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Biodegradation of Hydrocarbon Pollutants-A Microbial Perspective

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Abstract: Bioremediation, the removal of environmental pollutants by living organisms, has become a viable and promising means for restoring contaminated sites. It means giving nature a helping hand. Crude oil and its derivatives are recalcitrant molecules toxic to biotic factors and persists in environments for many years depending on the nature and quantity of oil spilled. This paper provided a review of the menace of petroleum hydrocarbon pollution and its biodegradation in the environment with the view of understanding the biodegradation processes for better exploitation in bioremediation challenges.

Key words: Crude Oil · Bioremediation · *Pseudomonas*

INTRODUCTION

Fossil fuels are the foundation of many world economics and reliance on this energy source is unlikely to wane in future decades. Oil accounts for about 31% of India's total energy consumption. India produced total oil roughly 954 thousand barrels per day (bbl/d) in 2010, in which approximately 86 thousand bbl/d was crude oil. In 2010, India consumed nearly 3.2 million bbl/d, making it the fourth largest consumer of oil in the world [1]. Energy information administration expected approximately 100 thousand bbl/d annual consumption growth through 2013. At present petroleum provides about 70% of the world's energy requirement and forms the source of 90% of world production of organic chemicals and plastics (Ref.??). The vast scale of the operations necessitated by the above demands renders the petroleum industry a potentially severe source of air, water and soil pollution [2].

The growing concerns and awareness of pollution problems have prompted many researchers and reviewers to assess the impacts of pollutants on ecosystems, human health's, agricultural produce and consequential losses [1-3] Environmental air pollution occurs by smoke and fumes from chimneys of industries. Auto emissions are generally considered to be the major source of atmospheric hydrocarbons and unleaded gasoline. Unleaded gasoline has a relatively high aromatic content and contributes more particulate carbon to the atmosphere than leaded gasoline [3-6]. In terrestrial ecosystem soil pollution may be caused by natural gas and petroleum seeps from underlying petroleum reservoirs, accidental spills resulting from transportation accidents, rupture of pipelines, blow-out, drilling activity, leaky joints, valves, faucets in oil refineries and deliberate disposal of petroleum wastes in soil. There are many sites through exploration and production operations at which soils have been negatively impacted by hydrocarbon spills or leakage from wellheads and surface facilities. In the United States, law prohibits haphazard dumping of oil on land [7]. Extensive damage to urban tree planting in the Netherlands was reported due to underground leakage from natural gas distribution systems [8].

In marine environment oceans and estuaries are the largest sites of spills because most spilled petroleum hydrocarbons ultimately will reach the sea. Oil spills due to incidents involving tankers account for 41.6%, urban and river run off for 27% and offshore oil production contributes only about 1.3% of the total oil input in the ocean. A study conducted recently by N.I.O., Goa, has revealed that over 80% of the oil pollution in the Arabian Sea is due to tankers carrying oil from Gulf Sea to South East Asia [9]. The collision between MSC Chitra and MV Khalijia on 9th August 2010, caused the spillage of 400 to 500 tons of oil and 31 containers of hazardous chemicals. BNHS (Bombay Natural history Society) reported a coastal area measuring 25-km radius is covered with oil, 1,273.24 hectares of mangrove cover was affected.

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Bioremediation: The apparently inevitable spillages, which occur during routine operations and as a consequence of acute accidents, have maintained a high research interest in this field. Even though bioremediation has been in existence since the beginning of life, it is only recently that scientists have begun to understand the complex nature of the process and the usefulness of a specific strain of bacteria. The use of microorganisms to control and destroy contaminants is one of the fastest growing sectors of the U.S. market for hazardous waste cleanup and it has become a $500 million per year industry in the year 2000 [10].

Bioremediation is a technique that enhances the natural rate of biodegradation of pollutants through reactions carried out by selected microorganisms [11]. Bioremediation of agricultural land polluted with crude oil using microorganisms to help in regaining the land's fertility can be achieved in two ways: by enhancing the growth and activity of microorganisms already present at the site of pollution through nutrient addition (biostimulation) and by adding more selected microorganisms (Bioaugmentation) to the pollution site [12,13].

