Microbial Bioremediation of Petroleum Hydrocarbon–Contaminated Marine Environments

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http://dx.doi.org/10.5772/intechopen.72207

Abstract

Petroleum pollution has become a serious environmental problem, which can cause harmful damage to the environment and human health. This pollutant is introduced into the environment from both natural and anthropogenic sources. Various physicochemical and biological treatments were developed for the cleanup of contaminated environments. However, bioremediation is based on the metabolic capabilities of microorganisms, and it is considered as the most basic and reliable way to eliminate contaminants, particularly petroleum and its recalcitrant compounds. It is more effective alternative comparing to classical remediation techniques. A high diversity of potential hydrocarbon degrader's microorganisms was reported, and bacteria constitute the most abundant group, which has been well studied for hydrocarbon degradation. Several bioremediation approaches through bioaugmentation or/and biostimulation have been successfully applied. The interest on the optimizing of different parameters to achieve successful bioremediation technologies has been increased. In this chapter, we summarize the diversity and the hydrocarbon degradation potential of microorganism involved in the remediation of contaminated environments. We also present an overview of the efficient bioremediation strategies used for the decontamination of polluted marine environments.

Keywords: bioremediation, hydrocarbonoclastic, hydrocarbon degradation, toxicity, biosurfactant

1. Introduction

Oil is a primary and necessary energy source, which is widely used in different fields, including industry, transport and daily human activities [1]. However, the widespread distribution and the overexploitation of hydrocarbons become a serious problem causing harmful impacts on the environment and health due to their toxicity and their carcinogenic and mutagenic properties [2]. Hydrocarbon pollution may occur through several sources that may be natural or anthropogenic. We can distinguish numerous anthropogenic sources such as oil extraction and treatment field, transportation fuels accidents, leakage from underground storage tanks, petrochemical industry activities and release from oil refinery sites [3–5]. To reduce this contamination, different chemical, physical and biological treatment methods are considered. The choice of remediation methods depends on several parameters such as the type of pollutant and its characteristics (the physicochemical nature of the pollutant and its toxicity), the properties of contaminated site (the pollution source, the nature of the site) and the type of the pollution (old or recent). The treatment of a polluted site is carried out only after evaluating the type of pollutant, the environmental and human-associated risks and the treatment feasibility and predicted efficiency.

Depending on its location, we distinguish in situ and ex situ contaminated site remediation methods [6–9]. However, bioremediation is considered as one of the best environment-friendly way to remove hydrocarbons presenting several advantages compared to other methods [9–11]. In fact it is a natural, efficient and economic method. Moreover, it converts hydrocarbon onto less toxic compound through metabolic and enzymatic reactions [10, 12]. The biodegradation is mainly carried out by bacteria, yeast and fungi. Bacteria represent the major class of microorganisms involved in the degradation of hydrocarbons [2, 13]. There are two approaches for bioremediation: bioaugmentation and biostimulation [14–16]. Bioaugmentation consists in the addition of highly efficient oil-degrading bacteria to improve and enhance the degradation. While biostimulation consists in the modification of the environment conditions in order to stimulate indigenous bacteria activity.

The aim of this chapter is to provide an overview on the diversity of hydrocarbonoclastic bacteria and their hydrocarbon degradation potential as well as an update on the different bioremediation strategies adopted for the treatment of polluted marine environments particularly the Mediterranean basin. The mechanism by which hydrocarbonoclastic bacteria degrade hydrocarbons will also be discussed.

2. Hydrocarbon pollution in the Mediterranean Sea

The Mediterranean Sea is highly exposed to hydrocarbon pollution due to its geographical situation taking into account the location of most oil-producing and oil-consuming countries, placed, respectively, on the Southern and Northern sides of the basin [17]. It is an enclosed basin and is a very strategic maritime route that hosts about 20% of the global oil tanker traffic, and its coasts host about 80 petroleum harbors. Every year approximately 150,000 tons of hydrocarbons are transported in the Mediterranean Sea [18, 19].

The Mediterranean Sea is highly exposed to several sources of hydrocarbon pollution particularly linked to maritime traffic as well as extraction and refinery activities can affect human health and disturb ecological balance. Moreover, this pollution seriously threatens the marine life [20]. A very high microbial diversity was detected and estimated at 17,000 different species in the Mediterranean Sea constituting a habitat and a reservoir for novel microorganisms [20].

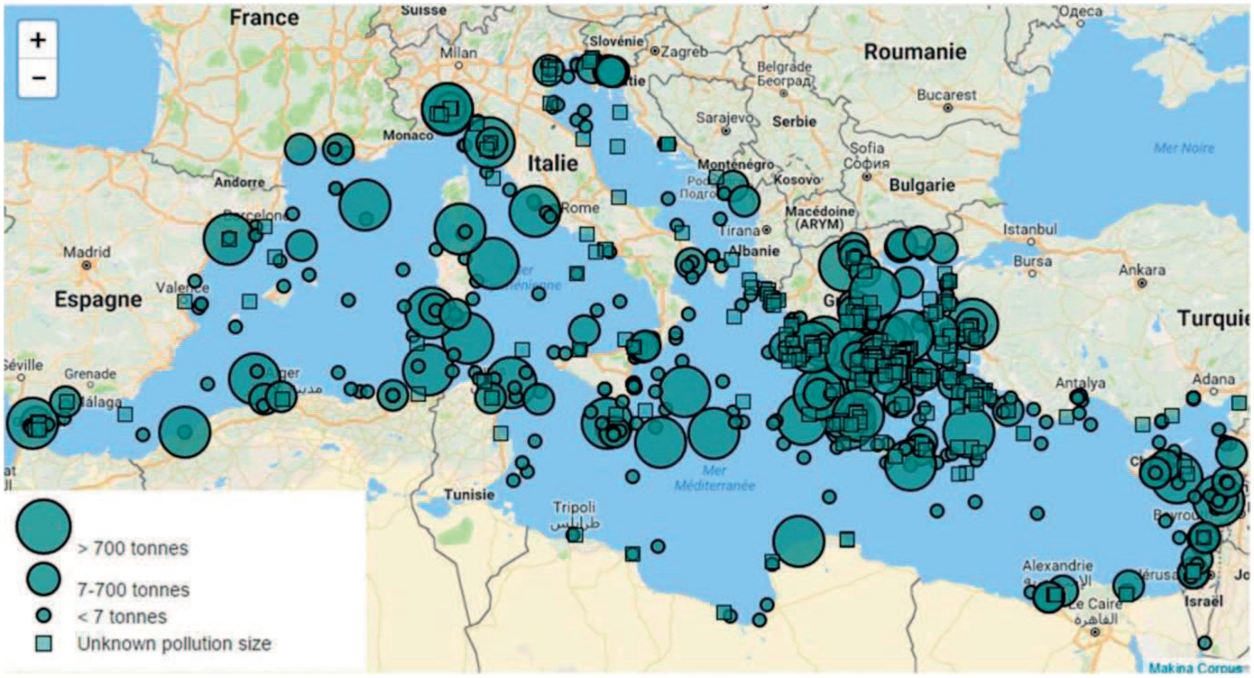

Figure 1. Accidents in the Mediterranean Sea, data from the REMPEC database from 1977 (http://www.rempec.org).

The Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea (REMPEC) was established in 1989. It aims the protection of areas and biological diversity in the Mediterranean Sea against pollution by oil and other harmful substances. According to the REMPEC database on alerts and accidents in the Mediterranean Sea, a high number of marine accidents that caused pollution of the Mediterranean Sea by oil were recorded since 1977. It is estimated at 659 accidents (**Figure 1**) that have led to the spillage of approximately 310,000 tons of oil since 1977 and at least 120,000 tons of hazardous and noxious substances since 1988 [21].

3. Different classes of hydrocarbons

Crude oil is an extremely complex mixture of tens of thousands of hydrocarbons (aliphatics and aromatics) and nonhydrocarbons (containing sulfur, nitrogen, oxygen and various trace metals). The composition of oil varies depending on their origin. Moreover, in the same source, we can also observe a time-dependent variation. For that reason, it is difficult to give a detailed composition of oil [22].

In general, the different compounds of crude oil can be classified into three groups as described in **Figure 2**.

3.1. Aliphatic hydrocarbons

Aliphatic hydrocarbons are composed of hydrogen and carbon, which can be linear, branched or cyclic. Aliphatic compounds can be saturated or unsaturated. There are several types of aliphatic hydrocarbons, including alkanes, alkenes and alkynes. Alkanes are the most abundant constituents in crude oil and are the first component to be degraded.

