Chapter 10
A Comprehensive Review on the Bioremediation of Oil Spills

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Abstract Oil spills are probable accidents occurring mostly during transportation and processing of oil that can contaminate marine, soil, sediments, and other environments. Oil spill is a special challenge to be remediated due to its several environmental, economic, and social threats. Several physical (mechanical), chemical, and biological methods are available as response to the oil spills. Among them bioremediation proved to be a promising technique for treatment of oil spills especially after being applied successfully for Exxon Valdez oil spill. Bioremediation is a greener approach in comparison with physicochemical methods, which is more cost-effective with less disruptive effect on the environments. In this method the natural or genetically manipulated microorganisms are applied to the polluted site and/or the polluted environment is enriched with nutrients, which are called bioaugmentation and biostimulation, respectively. These methods have been examined by researchers for treatments of oil spills mostly in laboratory scale and in less extent in real fields. One novel approach in this area of the research is focused on the novel material addition to the polluted environment for biostimulation of the treatment process. Novel materials include organic sources to provide nitrogen and phosphorus for the medium such as compost, biowastes, biofuel, etc. Biosurfactant addition is another promising method that improves the bioremediation by reducing the surface tension. Some polymeric materials can be added for improving the immobilization of microorganisms and consequently enhancing the degradation rate. Novel bioaugmentation approaches are conducted by manipulating microorganisms with the aim of modification of enzymatic characteristic, metabolic pathway design, expansion of substrate rate, enhancing the genes resistance toward catabolic activities, etc. However, still there are several resistances toward the application of these microorganisms to the real field, due to the environmental concerns. Another novel approach is the integration of electrochemical methods and biological routes. Several achievements were reported by researchers for the remediation of oil spills by using bioelectrochemical systems (BES). Microbial fuel cells are another

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technique to convert chemical energy into electricity concurrent with contaminant degradation. The future research on the oil spill bioremediation must be focused on these new aspects of the process and finally pave the way for application of bioremediation in real field to obtain promising pollutants degradation results.

10.1 Introduction

Oil spills occur when large quantities of petroleum hydrocarbons leak in the environment from storage tanks, pipelines, drilling process, non-suitable waste disposal practices, leaching from landfills, etc. (Pontes et al. 2013; Li et al. 2017). These can be originated from cleaning process of the equipment and unit or residues in containers and outdated chemicals and accidents during transportation (Helmy et al. 2015). Majorly, petroleum hydrocarbon spillage accident occurs during shipping, offshore and onshore exploration and production, and transportation (Atlas 1995).

Oil spills can occur in marine and terrestrial environments and threat the ecosystem and human health (Cheng et al. 2017). This environmental contamination can pollute the drinking water, cause fire and risk of explosion, ruin the water and air quality, destroy the recreational areas, waste the nonrenewable resources, and have huge economic costs. Oil spill's negative impacts have different economic, environmental, and social aspects. The consequence of oil spills on the ecosystem and natural resources are widespread and long term. Therefore, there is a need for a supportive logistic and trained workforce who can take the suitable responses in a short time after the occurrence of oil spills (Marzan et al. 2017).

Studies on oil spills treatment and removal strategies are usually considered in different mediums. Occurrence of oil spills in the sea and shoreline is the most common, since the petroleum is usually transported through marine transportation. The costs of these environmental disasters such as Exxon Valdez and Deepwater Horizon are incalculable and can influence the whole wildelife and human health. The carcinogenic and mutagenic effects of the oil spills in the sea have been proved. The marine oil spills, once occur, prevent light diffusion and oxygen penetration in the bottom layers of the sea (Bovio et al. 2017). Oily layer on the water surface threatens the existing marine flora and fauna (Jain et al. 2011). Destroying widelife, contamination of sea food, and reduction of tourism industry are all consequences of oil spills in the sea (Ng et al. 2015).

Some accidents have polluted the shorelines such as Amoco Cadiz spill which happened in Brittany shoreline (Atlas 1995). Shoreline contamination can occur due to the tidal and wave actions as well and adherence of the oil spillages to the soil (Lim et al. 2016).

The heavy components of the oil may sink to the sediments in the sea and form a tarry layer or get buried (Martin et al. 2015). The buried oil treatment is a totally different process than superficial oil. In the case of superficial spills, the treatment process is applied directly to the layers of the oil. These methods are not effective for the treatment of buried oil, since the materials used for treatment may not reach the polluted layer in bottom of the sea (Pontes et al. 2013).

When the oil spill occurs in soil, the contaminants are attached to the soil physically or chemically or trapped in the soil matrix. The severity of the problem of oil spills depends on the oil type. Heavy oil spreads slower in the soil and can reach the lower layers of the soil. Therefore, the faster the response to the oil spills is performed, there is more chance for stopping the contamination (Helmy et al. 2015). An indicator to quantify the contamination in the soil is total petroleum hydrocarbon (TPH) concentration, which in the case of oil spills can reach 20–50 g kg^{-1} in the soil. This level of contamination can threat human health and environment (Xu et al. 2017). Soil contamination occurs most of all in the petrol stations and refineries, where the soil is exposed to the small but constant leakage of petroleum (Rhykerd et al. 1995).

Several guidelines, regulations, and directives are available to take care of oil spill prevention, preparedness, management, and compensation. However, accidental oil spill are inevitable, and therefore the governments should be prepared to perform the best response in case of spill. Development of federal government's blueprint (National Contingency Plan (NCP) which is for responding to oil spills and release of hazardous substance), assigning a competent (national) authority, and response capability are among the duties of governments (Walker 2017). Oil spill prevention and preparedness are among the top priorities of United States Environmental Protection Agency (US EPA 2013). Several physical, chemical, and biological methods are available for remediation of oil spills; among them bioremediation is the most promising method, which is the scope of this chapter.

10.2 Oil Spill Accidents in History

The first oil spill happened in the year 1907 and 7400 tons of paraffin oil entered to the sea and coastline of United Kingdom as a consequence. After that, about 140 large spills occurred, and in total seven million tons of oil have entered freely in to the environment. However, more than 90% of the oil pollutions are either natural such as runoff from land-based sources or has anthropogenic sources (not necessarily accidents) such as normal ship operation and deballasting and tank washing (Mapelli et al. 2017).

The largest oil spills in the history occurred in the sea such as Gulf War, Deepwater Horizon, Ixtoc 1 oil well, Amoco Cadiz, and other famous oil spills (Lim et al. 2016).

Amoco Cadiz accident happened in 1978 and released 227,000 tons of crude oil and bunkers in marine and contaminated 320 km of the shoreline length up to as deep as 20 inches (Lim et al. 2016).

Ashland oil spill occurred in 1988 when a four million gallon tank containing diesel oil collapsed and the oil dumped into the Monongahela River (Miklaucic and Saseen 1989). In 1989, Exxon Valdez oil spill occurred when a tanker crashed a reef

Fig. 10.1 Image of Exxon Valdez oil spill 1989 ("The Exxon Valdez Oil Spill: 25 Years Ago Today – The Atlantic" n.d.)

in Alaska, which spilled thousands of tons of oil into the sea (Jain et al. 2011). The sea and shoreline contamination, caused sever localized ecological damage to nearby community (Atlas 1995). It was reported that more than 250 thousand seabirds were killed due to this spill (Mapelli et al. 2017). Figure 10.1 is a photo of Exxon Valdez oil spill, where bioremediation was applied effectively as a strategy.

The largest inland oil spill happened in 1992 in Fergana Valley Uzbekistan, when 88 million gallons of oil released from an oil well into land. The ground absorbed this spill, and no cleaning was possible ("10 Largest Oil Spills in History – Telegraph" n.d.).

Prestige accident occurred in 2002 by the sinking of tanker and affected kilometers of the coastline with loosing up to 66% of the species richness in the region as a consequence (Bovio et al. 2017).

One of the most famous oil spills was the Deepwater Horizon (DWH) accident which occurred in the year 2010 in Gulf of Mexico during the drilling rig explosion. During this disaster, more than 700 thousand tons of crude oil was released into Gulf of Mexico (Mapelli et al. 2017). This accident decreased the biodiversity of the vertebrates and metazoan meiofauna. The cleaning cost of that spill was estimated to be 10 billion USD (Alessandrello et al. 2017). This accident can be called the worst oil accident in the history (Ng et al. 2015).

10.3 Oil Spills Removal Strategies

The faster response to the spill leads to more chance to prevent and stop contamination (Helmy et al. 2015). The aim of oil spill responds is mitigating the adverse impacts of the oil spill rather than monitoring the contaminants and allowing its natural attenuation. The first aim of a respond to oil spill is controlling the source and preventing the oil spread. The respond could be any strategy, method, technology, or equipment to control the spill and remediate its negative consequences. In addition to the fast response, stewardship is necessary to monitor and foresee the movement of oil. Use of mechanical equipment for spill removal such as skimmers, booms, barriers and sorbents, dispersants, and controlled in situ burning are among the response strategies (Walker 2017).

The aim of environmental treatments and remediation methods is to degrade and transform the contaminants into less harmful and even harmless compounds; when not possible, treatments is done by reduction of contaminants mobility and migration to prevent their spreading into uncontaminated areas. With this approach, the contaminant toxicity does not change, but the probability of their further distribution to the environment is reduced. Several treatments and responses to the oil spills are available which include physical, chemical, and biological methods (Jain et al. 2011).

The common mechanical strategy used for marine oil spills is controlling the oil from spreading and reaching shorelines with the application of barriers and then concentrating the oil into thick layer by booms to facilitate the oil removal by different types of skimmers like suction skimmers oleophilic and weir. Natural or synthetic polymers are used as sorbent for small spills (Mapelli et al. 2017). After in situ burning, toxicity assessments must be performed on burned residues. This approach was applied in the case of Deepwater Horizon accident (Mapelli et al. 2017).

Solvents and dispersants can be applied to reduce the size of spill into small droplets (Mapelli et al. 2017). The use of dispersant is a strategy to reduce the size of oil droplets to make it consumable by microorganisms more easily. For this strategy, the consumption of 830,000 gallons of chemical dispersant in both below and above sea surface is needed. The bacterial growth is also enhanced by the use of dispersant (Martin et al. 2015). In order to be effective, the dispersants must be added immediately after spill, before volatilization of light hydrocarbons. The function of dispersants is affected by salinity of water, water temperature, and wave action. Size reduction of oil droplets increases the surface area and reduces the interfacial water-oil tension and accelerates the biodegradation (Mapelli et al. 2017).

Another factor that limits the hydrocarbon degradation rate is the solubility of hydrocarbons. Surfactants are used to modify the hydrophobicity of cell membrane and modulate the bioavailability. In order to make this approach more sustainable, the nontoxic biosurfactants are currently replacing chemical surfactants (Mapelli et al. 2017).

The choice of the best cleanup technique is quite complex and is based on several factors including type of oil, location and size of spill, and local regulations and standards. For selecting the best methods, various criteria including efficiency, time, cost, reliability, effect on oil characteristic, and necessity of the post-remediation treatment of the applied method must be considered (Marzan et al. 2017).

