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Bioremediation: A Sustainable Tool for Environmental Management of Oily Sludge

Meenakshi Nandal, Pooja Solanki, Mansi Rastogi and Rajni Hooda

Department of Environmental Sciences, M. D. University, Rohtak, Haryana, India

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ABSTRACT

Advances in technology and industrialization, bring with them, their unpleasant partners, pollution and degradation of the environment. The effects on the environment connected with industrial activities are mainly related to the production of industrial wastes. Because of problem associated with pollutant treatment by conventional methods, such as chemical treatment, incineration or landfills, an alternative approach, bioremediation, or the use of organisms for the removal of contamination or pollutants has become a popular option. Bioremediation is considered as one of the safer, cleaner, cost effective and environmental friendly technology for decontaminating sites which are contaminated with wide range of pollutants. As microorganisms show wide range of mechanisms, there are still few mechanisms which are not known, therefore, bioremediation is still considered as a developing technology. Thus, there is an urgent need for us to review and modify the available options for environmental clean-up. The objective of this paper is to conduct a comprehensive review on various indigenous microorganisms extracted from contaminated soil and capable of bioremediation of hydrocarbons present in the sludge.

INTRODUCTION

Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. Excess loading of hazardous wastes has led to scarcity of clean water and disturbances of soil, thus, limiting crop production (Chatterjee et al. 2008). The World demand for oil in 2010 was 94.3 million barrels per day and expected to be 101.6 million barrel per day in 2015 (Medjor et al. 2012). India is the World's 23rd largest producer of crude oil with 21 refineries (17 in Public sector, 3 in Private sector and 1 joint venture of BPCL) throughout the country with a total refining capacity of 193.386 MMTPA at present, and by expansion of existing refineries there will be an addition of 10.68 MMTPA to refining capacity (Ministry of Petroleum and Natural Gas, India 2007). Oil refineries generate large quantities of oily sludge, the safe disposal of which is major problem. At present, total sludge generated from all refineries is 28,200 tons per annum. The sludge is generated during cleaning of storage tanks, cleaning and desilting of oil separator basins, distillation column residues, exchanger tube bundle sludge and sludge generated from effluent treatment plant (ETP). Hydrocarbons are bio-persistent, bio-accumulative and can cause deleterious effects on flora and fauna as well as to humans (Benson et al. 2007). Petroleum hydrocarbon compounds bind to different soil components and are difficult to remove or degrade (Erdogan & Karaca 2011). Conventional disposal methods involve storing in

sludge pits, incineration, land-filling which are expensive to construct and add to the already limited land resource refineries. An alternative method of oily sludge management involves use of microorganisms for *in situ* degradation in the soil. The process called bioremediation is ecofriendly and attractive approach for better management of this huge amount of sludge and cleaning up of hydrocarbons from environment because it is simple technique, easy to maintain, applicable over large areas, cost-effective and leads to complete destruction of the contaminant (Bento et al. 2005, Achal et al. 2011).

Bioremediation: Bioremediation is the process by which living organisms degrade or transform hazardous organic contaminants to inorganic components, such as CO₂, H₂O and NO, (Luqueno et al. 2011). Bioremediation uses biological agents, mainly microorganisms, e.g. bacteria, fungi or yeast to clean up contaminated soil and water (Strong & Burgess 2008). The technologies employed are nature-compatible, reliable, cheaper and easy to adopt as compared to physical and chemical methods (Machin-Ramirez et al. 2008). Biological agents like microorganisms or plants transform the complex organic contaminants to other simpler organic compounds by their diverse metabolic capabilities for the removal and degradation of many environmental pollutants (Medina-Bellver et al. 2005). The major advantage of using microbes is that microbiological processes are flexible; they adapt to variable conditions (self regulation) and also to new molecules or combination of