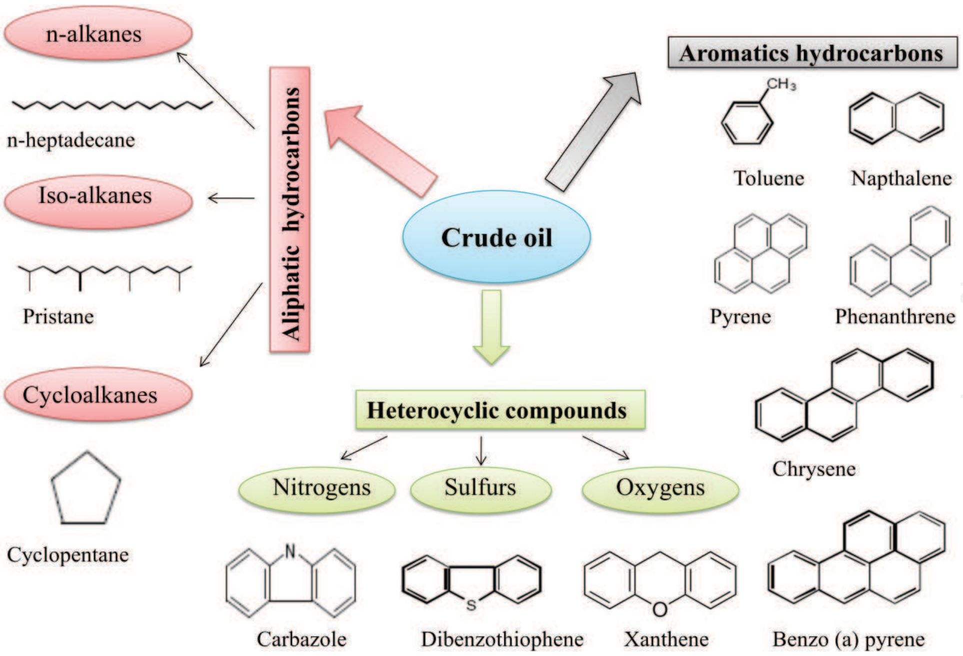

Figure 2. Different classes of hydrocarbon.

3.2. Aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a group of approximately 10,000 compounds that are atmospheric, water and soil pollutants containing one or more aromatic rings [23]. Among monoaromatic compounds, benzene, toluene and xylene were well reported and studied. Polycyclic aromatic hydrocarbons (PAHs), such as naphthalene, anthracene and phenanthrene, are also well documented. Due to their complex structure, PAHs are highly resistant to degradation and remain persistent in the ecosystem [24].

3.3. Heterocyclic compounds

Heterocyclic compounds are organic compounds containing at least one heterocyclic ring. They consist of compounds in which common heteroatoms are incorporated (oxygen, nitrogen and sulfur) into an organic ring structure in place of a carbon atom. They include polar compounds such as nitrogen (quinolines), sulfur (dibenzothiophenes) and oxygen (xanthene) atoms. Heterocyclic compounds are the most recalcitrant for degradation.

4. Risk hazards associated to hydrocarbon contamination

4.1. Impact on human health

Several human hazards are associated to the hydrocarbon dissemination. In fact, acute hydrocarbon exposure was reported to induce several pathologies including encephalopathy, arrhythmia, acidosis and dermatitis [25].

The most dangerous consequences of acute hydrocarbon ingestion likely arise from aspiration and result in pneumonitis [26]. Moreover, PAHs are well described for their toxic, mutagenic and/or carcinogenic properties [27–29]. Sixteen PAHs were identified by the US Environmental Protection Agency and the European Union as priority pollutant, and seven PAHs are regarded as carcinogens [30].

The American Association of Poison Control Centers Toxic Exposure Surveillance System studied 318,939 exposures to benzene, toluene/xylene, halogenated hydrocarbons, kerosene and lamp oil [31]. They demonstrated that hydrocarbons absorbed systemically and characterized by a low viscosity-induced higher hazard factors [31].

4.2. Hydrocarbon toxicity toward microorganisms

Considering the fact that hydrocarbon bioremediation is an important route for their environmental decontamination, these pollutants should not exert a toxic effect on the microbial community. However, several hydrocarbons and especially cyclic ones are known to be toxic to microbial cells. This toxicity is directly correlated to their partition coefficient between octanol and water (logP). The lipophilic compound accumulates into the lipid bilayers enhancing their availability to the cells. Due to hydrocarbon accumulation, the membrane loses its integrity and its permeability to protons and ions [32]. Due to those disturbances, dissipation of the proton motive force and impairment of intracellular pH homeostasis occur [32]. In order to overcome this toxicity and be able to degrade hydrocarbons, microbial strains should use a tolerance strategy toward those compounds. Sikkema and coworkers reported some adaptation mechanisms [32]. Microorganisms are thus able to increase structuring of the bilayer by changing the fatty acid conformation from cis to trans or by saturation of the fatty acid acyl chains. Another adaptation mechanism consists of the enhancement of the cross-linking degree between constituents of the cell wall and modifications of the cell wall hydrophobicity.

5. Hydrocarbon bioremediation: bioaugmentation, biostimulation and landfarming

Due to their high environmental and health-associated hazards, several physical and chemical hydrocarbon treatment methods have been investigated such as incineration, chlorination, UV oxidation and solvent extraction [24]. However, most of these approaches are associated to several drawbacks in terms of ecological impact and of removal efficiency.

In the meanwhile, the biological approach revealed to be of great interest for hydrocarbon removal. Several approaches were reported. We mainly distinguish (a) phytoremediation strategy that uses plants for decontamination purposes and (b) bioremediation that involves the use of microbial population for the cleanup of contaminated sites.

Bioremediation is considered as a tool for the decontamination of hydrocarbon polluted sites and as an extremely effective approach associated to eco-friendly features and relatively low cost. It is also considered as a safe approach. It is based on the catabolic activities of microorganisms and their ability to use pollutant as carbon and energy source by transforming it into less or non-toxic compounds.

Different bioremediation strategies are adopted depending on several factors such as site characteristics, type and concentration of pollutants [33]. Bioremediation can either be carried out ex situ or in situ. Ex situ methods involve the physical removal of the contaminated land for treatment process. However, in situ techniques involve treatment of the contaminated material in place [34].

The bioremediation efficiency depends mainly on the microbial structure, the sites to be decontaminated and the environmental conditions. These factors influence the degradation rate and the microbial activity. Moreover, bioremediation efficiency can be enhanced by three complementary approaches: bioaugmentation, biostimulation and landfarming [35].

5.1. Bioaugmentation

Bioaugmentation consists in the addition of selected microbial species, harboring specific catabolic abilities, into a contaminated environment. However, this technique involves the application of allochthonous or autochthonous microorganisms. The autochthonous bioaugmentation (ABA) consists on the exclusive introduction of indigenous microorganisms to the sites to be decontaminated [35, 36]. As described in **Figure** 3, the bioaugmentation treatment is detailed, where A is related to the cultivation of bacteria and/or consortium with high biodegradative capability in laboratory; B showed the addition on natural polluted environment; Cs explain the attack of bacteria and/or microbial consortium selected of pollutant (crude oil and/or hydrocarbons) and D showed the development of biodegradative process. The ABA approach consists on the addition of autochthonous microbial isolates or consortia that are adapted to the contaminated environments [37]. Different studies confirmed the efficiency of autochthonous microorganisms in the decontamination of hydrocarbon-polluted sites based on the fact that environmental conditions are suitable for their growth and metabolism [36, 38]. They significantly contribute to the biochemical activities in soil. Allochthonous microorganisms are defined as non-indigenous and originated from other site. When they are added into contaminated sites, they cannot easily integrate and adapt to the activities of the indigenous microbial community. Fodelianakis et al. [38] confirmed that bioaugmentation with allochthonous hydrocarbon-degrading bacterial consortia did not enhance the remediation process of hydrocarbon-contaminated sediments. Thus, the application of autochthonous microorganism is more effective [35]. Generally, Bioaugmentation is performed by using mixed consortia ([39]). Mrozik and Piotrowsk [40] showed the successful bioaugmentation of contaminated sites with a mixture of PAHs (naphthalene, phenanthrene, anthracene, pyrene, dibenzo[a] anthracene, benzo[a]pyrene) using a reconstructed microbial consortium. The addition of *Absidia cylindrospora* enhance the degradation of polycyclic aromatic hydrocarbons (more than 90% of fluorine was removed) more than of fluorine (a within 288 h) [41].

For a successful bioaugmentation treatment, environmental conditions, selected and indigenous microorganisms should be well controlled and characterized.

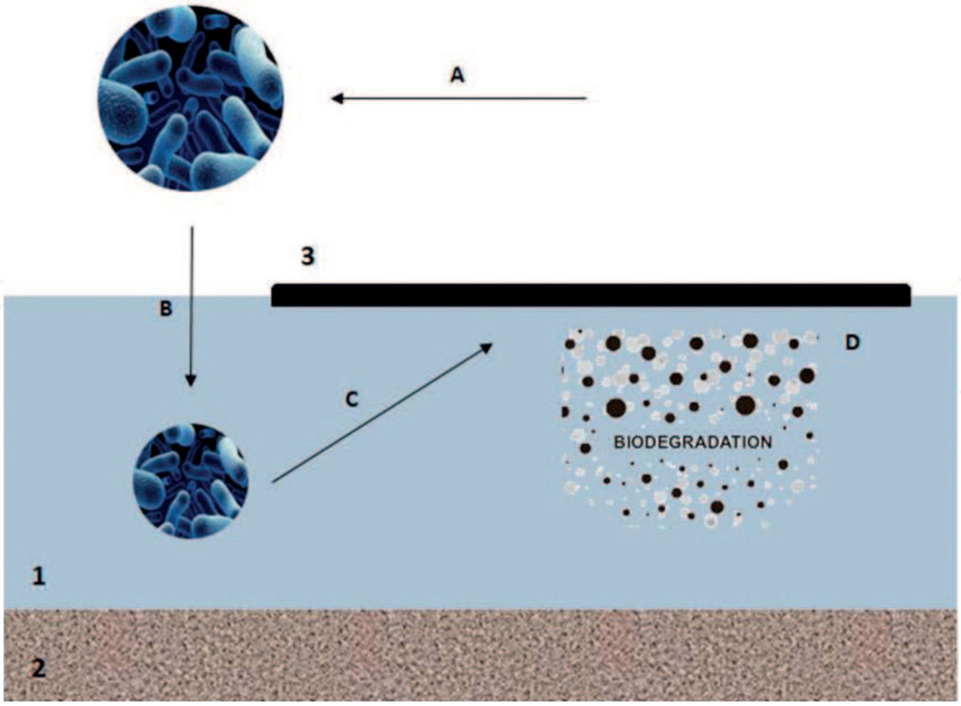

Figure 3. Schematic description of bioremediation (bioaugmentation) process. 1: Seawater; 2: marine sediment; 3: crude oil.