Hydrocarbons Degrading Microbes: The history of petroleum microbiology is more than a century old. Hydrocarbon using microorganisms have been known since Miyoshi [14] described the growth of the fungus *Botrytis cinera* on paraffins at room temperature and Sohngen [15] reported on the uptake of crude oil, gasoline and kerosene by microorganisms. Zo-Bell [16] reviewed the action of microorganisms on hydrocarbons. He recognized that many microorganisms have the ability to utilize hydrocarbons as sole sources of energy and carbon and that such microorganisms are widely distributed in nature. Soils contain tremendous number of microorganism. Most uncontaminated soils contain microbes capable of degrading hydrocarbons. Once hydrocarbons are applied to the soils, selective enrichment of hydrocarbon degrading microbes occurs; the number of hydrocarbon degrading microbes is greatly increased [17].

Bacteria and fungi are the principal agents of petroleum biodegradation in soil. The genera of hydrocarbon-degrading bacteria isolated are *Pseudomonas, Arthrobacter, Alcaligenes, Corynebacterium, Flavobacterium, Achromobacter, Micrococcus, Nocardia, Mycobacterium, Acinetobacter, Bacillus, Brevibacterium, Chromobacterium, Cytophaga, Erwinia, Proteus, Sarcina, Serratia, Spirillum, Streptomyces, Vibrio* and Xanthomonas [18-26]. *Pseudomonas* is most frequently reported and, so far, most studied hydrocarbon-degrading genus. *Pseudomonas putida, Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, were isolated from soil came from a sandpit heavily contaminated with oil refinery wastes [27]. *Pseudomonas saccharophila* and *Pseudomonas stutzeri*, strains were utilized for the degradation of polyaromatic hydrocarbons [28]. Several *Pseudomonas* strains were isolated from the surface water of Sunderban Biosphere reserve situated at the Hooghly river mouth [29].

The filamentous fungi can grow on hydrocarbons, with *Aspergillus* and *Penicillium* species being the most frequently reported [30-32]. Elshafiea *et al*. [33] showed that *Aspergillus* and *Penicillium* species are more active than the others. Molla *et al*. [34] reported that the strains/isolates Aspergillus *niger*, SS-T2008, WW-P1003 and RW-Pl 512 produce the highest dry biomass at higher sludge supplemented culture media from their respective group (*Aspergillus*, *Trichoderma*, *Penicillium* and *Basidiomycetes*, respectively). April *et al.* [35] that reported 22 species of *Penicillium* and 5 species of *Aspergillus* isolated from the flare pit soils in Northern and Southern Canada which show the ability to degrade hydrocarbons on solid medium amended with crude oil.

A number of actinomycetes also have been shown to have hydrocarbon-degrading abilities [36]. A unique group of hydrocarbon-degrading bacteria is methanotrophs. These organisms possess a highly specialized C1 metabolism. Since methane is biogenically generated, methanotrophs are quite ubiquitous in soil and play a vital role in the global carbon cycle [37]. A number of *Cyanobacteria* have been found to be capable of hydrocarbon degradation [38].

Crude Oil: Petroleum in its natural form is commonly known as crude oil. In Latin, 'Petra' means rock while 'oleum' means oil. Petroleum is thus the oil of rock. On molecular basis, petroleum is a complex mixture of hydrocarbons plus organic compounds of S, O and N, as well as compounds containing metallic constituents, particularly Vn, Ni, Fe, Cu etc. [39]. Crude oils may contain hundreds of thousands of components [40].

Petroleum hydrocarbons can be divided into four classes; saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters and porphyrins) and the resins (pyridines, quinolines, carbazoles, sulfoxides and amides) [41]. Hydrocarbons differ in their susceptibility to microbial attack and, have generally been

ranked in the following order of decreasing susceptibility: n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes (42-43). However, this pattern is not universal. The n-alkanes biodegradation in the sequence of C10 > C8> C7 > C6. Degradation of 100% C10, 97% C8, 74% C7 and 44% C6 has occurred in a mixture of n-alkanes [44].

API Gravity: It is an expression of the density of hydrocarbon. A high API gravity means a low density. American Institute of petroleum geologists has defined an equation for uniform presentation of the specific gravity data.

$$\text{APIGravity} = \frac{141.8}{\text{Sp.gravity of oil/60°F)}} - 131.8$$

Light oil has an API gravity higher than 40 and heavy oil has an API gravity of about 15-20 [45].

