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Review Article

Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview

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One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. Accidental releases of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Currently accepted disposal methods of incineration or burial insecure landfills can become prohibitively expensive when amounts of contaminants are large. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites have limited effectiveness and can be expensive. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants. This paper presents an updated overview of petroleum hydrocarbon degradation by microorganisms under different ecosystems.

1. Introduction

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year [1]. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [2]. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations [3]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants.

The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry [4]. In addition, bioremediation technology is believed to be noninvasive and relatively cost-effective [5]. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment [6] and is cheaper than other remediation technologies [7].

The success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment. Numerous scientific review articles have covered various factors that influence the rate of oil biodegradation [7–12]. One important requirement is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9. The physical and chemical characteristics of the oil and oil surface area are also important determinants of bioremediation success. There are the two main approaches to oil spill bioremediation: (a) bioaugmentation, in which known oil-degrading bacteria are added to supplement the existing microbial population, and (b) biostimulation, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting cosubstrates.

The success of bioremediation efforts in the cleanup of the oil tanker Exxon Valdez oil spill of 1989 [13] in Prince William Sound and the Gulf of Alaska created tremendous interest in the potential of biodegradation and bioremediation technology. Most existing studies have concentrated on evaluating the factors affecting oil bioremediation or testing favored products and methods through laboratory studies [14]. Only limited numbers of pilot scale and field trials have provided the most convincing demonstrations of this technology which have been reported in the peer-reviewed literature [15–18]. The scope of current understanding of oil bioremediation is also limited because the emphasis of most of these field studies and reviews has been given on the evaluation of bioremediation technology for dealing with large-scale oil spills on marine shorelines.

This paper provides an updated information on microbial degradation of petroleum hydrocarbon contaminants towards the better understanding in bioremediation challenges.

2. Microbial Degradation of Petroleum Hydrocarbons

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Petroleum hydrocarbons can be divided into four classes: the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) [19]. Different factors influencing hydrocarbon degradation have been reported by Cooney et al. [20]. One of the important factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Petroleum hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded [21]. Hydrocarbons differ in their susceptibility to microbial attack. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes [6, 22]. Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all [23].

Microbial degradation is the major and ultimate natural mechanism by which one can cleanup the petroleum hydrocarbon pollutants from the environment [24–26]. The recognition of biodegraded petroleum-derived aromatic hydrocarbons in marine sediments was reported by Jones et al. [27]. They studied the extensive biodegradation of alkyl aromatics in marine sediments which occurred prior to detectable biodegradation of n-alkane profile of the crude oil and the microorganisms, namely, *Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and Rhodococcus* were found to be involved for alkylaromatic degradation. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria was reported by Adebusoye et al. [28]. Nine bacterial strains, namely, *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus, and Corynebacterium* sp. were isolated from the polluted stream which could degrade crude oil.

Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi. The reported efficiency of biodegradation ranged from 6% [29] to 82% [30] for soil fungi, 0.13% [29] to 50% [30] for soil bacteria, and 0.003% [31] to 100% [32] for marine bacteria. Many scientists reported that mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil [33], fresh water [34], and marine environments [35, 36].

Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [37, 38]. Several bacteria are even known to feed exclusively on hydrocarbons [39]. Floodgate [36] listed 25 genera of hydrocarbon degrading bacteria and 25 genera of hydrocarbon degrading fungi which were isolated from marine environment. A similar compilation by Bartha and Bossert [33] included 22 genera of bacteria and 31 genera of fungi. In earlier days, the extent to which bacteria, yeast, and filamentous fungi participate in the biodegradation of petroleum hydrocarbons was the subject of limited study, but appeared to be a function of the ecosystem and local environmental conditions [7]. Crude petroleum oil from petroleum contaminated soil from North East India was reported by Das and Mukherjee [40]. *Acinetobacter* sp. was found to be capable of utilizing n-alkanes of chain length C_{10}–C_{40} as a sole source of carbon [41]. Bacterial genera, namely, *Gordonia*, *Brevibacterium*, *Aeromicrobium, Dietzia*, *Burkholderia,* and *Mycobacterium* isolated from petroleum contaminated soil proved to be the potential organisms for hydrocarbon degradation [42]. The degradation of polyaromatic hydrocarbons by *Sphingomonas* was reported by Daugulis and McCracken [43].