Technology	Examples	Technique details	Benefits	Limitations
In situ Bioremediation	Biosparging	Involves injection of air under pressure to enhance biological activity of microbes (Sharma 2012).	NonInvasive	Environmental constraints
	Bioventing	It involves supplying air and nutrients through well (Atlas & Phillip 2005)	Relatively passive	Extended treatment time
	Bioaugmentation	It involves supplying specialised microbes or genetically engineered microbes to target specific Pollutants (Thapa et al. 2012)	Natural attenuation processes	Monitoring difficulties
	Biostimulation	It involves the management of the natural environment to optimise the growth and activity of the natural microbial population (Crivelaro et al. 2010)	Natural attenuation process	Extended treatment time
<i>Ex situ</i> Bioremediation	Land Farming	Involve tilling of top soil and adding water and nutrients (Soccol et al. 2003)	Cost efficient, and low ground water contamination (Besalatpour et al. 2011)	Space requirements
	Composting	Anaerobic,convert's solidorganic wastes intohumus-likematerial (Nataraj et al. 2007)	Rapid reaction rate, Low cost	Requires nitro- gen supplemen- tationExtended treatment time
	Biopiles	It is a hybrid of land farming and composting (Wu & Crapper 2009)	Provides favourable environment for indigenous microbes	Need to control abiotic loss
	Bioreactors	These are basically tanks in which living organisms carry outbiological reactions (Chikere et al. 2011)	Better rate and extent of degradation (Sonawdekar 2012)	Highly expensive

Table 1: In situ and Ex situ bioremediation techniques.

new molecules (Kaplery & Purohit 2009). Increasing the ability of microorganisms to tolerate and degrade petroleum hydrocarbons can boost bioremediation efficiency of oil-polluted sites (Soleimani et al. 2011). The bioremediation can occur either in situ (at the site of contamination) or ex situ (contaminant taken out of the site of contamination and treated elsewhere) (Das & Mukherjee 2007). The biological degradation of hydrocarbons from a contaminated environment is their bioavailability (the degree of interaction of chemicals with living organisms) to an active microbial population (Abbasnezhad et al. 2011). The biodegradability of the oil components generally decrease in the following order: *n*-alkanes > branched-chain alkanes > branched alkenes > low-molecular-weight *n*-alkyl aromatics > monoaromatics > cyclic alkanes > polycyclic aromatic hydrocarbons (PAHs) > asphaltenes (Tyagi et al. 2011).

Factors to be considered while employing bioremediation are: biodegradative abilities of indigenous microbes, presence of metals and other inorganics, environmental parameters, geological factors, biodegradability of pollutants (Thapa et al. 2012).

Oil sludge: Oil sludge is a thick, viscous mixture of sediments, water, oil and high hydrocarbon concentration,

encountered during crude oil refining, cleaning of oil storage vessels and refinery-wastewater treatment (Ubani et al. 2013). During exploration, production, refining, transport and storage of petroleum and petroleum products, some accidental spills (Figs. 1 and 2) could occur (Mnif et al. 2009). The chemical composition of oil sludge is complex and depends on the source. Oil sludge is mainly composed of alkanes (40-70%), aromatics (15-30%) and heavy fractions like NSO (nitrogen, sulphur and oxygen fraction) asphaltenes and resin (5-15%) (Mandal et al. 2012). The two major sources of oil sludge are oil storage tanks and refinerywastewater treatment plants (Shie et al. 2004, Wang et al. 2010). Oil sludge found in crude oil storage tanks is typically made up of sulphides, phenols, heavy metals, aliphatic and polycyclic aromatic hydrocarbons (PAHs) of 4,5,6 and more rings, in over 10-20-fold concentration. Aromatic hydrocarbons are unsaturated ring type (complex polycyclic of three or more fused aromatic rings) compounds, which reacts readily because they have carbon atoms that are deficient in hydrogen (Sonawedkor 2012). All aromatic hydrocarbons have at least one benzene ring as part of their molecular structure. These components are highly recalcitrant under normal conditions. Such characteristics are attributed





Fig. 1: Oil sludge at Duliajan, Assam.

Fig. 2: Oil spill at south Gujarat (Source: www.ibnlive.in.com)

to their strong molecular bonds, high molecular weights, hydrophobicity and relative low solubility in water (Loick et al. 2009). Some important PAHs of environmental concern present in oil sludge include naphthalene, 1-methyl naphthalene, 2-methyl naphthalene, acenaphthylene, acenaphthene, fluorine, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(a) pyrene and indole(1,2,3-cd) pyrene. These PAHs which may be acute or chronic hazardous organic compounds are present in substantial quantities in oil sludge and are susceptible to microbial degradation (Boudia 2013).