Biological Markers in Petroleum: Alfred Treib's pioneering work of isolating porphyrins from crude oils in 1934 was the first evidence of biological origin of oil (46). However, the impetus to such studies was provided by the isolation and identification of pristane and phytane from crude oils. Pristane and phytane have chemical structures similar to the phytol side chain of the chlorophyll molecule because pristane and phytane originate from the phytol side chain of chlorophyll, during digenesis under reducing and oxidizing environments, respectively [47]. These molecules exist because their carbon skeletons survive through the processes of digenesis and maturation. Such molecules are called *"biological markers"* [48].

Biosurfactants: Microbial degradation of hydrocarbon is generally associated with biosurfactant production. Biosurfactants enhance emulsification of hydrocarbons and therefore increase their availability for microbial degradation. Biosurfactants, the surface-active substances of microbial origin were reported as 'emulsifying factor' or 'pseudosolubilizing factor' in some of the earliest publications (49).

Biosurfactants possess both hydrophilic and hydrophobic structural moieties, which imparts them properties to lower the surface tension of the water. At room temperature, the surface tension of water is approximately 72 dynes/cm and it results primarily from the effects of hydrogen bonding [50]. Many surfactants can reduce surface tension of water from 72 to approximately 30+5 dynes/cm depending on concentration and surfactant type. Surfactants achieve this effect by acting as bridge between the two materials meeting at the interface. Actually the term biosurfactant refers to compounds obtained from microorganisms that have some influence on interface. Thus it is also used for emulsifying and dispersing agents. At low concentration surfactants are present as individual molecules. However, as the concentration of surfactants is increased a concentration reached, where no further change takes place in interfacial properties. The amount of biosurfactant needed to reach this concentration is called critical micelle concentration (CMC). At the critical micelle concentration surfactant molecules aggregate to form monolayer (micelle) or bilayer (vesicle) structure that has the ability to encapsulate hydrocarbon molecule resulting in either solubilization or emulsification of hydrocarbon 51]. Biosurfactants have special advantage over their chemically manufactured counterparts because of their lower toxicity, biodegradable nature, effectiveness at extreme temperature, pH and salinity and ease of synthesis.

Taxonomic Diversity of Biosurfactant Producing Microbes in Response to Hydrocarbon Degradation: A wide variety of microorganisms can produce biosurfactants; mostly these are produced when microorganisms are cultured on hydrocarbons. Most of the biosurfactants, so far reported, are found to be produced by bacteria, however, a good number of yeasts and few fungi also have been reported as biosurfactant producers [52]. In table 1, a list of the major types of biosurfactants and the producing microorganisms was given, where it is clear that the production of biosurfactant is not limited to any specific group or class of microbes, rather, members of almost all saprophytic microbial groups are capable of producing it.

Growth Associated Production of Biosurfactants: A co-relationship between the substrate utilization, growth and biosurfactant production can be observed in hydrocarbon degradation. The carbon source plays an important role in production of biosurfactants. In some cases the chain length of the hydrocarbon substrates used had an effect on biosurfactant production. The normal production of cell-free 'Emulsan' by *Acinetobacter calcoacetius* RAG-1 is a mixed growth associated and non-growth associated process [53]. Accumulation of emulsan like polymer on the cell surface during the early exponential phase of growth has been reported and fermentative production of surface active agents from *B.cereus* IAF 346 and *Bacillus sp*. IAF-334 are found to be growth associated [54].

Table 1: Major types of biosurfactants and producing microorganisms reported

Biosurfactant	Microorganisms	Surface tension	CMC Interfacial d/cm	tension d/cm
(A) Glycolipds				
Trehaloselipids	R. erythropolis	32-26	400	14-17
	N.erythropolis	30	20	3.5
	Mycobacterium sp.	38	30	15
Rhamnolipids	P. aeruginosa	29	-	0.25
	Pseudomonas sps.	25-30	10-100	1
Sophorolipids	T. bombicola	33	-	1.8
	T.apicola	30	-	0.9
(B) Fatty Acids/Neutral Lipids				
Fatty acids (FA)	C.lepus	30	150	2
FA+Neutral lipids	N.erythropolis	32	-	3
Peptide lipid	B.licheniformis	27	20	0.1-0.3
Serrawettin	S.marcescens	28-33	-	-
Viscosin	P.fluroescens	26.5	15	-
Surfactin	B.subtilis	27-32	23-160	1
(D) Polymeric surfactants				
Carbohydrate protein-lipids	P. fluorescens	27	100	-

