved; 1) the non-heme iron oxygenases, 2) the soluble di-iron multi component, 3) the flavoprotein monooxygenases, and 4) the CoA ligases (Nishino *et al.*, 2000). Unlike aliphatic hydrocarbon

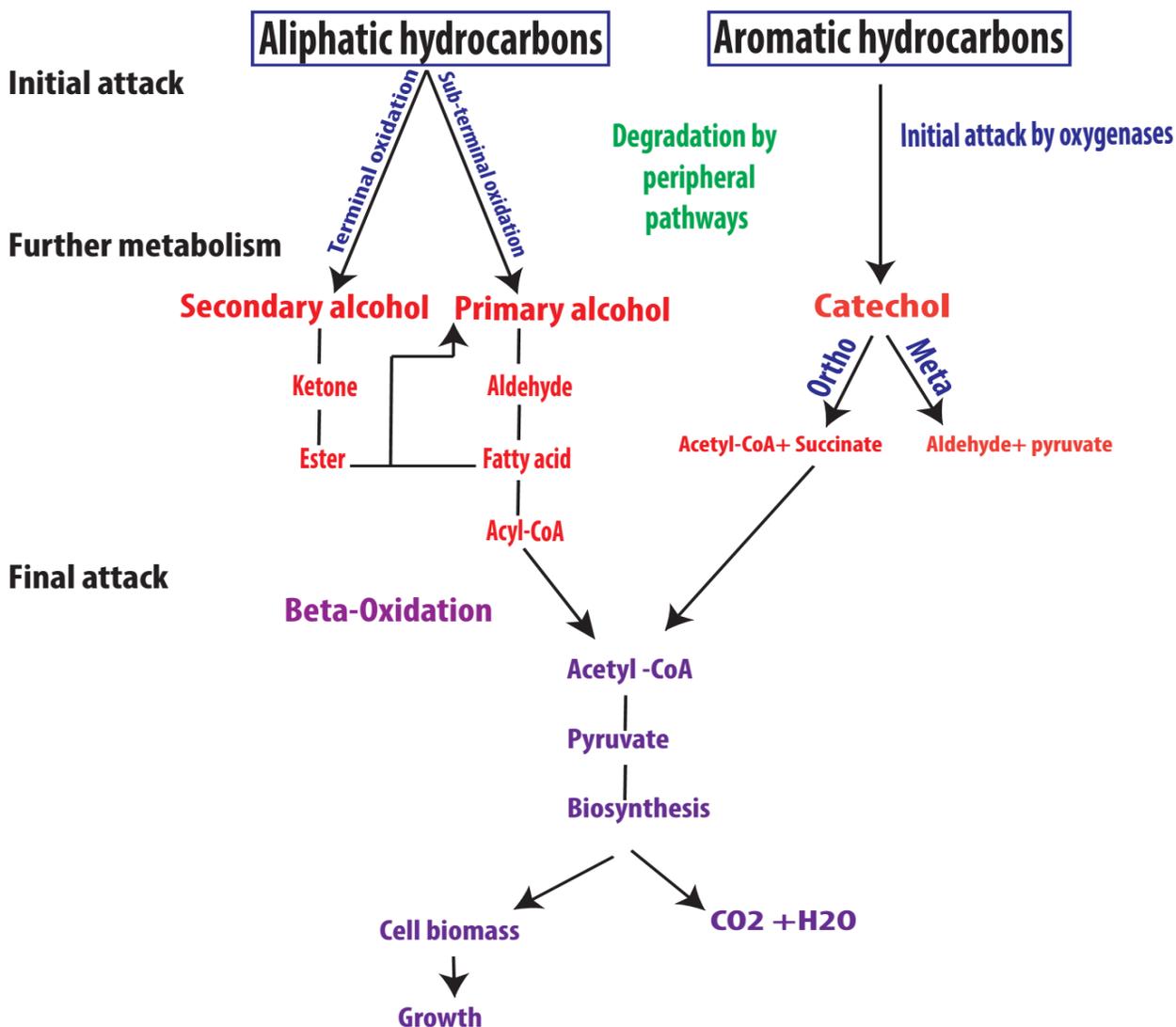


Figure 6: Schematic overview of metabolic pathways for hydrocarbons (aliphatic and aromatic) utilization by aerobic bacteria. Ortho: ortho cleavage pathway, meta: meta cleavage pathway, CoA: coenzyme A

into dicarboxylic acid and processed by β -oxidation pathway. Cell biomass is produced from the central precursor metabolites (acetyl-CoA and pyruvate).

