#### **REVIEW ARTICLE**



# Bioremediation perspectives and progress in petroleum pollution in the marine environment: a review

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#### Abstract

The marine environment is often affected by petroleum hydrocarbon pollution due to industrial activities and petroleum accidents. This pollution has recalcitrant and persistent compounds that pose a high risk to the ecological system and human health. For this reason, the world claims to seek to clean up these pollutants. Bioremediation is an attractive approach for removing petroleum pollution. It is considered a low-cost and highly effective approach with fewer side effects compared to chemical and physical techniques. This depends on the metabolic capability of microorganisms involved in the degradation of hydrocarbons through enzymatic reactions. Bioremediation activities mostly depend on environmental conditions such as temperature, pH, salinity, pressure, and nutrition availability. Understanding the effects of environmental conditions on microbial hydrocarbon degraders and microbial interactions with hydrocarbon compounds could be assessed for the successful degradation of petroleum pollution. The current review provides a critical view of petroleum pollution in seawater, the bioavailability of petroleum compounds, the contribution of microorganisms in petroleum degradation, and the mechanisms of degradation under aerobic and anaerobic conditions. We consider different biodegradation approaches such as biostimulation, bioaugmentation, and phytoremediation.

**Keywords** Petroleum pollution · Petroleum toxicity · Petroleum biodegradation · Environmental influence · Biodegradation techniques

# Introduction

Petroleum hydrocarbon has become an important source of energy in our life (Devan et al. 2020). Scientists have not yet found an effective alternative to petroleum products as an energy source (Varjani and Upasani 2016). Petroleum is a hydrocarbon mixture containing thousands of compounds with different structures (Nielson et al. 2020). Four categories of petroleum hydrocarbons exist: alkanes, aromatics, resins, and asphaltenes (Figure 1). Alkanes are the major components of petroleum that are the least toxic and easily biodegradable (Chen et al. 2019a, b).

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Polyaromatic hydrocarbon (PAH) compounds are characterized by solid materials because of their high melting and boiling points, low solubility, low and high molecular weight, and low vapor pressure. The increase in the PAH ring number decreases the solubility of PAHs in the aqueous phase but they are highly soluble in organic solvents because they are hydrophobic. These compounds are deposited in the sediments during sedimentation and partition because they diffuse in the sediment particles and are sorbed to the organic matter in the sediment particles and held together in the sediment particles (Khalaf et al. 2019; Mambwe et al. 2021). They are stable and steady in the environment and do not degrade easily and persist for a long time in the environment (Gennadiev et al. 2015; Abdel-Shafy and Mansour 2016). Asphaltenes and resins are hydrocarbon compounds that contain many elements such as oxygen, sulfur, and nitrogen, in addition to non-hydrocarbon compounds, and are characterized by their polar compounds (Sayed et al. 2021).

In 1967, the Liberian tanker Torrey Canyon collided with the rocks of the Cornwall coast in England. The result was the leakage of 119,000 tons of petroleum into the ocean, which caused the biggest pollution disaster in the sea and the

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Fig. 1 Petroleum hydrocarbon compounds categories

shoreline at this time. In addition, during the Gulf War in 1991, millions of petroleum barrels were released into gulf water make creating a huge disaster in the marine environment. These accidents have attracted the attention of the world to petroleum pollution and petroleum tanker accidents. These are the main sources of petroleum hydrocarbon contamination and derivatives in marine environments (Chen et al. 2019a, b; Bruckberger et al. 2020). Table 1 shows some petroleum spills caused by accidents during the twenty-first century, where large amounts of petroleum have leaked into seawater.

Every year, 1.5–10 million tons of petroleum hydrocarbons are spilled into seawater which cause changes in the physical and chemical properties and seawater viscosity, which threaten the marine environment (Bani-Hani et al. 2019; Yan et al. 2019; Mihankhah et al. 2020). The anthropogenic activities such as effluent release, municipal and industrial runoffs, and offshore and onshore petrochemical industry activities are causes of petroleum pollution in the marine environment (Varjani 2017), in addition to petroleum exploration, extraction, transportation, and petroleum refining (Ogunlaja et al. 2019; Iheonye et al. 2019).

When crude oil hydrocarbon is spilled into seawater, the spills spread horizontally over the water surface. The spreading depends on the water surface tension and oil viscosity. Many factors play a role in the movement of the crude oil spillage movement on the sea surface, including temperature and salinity because they cause density currents (Wang et al. 2017). The blowing wind contributes to the dispersion of the crude oil over the water surface and produces thick slicks of crude oil (Latimer and Zheng 2003). In 2018, the Iranian oil tanker Sanchi collided with a Chinese bulk carrier. Due to this collision, hundreds of metric tons of crude oil were leaked into the seawater (Pan et al. 2020). The sea currents, waves, and wind transported the crude oil far away to Japan and form a huge crude oil slick. The composition of crude oil spills changes over time due to weathering factors such as evaporation, emulsification, biological, and chemical degradation, photooxidation, and dissolution (Nordam et al. 2017). The low molecular weight compounds disappear because of the natural dispersant and evaporation process. The evaporated molecules form aerosols in the air and fall out with the rain (Tarr et al. 2016; Prior and Walsh 2018; Rajabi et al. 2020). The evaporation factor plays a vital role in diminishing the petroleum spill with different effects according to the petroleum type. Lee et al. (2003) reported that 5-10% of heavy petroleum, 40% of petroleum products, and 70% of light crude oil were evaporated within a few days of the spillage. The turbulence of seawater by waves causes dispersion of crude oil droplets and water and produces water in crude oil emulsions (Raya et al. 2020). The emulsion of water in crude oil eventually forms a "chocolate mousse" such as the "chocolate mousse" observed recently when the petroleum accident occurred in the Gulf of Mexico in 2010 (Warnock et al. 2015).

The "chocolate mousse" and oil slicks move toward beaches and may pollute the marshes and mangroves. In this form, the crude oil takes several years to biodegrade (Farrington 2014). In some cases, the "chocolate mousse" undergoes biodegradation or chemical degradation and is finally deposited in the seafloor sediments. The photooxidation reaction can change the composition of crude oil spills by the action of sunlight on oxygen and carbon atoms in hydrocarbon compounds to produce oxidized compounds (Shankar et al. 2015) such as aliphatic and aromatic ketones, aliphatic and aromatic alcohols, sulfones, sulfoxides, carboxylic and fatty acids, aldehydes, and anhydrides (Xu et al. 2018; Lee 2003). Magris and Giarrizzo (2020) reported many physical

Table T	seawater petroleum snips an	id tankers accidents on the sea and ocean water	

Year	Country	Seawater	Amount (barrels)	References
2010	Macondo-USA	Gulf of Mexico	5 million barrels	Mason 2019
2011	Brazil	coast of Rio de Janeiro	3700 barrels	Quinete et al. 2020
2011	China	Chines Bohai Sea	2620 barrels	Wang et al. 2017
2018	China	East China sea	1000.000 barrels	Sun et al. 2018a, b
2020	Mauritius	Southeast coast of Mauritius	23.748 barrels	Mauritius oil spill will have Wide-Ranging Effects 2020
2020	Russia	Arctic ocean	116.000 barrels	Arctic Wake-up Call. 2020

and chemical effects of petroleum contamination on the marine environment. Toxic chemicals cause a loss in biodiversity in marine conservation and severely impacts the coral reefs and marine animals (Robinson and Rabalais 2019).

