

Review

# Bioremediation of Diesel Contaminated Marine Water by Bacteria: A Review and Bibliometric Analysis

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**Abstract:** Oil pollution can cause tremendous harm and risk to the water ecosystem and organisms due to the relatively recalcitrant hydrocarbon compounds. The current chemical method used to treat the ecosystem polluted with diesel is incompetent and expensive for a large-scale treatment. Thus, bioremediation technique seems urgent and requires more attention to solve the existing environmental problems. Biological agents, including microorganisms, carry out the biodegradation process where organic pollutants are mineralized into water, carbon dioxide, and less toxic compounds. Hydrocarbon-degrading bacteria are ubiquitous in the nature and often exploited for their specialty to bioremediate the oil-polluted area. The capability of these bacteria to utilize hydrocarbon compounds as a carbon source is the main reason behind their species exploitation. Recently, microbial remediation by halophilic bacteria has received many positive feedbacks as an efficient pollutant degrader. These halophilic bacteria are also considered as suitable candidates for bioremediation in hypersaline environments. However, only a few microbial species have been isolated with limited available information on the biodegradation of organic pollutants by halophilic bacteria. The fundamental aspect for successful bioremediation includes selecting appropriate microbes with a high capability of pollutant degradation. Therefore, high salinity bacteria are remarkable microbes for diesel degradation. This paper provides an updated overview of diesel hydrocarbon degradation, the effects of oil spills on the environment and living organisms, and the potential role of high salinity bacteria to decontaminate the organic pollutants in the water environment.

**Keywords:** diesel; water pollution; bioremediation; organic pollutants; halophilic bacteria



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## 1. Introduction

The contamination of water bodies with petroleum hydrocarbons has become a serious environmental problem. This does not only increase concerns toward human health but also on the ecosystem. The potential sources of fresh and seawater contamination are believed to be oil spills from underground storage tanks, pipelines, land vehicles, accidental spills during transportation, drilling sites, and improper waste disposal practices [1,2]. In 2019, according to the statistics presented by International Tanker Owners Pollution Federation Ltd. (ITOPF), the total volume of persistent and non-persistent hydrocarbon spillages to the environment recorded was around 1000 tons. The recent statistics also reported two

medium spills and one large spill ranging from 7 to 700 tons that occurred in 2019 [3]. Nowadays, a lot of countries have strengthened the law enforcement for polluted water treatment. For example, Europe and some other countries have been using bioremediation technique to overcome the issue. This technique has shown a great impact, with notable achievements reported around the globe [4].

Bioremediation is an effective, cost-saving, and eco-friendly approach to re-establishing deteriorated environments [5]. There are several types of bioremediation, including microbial remediation (microbes), phytoremediation (plants), and mycoremediation (fungi) [6–8]. The meaning and implementation of each bioremediation type are totally different from one another in terms of mechanism. A study conducted by Lefebvre et al. [9] highlighted the advantages of using halophilic bacteria in bioremediation to substitute the expensive yet low yield of conventional methods. In fact, microbial remediation technology has helped to decontaminate the groundwater and clean up the oceans from environmental damages. Nevertheless, the growth and degradation rate of microbes influence the efficiency of bioremediation. Besides, parameters such as temperature, pH, salinity, and nitrogen or carbon sources contribute to the bacteria performance in utilizing hydrocarbons [10]. Studies carried out on bioremediation of water were not as many as compared to bioremediation in soils. Thus, more studies need to be conducted to restore the scarcity of clean water that already affects worldwide.

In recent years, halophiles have received the spotlight in industrial waste treatment as their unique physical and chemical components allow them to survive hypersaline environments and eliminate hazardous substances very well [11]. This group of microorganisms is categorized as good bioremediators for contaminated site clean-up due to their strong adaptability character. It has been reported that more than 2% of industrial effluents are saline and hypersaline [12], thus, making halophiles well suited for bioremediation and saline effluents removal. A study conducted by Le Borgne et al. [13] described the potential of halophilic bacteria, mainly *Cellulomonas* spp., *Bacillus marisflavi*, *Dietzia maris*, and *Halomonas eurihalina* to metabolize organic contaminants at 10% NaCl concentration. The use of oil and petroleum derivatives in many industrial sectors generates saline wastewater with high organic contaminants. Some examples of organic contaminants are phenols, aromatic hydrocarbons, nitroaromatics, and azo dyes [14].

## 2. Review Methodology

For the bibliometric analysis, data were collected from the online database named Scopus from the period 2011 to 2020. A total of 364 article-type documents were collected with the selected topics (TS) included the following combinations: (“diesel” AND “water pollution”) OR (“diesel” AND “marine pollution”). Software Biblioshiny and Vosviewer were used to analyze the descriptive data and conceptualize the thematic research and trend in the recent ten years [15,16].