Polyaromatic hydrocarbons such as biphenyls and naphthalene are more persistent in the environment than saturated hydrocarbons (Field *et al.*, 1995). Due to their toxic nature, they are the priority pollutants in

degradation, activated molecule is not transformed to alkanol but rather to intermediates of phenol (catechol). (Kulkarni and Chaudhari, 2007). Intradiol or extradioldioxygenases will further convert these phenol intermediates to di or tri-hydroxylated aromatic compounds that may enter into the Krebs cycle and completely metabolized into CO_2 (Abramowicz, 1990). Hydrocarbon degrading bacteria cleave benzene ring in diverse ways by



appropriate enzymes. In PAHs, benzene rings are degraded one after the other. But in case of cyclic alkanes, transition from alkane to alcohol takes place which is further dehydrogenated to ketones by an oxidase system. Alkenes may be attacked by (a) terminal oxygenase, (b) sub-terminal oxygenase, and (c) oxidation of double bond to resultant epoxide/diol.

Anaerobic biodegradation

Microbial degradation of various substrates, specifically obstinate hydrocarbons, is restricted under anaerobic conditions because O₂ is prerequisite for this process (Varjani and Upasani, 2017). Understanding of the mechanism of anaerobic degradation is more recent as compared to aerobic degradation. Therefore, less information is available about the genes and enzymes

hydrocarbons (up to n-C17) do not dissipate easily, so these compounds can develop and exert a harmful effect on the cell wall of bacteria, thus inhibiting their growth. Moreover, sulphate reducing bacteria degrade branched alkanes more efficiently than straight chain alkanes (Arulazhagan *et al.*, 2017; Ghattas *et al.*, 2017).

Anaerobic degradation is commonly established in deep and anoxic environments for example natural oil seeps on land/ocean and the sites polluted with oil. Likewise, this kind of biodegradation can happen beneath the surface of areas where aerobic biological activity has been ceased as all the oxygen is used. After oxygen exhaustion, there may be a consecutive employment of the electron acceptors (nitrate, ferric iron, sulphate, and hydrogen) to supply energy from the hydrocarbon mineralization (Aktas *et al.*, 2017; Arulazhagan *et al.*, 2017; Ghattas *et al.*, 2017;

Table 2: Bacterial and plant enzymes involved in alkane degradation

Enzyme	Catalytic action	Origin/Reference
Dehalogenase	Involved in release of chlorine and fluorine from halogenated straight chain and ring compounds	<i>Xantho bacterautotrophicus</i> , <i>Populus</i> spp and <i>Protobacteria</i> (Compant <i>et al.</i> , 2010)
Lacasse	Degrade numerous aromatic hydrocarbons	<i>Alfalfa</i> , <i>Trametesversicolor</i> and <i>Coriolorpsispolyzona</i> (Alaxender 1978; Reda <i>et al.</i> , 2010)
Dioxygenase	Degrade specific aromatic rings	<i>Pseudomonas</i> sp., <i>Mycobacterium</i> sp. (Mohan <i>et al.</i> , 2006)
Peroxidase	Involved in degradation of several aromatic compounds; dehalogenation of various n-alkanes	<i>Armoraciarusticana</i> , <i>Phanerochaetechrysosporidium</i> , <i>Phanerochaetelaavis</i> , <i>Medicago sativa</i> (Foght <i>et al.</i> , 1996)
Nitrilase	Cleaves cyanide group from aliphatic and aromatic nitriles	<i>Salix</i> spp., <i>Aspergillusniger</i> (Klein <i>et al.</i> , 2010)
Nitroreductase	Reduces nitro groups on nitro-aromatic compounds; removes N from ring structures	<i>Comamonassp</i> , <i>Pseudomonas putida</i> , <i>Populusspp</i> (Ijaz <i>et al.</i> , 2015)
Phosphatase	Cleaves phosphate groups from pesticides	<i>Spirodelapolyrhiza</i> (Bhatnagar and Kumari 2013)
Cytochrome p450 monooxygenase	Hydroxylation of ring and straight chain hydrocarbons	Bacteria, fungi and plants (Iori <i>et al.</i> , 2017)