Lumibao et al. (2018) reported that petroleum persists for a long time in marine plants, which affects animals feeding on these plants. In addition, petroleum contamination has lethal or sub-lethal effects on larvae and fish eggs or decreases egg hatching ability (Sørhus et al. 2016). Some studies have reported that fish exposed to petroleum contamination show slower movement, loss of balance and stability, and inconsistent swimming (Incardona et al. 2014; Mokarram et al. 2021). Pollution causes changes in fish and amphipod populations and may lead to death. These organisms play crucial roles in nutrient transport (Podlesińska and Dabrowska 2019). Petroleum contamination causes health problems after human consumption of these organisms by entering into cell and binding to proteins and DNA, which causes cell malformation and tumors. It binds to the exocyclic amino groups of guanine (G) and adenine (A) to form DNA and acts as an etiological agent in cancer disease (Gope et al. 2018; Abdelhaleem et al. 2019). PAH compounds cause increases in microbial hydrocarbon degrader numbers and decrease the number of other microbial cells, which lead to a loss in the functional capacity of the marine environment (Couto et al. 2016; Rodrigues et al. 2018).

Many chemical and physical techniques are used to clean up petroleum pollution from the marine environment such as in situ burning, skimmer, gelators, chemical dispersants, and sorbents, pumping, storing, booms incineration, UV oxidation, chlorination, and solvent extraction (Ghosal et al. 2016; Ben Jmaa and Kallel 2019; Abidli et al. 2020); all of these techniques still suffer from structural failure, low efficiency, high cost, low operations, and poor petroleum recovery. It is expensive and affected by the seawater currents and waves, which decrease the efficiency of these techniques (Al-Majed et al. 2012; Mapelli et al. 2017). Bioremediation is a promising technique involving in introducing newly developed (Muangchinda et al. 2020; Wei et al. 2020) or indigenous (Miettinen et al. 2019) microorganisms which are capable to degrade and utilize petroleum hydrocarbon compounds as the sole of carbon.

Microbial hydrocarbon degraders form less than 0.1–1% of total microorganism communities in the environment; when petroleum pollution occurs, the percentage of microbial hydrocarbon degraders increases to 10%, which explains the capability of these microbial cells to thrive and grow rapidly in petroleum contamination (Varjani 2017; Sun et al. 2018a, b; Neethu et al. 2019) because they can use petroleum hydrocarbons as a carbon source. Bioremediation techniques consider natural processes and have more advantages than chemical and physical techniques in that they are eco-friendly techniques, have fewer effects on the environment and humans, are low cost, produce fewer by-products, and exhibit good

performance (Bani-Hani et al. 2019; Yang et al. 2020). The main aims of biodegradation are the eliminating of toxic compounds, such as aromatic, polyaromatic, aliphatic, halogenated, heterocyclic, and nitro-substituted compounds from the environment (Martínková et al. 2009) or conversation to less toxic compounds that can be integrated into biogeochemical cycles (Varjani and Upasani 2017; Shi et al. 2019).

Several review papers have discussed and investigated the bioremediation petroleum of pollution in seawater. The novelty of the present review is to provide additional insights and visions into microbial bioremediation mechanisms to understand different strategies to remove petroleum contaminants more efficiently. Additionally, this review focuses on the quantity and contribution of metabolically active aerobic or anaerobic bacteria at the single or consortium level and also proposes different factors that affect bacterial activity to accelerate petroleum degradation in marine environments. No one technique can solve petroleum pollution in the marine environment; combination of different ideas is more effective to clean up seawater.

# Petroleum hydrocarbon biodegradation mechanisms

#### Aerobic biodegradation

Alkanes (paraffins) are inert compounds that need to be activated for further biodegradation (Chen et al. 2020a, b). In aerobic conditions, many enzymes assist in the introduction of oxygen molecules into alkane compounds. Microbial hydrocarbon degrader cells contain genes encoded by enzymes that catalyze hydrocarbon degradation reactions such as hydroxylases, cytochrome P450 hydroxylases, oxygenases (monooxygenases and dioxygenases) (Zampolli et al. 2019), and dehydrogenase enzymes (Imron et al. 2020). Rhodococcus strains produce the alkane 1-monooxygenase enzyme that oxidizes the n-alkane compounds by adding hydroxyl groups to produce alcohol compounds using electron carriers (Figure 2) (Zampolli et al. 2019). Several bacterial cells can also use monooxygenases enzymes to oxidize hydrocarbon compounds such as ammonia-oxidizing bacteria, propane-oxidizing bacteria, toluene-oxidizing bacteria, and methane-oxidizing bacteria (Abbasian et al. 2015).

The cytochrome P450 enzymes containing heme are produced by bacterial cells such as *Actinobacteria* and *Rhodococcus jostii* and play significant roles in the degradation of the alkane compounds, including the short chains (C5– C16) and long chains (C17–C28). They can catalyze monooxidations of the C–H bond by insertion of a single atom of oxygen using monooxygenase (Roberts et al. 2002). The monooxygenated alkane groups are the first step in alkane biodegradation under aerobic conditions. The *n*-alkane



compound is converted to alcohol by the added oxygen atom (Zampolli et al. 2019). The enzymes of alcohol dehydrogenase then convert the alcohol to aldehyde, and then to fatty acids. The  $\beta$ -oxidation process produces acetyl CoA which reacts with the fatty acids in the cytosol of prokaryotic cells (Figure 3). The acetyl CoA can also enter into the citric acid cycle (TCA) (Brzeszcz and Kaszycki 2018; Bulatović et al. 2020). A similar pathway is used to biodegrade iso-alkane compounds (Imron et al. 2020). Slightly different pathways are used by certain bacterial cells (such as *Rhodococcus aetherivorans*) to degrade cycloalkane compounds as a carbon

source (Presentato et al. 2018).

Biodegradation of cyclohexane compounds is initiated by the cyclohexane monooxygenase enzyme when an oxygen molecule is available. These enzymes attack the alkyl chain to produce alcohol, called a cyclohexanol compound. The cyclohexanol dehydrogenase enzyme converts cyclohexanol to cyclohexanone, cyclohexanone monooxygenase converts cyclohexanone to E-caprolactone, and E-caprolactone hydrolase converts this to the intermediate compound called adipic acid. Adipic acid is oxidized by  $\beta$ -oxidation to produce Acetyl-CoA, which enter the citric acid cycle (TCA) (Salamanca and Engesser 2014; Aalbers and Fraaije 2017). The biodegradation of the aromatic compound pathway follows the enzymes that are produced by microbial cells (Wen et al. 2020). For example, biodegradation of phenol is initiated by the production of catechol, which then undergoes fission to the meta or ortho-isomer (Khalil et al. 2021). Rajput et al. (2021) reported that complex and different pathways degrade these compounds. Therefore, different microorganisms are essential for complete biodegradation of BTEX compounds with different substrate specificities (Figure 4) (Kurniawan et al. 2018) such as Pseudomonas aeruginosa, Brochothrix thermosphacta, and Bacillus subtilis (Titah et al. 2018; Kurniawan et al. 2018).

PAH biodegradation is initiated by the oxygenase enzyme, which catalyzes the addition of oxygen atoms into aromatic compounds; therefore, the presence of oxygen is necessary to initiate this process (Rajput et al. 2021). Monooxygenase catalyzes the addition of one oxygen atom to the aromatic compounds, and the dioxygenase catalyzes the addition of two oxygen atoms to the aromatic compound. The result is the formation of catechol or protocatechol with hydroxyl groups. Dioxygenase enzymes function by cleaving the aromatic rings of catechol or protocatechol. These enzymes are classified into two groups: intra-diol enzymes cause ortho cleavage and extra-diol enzymes cause meta cleavage. The two enzymes differ in the site of cleavage: ortho cleavage cleaves between the two hydroxyl groups on the carbon atoms of the aromatic compounds, while the meta cleavage cleaves between two carbon atoms; one is hydroxylated and another non-hydroxylated (Patel et al. 2017; Abbasian et al. 2015). Catechol and/or protocatechol are catalyzed by chemical reactions to form 3-oxoadipate. Then, 3-oxoadipate enters further chemical reactions to form acetyl-coenzyme A (acetyl-Co A) and succinyl-coenzyme A before entering the citrate acid cycle. When acetyl Co-A enters the citric cycle, hydrocarbon compound degradation is completed by producing energy such as adenosine triphosphate (ATP) in addition to water and carbon dioxide (Martínková et al. 2009; Ukiwe et al. 2013).