## 3. Diesel Pollution in Water

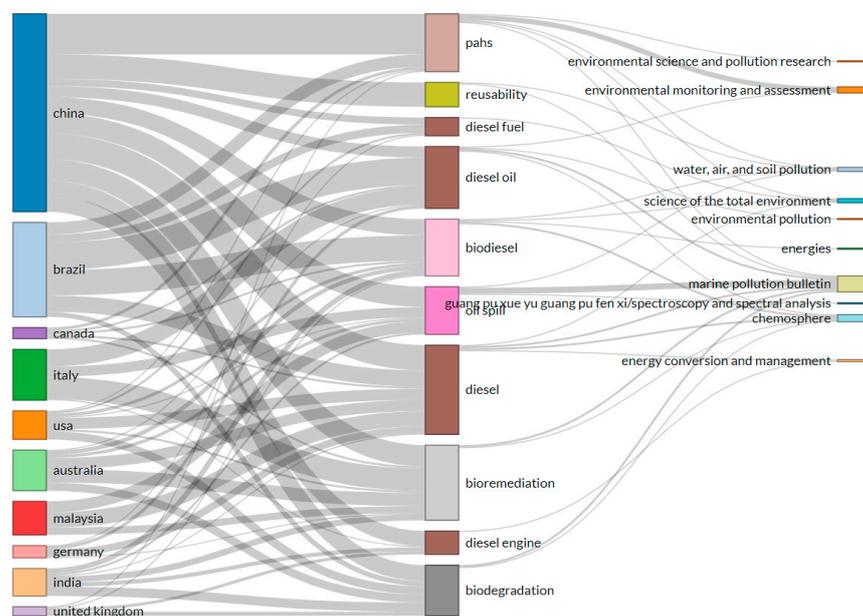
A mixture of hydrocarbon, diesel has been commonly utilized in industry and daily life as energy along with petrol, gasoline, kerosene, and natural gas [17]. The vast area of land and water bodies had to face negative impacts as a consequence of using approximately 40% of oil as a major power source [18]. Diesel spilled into groundwater and seawater will persist longer and spread immediately to a thin rainbow film and silver sheens. Spreading, dispersion, evaporation, dissolution, biodegradation, and emulsification are parts of the breaking down processes. The higher molecular fractions will sink into the deeper part of water [19–21]. In addition, diesel-polluted water is said to be more toxic than the originally spilled oil. This matter is driven by the event of physicochemical changes when mixing with water. Even though affected ecosystems have shown multiple signs of pollution, the responses and actions taken by authorities at particular sites were either too slow or too little.

Diesel comprises 64% aliphatic (mostly cycloalkanes and *n*-alkanes), 35% aromatic compounds, and 1% olefinic [22]. Diesel is always associated with hydrocarbons pollution coming from pipelines, transportation, storage tanks, and spills. From the incidents that occurred, various impacts of oil pollution pose a threat to the environment and living organisms, including flora and fauna. The insoluble and suspended oil blocks the entering of sunlight and supply of oxygen into the water causing the organisms to die, which leads to species extinction and reduced microbial population [23]. Table 1 shows the effects of oil spills on different organisms at different locations.

**Table 1.** Effects of oil spills on different organisms.

Location	Organism	Source	Effect	Reference
Tondiarpet, Chennai	Human	Benzene, toluene, ethylbenzene, xylene and naphthalene (BTEXN)	High carcinogenic risk	[24]
-	<i>Hippocampus reidi</i> (seahorse)	Benzene, toluene, ethylbenzene and xylene (BTEX)	Changes in pathological and gill epithelium morphology alteration	[25]
Van-Mijenfjorden, Svea	Bacteria, small phytoplankton and heterotrophic nanoflagellates	Polycyclic aromatic hydrocarbons (PAHs)	Changes in structure and major functional group biomass	[26]
Trondheimsfjorden, Norway	<i>Gadus morhua</i> L. (Atlantic cod)	Naphthenic crude oil	Acute and delayed toxicity, morphological deformation, spinal deformation, increased mortality rate, craniofacial, and jaw development alterations	[27]
Heishijiao, China	<i>Ulva pertusa</i> (macroalgae)	Fuel oil and diesel oil	Growth inhibition and fatty acids contents alteration	[28]
Obuaku, Abia State Nigeria	Livestock (cattle)	Polycyclic aromatic hydrocarbons (PAHs)	High non-carcinogenic risk	[29]
Skjervøy, northern Norway	<i>Mytilus edulis</i> spp. (blue mussels)	Polycyclic aromatic hydrocarbons (PAHs)	Oxidative stress and immune function modulation	[30]
Ring Road, Jos, Nigeria	Human	Benzo(a)pyrene and benzo(a)anthracene	Non-carcinogenic and carcinogenic health risk	[31]
Paranaguá, southern Brazil (subtropical Bay)	<i>Anomalocardia flexuosa</i> (clam)	Diesel oil and polycyclic aromatic hydrocarbons (PAHs)	Changes in antioxidant enzymes activity and lipid peroxides level	[32]
Iceland, Arctic	<i>Chlamys islandica</i> (icelandic scallops)	Marine diesel	Acetyl-cholinesterase activity inhibition and behavior response alteration	[33]

Within the period analyzed, several countries were quite prolific with a high number of author shares, such as China, Brazil, USA, India, Australia, and Malaysia. A three-field-plot (Sankey diagram) of Country, Keyword and Sources (journals) in Figure 1 depicts the proportion of research topics for each country and the publishing journals. Items in the top ten ranking from each metrics were selected. As shown in the figure, China has the highest publication in the past 10 years, showing a diverse range of topics. Chiefly, their research focus was heavily weighted in polycyclic aromatic hydrocarbons (PAHs) and demonstrated a strong relation with the Environmental Monitoring and Assessment journal. China is also the only country highlighting “reusability” in author’s keywords. On the other hand, bioremediation and biodegradation, which are regarded as the same subfield, received a fair share of attention from all countries. The sources where these studies were published were mainly distributed between Marine Pollution Bulletin and Chemosphere.



**Figure 1.** Relations between countries (left), author keywords (middle), and sources (right) represented in a three-field-plot. PAHs: Polycyclic aromatic hydrocarbons.

#### 4. Toxic and Hazardous Compounds in Diesel Contaminated Water

A study conducted by Ramadass et al. [34] detected the presence of main toxic compounds from diesel water accommodated fraction, surpassing the safe level limits for the aquatic environment, including benzene, toluene, ethylbenzene, and xylene (BTEX). In contrast to the long-chained hydrocarbons, these compounds have relatively low molecular weight. They are listed among harmful and important toxins that must be eliminated from industrial effluents before their release to the environment by The United States Environment Protection Agency (USEPA) [35]. Once BTEX are exposed to the environment, they will turn into vapor, while some compounds dissolve in water [36]. Higher water solubility may be detrimental and determine the rate of bioremediation uptake by respective organisms [37]. The physical and chemical composition of BTEX is shown in Table 2.

