Chapter 13
Microbial Bioremediation of Petroleum Hydrocarbon: An Overview

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Abstract Increased environmental toxicity due to extensive use of petroleum-based products gradually proves itself as a major issue of global concern. The release of petroleum products to the environment may cause catastrophic effect on aquatic habitats as well as barrens of fertile soil. Petroleum oil basically contains VOCs (volatile organic compounds), paraffin, gases (methane, ethane, propane, butane, etc.), metal ions (iron, nickel, copper, vanadium, etc.), etc., out of which VOCs may cause severe health problems such as lung, liver and kidney disease. Bioremediation is a process of treatment of contaminated environment with the help of living organisms to bring back to its natural state. Treatment of hydrocarbon-contaminated sites may be accomplished with the help of indigenous microorganisms with diverse groups present in the soil by augmenting with necessary nutrients or by adding external necessary microorganisms. Further, as the petroleum hydrocarbon pollutant creates a stressful environment for growth, the bacterial species having potential to tolerate stress conditions would be an added advantage.

13.1 Introduction

Fossil fuel merchandise increases the probabilities of soil contamination that becomes one of the most important worldwide environmental issues. Statistical report released by the International Tanker Owners Pollution Federation (ITOPF) Ltd. shows incidences of oil spillage in sea since the years 1970–2015 which shows more than 700 tonnes of oil spillage which occurred in year 2015 itself (ITOPF 2016).

NAS report shows over ninetieth of oil spillage incidence is directly or indirectly as a result of human activities together with deliberate oil waste disposal (USEPA 2000; NAS 1985, 2005). Large-scale oil spills, and oil spill accidents, have received a great deal of attention worldwide, as a result of their destructive result on the

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surroundings. Oil spillage on water body ends up in the transience of thousands of aquatic animals and causes major reduction in population of the many aquatic organisms and lots of long-standing environmental impacts (Spies et al. 1996; Campbell and Cary 2001; Van Hamme et al. 2003). Soil contamination primarily arises as a result of petroleum dumping or due to the rupture of underground storage tanks, etc. (Briganti et al. 1997; Butler and Mason 1997; Chang 1998). Minor oil spills and oil contamination as a result of non-point sources like urban runoff, etc. are not any less a threat to public health though they need but received nominal attention in the past. Such non-point sources of pollution remain the largest threat to water body as stated by the reports published by the *National Water Quality Inventory, USA* (Etkin 1998; USEPA 1999, 2000). It may conjointly cause severe health risks to the employees involved in treating of oil spillage areas once exposed to hydrocarbon fumes, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), material from controlled burns, etc. (Bach et al. 2005; Biddle et al. 2006; Campbell and Cary 2001; Chang et al. 2002, 2005; Christopher and Christopher 2004).

13.2 Composition of Petroleum Oil

Petroleum is recovered principally through oil drilling, and it's refined and fractioned into a range of commercial products.

In true sense, fossil oil includes inflammable liquid consisting of a fancy mixture of hydrocarbons of varied molecular weights and alternative liquid organic compounds; however, in common usage it includes all liquid (e.g. pentane and heavier ones), gaseous (e.g. methane, ethane, propane, butane, etc.) and solid (e.g. paraffin) hydrocarbons (Speight 1999). The hydrocarbons in fossil oil are largely alkanes, cycloalkanes and numerous aromatic hydrocarbons, whereas the other organic compounds contain varied metals like iron, nickel, copper, etc. (Speight 1999). Precisely, the molecular composition of hydrocarbons may vary from formation to formation. Chemically, fossil oil contains paraffin (15–60%), hydrocarbon (30–60%), aromatics (3–30%) and mineral (6%); however, the relative proportion of fossil oil largely varies from oil to oil (Mabro 2006). Although the constituents of fossil oil may vary, the elementary compositions may be presented as shown in Table 13.1 (Hyne 2001).