#### Anaerobic biodegradation

Anaerobic biodegradation occurs in deep seawater, where seafloors are contaminated by petroleum seeps or leakage (Mu et al. 2021). The oxygen is depleted in deep seawater because the oxygen is utilized by the aerobic activities of microbial cells. Depleted oxygen enhances the activity of anaerobic microbial hydrocarbon degraders (Singh et al. 2014). Under anaerobic conditions, the electron acceptors of sulfate and nitrate are employed to harvest energy from the hydrocarbon degradation process. The success of petroleum biodegradation under anaerobic conditions depends on the available electron acceptor system and energy (Mu et al. 2021).

Toluene biodegradation under anaerobic conditions is initiated by the oxygen-sensitive enzyme, benzylsuccinate synthase (BSS), which adds fumarate to toluene. This reaction converts toluene to (R)-benzylsuccinate (Abbasian et al. 2015). Then, (R)-benzylsuccinate is replaced by succinyl-CoA for further oxidation. During this reaction, succinate is released and succinate dehydrogenase is converted to fumarate. This reaction is aimed at regenerating the fumarate source. Succinyl-CoA is a donor added by the CoA transferase enzyme to activate (R)-benzylsuccinate. Lastly, by  $\beta$ oxidation benzyl-CoA is produced and succinyl-CoA is released to continue in the cycle. Benzyl-CoA is an important intermediate in the anaerobic degradation of aromatic hydrocarbons. Benzyl-CoA reductase oxidizes the benzyl-CoA by breaking down the aromatic ring leading to the conversion of benzoyl-CoA to aliphatic compounds. The aliphatic compounds are attacked by  $\beta$ -oxidation (Figure 5). These reactions are similar to those of other aromatic compounds, such as xylene (Dou et al. 2016).

# Factors affecting the microbial hydrocarbon degrader

Marine environments rich in microbial hydrocarbon degrader communities in sediment or seawater such as *Exiguobacterium* 



Fig. 3 Biodegradation mechanism of *n*-alkane hydrocarbons

sp., *Acinetobacter* sp., *Enterobacter* sp., *Haererehalobacter* sp., *Photobacterium* sp., *Oceanobacillus* sp., *Nesiotobacter sp., Ruegeria* sp., and *Pseudoalteromonas* sp. are able to degrade and utilize hydrocarbon compounds as a carbon source (McFarlin et al. 2014; Kumar et al. 2019; Muangchinda et al. 2020). Many adverse conditions can affect microbial activity to clean up petroleum pollution including environmental factors such as salinity, pH, temperature, sea level, sea surface, nutrition, petroleum solubility, oxygen availability, pressure, bioavailability of pollutant, and petroleum solubility (Figure 6) (Jiang et al. 2016; Marietou et al. 2018; Xu et al. 2018; Luis et al. 2019). In addition, high surface tension between petroleum and water can decrease the microbial efficiency of degradation (Nakama 2017). These conditions cause adverse impacts on microbial cells, microbial uptake, and the speed of cell lysis (He et al. 2007).

Marine bacteria can quickly respond to the changes in these harsh patterns. For example, it can adapt to the change in temperature by different physiological properties such as symbiosis with other marine organisms (Prazeres and Renema 2019) or penetrate epidermal cells, produce toxins that inhibit photosynthesis and adhesion to receptors in the coral (Banin et al. 2001). Therefore, microbial cells isolated from seawater or sediment are more suitable to use in the bioremediation of petroleum pollution in the marine environment (Lee et al. 2018). The bioremediation processes depend on the activities of microbial cells in various environmental conditions. Kumar et al. (2019) reported the ability of microorganisms isolated from the marine environment such as Exiguobacterium sp. Ruegeria sp., Oceanobacillus sp., Haererehalobacter sp. Acinetobacter sp., Photobacterium sp., Enterobacter sp., and Nesiotobacter sp. can grow under different temperatures (25–40°C); a wide range of pH (6–10) can tolerate high NaCl concentration (10%), and can adapt different catalytic pathways. Rhodococcus genus is thriving in the marine environment and exhibits unique cell wall, different enzymatic activities due to having large plasmid (extra



Fig. 5 Biodegradation of toluene under anaerobic condition



Fig. 6 Factors affecting microbial hydrocarbon degrader activities

DNA), and appropriate biotechnological properties, which can successfully be used in bioremediation of petroleum contamination in seawater. These genera have varied catabolic pathways to degrade different hydrocarbon compounds such as aromatic and polyaromatic compounds (Martínková et al. 2009). *Rhodococcus erythropolis* can resist and hydrolyze saturated and unsaturated aliphatic of nitrile compounds, because it can produce varied enzymes such as nitrile hydratase/ amidase which assess to tolerate and grow under high concentration of acetonitrile (900 mM) and salinity from 0 to 8% (Langdahl et al. 1996).

#### **Microbial physico-chemical properties**

The low bioavailability of hydrocarbon compounds can trap these compounds in regions with low permeability, contributed to low efficiency of the biodegradation process (Adadevoh et al. 2016). Many microbial cells possess chemotaxis properties to increase the affinity of microbial cells to hydrocarbon compounds. The chemotaxis is the ability of the microbial cells to move toward nutrition gradients in the environment such as hydrocarbon compounds, which display the carbon and energy source for these cells (Parales and Ditty 2018). This behavior provides the optimal condition for the microbial cells to survive and grow in sites contaminated by hydrocarbon compounds (Meng et al. 2019) such as adapting the differences in pH, temperature, and salinity (Wong-Ng et al. 2018).

Some cytoplasmic proteins act as sensory systems that play a role in microbial chemotaxis by producing signals that cause cell movement toward the substrates (Clerc et al. 2020). Several bacterial hydrocarbon degraders have a sensory system that detects and responds to hydrocarbon compounds, which suggests the role of this phenomenon in petroleum hydrocarbon bioremediation (Gkorezis et al. 2016; Ahmad et al. 2020; Ibrar and Zhang 2020). Samanta and Jain (2000) reported that *Pseudomonas putida* is a naphthalene-degrading bacterium that can be chemotactic toward naphthalene compounds. Krell et al. (2012) observed that an increase in hydrocarbon bioavailability led to an increase in cell chemotaxis.

When the bacterial cells come close to hydrocarbon compounds, the initial step of bioremediation, as reported by Bhatt et al. (2021), is the adsorption of these compounds. In their study, Yang et al. (2020) reported the bacterial cell *Halomonas* spp. isolated from a marine environment showed high diesel adsorption at the initial stage of bioremediation compared with the relatively low uptake process. This indicated that the bacterial cell quickly adsorbed diesel onto the cell surface and absorbed it in the second step (Figure 7).