Aside from BTEX, USEPA has also reported 16 compounds of polycyclic aromatic hydrocarbons (PAHs) available in the aquatic environment that should be removed. The 16 priority PAHs have been described in a study conducted at Yinma River Basin, China. From the water samples, a total of 16 compounds were successfully extracted. Naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo (a) pyrene, benz (a) anthracene, benzo (b) fluoranthene, benzo (k) fluoranthene, dibenz (a,h) -anthracene, indeno (1,2,3-cd) pyrene, and benzo(g,h,i)perylene were the listed candidates that are believed to be originated from oil related activities and oil spills [38]. The presence of numerous PAHs in the water may cause harm to microorgan-

isms and higher organisms, including humans, since they are not only cytotoxic but also mutagenic and carcinogenic [39]. Abdel-Shafy and Mansour [40] added that PAHs became the root of cancer and mutation emergence by disturbing the immune system and damaging/excretory system (kidney). Table 3 shows the physical and chemical composition of polycyclic aromatic hydrocarbons (PAHs).

**Table 2.** The physical and chemical composition of benzene, toluene, ethylbenzene, and xylene (BTEX).

Toxic Compound Structure	Molecular Formula	Molecular Mass (g/mol)	Density at 20 °C (g/mL)	Solubility in Water at 25 °C (mg/L)	EPA Carcinogen Classification <sup>1</sup>
Benzene	C <sub>6</sub> H <sub>6</sub>	78	0.8756	1790	A
Toluene	C <sub>7</sub> H <sub>8</sub>	92	0.8623	526	D
Ethylbenzene	C <sub>8</sub> H <sub>10</sub>	106	0.8626	170	D
<i>Ortho</i> -xylene	C <sub>8</sub> H <sub>10</sub>	106	0.8801	178	D
<i>Meta</i> -xylene	C <sub>8</sub> H <sub>10</sub>	106	0.8698	161	D
<i>Para</i> -xylene	C <sub>8</sub> H <sub>10</sub>	106	0.8610	162	D

<sup>1</sup> EPA carcinogen classification (group description): A-human carcinogen, B1 and B2-probable human carcinogen, C-possible human carcinogen, D-not classifiable as to human carcinogenicity and E-evidence of non-carcinogenicity for humans.

**Table 3.** The physical and chemical composition of polycyclic aromatic hydrocarbons (PAHs).

Toxic Compound Structure	Molecular Formula	Molecular Mass (g/mol)	Density at 20 °C (g/mL)	Solubility in Water at 25 °C (mg/L)	EPA Carcinogen Concentration <sup>1</sup>
Naphthalene	C <sub>10</sub> H <sub>8</sub>	128	1.162	0.031	C
Acenaphthylene	C <sub>12</sub> H <sub>10</sub>	154	1.222	3.90	D
Fluorene	C <sub>13</sub> H <sub>10</sub>	166	-	1.69	D
Phenanthrene	C <sub>14</sub> H <sub>10</sub>	178	1.179	1.10	D
Anthracene	C <sub>14</sub> H <sub>10</sub>	178	-	-	D
Fluoranthene	C <sub>16</sub> H <sub>10</sub>	202	-	-	D
Pyrene	C <sub>16</sub> H <sub>10</sub>	202	-	0.135	D
Chrysene	C <sub>18</sub> H <sub>12</sub>	228	1.274	2.0 × 10 <sup>-3</sup>	B2
Benzo (a) pyrene	C <sub>20</sub> H <sub>12</sub>	252	1.400	1.62 × 10 <sup>-3</sup>	A
Benz (a) anthracene	C <sub>18</sub> H <sub>12</sub>	228	-	9.4 × 10 <sup>-3</sup>	B2
Benzo (b) fluoranthene	C <sub>20</sub> H <sub>12</sub>	252	-	-	B2
Benzo (k) fluoranthene	C <sub>20</sub> H <sub>12</sub>	252	-	8.0 × 10 <sup>-4</sup>	B2
Dibenz (a,h) anthracene	C <sub>22</sub> H <sub>14</sub>	278	-	2.49 × 10 <sup>-3</sup>	B2
Indeno (1,2,3-cd) pyrene	C <sub>22</sub> H <sub>14</sub>	288	-	-	B2
Benzo (g,h,i) perylene	C <sub>22</sub> H <sub>14</sub>	276	-	2.6 × 10 <sup>-4</sup>	D

<sup>1</sup> EPA carcinogen classification (group description): A: Human carcinogen, B1 and B2: Probable human carcinogen, C: Possible human carcinogen, D: Not classifiable as to human carcinogenicity, and E: Evidence of non-carcinogenicity for humans.

A wide array of processes, including diesel and petrol leaks, marine oil, gas exploration, oil spills, combustions of fossil fuels, and even nature itself, deposit aliphatic hydrocarbons to the ecosystem. Alkanes (*n*-alkanes), cycloalkanes, alkenes, and alkynes group are referred to as four main classes of aliphatic hydrocarbons [41]. In Khark Island, Iran, the potential sources and ecotoxicological risks of 26 aliphatic hydrocarbons have

been studied. Petroleum contamination was found to be the root cause due to exposure to industrial wastewater discharges, petroleum inputs, and atmospheric depositions loaded with heavy metals and organic pollutants [42,43]. On the other hand, chlorinated aliphatic hydrocarbons represented by trichloroethylene (TCE) were ranked as the ultimate contaminant in the USA groundwater for its resistance against degradation, toxicity, and carcinogenic nature [44]. Early biodegradation studies on the oil-contaminated surface of ocean water have shown a lesser extent of cyclic alkanes degradation compared to *n*-alkanes. Meanwhile, higher toxicity of cyclic alkanes has been subjected to be the basis of low degradation [45]. In hydrocarbons principle, the more complex the bond between atoms, the lower the boiling point, polarity, and water solubility [46].