5.2. Biostimulation

Biostimulation consists in the addition of different substance to polluted environments to stimulate the natural biodegradation by indigenous degrading microorganisms. The biodegradation of hydrocarbons in marine environment is limited by low oxygen and nutriment availability, more specifically nitrogen and phosphorous [42]. The low bioavailability of pollutants is also considered as a limiting factor [42]. The biostimulation increases the growth of indigenous hydrocarbon degraders by the addition of growth-limiting nutrients. Moreover, the addition of specific substrate promotes and stimulates the degradation of the pollutant [43] (**Figure 4**). Adams et al. [44] showed the effectiveness of Biostimulation treatment with the addition of organic nutrients in contaminated soil containing more than 38,000 mg/kg TPH, and the result showed 100% removal of 2–3 ringed PAHs within the first 3 months.

5.3. Landfarming

Landfarming is a bioremediation approach, also known as land treatment or land application. This biotreatment process involves the spreading of excavated contaminated soils or sediments in a thin layer on a suitably prepared surface. In addition, the stimulation of the microbial activity in the soil is performed through the aeration and/or the addition of minerals and nutrients. Thus, the incorporated contaminated soil is periodically turned over or tilled to aerate the mixture. Various hydrocarbon contaminated soils were successfully

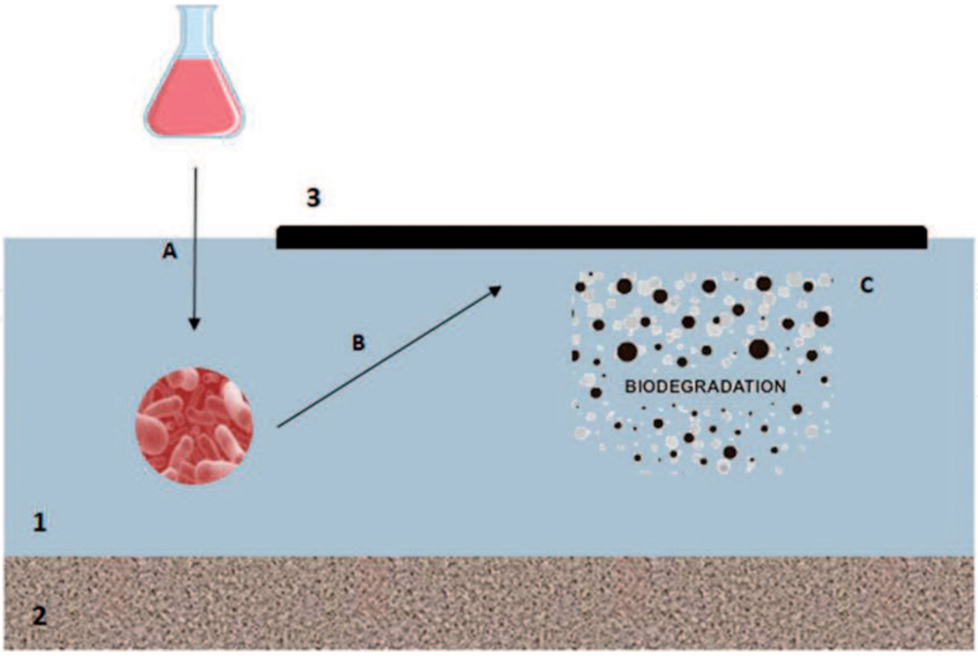

Figure 4. Schematic description of bioremediation (biostimulation) process. 1: Seawater; 2: marine sediment; 3: crude oil.

treated by landfarming [45]. Landfarming presents various advantages comparing to other treatment technologies. It has low cost, energy consumption and simple to implement. It has been successfully practiced for the remediation of hydrocarbon-contaminated soils [46]. The effectiveness of bioaugmentation in the bioremediation of oil-contaminated sediments, from the Mediterranean Sea, treated *ex situ* by landfarming was reported [38].

6. Factors affecting the bioremediation treatment

Various physical and chemical factors affect the rate of hydrocarbon degradation. Among many, we can cite the nature and amount of hydrocarbons, the type of polluted matrix (soil, sediment, water and effluents), the environmental conditions and the microbial community activity.

The environmental factors include the availability of nutrients, the temperature, the water content or humidity, oxygen, the bioavailability of the pollutant and the pH [47–49].

6.1. Nutrient availability

Inorganic sources such as nitrogen, phosphorus, potassium, hydrogen or oxygen are essential for microbial metabolism and affect the growth and the activity of microorganisms [48], whereas micronutrients, including zinc, manganese, iron, nickel, cobalt, molybdenum, copper and chlorine, are required but at a very low quantity. The ratios of carbon/nitrogen or carbon/phosphorus are considered as a determining factor of biodegradation rates and are high in hydrocarbon-contaminated sites which limit and affect the degradation rate [50].

Considerable effort has been devoted to the development of nutrient delivery systems that overcome the washout problems characteristic of open sea and intertidal environments; the use of slow release fertilizers and/or oleophilic nutrients can provide a continuous source of nutrients to polluted environment areas. Slow release fertilizers consist typically of inorganic nutrients in solid form coated with a hydrophobic compound like paraffin or vegetable oil. Successful alternative that overcomes the problem of quick dilution and wash out of water-soluble nutrients containing nitrogen and phosphorus is oleophilic biostimulants. The application of N and P sources in oleophilic form is considered to be the most effective nutrient application method, since oleophilic additives remain dissolved in the oil phase and thus are available at the oil-water or oil-sediment interface where they enhance bacterial growth and metabolism [43, 51].

6.2. Temperature

Temperature has a considerable effect on the ability of microorganisms to degrade hydrocarbons. At high temperatures, solubility, bioavailability, hydrocarbons distribution and diffusion rate increase, which promote the microbial biodegradation ability and enhance the biodegradation rate. On the other hand, very high temperature decreases oxygen solubility and limits the aerobic microbial biodegradation [52, 53].

Beside, Boopathy [34] confirmed that the degradation of pollutant in mesophilic temperatures is better and more efficient than the degradation at very low or high temperatures. However, it was reported that microorganisms are able to metabolize PAHs at extreme temperatures: for example, a high degradation rate of PAHs occurred in seawater at low temperatures (low as 0°C) and at 50°C [54]. Bargiela et al. [55] confirmed the correlation between the relative percentage of genes encoding enzymes participating in the biodegradation and temperature in oil-polluted sites along the coastlines of the Mediterranean Sea. The authors proved that low temperature increases bacterial richness with decreasing catabolic diversity. The relative percentage of gene sequences encoding enzymes for biodegradation ranged from 0.56 to 1.30% in low temperature (<3°C), whereas this number increased with site temperature 5.6-fold higher in the moderately warmer sites (from 13 to 26.5°C) [55].

6.3. Oxygen limitations

The initial steps in the catabolism of aliphatic, cyclic and aromatic hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases for which molecular oxygen is required. Although anaerobic biodegradation has been shown to occur in different ecosystems including marine environments, its ecological significance has been generally considered to be minor and the biodegradation rate is rather low [24]. Conditions of oxygen limitation normally exist in aquatic sediments and soils. Oxygen depletion can occur in the presence of easily utilizable substrates that increase microbial oxygen consumption. In several instances, the concentration of dissolved oxygen can be close to zero leading to practically zero aerobic biodegradation rates. Although oxygen can be successfully delivered (in various forms) to hydrocarbon-contaminated soils and groundwater, enhancing biodegradation rates, this is not the case in marine environments as it is very difficult to implement such technologies in the field. Tilling is essentially the only option in aerating the top layers of contaminated sediments during low tide [34, 48]. Thus, oxygen represents a very significant and potentially factor which limit the rate of hydrocarbon degradation.

6.4. pH

pH varies less in aquatic environments and most bacteria and fungi capable of degrading hydrocarbon require a neutral pH [52, 53]. In general, microbial activity is influenced by extremely low or high pH [53]. According to Bamforth and Singleton [48], 40% of phenanthrene degradation was observed for *Burkholderia cocovenenans* at pH 5.5. However, the degradation at neutral pH in the same conditions was 80%. Additionally, Leahy and Colwell [53] reported that microbial degradation of naphthalene decreased at pH 5.0 compared to the highest rate of degradation observed at pH 7. Moreover, other reports described the efficiency of some microorganisms, such as *Pseudomonas,* to degrade hydrocarbon at alkaline pH [48]. A degradation of PAH in acidic contaminated environment (pH 2) by indigenous microorganisms was reported [54]. The adequate pH depends on microorganisms to be used for the bioremediation.

6.5. Bioavailability of hydrocarbon

The bioavailability is defined as the rate of substrate mass transfer into the microbial cells. It is considered as one of the most determinant parameters regarding hydrocarbon degradation rate. PAHs are characterized by a low bioavailability due to their low aqueous solubility. That is the reason why they are reported to be resistant to the degradation process and persistent in the environment [24]. Unsuccessful remediation of PAH-contaminated sites was reported due to the low bioavailability of PAHs [24]. It has been reported that bioavailability of hydrocarbon decreases with time [24]. Although the photo-oxidation increased the biodegradation of petroleum hydrocarbon by increasing its bioavailability which promote microbial activities [56]. Hydrocarbon and particularly PAHs become more bioavailable when they are dissolved or evaporated [27]. The bioavailability of pollutant in contaminated environment may be increased by the application of surfactants.