Oil spill removal in the sea is done conventionally by using booms, skimmers, and big sponges as sorbents, skimming and mechanical removal using sorbents, vacuuming, in situ burning, and chemical dispersants (Marzan et al. 2017; Ng et al. 2015). However, all these methods have harmful environmental effects and endanger the ecosystem. The limitation of adsorbents application is the possibility of the erosion by the moving wave and lack of knowledge about its effectiveness (Helmy et al. 2015; Ng et al. 2015). Using dispersant as an oil spill removal method does not degrade the pollutants but just transforms it to another phase, which has still difficulty to be removed (Bovio et al. 2017).

Considering the case of soil, the available methods are solvent extraction, chemical oxidation, electrokinetic movement of contaminants, thermal desorption, flotation, washing with cosolvents or surfactants, using chemical agents for oxidation-reduction, physical removal such as ultrasonication, excavation of soil and sediment or groundwater pumping, and biological methods (Lim et al. 2016; Balba et al. 1998).

For remediation of sediments, different physicochemical methods are available such as ozonation, dredging, and electrochemical degradation. These methods have aggressive nature and are expensive and energy intensive (Li et al. 2017).

The conventional physical and chemical treatment methods are proved to be effective for removal of oil spills, but they produce several hazardous compounds which are still immunotoxicant and carcinogenic (Jain et al. 2011). Necessity of addition of chemicals for better removal makes chemical and physical treatment processes more costly (Marzan et al. 2017). Biologic methods detoxify hazardous substances, while physical methods usually transfer the hazardous substances to another environment. In addition, biological methods are less disruptive than excavation methods to the environment in the case of soil (Helmy et al. 2015).

10.4 Bioremediation of Oil Spills

Among the available methods, bioremediation is the most benign method which aims at enhancing the microbial metabolic activity and consequently stimulates the oxidation-reduction of the contaminants. During bioremediation, microorganisms degrade the organic contaminants (as their carbon and energy source) (Balba et al. 1998). However, the capability of microorganisms in degrading petroleum hydrocarbons is highly dependent on available chemical compounds and the conditions of the environment (Jain et al. 2011). This method has been developed in 1940s and became popular after Exxon Valdez oil spill in 1980s (Lim et al. 2016). Bioremediation is a quite slow process which requires weeks or months for effective cleanup. Although detailed economic analysis of this process is not performed yet, properly done bioremediation is a cost-effective method (Jafarinejad 2017). Not having significant adverse effects such as production of secondary contaminants (Cheng et al. 2017), minimal physical disruption of the site, effectiveness in removing toxic compounds, simpler mechanical technologies, and less economical cost are other advantages of this process. Necessity of the specific approach for each polluted site and each spill type is a disadvantage of this process. Bioremediation is a less effective treatment strategy in the sea (Jafarinejad 2017), and the available knowledge is still rough and mainly focused on the application of prokaryotic organisms (Bovio et al. 2017).

Microorganisms use enzymes and oxygen and break down the structure of hydrocarbons. They use the petroleum hydrocarbons as substrates to produce biomass and decompose pollutants into water (Martin et al. 2015), carbon dioxides, and other harmless compounds (Atlas and Barsa 1992) such as fatty acids (Marzan et al. 2017). When considering bioremediation as a treatment to the oil spills, the aim is addition of materials to the contaminated environment to accelerate the natural biodegradation process. As an example, the addition of nutrients enhances indigenous organism's growth and activity. Another approach is exposure of the polluted environment to nonindigenous microorganisms with enhanced ability for hydrocarbon degradation. Bioremediation is considered as a complementary treatment after conventional cleanup (Jafarinejad 2017). Auxiliary treatments such as aeration and temperature adjustment can improve the bioremediation process (Lim et al. 2016). During bioremediation, petroleum hydrocarbons are used either as growth medium or as co-metabolism. This means that the contaminants can be considered as carbon and energy sources and be totally degraded and mineralized or be used as extra nutrition source in combination of growth substrate (Lahel et al. 2016). However, long period is needed for an effective bioremediation, and in the case of highly polluted environment, the process is less effective (Soleimani et al. 2013).

When considering bioremediation for treatment of buried oil, one must ensure that the added materials (microorganism and nutrients) can reach the polluted environment (Pontes et al. 2013). In the case of shoreline, such as oil spill in Brittany coastline, bioremediation was reported to be fast and effective method. The reason for that could be the adaptation of the indigenous microorganism of that region to the release from ballast water tanks, constant aeration with wave action, and presence of nitrogen and phosphorus nutrients from the agricultural runoff. However, the formation of emulsion which is resistant to biodegradation can prevent the process, since the microorganisms may colonize on the surface of emulsion but cannot reach within the mass of emulsion (Atlas 1995). Limiting factors for bioremediation in marine environment are usually nonbiologic factors (e.g., oxygen, phosphate, and nitrogen concentration) (Atlas 1995). For soil bioremediation, the limiting factors are aging of the spill, ambiguity of the soil matrix type, and nature of the contaminants (Xu et al. 2017).

Research on the bioremediation of oil spills must consider all different aspects such as effects of environmental parameters, metabolic pathways, basis of hydrocarbon breakdown as substrate (dissimilation) from genetic point of view, and effects of hydrocarbon contaminants on microorganism. The basis of this study originated from monitoring the fate of hydrocarbon contaminants in the environment and search for the methods to accelerate the natural degrading process by overcoming the rate-limiting factors (Jain et al. 2011). Accelerating methods include addition of microbes with higher oil-degrading capacity or nutrients such as nitrogen and phosphorous (Marzan et al. 2017). For an effective bioremediation process, the presence of microorganism with desirable physiological characteristic and enzymatic capabilities, proper growth and activity conditions, and bioavailability of active microbial consortia play an important role (Lahel et al. 2016).

As already mentioned, several parameters can influence the bioremediation. Physical parameters (temperature, pressure, contaminant surface area) and chemical parameters (nutrient and oxygen availability, acidity, salinity, and contaminant nature and composition) have major effects on bioremediation. Among them most of the factors can be manipulated to accelerate natural biodegradation, while factors such as salinity are not adjustable in real field (Jafarinejad 2017). Among the biological factors metabolic parameters, mass transfer parameters in cell membrane and bioavailability must be considered (Gonzalez and Sanchez 2011).

Temperature can impact viscosity and consequently the toxicity; since at higher viscosity, the toxic light hydrocarbons are less volatile. The solubility of petroleum hydrocarbons changes with temperature as well. At low temperatures, alkanes with shorter chains are more soluble, while higher temperatures are favorable for solubility of several light aromatics. In all ranges of the normal seawater temperature (2–35 °C), biodegradation can take place. However, the rate decreases with decrease of temperature. The optimum temperature for biodegradation is 30–40 °C in soil and 20–30 °C in freshwater. For marine environment, it is reported to be 15–20 °C. Temperature has significant impact also on the microbial growth and activity. Dissolved oxygen is required for degradation and oxidization of the pollutants. Usually there is no oxygen limitation on superficial water in the sea and freshwater. However, oxygen may be limited in some subsurface sediments such as anoxic zones in water columns. Dense marine shorelines, tidal flats, coastal salt marshes, freshwater wetlands, and bottom layer of soil are other examples of the environment with lack of oxygen. The availability of oxygen also depends on water and wave turbulence, oil physical state, and availability of substrate. However, it was reported that anaerobic degradation of certain pollutants can occur in negligible extent as well (Rastegar et al. 2017; Nasirpour et al. 2015). Systems such as upflow anaerobic sludge blanket (UASB) are some bioreactor systems used for ex-situ bioremediation of petroleum hydrocarbons pollutants in wastewater obtained from a refinery. The advantages of anaerobic system over aerobic system is utilization of less space and no energy requirements (Rastegar et al. 2011).

Pressure can impact the rate of bioremediation. At higher pressure such as in the deep ocean, the rate of biodegradation decreases. The surface area of the contaminants can impact the interface of oil and water. Biodegradation rate improves with increase of surface area. In marine, turbulence of the sea surface can affect the process by influencing dispersion. This causes dilution of the available nutrients and spread of the oil (Jafarinejad 2017). Also the degree of spreading can affect the surface area. In aquatic system, oil normally spread and form a thin slick (Atlas 1991).

At higher pH, the rate of hydrocarbon degradation increases. Marine environments usually have alkaline conditions. The pH in salt marshes is lower (around 5), and pH in freshwater and soil is very variable (Jafarinejad 2017).

The presence of nutrients including nitrogen and phosphorous is a more limiting factor than oxygen. The nutrients are consumed not only by pollutant degrading microorganism but also other microorganism such as phytoplankton. Precipitation of phosphorus may also compete with oil-degrading microorganism (Jafarinejad 2017).

The adaptation skills of the microorganisms and their resistance to extreme environmental pollutions are an important factor as well (Bovio et al. 2017). However, even adapted microorganisms are not effective for biodegradation of extremely high amount of pollution. For instance, earthworms could not survive in the soil in which oil content contamination is more than 3%. At oil content of 1%, almost 100% inhibition of bacterial activity was observed (Lim et al. 2016). During oil spills, the concentration of petroleum hydrocarbons is far excess of tolerable limits (Atlas 1991).

Bioremediation in cold environments such as Alaska, northern Russia, and Canada need more studies and considerations. Between 1996 and 1999, 407 spills on average occurred annually in Alaska. Even higher risk of pipeline damage and petroleum hydrocarbon pollution is available there, in comparison with moderate climates. In cold zones, the oil spill impacts the microbial population, freeze-thaw processes, thermal and moisture regimes, as well as oxygen availability and pH of the soil. Environmental impact of the oil spill is harsher in the cold environments, since the cold ecosystems are more sensitive. Furthermore, low temperature results in higher viscosity, lower volatile evaporation rate, and higher water solubility of the oil which can delay the biodegradation process. However, successful bioremediation of oil spill was achieved in several cases such as arctic and subarctic regions (Montagnolli et al. 2015).

Bioremediation of pollutants in highly salinated areas is also particular due to the effect of salinity on microbial population (Si-Zhong et al. 2009).

Different microorganisms including bacteria, fungi, yeast, and microalgae are able to degrade petroleum hydrocarbons. The bioremediation can be performed in situ or ex situ (Lahel et al. 2016). In ex situ process, the contaminated matrix is extracted elsewhere to be treated, while during in situ treatment, the treatment occurs in the place of contamination (Balba et al. 1998). The bioremediation of soil was conducted effectively both with in situ and ex situ approaches (Lim et al. 2016). However, in situ approach is more cost-effective and safer than ex situ with less disruption of the polluted environment (Lahel et al. 2016).

Another common approach is supplying electron donors to stimulate the reduction reactions and degradation of halogenated compounds, or electron acceptors to stimulate the oxidation reactions and degradation of non-halogenated compounds (Balba et al. 1998).

Recently novel approaches have emerged that integrate physicochemical methods with biological approaches (Balba et al. 1998), which will be discussed thoroughly later on this chapter.

10.5 Microorganisms for Bioremediation of Oil Spills

It was reported that more than 200 different species of bacteria, fungi, and yeasts are able to degrade petroleum hydrocarbons. These microorganisms can be found naturally in marine, freshwater, and soil. The biodegradable hydrocarbon compounds range from methane to C_{40} compounds. To classify, almost 79 bacterial, 9 cyanobacterial genera, 103 fungi, 14 algae, and 56 yeasts are able to degrade the hydrocarbon pollutants (Jafarinejad 2017; Gonzalez and Sanchez 2011).