Table 13.1 Elementary compositions of fossil oil

Name of the constituents	Percentage (%)
Carbon	83–87
Hydrogen	10–14
Oxygen	0.05–1.5
Nitrogen	0.1–2
Sulphur	0.05–6
Metals	<0.1

13.3 Petroleum Oil Pollution, Environment and Health

The illegal disposal of crude oil is also one of the major causes of environmental hazard with international ramifications (Blodgett 2001; Guermouche et al. 2015). The discharge of oil into the surrounding causes environmental concern and attracts the general public attention (Roling et al. 2002). Petroleum oil and PAHs (polyaromatic hydrocarbons) have a widespread impact on the body, as prolonged exposure to crude oil might induce liver and excretory organ diseases and bone marrow injury or might cause the event of cancer (Crebelli et al. 1995; Mandri and Lin 2007; Guermouche et al. 2015). Processed engine oil contains additional heavy metals and serious PAHs and therefore contributes a lot more too chronic hazards as well as mutagenicity and carcinogenicity as compared to unused engine oil (Boonchan et al. 2000).

Unlike the claims created by the oil-exploring industries concerning the security measures taken for safe unleash of treated effluents to the surroundings, the digital and print media besides restricted scientific study claims environmental problems associated with oil contamination within the region notably within the abandoned drilling sites (Das et al. 2004; Yenn et al. 2014).

Oil contamination of soil and water isn't solely a regional issue but a worldwide issue of concern. Not only soil, the aquatic system, notably the marines, is the foremost at risk of oil spillage (Cairns and Buikema 1984). Marine oil spillage may have an effect on organisms present therein by direct toxicity or by physical stress (Perry 1980). Oil spills usually will cause varied damages to the marsh vegetation. Oil spill in water body forms a surface slick whose elements will follow several pathways. Some might pass into the mass of water, and proof suggests they will persist for an extended time before their degradation by microorganisms within the water body. The slick sometimes becomes additional viscous and forms water-in-oil emulsion. Oil in water causes depletion of dissolved gases because of transformation of the organic element into inorganic compounds, loss of biodiversity and eutrophication. Toxicity in fishes includes blood disease, dermal dysplasia and pasteurellosis (Beeby 1993). It also affects plant physiology in terms of stem height, photosynthetic rate, overall plant biomass, germination, etc. leading to their death (Krebs and Tanner 1981; Onwurah 1999). Oil spill may increase the mortality rate of a population by damaging the reproductive capacity of the respective population (Hall et al. 2006; Tiido et al. 2006).

It conjointly affects soil fertility by altering the mineral and organic matter content, ion exchange capability, salinity, pH, etc. However, the size of impact depends on the number and kind of oil spilled (Onwurah et al. 2007). As petroleum oil creates anaerobic condition in the soil, coupled to water logging and acidic metabolites, the result is high accumulation of aluminium and manganese ions, which are toxic to plant growth (Onwurah et al. 2007). Petroleum oil hydrocarbons may cause DNA damage, resulting in carcinogenesis, mutagenesis and impairment of reproductive capacity (Short and Heintz 1997). The risk of drinking water contaminated by crude oil can be extrapolated from its effect on rats that developed haemorrhagic tendencies after exposure to water-soluble components of crude oil (Onwurah 2002). Volatile components of crude oil after a spill may lead to asthma, bronchitis and other liver and kidney diseases (Kaladumo 1996; Anozie and Onwurah 2001).

13.4 Countermeasures for Oil Pollution

Conventionally physical, chemical and biological ways are used for the treatment of oil spillage.

13.4.1 Physical Methods

Removal of oil spill in soil and water body may involve mechanical removal methods such as skimming, manual removal (wiping), water flushing, etc. According to the recent USEPA 2015 report, current mechanical methods other than open burning of petroleum oil typically recover up to only 10–15% of oil after a massive oil spill, whereas burning of oil may reduce up to 98% but with a massive air pollution (OTA 1990; NAS 2005; USEPA 2015). Although standard ways, like physical removal of spilled oil, usually are the primary response choice, they rarely succeed in complete clean-up of oil spills.

13.4.2 Chemical Methods

Chemical ways, notably dispersants (synthetic surfactants), are more often utilized in several countries as a response choice (USEPA 1999). However, chemical ways haven't been extensively used due to the disagreement concerning their effectiveness (recover no over 8% of usable oil) and also the issues of their toxicity and long-run environmental effects because of their toxic formulation (USEPA 1999; https://www.restorethegulf.gov/; Hemmer et al. 2011). Synthetic surfactants possess both hydrophilic and hydrophobic groups which form oil-surfactant micelles by decreasing tension between water and oil interface which may lead to further degradation of droplets of the micelles in the water body towards physical and microbial degradation (Hemmer et al. 2011).

