

# Chapter 10

## Microbes: A Novel Source of Bioremediation for Degradation of Hydrocarbons



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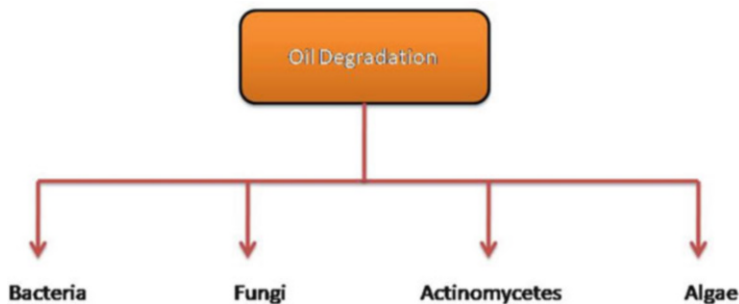
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**Abstract** In our daily life, the demand for liquid petroleum products is increasing day by day. Crude oil-derived hydrocarbons, the largest group of environmental pollutants found worldwide, pollute our environments severely. Oil or hydrocarbons cause drastic impacts on living organisms. The many reports about their toxicity emphasize the ultimate need to remove them from marine and terrestrial environments. For cleaning up pollution by these hydrocarbons, bioremediation seems to be the most acceptable and economically justified method. Bioremediation is considered one of the most sustainable cleanup techniques, but its potential has not been fully expressed in the field because it operates too slowly to meet the immediate demands of a given location. The process of bioremediation is carried out by various microorganisms. Therefore, in this review, we present information about methods of oil degradation by such microorganisms as bacteria, fungi, algae, and actinobacteria. These microbes can help degrade oil or hydrocarbons. This review presents the unique characteristics of oil-degrading microbes. In addition, it is a starting point for

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**Fig. 10.1** Microbes that degrade oil

wider debate about the limitations and possible improvements of currently employed hydrocarbon bioremediation strategies.

**Keywords** Hydrocarbons · Degradation · Microorganisms

## 10.1 Introduction

At the present time, petroleum and its constituent hydrocarbons are widely used as the main energy source in the industrial, transport, and domestic sectors (Varjani and Upasani 2016; Arulazhagan et al. 2010). However, use of these hydrocarbons produces a number of harmful chemical substances that widely affect human beings and the environment. The effectiveness of these substances depends upon the composition, concentration, and biological state of the affected organism at the time of contamination and also on such environmental factors as temperature (Obire and Ayanwu 2009).

In our environment, toxic components of hydrocarbons are released by transport, vehicle factories, thermal plants, oil spills, pipelines, oil well leakages, diesel stations, and contamination by vehicle garages (Costa et al. 2012). The petroleum hydrocarbons are categorized into two broad divisions, aromatic and aliphatic compounds. The simple aliphatic and aromatic compounds are degraded in the environment, but because of their complex structure, the large aliphatic and aromatic constituents of petroleum hydrocarbons are not degraded (Hasanuzzaman et al. 2007). Therefore, different strategies and approaches are used to degrade these hydrocarbons, broadly categorized into three groups: physical, chemical, and thermal approaches (Adnan et al. 2018). All these methods are very costly, and the chemicals required further greatly affect our environment. In the thermal process, large amounts of heat are generated that affect both the flora and fauna of a specific area (Ezeji et al. 2007).

