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RESEARCH ARTICLE

Crude oil pollution and biodegradation at the Persian Gulf: A comprehensive and review study

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Abstract

The Persian Gulf consider as the fundamental biological marine condition between the seas. There is a different assortment of marine life forms including corals, wipes, and fish in this marine condition. Mangrove timberlands are found all through this sea-going biological system. Sullying of the Persian Gulf to oil-based goods is the principle of danger to this marine condition and this contamination can effectively affect this differing marine condition. Numerous specialists examined the result of oil contamination on Persian Gulf marine creatures including corals sponges, bivalves, and fishes. These analysts affirmed this oil contamination on the Persian Gulf significantly diminished biodiversity. Diverse microorganisms fit to consume oil-based commodities detailed by various scientists from the Persian Gulf and their capacity to the debasement of unrefined petroleum has been examined. There has additionally been little exploration of cyanobacteria, yeast, and unrefined petroleum debasing organisms in this sea-going environment. Biosurfactants are amphipathic molecules that upgrade the disintegration of oil and increment their bioavailability to corrupt microscopic organisms. Additionally, biosurfactant-producing bacteria were discovered from the Persian Gulf, and the capability to degradation of crude oil in microscale was evaluated. The current review article aims to collect the finding of various researches performed in the Persian Gulf on oil pollution and crude-oil biodegradation. It is expected that by applying biological methods in combination with mechanical and chemical methods, the hazard consequences of crude-oil contamination on this important aquatic ecosystem at the world will be mitigated and a step towards preserving this diverse marine environment.

Keywords Bacteria · Biodegradation · Marine environment · Oil pollution · Persian gulf

Introduction

Life has begun from the sea and continues to this day. About 70% of the Earth is surrounded by the seawater, and marine organisms include the primary organism to complex and the most diverse species. Increasing human populations have put pressure on many natural sources, and to cope with this growing need, we can take refuge in the resources of the sea, which occupy one-third of the land [1].

The oceans comprise one-third of the Earth. There are various forms of life in the oceans. In recent years, due to human activities, various pollutants have entered the oceans and marine environments, leading to the change of life in these important aquatic ecosystems [2].

Petroleum pollution can enter the seas in several ways, which are divided into two categories: natural oil spill and artificial oil spill. Natural ways such as oil spills from reservoirs and volcanic processes in the deep ocean. Artificial processes include oil tanker accidents, oil transportation processes, oil refineries, oil extraction processes, and petroleum loading processes. In most cases, the main pollution way for contamination of sea with crude oil represents artificial roads [3].

Crude oil has an extremely complex composition of hydrocarbons. In general, crude oil compounds are divided into four fundamental categories and accordingly, there are two types of crude oil: light crude oil and heavy crude oil. Crude oil that has saturated hydrocarbons and polycyclic aromatic hydrocarbons (PAH) called light petroleum, and crude oil that has intenser amounts of resins and asphaltenes compounds than saturated hydrocarbons consider as heavy petroleum. In

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general, the environmental impacts of heavy crude oil are greater than light crude oil. [4].

Diverse strategies have therefore extremely been employed to remove crude oil from marine environments. Like physical, chemical, and mechanical methods. Each of these methods has advantages and disadvantages for crude-oil removal. The main advantage of these strategies is that they rapidly remove oil contamination from the sea surface, but the disadvantages of these methods are the creation of hazardous chemical intermediates, some of which are more harmful to the initial contamination and on the other hand, these strategies only eliminate pollution from the surface of the sea. However, many petroleum compounds are heavy and sediment deep in the sea.

The use of biological strategies to eliminate oil pollution has been considered in recent years. Biological techniques are slower than the above methods, but they have better advantages to use. Their benefits can be attributed to cost-effectiveness, lack of intermediate products, and complete degradation of pollutants from the marine environment. Biological strategies like biodegradation and bioremediation are the best technologies for conserve marine environments in the future [5–7].

The Persian Gulf has 68% of the world's oil reserves and also more than 40% of gas resources. Therefore, based on hydrocarbon resources, this marine environment is the richest in the world. As a result, the beaches and islands of southern Iran that are located in the Persian Gulf are constantly exposed to oil pollution. In this way, to study the oil spill in the Persian Gulf is important. Furthermore, the Persian Gulf has a vast diversity of marine invertebrates [8–10].

The Persian Gulf has experienced numerous and huge oil spills in recent years, each of which was enough to pollute any marine area, so the Persian Gulf can be considered the most pronounced victim of an oil spill. The most noticeable and paramount case of the Persian Gulf pollution and contamination is related to the Gulf War spill of 1991, which is considered the biggest oil spill in history. What has entered the Persian Gulf, which covers an area of approximately 239,000 km^2 and an average depth of only 35 m [11] or 36 m [12], to be the most polluted marine basin in the world is that the water there is warm and in heated waters, the toxicity level of oil is higher, and weathering takes place much faster [13]. Wars in the past two decades may have increased the pollution loads in the area [14].

Contamination of oil in the marine ecosystems

The pollution of crude oil in the ocean remains a hazardous threat to the existence of the planet earth, which can cause major damage to marine ecosystems and coastal areas. Annually, crude oil typically enters the environment through natural and anthropogenic sources, the former would be sufficient to pollute all of the marine ecosystems. According to an investigation, the annual rate of entered crude-oil in the waters of the world is about 1.3 million tones [1].

For the Persian Gulf that is one of the world's most significant water bodies, this rate represents 300 tones. Since approximately 60% of worldwide oil transportation takes place through this Gulf, oil contamination constitutes an acute and international threat in the Persian Gulf [15]. Since approximately 60% of worldwide oil transportation takes place through this Gulf, oil contamination constitutes an acute and international threat in the Persian Gulf [15]. An example of oil spill events caused by the human in this Gulf was the war between Iraq and the United States in 1991, known as the second Gulf war. In this conflict, the United States forced Iraq to release petroleum to the Persian Gulf from Mina Al-Ahmadi terminal for three successive days and burned about 700 oil wells. In a comparison between the Persian Gulf and the Gulf of Mexico, the rate of petroleum hydrocarbon concentrations was 1.2–542 ($\mu g\ l^{-1}$) and 0.4–66.8 ($\mu g.l^{-1}$) respectively [16–18].

Mixtures of crude oil

The petroleum is a natural, toxic, heterogeneous and complex organic compound and the mixture of hydrocarbons, its mass spectrometry showed more than 17,000 distinct chemical compounds including general alkanes with various chain lengths and branch points, cycloalkanes, mono-aromatic and polycyclic aromatic hydrocarbons. Sulfur, oxygen, and nitrogen can be excessively found in the structure of certain compounds, whereas phosphorus and heavy metals like vanadium and nickel are observed rarely [19]. Because of the considerable differences in the chemical and physical features of oil compounds (for instance solvability, viscosity, capacity to absorb and equally varying in its toxicity and bioavailability), their biodegradation potential and environmental fate are different.

In a classification, petroleum hydrocarbons are categorized in four general classes: Saturated, Aromatics, Asphaltenes (Porphyrins, Esters, Ketones, fatty acids, Phenols), and Resins (Amides, Sulfoxides, Carbazoles, Quinolines, Pyridines). In Fig. 1 the crude oil combination was illustrated. The rate of more polar chemical compounds such as asphaltenes and resins and also saturated and aromatic hydrocarbons are higher in light oils. The resistance of crude oil hydrocarbons to microbial attacks is as follows: n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes [20]. Biodegradation process of large branched aliphatic chain and high-molecular-weight aromatic hydrocarbons due to their structural complexity is harder than elementary hydrocarbons. Saturated hydrocarbons remain the most

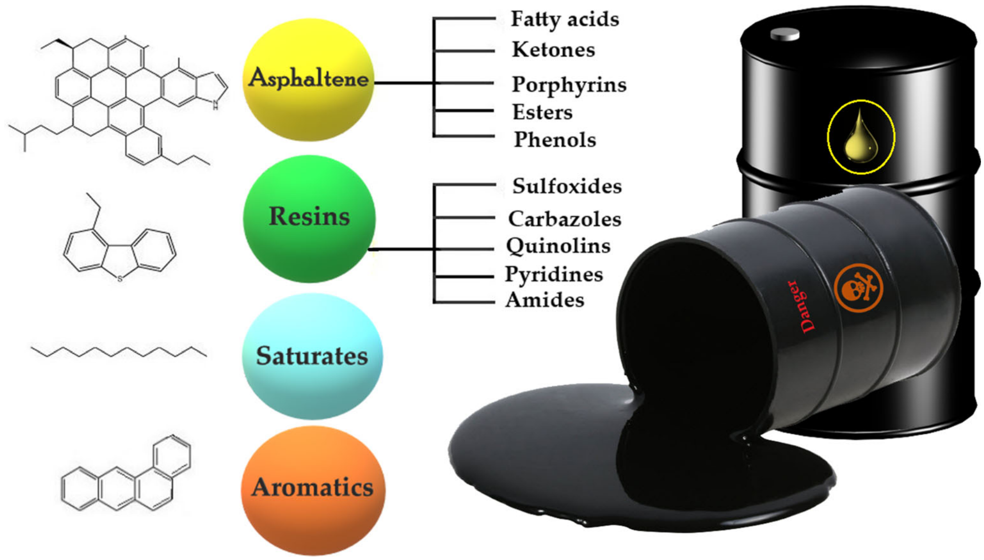


Fig. 1 The Chemical composition of crude oil

significant group of petroleum compounds which their biodegradation is environmentally significant. Because of the more toxicity and persistence of the aromatic compounds and polar components, they can cause long-time effects on the ecosystem. [21].