13.4.3 Biological Methods

Biological strategies have emerged as promising technology nowadays, notably as a secondary treatment choice for the clean-up of oil spillage. Bioremediation has been outlined as a treatment method of contaminated environment with the assistance of living organisms to bring back to its original state (OTA 1991; Ivanova et al. 2015). This technology is based on the fact that a considerable amount of oil components are mostly biodegradable in nature (Atlas 1981; Atlas 1984; Prince

1993) and hence can be augmented by adding nutrients. Bioremediation has many potential benefits over standard technologies such as it is very cost-effective and eco-friendly in terms of its finished product.

The success rate of bioremediation process is dependent on the capacity to establish favourable conditions in the contaminated site which enhances the rate of hydrocarbon degradation. The vital demand for the success of bioremediation is the presence of indigenous microorganisms with the suitable metabolic activities for degradation of specific type of fossil oil.

There are diverge group of microorganisms known to be capable of degrading variety of target constituents present in oily sludge (Eriksson et al. 2002; Barathi and Vasudevan 2001; Mishra et al. 2001). Out of so many reported microorganisms, various strains of *Pseudomonas* sp. are mostly reported by many researchers as a potential fossil oil degrader (Fall et al. 1979; Johnson et al. 1996; Guermouche et al. 2015). Some of the field trial reports claim successful bioremediation of oil-contaminated nearby areas of abandoned drill sites located particularly in Gelakey, Amguri, Lakwa and Borholla regions of Assam, India, by augmenting contaminated sites with *Pseudomonas* strains designated as N3 and N4 along with plant species *Gmelina arborea, Tectona grandis*, *Michelia champaca* and *Azadirachta indica* (Yenn et al. 2014).

Apart from *Pseudomonas* sp., there are some other bacteria which include *Yokenella* sp., *Alcaligenes* spp., *Roseomonas* sp., *Stenotrophomonas* sp., *Acinetobacter* spp., *Flavobacterium* sp., *Streptococcus* sp., *Providencia* sp., *Sphingobacterium* sp., *Capnocytophaga* sp., *Moraxella* sp. and *Bacillus* sp.; some fungi, viz. *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Eupenicillium* sp. and *Paecilomyces* sp.; etc. are known as potential hydrocarbon degraders (Rusansky et al. 1987; Sharidah et al. 2000; Bhattacharya et al. 2002; Juwarkar 2012; Bujang et al. 2013; Ameen et al. 2015). The component of media plays a vital role in microbial growth and proliferation. Hence, optimization and improvement of media components and growth parameters are crucial factors for obtaining enhanced rate of hydrocarbon degradation and are advocated by many researchers (Xia et al. 2006; Vieira et al. 2009; Dongfeng et al. 2011; Janani et al. 2014).

13.5 Microorganisms in Petroleum Degradation and Their Sources

Hydrocarbon bioremediation of contaminated sites by indigenous microbial population allows the conversion of toxic substances into less or nontoxic forms which might represent one of the primary mechanisms by which hydrocarbon products are removed from the contaminated environment inexpensively (Atlas 1981). The capability of microorganisms to emulsify hydrocarbon by producing surface-active agents is one of the most important characteristics of hydrocarbon-degrading bacteria. Such surface-active agents cause dispersion of hydrocarbons in water emulsions leading to the formation of micro-droplets (micelles) which may be ingested by the microbial cells for further degradation with the help of certain enzymes. One such important enzyme involved in hydrocarbon degradation is oxygenase which converts complex chain of hydrocarbons into smaller and simpler forms which finally enters into peripheral metabolic cycles as shown in Figs. 13.1 and 13.2 (Hommel 1990; Cerniglia 1992; Yakimov et al. 1995; Adebusoye et al. 2008; Ibrahim et al. 2013). A number of microorganisms, viz. *Bacillus* sp., *Corynebacterium* sp., *Edwardsiella* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Citrobacter* sp., *Micrococcus* sp., *Cladosporium* sp., *Acetobacterium* sp., *Mucor* sp., *Penicillus* sp., *Monosporium* sp., *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., etc., are reported to be hydrocarbon degraders by various researchers (Fall et al. 1979; Okpokwasili and Odokuma 1986; Okpokwasili and Okorie 1988; Rusansky et al. 1987; Johnson et al. 1996; Campbell and Cary 2001; Bhattacharya et al. 2002;