Regulatory Mechanisms Related to Biosurfactant Production: The growth of the microorganism and production of biosurfactant, these two seem to proceed as separate events. In the exponential phase of growth of cells, there is often a very low rate of surfactant production [55], over production of the biosurfactant then occurs as the cells stop growing. In fact the amount of surfactant needed to stimulate alkane dissolution and uptake is very small as the surfactant is not consumed by the producing cells in the uptake process. Once the signal to begin production of surfactant has been given by the presence of the alkanes, then the surfactant production will continue in an unregulated manner until the signal to stop is received. The microbial cells can be harvested at the surfactant producing state, maintained in the same state and can be employed for biosurfactant production. Thus, they do not multiply but continue to utilize carbon source for the synthesis of biosurfactants [56]. Siemann and Wagner [57] have reported the production of rhamnolipid by resting free and immobilized cells of *Pseudomonas sp.* DSM-2847 []. There are many other examples of biosurfactant production by resting cells by different microorganisms, like sophorolipid production by *T.* bombicola [58], trehalose tetraester production by *R.erythropolis* etc [59]. Using resting cells of *Arthrobacter sp.* DSM-2567 and various mono,-di, or terasaccharides as the carbon source, the production of corresponding glycolipids are observed [60]. Ramana and Karanth [61] reported a two-fold increase in rhamnolipid production when *P. aeruginosa* CFTR-6 is transferred after the growth phase into a medium devoid of phosphate. So far 'emalsan' produced by *Acinetobacter spp.,* is the only biosurfactant that has been commercialized [62].

Biodegradation of Crude Oil: Simply defined, biodegradation is a natural process in which microbes (bacteria fungi, yeasts, algae) breakdown hydrocarbons and produce biomass (cell growth), water, carbon dioxide and partially oxidized products. Oxygen is inserted into the hydrocarbons (oxidation) so that the molecule can be utilized in cellular metabolism [63]. Some hydrocarbons are completely oxidized to carbondioxide and water, while others may only be partially oxidized and incorporated in to cell biomass. Partially oxygenated biodegradation inter-mediates of hydrocarbons are fatty acids and phenolic substances [64]. This transformation is called mineralization. A compound, which will not degrade in laboratory, is called recalcitrant [65].

Laboratory Efficacy Testing: Laboratory experiments demonstrate the potential of a particular treatment, which may have to stimulate the removal of petroleum pollutants from a contaminated site [66]. Laboratory experiments that closely model real environmental conditions are most likely to produce relevant results [67-69]. In many cases this involves using samples collected in the field that contain the indigenous microbial populations. In such experiments it is important to include appropriate controls, such as sterile treatments, to separate the effects of

the abiotic weathering of oil from actual biodegradation. Such experiments do not replace the need for field demonstrations but are critical for establishing the scientific credibility of specific bioremediation strategies. They are also useful for screening potential bioremediation treatments.

The parameters typically measured in laboratory tests of bioremediation efficacy include enumeration of microbial populations, determination or fate of hydrocarbon degradation (disappearance of individual hydrocarbons and/or total hydrocarbons) [70]. The methodologies employed in these measurements are critical. It is assumed for example, that bioremediation of oil pollutants will result in elevated populations of hydrocarbon degraders. Undoubtedly, the most direct measure of bioremediation efficacy is the monitoring of hydrocarbon disappearance rates. When using this approach, the appropriate controls and the proper choice of analytical techniques become especially critical. The "disappearance" of hydrocarbons may occur not only by biodegradation but also by evaporation, photo degradation and leaching. In a laboratory setting, the later two are easily controlled, but the accurate control of evaporative losses is troublesome. Sealed incubation systems are incompatible with the high oxygen demand of hydrocarbon degradation. Poisoned controls in open systems notoriously underestimate biodegradation [71]. Normally, biodegradation and evaporation compete for the same hydrocarbons. If biodegradation is suppressed by metabolic poisons (usually $HgCl_2$), hydrocarbons that would otherwise be degraded eventually evaporate.