Sources of crude oil pollution and effect on marine ecosystems

The crude-oil hydrocarbons are the major pollutants to the environment (crude oil and intermediate products) for biological systems. Among of main biological sources that import hydrocarbons into the sea, can be mentioned to Wax materials of terrestrial organisms, degradation, burning of biological substances, hydrocarbons synthesis of plants, phytoplankton, bacteria, microscopic and large-scale algae. Anthropogenic sources of oil pollution are as follows: exploitation, transportation, refining, or damage to oil tankers. Damage to human health and ecological systems are recognized as the most destructive effects of the oil spill. Other destructive effects of oil pollution are influencing human lifestyle, carcinogenicity, mutagenicity, prevention of light diffusion, oxygen penetration, and consequently the death of organisms due to hypothermia [22–24]. For instance, in the Gulf of Mexico, the Deepwater Horizon explosion in April 2010 with a spill of about five million barrels of petroleum led to vertebrate and metazoan meiofauna reducing biodiversity [25–27].

At the same of an event in Spain over 66% of whole species (containing insects, crustaceans, mollusks, and polychaetes) was eradicated in oily coasts (Prestige, November 2002). Also in the Exxon Valdez spill in March 1989, nearly 41 million liters of petroleum were entered into the sea in the north of Alaska [28]. Hydrocarbons of crude oil can stick to the fur's mammals and feathers of marine birds, and these organisms have perished when they clean themselves due to overdose hydrocarbon consumption. Detriments caused by hydrocarbons specifically polycyclic aromatic hydrocarbons (PAHs) to fisheries and wildlife can be long-term; for instance, the pollution caused by the Brear spill (Shetland Islands, United Kingdom, 1993) because of polluted fish and shellfish, remained in place for more than six years. Moreover, physiological, behavioral, and genetic damages, declines in both development and fertility in fishes occur as a result of chronic contamination at supra-lethal concentrations [29]. The most extensive oil spill in human history caused by the Gulf War in 1991, in which oil and tar-covered an extensive area more than 770 km of the shoreline from Abu Ali Island in the south of Kuwait (Saudi Arabia) and destroyed the local plants and animals [30].

In cases where natural oil seepages occur, they penetrate from faults and cracks in seabed into water depth [31, 32]. Proverbially, California's coal oil point, the Timor Sea in Indonesia, and the north of the Persian Gulf are areas where such leakages occur. Coral reefs that are one of the most varied and complex systems in marine ecosystems, harbor an enormous variety of marine organisms and are extremely influenced by petroleum [33, 34]. Abnormalities in reproduction, produced few gonads and immature planulae, abortion, increase in the size of mucus cells (these cells are one of the remarkable defense agents against pathogens and stress which results in cell wall rupture and finally a decreased number of mucus cells can be attributed to petroleum. Numerous magnitude of oil can also have altered the primary composition of *Vibrio* with the hydrocarbon-degradation capacity of beneficial bacteria like *Alteromonas*, *Pseudoalteromonas*, and *Pseudomonas* [35, 36].

Persian Gulf and importance as a major marine environment in the world

The Persian Gulf is amongst the world's most various aquatic environments. There is a diverse variety of marine organisms including corals, sponges, and fish in this marine environment. Mangrove forests are found throughout this aquatic ecosystem. In mangrove forests, some complex ecological relationships between organisms live in intertidal regions. Other marine organisms in the Persian Gulf are also widely diversified [37, 38].

Conservation of this aquatic ecosystem in the world is, therefore, more important. In addition to its wide diversity, this marine environment poses a vast range of ecological threats, like the development of the fishing industry and the construction of artificial islands. The critical threat to this marine ecosystem is oil pollution. Oil pollution from various sources can enter the Persian Gulf and threaten the life of this ecosystem and cause the loss of marine life. These sources of contaminants in the Gulf include oil spills from oil reservoirs, shipping incidents, maritime transfers, and oil extraction processes.

Since nearly 60% of the worldwide oil is transferred to the Persian Gulf, oil contamination is inevitable. This has constructed the marine environment the most polluted sea in the world. Oil pollution is unmanaged in the Persian Gulf, many corals, sponges. Mangrove forests will be destroyed soon, and biodiversity will be considerably reduced.

Oil and marine sediments interactions

Oil spill takes place in the marine environment after that alter complex biological, physical, and chemical parameters such as spreading, dispersal, weathering (processes including evaporation, biodegradation, dissolution, and emulsification), drifting and stranding. Despite the dispersion of all oil droplets in the water column, monocyclic compounds (e.g., benzene and alkyl-substituted benzenes) with the log Kow values between 2.1 and 3.7 and selected lower molecular weight, 2–3 ring polycyclic aromatic hydrocarbons (PAHs) with the log Kow values between 3.7 and 4.8 can be partially dissolved [39–41]. Interactions of soluble and diffused oil components and sediment particles extremely affect such environmental processes. After an oil spill in the sea, sediments are accredited as significant vectors for transporting oil from one phase to another [7]. Muschenheim and Lee's [42] studies, has been shown the role of oil-sediments interactions in scattering and degradation of spilled oil. In nearshore waters, aggregations of oil droplets with suspended particulate material (SPM) are performed via connecting naturally dispersed oil droplets to SPM like organic matter and clay minerals. Following the oil spill, oil diffused in a mixture of sediments and seawater settled and is trapped on the bottom through adhering to sediments [7].

Several experiments have been shown the role of mineral-oil interactions in the natural cleaning of oiled shorelines in Prince William Sound, Alaska, after the Exxon Valdez spill [28, 43–46].

Diffused oil droplets and soluble oil components are two physical forms of oil in water. Also, sediments may come in two forms in water: SPM and settled aggregates. The reaction between diffused oil droplets or soluble oil components with sediments maybe happened in two forms of (1) direct aggregation to form OSAs, and (2) adsorption on or incorporation in the sediment phase [47, 48]. Oil-sediment interactions, one of the most important processes vis-à-vis oil spill, change the destiny and transport of petroleum from the aqueous phase by removing it [49]. It seems that the decrease in oil droplets size can be a factor for the formation of OSAs for oil droplets fixation and inhibition of their re-coalescing [50].

When the oil converts to droplets, increase its surface areas and increasing the availability of oil resulting in enhancement biodegradation rate. After the collision and adherence of oil adsorbed droplets or free phase liquid with solid particles in an aqueous suspension, form the oil-SPM aggregation which is called OSAs. Following electrical charge interactions between the polar oil compounds and particle surfaces, with the cations intermediary as electrical bridges form OSAs [49]. When an oil droplet attaches to solid particles with micrometer-sized, form the most recognizable and common type of OSAs and also sometimes with more than one droplet attachment form multi-droplet OSAs. Three forms of OSAs (droplets, solids, and flake aggregates) are recognized by UV Epi-fluorescence, bright-field microscopy, and scanning electron microscopy (SEM) techniques [51]. Thin sheets of flake aggregates are created by the ordered configuration of oil and solid particles.

Using confocal laser imaging to evaluate the oil-mineral structures, Omotoso et al. [52] the OSAs are classified based on their buoyancy: (1) Negatively floating flocs caused by interactions of hydrophilic minerals and low-viscosity oils (kaolinite and quartz). The minerals stabilize oil droplets in a water-continuous phase (oil dissipated in water), and (2) positively floating flocs consist of the mineral stabilized oil droplets as well as calcite in an oil-continuous phase (water dissipated in oil) [7, 53]. Steps of OSAs formation are as followed: breaking the oil film into small oil droplets constitutes the first step in such process, which through the outside disturbing forces created by flow fields like inertia or viscous forces and inner restoring forces of the oil like the interfacial tension to can maintain the oil droplet form [7]. For this step, the particular effective variables include the mixing energy, oil viscosity, and interfacial tension between oil and seawater. In the second step, the interaction of SPM and polar compounds in oil droplets leads to OSAs formation. This step can affect by factors like saltiness (or ion power), sort and concentration of oil and sort, and concentration of sediment particle [7]. Figure 2 presents the schematic presentation of the interaction between oil and marine sediment.

Hassanshahian et al. [54] studied the reactions of the natural microbial community in polluted and unpolluted sediments of the Persian Gulf and the Caspian Sea through microcosm experiments. Their results have shown the pollution of crude-oil hydrocarbons maintained a diversity of effects on microbial activity in sediment belongs to the Persian Gulf and Caspian. The rate of Persian Gulf contamination is more rapid than the Caspian Sea. These marine ecosystems from Iran have the ability for bioremediation as well as several bioremediation strategies based on molecular and enzymatic conditions of these two ecosystems need to be selected in the future.