7. Distribution of hydrocarbon-degrading microorganisms

Oil spill in the marine environment can be highly toxic to the marine microbial communities. However, a part of these communities resist to this type of pollution and are able to metabolize the pollutant.

Several studies have revealed a huge diversity of microorganisms (bacteria, fungi, archaea and algae), which were isolated from contaminated environments and were able to degrade hydrocarbons. Recent research reported that 79 bacterial, 9 cyanobacterial, 103 fungal and 14 algal are able to use hydrocarbons as a sole source of carbon and energy [39]. Moreover, in the marine environment, at least 25 bacterial genera of hydrocarbon degrader were found [57].

Bacteria are the most important and abundant group known for hydrocarbon degradation.

However, there is a class of bacteria that uses exclusively hydrocarbons as substrates and has been characterized as obligate hydrocarbonoclastic bacteria (OHCB) [39, 58, 59]. This group is present at low or undetectable levels in marine environment before a contamination with hydrocarbon. Hydrocarbon-degrading microorganisms are widely distributed and ubiquitous in marine environment especially after a contamination with hydrocarbons. Their number and activity increase and can represent 100% of the total microbial community. However, they can be found in non-contaminated environment but in very small proportion which can represent 0.1% of the total microbial community [53, 60, 61].

Based on microbial isolation and cultivation from hydrocarbon-contaminated environment, several microorganisms were isolated and characterized. However, this method can detect only 1–2% of the most dominant microbial species and only a small fraction of those communities are able to be cultivated [62]. In addition, different molecular approaches were used to study the composition and the structure of the bacterial community in different environment such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), automated ribosomal intergenic spacer analysis (ARISA) and 16SrRNA gene clone libraries [62–64].

With recent advances in genomics and sequencing technologies, more techniques based on high throughput sequencing of DNA (next generation sequencing technologies) are improved in metagenomic studies (**Figure 5**).

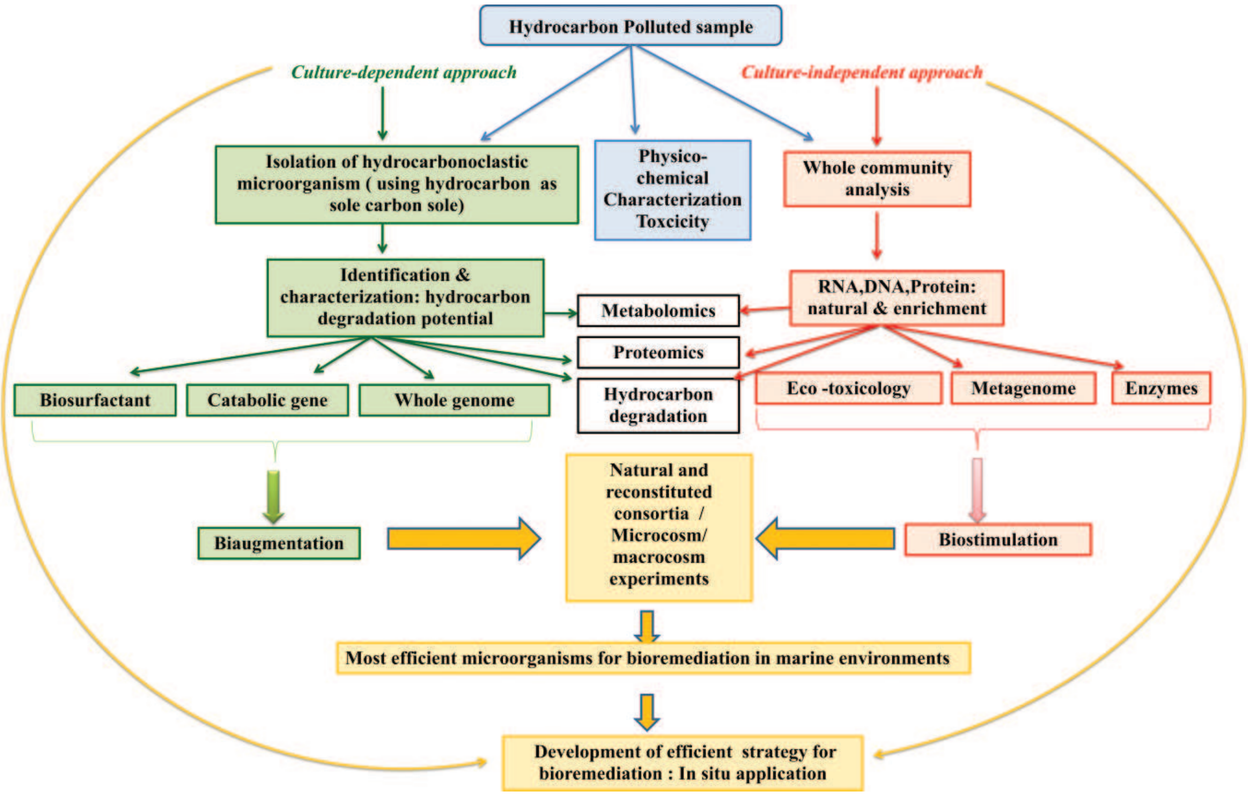

Figure 5. Schematic diagram explaining the different microbial hydrocarbon degradation strategy for bioremediation.

These hydrocarbonoclastic bacteria are of great importance since they belong to the key players in oil removal from contaminated marine sites. Among these OHCB, *Alcanivorax*, *Marinobacter*, *Thallassolituus*, *Cycloclasticus* and *Oleispira* are the most representative genera and they play a key role in the degradation of hydrocarbons [65]. As described in **Figure 6**, bacteria, which are able to degrade saturated and polycyclic aromatic hydrocarbons, were presented by red and blue, respectively, whereas the green organism is able to degrade dioxin compounds. The organisms shown in black color in **Figure 6** are able to degrade both saturated and PAH.

Among obligate hydrocarbonoclastic bacteria, *Alcanivorax borkumensis* is ubiquitous in hydrocarbon-polluted marine environment. This strain is able to metabolize linear and branched alkanes, but unable to degrade aromatic hydrocarbons [66, 67]. *Thallassolituus oleivorans* is highly specialized to degrade aliphatic hydrocarbons from C7 to C20 carbons [68]. Also, *Oleiphilus* and *Oleispira* are well known in the degradation of aliphatic hydrocarbons [58]. Whereas *Cycloclasticus* is reported to mineralize various PAHs such as naphthalene, phenanthrene, anthracene, pyrene and fluorene [69]. *Marinobacter* strains are able to degrade

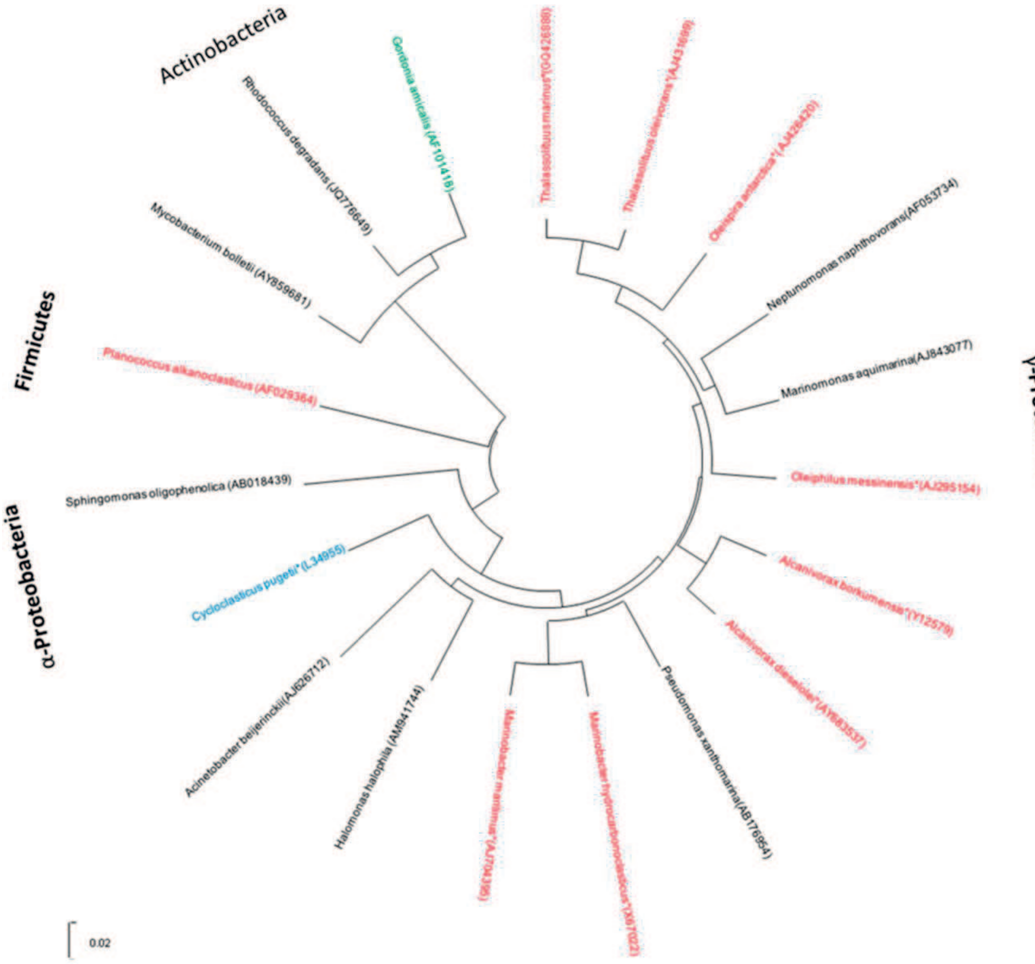

Figure 6. A phylogenetic tree of the distribution of hydrocarbon-degrading bacteria based on 16S rDNA partial sequences for type strains using MEGA 6.06. 16S rRNA sequence accession numbers of the reference type strains are indicated in parentheses. Microorganisms* showed obligate hydrocarbonoclastic bacteria.

hydrocarbon compounds efficiently [70]. Other non-obligate hydrocarbonoclastic bacteria have been described for their abilities to degrade different classes of hydrocarbons and were isolated from oil-contaminated environment. Among this group, able to degrade PAHs, we distinguish *Pseudomonas*, *Marinomonas*, *Halomonas* and *Micrococcus*. Moreover, *Acinetobacter* and *Sphingomonas* are well described in the degradation of aliphatic compounds [58].