The non-hydrocarbon components of diesel consist of vanadium, nickel, nitrogen, and sulfur [47,48]. Other pollutants include nitrogen oxides, particulate matter, unburned hydrocarbons, and carbon monoxide [49]. They are most likely similar to hydrocarbon components in terms of affecting human health and causing problems to the environment and global climate change. Nitrogen oxides formed by oxidation of atmospheric and nitrogen content in fuel at high temperatures are likely to produce smog and weak acid. This further leads to acid rain in waterways as well as crop pollution [50,51].

## 5. Sustainable Approach for Remediating Contaminated Water

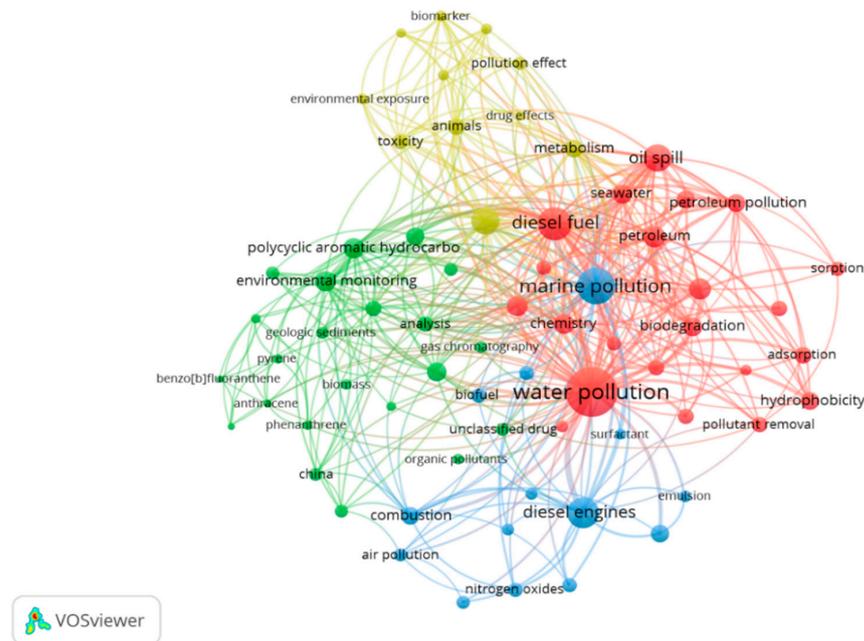
Water is the most vital among other natural resources and categorized as the crucial basic needed in life. The alarming rate of water pollution caused by nature and anthropogenic sources has become a major worry since the pollution poses a significant threat to the living creatures on earth and ecosystems. Over the years, researchers have implemented and tested diverse detoxification techniques and processes to mitigate and tackle the current issues [52]. A few examples of water recovery treatment conducted in several countries include advanced oxidation, adsorption, membrane bioreactor, neutralization of acid and base in the effluent as well as chemical treatment and incineration [53]. Unfortunately, such conventional and physical methods showed some serious drawbacks, such as slow progress, high cost, production of secondary intermediates, and inefficient removal of contaminants from the environment [54].

Therefore, in such cases, bioremediation is an acceptable practice or solution over conventional methods, which can be referred to as a natural purifier to reclaim contaminated environments using biological tools [55]. Moreover, bioremediation is the most promising biotechnology tool where the by-products (example: Carbon dioxide, water, fatty acids, and inorganic salts) formed are less hazardous than original compounds [56]. The technical aspect of bioremediation involves a number of mechanisms [57]. The mechanisms are biosequestration, biodegradation, phytohydraulics, biological extraction, and volatilization, in which microbes or plants are used to help immobilize the pollutants, remediating the water and soil. Following this, the ability of microbes, including bacteria, fungi, algae, and engineered microbes, to neutralize the soil and the aquatic environment for bioremediation purposes has been studied extensively [58].

At present, the application of both microbes and fungi has made a big impact by producing a combination of a clean and healthy environment as the end result [59]. According to Dangi et al. [60], microbes produce enzymes and play important roles when metabolic reactions take place. Microbes will attack, degrade, and transform the pollutants completely, hence making microbial bioremediation more favorable. Despite the successful results reported, bioremediation does lack in a way that is closely related to the biological and environmental factors [61–63]. The environmental factors affecting the rate of bioremediation are pH, temperature, pressure, as well as nutrient source [64]. Further limitations of bioremediation include the bioavailability, bioactivity and biochemistry of the systems [65].

From the pools of keywords collected in the database, co-occurrence mapping was created to conceptualize the research subfield. Distinct clusters can be identified in Figure 2 with four colors assigned to individual keywords of a high level of similarity. The size of the circle corresponded to the frequency of co-word while the thickness of the line

represents topic similarity and relative strength. Based on the clusters generated, four core topics can be deduced, which are red-bioremediation, yellow-diesel toxicity, green-environmental monitoring, and blue-diesel engine design. The figure demonstrates a considerable diversity of research themes in the relevant field.



**Figure 2.** Co-occurrence mapping based on keyword plus (min. number of occurrences: Five).

The trend of hot topics throughout the latest 10 years is tabulated in Table 4. During the period from 2011 to 2013, keywords related to diesel toxicity and toxicology screening reflected the on-trend study of diesel pollution effect on animals and other aquatic organisms. A more in-depth assessment was investigated in the following years involving source apportionment and the trend slowly evolved to the treatment of oil-water emulsion using adsorption. From 2016 onwards, bioremediation became the greatest interest among the scientific communities for its environmental sustainability in mitigating hydrocarbon pollution. In recent years, more innovative technologies, such as superhydrophobic materials, the discovery of new microorganisms in bioremediation, production of green energy, and optimization of diesel engines, emerged as the research front contributing to the stream of research.