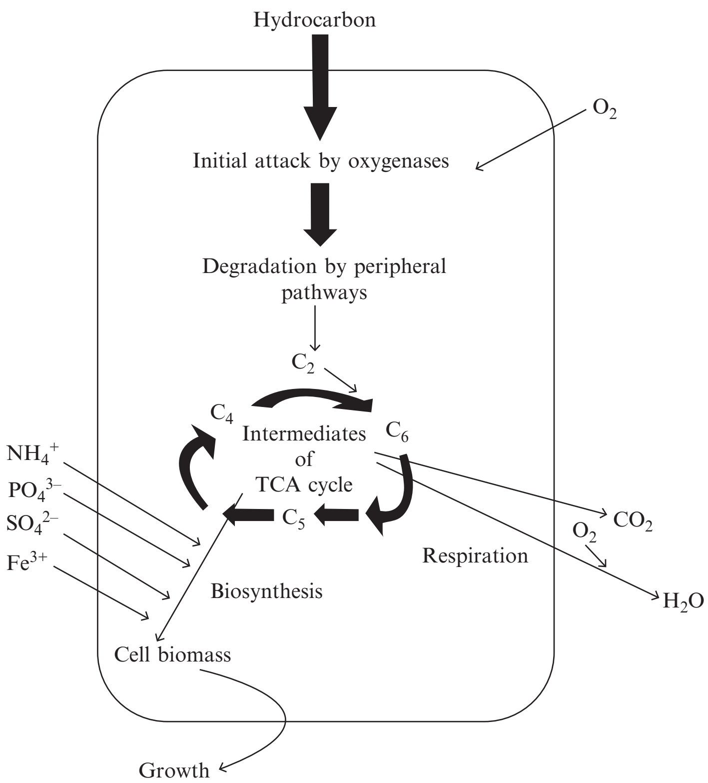

Fig. 13.1 Basic principle behind aerobic biodegradation of hydrocarbons by microorganisms. (Adopted from Das and Chandran 2011)

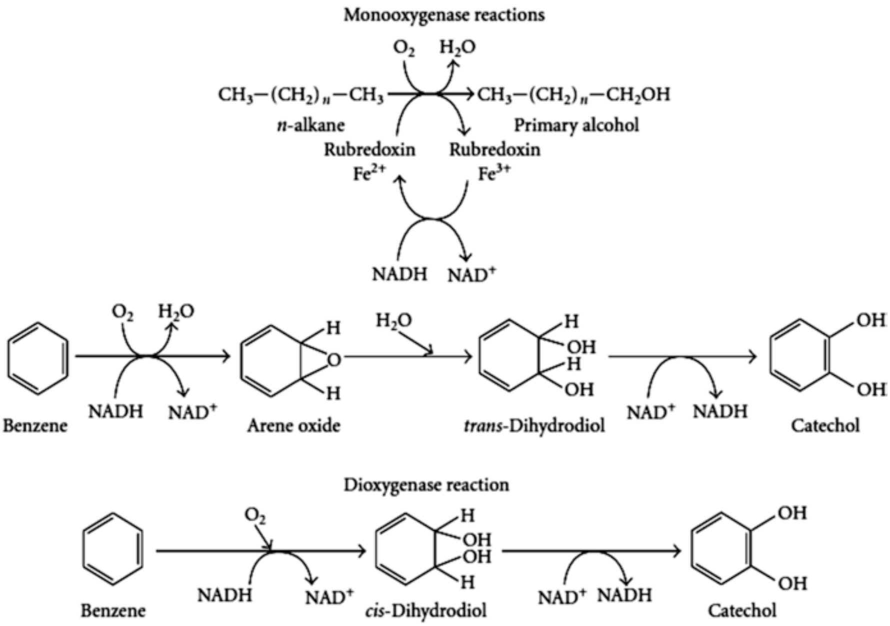

Fig. 13.2 Hydrocarbon degradation process by enzymatic action. (Adopted from Das and Chandran 2011)

Chang et al. 2002; Christopher and Christopher 2004; Chang et al. 2005; Bach et al. 2005; Biddle et al. 2006; Mandri and Lin 2007; Su et al. 2011; Luo et al. 2012; Yenn et al. 2014; Ameen et al. 2015; Jia et al. 2016).