Several studies reported that the efficiency of biodegradation ranged from: 6 to 82%, 0.13 to 50% and 0.003 to 100%, respectively, by fungi, soil bacteria and marine bacteria [71].

Among fungi, genera affiliated to *Amorphoteca*, *Neosartorya*, *Talaromyces*, *Aspergillus* and *Graphium* are able to degrade hydrocarbon. In addition, various studies reported the efficiency of hydrocarbon degradation by yeast belonging to *Candida lipolytica*, *Rhodotorula mucilaginosa*, *Geotrichum* sp. and *Trichosporon mucoides* [71].

Since crude oil is a complex mixture of hydrocarbon and individual microorganism is able to degrade only a limited number of crude oil components, several studies reported the efficiency of the degradation of crude oil by a whole consortium. It was defined as mixed cultures of hydrocarbon-degrading microorganism composed either by known hydrocarbon degrader microorganisms or by enrichment of indigenous microbial consortia. Several reports confirmed that the application of a consortium, with a broad enzymatic capacities to degrade a wide range of hydrocarbons, accelerate bioremediation treatment [33, 39, 44, 72]. A reconstructed consortium composed by a mixture of bacteria and fungi was able to achieve a high degradation of petroleum based on their complimentary metabolic potentials to metabolize the different components [72].

8. Mechanism of hydrocarbon degradation

The trend in biodegradation of petroleum hydrocarbons rate is performed in the following order: n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes. Resin and asphaltenes are the most recalcitrant compounds of the petroleum hydrocarbons [73].

8.1. Alkane-degrading bacteria

Alkanes are saturated hydrocarbons composed exclusively by carbon and hydrogen atoms. They can be n-alkanes (linear), cycloalkanes (cyclic) or isoalkanes (branched).

The major fraction of crude oil can be composed by alkanes which constitute more than 50% of crude oil depending on the type and source of oil.

Various aerobic and anaerobic microorganisms possess the metabolic capacity to use alkanes as sole carbon source and energy. Bacteria, fungi and yeasts were reported to degrade alkanes. The degradation of alkane by bacteria has been extensively studied [58]. The biodegradation of saturated hydrocarbons is essentially an aerobic process carried out by bacteria. Microorganism-degrading alkanes are widely distributed in the environment. The biodegradation of n-alkanes is better known than other classes of saturated hydrocarbons. Alkane hydroxylase is a key enzyme involved in alkane degradation [74].

Methane monooxygenase is involved in the degradation of short-chain length alkanes (C_2–C_4) [74]. The degradation of medium n-alkanes (C_5–C_{17}) was carried out via cytochrome P450 and monooxygenases including AlkB [73]. For the degradation of long n-alkanes > C_{18}, multiple alkane hydroxylases are reported [74].

Alcanivorax spp., initially undetectable in oil-contaminated sea water, were reported to constitute 90% of the whole bacterial community after nutrient addition in biostimulation treatment [39]. The detection of *alkB* genes, which encode the catalytic activity of alkane hydroxylase allowing the degradation of medium to long chain hydrocarbons, was also reported [74]. This gene was firstly discovered in *Pseudomonas putida* GPo1. Afterward, it was found in the majority of alkane-degrading bacteria. With advanced researches, 250 *alkB* gene homologs were reported in hydrocarbonoclastic bacteria [74].

8.2. Polycyclic aromatic hydrocarbons (PAHs)

PAHs represent a class of hazardous environmental pollutants that are accumulated in the environment from various origins and mainly from anthropogenic activities. PAHs are present in crude oil but in low concentrations. They are highly toxic and recalcitrant [60, 61].

Currently, there are many reports on aerobic catabolism of aromatics compounds and various catabolic pathways were investigated. In general, we distinguish two major strategies for PAHs degradation in the presence and absence of oxygen [74].

Usually, the degradation of PAHs by bacteria involves mono and dioxygenase since the first step is the hydroxylation of the aromatic ring *via* a dioxygenase which is a complex of enzymes composed by reductase, ferredoxin and terminal oxygenase subunits. Besides, several bacteria degrade PAHs through cytochrome P450 [24].

PAHs can be completely metabolized to carbon dioxide and water due to the presence of oxygen [61, 75].

The anaerobic biodegradation of PAHs is a slow process, and a few numbers of preliminary studies have reported the degradation pathways, catabolic genes and enzymes of this degradation. Among PAHs reported for their degradation in anoxic condition, we can cite naphthalene, anthracene, phenanthrene, fluorene, acenaphthene and fluoranthene [74].

9. Biosurfactant

The bioavailability of contaminants is considered as a major limit for the degradation and bioremediation of contaminated sites. This limit may be recovered by the use of biosurfactant produced by bacteria. Biosurfactant reduces the surface tension and facilitates the contact between microorganism and pollutants. Numerous studies reported the effect of biosurfactants on biodegradation of hydrocarbons and their industrial and commercial potentials [76, 77].

Biosurfactants are of great interest since these compounds are widely used in various fields, including industrial applications, agriculture, crude-oil recovery, cosmetics, pharmaceuticals, detergents, food processing and textile, and in various fields of biomedicine. Therefore, these compounds have advantages compared to chemical surfactant because these molecules are nontoxic, biodegradable and can be used under extreme conditions of temperature, pH and salinity [78–80].

Studies dealing with the biotechnological use of biosurfactants have significantly increased within the last years. Nowadays, with the research advance, the use of biosurfactants is being a potential alternative and promising tool for bioremediation.

Acinetobacter, *Stenotrophomonas*, *Pseudomonas*, *Koccuria* and *Bacillus*, isolated from hydrocarbon-contaminated sites in Tunisia, showed significant biosurfactant production and emulsification activities [2]. Several microorganism genera including *Corynebacterium*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Achromobacter* and *Ochrobactrum* were identified as efficient biosurfactant producers (**Table** 1) [83]. Therefore, these bacteria may constitute potential candidates for bioremediation and can be useful for biotechnological applications. *Pseudomonas* has been widely studied for their ability to produce biosurfactants with a glycolipid structure. Rhamnolipids are the better characterized biosurfactants and enhanced oil recovery and bioremediation process [84–86]. *Bacillus subtilis* is known for its ability to produce lipopeptide biosurfactant known as surfactin, which is used in commercial application [82, 87]. Additionally, the ability of *Brevibacillus* sp. to produce biosurfactant during phenanthrene degradation and an association between biodegradation and production of biosurfactants was shown [88]. Other report describes the rhamnolipids produced by *Burkholderia plantarii* and their application in pharmaceutical and industries [89]. According to Bordoloi and Konwar [81], a higher solubilization of pyrene and fluorine due to the addition of biosurfactant was observed. *Rhodococcus erythropolis* produced Trehalose lipids, biosurfactant which stimulate the solubilization of phenanthrene to facilitate the degradation of this hydrocarbon [90].

Types	Biosurfactants	Microorganisms	Application	References
Glycolipids	Rhamnolipid	*Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas chlororaphis, Renibacterium salmoninarum*	Bioremediation Biocontrol agent	Bordoloi and Konwar [81] and Banat et al. [78]
	Trehalolipid	*Rhodococcus, Mycobacterium, Corynebacterium, Gordonia, Arthrobacter*	Bioremediation Biocontrol agent	Banat et al. [78]
	Sophorolipid	*Candida bombicola*	Bioremediation	Whang et al. [82]
		Candida apicola	Emulsion	
Lipopeptides	Surfactin	*Bacillus subtilis*	Biomedical application	Banat et al. [78]
	Viscosin	*Pseudomonas fluorescens*		Banat et al. [78]
	Rhodofactin	*Rhodococcus erythropolis*		
Fatty acid	Fatty acid	*Corynebacterium lepus*		
Phospholipids	Phospholipid	*Corynebacterium insidiosum*		

Table 1. Main types of biological surfactants, producing microorganisms and application.

10. Case studies of bioremediation strategies used for the decontamination of polluted marine environments

Numerous and different studies can be carried out, for example, as application of bioremediation processes in the Mediterranean Sea, however, is particularly interesting is the example that can be extracted from the work of Cappello et al. [91], Genovese et al. [65] and Fodelianakis et al. [38].