Gravimetric determination of residual oil may overestimate biodegradation when volatile components are lost during evaporation of the extracting solvent. Conversely, biodegradation may be underestimated when nonhydrocarbon materials are co extracted by the solvent. Biodegradation intermediates that have incorporated oxygen also increase the residual weight and may contribute to the underestimation of biodegradative losses [2].

Because of the outlined problems with residual weight, most studies have turned to more definitive analytical procedures [73-75]. A wide verity of instrumental and noninstrumental techniques is currently used in the analysis of oil. Depending on chemical/physical information needs, the point of application and the level of analytical detail, the methods used for oil spill study can be in general, divided into 2 categories: nonspecific methods and specific methods for detailed component analysis [76]. In response to oil spill identification needs and specific site investigation needs, attention has recently focused on the development of flexible, tiered analytical approaches that facilitate the detailed compositional analysis by, gas chromatography with flame ionization detection (GC-FID), high pressure liquid chromatography (HPLC) and other analytical techniques. Many EPA and ASTM methods have been modified to improve specificity and sensitivity for measuring spilled oil and petroleum products in soils, waters and contaminated sites. A variety of diagnostic ratios, especially ratios of polycyclic aromatic hydrocarbons (PAH) and biomarker compounds have been proposed for interpreting chemical analysis results from spill samples. These advanced fingerprinting and data interpretation techniques are a clear advance over standard EPA methods because they can provide far more information directly useful for characterization and quantification of spilled oil hydrocarbons [77-81].

Successful oil fingerprinting involves appropriate sampling, analytical approaches and data interpretation strategies. In the nonspecific methods, only groups of fractions of chemical hydrocarbons are determined [82]. The data generated from these methods generally lack detailed individual component and petroleum source-specific information and therefore are of limited value in many oil spill investigation cases. In general, the petroleum-specific marker compounds selected must have the attributes of uniquely identifying spilled petroleum hydrocarbons from other sources and resist weathering and degradation over time. The major potential target analytes and hydrocarbon groups for biodegradation environmental assessment of spilled oil include the following.

- Individual saturated hydrocarbons including n-alkanes (C8-C40) and selected isoprenoids pristane and phytane [83].
- PAHs including the petroleum-specific (C1-C4) PAH homologues (that is, alkylated naphthalene, phenanthrene, dibenzothiophene, fluorine and chrysene series) and other EPA priority parent PAHs. These alkylated PAH homologues are the backbone of chemical characterization and identification of oil spills.

Polycyclic Aromatic Hydrocarbons (PAHs): Polycyclic aromatic hydrocarbons (PAHs) are fused ring aromatic compounds whose presence in contaminated soils and sediments poses a significant risk to the environment and they have cytotoxic, mutagenic and in some cases

carcinogenic effects on human tissue [84,85]. There are more than 70 compounds classed as polynuclear aromatic hydrocarbons (PAHs) and they have from 2 to 7 rings. During the last three centuries a relationship between the higher incidence of cancer in urban and industrial area than in rural areas and the exposure of human to polycyclic aromatic hydrocarbons (PAHs) has prompted considerable research on the source, occurrence, bioaccumulation, metabolism and deposition of these pollutants in atmospheric, aquatic and terrestrial ecosystems [86]. Because of their health risks to animals, including humans, PAHs are listed as priority pollutants by the Environmental Protection Agency (EPA) [87]. Although the ability of microorganisms to degrade various PAHs has been studied, chemical oxidation, photolysis and volatilization of PAHs has also been detected in nature [88].

Aromatic compounds are of special interest because they are relatively resistant to biodegradation and can therefore accumulate to substantial level in the environment. The bacterial utilization of several aromatic hydrocarbons with low water solubility's has been investigated. The microbial degradation of PAHs having two or three rings is well documented [89,90]. Only within the last decade have a number of bacteria that metabolize larger PAHs molecules been isolated. These include *Alcaligenes* denitrificans [91], *Rhodococcus species* strain UW1 [92], several *Pseudomonas* species [93,94] and various *Mycobacterium* species [95,96]. For example a *Beijerinckia* species has been found to metabolize benzo-(a)-pyrene and benz(a)-anthracene to cis-dihydrodiols [97] and two different *Mycobacterium strains* have been isolated which can mineralize the four-ring PAHs, pyrene [98-99].