Different groups of indigenous soil bacteria can degrade different compounds of petroleum hydrocarbons. These bacteria include *Pseudomonas* strains isolated from soil and aquifers to degrade polycyclic aromatic hydrocarbons (PAHs) (Atlas 1995). Other microorganisms with the ability to degrade petroleum hydrocarbons are *Yokenella* sp., *Alcaligenes* sp., *Alcanivorax* sp., *Microbulbifer* sp., *Sphingomonas* sp., *Micrococcus* sp., *Cellulomonus* sp., *Dietzia* sp., *Roseomonas* sp., *Stenotrophomonas* sp., *Gordonia* sp., *Acinetobacter* sp., *Corynebacterium* sp., *Flavobacter* sp., *Streptococcus* sp., *Providencia* sp., *Sphingobacterium* sp., *Capnocytophaga* sp., *Bacillus* sp., *Enterobacter* sp., and *Moraxella* sp. (Jain et al. 2011).

Alcanivorax sp. bacteria and *Cycloclasticus* sp. can use aliphatic and aromatic hydrocarbons, as their carbon source, respectively. Some bacteria can help to produce biosurfactants which can enhance the bioremediation by reducing surface tension and increase of crude oil uptake. However, factors such as availability of nutrients and nature of oil contaminants are influential in degrading the petroleum hydrocarbons (Bovio et al. 2017).

Some fungi are also capable of degrading petroleum hydrocarbons. However, they need longer time for effective degradation. Fungus belonging to *Aspergillus* sp., *Amorphoteca* sp., *Penicillium* sp., *Graphium* sp., *Neosartorya* sp., *Fusarium* sp., *Paecilomyces* sp., and *Talaromyces* sp. are among the microorganisms with the ability to degrade petroleum hydrocarbons. White rot fungi are reported to be able to degrade compounds such as polychlorinated biphenyls (PCBs) and PAHs (Baniasadi et al. 2018). Some yeasts including *Candida* sp., *Pichia* sp., and *Yarrowia* sp. also reported to have the potential to degrade the compounds available in oil contaminants (Jain et al. 2011). Some researchers suggests that in some specific circumstances, fungi can degrade petroleum better than bacteria. However, there is not much information available for fungal bioremediation of marine contaminated sites (Bovio et al. 2017).

Marzan et al. isolated bacteria for bioremediation from Shela River which was polluted with an oil spill in the year 2014 for their oil-degrading potential. They have isolated seven distinct bacterial colonies to degrade the furnace oil. Among the isolated bacteria, the top three with the oil-degrading capabilities were assessed to be *Pseudomonas aeruginosa*, *Bacillus* sp., and *Serratia* sp. (Marzan et al. 2017).

Using indigenous microorganisms available in the polluted site is suggested to be a promising method for bioremediation of petroleum hydrocarbon contaminants, since these native microorganisms are adapted to the available conditions. However, microorganisms with enzymatic ability for pollutant degradation may be absent which leads to very long process. For example, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and some other microorganisms are isolated from soil contaminated with petroleum hydrocarbons (Gonzalez and Sanchez 2011).

In the work of Bovio et al., fungal community capable of degrading oil spills were isolated from Mediterranean marine (67 strains) and sediments (17 strains). The fungal growth was stimulated by crude oil which was the carbon source. Among them *A. terreus*, *T. harzianum*, and *P. citreonigrum* yileded the highest dichlorization percentage, and *A. terreus* reported the highest yield in decreasing hydrocarbon compounds (Bovio et al. 2017).

When microorganism communities are exposed to contaminants (hydrocarbons), they adapt gradually and undergo selective genetic enrichment. After adaptation, the population of the bacteria capable of degrading hydrocarbons and plasmids of the bacterial cells that encode hydrocarbon catabolic genes is increased (Lahel et al. 2016). The increase in population of oil degrader microorganisms has been observed for *Alcanivorax* sp. and *Cycloclasticus pugetii* (Gonzalez and Sanchez 2011).

When the existing microbial population of the environment is not capable or sufficient for degradation of the pollution, the addition of oil-degrading microorganisms to the contaminated area is conducted. This approach is called bioaugmentation and is explained further in the next section (Jafarinejad 2017). Recently, the researchers are searching to manipulate the microorganisms genetically to enhance their oil-degrading ability (Martin et al. 2015).

Bioaugmentation is the method in which microorganisms with high oil-degrading ability are added to a contaminated environment as adjunct for the indigenous microbial population to achieve the effective biodegradation. It was reported that petroleum biodegradation is performed better in the presence of consortium of microorganism in comparison to monospecies activities (Jain et al. 2011). Singh et al. have used consortia of different bacterial strains (mixture of *Micrococcus* sp. GS2-22, *Flavobacterium* sp. DS5-73, *Corynebacterium* sp. GS5-66, *Bacillus* sp. DS6-86, and *Pseudomonas* sp. DS10-129) to perform bioremediation of petroleum hydrocarbon contaminated soil. In their work, oil degradation rate of 78% was achieved after 20 days (Singh et al. 2012).

10.6 Mechanism of Bioremediation of Oil Spills

Petroleum oil spill is complex mixture of different compounds. More than 17,000 chemical components have been identified in crude petroleum that contains large amounts of aliphatic, branched, and aromatic hydrocarbons. The majority of nonpolar fraction is composed of saturated and aromatic hydrocarbons. Petroleum hydrocarbons also contain halogenated hydrocarbons (Jain et al. 2011; Balba et al. 1998). The oil spill composition includes alkanes (both linear and branched), aromatics, and cycloalkanes and some NSO (nitrogen, sulfur, and oxygen)-containing compounds such as thiophene, phenol, and indole (Gonzalez and Sanchez 2011).

More methyl-branched compounds and/or condensed aromatic rings content make oil spill nature more complex and lead to slower degradation rate and possibility of accumulating of partially oxidized intermediary metabolites (Atlas 1995).

The enzymatic capability of microorganism enables them to degrade petroleum hydrocarbons. Some types of microorganisms are alkanes (linear, branched, and cyclic paraffins) degrader, and some are aromatics degrader and some both. The degradation of normal alkanes (C1-C26) is the easiest and fastest one. However, degradation of toxic light aromatics (like benzene, toluene, and xylene) by marine microorganisms is fast and easy as well (Ronald M. Atlas 1995). Low water solubility as well as high sorption capacity makes degradation of PAHs more difficult especially in cold climates (Si-Zhong et al. 2009).

The highest biodegradation rates are for saturates and then light aromatics. The order of petroleum hydrocarbon component degradation is normal alkanes followed by branched alkanes and alkenes, light n-alkyl aromatics, single aromatics, cyclic alkanes, polycyclic aromatic hydrocarbon, asphaltenes, and resins (Jafarinejad 2017; Si-Zhong et al. 2009).

Benzene, toluene, ethyl benzene, and xylene and in general aromatic hydrocarbons can be degraded by microorganisms such as *Pseudomonas*, *Rhodococcus*, and *Ralstonia*. The microorganisms suitable for degradation of polyaromatic hydrocarbons are *Pseudomonas* for naphthalene, *Pseudomonas* and *Haemophilus* for phenanthrene, *Rhodococcus* for anthracene, *Haemophilus* and *Mycobacterium* for pyrene, and *Rhodococcus* and *Mycobacterium* for benzo[a]pyrene (Gonzalez and Sanchez 2011).

It must be noted that the composition of oil spill may be changed by evaporation and dissolve of light aromatics and alkanes which are further metabolized by microorganisms. As a consequence, heavier components may remain (Jafarinejad 2017).

Crude oil never biodegrades completely. It was reported that in some days and weeks, more than half of the heavy oil can be degraded and a black complex residue is always left after biodegradation, mostly containing asphaltic compounds. However, bioremediation is still considered effective, since this residue is not toxic and has low bioavailability, and inasmuch as coating and suffocation of the polluted area do not occur, it can be considered environmentally inert (Helmy et al. 2015).

The bioremediation of petroleum oil can be conducted under aerobic as well as anaerobic conditions. During aerobic metabolism, oxygen-oxidizing enzymes which convert the O_2 to reduced substrate are needed. The lack of contact with water-insoluble hydrocarbons is a problem that bacteria can solve by two general strategies. The contact is enhanced by a particular adhesion mechanism in which emulsifying agents are produced extracellularly (Jain et al. 2011).

The mechanism of oil spill degradation is usually studied by using different model petroleum hydrocarbons. However, generally the biodegradation of petroleum hydrocarbons occurs via several sequential reactions initiating by attack of microorganism on petroleum structure and formation of intermediate substances. The intermediate compounds are utilized by different microorganisms and lead to further degradation (Jafarinejad 2017).

In the first step of degradation pathway, the hydroxyl group is added to the end of alkane chain. This group can be added on the unsaturated ring of PAH and form alcohol as well. The length of the chain is then reduced by oxidation of compound to aldehyde and later carboxylic acid. Finally, CO_2, H_2O, and biomass are formed. Oxygen addition to hydrocarbons makes them more polar and water soluble, with more biodegradable and less toxic structure (Jafarinejad 2017).

During the degradation of aliphatic hydrocarbons such as n-alkanes, firstly alcohols are produced, which is sequentially oxidized and dehydrogenated to form primary alcohols and aldehydes and a monocarboxylic acid consequently. The carboxylic acids then undergo β-oxidation (Jain et al. 2011) and form fatty acids and acetyl coenzyme and release carbon dioxide. The limiting step is the addition of oxygen to the hydrocarbon, and once carboxylic acid is formed, it can be metabolized rapidly. In the case of branched isoprenoid alkanes such as pristine, the hydrocarbon undergoes oxidation and forms dicarboxylic acids. The presence of methyl branches increases the resistance of hydrocarbons to microbial attack (Atlas and Barsa 1992).

The degradation pathway for aromatic and PAHs is through hydroxylation of the ring by enzymes which are mono- or dioxygenase. Consequently, diol is formed, and the ring is cleaved and undergoes further degradation (Jain et al. 2011).

10.7 Biostimulation and Bioaugmentation

As mentioned, physicochemical conditions (temperature, pressure, pollutant surface area, oxygen content, nutrient availability, pH, salinity, oil composition, etc.) influence the natural bioremediation process. When applying bioremediation as a response to the oil spill, two main approaches are available which are biostimulation (enhancing the nutrients availability – mostly nitrogen and phosphorus – to initiate the growth and accelerate the biodegradation) and bioaugmentation (inoculation of microorganisms with enhanced ability to degrade petroleum hydrocarbons in order to facilitate the process). However, a novel approach is also available, which is bioaugmentation with genetically engineered microorganisms (bioaugmentation with GEMs) (Jafarinejad 2017) (Lahel et al. 2016). It was reported that the effects of bioaugmentation can be observed much faster than biostimulation (Pontes et al. 2013). However, the most promising approach is combination of biostimulation and bioaugmentation with addition of biosurfactants (Gonzalez and Sanchez 2011).