**Table 4.** Trend topics from 2011 to 2020 based on author’s keywords. Items shown have word minimum frequency of 2.

Year	Items
2011	Toxicity, apoptosis, DNA damage
2012	Algae, biofuels, water-diesel emulsion, flow-cytometry-based in vitro micronucleus (MN) assay, soybean biodiesel
2013	Biomarker, heavy metals, PAH, <i>Pseudomonas aeruginosa</i>
2014	Pollution, source apportionment, sorption capacity, <i>Scirpus grossus</i> , oil sorption capacity
2015	Diesel engine, oxidative stress, biomarkers, emissions, kapok fiber
2016	Diesel, polycyclic aromatic hydrocarbons (PAHs), biodiesel, biodegradation, bioremediation
2017	Oil spill, diesel fuel, diesel oil, seawater, emission
2018	Water pollution, water, oil-water separation, oil removal, gasoline, sediment, risk assessment, water contamination
2019	Marine diesel engine, life cycle assessment, ozone, particles, sawdust, fish
2020	Oil/water separation, superhydrophobic, oil sorption, marine engine

## 6. Microbial Remediation Technologies

Indigenous microbial communities play an important role in diesel pollutant remediation as they fulfill the increasing demand for minimizing water pollution. Successful large-scale treatment of petroleum hydrocarbons contamination has been reported to be accomplished through microbial based bioremediation mostly in oceans, wastewater treatment in polar regions, removal of toxic pollutants from agro-industrial wastes, and treatment of polluted shorelines. Through the attenuation process, microbial remediation is generally able to degrade oil naturally. However, due to the nutritional imbalance in the ecosystem, the required and expected outcomes are yet to be achieved and certainly time-consuming [66].

Recently, several studies highlighted the bioremediation through microorganisms offering environmentally safe, cost-effective, and highly efficient techniques in degrading hydrocarbons as well as alkanes and polyaromatic compounds and several other pollutants. Microorganisms that manage to degrade hydrocarbons at a high rate have been reported in past research, namely *Acinetobacter haemolyticus* [67], *Mycobacterium* sp. [68], *Pseudomonas aeruginosa* [69], *Bacillus subtilis*, *Klebsiella* sp., *Acinetobacter junii*, *Acinetobacter* sp. [70], *Rhodococcus* sp., *Azotobacter vinelandii* [71], and *Bacillus megaterium*, *Acinetobacter iwoffii* [72]. The susceptibility of hydrocarbons to microbial degradation was ranked in a descending order. The order starts with linear alkanes followed by branched alkanes, small aromatics, cyclic alkanes and polyaromatic hydrocarbons [58,73].

## 7. Potential Role of Halophilic Bacteria as Bioremediatory Agent

In diesel bioremediation, diesel-degrading bacteria are used to eliminate pollutants from contaminated waters. Halophilic or salt-loving bacteria are very divergent. They are also those that can be found in water bodies with five times greater salt concentration than the ocean water. There are about 70 genera with over 150 species of them reported. They are classified into different groups based on the salt component requirements [74]. The groups have slight halophiles with 2–5% NaCl, moderate halophiles with 5–20% NaCl, extreme halophiles with 20–30% NaCl and halotolerant microorganisms, which are the strains that grow in between 0–5% salinity [75]. Additionally, they offer potential applications in various fields of biotechnology. The degradation or transformation of a range of organic and inorganic pollutants is among the application fields of these groups of halophiles. High salinity and nutrient availability (nitrogen and phosphorus) act as the factors limiting the biodegradation process by microorganisms in polluted areas.

Oil spillage commonly occurs in the water or sea via transportation. Consequently, scientists and researchers conducted countless studies on microbial degradation of hydrocarbon in the marine environment with varying NaCl concentration. Red Sea in Jeddah, Saudi Arabia was one of the marine environments that are greatly affected by petroleum hydrocarbons due to extensive human activities. Jamal and Pugazhendi [76] reported that the halophilic bacteria consortium, *Ochrobactrum halosaudis*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Mesorhizobium halosaudis* from water samples of Red Sea, had successfully degraded different PAHs (phenanthrene, fluorene and pyrene) with different molecular weights under 40 g/L of NaCl concentration. About 40% of diesel oil components were successfully removed by halophilic consortium and the dominant bacterial isolates belonged to the following genera: *Dietzia*, *Arthrobacter* and *Halomonas* [77].

*Halomonas* are the recognized and well-known moderately halophilic bacteria for their capability to degrade hydrocarbons under extreme conditions [78–82]. Apart from *Halomonas*, *Arthrobacter* sp. has been also reported in previous studies for its proficiency to degrade aliphatic and aromatic compounds [83]. An extremely halophilic bacteria, *Natrialba* sp. utilized phenol, naphthalene, and pyrene as sole carbon sources in the degradation at 25% NaCl salinity that was done via *ortho*-cleavage pathway [84]. Both chrysene and pyrene are not easily degraded, however, *Hortaea* sp. managed to achieve up to 77% and 92% rate of degradation for chrysene and pyrene in the aquatic environment [85]. Wang et al. [86] reported that *Marinobacter* contributed to phenanthrene degradation with

salinities ranging between 3–20% while maintaining the community structure in high salinity condition. Jamal [87] added that *Marinobacter* consortium, including *Marinobacter hydrocarbonoclasticus* and *Marinobacter* sp., utilized phenanthrene together with pyrene under 4% saline conditions. The phenanthrene was completely removed over time despite the low removal rate as the salinity increased.

Another halophile, *Vibrio alginolyticus* also optimized the diesel degradation as high as 98.20% and converted the initial compounds into carbon dioxide, water, and several intermediates [88,89]. *Alcanivorax* genera dominated marine environments suffering from oil contamination by utilizing hydrocarbons as the major carbon source, producing biosurfactants and forming biofilms around the oil droplets and oil-water interface [90]. *Halorientalis hydrocarbonoclasticus* sp. has been found as the first hexadecane-degrading strain among *Halorientalis* genus, with successful degradation reported at 57% in the presence of 3.6 M NaCl at oil-polluted hypersaline environments [91]. The low (naphthalene, phenanthrene, anthracene, and fluorene) and high (pyrene, benzo (e) pyrene, and benzo (k) fluoranthene) molecular weight PAHs have also been efficiently degraded (above 90%) by promising bacterial strains, such as *Ochrobactrum* and *Pseudomonas* under various saline conditions (4% to 30%) [92]. Interestingly, a number of reports claimed that the biodegradation of hydrocarbons is enhanced when salinity becomes higher [11]. Table 5 shows more examples of halophilic microorganisms that have been reported.