Oil sludge has been classified by the United States Environmental Protection Agency as a hazardous organic complex (US EPA 1997, Liu et al. 2010). This contaminant enters the environment as a result of human activities, which include deliberate dumping, improper treatments and management, storage, transportation and landfill disposal. This calls for concern because many of the oil sludge components have been found to be cytotoxic, mutagenic and potentially carcinogenic (Bojes & Pope 2007).

Harmful impacts of sludge: Oil sludge contains volatile organic carbons (VOCs) and semi volatile organic carbons (SVOCs) (for example, PAHs) which over the years have been reported as being genotoxic (Mishra et al. 2001, Bach et al. 2005, Bojes & Pope 2007). They have cumulative effect on the central nervous system (CNS) leading to dizziness, tiredness, loss of memory and headache, and the effect depends on duration of exposure (Lewis et al. 2008). In severe cases, PAH metabolism in human body produces epoxide compounds with mutagenic and carcinogenic properties that affects the skin, blood, immune system, liver, spleen, kidney, lungs and developing foetus (TERA 2008, API 2008, Bayoumi 2009). However, environmental regulations in many parts of the world have stressed on the necessity to decrease emission of volatile organic compounds as well as PAHs, and have placed more restriction on land disposal of oil sludge (Mahmoud 2004). The hydrocarbons spill impacts soil by affecting soil physical structure by coating soil aggregates, affecting soil water holding capacity, reducing and diverting water infiltration into the soil, reducing cation/anion exchange on soil aggregates and also result in an imbalance in C/N ratio in soil which cause a nitrogen deficiency leading to retardation of the growth of microbes (Dindar et al. 2013). Oil contaminated soil lose its fertility and decreases the agricultural productivity (Wang et al. 2008).

Treatment technologies for oil sludge: Safe disposal and treatment of huge quantity of oil sludge generated during the processing of crude oil are some of the major challenges faced by oil refineries and petrochemical industries (Srinivasarao et al. 2011). In recent years, most refineries treat oil sludge using conventional methods which include physical treatment (storage, land-filling, combustion, and incineration in a rotary kiln, lime stabilization and solidification) (Karamalidis & Voudrias 2001, Bhattacharyya & Shekdar 2003, Radetski et al. 2006, Beech et al. 2009, Liu et al. 2010), chemical treatment (oxidative thermal treatment, treatment with fly-ash, pyrolysis and solvent extraction treatment) (Taiwo & Otolorin 2009) and biological treatment (land farming, bio-reactor treatment and composting) (Singh et al. 2001, Hejazi et al. 2003, Mahmoud 2004, Dequing et al. 2007, Srivasarao et al. 2011, Udotong et al. 2011, Besalatapour et al. 2011). Some of these convert oil sludge into lighter products and reduce the quantity before disposal. Some may generate by-products that may need to be treated using other methods before disposal to a landfill (Liu et al. 2010), making them more expensive. These methods also generate toxic residues such as ash, scrubber water, sulphur dioxide, carbon monoxide and some organic compounds (Srinivasarao et al. 2011). It is observed that none of the conventional methods is environment friendly solution (Sood & Bamwari 2009). All physical and chemical methods for processing oil-containing wastes are expensive, power consuming and accompanied by the formation of secondary wastes, which are difficult to get rid of (Selivanovskaya et al. 2013).

Microbial biodegradation of oil sludge: Oil sludge exhibits some biodegradable properties in the environments such as transformation, conversion or mineralisation, specific adhesion mechanisms and production of extracellular emulsifying agent by microorganisms (Laskova et al. 2007, Paulauskiene et al. 2009). The hydrocarbons present at contaminated site can be used by microbes as substrate to fullfill their basic need of energy and carbon source, and these can be used to degrade or remediate environmental hazard (Sharma 2012). Microorganisms are known to attack specific compounds present in crude oil that is a complex mixture of saturates, aromatics and polar compounds (Boudia 2013). The organisms that are utilized vary, depending on

Technique	Working	Disadvantages
Incineration	The waste sludge is burnt at temperature from 980° C to 1200° C.	 Residue waste is more harmful. Expensive method.
Oxidative thermal treatment	Heated to remove organics and water from solids, the water is converted to steam to help strip off high boiling point semi-volatile compounds, which can be condensed for recovery and disposal.	 1) High energy Requirement 2) Complex process.
Pyrolysis	It is a technique for recovering oil and organic liquid gas by breaking down large molecules into smaller ones	 High energy Requirement. Residue waste disposal.
Lime stabilization Involves mixing a solid additive material to the oil sludge in order to produce a matrix within which the oil and metal are fixed and will not leach out		1) End moduate an expansive to treat
Solvent extraction	The oil sludge is extracted with a solvent to remove oil and other organics, the solvent is recovered and recycled.	 End products are expensive to treat. Can't remove heavy metal present in sludge.