10.7.1 Biostimulation

Biostimulation is a nutrient-enhanced bioremediation process to improve the indigenous biodegradation rate of petroleum hydrocarbons especially organic pollutants by providing the limiting nutrient material to the polluted medium (Soleimani et al.

2013). The nutrients include carbon, nitrogen, and phosphorus and some other growth-limiting cosubstrates. Modification of the conditions including temperature and aeration can be also done during the biostimulation. All these activities are performed with the aim of acceleration of oil degrader's growth and activity. This approach can be called fertilization or nutrient enrichment. (Jafarinejad 2017).

The microbial metabolic activity is improved due to nutrient supply. Electron acceptors and donors can be also added to stimulate the oxidation and reduction mechanism. However, their addition must be under control. The provided nutrients must be available and be in contact with the microorganisms (Balba et al. 1998). The conditions for enhancing natural biodegradation can be adjusted by manipulating of different parameters such as application of fertilizers, nutrients, biosurfactants, and biopolymers. Manipulation of all these parameters with the aim of improving natural bioremediation can be considered as biostimulation (Lim et al. 2016).

Another practice that is used for improving the conditions especially aeration is bioventilation process which is application of oxygen to soil porous with the aim of enhancing microorganisms growth and metabolism of organic matter by providing aerobic conditions. It was observed that using bioventilation the rate of bioremediation increases to 85% from 64% in natural attenuation process (Lim et al. 2016).

In marine environment or generally open systems, addition of N and P is quite difficult. Therefore, uric acid is added instead, which is the waste product of animals (birds, reptiles, insects, etc.). Uric acid by low water solubility can attach to the petroleum hydrocarbons and can be used by bacteria as nitrogen source or both nitrogen and carbon source (Gonzalez and Sanchez 2011). It was observed that for light crude oil degradation, addition of nitrate is more effective than ammonia in seawater, while in the salt-marsh soil, addition of ammonia is more effective than nitrate. Fortunately, no adverse impact, such as algal blooms was observed by nitrogen addition (Jafarinejad 2017).

Good results have been obtained by using this approach on sediments of the cost contaminated after Exxon Valdez spill in Alaska, and the rate of biodegradation increased three to five times by addition of fertilizers, such as iron, phosphorus, and nitrogen (Martin et al. 2015).

10.7.2 Bioaugmentation

Bioaugmentation is an approach which is used when the native microbial populations are inadequate for degrading the pollutant mixtures such as petroleum. This is done when the population of hydrocarbon-degrading microorganism is low or there is a need to degrade particular hydrocarbon which cannot be degraded by indigenous microbes. As an example, polynuclear aromatic hydrocarbons are usually hard to be degraded (Jafarinejad 2017).

In this approach, microorganisms with enhanced biodegradation ability are added to the polluted environment to supplement the naturally available microbes.

Different methods are available for this approach. Commonly the nonindigenous microbes from other polluted environments are used to be added to the target site (Jafarinejad 2017). Alternatively, microbes from the target site are separated and mass cultured under laboratory conditions in bioreactors and are used as inoculum to the target site. This method is called *autochthonous bioaugmentation* and is referred to the cases where the bioaugmentation is done by the native microbes of the contaminated site after enrichment to be reapplied to the site (Lim et al. 2016). Seeding of microorganisms to the contaminated site can reduce the lag period to start the biodegradation. When the seeding is done by the enhanced indigenous organisms taken from the target site, the adaptation problem is avoided (Jafarinejad 2017). The criteria for selection of the added microbes are based on their physiology and metabolic ability (Lim et al. 2016).

Bioaugmentation was done successfully in bench scale under controlled conditions. However, it must be considered that conditions in real fields may be uncontrollable (Jafarinejad 2017). It has been suggested that primary laboratory tests for microorganism selection before in situ application of the microorganism can increase the chance of successful bioremediation. In the work of Szulc et al., the most effective consortium (*Pseudomonas fluorescens* and *Pseudomonas putida* mixed with *Aeromonas hydrophila* and *Alcaligenes xylosoxidans*, in addition to *Xanthomonas* sp., *Gordonia* sp., *Stenotrophomonas maltophilia*, and *Rhodococcus equi*) for bioaugmentation was selected in the laboratory based on the quantity of CO_2 and dehydrogenase activity (Szulc et al. 2014). Kim et al. proposed a gene-based diagnostic technique that can reduce the needed time for microorganism selection. The DNA diagnostic method via oligonucleotide microarray method was applied to detect and observe genes with desirable ability to degrade aliphatic and aromatic hydrocarbons. In this work, the bioremediation of contaminated site was performed in field tests by bioslurping (Kim et al. 2014).

Researchers claim that the commercial bacterial blends can be produced with customized properties for each specific site and type of pollution in spill, considering the specific nutritional needs and limitations. Large quantities of the microbial blend can be produced in laboratory and be stored for emergency cases for up to 3 years (Jafarinejad 2017).

10.8 Novel Approaches for Bioremediation of Oil Spills

Current research in bioremediation of oil spills is mostly focused on novel material addition for biostimulation, using genetically modified microorganisms for bioaugmentation and integration of different physicochemical and biological approaches for treatment of oil spills. The novel approaches in bioremediation of oil spills are explained in following.

10.8.1 Novel Material Addition

As mentioned earlier, bioremediation is done normally with the addition of fertilizers and nitrogen and phosphorus materials. In novel approaches biowastes, inorganic materials, polymeric materials, etc. are added to enhance the bioremediation. Biosurfactant addition is another material that recently gained attention in the studies on bioremediation of oil spills.

Biosurfactants are produced extracellularly or as part of the cell membrane by different microorganisms including yeasts, bacteria, and filamentous fungi. Microorganism activity in the case of biosurfactant is due to the production of extracellular biosurfactant (e.g., trehalose lipids produced by *Rhodococcus* species) or cellular biosurfactants (e.g., mycolic acids) which cause the microbial cells to be attached to hydrophobic phases. Wide structural diversity of biosurfactants is available, including lipopeptides, glycolipids, fatty acids, lipoproteins, phospholipids, neutral lipids, and polymeric biosurfactants (Ayed et al. 2015).

Two groups of biosurfactants are available, which are low-molecular-weight surface active materials with the ability to lower the tension (both surface and interfacial) efficiently and polymers with high molecular weight (bioemulsifiers) that are used for stabilization of emulsions (Bezza et al. 2015).

Biosurfactants in comparison with chemical surfactants have less toxicity, biodegradability, and ecological acceptability. Biosurfactants act more effective in different pH, temperature, and salinity in comparison to chemical ones (Bezza et al. 2015). The biosurfactant-producing microorganisms are interesting for bioremediation especially for biodegradation of hydrophobic compounds. Recently, the application of biosurfactant microorganisms gained attention in the research due to offering superior biodegradability and being environmentally friendly in comparison with synthetic surfactants (Szulc et al. 2014).

Most of petroleum hydrocarbons are insoluble in water. Considering the case of oil spill in aqueous environments, the petroleum oil droplets are dispersed naturally by wave action in water column. Emulsification agents can be used for emulsification of diverse oil components. The ratio of surface to volume is an influential factor in bioremediation since the biodegradation process occurs at the hydrocarbon-water interface. Biosurfactant role is reducing the interfacial tension available between oil and water and enhancing the droplets dispersion in water column (Montagnolli et al. 2015).

Surface active materials by increasing the solubility remove hydrophobic compounds from soil and contribute to their biodegradation. Hydrophobic and hydrophilic moieties available in amphiphilic molecules can interact with interfaces that have different polarities. This leads to reduction of interfacial and surface tension and increase of bioavailability, transfer rate, and solubility of hydrophobic and insoluble organic compounds (Bezza et al. 2015). Generally, biosurfactant role in bioremediation is reduction of surface tension, increasing the solubility of hydrocarbons and making them available to microorganism. The hydrophobicity of the bacterial cell surface can be also of influence, and this allows substrates which are hydrophobic to be more in contact with bacterial cells (Ayed et al. 2015).

Low-molecular-weight surface active materials include glycolipids, lipopeptides, and phospholipids. The most common surfactant is lipopeptides which contain both fatty acid moiety (hydrophobic) and peptide moiety (hydrophilic). The critical micelle concentration (CMC), proper emulsification properties, powerful surface activities, and outstanding foaming characteristics are among the characteristics of low-molecular-weight surface active materials. Lipopeptide's physicochemical properties make them stable at diverse temperatures and pH levels. Famous biosurfactant-producing bacteria are *Pseudomonas*, *Bacillus*, *Acinetobacter*, and *Mycobacterium* (Bezza et al. 2015). Rhamnolipids is one common surface active compound of microbial origin since the congeners-constituents of this bioemulsifier are well described and investigated for efficient application during soil flushing and mobilization of resistant contaminants. These qualities make rhamnolipids a potential agent for improving bioremediation of polluted terrestrial environments. However, in the work of Szulc et al., no significant change was observed in the treatment process (both non-bio- and bioaugmented treatment) of diesel-contaminated soil by addition of rhamnolipids in real field (Szulc et al. 2014).

Another novel biosurfactant was produced by *Paenibacillus dendritiformis* that was isolated from the soil of the plant contaminated with creosote. This biosurfactant was identified as lipopeptide. The produced biosurfactant was analyzed and showed an amino acid (Cys-Gly-Ala-Gly-Ile-Asn-Leu as sequence) with long chain fatty acid (522 Da molecular mass). With hexane this biosurfactant showed 74% emulsification index and with cyclohexane 82%. High pH, thermal and saline stability was observed as well. The ability of this biosurfactant was tested in the work of Bezza and Chirwa in batch experiments for enhancing the bioremediation of PAHs from heavy oil-contaminated sands (Bezza et al. 2015).

Bacillus amyloliquefaciens was also reported to be a strong biosurfactant-producing bacteria in *Landy medium* (semisynthetic medium). The surface tension decreased to less than 30 from 72 mN/m by this biosurfactant and has CMC of 100 mg/L. The biosurfactant showed better solubilization efficiency toward diesel oil than SDS and Tween 80. Ayed et al. have investigated the ability of biosurfactant that was produced by *Bacillus amyloliquefaciens* in lowering the surface tension, improving solubility, and enhancing biodegradation (Ben Ayed et al. 2015).

In the work of Montagnolli et al. biosurfactant produced by *Bacillus subtilis* was investigated for biodegradation of simulated wastewater contaminated with crude oil, diesel, and kerosene. Mathematical models were used for demonstrating and predicting the effect of biosurfactant on kinetics of biodegradation process. Higher yield of CO_2 output was observed in the assays containing biosurfactants (Montagnolli et al. 2015).

The work of Hernández-Espriú et al. addressed the application of biosurfactants obtained from plants including locust bean, guar, and mesquite seed gums for the bioremediation of the soil that was contaminated with diesel after a pipeline accidental spill. Natural gums can be used in variety of industrial applications for their emulsifying, microencapsulating, thickening, and stabilizing properties. The results showed that natural gums are promising biosurfactant in bioremediation of oil contaminated soil. The obtained efficiencies were 54.38% and 53.46% for Guar gum and locust bean gum respectively which is higher than the efficiencies obtained by ionic and non-ionic surfactants. The best removal rate (82% for diesel) was obtained by application of a small amount of gum concentration (2 ppm) (Hernández-Espriú et al. 2013).