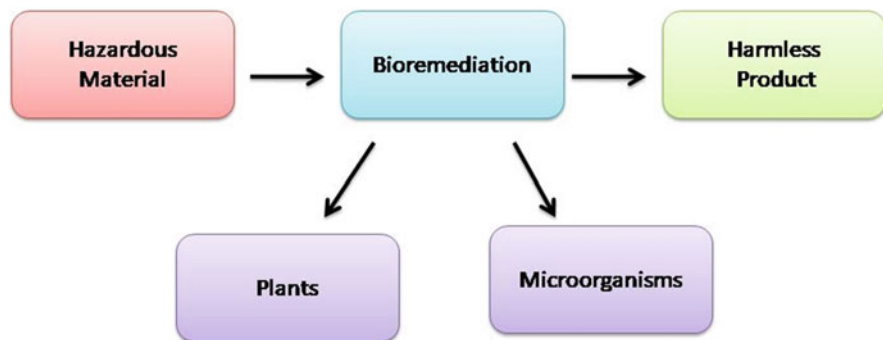


Fig. 10.2 Process of bioremediation

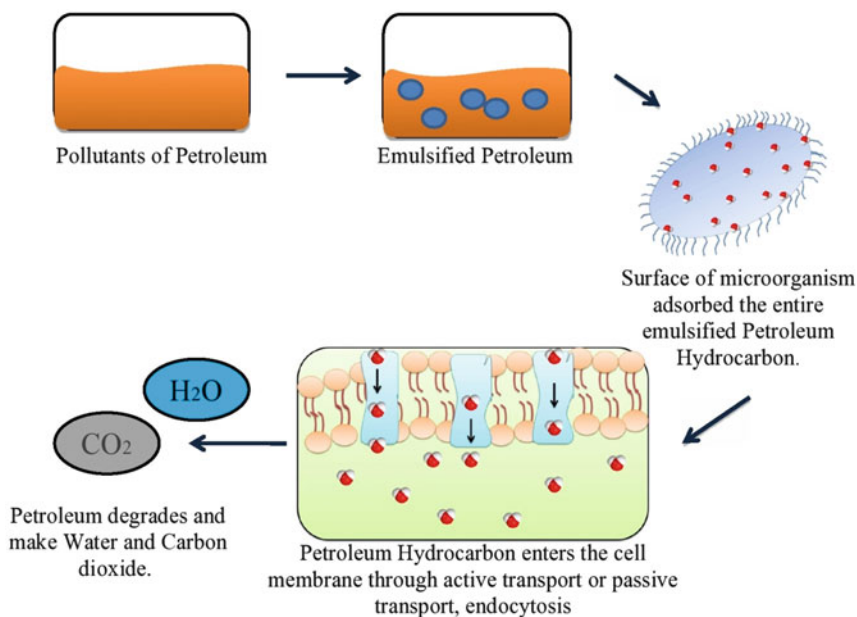


Fig. 10.3 Mechanism of oil degradation

Therefore, the preferred method for degradation of hydrocarbons is biological treatment because of reliability, feasibility, and the high potential for eco-friendly degradation. The biological methods are very simple to use and require low energy for operation. A variety of microorganisms can be used for the process in *in vitro* as well as in *in vivo* conditions (Fig. 10.1). Different types of microorganisms—bacteria, fungi, algae, and yeasts—degrade the hydrocarbons in a green revolution for removing hazardous contaminants from the environment (Zhang et al. 2013; Rahman et al. 2003). Native microorganisms have great potential for degradation as

compared to others because of the specific metabolic pathways that metabolize the oil content.

Crude oil is composed of several compounds, including aliphatic, aromatic, and polycyclic aromatic hydrocarbons (PAH) and also sulfur-, oxygen-, and nitrogen-containing compounds. PAH compounds are toxic and may be carcinogenic. High concentrations of such pollutants, by their poisonous and carcinogenic nature, can affect cellular metabolism (Tanti et al. 2009). The biodegradation of petroleum hydrocarbons may be contained by considering many factors. An essential limiting factor in the biodegradation of polluted soils is often the low bioavailability and solubility of the hydrocarbons. Crude oil is one of the most significant pollutants in the environment, able to cause extreme damage to human beings and ecosystems. Excessive oil concentration causes serious problems in our body such as liver or kidney disorders, visible harm to bone marrow, and an increased risk of cancer (Mishra et al. 2001). The use of microorganisms in degradation of petroleum and its products has been established as a green, cost-effective, flexible, and environmentally sound remedy. The search for effective and green strategies of oil removal from polluted infected sites has intensified in recent years because the microbial cleanup of untreated oil spills is a slow process (Grangemard et al. 2001). In microbial