Table 2: Conventional methods of sludge treatment. Source: Ubani et al. (2013)

Table 3: Microbes useful in biodegradation of oily sludge. Source: Desai & Vyas (2006)

Crude oil component	Microorganisms
Saturates	Arthrobacter sp., Acinetobacter sp., Candida sp., Pseudomonas sp., Rhodococcus sp., Streptomyces sp., Bacillus sp., Aspergillus japonicas
Monocyclic aromatic hydrocarbons	Pseudomonas sp., Bacillus sp. B. stereothermophilus, Vibrio sp., Nocardia sp., Corynebacterium sp., Achromobacter sp.
Polycyclic aromatic hydrocarbons	Arthrobacter sp., Bacillus sp., Burkholderia cepacia, Pseudomonas sp., Mycobacterium sp., Xanthomonas sp., Phanerochaete chrysosporium, Anabena sp., Alcaligenes
Resins	Pseudomonas sp., Members of Vibrionaceae., Enterobacteriaceea, Moraxella sp.

the chemical nature of the polluting agents, and are to be selected carefully as they only survive within a limited range of chemical contaminants (Prescott et al. 2002, Dubey 2004). They must be able to synthesize enzymes that can catalyse the reaction in which these contaminants are degraded to simpler, lower molecular chains and less toxic compounds (CO_2 and H_2O), through obtaining the nutrients and energy necessary for their survival in the process (Ho & Rashid 2008). The initial step in this mechanism is the catabolism of oil sludge by bacteria and fungi, which involve the oxidation of the substrate by oxygenases, in which molecular oxygen is required (Atlas 2011).

Aerobic conditions are necessary for this route of microbial oxidation of hydrocarbons to take place (Marin Millan 2004). The characteristics and fate of oil sludge is dependent on its molecular size and topology or stoichiometry (Mukred et al. 2008). The removal of low molecular weight petroleum hydrocarbons (4-ring or less), is first done through evaporation. As the molecular sizes increase, biodegradation rates become slower (Fig. 3).

Oil sludge, albeit very slow, is susceptible to degradation by naturally occurring microflora, but this process reduces nutrient and oxygen level in soil, which in turn impedes other environmental processes such as transformation or mineralisation. In order to enhance the oil sludge biodegradation processes and make it economically realistic and fast, it is necessary that the bioavailability of hydrocarbons present in the oil sludge matrix be increased. This may be done by bio-stimulation, which is simply the addition of nutrients to stimulate the growth and degradative capabilities of the indigenous microorganisms present (Piskonen & Itavarra 2004). Most of the microbial strains are capable of degrading only specific components of oil sludge (Fig. 4). However, oil sludge is a complex mixture of different petroleum hydrocarbon (Mac Naughton et al. 1999). Natural organisms, either indigenous or extraneous (introduced), are the prime agents used for bioremediation (Prescott et al. 2002). A mixture of different bacterial species that can degrade a broad range of the hydrocarbon constituents such as present in oil sludge would show more potential (Oteyza et al. 2006). Liu et al. (2010) suggested that indigenous microorganisms isolated from a contaminated site will assist in overcoming this problem, as the microorganisms can degrade the components and have a higher tolerance to toxicity that may wipe off other introduced species. Petrophiles are very unique organisms that

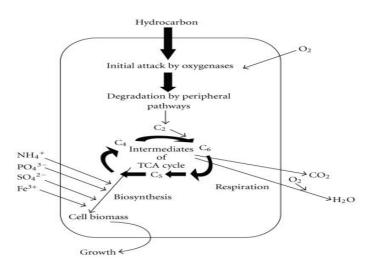


Fig. 3: Main principle of Aerobic degradation of hydrocarbons (Das & Chandran 2011).

can naturally degrade large hydrocarbons and utilize them as food source (Tabassum et al. 2010) (Fig. 5).