Several studies have shown that there is a correlation between the bacterial cell surface and the degradation of hydrocarbon compounds (Liao et al. 2015; Yu et al. 2020; Mambwe et al. 2021). These compounds are attached to the active sites of chemical groups such as carboxyl, hydroxyl, and phosphate groups on the bacterial surface, and the bacterial cells absorbed compounds into the cytoplasm (Liao et al. 2015)

Pyrene is the major portion of PAH compounds found and distributed in marine environments and classified as high molecular weight and low bioavailable, and resists biological attack. Thus, it has a toxic impact on both humans and organisms in the environment (Laothamteep et al. 2021; Zada et al. 2021). Liao et al. (2015) reported that pyrene could be degraded by Brevibacillus brevis; they observed pyrene molecules are highly soluble in lipids. Therefore, it interacts with the phospholipid of the cell membrane by hydrophobic bound and forms stable bounds. Then, the pyrene enters the cytoplasm by transmembrane to transport and degrade gradually in the cytoplasm. They found the pyrene was decreased from 1 to 0.093 mg/L during 168 h. Rhodococci, Mycobacterium, Nocardia, and Corynebacterium are genera of bacteriacontaining mycolic acids compounds in their cell walls. These compounds contain long aliphatic chains, which facilitate the uptake of hydrocarbon compounds into cells (Martínková et al. 2009) and play the main role in bacterial adhesion onto other compounds (Zhang et al. 2010).

Bacteria with chemotaxis properties have the advantage to sense and locate environmental pollution and increase bacterial hydrocarbon degrader within contamination vicinity which enhance pollution bioremediation (Zheng et al. 2020). Thus, chemotaxis is an important factor and plays a crucial role in hydrocarbon bioremediation. In some cases, the opposite is happening; some microbial hydrocarbon degrader such as bacteria (Varjani 2017), algae (Rahman et al. 2019), and fungi (Sena et al. 2018) can release compounds such as extracellular polymer (EPS) (Meng et al. 2019) lipids and proteins which are able to absorb hydrocarbon compounds such as



Fig. 7 Sketch picture of bacterial chemotactic biosorption

PAH in the aquatic environment. Then, subsequently, uptake and degradation by bacterial enzymes intracellularly such as monooxygenase and cytochrome P450 (Gao et al. 2014) contribute to accelerating hydrocarbon biodegradation from the marine environment.

#### **Nutrient availability**

Nutrients such as phosphorus, nitrogen, carbon, oxygen, hydrogen, and sulfur are essential for microbial growth because they are necessary to build microbial structures and produce energy (Abdel-Aziz et al. 2016). The main problem during petroleum bioremediation is the imbalance in nutrient elements because of the increase in carbon level with a decrease in other nutrient elements such as nitrogen (N) and phosphorus (P) in the petroleum spills. The petroleum mixture is rich in carbon (C) and poor in P and N, which causes decreased microbial growth to support petroleum degradation (Simpanen et al. 2016; Varjani 2017). The addition of nutrients to the contaminated environment, which aims to treat the imbalance in the C:N:P ratio, is a process called biostimulation (Muangchinda et al. 2020). The change in these elements can affect the microbial hydrocarbon degrader growth stimulation (Ortega et al. 2018). Beskoski et al. (2011) reported that a suitable fertilizer (C:P:K) ratio to enhance microbial growth must be 100:10:1 in the environment. Varjani (2017) observed that microbial hydrocarbon degraders only introduced into a contaminated area are not sufficient to support the degradation rate, and nutrients (such as nitrogenous fertilizers) must be added to enhance biodegradation significantly.

Zaidi and Imam (1999) reported that the addition of inorganic nitrogen sources such as KNO<sub>3</sub> causes increased phenanthrene biodegradation in Caribbean Coastal Water by microbial indigenous to 10 times and reduce the time of crude oil biodegradation. Xia et al. (2006) used 10NO<sub>3</sub>-P as a fertilizer to decrease remediation time to 14 days comparing with 78 days without using fertilizer. Nutrition has another advantage in bioremediation by enhancing biological molecules syntheses such as exopolysaccharides (EPS) and bioflocculant compounds (Staudt 2010), which is important in emulsifying the low solubility hydrocarbon compounds in water to increase the bioavailability of these compounds for biodegradation (Zeng et al. 2018). These compounds are varying from microbial to another microbial cell and are affected by nutrient composition (Staudt 2010).

Nutrient elements are found naturally and the ratio is varying from an environment to another. McFarlin et al. (2014) reported that the natural nutrients in Arctic seawater support the bioremediation of crude oil at  $-1^{\circ}$ C (Delille et al. 1998) while adding a fertilizer to enhance the biodegradation of crude oil pollution in Antarctic coastal, which indicated the limited nutrient content. Santas et al. (1999) reported the feasibility of using natural products as fertilizers such as modified fishmeal with C:N:P ratio (24:18:3.5) in enhancing the bioremediation process when they mix with anipol fertilizer. The result shows increased hydrocarbon contaminant reduction to 70% in seawater. This type of fertilizer is reported as rich in phosphor and nitrogen comparing with other fertilizers. The excess nutrition in seawater may cause adverse effects on the bioremediation because it enhances algae blooms. These organisms cause oxygen depletion in seawater (Lu et al. 2018). whereas Haule et al. (2015) prove the oxygen element is a very important factor that can enhance hydrocarbon degradation.

#### **Biosurfactant production**

Hydrocarbon compounds (non-polar) are not soluble in water unless these compounds have functional groups attached, such as hydroxyl or carboxyl groups (Imron et al. 2020). The most commonly used method to improve crude oil hydrocarbon bioremediation in seawater is the addition of surfactants, which reduce the surface tension between water and petroleum compounds (Nakama 2017). This dissolves the crude oil in water, breaks it down into small droplets, and causes mixing of the two phases together to increase its bioavailability for microbial cells (Dwivedi et al. 2019). Bacterial biosurfactant producers have two physiological approaches to

increase the availability of petroleum for degradation: enhance petroleum solubilization and increase the affinity between microbial cell membranes, and hydrocarbon compounds (Hou et al. 2018). Many microbial cells can produce various biosurfactant groups such as rhamnolipids, trehalolipids, and sophorolipids which belong to glycolipid groups mainly produced by Pseudomonas aeruginosa, Nocardia spp., Mycobacterium spp., Corvnebacterium spp., and Candida spp. (Santos et al. 2016; Mnif et al. 2018). Surfactin belongs to the lipopeptide group produced mainly by Bacillus strains, such as B. subtilis (Fei et al. 2020). Liposan and emulsan belong to the polymeric biosurfactants produced by Candida lipolytica (Santos et al. 2016). Some surfactants are produced synthetically (chemical surfactants) such as linear alkylbenzene sulfonate (LAS) and sodium dodecyl sulfate (SDS). Synthetic surfactants are widely used in large marine crude oil spills such as the accident of the Deepwater Horizon in the Gulf of Mexico in 2010. The feasibility and effects of using synthetic surfactants in the bioremediation of crude oil in the environment were investigated (Johnson et al. 2021). Staninska-Pieta et al. (2019) proved the use of chemical dispersants to remediate crude oil pollution and it can change microbial communities by inhibited microbial hydrocarbon degrader growth and increased the non-hydrocarbon degrader growth. That is because the chemical surfactants cause inhibited the microbial enzymes' activity while biosurfactants cause increased bacterial enzyme activity significantly (Couto et al. 2016). Staninska-Pieta et al. (2019) suggested using biosurfactants produced by Pseudomonas genus rather than synthetic surfactants such as polysorbate-80 to enhance diesel oil biodegradation. The biosurfactants showed enhanced degradation (47%) of diesel and were considered to be environmentally friendly and less toxic. Zeng et al. (2018) reported the ability of surfactants to interact with microbial membrane lipids and proteins disrupts the cell membrane and reduces microbial cell function. In contrast, these effects were less significant when biosurfactants were used. Couto et al. (2020) reported that biosurfactants isolated from the B.velezensis strain are active under different environmental conditions of salinity, pH, and temperature during the bioremediation of crude oil-contaminated seawater. Durval et al. (2018) proved that lipopeptide biosurfactants isolated from B. cereus showed fewer toxic effects on marine organisms such as the bivalve Anomalocardia brasiliana and fish Poecilia vivipara, which showed a survival rate of 55% and 90%, respectively.