**Table 10.1** List of hydrocarbon- or oil-degrading bacteria

Sample no.	Bacteria	Degradation	Reference
1	<i>Pseudomonas</i>	Hydrocarbons	Leahy and Colwell (1990)
2	<i>Acinetobacter</i>	Hydrocarbons	Adebusoye et al. (2007)
3	<i>Alcaligenes</i>	Hydrocarbons	Floodgate (1995)
4	<i>Vibrio</i>	Hydrocarbons	Leahy and Colwell (1990)
5	<i>Flavobacterium</i>	Hydrocarbons	Adebusoye et al. (2007)
6	<i>Achromobacter</i>	Hydrocarbons	Floodgate (1995)
7	<i>Micrococcus</i>	Hydrocarbons	Leahy and Colwell (1990)
8	<i>Nocardia</i>	Hydrocarbons	Adebusoye et al. (2007)
9	<i>Corynebacterium</i>	Hydrocarbons	Floodgate (1995)
10	<i>Pseudomonas stutzeri</i>	<i>n</i> -Tetradecane	Adel et al. (2012)
11	<i>Bacillus thuringiensis</i>	<i>n</i> -Tetradecane	Abou-Shanab et al. (2016)
12	<i>Bacillus pumilus</i>	<i>n</i> -Tetradecane	Awad et al. (2011)
13	<i>Bacillus cereus</i>	<i>n</i> -Tetradecane	Bayoumi et al. (2010)
14	<i>Pseudomonas</i> sp.	Hydrocarbons	Brito et al. (2006)
15	<i>Marinobacter</i> sp.	Hydrocarbons	Akpoveta et al. (2011)
16	<i>Alcanivorax</i> sp.	Hydrocarbons	Juhasz and Naidu (2000)
17	<i>Microbulbifer</i> sp.	Hydrocarbons	Bishnoi et al. (2008)
18	<i>Sphingomonas</i> sp.	Hydrocarbons	Snape et al. (2001)
19	<i>Micrococcus</i> sp.	Hydrocarbons	Lloyd and Cackette (2001)
20	<i>Cellulomonas</i> sp.	Hydrocarbons	Chaillan et al. (2004)
21	<i>Dietzia</i> sp.	Hydrocarbons	Akpoveta et al. (2011)
22	<i>Gordonia</i> sp.	Hydrocarbons	Bishnoi et al. (2008)

remediation, organization of numerous microbes present in the soil can degrade a wide range of oily sludge (Barathi and Vasudevan 2001).

Oil spills affect many species of plants and animals within the surrounding areas as well as humans. The search for green and powerful approaches to defining the rate and overall extent of biodegradation of waste lubricating oil in soils or contaminated sites has intensified in current years (Umar et al. 2013). Microorganisms can metabolize oil much as humans convert their food into energy or power. The soil is the habitat of many organisms, so any changes or variations in soil may further destroy our environment. The impact of an oil spill is enrichment of the soil-degrading microbial populations. No single microorganism has been observed to completely degrade a petroleum hydrocarbon molecule, but particular species or traces of equal species may be capable of degrading concentrations of oil hydrocarbons (Facundo et al. 2001). Species of *Pseudomonas* are known for their capability of hydrocarbon degradation (Jewetz et al. 1999) (Fig. 10.2).

## 10.2 Mechanism of Oil Degradation by Microorganism

The biodegradation of hydrocarbons by microorganisms in nature has four main steps (Fig. 10.3).

In the first step, pollutants of petroleum are emulsified by surfactant secreted by a microorganism. Then, the surface of the microorganism adsorbs the entire emulsified petroleum hydrocarbon. Now, the petroleum hydrocarbon, which is adsorbed onto the surface of the cell membrane, enters the cell membrane through active transport or passive transport, endocytosis. In the last step, the petroleum hydrocarbon enters into the cell, and undergoes an enzymatic reaction that causes its degradation (Li et al. 2019).

### 10.2.1 Degradation of Oil and Hydrocarbon by Bacteria

Different species of bacteria are widely used to biologically degrade petroleum hydrocarbons and also to help remove oil spills by degradation (Abou-Shanab et al. 2016). Many studies have shown that bacteria can degrade hydrocarbons such as asphaltenes (phenols, ketones, esters, porphyrins, fatty acids), resins (carbazoles, sulfoxides, pyridines, quinolines, amides) (Steliga 2012), and aliphatics, aromatics, and resins (carbazoles, sulfoxides, pyridines, quinolines, amides) (Table 10.1). The bacterial strains *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffii*, *Flavobacterium* sp., *Micrococcus roseus*, and *Corynebacterium* sp. isolated from polluted areas in Nigeria were observed for degradation of crude oil (Adebusoye et al. 2007).

Petroleum bioremediation is completed by microorganisms that can utilize hydrocarbons as a source of energy (Rosenberg et al. 1998). These bacteria are ubiquitous