In the work of Cappello et al. [91] and Genovese et al. [65], a modular system aimed at optimizing the biodegradation process was implemented and tested for the remediation of hydrocarbon-contaminated sediments collected in Messina harbor (Italy). The system was designed to operate directly in the field but retains the advantages of controlled methods that do not impact the surrounding environment. The effects of the addition of air and/or slow-released fertilizer, temperature regulation and the addition of oil sorbents on the efficiency of the system were evaluated. The abundance of the indigenous microbial communities was monitored for 30 days of treatment. Measures of microbial activity, biochemical oxygen demand (BOD), screen of functional genes, quantitative of petroleum hydrocarbon degradation and ecotoxicological bioassays using the organism *Corophium orientale* were carried out.

The proposed system was designed according to the two needs highlighted by Nikolopoulou and Kalogerakis: (a) the development of low-cost oxygenation systems for aerobic bioremediation of contaminated anoxic sediments and (b) the development of novel biostimulant methods that are nontoxic to the marine environment (**Table 2**).

The results showed the effect of the air injection in the development of bacterial biomass with an increase of bacterial population by the DAPI and MPN counts with air insufflations comparing to the control (untreated sediments). The addition of nutrients and the regulation of temperature enhance the degradation rate to achieve a high degradation in the shortest possible time. In the mesocosm system, ~70% of the total oil and linear hydrocarbons were degraded with the development of mesophilic bacteria. Besides, the addition of fertilizers promoted the degradation of crude oil improving the degradation efficiency within the 30 days of the treatment when >90% of n-alkanes (C_{15}–C_{30}) and >60% of (alkyl)naphthalenes were degraded.

However, the authors found that biostimulation treatment through nutrient amendment to hydrocarbon-contaminated sediments enhanced the biodegradation activity of the indigenous microbial community and especially hydrocarbonoclastic bacteria such as *Alcanivorax*, *Marinobacter* and *Cycloclasticus*. In fact, sediment toxicity was evaluated by ecotoxicological method using *Corophium orientale*, an endemic and widespread Mediterranean amphipod, used as an indicator species in toxicity of harbor sediments [94]; a decrease of toxicity in treated sediments was observed (~30%) comparing to untreated system (95%). Thus, the success of the bioremediation treatment was explained by both reduction of the hydrocarbon in sediment and shift of toxicity.

As another example for bioremediation strategy to evaluate the effectiveness of bioaugmentation with allochthonous bacterial consortia in the bioremediation of oil-contaminated sediments collected from a coast adjacent to an oil refinery (Elefsina bay, Greece) treated ex

Type of bioremediation treatment	**Microorganisms/nutrients added**	**Type of contaminant**	**Percentage removal (%)**	**Duration (days)**	**References**
Bioaugmentation	*Acinetobacter baumannii*	Crude oil	43	35	Adams et al. [44]
	Candida tropicalis	Diesel	83	120	Adams et al. [44]
	Pseudomonas arthrobacter	Diesel	32	32	Adams et al. [44]
	Alcanivorax, Marinobacter Cycloclasticus and Thalassolituus	TERHC	79	14	Crisafi et al. [92]
	Acinetobacter	TPH	34	42	Wu et al. [93]
	Alcanivorax and *Thalassolituus*	Crude oil	70	20	Hassanshahian et al. [16]
	Absidia. Cylindrospora	Fluorene	90	12	Garon et al. [41]
	A bacterial consortia	Diesel	72	84	Adams et al. [44]
Biostimulation	Nitrogen and phosphorus	TPH	60	42	Wu et al. [93]
	Inorganic nutrients KH_2PO_4, NH_4Cl and $NaNO_3$	Crude oil	80	20	Hassanshahian et al. [16]
	Mixed of $(NH4)_2$, SO_4 and K_2HPO_4	Diesel	45	84	Adams et al. [44]
	Slow release fertilizers	Crude oil	90% of n-alkanes (C15–C30) and 60% of PAHs	30	Nikolopoulou and Kalogerakis [43]
	Inorganic nutrients (KNO_3 and K_2HPO_4)	Crude oil	97% of C12–C35 n-alkanes and 45% of PAH	45	Nikolopoulou and Kalogerakis [43]
	Organic nutrients (uric acid and lecithin) and biosurfactants (rhamnolipids)	n-Alkanes	97	45	Nikolopoulou et al. [36]
Bioaugmentation and biostimulation	*Rhodococcus* sp. and chemical surfactant and Tween 80	TPH	50	46	Adams et al. [44]
	Pseudomonas aeruginosa, Bacillus subtilis and fertilizer	TPH	75	70	Adams et al. [44]
	Inorganic nutrients + rhamnolipids and autochthonous bacterial consortia	Crude oil	80	30	Nikolopoulou et al. [36]

Table 2. Example of successful bioremediation strategies.

situ by landfarming. For the bioaugmentation treatment, a bacterial consortia composed by hydrocarbonoclastic strains isolated from different oil-contaminated sediments collected from North Africa (Morocco, Tunisia, Egypt) and Red Sea (Jordan) were used. Besides, the biostimulation was performed with the addition of N/P in the form of $KNO3/KH2PO4$ to a final ratio of C:N:P (100:10:1). The experiments were carried out for 56 days. The result showed that biodegradation was mostly performed by the autochthonous degraders, while bioaugmentation did not enhance the remediation process.

11. Conclusion

Petroleum hydrocarbons are the most common pollutants in marine environment and the Mediterranean Sea is particularly endangered by hydrocarbon pollution. This pollution generates a great hazard to marine ecosystems. Bioremediation through bioaugmentation or biostimulation constitutes a promising strategy for the cleanup of oil contamination. It was based on the presence of catabolic genes and enzymes, which allow microorganisms to utilize hydrocarbons as carbon and energy source. This chapter presents an overview of different efficient strategies used for the decontamination of polluted marine environments. There are various factors that affect bioremediation efficiency such as oxygen, pH, temperature and nutrient availability. Thus, it has been confirmed that the combination of autochthonous bioaugmentation and biostimulation constitutes the best and efficient strategy for the decontamination of petroleum-contaminated marine environment. However, further researches are still required to answer multiple questions to develop a successful system for bioremediation.

Acknowledgements

The authors thank for financial support the European Union in the ambit of research projects: ULIXES (European Community's Seventh Framework Programme, FP7-KBBE-2010-4, CP-FP-SICA under grant agreement no. 266473) and MADFORWATER (European Union's Horizon 2020, WATER-5c-2015 under grant agreement no.688320) and the Tunisian Ministry of Higher Education and Scientific research in the ambit of the laboratory project LR11ES31.

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References

[1] Turner NR, Renegar DA. Petroleum hydrocarbon toxicity to corals: A review. Marine Pollution Bulletin. 2017;**2**(119):1-16

[2] Mahjoubi M, Jaouani A, Guesmi A, Amor SB, Jouini A, Cherif H, Najjari A, Boudabous A, Koubaa N, Cherif A. Hydrocarbonoclastic bacteria isolated from petroleum contaminated sites in Tunisia: Isolation, identification and characterization of the biotechnological potential. New Biotechnology. 2013;**30**(6):723-733

[3] Cappello S, Caruso G, Zampino D, Monticelli LS, Maimone G, Denaro R, Tripodo B, Troussellier M, Yakimov MM, Giuliano L. Microbial community dynamics during assays of harbor oil spill bioremediation: A microscale simulation study. Journal of Applied Microbiology. 2007;**102**:184-194

[4] Cappello S, Santisi S, Calogero R, Hassanshahian M, Yakimov MM. Characterisation of oil-degrading bacteria isolated from bilge water. Water, Air, & Soil Pollution. 2012;**223**(6):3219-3226

[5] Jiang Z, Huang Y, Xu X, Liao Y, Shou L, Liu J, Zeng J. Advance in the toxic effects of petroleum water accommodated fraction on marine plankton. Acta Ecologica Sinica. 2010;**30**(1):8-15

[6] Costa AS, Romão LPC, Araújo BR, Lucas SCO, Maciel STA, Wisniewski A, Alexandre MDR. Environmental strategies to remove volatile aromatic fractions (BTEX) from petroleum industry wastewater using biomass. Bioresource Technology. 2012;**105**:31-39

[7] De Vasconcellos SP, Crespim E, da Cruz GF, Senatore DB, Simioni KCM, dos Santos Neto EV, Marsaioli AJ, de Oliveira VM. Isolation, biodegradation ability and molecular detection of hydrocarbon degrading bacteria in petroleum samples from a Brazilian offshore basin. Organic Geochemistry. 2009;**40**(5):574-588

[8] Greenwood PF, Wibrow S, George SJ, Tibbett M. Hydrocarbon biodegradation and soil microbial community response to repeated oil exposure. Organic Geochemistry. 2009;**40**(3):293-300

[9] Lin TC, Pan PT, Cheng SS. Ex situ bioremediation of oil-contaminated soil. Journal of Hazardous Materials. 2010;**176**(1):27-34

[10] Bacosa HP, Suto K, Inoue C. Bacterial community dynamics during the preferential degradation of aromatic hydrocarbons by a microbial consortium. International Biodeterioration & Biodegradation. 2012;**74**:109-115

[11] Liu PWG, Chang TC, Whang LM, Kao CH, Pan PT, Cheng SS. Bioremediation of petroleum hydrocarbon contaminated soil: Effects of strategies and microbial community shift. International Biodeterioration& Biodegradation. 2011;**65**(8):1119-1127

[12] Roy AS, Baruah R, Borah M, Singh AK, Boruah HPD, Saikia N, Deka M, Dutta N, Bora TC. Bioremediation potential of native hydrocarbon degrading bacterial strains in crude oil contaminated soil under microcosm study. International Biodeterioration& Biodegradation. 2014;**94**:79-89