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Many recent studies proved that bacterial communities in seawater are changed by the decrease of bacteroid and increase of Proteobacteria comparing with the original sample. Proteobacteria is the most abundant bacteria in the contaminated marine environment. Most microorganisms prefer neutral or low alkaline conditions to degrade hydrocarbon compounds (Wang et al. 2016; Muangchinda et al. 2018). Any changes in pH can cause changes in the microbial communities in seawater (Louvado et al. 2018). The optimal pH is the value where the microbial cells showed good growth and high enzymatic activity where it is different among the microbial cells (Wang et al. 2016; Neina 2019). Obahiagbon et al. (2014) reported that the high biodegradation rate of crude oil was at pH 6 by microbial consortium culture and they showed decreased degradation activity at high acidic or alkaline pH. Extremely high or low pH values generally result in complete loss of activity for most enzymes which inhibit cell growth and decrease biodegradation (Geng et al. 2020; Laothamteep et al. 2021). Muangchinda et al. (2018) proved that alkaline pH value up to 9 decreased the efficiency of biodegradation. In contrast, Patel et al. (2012) showed that the naphthalene-degrading bacterial consortium preferred pH 8-10 to degrade 100% of naphthalene contamination and the degradation activity decreased to 88% at pH 7, which indicated that the consortium culture preferred the alkaline conditions. Over acidity conditions in the ocean caused by the increase in atmospheric CO<sub>2</sub> concentration dissolved in water (Zhang et al. 2017) and accumulation of acidic intermediate products due to the degradation process during the microbial activity in seawater (Lin and Cai 2008; Patel et al. 2012) caused a decrease in microbial uptake and speeds cell lysis.

# Salinity

High salt concentration reduces microbial growth by inhibiting enzyme secretion due to high osmotic pressure of the surrounding environment (Qin et al. 2012; He et al. 2017), reducing available oxygen to microorganisms (Liang et al. 2017) and hydrocarbon compound solubility (Truskewycz et al. 2019); it also causes cell membrane lysis. Muangchinda et al. (2018) reported that salinity causes a change in the microbial communities; they found an increase of Alcanivorax, Thalassospira, and Pseudidiomarina, and a decrease of Methylophaga and Pseudomonas in seawater samples at high salinity. Laothamteep et al. (2021) emphasized the ability of bacterial consortium culture to adapt salinity (>5%) and enhanced pyrene biodegradation. They observed that the increased salinity caused increasing lag time, decreasing diesel oil absorbance and biodegradation rate. Thavasi et al. (2007) proved that the optimum salinity for crude oil biodegradation by Pseudomonas aeruginosa was at 35g/l while Minai-Tehrani et al. (2009) reported that it was 30g/l for PAH degradation when the increase of the salinity to 50g/l inhibited the biodegradation activity.

#### **Concentration of hydrocarbon**

The accumulation of contamination effects on cell viability, internal granule, cell size, and cell surface morphology also changes intracellular particle (Liao et al. 2015), cell surface hydrophobicity (Kaczorek et al. 2010), and membrane permeability (Shi et al. 2013) by the increase of ion and proton permeability. It impacts membrane-embedded protein, membrane integrity, membrane excretion system, and loss intracellular pH hemostasis (Sikkema et al. 1995). The high concentration of hydrocarbon pollution led to limited microbial growth due to limited nutrition, which causes microbial death with extension exposure time (Alegbeleye et al. 2017; Yang et al. 2020) and changes microbial communities (Hamdan and salam 2020).

The inhibitory effects of highly toxic compounds' concentration are found in crude oil such as naphthalene (Pathak et al. 2009; Patel et al. 2012). Awasthi et al. (2018) reported that increasing hydrocarbon concentration decreases microbial biodegradation activity due to increasing the number of toxic compounds and decreasing the aeration. Koolivand et al. (2019) observed a decline in biodegradation activities of petroleum hydrocarbon when the concentration of hydrocarbon increased from 1 to 5%. In addition, the low concentration of hydrocarbon compounds reduces microbial biodegradation activity due to limited carbon sources, which limited bacterial growth (Varjani and Upasani 2017).

#### **Temperature effects**

Temperature plays a critical role in microorganism's growth, activity, and communy structure (Qin et al. 2018; Siles and Margesin 2018). The low temperature caused decreased biodegradation activities (Horel and Schiewer 2011) due to the increase in crude oil viscosity, decreased its content volatilization, and decreased its solubility in water (Sharma and Schiewer 2016). Hamdan and Salam (2020) reported that the change in seasons caused a change in microbial communities' structure due temperature change. That is because different temperature impacts microbial adaptation (Gutiérrez et al. 2018). Techtmann et al. (2017) found that microbial hydrocarbon communities isolated from seawater surface degrade crude oil faster than deep microbial communities due to temperature differences. Yang et al. (2011) reported that the increased seawater temperature from 4 to 18°C causes increased biodegradation rate 4 times due to activating the microbial enzymes that are involved in crude oil biodegradation activities. Therefore, at mesophilic temperature, the biodegradation of crude oil is faster (Ferguson et al. 2017). Patel et al. (2012) observed the optimum temperature to degrade 100% of naphthalene in marine sediment using bacterial consortium is 37°C and decreased to 94% at 30°C and reach 76% at 20°C, but the increase in temperature more than 37°C causes decreased consortium growth. That is because the high temperature denatures and inactivates microbial nucleic acids, proteins, and enzymes, which inhibited microbial growth (Yadav and Hassanizadeh 2011). Thus, the crude oil spills in cold regions take a long time to remediate.

#### Pressure

Approximately 3-31% of crude oil spills are precipitated in seafloor (Chanton et al. 2015). This area is characterized by high pressure and low temperature, which creates a challenge for biodegradation by microbial cells (Fasca et al. 2018). Marietou et al. (2018) suggested that increasing pressure causes decreased microbial growth and increased biodegradation time, which showed the effects of pressure in decreased biodegradation activities. The biodegradation of hydrocarbon compounds at the sea surface is greater than that in deep water, and the optimum pressure for each microbial cells plays a significant role to enhance microbial activity (Nguyen et al. 2018). When going to the seafloor, the pressure increase is 1 MPa per 100 m (Kostka et al. 2019). The loss of biodegradation activity was inversely proportional with increased pressure, where 4% of n-alkanes and PAH biodegradation activity is lost when the pressure increased 1MPa. Schwarz et al. (1974) reported that the microbial growth and biodegradation of hexadecane decreased 10 times when the pressure increased from 0.1 to 50MPa, while Grossi et al. (2010) proved that piezotolerant organisms (organisms pressure loving) have no effect on their growth and biodegradation of hexadecane at high pressure 35 MPa and showed slightly inhibition at lower pressure 15 MPa. Schedler et al. (2014) observed the effects of different pressure on two hydrocarbon degrader strains; Rhodococcus qingshengii is alkane hydrocarbon degrader, and Sphingobium yanoikuyae is aromatic hydrocarbon degrader. R. qingshengii showed good growth and biodegradation for n-hexadecane at pressure 15MPa, while the S. yanoikuyae showed no growth at pressure more than 12 MPa. These results showed the ability of some microbial cells to tolerate high pressure rather than others. The high pressure causes leakage of cell intracellular components, increased uptakes of degraded hydrocarbon compounds (Smułek et al. 2019), impaired microbial replication, cell division, microbial membrane fluidity, membrane protein function, protein, RNA synthesis, and nutrient uptake, and could kill the microbial cell if reach 200 MPa (Jebbar et al. 2015).