Polyaromatic hydrocarbons (PAHs) are relatively insoluble in water. Banerjee [100] calculated the solubility of a wide range of PAHs and found them to vary greatly. For example, solubility in 25°C distilled water varies from 31.7mg/l for naphthalene to 0.002 mg/l for the four-ring chrysene. In general PAHs solubility decreases with increasing molecular weight or number of rings [101]. Naphthalene has often been selected as a model compound for the study of PAHs degradation because of its high aqueous solubility and the ease of isolation of microbes capable of its degradation [102].

General Concept in the Bacterial Degradation of Polyaromatic Hydrocarbons (PAHs): The degradation of PAHs containing up to four or five rings by bacteria has been well documented. The process involving biodegradation are inversely proportional to ring size of the PAH molecule. The lower weight of PAHs is degraded more rapidly than the higher weight PAHs containing three or more fused rings. Normally the higher molecular weight PAHs do not serve as amenable substrates for bacterial metabolism. Bacteria initially oxidize aromatic hydrocarbons by incorporating two atoms of molecular oxygen into the substrate to from a dihydrodiol by a cis-configuration [103]. This reaction is catalyzed by a dioxygenase, which is a multicomponent enzyme system, consisting of a flavoprotein, an iron-sulfur protein and a ferredoxin [104]. Further oxidation of cis-dihydrodiols leads to the formation of catechols. Another highly stereoselective reaction during bacterial oxidation is the rearomatization of the cis-dihydrodiol by dehydrogenases to form a dihydroxylated intermediate [105] (Figure 1). Dihydroxylation of the benzene nucleus has been found to be essential for cleavage of the aromatic ring [106].

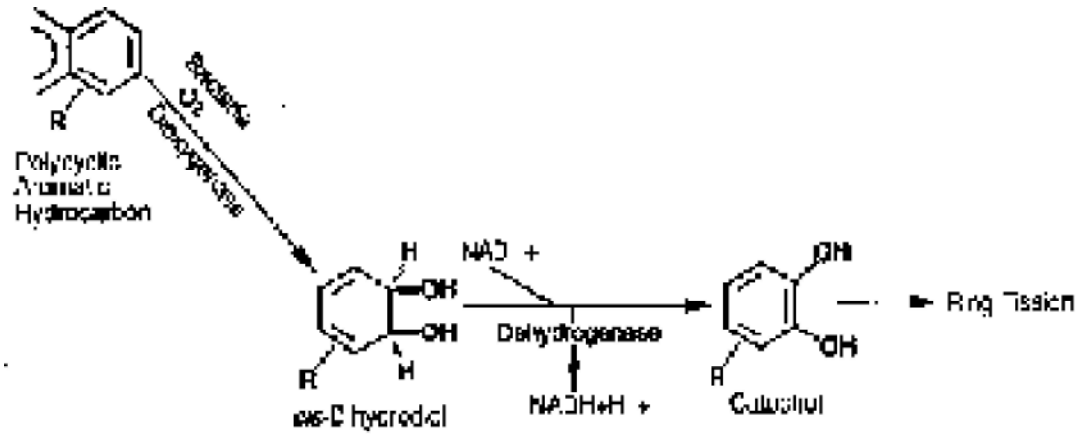

Fig. 1: Microbial oxidation of PAHs via dioxygenase pathway, [107].

Microcosm Study: There are many definitions of 'microcosm' a typical one is that of an intact, minimally disturbed piece of an ecosystem brought into the laboratory for study in its natural state [108]. Microcosms can vary in complexity from simple static soil jars of contaminated soil to highly sophisticated systems designed to enable variations in various environmental parameters encountered on site to be more accurately simulated in the laboratory.

To determine the rate of hydrocarbon biodegradation, accurate and reliable analyses are critical. One of the recommended standard analyses for the total petroleum hydrocarbons (TPHs) is based on the degradation in the treated soil was accompanied by significant reduction in the ratio, compared to little or no change in the control tests [109].