Microorganisms, which are capable of hydrocarbon degrading, are believed to be capable of tolerating stress conditions to some extent, as hydrocarbon-contaminated sites itself provide hostile situations for the growth and proliferation of such microorganisms. Besides, very limited numbers of scientific reports dealing with microbial hydrocarbon degradation under stressed conditions are available (Sorensen et al. 2010; Tapilatu et al. 2010; Martino et al. 2012; Abed et al. 2014; Li et al. 2016). One such literature shows the existence of hydrocarbon degradation *Pseudomonas* sp. and *Variovorax* sp. at a temperature as low as 0 °C in fuel-contaminated soils of Greenland high Arctic region (Sorensen et al. 2010). Selection of such bacteria with stress-tolerant capability to degrade mono- or polyaromatic compounds by synthesizing biosurfactants and biopolymers that may play a certain role in enhanced stress tolerance could be a good approach to find a suitable bioaugmentation agent (Martino et al. 2012; Li et al. 2016). A detailed list of hydrocarbon-degrading microorganisms and their source of isolation is given in Table 13.2.

Table 13.2 Name of the microorganisms and their source of isolation along with the reported values of percentage hydrocarbon degradation are shown

Name of the microorganisms	Source	Hydrocarbon degradation (%)	References
Alcaligenes faecalis, *Candida tropicalis* (*the first report on the isolation of hydrocarbon-degrading microorganisms from Amazonian soil)	Artificially augmented Amazonian rainforest soil samples	–	Bastos et al. (2000)
Bacillus subtilis	Contaminated soil of Kuwait	–	Sharidah et al. (2000)
Alcanivorax borkumensis	Marine water	–	Golyshin et al. (2003)
Gordonia sp., *Aeromicrobium* sp., *Brevibacterium* sp., *Dietzia* sp., *Paecilomyces* sp., *Burkholderia* sp., *Yarrowia* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Neosartorya* sp., *Talaromyces* sp., *Graphium* sp., *Pichia* sp. and *Amorphoteca* sp.,	Contaminated soils of Indonesia	4.9–22%	Chaillan et al. (2004)
Microcosm of six microbes (names not mentioned)	Contaminated oil refinery plant in China	63.2 ± 20.1%	Ma et al. (2015)
Alkanindiges sp., *Arthrobacter* sp., *Pseudomonas* sp., *Mycobacterium* sp. and *Rhodococcus* sp.	Contaminated soils of Northern China	–	Sun et al. (2014)
Alternaria alternata, *Cladosporium* sp., *Aspergillus terreus*, *Eupenicillium hirayamae*, *Sphaerospermum* sp., *Paecilomyces variotii*	Contaminated mangrove sediments from Red Sea coast of Saudi Arabia	28–56%	Ameen et al. (2015)
Stenotrophomonas sp., *Pseudomonas* sp.	Dredged sediments of a river estuary in Italy	43–95%	Gregorio et al. (2016)
Polaromonas sp., *Sphingomonas* sp., *Alcaligenes* sp., *Caulobacter* sp. and *Variovorax* sp.	Uncontaminated Arctic soil	–	Eriksson et al. (2002)
Nocardia otitidiscaviarum	Contaminated desert soil of Iran	–	Zeinali et al. (2007)
Mycobacterium sp.	Soil	–	Miller et al. (2004)
Mycobacterium sp.	Uncontaminated Natural Park Soil of Schwa of Germany	–	Kim et al. (2005)
Acinetobacter sp., *Aeromonas* sp., *Alcaligenes* sp., *Bacillus* sp., *Kocuria* sp. (*Micrococcus*), *Ochrobactrum* sp., *Pseudomonas* sp. and *Xanthomonas* sp.	Contaminated Patagonian soil	0.028–100%	Peressutti et al. (2003)

(continued)

Table 13.2 (continued)