#### Oxygen demand

Oxygen is an important element for petroleum bioremediation (Nguyen et al. 2019). When crude oil leakage goes into seawater, the crude oil slicks on seawater surface decreases light penetration and oxygen dissolving in water that causes negative effects on the marine ecosystem by reducing the autotrophic organism's photosynthesis which is considered the main food source. It also decreases the rate of crude oil biodegradation under aerobic condition because most of the hydrocarbon compounds are degraded under aerobic condition (Riser-Roberts 1998; Haule et al. 2015). Mille et al. (1988) observed a decrease in petroleum biodegradation 2–3 times when the dissolved oxygen decreased from 8 to 2–3 ppm. Oxygen plays a crucial role in hydrocarbon compound degradation as an electron acceptor. The microbial hydrocarbon degrader uses oxygen to oxidize the hydrocarbon compounds under aerobic conditions using oxygenase enzymes (Abbasian et al. 2015).

The process initiated by the enzyme monooxygenated, which added the oxygen atom to hydrocarbon compounds, is essential to produce the alcohol compounds (Imron and Titah 2018) also, PAH compounds are oxidized by multicomponent enzymes to produce dihydrodiol in presence of oxygen (Kadri et al. 2017). The limited oxygen causes inhibit the function of oxidation enzymes such as the laccase enzyme, that led to prevent chrysene degradation (Hadibarata et al. 2009) and prevent oxidize phenolic compounds (Kadri et al. 2017). Laccase enzymes oxidize aromatic compounds by reducing the oxygen molecules to water (Taha et al. 2020).

# Petroleum hydrocarbon bioremediation strategies in the marine environment

The greatest challenge for bioremediation applications in marine environments is the adaptation of microorganisms to environmental conditions and overcoming the conditions that affect microbial hydrocarbon degrader growth, and activities (Patel et al. 2012; Chen et al. 2017). Several bioremediation strategies have been proposed to enhance the bioremediation of crude oil pollution in marine environments. We distinguish between three main bioremediation strategies that involve (1) biostimulation, (2) bioaugmentation, and (3) phytoremediation strategies for remediation purposes Figure 8.

## **Biostimulation**

Biostimulation involves the addition of growth-limiting nutrients or specific substrates that enhance microbial hydrocarbon degrader growth and stimulate biodegradation activities (Brakstad et al. 2017; Hamdan and Salam 2020). The biodegradation of petroleum contamination using only indigenous microbial cells without the addition of stimulants is a timeconsuming process. The addition of biosurfactants (such as rhamnolipids or synthetic surfactants) has been shown to enhance the *n*-hexadecane reduction in marine environments (Ghafari et al. 2019). Goveas and Sajankila (2020) demonstrated that the addition of yeast extract (0.05g/L) as a growth stimulator for Acinetobacter baumannii in seawater samples at pH 8.1 and temperature 27°C has resulted in enhanced hydrocarbon degradation in seawater. The bacterial cell growth was  $2.35 \pm 0.12$  g/L in the absence of the yeast extract and  $5.42 \pm 0.17$  g/L in the presence of the yeast extract with degradation efficiency 17.48-3.55% of crude oil composition (C8-C14) within 15 days. Chen et al. (2020a, b) observed an increase in crude oil degradation efficiency to 76.92% and 115.38% when 0.02 and 2.00 g/L of nutrients consisting of NH<sub>4</sub>Cl, NaNO<sub>3</sub>, and K<sub>2</sub>HPO<sub>4</sub> were added at temperature 25 °C compared to the control within 32 weeks. Crisafi et al. (2016) reported the addition of inorganic nutrients consisting of 0.077 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.2 g/L NH<sub>4</sub>Cl, and 0.1 g/L NaNO<sub>3</sub> enhanced the bioremediation of crude oil pollution in the Taranto Gulf and 73% of crude oil pollution was removed within 14 days. Many inorganic nutrients are used to stimulate crude oil bioremediation, such as mineral nutrient salts (NH<sub>3</sub>NO<sub>3</sub>, Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>, MgNH<sub>4</sub>PO<sub>4</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, K<sub>2</sub>HPO<sub>4</sub>, KNO<sub>3</sub>), and commercial inorganic fertilizer (Gwenzi et al. 2018). The addition of nutrients to enhance bioremediation does not change the microbial hydrocarbon degrader communities in contaminated areas, but the season affects microbial communities. Hamdan and Salam (2020) reported that biostimulation has a high degradation rate of PAHs during the dry season compared to the wet season. The different temperatures between the wet and dry seasons affect the microbial adaptation of the environment, which affects the microbial composition.

Biostimulation is effective even under anaerobic conditions. Bianco et al. (2020) reported biodegradation of 55% of PAHs under anaerobic conditions after 120 days with 37°C degree by adding an organic fraction of nutrients called fresh organic fraction of municipal solid waste (OFMSW) which consist of heterogeneous material a mixture of food, garden wastes, and paper), nutrient solution (KNO<sub>3</sub>, Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, MgSO<sub>4</sub>·7H<sub>2</sub>O, KH<sub>2</sub>PO<sub>4</sub>, EDTA iron (III) as macronutrients, and H<sub>3</sub>BO<sub>3</sub>, MnCl<sub>2</sub>·4H<sub>2</sub>O, ZnSO<sub>4</sub>·7H2O, CuSO<sub>4</sub>·5H<sub>2</sub>O, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O) and Digestate (a semistabilized by-product of anaerobic digestion). Nutrient addition stimulates crude oil bioremediation in coasts and enclosed marine environments (Sakaya et al. 2019), but not in open seawater, because the addition of fertilizer may be diluted (Chen et al. 2020a, b). Slow-release fertilizer is a new technique used to overcome the problem of the washing out of nutrition in intertidal and open seawater. It is performed by providing a continuous source of nutrients for indigenous microbial cells in the contaminated site. It consists of a solid surface coated with a mixture of inorganic nutrients. Slow-release fertilizer is considered to be cost-effective compared to water-soluble nutrients (Gwenzi et al. 2018).

The use of slow-release fertilizer can remediate 90% of nalkanes and 60% of (alkyl) naphthalenes from seawater within 30 days (Kasai et al. 2002). Becker et al. (2016) found that biostimulation using the slow-release nutrient, Osmocote, sustained high amounts of nutrients such as potassium, nitrogen, and phosphorus in the sediment pore water, which enhanced the metabolic activity of the microbial biomass, and increased crude oil biodegradation. Swannell et al. (1996) Fig. 8 Scheme of bioremediation of marine environment contaminated with petroleum hydrocarbons



suggested using a slow-release fertilizer called Customblen, consisting of calcium phosphate and ammonium nitrate with vegetable oil as a polymerization coat to enhance petroleum contamination removal from marine environments. It performed better than the water-soluble fertilizer. Agarry (2017) used an adsorption biostimulation strategy to enhance crude oil bioremediation in marine environments. The combination includes first Osmocote slow-release inorganic NPK fertilizer which consists of 14:14:14, ammoniacal nitrogen with nitrate nitrogen, available phosphate and soluble potash, second organic fertilizer mixture (poultry droppings, cattle dung, and pig dung), and third Tween 80 as surfactants, and commercial activated carbon. The results showed that combined biostimulation strategy enhanced crude oil removal with 98.5% within 4 weeks.