The first patent for a biological remediation agent was registered in 1974, being a strain of Pseudomonas putida (Chatterjee et al. 2008) that was able to degrade petroleum. Many native strains including ligninolytic fungi have great potential for remediation of polycyclic aromatic hydrocarbons from contaminated soils in oil refinery sites (Low et al. 2008). Bacterial genera, namely, Gordonia, Brevibacterium, Aeromicrobium, Dietzia, Burkholderia, Mycobacterium, Psedomonas, Sphingomonas and Enterobacter isolated from petroleum contaminated soil proved to be the potential organisms for hydrocarbon degradation (Daugulis & Mc Cracken 2003, Chaillan et al. 2004, Pathak et al. 2008, Igwo-Ezikpe et al. 2010, Jain et al. 2010a, b). Species of Pseudomonas, Mycobacterium, Haemophilus, Rhodococcus, Paenibacillus and Ralstonai are some of most extensively studied bacteria for bioremediation of hydrocarbons (Farhadian et al. 2008) (Fig. 6).

Fungal genera, namely, Amorphoteca, Neosartorya, Talaromyces and Graphium and yeast genera, namely, Candida, Yarrowia and Pichia were isolated from petroleumcontaminated soil and proved to be the potential organisms for hydrocarbon degradation (Chaillan et al. 2004). Singh (2006) also reported a group of terrestrial fungi, namely, Aspergillus, Cephalosporium and Penicillium which were also found to be the potential degrader of crude oil hydrocarbons. Aspergillus flavus, Alternaria sp., Penicillium sp. and Fusarium sp. isolated from the contaminated soil of Panipat Refinery showed better hydrocarbon degradation (Chaudhry et al. 2012). The yeast species, namely, Candida lipolytica, Rhodotorula mucilaginosa, Geotrichum sp., Rhodosporidium toruloides and Trichosporon mucoides, isolated from contaminated sites, were noted to degrade petroleum compounds (Boguslawska-Was & Dabrowski 2001, Kumari & Abraham 2011). Bacterial genera, namely, Acinetobacter sp., Gordonia, Brevibacterium, Aeromicrobium, Dietzia, Burkholderia and Mycobacterium were isolated from petroleum contaminated soils in north-east India. Among them Acinetobacter sp. was found to be capable of utilizing n-alkanes of chain length C_{10} – C_{40} as a sole carbon source (Das & Mukherjee 2007). Mancera-López et al. (2007) isolated 37 hydrocarbon-degrading, but only six strains showed a high ability to



Fig. 4: Mechanism of degradation of oil by microbes.

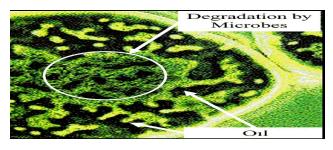


Fig. 5: Degradation of oil by microbes (Source: www.ebsinfo.com)

Parameter	Condition required for microbial activity	Optimum value for oil degradation
Soil moisture	25-28% of water holding capacity	30-90%
Soil pH	5.5-8.8	6.5-8.0
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10-40%
Nutrient content	N and P for microbial growth	C:N:P = 100:10:1
Temperature (°C)	15-45	20-30
Contaminants	Not too toxic	Hydrocarbon 5-10% of dry weight of soil
Heavy Metals	Total content 2000ppm	700ppm

Table 4: Optimum conditions for microorganisms activity. Source: Vidali (2001)

degrade PAHs, AHs and TPH. These strains were identified as Pseudomonas pseudoalcaligenes, Bacillus firmus, Bacillus alvei, Penicillium funiculosum, Aspergillus sydowii and Rhizopus sp., and they removed 79%, 80%, 68%, 86%, 81% and 67% of TPH, respectively. P. pseudoalcaligenes and P. funiculosum removed 75% of PAHs, while B. firmus and P. funiculosum removed 90% and 92% of AHs, respectively. Bioremediation of petroleum oil contaminated sites has become possible in India. Recently, The Energy and Resources Institute (TERI, New Delhi) developed TERI's Oilzapper (3 strain Acinetobacter baumannii, one strain Burkholdia cepacia, one strain Pseudomonas sp.) (Lal et al. 2000). It is a crude oil degrading bacterial system that has been developed to reclaim contaminated sites. TERI has stocks of bacteria that were isolated from the natural environment and have capacity to eat up the harmful compounds in oil spill sites. These bacteria are cultivated in the laboratory and then mixed with specific carrier material and packed in poly-bags for easily transportation to petroleum contaminated sites (Jain & Bajpai 2012). The name 'Oilivorous-S' suggests an affinity to 'Oilzapper'. It belongs to the same family of oily sludge-degrading consortia of