Fungal genera, namely, *Amorphoteca*, *Neosartorya*, *Talaromyces,* and *Graphium* and yeast genera, namely, *Candida, Yarrowia,* and *Pichia* were isolated from petroleum-contaminated soil and proved to be the potential organisms for hydrocarbon degradation [42]. Singh [44] also reported a group of terrestrial fungi, namely, *Aspergillus*, *Cephalosporium,* and *Pencillium* which were also found to be the potential degrader of crude oil hydrocarbons. The yeast species, namely, *Candida lipolytica, Rhodotorula mucilaginosa, Geotrichum* sp, *and Trichosporon mucoides* isolated from contaminated water were noted to degrade petroleum compounds [45].

Though algae and protozoa are the important members of the microbial community in both aquatic and terrestrial ecosystems, reports are scanty regarding their involvement in hydrocarbon biodegradation. Walker et al. [51] isolated an alga, *Prototheca zopfi* which was capable of utilizing

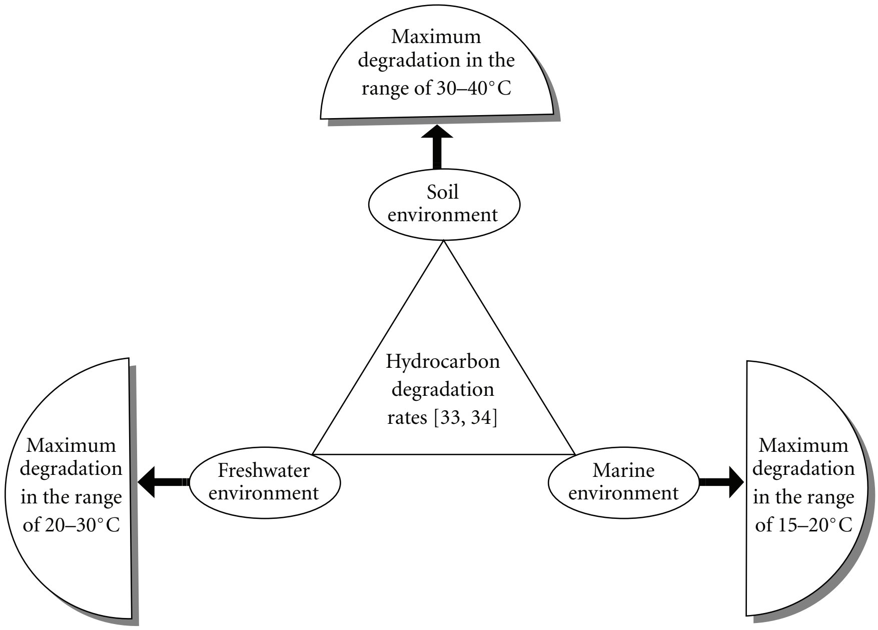

Figure 1: Hydrocarbon degradation rates in soil, fresh water, and marine environments.

crude oil and a mixed hydrocarbon substrate and exhibited extensive degradation of n-alkanes and isoalkanes as well as aromatic hydrocarbons. Cerniglia et al. [52] observed that nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms could oxidize naphthalene. Protozoa, by contrast, had not been shown to utilize hydrocarbons.

3. Factors Influencing Petroleum Hydrocarbon Degradation

A number of limiting factors have been recognized to affect the biodegradation of petroleum hydrocarbons, many of which have been discussed by Brusseau [53]. The composition and inherent biodegradability of the petroleum hydrocarbon pollutant is the first and foremost important consideration when the suitability of a remediation approach is to be assessed. Among physical factors, temperature plays an important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Atlas [54] found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation.

Temperature also affects the solubility of hydrocarbons [62]. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. Figure 1 shows that highest degradation rates that generally occur in the range 30–40°C in soil environments, 20–30°C in some freshwater environments and 15–20°C in marine environments [33, 34]. Venosa and Zhu [63] reported that ambient temperature of the environment affected both the properties of spilled oil and the activity of the microorganisms. Significant biodegradation of hydrocarbons have been reported in psychrophilic environments in temperate regions [64, 65].

Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus, and in some cases iron [34]. Some of these nutrients could become limiting factor thus affecting the biodegradation processes. Atlas [35] reported that when a major oil spill occurred in marine and freshwater environments, the supply of carbon was significantly increased and the availability of nitrogen and phosphorus generally became the limiting factor for oil degradation. In marine environments, it was found to be more pronounced due to low levels of nitrogen and phosphorous in seawater [36]. Freshwater wetlands are typically considered to be nutrient deficient due to heavy demands of nutrients by the plants [66]. Therefore, additions of nutrients were necessary to enhance the biodegradation of oil pollutant [67, 68]. On the other hand, excessive nutrient concentrations can also inhibit the biodegradation activity [69]. Several authors have reported the negative effects of high NPK levels on the biodegradation of hydrocarbons [70, 71] especially on aromatics [72]. The effectiveness of fertilizers for the crude oil bioremediation in subarctic intertidal sediments was studied by Pelletier et al. [64]. Use of poultry manure as organic fertilizer in contaminated soil was also reported [73], and biodegradation was found to be enhanced in the presence of poultry manure alone. Maki et al. [74] reported that photo-oxidation increased the biodegradability of petroleum hydrocarbon by increasing its bioavailability and thus enhancing microbial activities.

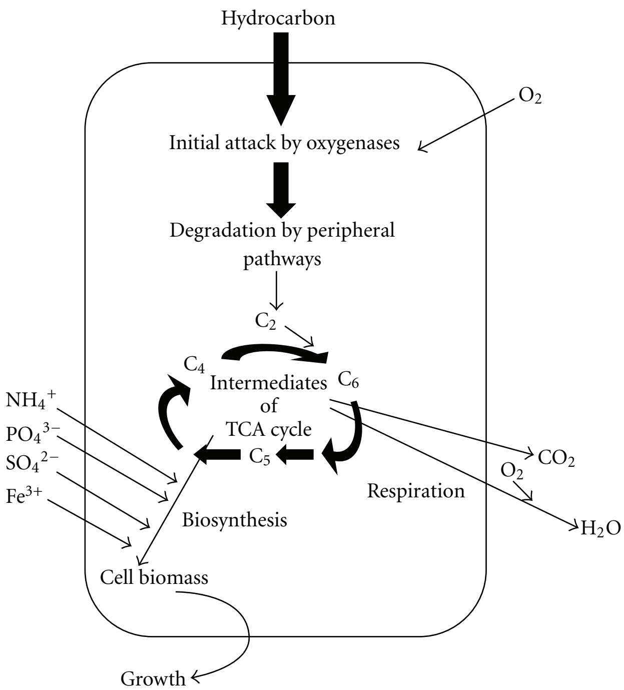

Figure 2: Main principle of aerobic degradation of hydrocarbons by microorganisms.

4. Mechanism of Petroleum Hydrocarbon Degradation

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. Figure 2 shows the main principle of aerobic degradation of hydrocarbons [75]. The initial intracellular attack of organic pollutants is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases. Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various biosyntheses and growth are synthesized by gluconeogenesis.

The degradation of petroleum hydrocarbons can be mediated by specific enzyme system. Figure 3 shows the initial attack on xenobiotics by oxygenases [75]. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants [76]. The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but production of biosurfactants has been well studied.

5. Enzymes Participating in Degradation of Hydrocarbons

Cytochrome P450 alkane hydroxylases constitute a super family of ubiquitous Heme-thiolate Monooxygenases which play an important role in the microbial degradation of oil, chlorinated hydrocarbons, fuel additives, and many other compounds [77]. Depending on the chain length, enzyme systems are required to introduce oxygen in the substrate to initiate biodegradation (Table 1). Higher eukaryotes generally contain several different P450 families that consist of large number of individual P450 forms that may contribute as an ensemble of isoforms to the metabolic conversion of given substrate. In microorganisms such P450 multiplicity can only be found in few species [78]. Cytochrome P450 enzyme systems was found to be involved in biodegradation of petroleum hydrocarbons (Table 1). The capability of several yeast species to use n-alkanes and other aliphatic hydrocarbons as a sole source of carbon and energy is mediated by the existence of multiple microsomal Cytochrome P450 forms. These cytochrome P450 enzymes had been isolated from yeast species such as *Candida maltosa*, *Candida tropicalis*, and *Candida apicola* [79]. The diversity of alkaneoxygenase systems in prokaryotes and eukaryotes that are actively participating in the degradation of alkanes under aerobic conditions like Cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g., *alkB*), soluble di-iron methane monooxygenases, and membrane-bound copper containing methane monooxygenases have been discussed by Van Beilen and Funhoff [80].