Marine crude oil-degrading bacteria isolated from the Persian Gulf

Crude oil-utilizing bacteria are distributed in marine environments. Many researchers isolated these bacteria in different oceans in the world. Some of these bacteria solely degraded crude oil and were known as Hydrocarbonoclastic bacteria (HCB). The term of Hydrocarbonclastic means eating, breaking, and decrease of hydrocarbon molecules. Diazotrophic bacteria that are scarcely capable of degrading hydrocarbon are called Hydrocarbonoclasticus. Such bacteria would be ideal for bioremediation. The marine hydrocarbon-degrading bacteria namely the genera: *Alcanivorax*, *Cycloclasticus*, *Oleispira*, *Oleiphilus*, and *Thalassolituus* can only inhabit on hydrocarbons in marine ecosystems [55].

Several investigations in the past two decades have been accomplished on non-symbiotic hydrocarbonoclasticus bacteria and their function in its self-cleaning in the Persian Gulf [56–59].

Picocyanobacteria exist in abundance in surface layers of the whole saltwater bodies such as the Persian Gulf and are associated with hydrocarbonoclasticus bacteria. Such communities are effectively involved in the bioremediation of oil spills. The initial hydrocarbon-utilizing bacteria were isolated nearly one century ago. In recent years, surveys revealed that 14 algal genera, 103 fungal genera, 79 bacterial genera, and nine Cyanobacterial genera demonstrate the potential of degrading and transform hydrocarbons [58, 59]. In anaerobic, oil-degrading sulfate-reducing bacteria carry out this role. To discover genetic, biochemistry basis, and arrangement of hydrocarbon-utilizing pathways some oil-utilizing bacteria have been applied as a model [60]. Such models (e.g. *Alcanivorax borkumensis*) degrade hydrocarbon during lack of nutrients like nitrogen and phosphorus. Various hydrocarbon-utilizing bacteria have been isolated from the Persian Gulf which Proteobacteria, for instance, Gamaproteobacterial genera (e.g. *Acinetobacter*, *Marinobacter*, and *Alcanivorax*) and Alphaproteobacterial genera (e.g. *Tissrella* and *Zavarzina*) and also Actinobacteria are the dominant cultivable marine bacteria [61–64].

Marine ecosystems all around the world such as the Persian Gulf are considered as appropriate habitat for native hydrocarbon-degrading bacteria with the potential of stick to animate and inanimate substrates and biofilms formation [65–67]. These bacteria belong to the genera *Marinobacter, Microbacterium, Rhodococcus, Kocuria, Alcanivorax, Microbacterium, Pseudomonas, Pseudoalteromonas, Dietzia* and some others which approximately all of them are found in the aerobic zone of marine sediments [56, 57, 68–70, 86].

In the past decade, various hydrocarbon-utilizing bacteria, for example, *Thalassolituus* spp., *Oleiphilus* spp., *Oleispira* spp., *Cycloclasticus* spp., *Alcanivorax* spp. and some genera belong to *Planomicrobium* have been isolated formerly known as Planococcus [55, 71]. Among the above-mentioned cases, *Thalassolituus* spp., *Oleiphilus* spp.,

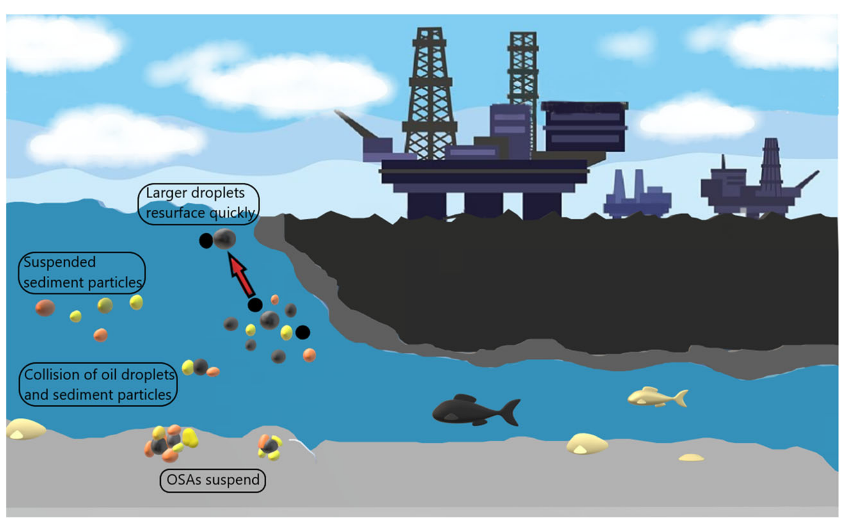


Fig. 2 The interaction between crude oil and marine sediments

Oleispira spp., *Alcanivorax* spp. possess the potential of degrading saturated hydrocarbons with straight-chain and or branched-chain whereas *Cycloclasticus* spp. degrade a variety of polycyclic aromatic hydrocarbons [21]. The alkaliphilic oil-utilizing bacteria include *Marinobacter, Citricoccus, Oceanobacillus, Micrococcus, Bacillus* and *Dietzia* and also the halophilic oil-degrading bacteria comprise *Microbacterium, Cellulomonas, Stappia, Marinobacter, Isoptericola, Bacillus*, and *Georgia* [72].

Such alkaliphilic and halophilic bacteria were isolated from the Persian Gulf and then recognized by their 16S ribonucleic acid sequences. Most of these bacteria are capable of developing an extensive range of aromatic compounds and pure n-alkanes. Quantitative Gas-Liquid Chromatographic analysis (GLC) indicated that individual isolated bacteria may attenuate petroleum and represent pure hydrocarbons in the culture medium [72]. In a review of marine sediments, incubation of sediments in the presence of phenanthrene and bromodeoxyuridine (BDU) followed by an analysis of BDU-labelled DNA determined considerable variety of PAH-degrading bacteria belong to the genera *Bacteroides*, *Shewanella*, *Pseudomonas*, *Methylomonas*, *Exiguobacterium* and also Deltaproteobacteria and Gammaproteobacteria that were nearly unaffiliated to cultivated organisms [73].

In the same way, stable-isotope probing (SIP) of DNA was applied to determine the involvement of a novel clade of Rhodobacteraceae in biodegradation of low molecular weight (LMW) PAHs in marine algal bloom [74]. In the course of investigation in the Persian Gulf reported slurry and microbial mat samples rich in filamentous Cyanobacteria, Picocyanobacteria, and cultivable oil-degrading bacteria, according to their 16S rRNA gene sequences were associated to *Marinobacter hydrocarbonoclasticus, Halomonas aquamarina, Marinobacter* sp.; *Dietzia maris* and *Alcanivorax* sp. [75, 76].

These bacteria are diazotrophic and consume a high variety of individual aliphatic and aromatic hydrocarbons. In a report illustrated *Halomonas* genetically close to *Marinobacter* [77] besides the bacteria referred above, certain uncultivable bacteria can also degrade crude oil. The predominant *Halomonas* bacteria couldn't grow on all the aliphatic and aromatic sectors unlike other cultivable utilizing-oil bacteria in pristine slurry samples. Heretofore, various hydrocarbon-degrading bacteria namely *Pseudomonas, Nocardia, Vibrio, Acinetobacter, Achromobacter, Alcanivorax, Marinobacter, Sphingomonas, Micrococcus* and MS1 (Halomonas) have been caught from Kish Island in Iran [78]. The genus *Halomonas*, includes more than 20 species, are among the larger moderate halophilic bacterial groups with biodegradation potential of hydrocarbon pollutants for the first time proposed by Vreeland. The diverse arrangement of alkane hydroxylase systems in *Acinetobacter* spp., that generally isolates from oil-polluted marine environments enables them to metabolize both long and short-chain alkanes. Proverbially, the consumption of C_{32} and C_{36} n-alkanes by *Acinetobacter* strain DSM 17874 is due to a flavin-binding monooxygenase (AlmA). Also, this gene has been observed in *Alcanivorax dieselolei* B-5 which is caused by long-chain *n*-alkanes of C_{22} - C_{36} [79].

The genus of *Alcanivorax* can degrade branched and straight-alkanes. The species of *Alcanivorax borkumensis* despite the lack catabolic versatility, it has several alkane-catabolism pathways with key enzymes like alkane hydroxylases (a non-heam diiron monooxygenase, AlkB1, and AlkB2) and three cytochrome P450-associated alkane monooxygenases and almost exclusively utilizes alkanes as carbon and energy sources [80]. This bacterium has been adapted for the availability of oil through the synthesis of emulsifiers and producing of biofilm as well as for survival in distinct marine ecosystems (e.g. scavenging nutrients and ultraviolet resistance). Similarly, in oil-spill bioremediation experiments carried out in laboratory microcosms and in the field, 16S ribosomal RNA (rRNA)-gene sequences from *Alcanivorax* spp. were undetectable in control experiments in which samples were untreated with oil, but within 2 weeks of oil treatment, they consisted of more than 30% of the sequences in libraries of 16S-rRNA gene clones constructed from oil-treated sediments and more than 70% of the sequences recovered from sediment treated with oil and inorganic nutrients [81].