**Table 5.** List of halophilic microorganisms and the hydrocarbon degraded.

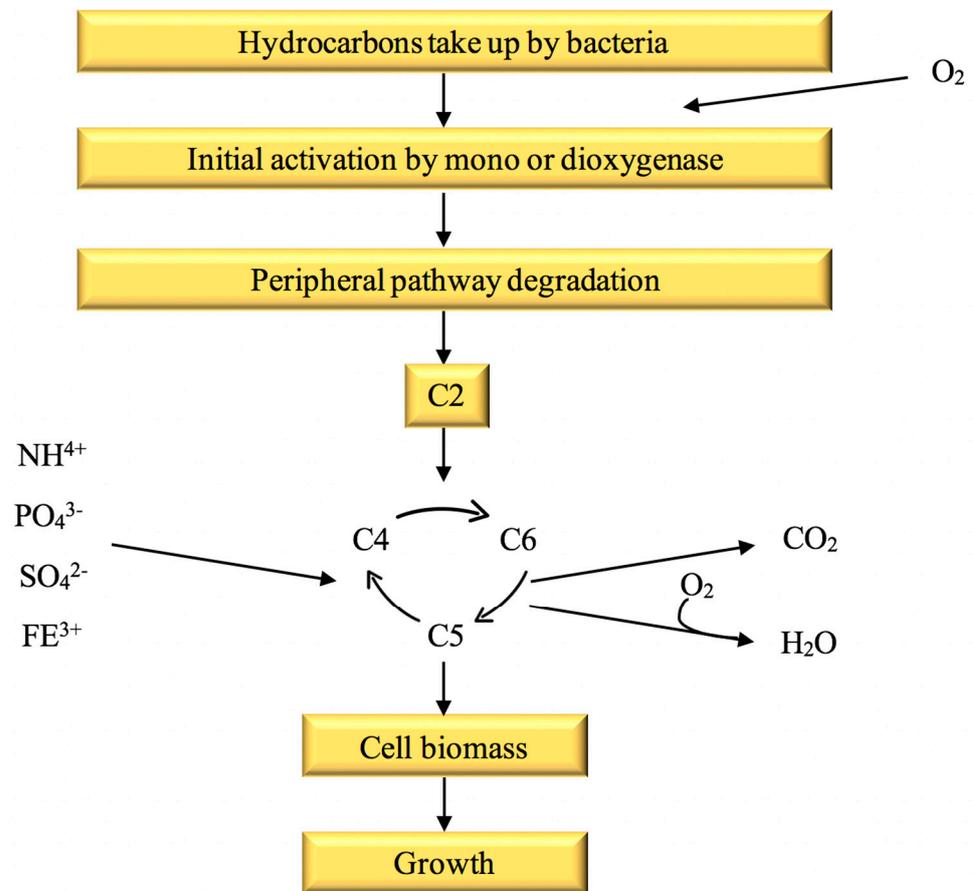
Microbial Species	Salinity Condition g/L	Hydrocarbon Degraded	Reference
<i>Martellella</i> sp.	1–150	Phenanthrene	[93]
<i>Halobacterium piscisalsi</i> <i>Halorubrum ezzemoulense</i> <i>Halobacterium salinarium</i> <i>Haloarcula hispanica</i> <i>Haloferax</i> sp. <i>Halorubrum</i> sp. <i>Haloarcula</i> sp.	200	<i>p</i> -hydroxybenzoic acid Naphthalene Phenanthrene Pyrene	[94]
<i>Achromobacter</i> sp. <i>Marinobacter</i> sp. <i>Rhodanobacter</i> sp.	30, 60, and 90	Benzo (e) pyrene Phenanthrene	[95]
<i>Chromohalobacter</i> sp.	200	<i>p</i> -hydroxybenzoic acid Naphthalene Phenanthrene Pyrene	[96]
<i>Pseudomonas</i> sp. <i>Staphylococcus</i> sp.	10–50	Phenanthrene	[97]
<i>Modicisalibacter</i> sp. <i>Brevibacterium</i> sp. <i>Idiomarina</i> sp.	90	Pyrene Benzopyrene Phenanthrene Naphthalene Phenol Hexadecane	[98]
<i>Marinobacter</i> sp. <i>Halomonas</i> sp.	50	Phenanthrene	[99]
<i>Staphylococcus pasteurii</i>	100	Phenanthrene Fluoranthene Pyrene	[100]
<i>Rhodococcus erythropolis</i>	36	<i>n</i> -alkane	[101]

## 8. Mechanisms Involved in Diesel Bioremediation by Bacteria

Degradation of hydrocarbons by microorganisms occurs as a result of catalysis by intracellular enzymes that involves four important steps [102]. The first crucial step of the process starts with microorganisms taking up pollutants and secreting surfactants to facilitate emulsification [103,104]. Next, the emulsified pollutants are adsorbed on the surface of the cell membrane. Then, they will directly enter the cell membrane by endocytosis in the form of active or passive transport and undergo an enzymatic reaction with the respective enzymes as catalysts to complete the overall process [105].