Nikolopoulou and Kalogerakis (2010) reported that this technique needs to control the release of nutrients to ensure the providing of continuous nutrients for a long time in the marine environment; that is, the very slow release of nutrients does not provide sufficient nutrients for biodegradation, and the fast release of nutrients does not support biodegradation for a long time (Kenneth et al. 1993). In addition, low temperatures decrease or suppress the permeability of nutrients on the coat of slow-release nutrient complexes. Thus, this technique requires excessive amounts of fertilizer to enhance the biodegradation process or it will cease after a short time (McKew et al. 2007). Gertler et al. (2009) suggested using oleophilic fertilizer such as paraffinized urea to support the bioremediation process because the paraffinized urea has a high affinity for crude oil, which supports microbial hydrocarbon degraders selectively and prevents algal blooms in seawater (Gertler et al. 2015). Inipol EAP22 is an oleophilic fertilizer containing urea as a nitrogen source, lauryl phosphate as a phosphorous source, a surfactant, and oleic acid to make the compound hydrophobic. The fertilizer successfully supports bacterial growth and bioremediation activities in marine environments and shorelines (Jiménez et al. 2007; Røberg et al. 2011). Knezevich et al. (2006) observed that using guano oleophilic fertilizer supported the growth of hydrocarbon degrader Alcanivorax and Alteromonas strains to 10<sup>8</sup> cells/mL with 70% of crude oil in seawater removed because this fertilizer contains uric acid, which can selectively bind to crude oil. The uric acid content of guano oleophilic fertilizer is also degraded by bacterial cells, which use it as a carbon or nitrogen source (Koren et al. 2003), or it may be converted into ammonium and utilized by marine microbial cells, such as Halomonas as nitrogen sources (Gertler et al. 2015).

## **Bioaugmentation**

Biostimulation is an effective strategy to remediate crude oil pollution from marine environments because indigenous microbial cells are better adapted to the marine environment. Because of the low number of indigenous microbial hydrocarbon degraders due to environmental complexity (Nuñal et al. 2014; Fu et al. 2020), biostimulation takes a long time in the environment, and bioremediation needs to be enhanced by introducing more microbial cells (Nuñal et al. 2014). Hosokawa et al. (2009) suggested introducing exogenous microbial cells with known degradation capability, a process called bioaugmentation. It aims to increase microbial

populations that are capable of degrading pollutants in contaminated areas (Almeida et al. 2013). Crisafi et al. (2016) observed the bioremediation of crude oil pollution by introducing bacterial consortium culture consisting of *Alcanivorax dieselolei, Cycloclasticus* spp., *Thalassolituus oleivorans*, *A.borkumensis*, and *Marinobacter hydrocarbonoclasticus* into seawater. This achieved better performance in crude oil degradation (79%) than the biostimulation of indigenous microbial cells by yeast extract (73%).

Most bioaugmentation processes are still in vitro (Fantroussi and Agathos 2005), because the introduced microbial cells may have unknown effects on environmental microbial communities. In addition, this method may not be successful because the introduced microbial cells are unable to compete with the indigenous microbial cells and their survival depends on the field conditions (Mrozik and Piotrowska-Seget 2010). To ensure the efficiency of the bioaugmentation process, Muangchinda et al. (2018, 2020) proposed using isolated bacterial cells from contaminated seawater and sediment to degrade hydrocarbon compounds rather than introducing bacterial cells from different environments because these cells are better adapted to a wide range of environmental conditions, such as pH (4.0–9.0), temperatures (25 °C–37 °C), and salinities (0-10 NaCl g/L), and have high efficiency in degrading PAH compounds such as phenanthrene, pyrene, fluoranthene, and anthracene. Yang et al. (2020) observed high biomass production in marine bacterial cells (Halomonas sp. nmyj-1) during the bioremediation of crude oil with salt concentration (5.99%) including NaCl 5g, (NH<sub>4</sub>)2SO<sub>4</sub> 1g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.25g, NaNO<sub>3</sub> 2g, K<sub>2</sub>HPO<sub>4</sub>· 3H<sub>2</sub>O 10g, KH<sub>2</sub>PO<sub>4</sub> 4g, and pH 8.13 with the rotation time 179.85r/min. Muangchinda et al. (2020) reported that the bioaugmentation of single bacteria isolated from contaminated sediment (Exiguobacterium sp. AO-11) degraded 75% of the crude oil pollution.

The introduced microbial cells may be diluted in the open ocean or seawater and have different nutrient demands in different microbial cells (Sun et al. 2018a, b; Chen et al. 2020a, b). Therefore, Chen et al. (2017) suggested immobilizing bacterial cells onto a solid surface to overcome this problem. Bioaugmented immobilized microbial hydrocarbon degraders have been widely used in environmental pollution treatments (Lin et al. 2014; Chen et al. 2017; Xue et al. 2017). It depends on the use of carriers to deliver the hydrocarbon degrader to the contaminant site (Chen et al. 2016). Carrier materials provide an appropriate niche for microbial cells to protect them from adverse conditions through pore space on the carrier surface or by providing nutrients to support microbial growth, which provides a favorable environment for microbial survival and functioning (Ronchi and Ballatti 1996; Gentili et al. 2006; Xue et al. 2017). In addition, the ability of the carrier to absorb crude oil is degraded by the immobilized cell (Fu et al. 2020). Chen et al. (2017) reported the ability of immobilized consortium bacterial cells consisting of *Pseudomonas aeruginosa*, *Alcaligenes* spp., *Exiguobacterium* spp., and *Bacillus* spp., on calcium alginate perform crude oil degradation 65% within 5 days comparing with free cells 57% within 7 days under different environmental conditions of pH, temperature, salinity, and different crude oil concentrations. Immobilization technology is a way to reestablish a favored environment for petroleum biodegradation (Fu et al. 2020). Bioaugmentation is a way to improve persistent polyaromatic hydrocarbon biodegradation. Dellagnezze et al. (2016) reported that 99% of Benzo (K) fluoranthene was degraded using immobilized consortium culture including metagenomics clones and *Bacillus subtilis* strain in chitosan beads within 30 days comparing with 70% by free bacterial cells.

Kumar et al. (2019) proposed immobilizing indigenous bacterial cells including Oceanobacillus sp., Ruegeria sp., Haererehalobacter sp., Pseudoalteromonas sp, Photobacterium sp., Acinetobacter sp., Enterobacter sp., Nesiotobacter sp., and Exiguobacterium sp. onto wheat bran as a natural material improved crude oil degradation in deep seawater to 84% while 70% degraded by free cells within 10 days. In this study, the immobilized bacterial cells proposed for further biodegradation because of the increased bacterial cell load from  $3.5 \pm 0.28 \times 10^4$  to  $1.31 \pm 0.18 \times 10^8$  CFU/g in the carrier at 10 days. The immobilized cells showed good growth and stable degradation activity for 50 days compared to free cells. Immobilization technology enhances microbial growth, which provides a high bacterial mass for bioremediation, decreases microbial loss to the environment, and increases bacterial cell adaptation to various conditions (Bao et al. 2013; Shi et al. 2018). This increases the biodegradation rate and retains microbial cell viability for a long time. Many natural materials can be used to immobilize bacterial cells to reduce costs such as cocopeat and rice hull powder (Nuñal et al. 2014), chitin and chitosan flakes (Gentili et al. 2006), sawdust, and oil palm empty fruit bunches (Hazaimeh et al. 2014). Bacterial cells immobilized onto the carrier surface indicate the ability of these bacterial cells to produce exopolysaccharides (EPS) to create biofilms on the carrier surface. EPS is a component of biosurfactants that increases the solubility and emulsification of immiscible phases of crude oil in water to increase crude oil viability for bioremediation (Hazaimeh et al. 2014).