6. Uptake of Hydrocarbons by Biosurfactants

Biosurfactants are heterogeneous group of surface active chemical compounds produced by a wide variety of microorganisms [57, 58, 60, 81–83]. Surfactants enhance solubilization and removal of contaminants [84, 85]. Biodegradation is also enhanced by surfactants due to increased bioavailability of pollutants [86]. Bioremediation of oil sludge using biosurfactants has been reported by Cameotra and Singh [87]. Microbial consortium consisting of two isolates of *Pseudomonas aeruginosa* and one isolate *Rhodococcus erythropolis* from soil contaminated with oily sludge was used in this study. The consortium was able to degrade 90% of hydrocarbons in 6 weeks in liquid culture. The ability of the consortium to degrade sludge hydrocarbons was tested in two separate field trials. In addition, the effect of two additives (a nutrient mixture and a crude biosurfactant preparation on the efficiency of the process was also assessed. The biosurfactant used was produced by a consortium member and was identified as being a mixture of 11 rhamnolipid congeners. The consortium degraded 91% of the hydrocarbon content of soil contaminated with 1% (v/v) crude oil sludge in 5 weeks. Separate use of any one additive along with the consortium brought about a 91–95% depletion of the hydrocarbon content in 4 weeks, with the crude biosurfactant preparation being a more effective enhancer of degradation. However, more than 98% hydrocarbon depletion was obtained when both additives were added together with the consortium. The data substantiated the use of a crude biosurfactant for hydrocarbon remediation.

Pseudomonads are the best known bacteria capable of utilizing hydrocarbons as carbon and energy sources and

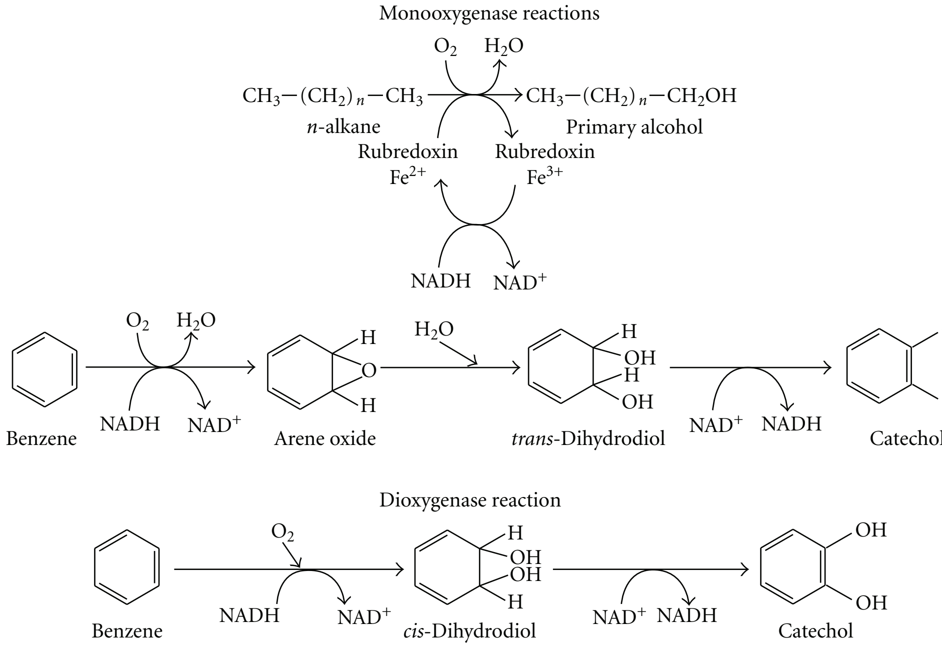

Figure 3: Enzymatic reactions involved in the processes of hydrocarbons degradation.