Compost addition can be considered as a method to supply nutrients to the medium. Therefore, several researchers added compost for improving the bioremediation (Gomez and Sartaj 2013; Bastida et al. 2016; Dadrasnia and Agamuthu 2014).

Bastida et al. conducted the bioremediation of hydrocarbon polluted soil in semi-arid areas where the soil nutrients and organic matter are poor. This makes the microbial development of soil problematic. The results showed enhanced (88%) removal of PAHs and alkanes after 50 days with compost, while the biodegradation without compost was not significant. Bioremediation in the presence of compost was conducted by *Sphingomonadales* and uncultured bacteria and led to secretion of catabolic enzymes such as 2-hydroxymuconic semialdehyde, cis-dihydrodiol dehydrogenase, and catechol 2,3-dioxygenases (Bastida et al. 2016).

Gomez and Sartaj performed combined biostimulation and bioaugmentation by inoculation of microbial consortia and addition of mature organic compost in cold environment. The bioremediation results were the best, having both consortia and compost in comparison with their individual use (Gomez and Sartaj 2013).

Besides application of nutrients and fertilizer to biostimulate the bioremediation process, some researchers suggested to use agricultural biowaste for biostimulation with organic matter. Rice husk, chicken manure, and other biowastes were used for this purpose (Dadrasnia and Agamuthu 2014; Adams et al. 2017). Manure addition have advantages including soil alteration, improving organic matter, increase of water holding capacity and advantageous biota (Adams et al. 2017). The application of biowaste in the soil-contaminated with diesel fuel showed enrichment value of $\delta^{13}C$ in treatments amended with organic waste (Dadrasnia and Agamuthu 2014).

In the work of Horel et al., the addition of organic nutrients plant material and fish tissue (*Spartina alterniflora* and *Chloroscombrus chrysurus*, respectively) was investigated for bioremediation of sandy beach sediments available in coastal region of Alabama, and the results were compared with the cases where inorganic nitrogen and phosphorus were added. The highest degradation rate was obtained by fish tissue which led to 104% increase of degradation rate. Inorganic nutrients addition increased the degradation rate 57%. Plant material only improved the degradation rate in low extent (7%) (Horel et al. 2015).

Dias et al. (2012) have compared the results obtained for bioremediation of soil with addition of different organic and inorganic materials. In their study, they have studied samples with inorganic salt, chemical surfactant (Brij700), fish meal, and a special commercial product. The inorganic salt was used as an example of component with solubility in water. Fish meal was a slow release source of N and P, and the used commercial product was OSEII (Oil Spill Eater International, Corp.) which is an oleophilic rich in nitrogen and phosphorus that can delay the washing process as reported. This commercial product is mentioned in EPA's National Contingency Plan for Oil Spills as a supplementary material that contains phosphorus, nitrogen, carbon and some vitamins which are helpful for fast colonization of natural bacteria. Although the fish meal enhanced the bacterial growth and activity, it did not help the hydrocarbon removal. Organic salts evidence no significant decrease in the pollutant, while, commercial products caused around 50% increase in hydrocarbon removal after 45 days.

Ng et al. (2015) investigated the biodegradation of petrodiesel by using biodiesel obtained from *Jatropha*, soybean, and palm as an additive for biostimulation. Biodiesel addition enhanced the biodegradation rate of the mixture, respectively, 12.8%, 19.4%, and 17.5% (from different biomass sources). The efficiency of biodegradation was evaluated by CO_2 evaluation test. The enhancement was reported to be mostly related to co-metabolism and solvation. The co-metabolism effect of biodiesel is its potential to act as nutrient source with providing the energy for microorganisms that consume hydrocarbon and consequently increasing microbial activity. The solvation effect is due to increased exposure area that is caused by solubilizing effect that biodiesels has on petrodiesel. Petrodiesel when mixed with biodiesel enhances solvating and ease of dispersion which prevent the pollutants from integration into sediments and facilitates recovery.

Immobilization of the microorganisms used for bioaugmentation on a career is another effective method for enhancing biodegradation. The most common immobilization technique is formation of biofilm or entrapment and encapsulation of microorganisms using polymeric gels. Microbial immobilization in oil sorbents can produce series of synergetic sorption-biodegradation reaction. Alessandrello et al. immobilized coculture of *Pseudomonas monteilii P26* and *Gordonia* sp. *H19* on polyurethane foam and further used the immobilized cell for the removal of petroleum oil from artificial seawater. Polyurethane foam was selected as a carrier for being economic and readily available and presenting good buoyancy and oleophilic properties. In this work, different temperatures have been tested. The best oil removal was achieved at 30 °C with immobilized mixed biofilm on polyurethane foam after 7 days. The oil removal was due to both biological activity and sorption on the biofilm/carrier system. The immobilized cell can be also stored. Their storage at 4 °C enhanced oil bioremoval at low temperature even though bacterial viability of *P. monteilii* P26 in the biofilm decreased. They have concluded that bacterial acclimatization occurred during the storage improving their metabolic activity at low temperature (Alessandrello et al. 2017).

10.8.2 Genetically Modified Microorganisms

The first genetically engineered microorganism (GEM) was built in 1970 which got the name of "superbug" and was able to degrade oil. This was done with plasmid transfer to utilize some toxic hydrocarbons including hexane, octane, toluene, xylene, camphor, and naphthalene (Kulshreshtha 2013).

The development of GEMs became more important in the early 1980s after improvement of genetic engineering methods and thorough research on metabolic capabilities of microorganisms. It was in 1981 that the first two strains which were modified genetically were patented. These two strains are *Pseudomonas aeruginosa* (NRRL B-5472) and *Pseudomonas putida* (NRRL B-5473) containing genes that give them the ability to degrade naphthalene, salicylate, and camphor. Two operons available in these strains (xylUWCMABN and xylXYZLTEGFJQKIHSR) are responsible for metabolism of toluene, *m*-ethyltoluene, and *m*- and paraxylene (Wasilkowski et al. 2012).

The limitation of natural microorganisms for bioremediation of contaminants is the slow degradation rate. Another limitation of natural attenuation is toxicity of some of organic pollutants for microorganisms in combination of complexity caused by diversity of pollutants. This is more severe about new man-made contaminants released into nature, since the microorganisms have not evolved the proper catabolic pathway for their degradation in such a short time (Chai et al. 2015). This is the main focus of genetic engineering and manipulation of microorganism for bioaugmentation with GEMs process. Recent advances in molecular biology promoted this area of research in the field of engineering microorganisms for specific bioremediation. During genetic modification, microorganisms are supplemented with new genetic properties to be capable of biodegradation of specific pollutants that are not degraded by natural microorganism proper and fast enough. Microbiological information in addition to knowledge on ecological and biochemical mechanisms are needed for combining various desirable metabolic characteristics of organisms and manipulation of important genetic parameters (Jafari et al. 2013). In order to develop genetically manipulated bacteria, there is a need for understanding the way that bacteria break down petroleum compound molecules for removal of the oil spills. For proper design of GEMs, information about interaction of microbes and contaminants, the genetic basis of the interactions, biochemical paths, operon arrangement, and molecular biology must be considered (Kulshreshtha 2013).

Researchers at the University of Texas, Austin, have revealed the genetic code of petroleum hydrocarbon degradation during the Deepwater Horizon oil spill. They have revealed that the ability of some bacteria for oil degradation is far greater than what was expected especially for aromatic hydrocarbon (as an example *Alcanivorax* was formerly considered to be incapable of oil degradation). In this research they have sequenced the DNA of the microbes that have oil degradation ability to uncover genetic characteristic of several bacterial species. The gene sequencing also revealed the method that the genetic potential of the microbial consortia increased (Dombrowski et al. 2016).

The construction of GEMs with enhanced ability for biodegradation of organic compounds is possible since the degradative mechanism, the enzymes, and the relevant genes are understood and biochemical reactions are explained thoroughly (Wasilkowski et al. 2012). The limitation of this method is on one hand the survival of GEMs in the environment and public acceptance on the other hand, which hinder their wide application (Jafarinejad 2017).

For the purpose of bioremediation, different genetic engineering methods are available including improving specificity and affinity of enzyme, metabolic pathway design, and its regulation, expansion of the range of substrate for existing pathway, preventing the production of toxic intermediates which inhibit the path by redirection of carbon flux, enhancing of genetic stability of catabolic activities, identification of genetically modified bacteria in polluted environment by marker gene, and utilization of biosensor for monitoring specific chemical compound. The most common method for creation of GEMs is engineering of one gene or operons and construction of pathways and modification of the existing genetic sequence. For GEM construction, the first step is identification of microorganisms for modification with relevant genes (Kulshreshtha 2013; Chai et al. 2015). By genetic manipulation, rate-limiting steps in metabolic pathways are modified to increase the degradation rate. Incorporation of totally new metabolic pathways into bacterial strains is also possible. Genetic engineering can help for elaborating strategies to monitor, control, and assess the toxicity (Sayler and Ripp 2000). As an example, microbes are limited to aerobic catabolic and co-metabolic biodegradation pathway, and there are limitations for their application in anaerobic environments. By inserting oxygenase genes, this microorganism can undergo anaerobic pathways as well (Kulshreshtha 2013).

For multiplying or expressing specific genes, there is a need for a cloning vector which is commonly plasmids. Vectors are genetic molecules using for transfer of target genetic information to cell to be modified. In the new cell, they can replicate their chromosomal DNA independently. Vectors contain a set of diverse gene such as antibiotic resistance genes. Transposons are other type of genetic elements that act as vectors. Currently, the artificial plasmid vectors are used as well for construction of GEMs. Expression plasmids are also used widely since they facilitate production of desired protein in large quantity very quickly. Another genetic engineering tool for cut-and-paste techniques is enzymes including restriction endonuclease by cleaving DNA in a specific site and DNA ligases which facilitates the joining of DNA strands together and formation phosphodiester as backbone of DNA (Wasilkowski et al. 2012).

The object of genetic manipulation is mostly bacteria especially from genus *Pseudomonas*. These bacteria are available in most of environments and are potent degraders of toxic contaminants. They carry genes for metabolism of contaminants both in their chromosome and plasmids. This makes these microorganisms the main source for obtaining catabolic genes for genetic manipulation (Wasilkowski et al. 2012).

For construction of a proper GEM, there is a need to have a bank of genetic groups and encoding the properties to generate microorganism with improved degradation capabilities. One strategy for doing so is the logical integration of catabolic segments obtained from diverse organisms within one target strain (Jafari et al. 2013). The single constructed GEM has the capability of different microbial community due to insertion of different genes in it and can improve the efficiency and efficacy of the metabolic pathway (Wasilkowski et al. 2012). A successful example was used for bioremediation of a plant which was contaminated with polychlorinated biphenyls. In this case genetic engineering methods were applied to change biphenyl dioxygenase enzyme available in *Pseudomonas alcaligenes* KF707 and *Pseudomonas* sp. LB400 by modifying their substrate specificity. The substrate range of these microorganisms were combined, and various biphenyl dioxygenase were created that can oxidize double ortho- and double para-substituted PCBs (Jafari et al. 2013).