**Table 10.2** List of oil- or hydrocarbon-degrading fungi

Sample no.	Fungus	Degradation	References
1	<i>Aspergillus flavus</i>	Petroleum oil	Adekunle and Oluyode (2002)
2	<i>A. niger</i>	Petroleum oil	Bartha and Atlas (1997)
3	<i>Mucor</i>	Petroleum oil	Battelle (2000)
4	<i>Rhizopus</i>	Petroleum oil	Nwachukwu (2000)
5	<i>Talaromyces</i>	Petroleum oil	Ojo (2005)
6	<i>Penicillium</i>	Hydrocarbons	Ahmad et al. (2016)
7	<i>Amorphoteca</i>	Hydrocarbons	Throne-Holst et al. (2007)
8	<i>Candida</i>	Hydrocarbons	Farag and Soliman (2011)
9	<i>Fusarium</i>	Hydrocarbons	Al-Nasrawi (2012)
10	<i>Neosartorya</i>	Hydrocarbons	Jawhari (2014)
11	<i>Mycotypha</i>	Hydrocarbons	Okafor et al. (2009)
12	<i>Rhizopus</i>	Hydrocarbons	Mittal and Singh (2009)
13	<i>Botrytis</i>	Hydrocarbons	Joshi and Pandey (2011)
14	<i>Polyporus</i> sp.	Crude oil	Kristanti et al. (2011)
15	<i>Amorphoteca</i> sp.	Hydrocarbons	Jones et al. (2001)
16	<i>Neosartorya</i> sp.	Hydrocarbons	Chaillan et al. (2004)
17	<i>Paecilomyces</i> sp.	Hydrocarbons	Ramasamy et al. (2014)
18	<i>Talaromyces</i> sp.	Hydrocarbons	Wang et al. (1998)
19	<i>Graphium</i> sp.	Hydrocarbons	Balaji et al. (2014)
20	<i>Popularia</i> sp.	Oil	Sandhu et al. (2016)
21	<i>Geotrichum</i> sp.	Oil	Sandhu et al. (2016)

in nature and able to degrade numerous hydrocarbons including short-chain, long-chain, and numerous aromatic compounds, including PAHs. These compounds have low solubility in water. Thus, as the first step in hydrocarbon degradation entails a membrane-bound oxygenase, it is important for microorganisms to be in direct contact with the hydrocarbon substrates. One biological approach to accomplish contact between the microorganisms and water-insoluble hydrocarbons is emulsification of the hydrocarbon. Therefore, it is not unexpected that microorganisms growing on petroleum typically produce emulsifiers. These surfactants assist to disperse the oil and to detach the bacteria from the oil droplets after utilizable hydrocarbon has been depleted (Ron and Rosenberg 2002).

### 10.2.2 Biodegradation of Oil and Petroleum by Fungi

Crude oil is a primary source of profits for Iraq, which is certainly one of the most important international oil producers and exporters, ranked nearly fourth internationally in terms of oil reserves. Incidental spills of crude oil and frequent illegal disposal of oil wastes lead to serious damage to environments. Cleaning up oil contaminants is a priority project for the restoration of our natural environment.

Chemical, physical, and thermal strategies are available but these methods are very costly and require site recovery. Several physicochemical and biological methods have been assessed for treating oil-contaminated environments (Ezeji et al. 2007). Organic treatment is desired for physicochemical strategies for reasons of its feasibility, reliability, and capability to achieve high elimination efficiency with low price. Other reasons include the simplicity of its low-power layout, creation, operation, and use; biodegradation of hydrocarbons is a cost-effective method compared to chemical methods (Liu et al. 2013). In a biological technique, microorganisms can use hydrocarbons as their sole energy and carbon source and degrade them instead of gathering them at every other level (Zhang et al. 2015). Biological treatment may have an advantage over physicochemical treatment in the removal of spills because it affords crucial biodegradation of oil parts through microorganisms, is a “green” alternative for treating risky contaminants without environmentally degrading effects, and may be cheaper than other strategies (Zhang et al. 2011). Diverse microorganisms, including bacteria, algae, yeasts, and fungi, can degrade hydrocarbons. Indigenous microorganisms with particular metabolic capacities have a considerable role in the biodegradation of crude oil (Rahman et al. 2003). Rahman et al. (2002) suggested that bacterial consortia isolated from crude oil-infected soils have the potential to degrade crude oil fractions. In addition to bacteria, fungi are one of the best oil-degrading organisms. Numerous studies have identified many fungal species able to use crude oil as their sole source of energy, including *Cephalosporium*, *Rhizopus*, *Paecilomyces*, *Torulopsis*, *Pleurotus*, *Alternaria*, *Mucor*, *Talaromyces*, *Gliocladium*, *Fusarium*, *Rhodotorula*, *Cladosporium*, *Geotrichum*, *Aspergillus*, and *Penicillium* (Jawhari 2014). Hanafy et al. (2017) observed that the *Aspergillus* and *Penicillium* isolated from oil-contaminated sites close to the Red Sea within the Yanbu region have been extremely useful in crude oil degradation. Using fungi as a means of bioremediation gives a powerful alternative for cleansing the environment of contaminants (Hanafy et al. 2017). Data are shown in Table 10.2.