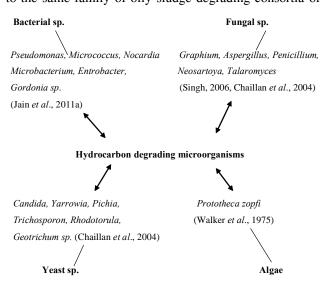


Fig. 6: Different microbes showing degradation of hydrocarbons.

microbes. Oilivorous-S a tad different from Oilzapper is an additional bacterial strain that makes the former more effective against sludge and crude oil with high-sulphur content (www.teriin.org).

Factors affecting the biodegradation mechanisms: There are many factors, including physical, chemical and biological that may ultimately determine the effectiveness of strategies for microbial bioremediation of oil sludge (van Hamme et al. 2003). These include: Biosurfactants, effects of pH, nutrients, salinity, oxygen, temperature and water activity/ moisture contents (Agarry & Ogunleye 2012). Biosurfactants are important agents that enhance the effective uptake of petroleum hydrocarbons by bacteria and fungi (Cort & Bielefeldt 2000a; b; Shiohara et al. 2001). Bacteria are known to produce biosurfactants, which they use to form emulsions of oil substrate (Calvo et al. 2004, Bayoumi 2009, Liu et al. 2011, Plaza et al. 2011). The biosurfactant they produce can emulsify petroleum hydrocarbon in oil sludge so that they can be bioavailable to bacteria for biodegradation in the system. They do this by increasing the surface area of the substrates therefore, increased their solubility (Ahimou et al. 2000, Maier 2003, Mukherjee & Das 2005). Biosurfactant production by bacteria comes with the advantage of being natural, non-toxic, and bio-degradable and a cost effective approach that can help in solubilisation (Sim et al. 1997, Calvo et al. 2004, Bayoumi 2009, Liu et al. 2011, Plaza et al. 2011). Hydrocarbon catabolism in the environment can be enhanced by the production of biosurfactants and supplementary application of additives and bulking agents (Ward et al. 2003). The growth of heterotrophic bacteria and fungi depends on a number of nutrient elements, an electron acceptor and organic compounds that serve as the source of carbon and energy (Adriano et al. 1999, Boettcher et al. 2001). Aerobic microorganisms use oxygen as electron acceptor. Some microorganisms also utilise inorganic compounds such as nitrates, sulphates, carbon dioxide, ferric ion and some organic compounds. According to report by van Hamme et al. (2003), nitrogen and phosphorus contents have great effects on microbial degradation of oil sludge. Hyper salinity will result in the de-



Fig. 7: Pre-bioremediation: A site in Mehsana, Gujarat (Source: www.teriin.org)

crease in microbial metabolic rates (Micky 2006). Great efficiency of natural microbial hydrocarbon degradation occurs mostly when oxygen is available because it involves oxygenase (Ward et al. 2003). Although anaerobic degradation of PAHs by microorganisms has been shown to occur, the rates are somewhat negligible and limited to halogenated aromatics compounds such as the halobenzoates, chlorophenols and alkyl-substituted aromatics (Angelidaki et al. 2000). Most bacteria are mesophilic and are active between 5 and 40°C. The optimal activity generally occurs at 35°C. A few mesophilic bacteria can survive up to 60° C, but only thermophilic microorganisms can grow above this temperature (El Naggar et al. 2012). According to Vinas et al. (2005), the rate at which PAHs are degraded are also determined by moisture level because water is needed for microbial growth and enzymatic/biochemical activities. Optimal activity occurs when the soil moisture and water content for aerobic bioremediation treatment matrix is usually between 50 and 80% of saturation (moisture holding capacity) (Kosaric 2001) (Figs. 7 and 8).