Another strategy is protein engineering that is utilized for improving the stability of the enzyme specificity of substrate and the kinetic properties. This is done through site-directed mutagenesis or oligonucleotide-directed mutagenesis. For this molecular biology method, study of the molecule structure-function relationships and 3D structure of the enzyme or any other protein in protein family is needed to model the structure of the protein (Jafari et al. 2013). Different steps of metabolic pathway are triggered by translation and transcription of genes that lead to enzyme production. Therefore, hybrid gene clusters of GEMs change their enzymatic activity and enzyme substrate specifications (Kulshreshtha 2013).

The major limitation of protein design is that only the structure of few numbers of degradative enzymes is elucidated. By phenotypic selection, unconventional natural or induced mutants can happen. If not possible more efficient approaches are needed. The exchange of subunits or subunit sequences is a method to combine the best attributes of different enzymes. Production of hybrid genes is done by technology of recombinant DNA and in vitro mutagenesis (in which a mutation is generated in a part of cloned DNA). The hybrid genes then encode fusion proteins having improved properties and provide promoters for transcription and translational start sites to induce expression of enzyme (Jafari et al. 2013; Chai et al. 2015). Shuffling DNA sequences is another recently developed approach for obtaining novel proteins which is the random fragmentation and random reassembly. This leads to creation of a broad range of fusion proteins suitable for bioremediation applications (Jafari et al. 2013). Gene transfer encoding homologous (dissimilar) subunits, site-directed mutagenesis (SDM) of important amino acids, and DNA shuffling are among these methods (Chai et al. 2015).

The recombinant bacteria for metabolizing toxic pollutants are obtained in laboratory scale by transformation. Genetic transfer is the mechanism that is used for DNA transformation from a donor to recipient. The gene transfer is obtained by receive of free naked fragments of DNA from environment by the cell of recipient bacteria. The first step is insertion of DNA fragment into a vector and its introduction to the host cell. This is followed by production of multiple copies of a single gene and selection of recombinant DNA. DNA screening for desired biological properties is the final step. Another possibility is conjugation in which genetic material are transferred to another cell by direct contact. This process is done only in one direction (Wasilkowski et al. 2012).

Some modern molecular techniques are used for selection and identification of genetically modified microorganisms which are through detection of specific DNA or RNA sequences. These methods include fluorescent in situ hybridization (FISH, techniques to identify the positions of genes on chromosomes), polymerase chain reaction (PCR, laboratory technique to make billions copies of specific part of DNA), denaturing gradient gel electrophoresis (DGGE, applying a DNA or RNA sample to an electrophoresis gel containing denaturing agent), and terminal restriction fragment length polymorphism (T-RFLP, a technique for describing microbial communities on the basis of the position of a restriction site) and amplified rDNA restriction analysis (ARDRA, extension of RFLP technique) (Wasilkowski et al. 2012).

Several efforts have been done to conduct genetic manipulation on microbes to enhance their oil chewing ability both on land and sea (Martin et al. 2015). The aim is creating microorganism that are more efficient than natural ones in degrading petroleum fractions. Some multiplasmid *P. putida* strain with the simultaneous ability to degrade light alkanes and aromatics has been created by genetic modification (Jafarinejad 2017).

The breakdown of crude oil components was tested with GEMs known as "metagenomic clones" to treat simulated seawater. Genetically modified microorganisms have DNA fragments cloned from the DNA of microbes extracted from oil-contaminated environments. Among them three metagenomic clones combined the metabolic pathways in a way that can be found in nature. They used metabolic machinery derived aerobic and anaerobic bacteria simultaneously. The results obtained for biodegradation of petroleum hydrocarbons by genetically modified bacteria were compared with the results obtained by bacterial strains isolated from reservoir-derived. For saturated hydrocarbons, 31% and 47% were obtained by two metagenomic clones and 99% with natural bacteria. For aromatic hydrocarbon, the degradation was more with metagenomic clones (94%) in comparison with natural strains (63–99%) (Dellagnezze et al. 2014).

Kim et al. have developed a DNA diagnostic method that enables the selection of contaminated sites which are suitable for bioremediation. In this work they have used an oligonucleotide microarray method and identified the genes that are suitable for degradation of aliphatic and aromatics. After that the bioremediation of the contaminated site was performed by applying bioslurping in the field. Bioslurping is an enhanced dewatering technology that is used for the bioremediation of soil and water. The advantages of this system include minimization of discharge of groundwater and soil (Kim et al. 2014).

Das et al. (2015) performed the genome sequence analysis for a strain with high contaminate degradation ability (*Pseudomonas aeruginosa* N002) isolated from the soil contaminated with crude oil. In this work gene sequencing was performed by shotgun sequencing. The catabolic genes encoding the enzymes contributing to hydrocarbon degradation pathways and expression include alkane monooxygenase of *Pseudomonas putida*, alkM from *Acinetobacter* sp. strain, alkane monooxygenase from *Rhodococcus* sp., catechol 2,3-dioxygenase of *P. putida*, naphthalene dioxygenase of *P. putida*, and pyrene dioxygenase from *Mycobacterium* sp. strain PYR-1.

Limitations of application of GEMs in the environment are due to the species classification ambiguities, probable gene transfer to other microorganisms and co-release of antibiotic resistance markers. The concerns about environment and public health safety limit the research with application of GEMs in real fields. Some regulation and limitation were established by US Environmental Protection Agency to control the release of GEMs in the environment (Sayler and Ripp 2000).

The investigation of GEMs application for bioremediation was done mostly in the laboratory experiments. However, for understanding the real effect of GEMs, long-term bioremediation in real field must be done. This is necessary for determining the overall effectiveness and their potential risk to ecosystem (Sayler and Ripp 2000). The survival of GEMs depends strongly on the environmental condition of the field such as clay content, pH, moisture, presence of competing microorganism, etc. (Urgun-Demirtas et al. 2006).

Pseudomonas fluorescens HK44 was the first GMM that was approved to be used for bioremediation in real field. This study was done with the aim of long-term bioremediation of naphthalene-contaminated soil. The used GEMs contained plasmid pUTK21 which made by inserting transposon Tn4431 into NAH7 plasmid obtained from *P. fluorescens* 5R. Simultaneous degradation of naphthalene and luminescent signal was due to the genes which promote pathway for naphthalene decomposition and gene cassette (lux) (Wasilkowski et al. 2012). The parental strain from which NK44 strain was derived was a strain isolated from gas plant facility that was severely polluted with PAHs. In this work a system was developed in which an environmental pollutant was sensed and the proper response was made through an easily detectable signal (bioluminescence) (Sayler and Ripp 2000).

Several authorities are reluctant to accept the release of genetically modified microorganisms due to their adverse environmental impact such as gene transfer. However, it must be noted that GEMs do not add new genes to the environment and are taken from another microorganisms, and usually the introduced engineered microorganism will not survive for a long time after exhaust of its specific substrate. On the other hand, transfer of gene materials among native organism is a common phenomenon. In addition, several methods are available for mitigation of the potential risk of genetically modified organisms (Jafari et al. 2013; Chai et al. 2015).

In general, a successful application of GEMs for bioremediation is based on establishment of capable microorganism for biodegradation and appropriate mechanism for their removal afterward (Kuhad and Singh 2013).

Some methods are available to reduce the potential risk of GEMs in the real field environments. One method is using some genetic barriers that restrict the recombinant bacteria survival and gene transfer in the environment. The restriction can be achieved by kind of transposons which are free from transposase gene or by elimination of conjugation gens from plasmid (Wasilkowski et al. 2012).

A novel strategy is construction of suicidal GEMs that can be achieved by addiction system with antisense RNA and proteic plasmid and application of degradative operons of bacteria. This novel GEMs makes microbes susceptible to death after finishing the degradation of contaminants and reduce their risk to human and environment. In the future, by having more information on microorganism, genomes, and biochemical mechanism, the development of suicidal GEMs would be the most efficient method of using GEMs in real fields (Kulshreshtha 2013).

10.8.3 Integrated Methods for Bioremediation of Oil Spills

As mentioned earlier the strategies to remediate the oil spills are based on physico-chemical or biological technologies. These methods could be used individually or in integrated approach. Supplying electron donors and acceptors is a common approach that can enhance the bioremediation of petroleum hydrocarbons. This is mostly helpful for the degradation of halogenated compounds. Supplying electron acceptors stimulates the biodegradation of non-halogenated compounds. Common electron acceptors are hydrogen and acetate that are delivered directly or through passive dissolution by hollow fiber membranes. Organic substrates such as butyric, lactic, and humic acids as well as ethanol can be used for indirect supply of hydrogen. However, there is challenge in this approach which is the rapid consumption of reagents and their migration from the contaminated area. Therefore, there is need for constant reagent supplement, which is costly and problematic (Daghio et al. 2017).

In bioelectrochemical systems (BES) which is the integration of electrochemical and biological techniques, an electrical current is used both as electron donor and electron acceptor in bioremediation of oil spill by active bacteria (called also exo-electrogens, electricigens, or anode respiring) while they oxidize the substrates anaerobically (Balba et al. 1998; Lu et al. 2014). This technique is controllable and enables the real-time monitoring of the degradation process. Controlling the supply of electron donors is also helpful to avoid unwanted side reactions. For effective BES process, especially in the field applications, several aspects of system design, material selection, and radius of influence must be considered. Mode of action and operational parameters must be assigned effectively. For this process, the knowledge about the microbial process is limited in comparison with the knowledge about the mechanism of electron transfer. The effect that environmental parameters can have on the activity of pollutant-degrading microorganism is another limitation for real-field applications (Daghio et al. 2017; Mapelli et al. 2017). Knowing the microbial mechanism is helpful for understanding the two simultaneous activities taking place in the bioremediation which are the natural attenuation process with native electron acceptors in the environment, and exoelectrogen bacterial consortia that take advantages of the electrodes (Lu et al. 2014).

Having non-exhaustible electron acceptors and donors, this method does not consume large amount of energy and chemicals which makes the remediation process economical for long runs (Lu et al. 2014). In this process the microorganisms catalyze the oxidation reduction reactions near or on the surface of the electrodes. The system includes an anode and a cathode divided by a matrix. The microorganism can exchange electrons with the electrodes directly or indirectly by using a chemical compound as an electron shuttle. The chemical compound is secreted by the microorganisms such as *Pseudomonas* or added exogenously. The anode collects the electrons produced from the oxidation of organic compounds. In the benthic sediments or contaminated aquifer, the anode is buried and is electrically connected to a cathode which is located in the water. The collected electrons are transferred to the cathode via electrical connection and can be used to reduce the oxygen in anaerobic water environment. Compounds available in oil spills such as alkanes and aromatic hydrocarbons could be removed by BES system effectively (Daghio et al. 2017). The biocatalyzing of oxidation reactions of highly concentrated organic compounds is reported to be thermodynamics favor reaction and leads to double benefits which are pollutants degradation and electricity production (H. Li et al. 2017).