Alkanes generally undergo terminal or subterminal oxidation mediated by diverse alkane hydroxylase systems, including methane monooxygenases (mmo genes, pmoA [106]), integral membrane non-heme iron alkane hydroxylases (or alkane monooxygenases, alkB [107,108]), and cytochrome P450-type alkane hydroxylases (CYP153 genes [109]). Oxidation products are further oxidized by alcohol and aldehyde dehydrogenases, while the resulting fatty acids enter the tricarboxylic acid (TCA) cycle. Anaerobic degradation pathways in terrestrial environments have received less attention. In anaerobic oxidation of alkanes, sulfate or nitrate acts as an electron acceptor and it has been suggested that alkane activation occurs by the addition of a C group. Principally, one important function of aerobic bioremediation is the clean-up of spilled oil in ocean water. The quick and whole degradation of most organic contaminants occurs under aerobic conditions, which is mostly influenced by the nature and quantity of the contaminants present. Another significant influence could be the limited availability of microorganisms, either low biodiversity of local microbes or the scarcity of local specialized microbes at polluted sites. These microbes have been said to have the supplementary substrate properties required for multiple hydrocarbons degradation at the site [110]. In addition, the consortium bacteria have shown a higher possibility to metabolize hydrocarbons compared to the individual cultures that belong to the same or different genera [111]. For example, *Pseudomonas* sp., *Micrococcus* sp., *Staphylococcus* sp., *Bacillus* sp., *Flavobacterium* sp., *Achromobacter* sp., *Klebsiella* sp., *Actinomyces* sp., *Acetobacter* sp., and *Rhodococcus* sp. were the consortium isolated from diesel-contaminated environments. They went through a couple of processes to break down the hydrocarbons in diesel and utilize them as their carbon sources [112,113].

Polycyclic aromatic hydrocarbons (PAHs) are notable as one of the most dangerous aspects of spilled oil toward environment. Interestingly, bacteria favor the degradation of PAHs under aerobic conditions. The aerobic bacterial PAHs degradation begins with hydrocarbons making the first intracellular attack via oxidation, activation, and incorporation of oxygen [114]. Through hydroxylation of an aromatic ring from substrate, a *cis*-dihydrodiol was formed. This event is catalyzed by dioxygenase. Afterward, *cis*-dihydrodiol gets subsequently dehydrogenated to a diol intermediate by an enzyme action that is dehydrogenase. The metabolites may then be cleaved by ring-cleaving dioxygenases through *ortho*-cleavage or *meta*-cleavage pathway. The previous reaction leads to the production of other intermediates, such as salicylic acid and catechol, whereas the following conversion leads to TCA cycle intermediates production [115–119]. At the end of PAHs' aerobic metabolism, intermediates are not the only product, but also biomass and final metabolic products, such as oxygen and carbon dioxide. The basic principle of aerobic degradation of PAHs is shown in Figure 3.



**Figure 3.** The principle of polyaromatic hydrocarbons (PAHs) aerobic degradation.

According to Dhar et al. [120] and Boll et al. [121], aside from aerobic degradation, certain bacteria can also degrade PAHs under anaerobic, nitrate-reducing, sulphate-reducing, metal-reducing, and methanogenic conditions [122]. Koshlaf and Ball [123] added another common method use for bioremediation of PAHs, which is through the cytochrome P450 monooxygenase pathway.

Alkanes are energy-rich organic compounds that are widespread in nature and also listed as the dominant constituents of diesel fuel. For *n*-alkanes ( $C_8$ – $C_{40}$ ), the aerobic degradation is divided into three oxidation modes [124]. These modes are known as terminal oxidation [125], subterminal oxidation [126], and biterminal oxidation [127]. The oxidation modes take place at different carbon positions and form various end products. For instance, the sequential oxidation of terminal carbon produces primary alcohol, which is further oxidized into aldehydes and fatty acids before entering beta oxidation [128]. Fatty acids are conjugated into CoA beforehand while acetyl-CoA is produced from beta oxidation [129]. The summary of three degradation pathways of *n*-alkanes is shown in Figure 4.