Name of the microorganisms	Source	Hydrocarbon degradation (%)	References
Rhodococcus sp. and *Pseudomonas* sp.	Contaminated soils of Kaluga, Kirov, Moscow	0–95%	Baryshnikova et al. (2001)
Not defined	Uncontaminated soils of Western Siberia (Arctic region)	3.8–51.2%	Belousova et al. (2002)
Alcanivorax borkumensis	North Sea, Atlantic Ocean, Mediterranean Sea, Sea of Japan, South China Sea and the Antarctic	80–90%	Golyshin et al. (2003)
Nocardia otitidiscaviarum	Soil contaminated with wastewater of a petroindustrial site in Iran	10–55%	Zeinali et al. (2007)
Afipia sp., *Janthinobacterium* sp., *Leptothrix* sp., *Massilia* sp., *Methylobacterium* sp., *Rhizobium* sp., *Sinorhizobium* sp. and *Thiobacillus* sp.	Uncontaminated soil of Arizona	88%	Bodour et al. (2003)
Mucor mucedo	Uncontaminated soils of Shenfu irrigation area, China	87%	Jia et al. (2016)
Haloarcula sp., *Haloferax* sp. (*the first report on the potential role of halophilic archaea belonging to the genera *Haloarcula* and *Haloferax*)	Uncontaminated pond water of Camargue, France	32–95%	Tapilatu et al. (2010)
Marinobacter sp., *Pseudomonas* sp., *Halomonas* sp., *Hahella* sp. and *Alcanivorax* sp.	Contaminated sediments from coastal region in Oman	67%	Abed et al. (2014)
Pseudomonas sp. and *Variovorax* sp.	Contaminated soils from Station Nord (St. Nord) in Greenland high Arctic region	70%	Sorensen et al. (2010)

13.6 Microbial Degradation of Petroleum Hydrocarbon

13.6.1 Enzymatic Pathways

Short-chain hydrocarbons are considered as the most biodegradable petroleum hydrocarbons. Hydrocarbons with C_5–C_{10} carbon numbers are homologues of solvents that tend to disrupt lipid membranes of hydrocarbon-degrading microorganisms, and hence, they are reported to have inhibitory action. Waxes, with alkane of C_{20}–C_{40}, are solids with hydrophobic nature at room temperature and hence are less biodegradable in nature (Atlas and Bartha 1973). Literature suggests that the majority of organic pollutants completely or optimally degrade under aerobic condition. Enzymes such as oxygenases and peroxidases play a vital role in hydrocarbon degradation, and these are responsible for initial intracellular oxidation of organic pollutants. The organic pollutants are then converted into intermediates of central intermediary metabolism such as the Krebs cycle in a stepwise manner. Cell biomass are then synthesized by central precursor metabolites such as acetyl CoA, succinate, pyruvate, etc. Sugars are the essential source of carbon, which is required for different types of biosynthesis and growth of the microbes, and are synthesized by gluconeogenesis (Fritsche and Hofrichter 2000). Figures 13.1 and 13.2 show the initial action on hydrocarbon by oxygenases (Das and Chandran 2011).

13.6.2 Role of Biosurfactant in Petroleum Degradation

As discussed earlier, the ability to produce biosurfactants by microbes is the most important feature of a potential hydrocarbon degrader. Biosurfactants are a complex mixture of biomolecules such as proteins, exopolysaccharides, fatty acids, amino acids, glycolipids, etc. with hydrophobic and hydrophilic components that emulsify hydrocarbon by minimizing the interstitial surface tension which makes them suitable for bioremediation and microbial enhanced oil recovery process (Ibrahim et al. 2013). Thus, surfactant subsequently increases the aqueous solubility of oily contaminants and makes them available for microorganisms for further degradation (Karanth et al. 1999). On the other hand, synthetic surfactants such as SDS (sodium dodecyl sulphate), LAS (linear alkylbenzene sulphonate), Brij 30, Tween 80, etc. used for the treatment of oil contaminants are often toxic, which also act as an additional source of contamination (Bognolo 1998). Microbial surfactants have analogous properties to that of synthetic surfactants but are biodegradable in nature and can be produced at the contaminated site (in situ) (Cha 2000). Isolation of microorganisms with desired capabilities to emulsify and solubilize hydrophobic contaminants both ex situ and in situ is a major benefit over competitors in contaminated areas (Reddy and Singh 1982; Vecchioli et al. 1990; Chattre et al. 1996; Cassidy and Hudak 2001). Nowadays, these are not only important for bioremediation purpose but also area of research for microbial enhance oil recovery from wells. Such process involves the implementation of microbes directly or microbial surfactants in the well which helps in reducing the viscosity of oil to increase the free flow through the pipelines and also stabilizes fuel water–oil emulsions (Ghurye and Vipulanandan 1994; Makkar and Cameotra 1997; Bognolo 1998).