These results were mirrored by the detection of *alkB* genes, which encode the catalytic component of alkane hydroxylase, only in samples in which *Alcanivorax* spp. 16S-rRNA genes were abundant [21]. It is traditionally believed that these organisms are normally present in very small numbers, and utilizing of the hydrocarbons as a carbon and energy source causes their growth and reproduction rapidly. *Alcanivorax*-like bacteria have directly been detected in oil-impacted environments across the globe. They have been isolated or detected in culture-independent bacterial community surveys from the United States, Germany, the United Kingdom, Spain, Italy, Singapore, China, the West Philippines, Japan, the Mid-Atlantic Ridge near Antarctica, and from deep-sea sediments from the eastern Pacific Ocean [36, 81, 82]. Presumably, the importance of *Alcanivorax* in contrast to other hydrocarbon-degrading bacteria is due to its capability to branched-chain alkanes degradation efficiently. Because of Pristine entering the sea through various sources such as oil spills and marine plankton, *Alcanivorax* with the potential of consumption these hydrocarbons maintain extensive distribution. There is a similar status for *Cycloclasticus* spp., which carry out a worldwide and significant role in biodegradation through the consumption of oil spilled aromatic hydrocarbons in marine environments [21].

A study realized that *Cycloclasticus* increase by amendment of oil-contaminated gravel with inorganic nutrients. Likewise, culture-based researches have indicated an abundance of *Cycloclasticus* than other polycyclic aromatic hydrocarbon utilizing bacteria (PAHs) in the Gulf of Mexico and Puget Sound (United States), particularly in oily sites. In addition to *Cycloclasticus* that regarded as the most dominant marine PAH-degrading microbe, the genera of *Vibrio, Pseudoalteromonas, Marinomonas, marinobacter, Cycloclasticus* and *Halomonas* isolated from San Diego Bay sediments also can grow on chrysene or phenanthrene [83].

Hassanshahian et al. [58] isolated different petroleum utilizing bacteria from the Persian Gulf. They isolate 25 petroleum utilizing bacteria from the Persian Gulf and the Caspian Sea sediments in their research. The molecular recognition confirmed that these degrading bacteria belong to these genera: *Acinetobacter, Cobetia, Gordonia, Rhodococcus, Pseudomonas, Halomonas, Microbacterium, Marinobacter,* and *Alcanivorax*. Their results confirmed that petroleum degrading bacteria in Iran are capable of biodegradation at a high level. Using such bacteria for bioremediation goals can easily reduce the rate of oil contamination in the Persian Gulf and the Caspian Sea.

Hassanshahian et al. [8–10] screened fifteen petroleums utilizing bacteria from oil-polluted sites in the Persian Gulf at Khorramshahr provenance. One strain that shows high crude oil degradation belongs to the *Corynebacterium variable*. Their results indicate that effective petroleum degrading bacteria exist in the Persian Gulf. Besides, they found that petroleum biodegradation by *C. variable* strain PG-Z maintains a direct relationship with the production of biosurfactant. The Table 1 shows the crude oil-degrading bacteria isolated from the Persian Gulf.

The marine cyanobacteria of the Persian Gulf and their importance in biodegradation

Cyanobacteria represent ancient photosynthetic prokaryotes, autotroph, anaerobic and often mobilizing, which also known as turquoise bacteria and cyanophytes. The effect on biodegradation of petroleum hydrocarbons considers as the most remarkable function of cyanobacteria. These organisms are both involved in hydrocarbon oxidation and in limiting the growth of oxygen-dependent oil-degrading bacteria [15, 87, 88]. In the intertidal zone, cyanobacteria colonized in the presence of oil and started growing on top of the oiled sediments [89]. Increasing the amount of cyanobacteria is offered as the first step of bioremediation [89]. The growth of cyanobacteria is limited through grazing pressure by benthic animals and where bioturbation caused by crabs and polychaetes leads to destabilization of sediment surface but after the resettlement of sediments their growth resumed and a deep layer was created [30, 90].

Such layers inhibit oil degradation and resettlement by macro-fauna through the attachment of sediments and producing an anaerobic environment. On the other hand, these mats carry out an indirect and significant role in biodegradation which amplifies the rate of activity and growth of aerobic oil-degrading bacteria. In a study, was examined the ability of ten non-axenic typical mat-forming cyanobacterial strains to attenuate dibenzothiophene, n-octadecane, pristane and phenanthrene. Five strains (*Aphanothece halophyletica, Dactyolococcopsis salina, Halothece* strain EPUS, *Oscillatoria* strain OSC, and Synechocystis strain UNIGA) had the potential to degrade n-alkanes. Whereas in the other five strains (*Microcoleus chthonoplastes, Oscillatoria* sp. MPI 95 OS 01, *Halothece* strain EPUG, *Halomicronema excentric*, and *Phormidium* strain UNITF) were not observed significant results [91].

Cyanobacterial mats that isolated from a bloom of *Phormidium* spp. and *Oscillatoria* spp. In oily sabkhas along the Gulf of Suez coasts (Africa) and the pristine solar lake (Sinai) indicated effective petroleum degradation in the light. Cyanobacteria in the light contribute to biodegradation of hydrocarbons by the production of oxygen for the aerobic heterotrophic bacteria such as *Marinobacter*, and in lack of light (anaerobic sulfide-rich habitat) with affecting on sulfate-degrading bacteria. Two picocyanobacterial strains related to *Acaryochloris*, isolated from the north and south shore of Kuwait, in three meters below the water surface. Both strains were ultrastructurally, morphologically and phylogenetically (to a lesser extend) similar to *Acaryochloris*. However, in both strains, chlorophyll a is the particular photosynthetic pigment and missed chlorophyll d.

Both picocyanobacterial isolates were related to oil-degrading bacteria in the magnitude of 10^5 cells g^{-1}. Their 16S rRNA gene sequences indicated that related bacteria isolated from the north were associated with *Paenibacillus* sp., *Bacillus pumilus*, and Marinobacter aquaeolei, but those related that isolated from the south were associated to Bacillus asahii and Alcanivorax jadensis. These bacterial diversities occur presumably because of environmental variations [92].

The oil-degrading yeasts and fungi at the Persian Gulf

Fungi can adapt and resist extreme environments. Fungi are considered to remain notable factors in the process of bioremediation. The process of remediation by fungi is preferred to approaches microbial degradation due to their potential to cultivate large groups of substrates. Fungi nonspecifically action in PAHs degradation and can hydroxylate numerous xenobiotic. The process of biodegradation occurs more gradually by fungi in contrast to bacteria [93]. Also, the ability to the consumption of PAHs like Benzo[α] pyrene and biosurfactant production increases the importance of these organisms. Among oil hydrocarbons-degrading, fungi can imply to

Table 1 The crude oil degrading bacteria that isolated from the Persian Gulf

Bacteria name	Isolation source	Reference
	Bacteria	
Alcanivorax	Persian Gulf	Rosenberg et al. 2006
Isoptericola	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Cellulomonas	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Bacillus	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Oceanobacillus	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Citricoccus	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Stappia	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Georgenia	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Marinobacter	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Micrococcus	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Microbacterium	Kuwait,Persian Gulf	Al-Awadhi et al. [57]
Psychrobacter	Kuwait,Persian Gulf	Radwan et al. [84]
Vibrio	Kuwait,Persian Gulf	Radwan et al. [84]
Planococcus	Kuwait,Persian Gulf	Radwan et al. [84]
Pseudomonas	Kuwait,Persian Gulf	Radwan et al. [84]
Actinobacterium	Kuwait,Persian Gulf	Radwan et al. [84]
Dietzia maris	Persian Gulf	Al-Mailem et al. [61, 85]
Halomonas aquamorina	Persian Gulf	Al-Mailem et al. [61, 85]
Gordonia bronchialis	Persian Gulf	Al-Mailem et al. [61, 85]
Zavarzinia	Persian Gulf	Al-Mailem et al. [61, 85]
Flavobacterium	Persian Gulf	Al-Mailem et al. [61, 85]
Gaetbulibacter	Persian Gulf	Al-Mailem et al. [61, 85]
Owenweeksia	Persian Gulf	Al-Mailem et al. [61, 85]
Tistrella	Persian Gulf	Al-Mailem et al. [61, 85]
Nocardia	Kish Island, Iran	Hassanshahian et al. [86]
Achromobacter	Kish Island, Iran	Hassanshahian et al. [86]
Sphingomonas	Kish Island, Iran	Hassanshahian et al. [86]
Halomonas sp. MS1	Kish Island, Iran	Hassanshahian et al. [86]

Aspergillus, Penicillium, Fusarium, Amorphotheca, Neosartoria, Paecilomyces, Talaromyces, Graphium Cunninghamella [94]. Such function can also observe in yeasts such as *Candida, Clavispora, Debariomyces, Sporobolomyces, Leucosporidium, Lodderomyces, Rhodosporidium, Rhodotorul. Esporidiobolus, Trichosporium.*

Fungal extracellular enzymes penetration into the contaminated soils is known as one of the removing pollutant approaches [95]. For fungi, the function of degrading enzymes depends on agents such as accessibility of the nutrient, oxygen, pH, temperature, and chemical structure [96]. In the past few years, ligninolytic fungi have been extremely investigated for their ability to degradation, hydrocarbons mineralization, and irregular structure of lignin [97]. Major extracellular enzymes in the lignin system containing lignin peroxidases, manganese peroxidases, phenol oxidases involve in the PAHs biodegradation. Manganese peroxidases oxidize PAHs using other enzymes whereas lignin peroxidases directly lead to PAHs oxidation [98].