involved in these pathways. During degradation of PHs, anaerobic bacteria offer nutrients required for the growth of other catabolizing bacteria (Varjani, 2017). A large variety of microorganisms (bacteria and archaea) have been identified with the capability to degrade hydrocarbon molecules anaerobically. These bacteria exploit anaerobic respiration via nitrate, nitrite, and metal ions or fermentation during substrate catabolism (Lueders, 2017; Portugal *et al.*, 2017).

For anaerobic bacteria, alkanes with smaller chain length are difficult to degrade than alkanes having mid- to long-chain lengths. In anaerobic conditions, short-length

Lueders, 2017).

Enzymatic biodegradation

In order to explore functional genes involved in degradation of hydrocarbons, one must have knowledge about the enzymes involved in biodegradation. Though there are few bacteria that can fully mineralize the particular organic pollutant, single species usually do not have the capability to degrade PHs or lack entire degradation pathways (Peixoto *et al.*, 2011). However, consortium of heterogeneous bacterial strains can effectively degrade these recalcitrant compounds fully.



Details of bacterial and plant based degradative enzymes (Van Beilen and Funhoff, 2007) are depicted in Table 2.

The prokaryotic monooxygenases isolated are catalogued into two sets on the basis of their electron transport system and the microorganisms in which they are available: (a) enzyme dependent on rubredoxin (2FeO), in most of bacteria this enzyme is encoded by *alkB* gene and *alkM* specifically in *Acinetobacter* sp., (b) cytochrome P450 monooxygenase belonging to CYP153 family of microbes. Alkane hydroxylase enzyme was firstly described in *Pseudomonas putida* GPo1 where it was located on the OCT plasmid and was reported to be organized in two operons: *alkBFGHJKL* and *alkST* (Throne-Holst *et al.*, 2007).

The cytochrome P450 enzymes are set of heme (iron protoporphyrin IX) comprising monooxygenase enzymes that work in association with sub-atomic oxygen, and an electron-transfer system to oxidize diverse range of compounds (Muangchinda *et al.*, 2015). Rather than eukaryotes, the bacterial cytochrome P450 are soluble in the cytoplasm. The structures of these enzymes vary from species to species.

Moving on, with extensive distribution ranges, various genotypes, and sufficient previous research results, *alkB* gene is considered an encouraging useful biomarker to monitor potential of bioremediation at a site of oil pollution. A number of studies attempted to communicate the degradation practices or contaminant mineralization to kinetic changes of *alkB* gene diversity, abundance, or expression *in situ* (Van Beilen and Funhoff, 2007; Peixoto *et al.*, 2011; Muangchinda *et al.*, 2015).

Conclusion and future prospects

Soil pollution might increase in coming years due to speedy population growth and as a result an enhancement in industrialization, urbanization and intensive agriculture all over the world. To alleviate the harsh belongings of soil pollution by organic chemicals, phytoremediation might be an efficient and inexpensive methodology to remediate contaminated soil and water. Nevertheless, several features still need to be studied to make this technology effective. In particular, consideration should be paid to the exploitation of plant-endophyte synergisms for the clean-up of polluted soil and water. In the case of remediation of soil and water polluted with organic chemicals, endophytes harboring the suitable degradation pathway(s) can support their host plant by degrading pollutants that are freely absorbed by plants. To investigate the whole microbial diversity in a particular environment, the isolation and identification of microorganisms is not an appropriate technique. The modern metagenomic approach facilitate scientists to

analyze the complete bacterial diversity, and the genetic capability for the active metabolic pathways existing in a specified environment. This practice is particularly effective for the study of the biodiversity and genome analysis of complex environmental samples where most of microorganisms cannot be cultured under normal laboratory conditions.

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