CONCLUSION

It is evident that researchers and most refineries have tried to treat oil sludge using conventional methods. Since these methods may require expensive equipments and high energy to treat the oil sludge, bioremediation is considered to be very safe and helpful technology as it relies on microbes that occur naturally in the soil and pose no threat to environment and the people living in that area. The process of bioremediation can be easily carried out on site without causing a major disruption of normal activities and threats to human and environment during transportation. Bioremediation is less expensive than other technologies that are used for clean-up of hazardous sludge. The pathway



Fig. 8: Post-bioremediation: the same site after 2 Months (Source: www.teriin.org)

of aerobic transformation has been reported and it is established that microorganisms capable of degrading oil sludge could be found in the contaminated environment. Even though various sources of bioremediation such as bacteria, archaebacteria, yeasts, fungi, algae and plants are available but, the biological treatment alone is not sufficient enough to treat the pollutants or contaminated sites. Every biological form has a different growth requirements (temperature, pH and nutrients) so we need to isolate those forms, which can cultured easily in the lab, with minimal requirement and can be useful in treating variety of hydrocarbons present in the sludge. Use of genetically engineered microorganisms is probably not needed in most cases because of wide availability of naturally occurring microbes. Besides using these natural or genetically engineered microbes, there is an urgent need for us to educate and aware local people about the various life forms, their potential applications and tendencies to absorb/adsorb the contaminants whose existence can harm our environment. A detailed study of area-wise and hydrocarbon type data base is much needed to finalize the priority area and the need for the effective removal of the pollutant from the contaminated sites. As natural resources are major assets to humans their contamination resulted in long term effects. The decontamination of these natural resources is essential for the conservation of nature and environment using bioremediation process. Thus, there is an urgent need to study the effect of various microorganisms in combination against various pollutants for the conservation of natural resources and environment management.

REFERENCES

Abbasnezhad, H., Gray, M. and Fought, J.M. 2011. Information of adhesion on anaerobic biodegradation and bioremediation of liquid hydrocarbon. Applied Microbiol. Biotech., 92: 653-675.

- Achal, V.D., Kumari, D. and Pan, X. 2011. Bioremediation of chromium contaminated soil by a brown rot fungus, *Gloephyllum sepiarium*. Res. J. of Microbiol., 6: 166-171.
- Adriano, D.C., Bollag, J.M., Frankenberger, Jr. W.T. and Sims, R.C. 1999. Bioremediation of contaminated soil. Agronomy, No. 37, American Society of Agronomy.
- Agarry, S.E. and Ogunleye, O.O. 2012. Box-Behnken design application to study enhanced bioremediation of soil artificially contaminated with spent engine oil using biostimulation. International J. of Energy and Environ. Engg., 3: 31.
- Ahimou, F., Jacques, P. and Deleu, M. 2000. Surfactin and Iturin A effects on *Bacillus subtilis* surface hydrophobicity. Enzyme Microb. Technol., 27: 749-754.
- Angelidaki, I., Mogensen, A.S. and Ahring, B.K. 2000. Degradation of organic contaminants found in organic waste. Biodeg., 11: 377.
- Atlas, R.M. 2011. Oil biodegradation and bioremediation: A tale of the two-worst spills in U.S. history. Environ. Sci. and Technol., American Chemical Society, 45: 6709-671.
- Atlas, R.M. and Philip, J. 2005. Bioremediation: Applied microbial solutions for real-world environmental cleanup. ASM Press, Washington, D.C., 1-292.
- Bach, Q.D., Kim, S.J., Choi, S.C. and Oh, Y.S. 2005. Enhancing the intrinsic bioremediation of PAH-contaminated anoxic estuarine sediments with biostimulating agents. J. of Microbiol., 43: 319.
- Bayoumi, R.A. 2009. Bacterial bioremediation of polycyclic aromatic hydrocarbons in heavy oil contaminated soil. J. of Appl. Sci. Res., 5(2): 197-211.
- Beech, J.F., Elder, S. and Weeks, N. 2009. Case-study: Use of circulating fluidized bed boiler by-product to solidify oil sludge. World of Coal Ash (WOCA) Conference, May 4-7, in Lexington, KY, USA.
- Benson, N.U., Essien, J.P., Williams, A.B. and Ebong, G.A. 2007. Petroleum hydrocarbons accumulation potential of shellfishes from littoral waters of the Bight of Bonny, Niger Delta, Nigeria. Res. J. of Environ. Sci., 1:11-19.
- Bento, F.M., Camargo, F.A.O., Okeke, B.C. and Frankenberger, W.T. 2005. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresour. Technol., 96(9): 1049-1055.
- Besalatpour, A., Hajabbasi, M.A., Khoshgoftarmanesh, A.H. and Dorostkar, V. 2011. Landfarming process effects on biochemical properties of petroleum-contaminated soils. Soil and sediment contamination. 20: 234-248.
- Bhattacharyya, J.K. and Shekdar, A.V. 2003. Treatments and disposal of refinery sludges: Indian scenario. Waste Manage. Res., 21: 249-261.
- Boettcher, G., Nyer, E.K., Palmer, P.L., Carman, E.P., Bedessem, J., Kidd, D.F., Lenzo, F., Rorech, G.J. and Crossman, T.L. 2001. *In*situ Bioremediation. *In-situ* Treatment Technology. Second Edition, CRC Press.
- Boguslawska-Was, E. and Dabrowski, W. 2001. The seasonal variability of yeasts and yeast-like organisms in water and bottom sediment of the Szczecin Lagoon. International Journal of Hygiene and Environmental Health, 203(5-6): 451-458.
- Bojes, H.K. and Pope, P.G. 2007. Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas. Regul. Toxicol. Pharmacol., 47(3): 288-295.
- Boudia, A.O. 2013. Bioremediation of crude oils/complex mixture of hydrocarbons (CMH) contaminants in seawater by a halotolerant bacteria under aerobic conditions: *Enterobacter cloacae, Pseu*domonas spp. and *Escherichia coli*. Global Advanced Res. J. of