In addition to the mentioned cases, fungi's enzymes like epoxide hydrolases, proteases, monooxygenases, dioxygenases, and lipases are capable of PAHs degradation [99]. An experiment by Novotny et al. proved the importance of MnP and laccase in biodegradation some compounds such as pyrene and anthracene by *Tramtes versicular, Pleurotus ostereatus and Phanerachaeta chrisosporium.* Studies on *Aspergillus niger, A. ochraceus* and *Penicillium chrisogenum* isolated from the coasts of Oman (Persian Gulf) by Snellman et al. [100] revealed significant differences between these species in the consumption of C_{15}, C_{16}, C_{17}, and C_{18} and also in magnitude of produced biomass on C_{13}, C_{17}, C_{18}, and crude oil. Statistically the biomass and petroleum degradation coefficient for these species is as follows the correlation coefficient of biomass and oil utilization for these species is as follows: *A. niger* > *A. terreus* > *P. chrysogenum* [101].

Hassanshahian et al. [59] studied petroleum degrading yeast from the Persian Gulf. They isolated six degrading yeast from the petroleum polluted region at the Persian Gulf and after screening analysis two strains were selected because of show high oil degradation. These two strains belong to *Yarrowia lipolytica*. Their results confirmed there was a relationship was between both the cell the yeast strains hydrophobicity and their emulsification and petroleum degradation and a reduction in surface tension. Ultimately, we concluded that these 6 strains could be useful in the Persian Gulf bioremediation cycle and the reduction of petroleum contamination in this aquatic environment.

Isolation of oil-degrading bacteria from marine organisms at the Persian Gulf

Various marine organisms including aquatic fauna, blue-green algae [84, 89], epilithic algal biomass [102] contain the most levels of hydrocarbon-degrading bacteria. In studies on 10 species of fish isolated from the Persian Gulf and two species from Fish, farms confirmed that millions of hydrocarbon-degrading bacteria exist per square centimeter of Fish surface and per gram of fish, that these bacteria belong to *Psychrobacter*, *Vibrio*, *Planococcus*, *Pseudomonas* and *Actinobacterium* [103]. Actinobacteria have recently been thought to be endemic to the marine ecosystem and their existence is due to be washed into the sea from nearby territories [103]. Although, Bull et al. [104] find Actinobacteria (Rhodococcus and Dietzia), and Austin [105] finds Microbacterium to be marine microflora indigenous.

These three bacteria, which are Fish-associated, could degrade petroleum [106–108]. In addition to fish isolates, planktonic and benthic microflora also accommodated oil-utilizing bacteria. Phytoplankton provides a better-aerated environment for bacteria in contrast to fishes consequently, bacterial diversity of phytoplanktonic and fish samples differs because of various environmental conditions. Whole samples could grow on many aliphatic and aromatic hydrocarbons as single sources of carbon and energy [103].

Corals, one of the bacterial hosts, harbor fewer bacteria than marine plants and animals. In an experiment by [109], determined isolated coral samples from the petroleum-contaminated beaches of Qaro and Umm Al-Maradim Islands contained oil-utilizing bacteria. Oil-degrading bacteria in produced mucose by *P. compressa* and *A. clathrata* are more abundant than tissue samples because of their adhesion properties [109]. Grossart [110, 111] expressed that *Thalassiosira rotula* (diatom), and a solitary copepod can accommodate 10^8 and 10^9 hydrocarbons-degrading bacteria, respectively [112].

Mussels, oysters, and clams (filter-feeding bivalves) remain extremely significant components of the marine ecosystem bioremediation cycle. They are capable of collecting and storing a significant number of small particulate matter such as phytoplankton, zooplankton, microorganisms, and other organic particulate material. Otherwise, organic components in suspended cases may be snared and used by filter-feeding bivalves. Suspended matters can be trapped and applied by filter-feeding bivalves.

Bayat et al. [113, 114] analyzed relationships between mussels and bacteria in oil-utilizing aquatic ecosystems. They screen petroleum-utilizing bacteria from numbers of mussels that are isolated from hydrocarbon-polluted zones of Qeshm island in the Persian Gulf. 28 petroleum-utilizing bacteria were collected from three mussels isolated from the hydrocarbon-polluted region at Qeshm Island. According to more growth and degradation of petroleum, four samples were selected between these twenty-eight strains for research. These strains were *Shewanella algae*, *Micrococcus luteus*, *Pseudoalteromonas* sp., and *Shewanella haliotis*.

In another study, Bayat et al. [115] research on symbiosis relationship between petroleum-utilizing bacteria and *Mactra stultorum* (mussel). They select bivalves according to their importance for estuarine and coastal communities. Bivalves filter vast quantities of water to satisfy their food needs and collect dissolved oil components and hydrocarbon-containing particles found in hydrocarbon-contaminated water regions. Bivalves can be filtered and concentrate bacteria may lead to raising the concentration of bacteria in the marine environment. In the mussel samples, bacterial concentrations were more elevated than the seawater during the year, thereby carry out a critical role in the marine environment's bioremediation cycle. They succeeded to isolate *Alcanivorax dieselolei* and *Idiomarina baltica* from this mussel at the Persian Gulf.

Isolation of crude-oil degrading microorganisms from a harsh environment

A harsh environment can universally be considered as a setting in which an organism's survival is difficult or impossible. A harsh environment called the Persian Gulf, Owing to temperatures of up to 50 °C and high evaporation, the water salinity can exceed 16%. Various microbial mat systems coat the coastal flats of the Gulf [116], which These systems undergo varying environmental conditions daily from mild to an extreme due to tidal conditions [117].

Hypersaline offshore areas in Kuwait are considered the super tidal "Sabkhas", where the rate of degradation hydrocarbon and growth of extremely halophilic and oil-degrading bacteria and archaea [61, 62, 118] increase using special modifications including salts (K^+ and Mg^{2+}) and pure vitamins [61, 62]. The variations in environmental conditions can lead to diversification of microorganisms. For instance, the differences in the Denaturing Gradient Gel Electrophoresis (DGGE) profiles at various tidal positions demonstrate that the desiccation effect on the mats bacterial composition is different from their wetting effect [91].

Harsh environmental conditions may be considered as a natural obstacle to hydrocarbon degradation. Proverbially, enhancing salinity more than 15% prevents hydrocarbon biodegradation. The cyanobacterium *Microcoleus chthonoplastes* isolated from hypersaline areas in the Persian Gulf [119] have been indicated that able to survive even at salinities 12% in culture media [120]. Also, a study on *Deinococcus* showed the presence of microbial communities that are UV resistant [121].

In a work, oil-utilizing bacterium *Bacillus licheniformis* collected from Dagang oilfield that would be ideal for biosurfactant production, indicated resistance to high salinity and temperature [122]. Hambrick et al. [123] and Peyton et al. [124] have been investigated the biodegradation of hydrocarbon in alkaline environments. Sarnaik and Kanekar [125], Maltseva et al. [126], Maltseva and Oriel [127], Kanekar et al. [128] and Yumoto et al. [129, 130] showed alkaliphilic hydrocarbon-utilizing bacteria are capable of biodegradation of organic compounds. In an experiment on two diazotrophic, halophilic and hydrocarbonoclasics bacteria, *Marinobacter sedimentarum* and *M. flavimarsis,* isolated from shores of Kuwait were found these strains didn't survive without NaCl and illustrated the rate of the highest growth and hydrocarbon degradation at 5 M NaCl concentration. Further, these strains mineralized crude oil in hypersaline media in the absence of any azote (N) additive. Among halophilic microorganisms that are capable to degrade many types of hydrocarbons at high salinities can be mentioned to bacteria such as *Marinobacter sedimentalis, Halomonas salina* and *Pseudomonas* sp., Archaea like *Halobacterium salinarum* and *Haloferax larsenii* and also fungi [131].

Fakhrzadegan et al. [132] studied petroleum-utilizing bacteria in mangrove forests at the Persian Gulf. Mangroves forests consider a harsh marine environment because these forests are environments discovered in tropical and subtropical areas all over the world. There are these forests in the regions between terrestrial and aquatic environments; because of their geographical distribution, these ecosystems have been seen in the Americas, Africa, Asia, and Oceania. Mangroves are remarkably resistant to harsh conditions such as low nutrient and oxygen levels and uncommon temperatures.

These forests are placed along the Iranian shores and around Bahrain, Qatar, Saudi Arabia, and the United Arab Emirates. Mangrove forests in Iran are filled with two species of trees: *Avicennia marina* and *Rhizophora mucronata*. *A. marina* represents the most abundant species, that covering more than 90% of the mangrove ecosystems of the Oman Sea and the Persian Gulf. The production of *R. mucronata* is restricted and principally confined to the rivers of the Syriac region (including Hara and gas creeks).

They concluded that these forests are the most susceptible marine ecosystem against hydrocarbon pollution, and therefore preservation of these habitats is significant for protecting the marine animal and plant species. Researches confirmed that in this Persian Gulf ecosystem petroleum-using bacteria have ample diversity and density. These bacteria can reduce petroleum contamination in this significant marine environment by using some strategies such as biostimulation and bioaugmentation. They characterized these genera as petroleum-utilizing bacteria in the mangroves around the Persian Gulf: *Vibrio sp, Idiomarina sp, Kangiella sp, Marinobacter, Halomonas sp,* and *Vibrio sp.*

Biosurfactant and their role in biodegradation

Biosurfactants are amphiphilic and active compounds with biodegradation ability, good adaptation to the environment, higher foaming, lower toxicity, high selectivity, stability and specificity at excessive temperatures, pH and salinities, which are produced by microorganisms including bacteria, fungi, and yeasts [133]. 3 analyses namely Using nuclear magnetic resonance spectroscopy (NMR), X-ray diffraction (XRD), and thermal gravimetric (TG) respectively, indicated the functional groups, surface nature, and biosurfactant thermos-stability [134].