[13] Dell'Anno A, Beolchini F, Rocchetti L, Luna GM, Danovaro R. High bacterial biodiversity increases degradation performance of hydrocarbons during bioremediation of contaminated harbor marine sediments. Environmental Pollution. 2012;**167**:85-92

[14] Bento F-M, Flávio AO, Camargo F-V-A, Okeke B-C, Frankenberger W-T. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Applied Bioresource Technology. 2005a;**96**:1049-1055

[15] Liu PWG, Chang TC, Chen CH, Wang MZ, Hsu HW. Bioaugmentation efficiency investigation on soil organic matters and microbial community shift of diesel-contaminated soils. International Biodeterioration& Biodegradation. 2014;**95**:276-284

[16] Hassanshahian M, Emtiazi G, Caruso G, Cappello S. Bioremediation (bioaugmentation/ biostimulation) trials of oil polluted seawater: A mesocosm simulation study. Marine Environmental Research. 2014;**95**:28-38

[17] Amer RA, Mapelli F, El Gendi HM, Barbato M, Goda DA, Corsini A, Lucia Cavalca L, Fusi M, Borin S, Daffonchio D, Abdel-Fattah YR. Bacterial diversity and bioremediation potential of the highly contaminated marine sediments at El-Max district (Egypt, Mediterranean Sea). BioMed Research International. 2015;**2015**:17

[18] Daffonchio D, Ferrer M, Mapelli F, Cherif A, Lafraya Á, Malkawi HI, Yakimov MM, Abdel-Fattah YR, Blaghenh M, Golyshini PN, Kalogerakis N, Boon N, Magagnini M, Fava F. Bioremediation of southern Mediterranean oil polluted sites comes of age. New Biotechnology. 2013;**30**(6):743-748

[19] Daffonchio D, Mapelli F, Cherif A, Malkawi HI, Yakimov MM, Abdel-Fattah YR, Boon N, Magagnini M, Fava F. ULIXES, unravelling and exploiting Mediterranean Sea microbial diversity and ecology for xenobiotics' and pollutants' clean up. Reviews in Environmental Science and Bio/Technology. 2012;**11**(3):207-211

[20] Coll M, Piroddi C, Steenbeek J, Kaschner K, Lasram FBR, Aguzzi J, Ballesteros E, Bianchi CN, Corbera J, Dailianis T, Danovaro R, Estrada M, Froglia C, Galil BS, Gasol JM, Gertwagen R, Gil J, Guilhaumon F, Kesner-Reyes K, Kitsos MS, Koukouras A, Lampadariou N, Laxamana E, Lopez-Fe de la Cuadra CM, Lotze HK, Martin D, Mouillot D, Oro D, Raicevich S, Rius-Barile J, Saiz-Salinas JI, San Vicente C, Somot S, Templado J, Turon X, Vafidis D, Villanueva R, Voultsiadou E. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. PLoS One. 2010;**5**(8):e11842

[21] REMPEC: Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea. http://www.rempec.org.

[22] Singh SN, Kumari B, Mishra S. Microbial degradation of alkanes. In: Microbial Degradation of Xenobiotics. Berlin Heidelberg: Springer; 2012. pp. 439-469

[23] Hassanshahian M, Abarian M, Cappello S. Biodegradation of Aromatic Compounds. In Biodegradation and Bioremediation of Polluted Systems-New Advances and Technologies. InTech; 2015

[24] Ghosal D, Ghosh S, Dutta TK, Ahn Y. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Frontiers in Microbiology. 2016;**7**:1369

[25] Tormoehlen LM, Tekulve KJ, Nanagas KA. Hydrocarbon toxicity: A review. Clinical Toxicology. 2014;**52**(5):479-489

[26] Mickiewicz M, Gomez HF. Hydrocarbon toxicity: General review and management guidelines. Air Medical Journal. 2006;**20**(3):8-11

[27] Abdel-Shafy HI, Mansour MS. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum. 2016;**25**(1):107-123

[28] Rengarajan T, Rajendran P, Nandakumar N, Lokeshkumar B, Rajendran P, Nishigaki I. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific Journal of Tropical Biomedicine. 2015;**5**(3):182-189

[29] Yu H. Environmental carcinogenic polycyclic aromatic hydrocarbons: Photochemistry and phototoxicity. Journal of Environmental Science and Health, Part C. 2002;**20**(2):149-183

[30] Sany SBT, Hashim R, Rezayi M, Salleh A, Rahman MA, Safari O, Sasekumar A. Human health risk of polycyclic aromatic hydrocarbons from consumption of blood cockle and exposure to contaminated sediments and water along the Klang Strait, Malaysia. Marine Pollution Bulletin. 2014;**84**(1):268-279

[31] Cobaugh DJ, Seger DL, Krenzelok EP. Hydrocarbon toxicity: An analysis of AAPCC TESS data. Przeglad Lekarski. 2006;**64**(4-5):194-196

[32] Sikkema J, De Bont JA, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews. 1995;**59**(2):201-222

[33] Santisi S, Cappello S, Catalfamo M, Mancini G, Hassanshahian M, Genovese M, Giuliano L, Yakimov MM. Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium. Brazilian Journal of Microbiology. 2015;**46**(2):377-338

[34] Boopathy R. Factors limiting bioremediation technologies. Bioresource Technology. 2000;**74**(1):63-67

[35] Nikolopoulou M, Pasadakis N, Kalogerakis N. Evaluation of autochthonous bioaugmentation and biostimulation during microcosm-simulated oil spills. Marine Pollution Bulletin. 2013a;**72**(1):165-173

[36] Nikolopoulou M, Eickenbusch P, Pasadakis N, Venieri D, Kalogerakis N. Microcosm evaluation of autochthonous bioaugmentation to combat marine oil spills. New Biotechnology. 2013b;**30**(6):734-742

[37] Hosokawa R, Nagai M, Morikawa M, Okuyama H. Autochthonous bioaugmentation and its possible application to oil spills. World Journal of Microbiology and Biotechnology. 2009;**25**(9):1519-1528

[38] Fodelianakis S, Antoniou E, Mapelli F, Magagnini M, Nikolopoulou M, Marasco R, Barbato M, Tsiola A, Tsikopoulou I, Mahjoubi M, Jaouani A, Amer R, Hussein E, Al-Horani FA, Benzha F, Blaghen M, Malkawi HI, Abdel-Fattah Y, Cherif A, Daffonchio D, Kalogerakis N. Allochthonous bioaugmentation of crude oil-polluted sediments is inefficient in the presence of an effective degrading indigenous microbiome. Journal of Hazardous Materials. 2015;**287**:78-86

[39] Head IM, Jones DM, Röling WF. Marine microorganisms make a meal of oil. Nature Reviews. Microbiology. 2006;**4**(3):173

[40] Mrozik A, Piotrowska-Seget Z. Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Microbiological Research. 2010;**165**(5):363-375

[41] Garon D, Sage L, Wouessidjewe D, Seigle-Murandi F. Enhanced degradation of fluorene in soil slurry by Absidia cylindrospora and maltosyl-cyclodextrin. Chemosphere. 2004;**56**(2):159-166

[42] Chandra S, Sharma R, Singh K, Sharma A. Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. Annals of Microbiology. 2013;**63**(2):417-431

[43] Nikolopoulou M, Kalogerakis N. Biostimulation strategies for fresh and chronically polluted marine environments with petroleum hydrocarbons. Journal of Chemical Technology and Biotechnology. 2009;**84**(6):802-807

[44] Adams GO, Fufeyin PT, Okoro SE, Ehinomen I. Bioremediation, biostimulation and bioaugmention: A review. International Journal of Environmental Bioremediation & Biodegradation. 2015;**3**(1):28-39

[45] Morais EBD, Tauk-Tornisielo SM. Biodegradation of oil refinery residues using mixed-culture of microorganisms isolated from a landfarming. Brazilian Archives of Biology and Technology. 2009;**52**(6):1571-1578

[46] Nikolopoulou M, Kalogerakis N. Biostimulation strategies for enhanced bioremediation of marine oil spills including chronic pollution. In: Handbook of Hydrocarbon and Lipid Microbiology. Berlin, Heidelberg: Springer. 2010;2521-2529

[47] Andreoni V, Gianfreda L. Bioremediation and monitoring of aromatic-polluted habitats. Applied Microbiology and Biotechnology. 2007;**76**(2):287-308

[48] Bamforth SM, Singleton I. Bioremediation of polycyclic aromatic hydrocarbons: Current knowledge and future directions. Journal of Chemical Technology and Biotechnology. 2005;**80**(7):723-736

[49] Haritash AK, Kaushik CP. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. Journal of Hazardous Materials. 2009;**169**(1):1-15

[50] Breedveld GD, Sparrevik M. Nutrient-limited biodegradation of PAH in various soil strata at a creosote contaminated site. Biodegradation. 2000;**11**(6):391-399

[51] Silva-Castro GA, Uad I, Gónzalez-López J, Fandino CG, Toledo FL, Calvo C. Application of selected microbial consortia combined with inorganic and oleophilic fertilizers to

recuperate oil-polluted soil using land farming technology. Clean Technologies and Environmental Policy. 2012;**14**(4):719-726

[52] Margesin R, Schinner F. Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology. 2001;**56**(5):650-663

[53] Leahy JG, Colwell RR. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews. 1990;**54**:305-315

[54] Shallu S, Pathak H, Jaroli DP. Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. International Journal of Pure and Applied Bioscience. 2014;**2**:185-202