For the future perspective, Dangi et al. (2019) suggest using metabolic engineering techniques to overcome bioaugmentation problems such as the efficiency and process slow. The metabolic engineering process depends on improving the biodegradation functions of introducing microbial cells by engineering the existing metabolic pathways or introducing new catabolic pathways. It depends on introducing many identified genes involved in biodegradation pathways, which were encoded to enzymes that can achieve complete bioremediation for the pollutants (Shahi et al. 2017; Li et al. 2016).

## Phytoremediation

Phytoremediation is a bioremediation technology used to remove petroleum contamination from soil (Radwan et al. 2000). In marine environments, phytoremediation can be used by bacterial hydrocarbon degraders associated with macroalgae or cyanobacteria to enhance petroleum degradation in seawater. Radwan et al. (2002) found that microalgae surface is rich in bacterial hydrocarbon degraders that grow on them. Several studies have reported the immobilization of bacterial cells on the algal surface (Gaur et al. 2018; Zhang et al. 2018a, b; Zhang et al. 2020). Microalgae such as Enteromorpha, Iyengania, and Padina spp. grow naturally in seawater and play important roles in bacterial hydrocarbon degrader growth, such as Acinetobacter. They also found that macroalgae increased bacterial growth 32 to 490 times and a lower number of bacterial cells were released into seawater when this technique was used, which indicates the high stability of immobilized bacterial cells on macroalgal surfaces. The algal cells show the ability to absorb hydrocarbon compounds. It contains active sites such as carboxyl, hydroxyl, and amine groups in their cell wall (Flores-Chaparro et al. 2017; Ghadiryanfar et al. 2016). Kalhor et al. (2017) reported the ability of Chlorella vulgaris to remediate 94% and 88% of light and heavy hydrocarbon compounds, respectively, in 14 days. Algal materials Enteromorpha and kelp residues have the ability to absorb 411% and 273% respectively of diesel oil after 2 h (Zhang et al. 2018a, b). The immobilized bacterial cells (Bacillus sp. E3) onto the two carriers degraded 70% of alkane and PAH compounds comparing with 63% and 66% respectively using free bacterial cells after 21 days. This study showed the efficiency of algal cells to improve oil biodegradation in the marine environment.

Al-Hasan et al. (1994) used the same technology in the Arabian Gulf coast and reported that cyanobacteria could enhance indigenous microbial cells to degrade hydrocarbon compounds. Additionally, they showed that hydrocarbon pollution enhances cyanobacteria growth, which protects bacterial hydrocarbon degraders from washing out and harsh condition such as excessive light and dryness (Garcia-Pichel and Pringault 2001; Des Marais 2003), and increases the number of immobilized hydrocarbon degrader cells on the surface, leading to increase microbial growth and petroleum degradation activities because the cyanobacteria supply the hydrocarbon-degrading bacteria with oxygen and nutrients (Schuurmans et al. 2015; Zhang et al. 2020). In addition to oxygen and nutrition, macroalgae can supply microbial hydrocarbon degraders with vitamins, which are necessary for optimal activities (Radwan and Al-Muteirie 2001). In addition, bacterial cells also produce many vitamins that support algal cells growth, such as vitamins B1, B7, and B12 (Yong et al. 2021).

Radwan and Al-Hasan (2000) and Chaillan et al. (2005) reported the cyanobacteria are unable to degrade or utilize

crude oil. The blooms of cyanobacteria in the hydrocarboncontaminated area can intensively grow due to the accumulation of the organic acid produced during crude oil degradation (Hoehler et al. 2001). Baghour (2019) reported the capability of algae to degrade organic compounds in a process called phycoremediation, which showed a high capability to enhance the biodegradation of PAHs and a wide range of n-alkane compounds. It is a natural process with many advantages, such as sustainability, low impact on the environment, and environmental friendliness. Algae have many enzymes involved in the bioremediation process and produce biosurfactants, which play a critical role in accelerating the bioremediation process. Warshawsky et al. (1988) reported green algal (Selenastrum capricornutum) synthesis of dioxygenase enzymes are involved in hydrocarbon metabolic pathways, such as in the degradation of PAH compounds (Chekroun et al. 2014). Hao et al. (2020) observed the ability of marine algae Isochrysis galbana to remove 50 and 100 mg/ L of phenol within 14 and 24 h respectively under temperature 20–25°C and light intensity 180  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> in seawater. Thus, algae can enhance petroleum degradation either by direct degradation or by enhancing bacterial potential degradation (Zhang et al. 2020). The high biomass produced by algae during bioremediation is not a problem for the marine environment because it can be used to produce biofuel, which is an effective, sustainable, renewable, and ecologically friendly energy source that can mitigate climate change (Vassilev and Vassileva 2016), and reduce the amount of  $CO_2$  emitted to the atmosphere (Zhang et al. 2020), and the algal residue in seawater is easily biodegraded by microbial cells (Zhang et al. 2018a, b). Thus, the bacterial hydrocarbon degrader immobilized on algal cells. The algal cells can degrade crude oil from seawater and benefit from algal growth in seawater as a friendly-ecological energy source.

The selection of bioremediation techniques primarily depends on various factors that affect remediation efficiency and suitability. Figure 6 depicts the various factors that impact the remediation processes and the lack of information regarding the factors that can affect the process and often reduce the efficacy of the process when implemented. Hence, knowledge of the microbial composition, environmental conditions, remedial approach and method, and extended treatment time all influence the suitability and feasibility of the selected technique.

The combination of the bioremediation technique in marine environments makes the process of petroleum degradation efficient (Gaur et al. 2018; Zhang et al. 2018a, b; Zhang et al. 2020) where the environmental conditions are suitable for merging them, such as using both algal and bacterial cells together. There is a mutual relationship between the algal and bacterial cells. The algal cells provide oxygen and nutrients to bacterial cells, which support and induce bacterial cell growth and increase petroleum biodegradation, and provide algal cells with some vitamins (Xie et al. 2013). The combination of bacterial cells and algae may be better than adding nutrients to biostimulate biodegradation because the added nutrients may cause algal blooms that impact marine life and intoxicate many levels of life, influencing trophic chains (Yong et al. 2021).

# Conclusions

Marine environments polluted by petroleum hydrocarbons and their products are priority pollutants as they extend from marine organisms to human health. Bioremediation is recognized as an effective performance technique (low cost, and low in products), used to remove crude oil contamination from marine environments. Many factors affect the bioremediation of crude oil, such as the nature and composition of petroleum, distribution, and fate in marine environments, mechanisms of degradation (aerobic or anaerobic), oxygen availability, temperature, pH, pressure, biosurfactant production, and petroleum availability. All of these factors influence the success of the bioremediation process. Biodegradation is initiated when favorable environmental conditions are present. Owing to the low number of microbial hydrocarbon degraders in marine environments and the variable conditions, many strategies have been proposed to enhance bioremediation, such as biostimulation, bioaugmentation, and phytoremediation. All these techniques can be used successfully in the bioremediation of marine oil pollution. However, the environmental conditions that affect microorganism growth and activity are critical in determining the most suitable technique.

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Availability of data and materials The datasets generated and/or analyzed during the current study are available in the Saudi digital library repository (https://sdl.edu.sa/SDLPortal/ar/Publishers.aspx).

# Declaration

Ethical approval NA

**Consent to publish** All authors contributed to the research. Their contributions are attached in the author contribution file. They agreed to publish this research in Environmental Science and Pollution Research journal.

**Consent of the participant** In this research, no living participant is involved. Therefore, this is not applicable.

Human and animal rights This article does not contain any studies with human or animal subjects.

**Conflict of interest** The authors declare that they have no conflict of interest.

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