The produced biosurfactant by these microorganisms either binds to the cell surface or is sending to extracellular in the growing culture media. Such compounds are employed in food, pharmaceutical [135, 136] and cosmetic industries, also increasing spilled oil and aromatic hydrocarbons biodegradation by microorganisms [137]. Glycolipids consist of rhamnolipid, trehalose, and sophorolipids, also lipopeptides including Surfactin, Gramicidin S, and Polymyxin represent extensive groups of low molecular-weight biosurfactant [138–140].

Substantially, rhamnolipid leads to increasing hydrocarbon bioavailability and its degradation. But according to a report rhamnolipid inhibited only *Sphingomonas* sp. In pure culture, whereas limited phenanthrene degradation by a group of 2 species bacteria namely *Sphingomonas* and *Paenibacillus* sp. [141]. Stress enhanced due to the solubilized phenanthrene, or the rhamnolipid and phenanthrene together [1]. High molecular weight EPS is a heterogeneous polymer consist of polysaccharides, proteins, lipopolysaccharides, lipoprotein or such biopolymers 'complex mixtures which can serve a role corresponded to biosurfactants. Biosurfactant production is responsible for enhancing the bioavailability of PAHs. Despite the inhibition of specific microbes, biosurfactants are benefiting others through increasing the hydrophobic compound bioavailability and consequently recognizing as "common goods" [142].

Biosurfactants could act as antagonists and are known in many pathogens as virulence factors. They alter the hydrophobic property of surface cells. The hydrophobic substrates surface via diminishing the culture surface tension enhances by biosurfactant results to increase bioavailability. Hydrocarbon-degrading bacteria including *Bacillus licheniformis* isolated from Dagang oilfield [122], *Rhodococcus erythropolis* M-25 and *Alcanivorax borkumensis* are capable of biosurfactant production [143].

In an investigation on hydrocarbon-degrading bacterium with the potential of biosurfactant production, *Bacillus methylotrophicus* USTBa, observed that produced biosurfactant by this strain could drastically reduce the water surface tension [134]. It displayed a 90% emulsification activity on petroleum and had caused antibiotic activity inhibition of many bacteria even though it is not an inhibitor for various vegetables [134].

Pseudomonas aeruginosa sp. ZN, biosurfactant producing bacterium with high-ability, was selected from oil-contaminated soils at Ahvaz City (southern of Iran). The synthesis of biosurfactant in BH2 culture medium modified with 1% n-hexadecane occurred in the course of the exponential process causing a decrease in surface tension. This biosurfactant possesses distinct properties to other *Pseudomonas* strains. The created biosurfactant was unable to separate stable emulsion of span-80-kerosene: Tween80-distilled water within 1 day. The created biosurfactants could be enhancing the bacterial cell hydrophobicity [144]. In terms of Chirwa and Bezza, experiments occur an enhancing in the rate of hydrocarbon biodegradation when the biosurfactant was present. Investigation of a group of eight distinct species isolated from the French beach by [145] determined that biosurfactant only using single pure strain didn't exhibit emulsification petroleum and rapid hydrocarbon degradation required the whole bacterial communities. The Schematic representation of biosurfactant function on enhancing crude-oil degradation was shown in Fig. 3.

Hassanshahian et al. [8, 9] reported some of the bacteria that produce biosurfactants from the Persian Gulf. He used some screening techniques to select the best bacteria in terms of biosurfactant production from samples that were isolated from petroleum-contaminated areas of the Persian Gulf (coastline of Bushehr provenance). The genera that he named as best biosurfactant producers were *Shewanella alga, Shewanella upenei, Vibrio furnissii, Gallaecimonas pentaromativorans, Brevibacterium epidermidis, Psychrobacter namhaensis,* and *Pseudomonas fluorescens* bacteria.

Factors influencing the degradation of crude oil in marine environments

Several agents including temperature, oxygen, pH, and nutrients can dramatically be useful on microbial activity that is described as follows. Also in Fig. 4 as schematic representation, these factors were shown.

Temperature

Temperature results in alteration of the physical and chemical nature of oily compounds [94]. At low temperature, due to reducing the enzymatic activities notably decrease the degradation rate [146]. The maximum biodegradation in the different ecosystems (land, sea, and freshwater) happened at the range of 30–40°C, 20–30°C and 15–20°C [94] Development of metabolic capabilities through genetic modifications, infusion of different enzymes and eclectic enrichment of microorganisms can successfully lead to biodegradation. Reportedly, The degradation of Metula crude oil using mixed marine bacterial cultures is likely at 30°C [94]. Also, Hassanshahian et al. [147] expressed that the crude-oil degraded in the soil at 30°C. Also, the degradation of hydrocarbon in sediments is restricted at low temperatures in winter [94].

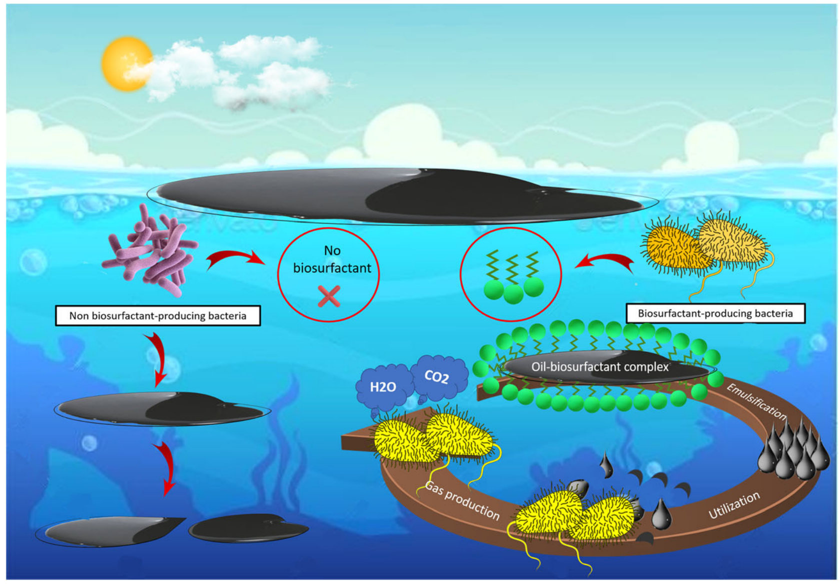
Oxygen

Hydroxylases (oxygenases), key enzymes in the biodegradation process, via oxygen production and oxidation of hydrocarbon substrates that are dependent on molecular oxygen for microbial mats involved in the decomposition of all hydrocarbons [148–150]. The rates of oxygen consumption by microorganisms, soil type, and the usable substrate presence affect the concentration of oxygen in the soil. In the molecular oxygen absence, alkyl-substituted aromatics and non-substituted metabolizing to benzene, 1,3-dimethyl benzene, and acenaphthene, naphthalene, toluene and xylene by soil microbial consortia [151]. In anaerobic conditions, hydrocarbons biodegradation is slower compared with aerobic conditions [152].

pH

The rate of pH can be extremely variable. The environmental pH change alters enzyme activities, Transport of cell membranes, and balancing catalytic reactions. Heterotrophic fungi and bacteria prefer the natural to alkaline acidity (in comparison with pH of the other aquatic environments) and fungi are more resistant than bacteria in acidic conditions. The rate of soil pH is 2.5–11 in alkaline lands [94]. The pH rate matters in improving biological treatment methods. The theory of biological treatment is to remove toxins and contaminants from enclosed sites by consuming

Fig. 3 The effect of biosurfactant on crude oil biodegradation

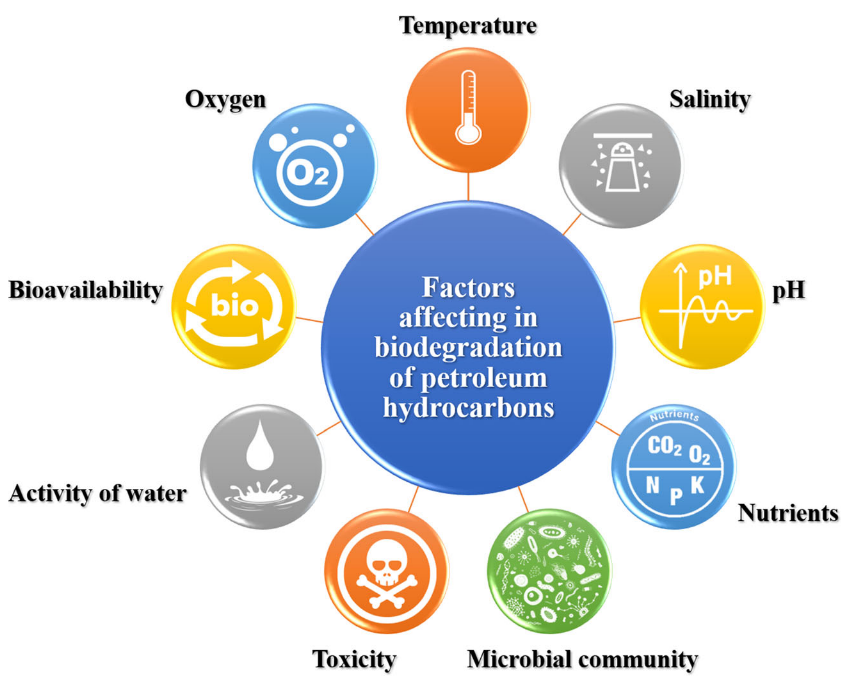


microorganisms. Naphthalene and octadecane are mineralized by microorganisms at a pH of 6.5 [94]. It equally found that increasing in pH from 6.5 to 8.0 did not affect the mineralization rate of naphthalene whereas this parameter notably increased in octadecane [94]. Thavasi et al. [153] reported pH 8.0 was the most appropriate acidity for the highest petroleum biodegradation in water by *Pseudomonas aeruginosa*. Because of Pawar [154], the most suitable for pHs biodegradation was at pH 7.5. *Burkholderia cocovenenas* bacterium isolated from contaminated soils ranging from pH 6.5–7.0 shown the maximum rate of phenanthrene degradation in liquid media (Hassanshahian et al., 2014c).