### ***10.2.3 Biodegradation of Oil and Petroleum by Algae***

Natural contamination has been stated to be the most significant issue affecting the world (Reyes et al. 2016). One of the main causes of environmental pollution is hydrocarbon contamination in soil and water (El-Sheekh et al. 2013). Unrefined petroleum, also called dark gold, is the most significant asset in industrialized nations; however, its handling and transport can cause genuine ecological contamination and interfere with many populations of organisms (Xaaldi et al. 2017). Many recorded data attest to the real genuine harm brought about by oil slicks in ecosystems and to marine creatures, silt, higher-level organisms, fish, coral reefs, avian species, reptiles, and surface water bodies (Afshar-Mohajer et al. 2018). When oil is spilled in the ocean or other waterways, it creates a film that decreases the proportion of daylight reaching the underwater world, which affects the process of

**Table 10.3** List of oil- and hydrocarbon-degrading algae

Sample no.	Algae	Degradation	References
1	<i>Amphora</i> sp.	Crude oil	Kvenvolden and Cooper (2003)
2	<i>Prototheca zopfii</i>	Crude oil and hydrocarbons	Aditi et al. (2015)
3	<i>Porphyridium</i> sp.	Petroleum waste	Vidyashankar and Ravishankar (2016)
4	<i>Microcoleus</i> sp.	Hydrocarbons	Yakimov et al. (2007)
5	<i>Agmenellum</i> sp.	Petroleum waste	Walker et al. (1975)
6	<i>Anabaena</i> sp.	Hydrocarbons	Cerniglia et al. (1980)
7	<i>Coccochloris</i> sp.	Hydrocarbons	Bibi et al. (2017)
8	<i>Nostoc</i> sp.	Hydrocarbons	Lohitesh et al. (2013)
9	<i>Cylindrotheca</i> sp.	Petroleum waste	Srivastav et al. (2013)
10	<i>Aphanocapsa</i> sp.	Hydrocarbons	Shankar and Suneetha (2013)
11	<i>Chlorella</i> sp.	Petroleum waste	Rath et al. (2012)
12	<i>Chlamydomonas</i> sp.	Crude oil	Venkata Gopichand et al. (2013)
13	<i>Ulva</i> sp.	Hydrocarbons	Lohitesh et al. (2013)
14	<i>Petalonia</i>	Crude oil and hydrocarbons	Aditi et al. (2015)

photosynthesis. Additionally, total petroleum hydrocarbon (TPH), a natural toxin in the Earth, is poisonous for all human beings and numerous other organisms (Lee et al. 2015). Polycyclic aromatic hydrocarbons (PAHs) are the most lethal components of unrefined petroleum and are related to cancer-causing agents (Duran and Cravo 2016). Bioremediation suggests the utilization of living organisms and their biochemical apparatus to debase or change poisons into less dangerous forms, which has been demonstrated to be a powerful, confined, and more affordable technique (Sharma et al. 2018). In any case, a limitation of the bioremediation procedure with microorganisms is the accessibility of supplements, for example, nitrogen and phosphorus, which influences the speed of oil degradation (Ron and Rosenberg 2014), although advances in atomic innovations on recombinant DNA have permitted the hereditary improvement of numerous organisms and support the speed of remediation. The fundamental segments of raw petroleum are naphthenes, asphaltenes, waxes, pavements, aromatic hydrocarbons, tars, and other unstable mixes, for example, benzene, toluene, ethylbenzene, and xylene. Many mixes, for example, pyrene, benzo(a)pyrene and chrysene, are cancer causing, mutagenic, and teratogenic (Sammarco et al. 2013). Numerous microorganisms, including a few types of microalgae (*Monoraphidium braunii*, *Chlamydomonas reinhardtii*, *Chlorella* sp.), parasites (*Trametes versicolor*, *Pleurotus eryngii*, *Phanerochaete chrysosporium*), and bacteria (*Pseudomonas aeruginosa*, *Rhodococcus erythropolis*), have catabolic pathways for the debasement of contaminants (Sharma et al. 2018). Algal growth is fundamental in seagoing biological systems and in light of the fact that they are essential markers, are important in the trophic chain,