The lack of electron acceptor is an important problem in the case of bioremediation of underwater sediments; therefore, application of BES is a promising alternative for the conventional remediation process to be applied to benthic microbial electrochemical system (Li et al. 2017). However, this method can be applied effectively for bioremediation of oil spills in soil and water too (Balba et al. 1998). The bottleneck of the process under anaerobic conditions such as benthic environments is the initiation of the degradation process. In aerobic conditions, the process is started by catalyzing the addition of hydroxyl groups by an oxygenase, which is a less efficient process in anaerobic conditions. In such cases, the anode apart from being electron acceptor contributes in initiation of the process by production of oxygen and modifying the pH. This ability depends on salinity, ion species and concentration, pH, temperature, and electrode properties. The reduction reactions at the cathodes are exploited for the reduction of oxidized compounds (Daghio et al. 2017). Inefficient mass transfer is another limitation of the BES techniques (Li et al. 2015). In the subsurface environment, usually graphite is used as electron acceptor in BES (Viggi et al. 2015).

This approach can be applied in a microbial fuel cell that is an electrochemical device to convert chemical energy into electricity using exoelectrogenic bacteria as biocatalysts. This approach was firstly applied for the wastewater treatment and further developed for recalcitrant compounds removal such as petroleum hydrocarbons (Adelaja et al. 2015). Simultaneous pollutant biodegradation (due to secondary reactions) and energy production can be achieved in this method. In microbial fuel cells, the electrons obtained by exoelectrogenic bacteria are transferred through external circuit from anode to cathode for oxygen reduction (Wang et al. 2012; Chandrasekhar and Venkata Mohan 2012). In this approach, non-exhaustible electron acceptors are used, and the necessity of aeration in the subsurface is eliminated. However, a semi-aerobic metabolic pathway on the cathode is sustained (Lu et al. 2014).

In the recent approaches, the application of electrodes colonized with mixed consortia has been used for their better stability and performance both in degradation and electricity generation (Venkidusamy et al. 2016).

For the microbial fuel cell application in the real field, there is a need to study the robustness of the system in different operating conditions. Temperature is effective, since low temperature inhibits the methanogenic bacteria growth. However, at low temperature electrogenesis is promoted, while high temperature improves the thermodynamics of the system and rate of substrate utilization and increase biokinetics by improving mass transfer and activation energy. The use of exogenous redox mediators enhances the electron transfer rate and improves the electrochemical performance of the system. The influencing parameters are toxicity of the redox mediator, the ratio of redox potential of the mediator to the redox potential of the substrate, and permeability characteristics of the cell membrane for the molecules of redox mediator (Adelaja et al. 2015). However, the application of this system in real field has not been tested yet, and there are several facts to be verified according to different conditions and the actual scalability (Daghio et al. 2017).

A novel bioelectrochemical approach is a simpler approach called “Oil-Spill Snorkel” used for bioremediation of soil and sediments contaminated with petroleum hydrocarbons. This system is composed of a snorkel (which is the electrode made of conductive material) placed for providing electrochemical connection. The snorkel is the electrode (acting as both anode and cathode) which is a conductive rod which makes a bridge between aerobic and the anaerobic zones. However, in this method, the electricity cannot be harvested or monitored. The electrons derived from oxidation of contaminants are accepted by an anode electrode buried in sediments. These electrons are transferred through snorkel to the cathode where aerobic conditions are available. There, the reduction of oxygen is occurs to form water (Daghio et al. 2017; Viggi et al. 2015).

The snorkel provides link to connect an anoxic zone (polluted sediments) and the oxic zone (top oxygen-containing water). The bottom part of the snorkel buried in the target sediment is the anode acceptor. In this system the electric resistance available in conventional BES and microbial fuel cells resulting from the separate electrodes is eliminated. This way the bacterial community in the sediments can access the high redox potential electron acceptor (oxygen). This method was used by Viggi et al. for bioremediation of marine sediments contaminated with petroleum hydrocarbons (Viggi et al. 2015). The schematic figure of oil spill snorkel can be seen in Fig. 10.2.

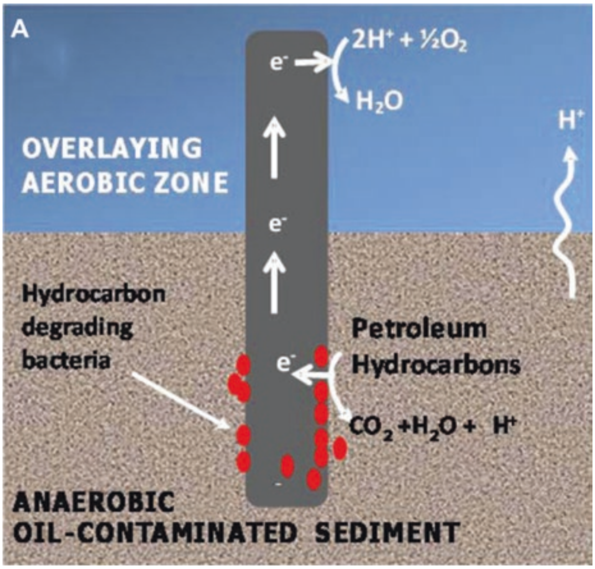

Fig. 10.2 Illustration of oil spill snorkel (Viggi et al. 2015)

For the electricity production, the type and amount of the contaminants can influence the potential current and power density produced (Li et al. 2017). The anodic solution conductivity was higher in more salinity condition. The internal resistance of microbial fuel cell was also decreased. However, the microbial activity and growth can be affected adversely by high salinity (Adelaja et al. 2015).

In the work of Cheng et al., the microbial fuel cell for bioremediation of oil spill in soil was used. In this work, the voltage of 190 mV and 24% total petroleum hydrocarbon removal was achieved in 66 days. The scanning electron microscopy images on the anode electrodes (carbon fibers) revealed the formation of biofilm which build the link between carbon fibers and can improve electron transmission (Cheng et al. 2017).

Li et al. used microbial fuel cell, for bioremediation of sediments contaminated by hydrocarbons. Sand was mixed with the contaminated soil to enlarge the pore size of soil in order to accelerate the ion and substrate transfer. Electricity generation and degradation rate were improved using this method (Li et al. 2015).

Bioelectrochemical remediation system in the work of Venkidusamy et al. was performed with pre-cultured anodes. The performance of enriched biofilm anodes was compared with the performance of freshly inoculated anode. It was reported that enrichment of anode had significant effects on the results obtained from microbial fuel cell both for contaminant removal and current generation (Venkidusamy et al. 2016).

The future studies in the field of BES must be focused on the physicochemical conditions that can lead the effective real-field application of this system. As an example, pH of the field can affect not only microbial activity but also the availability of the alternative electron acceptors which can affect the bioelectrochemical anode reduction reactions (Daghio et al. 2017).

A pilot-scale benthic microbial electrochemical system was built by Li et al. for bioremediation of polluted river sediments. The anode in this system was carbon mesh with honeycomb structure supports as anode, and the cathode was activated carbon. The river water was simulated with wastewater. Removal of polycyclic aromatic hydrocarbons reached 74%, and a maximum power density of 63 ± 3 mW m^2 was achieved. The power density decreased to 42 ± 2 mW m^2 due to cathode degradation and at the end of the operation reduced to 30 ± 3 mW m^2 due to substrate limitation (Li et al. 2017).

The effects of temperature, salinity, presence of redox mediators, and fed-batch system on the degradation efficiency and electrochemical functionalities were studied in the work of Adelaja et al. for bioremediation of mixture of petroleum hydrocarbon in a microbial fuel cell. The optimum condition was salinity of 2.5%w/v and temperature of 40 °C (Adelaja et al. 2015).

10.9 Conclusion

Oil spill occurrence is not a new problem and has been the issue for more than a century. This problem whether occurring in water or soil is a huge threat for ecosystem, fauna and flora. Bioremediation as an economical and environmentally friendly approach is based on microorganism's capabilities to degrade petroleum hydrocarbons. This method aims at biostimulation and bioaugmentation of the natural attenuation of the contaminants with indigenous microorganisms. In comparison with physicochemical methods (application of skimmers, booms, barriers and sorbents, dispersants, and controlled in situ burning), bioremediation is a more effective approach without disrupting the polluted environments. Although several aspects of this approach had been studied by different researchers and quite high hydrocarbon removal rate were reported specially in laboratory scale, the real-field applications are not developed thoroughly. Novel approaches for bioremediation including addition of novel materials, using GEMs, and integration of electrochemical strategies with biological methods are new fields of research for bioremediation of oil spills.

References

Adams FV, Niyomugabo A, Sylvester OP (2017) Bioremediation of crude oil contaminated soil using agricultural wastes. Proc Manuf 7:459–464

Adelaja O, Keshavarz T, Kyazze G (2015) The effect of salinity, redox mediators and temperature on anaerobic biodegradation of petroleum hydrocarbons in microbial fuel cells. J Hazard Mater 283:211–217

Alessandrello MJ, Juárez Tomás MS, Raimondo EE, Vullo DL, Ferrero MA (2017) Petroleum oil removal by immobilized bacterial cells on polyurethane foam under different temperature conditions. Mar Pollut Bull 122(1–2):156–160

Atlas RM (1991) Microbial hydrocarbon degradation—bioremediation of oil spills. J Chem Technol Biotechnol 52(2):149–156

Atlas RM (1995) Petroleum biodegradation and oil spill bioremediation. Mar Pollut Bull 31:178–182

Atlas RM, Barsa R (1992) Hydrocarbon biodegradation and oil spill bioremediation. Plenum Press, New York, p 12

Ayed HB, Jemil N, Maalej H, Bayoudh A, Hmidet N, Nasri M (2015) Enhancement of Solubilization and biodegradation of diesel oil by biosurfactant from Bacillus Amyloliquefaciens An6. Int Biodeter Biodegr 99:8–14

Balba MT, Al-Awadhi N, Al-Daher R (1998) Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. J Microbiol Method 32(2):155–164

Baniasadi M, Mousavi SM, Zilouei H, Shojaosadati SA, lRastegar SO (2018) Statistical evaluation and process optimization of bioremediation of polycyclic aromatic hydrocarbon in a bioreactor. Environ Eng Manag J 17(8):1782–1790

Bastida F, Jehmlich N, Lima K, Morris BEL, Richnow HH, Hernández T, von Bergen M, García C (2016) The ecological and physiological responses of the microbial community from a semi-arid soil to hydrocarbon contamination and its bioremediation using compost amendment. J Proteome 135:162–169

Bezza FA, Evans M, Nkhalambayausi C (2015) Biosurfactant from Paenibacillus Dendritiformis and its application in assisting polycyclic aromatic hydrocarbon (PAH) and motor oil sludge removal from contaminated soil and sand media. Process Saf Environ 98:354–364

Bovio E, Giorgio G, Prigione V, Spina F, Denaro R, Yakimov M, Calogero R, Crisafi F, Cristina Varese G (2017) The culturable mycobiota of a Mediterranean marine site after an oil spill: isolation, identification and potential application in bioremediation. Sci Total Environ 576:310–318

Chai L, Jiang X, Zhang F, Zheng B, Shu F, Wang Z, Cui Q, Dong H, Zhang Z, Hou D, She Y (2015) Isolation and characterization of a crude oil degrading bacteria from formation water: comparative genomic analysis of environmental Ochrobactrum intermedium isolate versus clinical strains. J Zhejiang Uni SCI B 16(10):865–874