Table 1: Enzymes involved in biodegradation of petroleum hydrocarbons.

Enzymes	Substrates	Microorganisms	References
Soluble Methane Monooxygenases	C_1–C_8 alkanes alkenes and cycloalkanes	*Methylococcus* *Methylosinus* *Methylocystis* *Methylomonas* *Methylocella*	McDonald et al. [46]
Particulate Methane Monooxygenases	C_1–C_5 (halogenated) alkanes and cycloalkanes	*Methylobacter* *Methylococcus,* *Methylocystis*	McDonald et al. [46]
AlkB related Alkane Hydroxylases	C_5–C_{16} alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	*Pseudomonas* *Burkholderia* *Rhodococcus,* *Mycobacterium*	Jan et al. [47]
Eukaryotic P450	C_{10}–C_{16} alkanes, fatty acids	*Candida maltosa* *Candida tropicalis* *Yarrowia lipolytica*	Iida et al. [48]
Bacterial P450 oxygenase system	C_5–C_{16} alkanes, cycloalkanes	*Acinetobacter* *Caulobacter* *Mycobacterium*	Van Beilen et al. [49]
Dioxygenases	C_{10}–C_{30} alkanes	*Acinetobacter sp.*	Maeng et al. [50]

producing biosurfactants [37, 87–89]. Among *Pseudomonads*, *P. aeruginosa* is widely studied for the production of glycolipid type biosurfactants. However, glycolipid type biosurfactants are also reported from some other species like *P. putida* and *P. chlororaphis*. Biosurfactants increase the oil surface area and that amount of oil is actually available for bacteria to utilize it [90]. Table 2 summarizes the recent reports on biosurfactant production by different microorganisms. Biosurfactants can act as emulsifying agents by decreasing the surface tension and forming micelles. The microdroplets encapsulated in the hydrophobic microbial cell surface are taken inside and degraded. Figure 4 demonstrates the involvement of biosurfactant (rhamnolipids) produced by *Pseudomonas sp*. and the mechanism of formation of micelles in the uptake of hydrocarbons [75].

7. Biodegradation of Petroleum Hydrocarbons by Immobilized Cells

Immobilized cells have been used and studied for the bioremediation of numerous toxic chemicals. Immobilization not only simplifies separation and recovery of immobilized cells

Table 2: Biosurfactants produced by microorganisms.

Biosurfactants	Microorganisms
Sophorolipids	*Candida bombicola* (Daverey and Pakshirajan, [55])
Rhamnolipids	*Pseudomonas aeruginosa* (Kumar et al. [56])
Lipomannan	*Candida tropicalis* (Muthuswamy et al. [57])
Rhamnolipids	*Pseudomonas fluorescens* (Mahmound et al. [58])
Surfactin	*Bacillus subtilis* (Youssef et al. [59])
Glycolipid	*Aeromonas* sp. (Ilori et al. [60])
Glycolipid	*Bacillus sp*. (Tabatabaee et al. [61])

but also makes the application reusable which reduces the overall cost. Wilsey and Bradely [91] used free suspension and immobilized *Pseudomonas* sp. to degrade petrol in an aqueous system. The study indicated that immobilization resulted in a combination of increased contact between cell and hydrocarbon droplets and enhanced level of rhamnolipids production. Rhamnolipids caused greater dispersion of water-insoluble n-alkanes in the aqueous phase due to their amphipathic properties and the molecules consist of hydrophilic and hydrophobic moieties reduced the interfacial tension of oil-water systems. This resulted in higher interaction of cells with solubilized hydrocarbon droplets much smaller than the cells and rapid uptake of hydrocarbon in to the cells. Diaz et al. [92] reported that immobilization of bacterial cells enhanced the biodegradation rate of crude oil compared to free living cells in a wide range of culture salinity. Immobilization can be done in batch mode as well as continuous mode. Packed bed reactors are commonly used in continuous mode to degrade hydrocarbons. Cunningham et al. [93] used polyvinyl alcohol (PVA) cryogelation as an entrapment matrix and microorganisms indigenous to the site. They constructed laboratory biopiles to compare immobilised bioaugmentation with liquid culture bioaugmentation and biostimulation. Immobilised systems were found to be the most successful in terms of percentage removal of diesel after 32 days.