Contact angle of a fluid with a solid surface is an index used to examine the action of biosurfactant on the rock surface wettability (i.e. the interaction between fluid and solid surface) (Ibrahim et al. 2013). It is defined as the angle between the tangent to the periphery of the point of fluid contact with the solid and the surface of the solid in the direction where the droplet exists as shown in Fig. 13.3. Contact

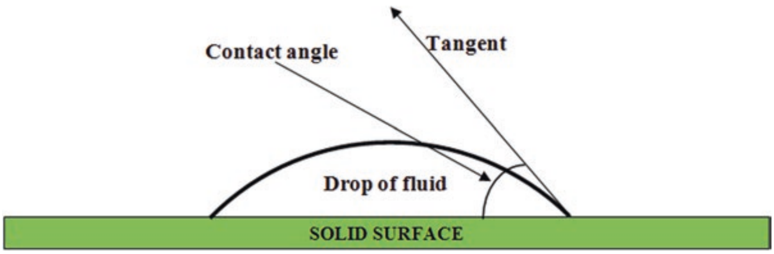

Fig. 13.3 The contact angle formed by a drop of fluid on a solid surface

angle less than 90° for surfactant-containing liquid is considered to be wet on solid surface, and a contact angle greater than 90° is said to be non-wetting (i.e. the liquid which does not wet the solid surface). The alteration in wettability has been projected as one of the mechanisms of MEOR (Alvarez et al. 2015).

Some of the field trials carried out by researchers in Poland, Holland, the Czech Republic, the United States, Romania and Hungary reported significant enhancement in oil recovery by MEOR process (Karanth et al. 1999). Apart from the cost-effective nature of biosurfactants, they are also widely used in food, pharmaceutical and cosmetic industry which makes it an important microbial product of commercial importance (Batista et al. 2006).

A review work carried out by Satpute et al. (2010) represents marine biosphere as a wealthy natural source of flora and fauna with functional commercial-grade bioactive compounds, biosurfactants/bioemulsifiers, etc. which may be used for the purpose of bioremediation. Marine microorganisms such as *Bacillus*, *Acinetobacter*, *Halomonas*, *Myroides*, *Pseudomonas*, *Corynebacterium*, *Arthrobacter* and *Alteromonas* sp. were reported for their potential application for the production of biosurfactants and exopolysaccharides. (Filonov et al. 2004; Coral and Karagoz 2005; Das and Mukherjee 2007; Simpson et al. 2011).

Various species of the genus *Pseudomonas* are probably the most widely studied hydrocarbon-degrading bacteria. Existing literature shows the role of various species of *Pseudomonas* isolated from contaminated sites in hydrocarbon degradation (Baryshnikova et al. 2001; Filonov et al. 2004; Shin et al. 2006; Niepceron et al. 2010; Aresta et al. 2010; Nie et al. 2010; Singh and Malik 2013; Li et al. 2016). Their source of isolation even ranges from hot springs (Perfumo et al. 2006), marine water (Satpute et al. 2010) and cow dung (Singh and Fulekar 2010) up to the Arctic region (Sorensen et al. 2010).

Some of the researchers studied the capability of various *Pseudomonas* strains in BTEX (benzene, toluene, ethylene and xylene), naphthalene and TPH degradation, surface tension reduction of petroleum oil, formation of biofilm on hydrocarbon-supplemented medium, formation of rhamnolipid, glycolipid-based surfactants, etc. (Baryshnikova et al. 2001; Perfumo et al. 2006; Kim and Jaffé 2008; Zhang et al. 2011; Cébron et al. 2011; John and Okpokwasili 2012; Martino et al. 2012; Li et al. 2013; Oyetibo et al. 2013; Pedetta et al. 2013; Dudášová et al. 2014;

Pacwa-Płociniczak et al. 2014; Ma et al. 2015). This report advocates their capability to achieve 20–100% of hydrocarbon degradation within 7–30 days of incubation. But physicochemical parameters such as pH, temperature, salinity, type of culture medium used, carbon source, etc. may influence the yield of biosurfactant production in industrial scale (Lang 2002; Batista et al. 2006).

13.7 Genes Involved in Petroleum Degradation

Molecular techniques not only play a vital role for the identification of petroleum degraders but are also largely used for the characterization and identification of different genes involved in hydrocarbon degradation. Genes responsible for hydrocarbon degradation may be present in either genomic or plasmid DNA or may be in both at the same time in a certain microorganism. Plasmid curing is a convenient tool for the determination of the involvement of plasmid-encoded genes in hydrocarbon degradation (Karpagam and Lalithakumari 1999; Liu et al. 2004; Vasudevan et al. 2007; Kumar and Gopal 2015; John and Okpokwasili 2012). Reports also show presence of certain genes in the genomic DNA of some microbes that are involved in hydrocarbon degradation (Kim et al. 2007; Quatrini et al. 2008; Weelink et al. 2009; Li et al. 2011; Sun et al. 2014).