**Table 10.4** List of oil- and hydrocarbon-degrading actinomycetes

Sample no.	Actinobacteria	Degradation	References
1	<i>Actinoplanes</i>	Oil	Cappuccino and Sherman (2002)
2	<i>Nocardia</i>	Hydrocarbons	George et al. (2011)
3	<i>Streptomyces</i>	Oil	Rahman et al. (2002)
4	<i>Streptosporangium</i>	Hydrocarbons	Rifaat and Yosery (2004)
5	<i>Rhodococcus</i>	Oil	George et al. (2011)
6	<i>Nocardia</i>	Hydrocarbons	Watanabe et al. (2002)
7	<i>Gordonia</i>	Oil	Essien and Udosen (2000)
8	<i>Dietzia</i>	Oil	Beerka and Steinbuchel (2000)
9	<i>Micromonospora</i>	Hydrocarbons	George et al. (2011)
10	<i>Actinomyces octodloyts</i>	Petroleum Hydrocarbons	
11	<i>Saccharomyces cerevisiae</i> (yeast)	Petroleum Hydrocarbons	

providing oxygen and natural substances to other living things. *Chlorella vulgaris* is a significant species because it adsorb an assortment of natural pollutants (Kong et al. 2010), so the development of microalgae in wastewater treatment is spreading widely for the disposal of supplements, control of physical substance parameters, as feedstock for the generation of biofuel, and expulsion of phenol and polycyclic aromatic compounds, because of its high adsorption limit, bioaccumulation, bio-transformation, and biodegradation (He et al. 2016). For this reason, it was proposed here to determine the capability of biodegradation of unrefined petroleum by the microalgae *Chlorella* sp. (Deimer et al. 2018). Data are shown in Table 10.3.

#### 10.2.4 Biodegradation of Oil and Hydrocarbons by Actinomycetes

The tragic history of soil and water pollution by way of oil spillage from the oil industry, tankers, offshore systems, related pipelines, garage tanks and wells, and unlawful oil bunkering has caused essential environmental and fitness defects in oil-structured countries (Ordinioha and Brisibe 2013). Pollution through crude oil, inclusive of oil spills and toxic wastes, is a persistent struggle that has prompted serious threats to human fitness with issues regarding the viability and productiveness of ecosystems (Okoh and Trejo-Hernandez 2006). Mechanical and chemical techniques for the remediation of hydrocarbon-polluted surroundings are frequently costly and technologically complex. Increasing attention has been paid to the growing innovative era for cleaning up this contaminant, with bioremediation being a completely useful method (Vidali 2001). There are many herbal and natural

microorganisms that thrive on the decomposition of those toxic compounds. Usage of microorganisms for cleanup efforts, referred to as bioremediation, has been shown to be a successful method for the cleanup of marine regions suffering from oil spills (Coulon et al. 2006). Bioremediation strategies are currently receiving favorable exposure as low-cost and promising environmentally friendly technologies for the remediation of crude oil hydrocarbons without difficulty. Biodegradation of crude oil and derived aromatic hydrocarbons in marine sediments has been reported (Jones et al. 2008). The maximum fast and complete degradation of general organic pollution is introduced under cardiac conditions and the biodegradation system is mediated by unique enzyme structures (Das and Chandran 2011). Extracellular and intracellular assault of organic pollution by microbes through oxidation is catalyzed by peroxidases and oxygenases. The cleanup of toxic natural compounds through numerous microorganisms and fungi takes place through oxidative coupling mediated via oxidoreductases together with peroxidases (Karigar and Rao 2011). Microbes derive power via power-yielding biochemical reactions mediated by these enzymes to cleave chemical bonds and help transfer of electrons from a reduced natural substrate (donor) to some other chemical compound (acceptor). For this reason, it is essential to analyze the function and organization of enzymes for crude oil biodegradation. Actinobacteria have several characteristics that are vital for surviving in extreme situations, including dry environments and nutrient lack, and produce biosurfactants that boost contaminant bioavailability and facilitate the manner of biodegradation (Beilen and Funhoff 2005): these promote the prevalence of Actinobacteria in pristine and hydrocarbon-polluted soil (Quatrini et al. 2008). Consequently, it is important to observe crude oil biodegradation of actinobacterial isolates, particularly from oil-contaminated sites (Table 10.4).

### 10.3 Conclusion

Bioremediation is the main natural mechanism that can cleanse petroleum and oil pollutants from the environment. This process uses microscopic organisms such as bacteria, fungi, algae, and actinomycetes that live in soil and consume oil or hydrocarbons. A number of factors influencing degradation have been identified to reduce the toxicity of oil contamination in the environment by removing, degrading, or transforming contaminants. Therefore, successful bioremediation treatment requires understanding of those factors.

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