[55] Bargiela F, Mapelli F, Rojo D, Chouaia B, Tornes J, Borin S, Richter DPM, Simone S, Christoph C, Genovese M, Denaro R, Martinez M, Fodelianakis S, Amer R, Bigazzi D, Han X, Chernikova J, Golsyhina O, Mahjoubi M, Jaouani A, Benzha F, Magagnini M, Hussein E, Al-Horani F, Cherif A, Blaghen M, Abdel-Fattah Y, Kalogerakis N, Barbas C, Malkawi H, Golyshin P, Yakimov M, Daffonchio D, Ferrer M. Bacterial population and biodegradation potential in chronically crude oil-contaminated marine sediments are strongly linked to temperature. Scientific Reports. 2015;**5**:11651

[56] Maki H, Hirayama N, Hiwatari T, Kohata K, Uchiyama H, Watanabe M, Yamasaki F, Furuki M. Crude oil bioremediation field experiment in the Sea of Japan. Marine Pollution Bulletin. 2003;**47**:74-77

[57] Kimes NE, Callaghan AV, Suflita JM, Morris PJ. Microbial transformation of the Deepwater horizon oil spill—Past, present, and future perspectives. Frontiers in Microbiology. 2014;**5**:603

[58] Harayama S, Kasai Y, Hara A. Microbial communities in oil-contaminated seawater. Current Opinion in Biotechnology. 2004;**15**(3):205-214

[59] Yakimov MM, Timmis KN, Golyshin PN. Obligate oil-degrading marine bacteria. Current Opinion in Biotechnology. 2007;**18**(3):257-266

[60] El Fantroussi S, Agathos SN. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Current Opinion in Microbiology. 2005;**8**(3):268-275

[61] Rodrigues EM, Kalks KH, Fernandes PL, Tótola MR. Bioremediation strategies of hydrocarbons and microbial diversity in the Trindade Island shoreline—Brazil. Marine Pollution Bulletin. 2015;**101**(2):517-525

[62] Zhang DC, Mörtelmaier C, Margesin R. Characterization of the bacterial archaeal diversity in hydrocarbon-contaminated soil. Science of the Total Environment. 2012;**421**:184-196

[63] Berry D, Gutierrez T. Evaluating the detection of hydrocarbon-degrading bacteria in 16S rRNA gene sequencing surveys. Frontiers in Microbiology. 2017;**8**:896

[64] Brightwell G, Boerema J, Mills J, Mowat E, Pulford D. Identifying the bacterial community on the surface of Intralox™ belting in a meat boning room by culture-dependent and culture-independent 16S rDNA sequence analysis. International Journal of Food Microbiology. 2006;**109**(1):47-53

[65] Genovese M, Crisafi F, Denaro R, Cappello S, Russo D, Calogero R, Santisi S, Catalfamo M, Modica A, Smedile F, Genovese L, Golyshin PN, Giuliano L, Yakimov MM. Effective bioremediation strategy for rapid in situ cleanup of anoxic marine sediment in mesocosm oil spill simulation. Frontiers in Microbiology. 2014;**5**:162

[66] Schneiker S, dos Santos VAM, Bartels D, Bekel T, Brecht M, Buhrmester J, Chernikova N, Denaro R, Ferrer M, Gertler C, Goesmann A, Golyshina OV, Kaminski F, Khachane AN, Lang S, Linke B, McHardy AC, Meyer F, Nechitaylo T, Pühler A, Regenhardt D, Rupp O, Sabirova JS, Selbitschka W, Yakimov MY, Timmis KN, Vorhölte F, Weidner S, Kaiser O, Golyshin PN. Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium *Alcanivorax borkumensis*. Nature Biotechnology. 2006;**24**(8):997-1004

[67] Yakimov MM, Golyshin PN, Lang S, Moore ER, Abraham WR, Lünsdorf H, Timmis KN. Alcanivoraxborkumensis gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. International Journal of Systematic and Evolutionary Microbiology. 1998;**48**(2):339-348

[68] Yakimov MM, Giuliano L, Denaro R, Crisafi E, Chernikova TN, Abraham W, Luensdorf H, Timmis KN, Golyshin PNR, Golyshin PN. *Thalassolituus oleivorans* gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons. International Journal of Systematic and Evolutionary Microbiology. 2004a;**54**(1):141-148

[69] Kasai Y, Kishira H, Harayama S. Bacteria belonging to the genus Cycloclasticus play a primary role in the degradation of aromatic hydrocarbons released in a marine environment. Applied and Environmental Microbiology. 2002;**68**(11):5625-5633

[70] Yakimov MM, Gentile G, Bruni V, Cappello S, D'Auria G, Golyshin PN, Giuliano L. Crude oil-induced structural shift of coastal bacterial communities of Rod Bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. FEMS Microbiology Ecology. 2004b;**49**(3):419-432

[71] Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnology Research International. 2010;**2011**:1-13

[72] Olajire AA, Essien JP. Aerobic degradation of petroleum components by microbial consortia. Journal of Petroleum & Environmental Biotechnology. 2014;**5**(5):1

[73] Rojo F. Degradation of alkanes by bacteria. Environmental Microbiology. 2009;**11**(10): 2477-2490

[74] Wang W, Shao Z. Enzymes and genes involved in aerobic alkane degradation. Frontiers in Microbiology. 2013;**4**:116

[75] Darmawan R, Nakata H, Ohta H, Niidome T, Takikawa K, Morimura S. Isolation and evaluation of PAH degrading bacteria. Journal of Bioremediation & Biodegradation. 2015;**6**(3):1

[76] Batista SB, Mounteer AH, Amorim FR, Totola MR. Isolation and characterization of biosurfactant/bioemulsifier-producing bacteria from petroleum contaminated sites. Bioresource Technology. 2006;**97**(6):868-875

[77] Bento FM, de Oliveira Camargo FA, Okeke BC, Frankenberger WT. Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. Microbiological Research. 2005b;**160**(3):249-255

[78] Banat IM, Makkar RS, Cameotra SS. Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology. 2000;**53**(5):495-508

[79] Cameotra SS, Makkar RS. Recent applications of biosurfactants as biological and immunological molecules. Current Opinion in Microbiology. 2004;**7**(3):262-266

[80] Owsianiak M, Chrzanowski Ł, Szulc A, Staniewski J, Olszanowski A, Olejnik-Schmidt AK, Heipieper HJ. Biodegradation of diesel/biodiesel blends by a consortium of hydrocarbon degraders: Effect of the type of blend and the addition of biosurfactants. Bioresource Technology. 2009;**100**(3):1497-1500

[81] Bordoloi NK, Konwar BK. Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. Journal of Hazardous Materials. 2009;**170**(1):495-505

[82] Whang LM, Liu PWG, Ma CC, Cheng SS. Application of rhamnolipid and surfactin for enhanced diesel biodegradation—Effects of pH and ammonium addition. Journal of Hazardous Materials. 2009;**164**(2):1045-1050

[83] Huang XF, Guan W, Liu J, Lu LJ, Xu JC, Zhou Q. Characterization and phylogenetic analysis of bioemulsifier-producing bacteria. Bioresource Technology. 2010;**101**(1):317-323

[84] Aparna A, Srinikethan G, Smitha H. Production and characterization of biosurfactant produced by a novel pseudomonas sp. 2B. Colloids and Surfaces B: Biointerfaces. 2012;**95**:23-29

[85] Das K, Mukherjee AK. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technology. 2007;**98**(7):1339-1345

[86] Salihu A, Abdulkadir I, Almustapha MN. An investigation for potential development on biosurfactants. Biotechnology and Molecular Biology Reviews. 2009;**4**(5):111-117

[87] Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews. 1997;**61**(1):47-64

[88] Reddy MS, Naresh B, Leela T, Prashanthi M, Madhusudhan NC, Dhanasri G, Devi P. Biodegradation of phenanthrene with biosurfactant production by a new strain of Brevibacillus sp. Bioresource Technology. 2010;**101**:7980-7983

[89] Hörmann B, Müller MM, Syldatk C, Hausmann R. Rhamnolipid production by *Burkholderia plantarii* DSM 9509T. European Journal of Lipid Science and Technology. 2010;**112**(6):674-680

[90] Marqués AM, Pinazo A, Farfan M, Aranda FJ, Teruel JA, Ortiz A, Manresaa A, Espuny MJ. The physicochemical properties and chemical composition of trehalose lipids produced by Rhodococcus erythropolis 51T7. Chemistry and Physics of Lipids. 2009;**158**(2):110-117

[91] Cappello S, Calogero R, Santisi S, Genovese M, Denaro R, Genovese L, Giuliano L, Mancini G, Yakimov MM. Bioremediation of oil polluted marine sediments: A bio-engineering treatment. International Microbiology. 2015;**18**:127-134

[92] Crisafi F, Genovese M, Smedile F, Russo D, Catalfamo M, Yakimov ML, Denaro R. Bioremediation technologies for polluted seawater sampled after an oil-spill in Taranto Gulf (Italy): A comparison of biostimulation, bioaugmentation and use of a washing agent in microcosm studies. Marine Pollution Bulletin. 2016;**106**:119-126

[93] Wu M, Dick WA, Li W, Wang X, Yang Q, Wang T, Xu L, Zhang M, Chen L. Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil. International Biodeterioration & Biodegradation. 2016;**107**:158-164

[94] American Public Health Association (A.P.H.A.). Standard Methods for the Examination of Water and Waste Water. 18th ed. Washington, DC: American Public Health Association; 1992