In contrary to the above, there are also reports which show contribution of both genomic and plasmid DNA-encoded genes in the same organism in hydrocarbon degradation (Fondi et al. 2013). A brief list of important genes responsible for microbial hydrocarbon degradation is shown in Table 13.3.

13.8 Conclusions

Even though widespread research has been conducted on petroleum bioremediation during the last decade, the usefulness of these tools has only rarely been convincingly established, and on the commercially available bioremediation products, the literatures are nearly lacking with supportive evidence of success. Existing literatures chiefly demonstrated the assessment of factors affecting bioremediation under laboratory condition. Out of these, very few numbers of literature reports their implementation in field trials at pilot-scale convincingly demonstrated this technology. Only 27 commercially available bioremediation agents such as Inipol EAP22, BIOREN 1 and 2, Oil Spill Eater II® (OSE II), ENVIROZYME BR, BioCATalystIOS-500, Petro-Clean, IOS-500, Micro-Blaze, WMI-2000, etc. are listed on the NCP schedule of USEPA till March 2015 (USEPA 2015). But the scientific community is still trying to screen out more effective microorganisms of the same type or sometimes a consortium of different types of microbes for bioremediation of a hydrocarbon-contaminated site considering their advantages over commercially available synthetic bioremediation agents. In conclusion, as the petroleum

Table 13.3 A brief list of hydrocarbon-degrading genes and their source organisms

Name of the microbes	Genes involved	Encodes for	References
Rhodococcus sp., *Gordonia* sp.	alkB	Non-haem iron-containing alkane monooxygenases and hydroxylases	Kim et al. (2007), Quatrini et al. (2008)
Acinetobacter sp., *Staphylococcus haemolyticus*	C23O	Cytochrome C230	Onur et al. (2015)
Betaproteobacteria sp., *Sterolibacterium denitrificans*	bssA	α-Subunit of Bss enzyme	Weelink et al. (2009)
Proteobacteria sp., *Firmicutes* sp., *Acidobacteria* sp., *Actinobacteria* sp., *Deferribacteres* sp., *Bacteroidetes* sp., *Thauera chlorobenzoica*	bamA	6-oxocyclohex-1-ene-1-carbonyl-CoA hydrolase	Sun et al. (2014), Li et al. (2011)
Gammaproteobacteria sp.		Catechol	Sei and Fathepure (2009)
Acinetobacter sp., *Alcanivorax* sp.	almA	Flavin-binding monooxygenase	Wang and Shao (2012)
	arfA	Arthofactin	Roongsawang et al. (2003), Das et al. 2008
B. tequilensis, E. coli, B. subtilis, *Aeromonadaceae* sp., *Bacillaceae* sp., *Enterobacteriaceae* sp., *Gordoniaceae* sp., *Pseudomonadaceae* sp.	*sfp*	4′-phosphopantetheinyl transferase protein	Porob et al. (2013), Anburajan et al. (2015), Ndlovu et al. (2016)
	*sfp*O	Phosphopantetheinyl transferase	Anburajan et al. (2015)
Aeromonadaceae sp., *Bacillaceae* sp., *Enterobacteriaceae* sp., *Gordoniaceae* sp. and *Pseudomonadaceae* sp.	*rhl*B	Rhamnosyltransferase subunit B	Ndlovu et al. (2016)
Aeromonadaceae sp., *Bacillaceae* sp., *Enterobacteriaceae* sp., *Gordoniaceae* sp. and *Pseudomonadaceae* sp.	*bam*C	Bacillomycin C	Ndlovu et al. (2016)
Pseudomonas sp.	*rhl*	Rhamnolipid	Pacwa-Płociniczak et al. (2014)
A. xylosoxidans	bphA		Pacwa-Płociniczak et al. (2014)
Pseudomonas putida	ndoB	Iron–sulphur protein	Hamann et al. (1999)
Rhodococcus sp.	thmA		Kim et al. (2007)
Rhodococcus sp.	PrmA		Kim et al. (2007)

hydrocarbon pollutant creates a stressed environment for the growth and proliferation of indigenous microbial species, hence research on finding out novel hydrocarbon-degrading microorganisms with stress-tolerant potential would be an added advantage.

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