Rahman et al. [94] conducted an experiment to study the capacity of immobilized bacteria in alginate beads to degrade hydrocarbons. The results showed that there was no decline in the biodegradation activity of the microbial consortium on the repeated use. It was concluded that immobilization of cells are a promising application in the bioremediation of hydrocarbon contaminated site.

8. Commercially Available Bioremediation Agents

Microbiological cultures, enzyme additives, or nutrient additives that significantly increase the rate of biodegradation to mitigate the effects of the discharge were defied as bioremediation agents by U.S.EPA [95]. Bioremediation agents are classified as bioaugmentation agents and biostimulation agents based on the two main approaches to oil spill bioremediation. Numerous bioremediation products have been proposed and promoted by their vendors, especially during early 1990s, when bioremediation was popularized as “the ultimate solution” to oil spills [96].

Table 3: Bioremediation agents in NCP product schedule (Adapted from USEPA, 2002).

Name or Trademark	Product Type	Manufacture
BET BIOPETRO	MC	BioEnviro Tech, Tomball, TX
BILGEPRO	NA	International Environmental Products, LLC, Conshohocken, PA.
INIPOL EAP 22	NA	Societe, CECA S.A., France
LAND AND SEA	NA	Land and Sea Restoration LLC, San Antonio, TX
RESTORATION MICRO-BLAZE	MC	Verde Environmental, Inc., Houston, TX
OIL SPILL EATER II	NA/EA	Oil Spill Eater International, Corporation, Dallas, TX
OPPENHEIMER FORMULA	MC	Oppenheimer Biotechnology, Inc., Austin, TX
PRISTINE SEA II	MC	Marine Systems, Baton Rouge, LA
STEP ONE	MC	B & S Research, Inc., Embarrass, MN
SYSTEM E.T. 20.	MC	Quantum Environmental Technologies, Inc(QET), La Jolla, CA
VB591TMWATER, VB997TMSOIL, AND BINUTRIX	NA	BioNutraTech, Inc., Houston,TX
WMI-2000	MC	WMI International, Inc

Abbreviations of product type:
MC: Microbial Culture
EA: Enzyme Additive
NA: Nutrient Additive.

The U.S. EPA compiled a list of 15 bioremediation agents [95, 97] as a part of the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule, which was required by the Clean Water Act, the Oil Pollution Act of 1990, and the National Contingency Plan (NCP) as shown in Table 3. But the list was modified, and the number of bioremediation agents was reduced to nine.

Studies showed that bioremediation products may be effective in the laboratory but significantly less so in the field [14, 17, 18, 98]. This is because laboratory studies cannot always simulate complicated real world conditions such as spatial heterogeneity, biological interactions, climatic effects, and nutrient mass transport limitations. Therefore, field studies and applications are the ultimate tests or the most convincing demonstration of the effectiveness of bioremediation products.

Compared to microbial products, very few nutrient additives have been developed and marketed specifically as commercial bioremediation agents for oil spill cleanup. It is probably because common fertilizers are inexpensive, readily