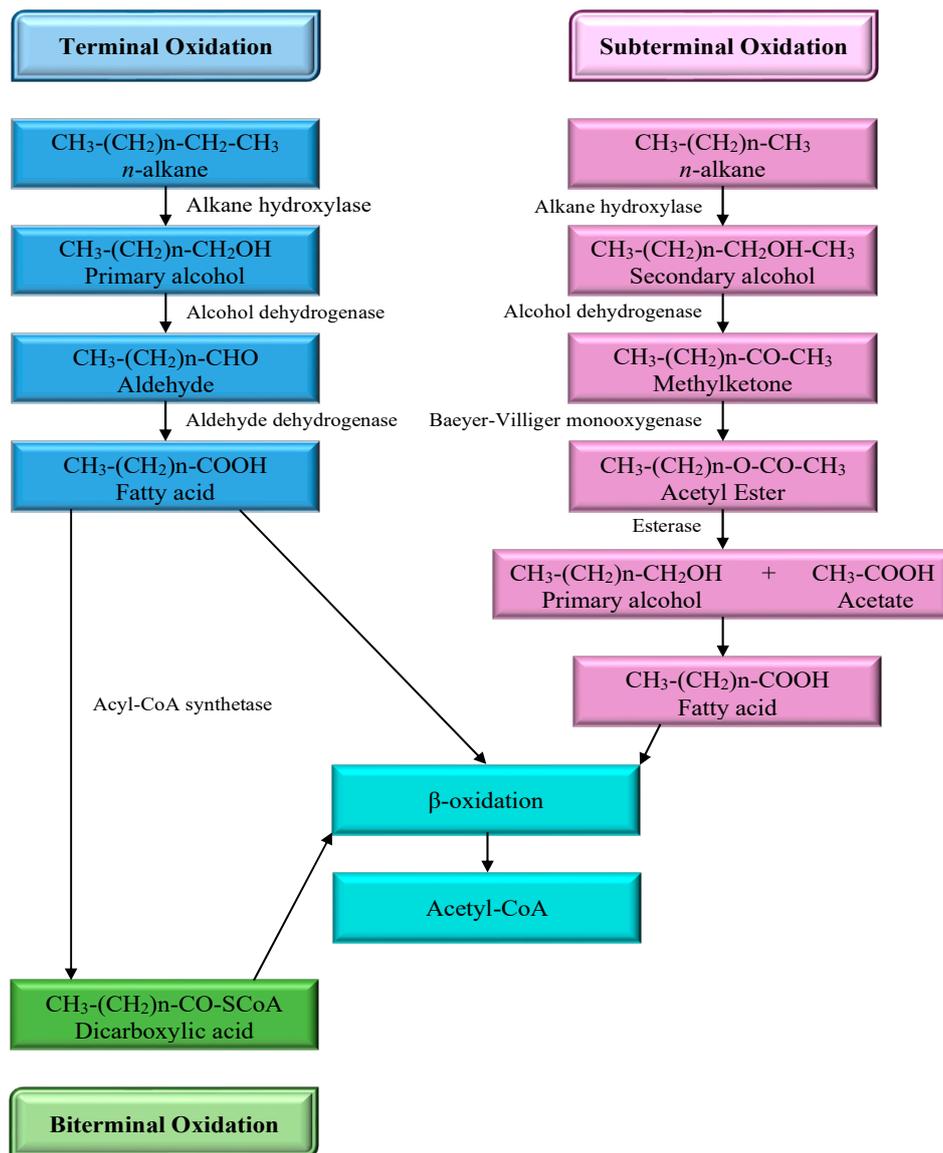
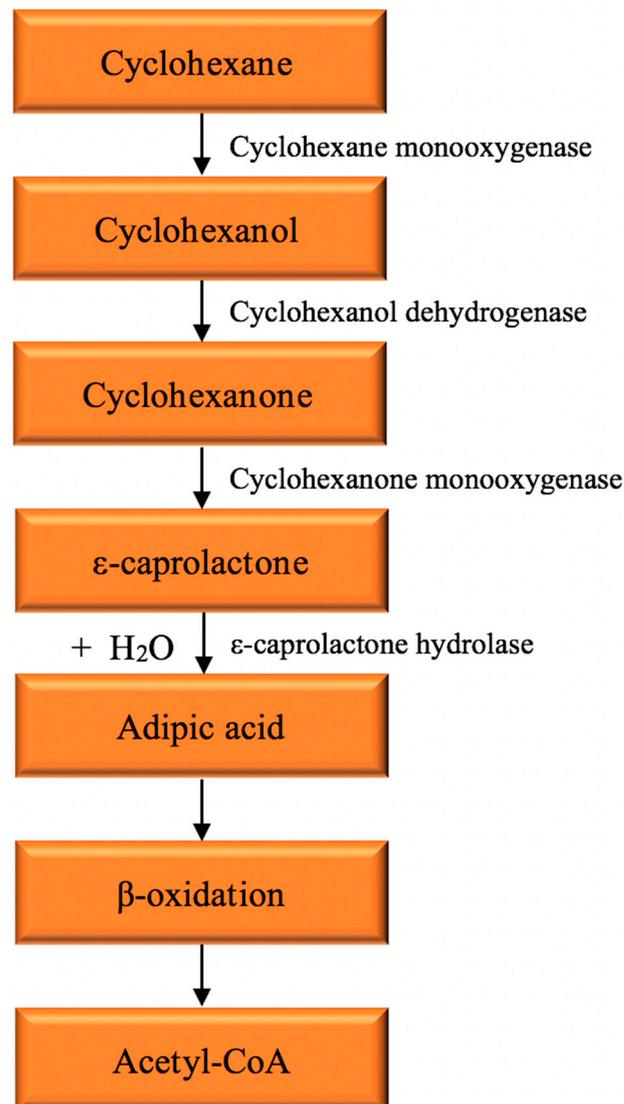


Figure 4. The summary of three degradation pathways of *n*-alkanes.

Referring to Figure 5, cycloalkane degradation has a slight contrast mechanism with *n*-alkanes. The cycloalkane is firstly oxidized by the enzyme called hydroxylase to form a cycloalkanol. Cycloalkanol is produced from the reaction of enzyme attacking the alkyl group when oxygen is present. The dehydrogenation of cycloalkanol then produces naphthenic ketone. Next, with the help of oxygenase enzyme, the naphthenic ketone undergoes conversion to produce  $\epsilon$ -caprolactone. The addition of water molecule ( $H_2O$ ) breaks the ring of  $\epsilon$ -caprolactone and generates adipic acid as an intermediate compound. The adipic acid then goes through beta oxidation and generates Acetyl-CoA that could be used by bacteria to maintain their life activities [130–132]. However, only certain bacteria species can use cycloalkanes directly as a sole carbon source for their metabolism.



**Figure 5.** The mechanism of cycloalkane degradation.

## 9. Conclusions

Water pollution is not a localized problem, but rather a genuine global problem. Clean water will surely help to maintain survival with less rendering toxic and risk toward human health and the aquatic environment. Hence, it is highly necessary to decontaminate diesel hydrocarbons, which mainly incorporate aromatic and aliphatic compounds deposited in the water bodies. Bioremediation is one of the possible solutions to reclaim polluted water. Evidently, the high salinity bacteria could be effectively used to degrade various organic pollutants in hypersaline or saline environments. The aid of microbes in the removal of spilled oil is possible since they acquire the vital enzymes (e.g.,: oxygenase and dehydrogenase) responsible as catalysts to degrade and utilize the hydrocarbons as a carbon source and energy. Based on previous research, the advantages from the implication of microbes, notably halophiles, are significant. A deeper and better understanding of biodegradation mechanisms holds significant ecological importance that highly dependent on the actions of microbes to convert or mineralize the organic pollutants. Even so, the factors that may influence the biodegradation process should be considered to prevent manipulating factors from monopolizing the process. In conclusion, based on the present review, microbial degradation could be a key player in the cleaning measures for diesel hydrocarbon remediation.

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