Chandrasekhar K, Venkata Mohan S (2012) Bio-electrochemical remediation of real field petroleum sludge as an electron donor with simultaneous power generation facilitates biotransformation of PAH: effect of substrate concentration. Bioresour Technol 110:517–525

Cheng Y, Wang L, Faustorilla V, Megharaj M, Naidu R, Chen Z (2017) Integrated electrochemical treatment systems for facilitating the bioremediation of oil spill contaminated soil. Chemosphere 175:294–299

Dadrasnia A, Agamuthu P (2014) Biostimulation and monitoring of diesel fuel polluted soil amended with biowaste. Pet Sci Technol 2:2822–2828

Daghio M, Aulenta F, Vaiopoulou E, Franzetti A, Arends JBA, Sherry A, Suárez-Suárez A, Head IM, Bestetti G, Rabaey K (2017) Electrobioremediation of oil spills. Water Res 114:351–370

Das D, Baruah R, Roy AS, Singh AK, Boruah HPD, Kalita J, Bora TC (2015) Complete genome sequence analysis of Pseudomonas aeruginosa N002 reveals its genetic adaptation for crude oil degradation. Genom 105(3):182–190

Dellagnezze BM, de Sousa GV, Martins LL, Domingos DF, Limache EEG, de Vasconcellos SP, da Cruz GF, de Oliveira VM (2014) Bioremediation potential of microorganisms derived from petroleum reservoirs. Mar Pollut Bull 89(1–2):191–200. https://doi.org/10.1016/j.marpolbul.2014.10.003

Dias RL, Ruberto L, Hernández E, Vázquez SC, Balbo A, Del Panno MT, Mac Cormack WP (2012) Bioremediation of an aged diesel oil-contaminated Antarctic soil: evaluation of the ‘on site’ biostimulation strategy using different nutrient sources. Int Biodeter Biodegr 75:96–103

Dombrowski N, Donaho JA, Gutierrez T, Seitz KW, Teske AP, Baker BJ (2016) Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the deepwater horizon oil spill. Nat Microbiol 1:1–7

Gomez F, Sartaj M (2013) Field scale ex-situ bioremediation of petroleum contaminated soil under cold climate conditions. Int Biodeter Biodegr 85:375–382

Gonzalez P, Sanchez Y (2011) Bioremediation of oil spills. Escuela Tecnica Superior de ingenieros de Minas

Helmy Q, Laksmono R, Kardena E (2015) Bioremediation of aged petroleum oil contaminated soil: from laboratory scale to full scale application. Proc Chem 14:326–333

Hernández-Espriú A, Sánchez-León E, Martínez-Santos P, Torres LG (2013) Remediation of a diesel-contaminated soil from a pipeline accidental spill: enhanced biodegradation and soil washing processes using natural gums and surfactants. J Soils Sediments 13(1):152–165

Horel A, Mortazavi B, Sobecky PA (2015) Input of organic matter enhances degradation of weathered diesel fuel in sub-tropical sediments. Sci Total Environ 533:82–90

Jafari M, Rezaee Danesh Y, Mohammadi Goltapeh E, Varma A (2013) Bioremediation and genetically modified organisms. In: Goltapeh EM, Danesh YR, Varma A (eds) Fungi as bioremediators, vol 32. Springer, Berlin/Heidelberg, pp 433–451

Jafarinejad S (2017) Oil-spill response. Petroleum waste treatment and pollution control. Elsevier, Oxford, pp 117–148

Jain PK, Gupta VK, Gaur RK, Lowry M, Jaroli DP, Chauhan UK (2011) Bioremediation of petroleum oil contaminated soil and water. Acad J Inc 5(1):1–26

Kim S, Krajmalnik-Brown R, Kim JO, Chung J (2014) Remediation of petroleum hydrocarbon-contaminated sites by DNA diagnosis-based bioslurping technology. Sci Total Environ 497–498:250–259

Kuhad RC, Singh A (2013) Biotechnology for environmental management and resource recovery. Springer, New Delhi

Kulshreshtha S (2013) Genetically engineered microorganisms: a problem solving approach for bioremediation. J Bioremed Biodegr 04(04):e133

Lahel A, Fanta AB, Sergienko N, Shakya M, Estefanía López M, Behera SK, Rene ER, Park HS (2016) Effect of process parameters on the bioremediation of diesel contaminated soil by mixed microbial consortia. Int Biodeter Biodegr, vol 113, pp 375–385

Li X, Wang X, Jason Ren Z, Zhang Y, Li N, Zhou Q (2015) Sand amendment enhances bioelectrochemical remediation of petroleum hydrocarbon contaminated soil. Chemosphere 141:62–70

Li H, He W, Qu Y, Li C, Tian Y, Feng Y (2017) Pilot-scale benthic microbial electrochemical system (BMES) for the bioremediation of polluted river sediment. J Power Sources 356:430–437

Lim MW, Von Lau E, Poh PE (2016) A comprehensive guide of remediation technologies for oil contaminated soil – present works and future directions. Mar Pollut Bull 109(1):14–45

Lu L, Huggins T, Jin S, Zuo Y, Ren ZJ (2014) Microbial metabolism and community structure in response to bioelectrochemically enhanced remediation of petroleum hydrocarbon-contaminated soil. Environ Sci Technol 48(7):4021–4029

Mapelli F, Scoma A, Michoud G, Aulenta F, Boon N, Borin S, Kalogerakis N, Daffonchio D (2017) Biotechnologies for marine oil spill cleanup: indissoluble ties with microorganisms. Trends Biotechnol 35(9):860–870

Martin CW, Hollis LO, Turner RE (2015) Effects of oil-contaminated sediments on submerged vegetation: an experimental assessment of *Ruppia maritima*. PLoS One 10(10):e013879

Marzan LW, Sultana T, Mahbub Hasan M, Akter Mina S, Islam R, Rakibuzzaman AGM, Hassan Khan I (2017) Characterization of furnace oil bioremediation potential of hydrocarbonoclastic bacteria isolated from petroleum contaminated sites of the Sundarbans, Bangladesh. J Genet Eng Biotechnol 15(1):103–113

Miklaucic EA, Saseen J (1989) The Ashland oil spill, Floreffe, PA – CASE history and response evaluation. In: International oil spill conference

Montagnolli RN, Matos Lopes PR, Dino Bidoia E (2015) Assessing Bacillus subtilis biosurfactant effects on the biodegradation of petroleum products. Environ Monit Assess 187(1):4116

Nasirpour N, Mousavi SM, Shojaosadati SA (2015) Biodegradation potential of hydrocarbons in petroleum refinery effluents using a continuous anaerobic-aerobic hybrid system. Korean J Chem Eng 32(5):874–881

Ng YF, Ge L, Chan WK, Tan SN, Hong Yong JW, Tan TTY (2015) An environmentally friendly approach to treat oil spill: investigating the biodegradation of petrodiesel in the presence of different biodiesels. Fuel 139:523–528

Pontes J, Mucha AP, Santos H, Reis I, Bordalo A, Basto MC, Bernabeu A, Almeida CMR (2013) Potential of bioremediation for buried oil removal in beaches after an oil spill. Mar Pollut Bull 76(1–2):258–265

Rastegar SO, Mousavi SM, Shojaosadati SA, Sheibani S (2011) Optimization of petroleum refinery effluent treatment in a UASB reactor using response surface methodology. J Hazard Mater 197:26–32

Rastegar SO, Mousavi SM, Shojaosadati SA, Sheibani S (2017) Kinetic constants determination of petroleum refinery effluent treatment in a UASB reactor using RSM. Environ Eng Manag J 16(1):121–130

Rhykerd RL, Weaver RW, McInnes KJ (1995) Influence of salinity on bioremediation of oil in soil. Environ Pollut 90:127–130

Sayler GS, Ripp S (2000) Field applications of genetically engineered microorganisms for bioremediation processes. Curr Opin Biotechnol 11:286–289

Singh B, Bhattacharya A, Channashettar VA, Jeyaseelan CP, Gupta S, Sarma PM, Mandal AK, Banwari L (2012) Biodegradation of oil spill by petroleum refineries using consortia of novel bacterial strains. B Environ Contam Toxicol 89(2):257–262
Si-Zhong Y, Hui-Jun JIN, Zhi WEI, Rui-Xia HE, Yan-Jun JI, Xiu-Mei LI, Shao-Peng YU (2009) Bioremediation of oil spills in cold environments: a review. Pedosphere 19(3):371–381
Soleimani M, Farhoudi M, Christensen JH (2013) Chemometric assessment of enhanced bioremediation of oil contaminated soils. J Hazard Mater 254–255:372–381
Szulc A, Ambrożewicz D, Sydow M, Ławniczak Ł, Piotrowska-Cyplik A, Marecik R, Chrzanowski Ł (2014) The influence of bioaugmentation and biosurfactant addition on bioremediation efficiency of diesel-oil contaminated soil: feasibility during field studies. J Environ Manag 132:121–128
Telegraph, 10 Largest Oil Spills in History (n.d.) Retrieved 18 October 2017, from web site: http://www.telegraph.co.uk/news/worldnews/australiaandthepacific/newzealand/8812598/10-largest-oil-spills-in-history.html
The Atlantic, The Exxon Valdez Oil Spill: 25 Years Ago Today (n.d.) Retrieved 20 November 2017, from web site: https://www.theatlantic.com/photo/2014/03/the-exxon-valdez-oil-spill-25-years-ago-today/100703/
Urgun-Demirtas M, Stark B, Krishna P (2006) Use of genetically engineered microorganisms (GEMs) for the bioremediation of contaminants. Crcit Rev Biotechnol 26(3):145–164
US EPA, Oil Spills Prevention and Preparedness Regulations (2013) Retrieved 29 November 2017, from web site: https://www.epa.gov/oil-spills-prevention-and-preparedness-regulations
Venkidusamy K, Megharaj M, Marzorati M, Lockington R, Naidu R (2016) Enhanced removal of petroleum hydrocarbons using a bioelectrochemical remediation system with pre-cultured anodes. Sci Total Environ 539:61–69
Viggi C, Enrica Presta C, Bellagamba M, Kaciulis S, Balijepalli SK, Zanaroli G, Papini MP, Rossetti S, Aulenta F (2015) The 'oil-spill snorkel': an innovative bioelectrochemical approach to accelerate hydrocarbons biodegradation in marine sediments. Front Microbiol 6(September):881
Walker AH (2017) Oil spills and risk perceptions. In: Oil spill science and technology. Elsevier, pp 1–70
Wang X, Cai Z, Zhou Q, Zhang Z, Chen C (2012) Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. Biotechnol Bioeng 109(2):426–433
Wasilkowski D, Swędziol Z, Mrozik A (2012) Przydatność Genetycznie Modyfikowanych Mikroorganizmów Do Bioremediacji Zanieczyszczonych Środowisk. Chemik 66(8):817–826
Xu J, Kong F, Song S, Cao Q, Huang T, Cui Y (2017) Effect of Fenton pre-oxidation on mobilization of nutrients and efficient subsequent bioremediation of crude oil-contaminated soil. Chemosphere 180:1–10