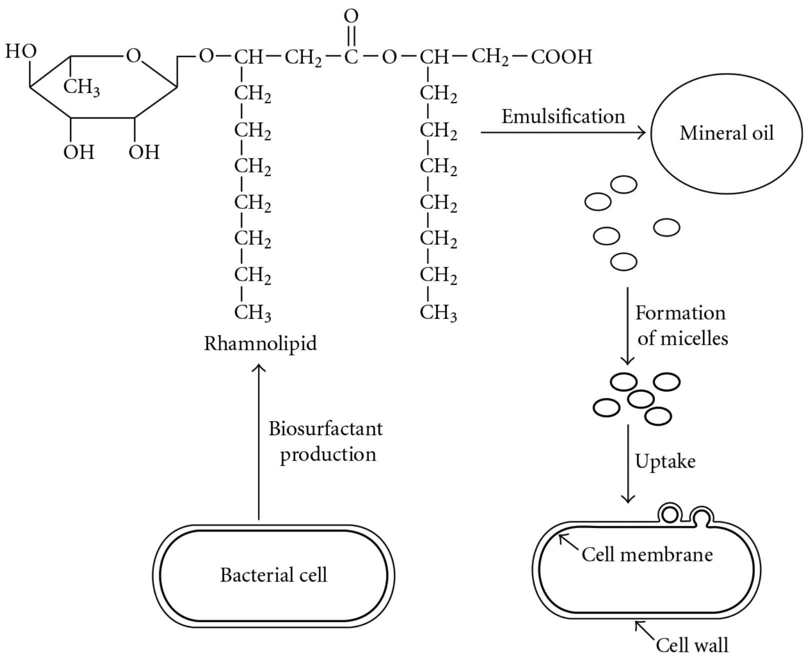

Figure 4: Involvement of biosurfactant (rhamnolipid) produced by *Pseudomonas* sp in the uptake of hydrocarbons.

Table 4: Advantages and disadvantages of phytoremediation over traditional technologies.

Advantages	Disadvantages
Relatively low cost	Longer remediation times
Easily implemented and maintained	Climate dependent
Several mechanisms for removal	Effects to food web might be unknown
Environmentally friendly	Ultimate contaminant fates might be unknown
Aesthetically pleasing	Results are variable
Reduces landfilled wastes	
Harvestable plant material	

source, 2-butoxy-1-ethanol as a surfactant, and oleic acid to give the material its hydrophobicity. The claimed advantages of Inipol EAP22 include (1) preventing the formation of water-in-oil emulsions by reducing the oil viscosity and interfacial tension; (2) providing controlled release of nitrogen and phosphorus for oil biodegradation; (3) exhibiting no toxicity to flora and fauna and good biodegradability [99].

Oil Spill Eater II (Oil Spill Eater International, Corp.) is another nutrient product listed on the NCP Schedule [97]. This product is listed as a nutrient/enzyme additive and consists of “nitrogen, phosphorus, readily available carbon, and vitamins for quick colonization of naturally occurring bacteria”. A field demonstration was carried out at a bioventing site in a Marine Corps Air Ground Combat Center (MCAGCC) in California to investigate the efficacy of OSEII for enhancing hydrocarbon biodegradation in a fuel-contaminated vadose zone [106].

Table 5: Genetic engineering for biodegradation of contaminants.

Microorganisms	Modification	Contaminants	Reference
Pseudomonas. putida	pathway	4-ethylbenzoate	Ramos et al. [100]
P. putida KT2442	pathway	toluene/benzoate	Panke and Sanchezromero [101]
Pseudomonas sp.FRI	pathway	chloro-, methylbenzoates	Rojo et al. [102]
Comamonas. testosteroni VP44	substrate specificity	*o*-, *p*-monochlorobiphenyls	Hrywna et al. [103]
Pseudomonas sp. LB400	substrate specificity	PCB	Erickson and Mondello [104]
P. pseudoalcaligenes KF707-D2	substrate specificity	TCE, toluene, benzene	Suyama et al. [105]

Table 6: Application of genetically modified bacteria for assessing the biodegradation process efficiency.

Microorganisms	Application	Contaminants	Reference
A. eutrophus H850Lr	process monitoring	PCB	Van Dyke et al. [107]
P. putida TVA8	process monitoring	TCE, BTEX	Applegate et al. [108]
P. fluorescens HK44	process monitoring	naphthalene, anthracene	Sayler and Ripp [109]
B. cepacia BRI6001L	strain monitoring	2,4-D	Masson et al. [110]
P. fluorescens 10586s/pUCD607	stress response	BTEX	Sousa et al. [111]
Pseudomonas strain Shk1	toxicity assessment	2, 4-dinitrophenol hydroquinone	Kelly et al. [112]
A. eutrophus 2050	end point analysis	non polar narcotics	Layton et al. [113]

Conclusion

Cleaning up of petroleum hydrocarbons in the subsurface environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transformormineralize the organic contaminants.Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. The use of genetically modified (GM) bacteria represents a research frontier with broad implications. The potential benefits of using genetically modified bacteria are significant. But the need for GM bacteria may be questionable for many cases, considering that indigenous species often perform adequately but we do not tap the full potential of wild species due to our limited understanding of various phytoremediation mechanisms, including the regulation of enzyme systems that degrade pollutants. Therefore, based on the present review, it may be concluded that microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation.

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