The most effective nutrients in biodegradation include nitrogen, phosphorus, and iron (Al − Hawash et al. 2018). Furthermore, certain nutrients may become a limiting biodegradation factor. A remarkable enhance of carbon and reduction of phosphorus and nitrogen caused by oil spills can be effective in the biodegradation process. In wetlands, the biodegradation by microorganisms requires adding nutrients since nutrient consumption by plants. In other words, nutrient accumulation can limit the biodegradation (Al − Hawash et al. 2018).

Also, the abundance of PAHs limits the growth of microorganisms that developed a response to hydrocarbons concerning the cell membrane structure, sporulation

Fig. 4 Some factors that affect crude oil biodegradation on marine environment



alterations and pigmentation of mycelia [156]. A study on fungal strains including *Penicillium chrysogenum, Lasiodiplodia, Theobromae,* and *Mucor racemosus* determined that sucrose and cellulose include the best carbon sources for lipase production [99]. The best catalyst of high extent of lipase production in this fungy is yeast extract. Also, the degradation of hydrocarbons by Trichoderma hypocrea was investigated by pyrene as a carbon source [157].

After adding the extract of yeast, lactose or sucrose, the strain grows and degrading of pyrene interestingly increased after 1–2 weeks of incubation [157]. Fish peptides and amino acids can provide nitrogen for oil-utilizing bacteria [103].

Bioavailability

The effect of microbiological, physical and chemical agents on the level and rate of biodegradation defines as "bioavailability".

The bioavailable part of the hydrocarbons in the area that can be access to microorganisms. Hydrophobic organic pollutants such as PHs have low bioavailability and their little water solubility leads to resistance to photolytic breakdown and chemical-biological [158]. Low local microbial biodiversity or the lack of local hydrocarbon-degrading microbes restrict biodegradation and presumably result in incomplete degradation of high molecular weight hydrocarbons [159].

Water activity

The growth and movement of microorganisms directly depend on water availability. Consequently, in terrestrial environments the biodegradation of hydrocarbons restricted since the scarcity of water. Illustrated the biodegradation was maximum when oil sludge saturate with 30–90% water [94].

Biodegradation mechanism of crude oil

The most organic pollutants quickly and completely degrade under aerobic conditions. Instantly, these pollutants take oxide and active states, and oxygen is formed through peroxidases and oxygenases. Peripheral degradation mechanisms of hydrocarbon pollutants include several steps which in the course of are produced intermediates like the tricarboxylic acid cycle [94]. The Key Precursor Metabolites, such as the pyruvate, succinate, and acetyl-CoA lead to the cell biomass biosynthesis.

The Gluconeogenesis process provides the saccharides necessary for growth and biosynthesis. The mechanisms like attachment of microbial cells to substrates and production of biosurfactant and also enzyme systems activities involve in PHs degradation [160]. Selectively metabolizing PHs may be possible both through a particular strain of microorganisms and through a microbial strain-relevant consortium of the same or dissimilar genera [161], but in the consortium occurs more degradation compare with the distinct cultures [162].

Enzymes are included as one of the efficient key factors for PHs degradation. The microbial degradation of many compounds such as chlorinated oil, PAHs and other hydrocarbons happen by cytochrome P450 hydroxylases [163]. Scheller et al. [164] extracted the cytochrome P450 enzymes from *Candida* species such as *Candida apicola, C. maltose,* and *C. tropicalis.* Among alkanes-degrading enzymes under aerobic conditions can be mentioned to alkane oxygenases, including cytochrome P450, membrane-bound copper-containing methane monooxygenases, soluble di-iron methane monooxygenases, and integral membrane di-iron alkane hydroxylase enzymes (e.g., alkB) which are various in prokaryotes and eukaryotes [165]. Aromatic and aliphatic degradation schematic were illustrated in Fig. 5.

The efficiency of biological approaches in crude oil removal

Oil spill takes place annually in the marine environment. Diverse techniques have been creating for crude-oil removal from the environment in which the user of these techniques requires an exact evaluation of several factors particularly, site characteristics, type of contaminant, and environmental conditions. Generally, preliminary phases of emergency plans including oil smudge limiting by afloat obstacles, eliminate pollution using absorbents material and skimmers using nanotechnology. Moreover, if the environmental condition is suitable surfactants can be applied that reduce the pollution damage [166].

The latter operation is performed using products with environmentally friendly properties and low toxicity, which has been confirmed by national authorities. Detergents agents increase the bioavailability to oil via their emulsification and fragmentation. Oil-diluting solvents that make possible elimination with physical strategies and surfactants with the potential of dispersing oil into the water are recognized as two major groups of these compounds [166].

In recent studies, the consequence of these materials has been investigated on the population and dynamic of seawater microorganisms, and bacterial isolated strains [167, 168]. The first reaction of surfactants remains the stimulation of the oil slick from the surface to the water which presumably increases the biodegradation by enhancing in microbial bioavailability [168–170].

The use of dispersant leads to an increase in growth rates and viability of non-hydrocarbon-degrading bacteria like *Vibrio* due to decreasing in the toxic effect of petroleum, whereas these parameters decrease in oil-degrading bacteria

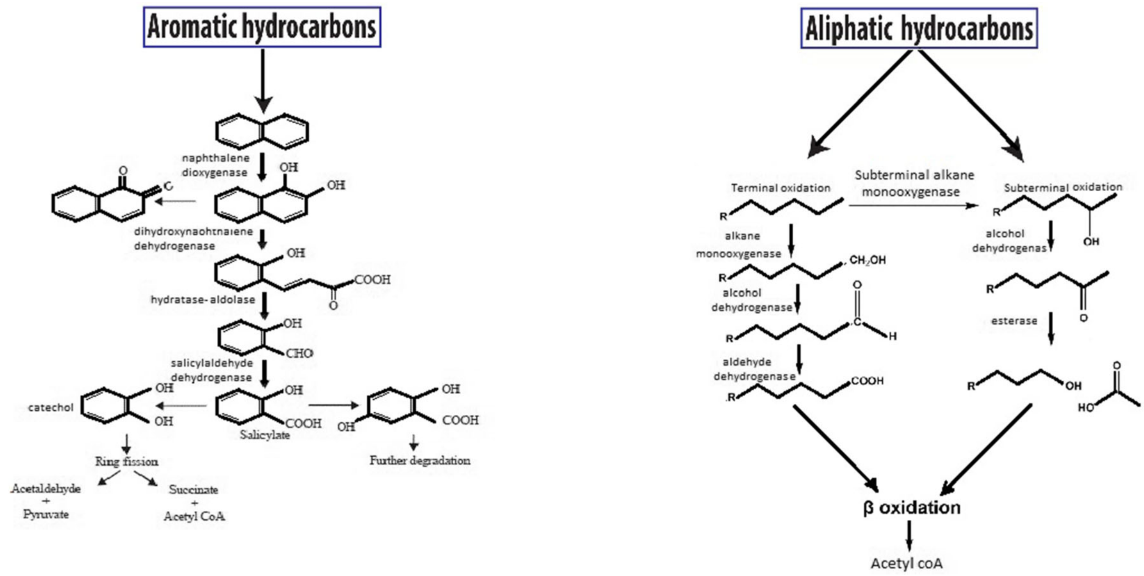


Fig. 5 The metabolic pathway of aromatic and aliphatic hydrocarbon biodegradation present in crude oil

including *Marinobacter acinetobacter* because of diminishing carbon source. Obligate hydrocarbonoclastic bacteria (OHCB) are the major of the representative of hydrocarbon mineralization in the marine environment [70]. In recent years, using OHCB such as *Marinobacter* the concept of "autochthonous bioaugmentation" has been defined for oil bioremediation [171, 172]. Despite the increase in the biodegradation by the chemical dispersants, in an investigation on *Rhodococcus erythropolis* M-25 using the Corexit 9500A another result observed. This surfactant had not any enhancing effect on the degradation of crude oil, on the contrary, it reduced degradation from 60.7% to 32.8% [143].