Bioremediation was made simpler and more practical in the late 1980s by a technique that induces colonies of oil-eating bacteria to enter a state of suspended animation-an inactive mode that the microbes normally adopt during extended periods of drought or freezing. In this state, the bacteria can be air-dried, packaged and stored as a high concentrate powder with 90 percent survival rate, to be used in the field as needed. The dried bacteria can be quickly restored to normal function at polluted sites by adding liquid nutrients and biological catalysts [110]. Many carrier materials, mostly agricultural byproducts, are used to transfer the bacterial consortium to the field effectively [111-112]. The carrier material provides nutrients, moisture and physical support for the increased aeration needed by the microorganisms and also assists in extending the survival of the microorganisms until they are applied in the field. Extended survival of the microorganisms under field conditions is essential for efficient degradation of the toxic hydrocarbons, especially of the multi-ringed aromatic hydrocarbons and the recalcitrant ones, because they are not degraded during the early stage of the process [113-115]. For example: The microbial consortium named as oilzapper was developed by the Microbial Biotechnology Laboratory at TERI in collaboration with Indian Oil (R and D). This bacterial consortium was developed by mixing five bacterial strains, which could degrade aliphatic, aromatic, asphaltene and NSO (Nitrogen, Sulphur and Oxygen compounds) fractions of crude oil and oily sludge. It was immobilized on a suitable carrier material namely powdered corncob; which is an environment-friendly, biodegradable product. Survivability of the consortium in the immobilized condition was determined and found to be three months at ambient temperatures. The immobilized culture was put into sterile polythene bags and sealed aseptically and transported to the place of requirement [116].

CONCLUSION

A variety of technologies are currently available to treat soil contaminated with crude oil including excavation and containment in secured landfills, vapor extraction, stabilization and solidification, soil flushing, soil washing, solvent extraction, thermal desorption, vitrification and incineration. Many of these technologies, however, are either costly or do not result in completed destruction of contamination. On the other hand, biological treatment 'bioremediation' appears to be among the most promising methods for dealing with oil spills, particularly petroleum hydrocarbon. The technology is also environmentally sound, since it simulates natural processes and since it can result in the complete destruction of hazardous compounds into innocuous products.

No national coordinated response plan for oil spill bioremediation currently exists. A subcommittee of the EPA's Biotreatment Action Committee Task Force, therefore, was formed in June 1990 to discuss the development of a National Bioremediation Spill Response Plan. Such a plan would attempt to maximize the potential for the biotreatment of oil spills by focusing and channeling the sundry research and development efforts into a single coordinated plan.

Bioremediation offers several major advantages over conventional remediation techniques. The costs of bioremediation of soils and sludges at refineries are nearly half the costs of conventional land farming. In addition bioremediation uses one-third to one-tenth the amount of land and is significantly faster. Another study showed that costs of bioremediation could be as little as 1 percent of off-site incineration. One of the major advantages of in-situ bioremediation is that it is nondestructive. In many cases excavation is impossible due to the presence of buildings and other structures. Also, the extent of the contamination may make excavation unreasonable.

Former U.S. Environmental Protection Agency (EPA) Director, William Reilly supports bioremediation and considersing it to be one of the most promising treatment options. The EPA regards bioremediation as an innovative technology and is encouraging industry participation. In fact, the EPA has created the Bioremediation Action committee composed of 150 leading biotreatment experts from government, industry and academia.

During the past decade, the need to deal with oil spills of increasing frequency and magnitude created a whole new field of engineering and technology. The proceeding of conferences on "Prevention and control of oil spills" (1969, 1971 and 1973, American Petroleum Institute, Washington, D.C.) record many of these developments. The current wave of scientific and commercial interest in this subject was heralded by a feature article in chemical and engineering News. A literature study of the subject was commissioned by the U.S. coast guard and a workshop on "the microbial degradation of oil pollutants" was held (December 4-6, 1972, Atlanta, Georgia). Several research groups proceeded to isolate and study highly effective strains or mixed enrichments of hydrocarbon degraders. Various commercial inocula, such as "Petrodeg," "Petrobac", "Ekolo-Gest" and "DBC-Bacterial" appeared on the market and were promoted as being effective for oil cleanup.

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