The chemical dispersants do not solve the problem, but only resulting in oil transformation into a state that can't be easily removed from the environment, so is not suitable economically, ecologically and technically [173, 174]. Consequently, innovative and sound technologies significantly have been regarded for the elimination of contamination. The technique of microbial remediation produces lower secondary pollution compare with the traditional methods and is extensively applied because it has better performance and environmental adaptation [175–184]. But low bioavailability of hydrocarbons to microorganisms limits the effectiveness of this technique. The success in biodegradation strategies related to some factors such as type of degrading microorganisms, bioavailability and optimize the environmental condition. In addition to the cases mentioned above, there are other strategies for bioremediation including bioaugmentation (i.e. inoculation) with exogenous hydrocabonoclastic microorganisms, biostimulation of microorganisms, laser technique and evaporation of oily compounds [85].

The efficiency of bio-stimulation and bio-augmentation strategies on the degradation of crude oil at field scale

Mesocosm is medium-sized and closed laboratory ecosystems that have been widely used as tools in ecological, applied, and developmental research. They have combined environmental management, technology, and live population change control. The mesocosm design can be in the form of tanks, cylinders, circulators, tubes, and so on. The type of material used is varied in structure and can be fiberglass, steel, and so on. The type and size of the reactor used to depend on the type of environment we intend to simulate and also depend on the chemical, physical, and biological processes studied [85].

Mesocosm reflects the development of microcosms. Their relatively massive size allows for more extensive use of ecological complexity. During experiments on mesocosms, it would be possible to allow interactions between chemical, physical, and biological parameters with a control. Mesocosm can largely mimic real-life conditions in aquatic ecosystems and serve as a bridge between the experimental scaling slopes that are difficult to control [185–187].

Numerous studies have shown the potential and power of these systems in mimicking and producing natural processes. Mesocosm systems are designed to examine the dynamics of the microbial community in marine environments, analyze diatom blooms, assess the effects of radioactive radiation, and study the effects of pollution on the ecosystem and so on [56, 57, 117, 188].

Hydrocarbon-degrading microorganisms maintain low distribution in marine environments. Contamination by petroleum hydrocarbons will stimulate the growth of such organisms and lead to changes in the structure of the microbial community in contaminated areas. Identification of key organisms that play a role in biodegradation of hydrocarbons is primary for understanding the evolution and development of biological treatment strategies. For this reason, numerous researches have been done to characterize bacterial communities to identify the responsible degrader and determine the catalytic potential of these degraders. In natural marine environments, nutrients, especially nitrogen and phosphorus, are inadequate to support the microbial needs for growth, especially after the sudden increase in the level of hydrocarbons with oil spills. Therefore, the addition of nitrogen and phosphorus to the contaminated media to stimulate the growth of hydrocarbon-degrading microorganisms results in increased biodegradation of the hydrocarbon pollutant [166].

Bioaugmentation is defined as a method to improve the ability of contaminated sites (soil, marine environments, etc.) to remove contaminants by introducing susceptible bacterial strains into a separate or mixed culture. The basis of this method is based on the increased metabolic capacity of the internal population by foreign microorganisms inoculated leading to a high biodegradation reaction. Bioaugmentation on a mesocosm scale comprises a combination of microbiology, microbial ecology, molecular biology, and bioengineering. Bioaugmentation has remained the subject of many biodegradation studies of crude oil and other pollutants in the last decade [23, 172, 189].

Ruberto et al. [190] designed mesocosm systems to analyze the biodegradation of oil-contaminated soils in the Antarctic region, which is a cold ecosystem. They inoculated the cold-tolerant bacterium *Acinetobacter* strain B22 into contaminated soils in the mesocosm for biological evaluation. They concluded that biodegradation with strain B22 removes 75% of the hydrocarbons from the contaminated soil and also increases the number of indigenous heterotrophic bacteria but reduces soil microbial diversity. They suggested biological evaluation as a way to improve biological treatment.

Maa et al. [191] investigated the bioaugmentation efficiency of an activated sludge system for petrochemical plant effluent analysis. They found that the impact of bioaugmentation of activated sludge on the degradation of toxic petrochemical compounds depends on several factors such as the pollutant's chemical properties and the activity of the bacteria used to evaluate it. There is much evidence in the literature that the best way of bioaugmentation is the use of microorganisms derived from the similar ecological location of the contaminated environment.

Hassanshahian et al. [192] designed three types of mesosomes for biodegradation of oil in the marine environments. Two of these mesosomes had a bioassay and one bio-stimulation. In this section, we discuss the effects of these treatments on the typical microbial community, oil degradation, and comparison of their efficacy with each other. This is the first report on the design of bioaugmentation and biostimulation mesocosm systems for the evaluation of crude-oil biodegradation and ecological effects in marine environments by newly hydrocarbonoclasticus bacteria.

Flavia et al. [193] investigated the effects of oil pollution and biostimulation on soil biodiversity in the microbial community. They used both culture-dependent methods such as enumeration the number of heterotrophic bacteria and a molecular method like PCR-DGGE to perceive this effect. Their results showed that crude-oil contamination increased the number of hydrocarbon degraders in the soil that received the biostimulation treatment. Their DGGE pattern in soil with oil contamination and biostimulation showed biostimulation had a decreasing effect on the diversity of the natural microbial community. The number of phylogenetic groups and bands decreased sharply after 15 days of biostimulation treatment but increased from 15 days to 90 days and the band pattern remained constant until the end of incubation (360 days). They concluded the addition of mineral nutrients to oil-contaminated soil caused a more enormous effect on the bacterial community than on oil-only contaminated soil.

Hassanshahian et al. [192] studied the efficiency of two bioaugmentation mesocosms degradations of oil from marine environments. In the first mesocosm, bioaugmentation was studied by a unique culture of *Alcanivorax* and in the two mesocosms, the bioaugmentation by mixed culture of *Alcanivorax* and *Thalassolituus* bacteria was studied. The results showed that bioaugmentation by single culture was more effective in biodegradation of crude oil (95%) than bioaugmentation with mixed culture (70%). On the other hand, single cultures added to mesocosm caused less effect on indigenous marine microbial communities than mixed cultures, so that a remarkable decrease in biodiversity index and phylogenetic groups was observed in the bioaugmentation with mixed culture. However, biodiversity decreased in both cases, which is consistent with the results of other researchers. Adding food to contaminated sites to stimulate the growth of the indigenous microbial community is referred to as biostimulation. Biostimulation and application of nutrients for biodegradation purposes can be in three forms: (A) adding soluble mineral nutrients like nitrogen and phosphorus (B) adding organic nutrients such as acetate and fumarate (C) fertilizer additives. Slowly releasing minerals such as nitrogen and phosphorus are gradually released. Numerous studies have demonstrated the efficacy of using biostimulation as a method of conserve marine and onshore environments contaminated with oil and other pollutants, but our knowledge of the effects of this process on natural ecosystems is limited [10].

In a study by Hassanshahian et al. [192] the effect of biostimulation was investigated by including nitrogen and phosphorus nutrients on the oil-contaminated marine microbial community at the mesocosm level. The results showed biostimulation reduced biodiversity and reduced the number of DGGE bands by day 10 and then the phylogenetic groups returned to baseline at the terminal the incubation period (day 20) and according to with the results obtained by Flavia et al. [193] that the increase in the quantity of crude oil-degrading bacteria in a mesocosm.

Some researchers have compared the efficacy of two methods of biodegradation, including biostimulation and bioaugmentation, to develop a suitable strategy for removing crude oil from marine environments. Ruberto et al. [194] investigated the effect of adding nitrogen and phosphorus mineral nutrients (biostimulation) and inoculation with degrading bacteria such as *Rhodococcus, Pseudomonas, Sphingomonas* (bioaugmentation with mixed culture) on the removal of oil from contaminated soils (Antarctica). Their results showed that the number of heterotrophic bacteria and degrading bacteria increased and 86% of the oil degraded in the soil. But in the bioaugmentation, there was no significant difference between the soil that was added with the bacterium and the soil that was not bacterial inoculated, with only 56% of the oil degraded in the soil. They conclude that bio-stimulation is more effective than bioaugmentation when the soil has chronic oil pollution.

Hassanshahian et al. [195] evaluated two methods of bioaugmentation with a single bacterium and mixed culture, and compare it with the biostimulation method to degradation of crude oil in marine environments at mesocosm scale. The results of this study showed bioaugmentation with a single bacterium showed the best oil removal and biostimulation method is more efficient than bioaugmentation with a mixed culture, which is consistent with the above-mentioned results. Comparing the effect of these methods on the reduction of marine microbial community diversity, revealed the biostimulation method had the least decreasing effect on the diversity of the marine microbial community and the greatest reduction effect of biodiversity was related to bioaugmentation evaluation with mixed culture [86, 147, 155, 196–202].

Conclusion and future perspective

In this article, we tried collecting all the studies on crude-oil degrading microorganisms in the Persian Gulf. The sum of all these articles is that crude-oil degrading microorganisms, including bacteria, yeast, and fungi, maintain sufficient diversity in the Gulf. However, further studies are required, especially concerning marine organisms and bacteria. By developing field methods and evaluating bioaugmentation and biostimulation methods on a mesocosm scale and then extending it to the field experiment, these bacteria can be used to reduce oil pollution in the Persian Gulf and decrease the harmful effects of oil pollution on marine life in the Gulf. So one of the solutions to conserve the marine environment of the Persian Gulf is to develop biodegradation and bioremediation strategies.

Compliance with ethical standards

Conflicted of interest There is not any conflicted of interest between the authors.

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