Microbiol., 2(8): 131-136.

- Calvo, C., Toledo, F.L. and Gonzalez-lopez, J. 2004. Surfactant activity of a naphthalene degrading *Bacillus pumilus* strain isolated from oil sludge. J. of Biotechnol., 109: 255-262.
- Chaillan, F., Fleche, A. Le., Bury, E., Phantavong, Y., Grimont, P., Saliot, A. and Oudot, J. 2004. Identification and biodegradation potential of tropical aerobic hydrocarbon degrading microorganisms. Res. in Microbiol., 155(7): 587-595.
- Chatterjee, S., Chattopadhyay, P., Roy, S. and Sen, S.K. 2008. Bioremediation: A tool for cleaning polluted environment. J. of App. Bioscience, 11: 594-601.
- Chaudhry, S., Luhach, J., Sharma, V. and Sharma, C. 2012. Assessment of diesel degrading potential of fungal isolates from contaminated soil of petroleum refinery, Hariyana. Res J. of Microbiol., 1816-4935.
- Chikere, C.B., Chikere, B.O. and Okpokwasili, G.C. 2012. Bioreactorbased bioremediation of hydrocarbon-polluted Niger Delta marine sediment, Nigeria. Biotech., 2: 53-66.
- Cort, T. and Bielefeldt, A. 2000. Effects of surfactants and temperature on PCP biodegradation. J. of Environ. Engg., 126: 635-643.
- Crivelaro, S.H.R., Mariano, A.P., Furlan, L.T., Gonçalves, R.A., Seabra, P.N. and Angelis, D.F. 2010. Evaluation of the use of vinasse as a biostimulation agent for the biodegradation of oily sludge in soil. Int. J. of Brazil Biol. Technol., 53(5): 1217-1224.
- Das, N. and Chandran, P. 2011. Microbial degradation of petroleum hydrocarbon contaminants: An overview, SAGE-Hindawi access to research. Biotechnology Res. Int., 1-13.
- Das, K. and Mukherjee, A.K. 2007. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from northeast India. Bioresource Technol., 98(7): 1339-1345.
- Daugulis, A.J. and Mc Cracken, R.M. 2003. Microbial degradation of high and low molecular weight polyaromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of *Sphingomonas* sp. Biotechnol. Letters, 25(17): 1441-1444.
- Desai, A. and Vyas, P. 2006. Petroleum and hydrocarbon microbiology. MS University, Baroda, India.
- De-qing, S., Jain, Z., Zhao-long, G., Jian, D., Tian-Li, W., Murygina, V. and Kalyuzhnyi, S. 2007. Bioremediation of oil sludge in Shengli oilfield. Water Air Soil Pollut., 185: 177-184.
- Dindar, E., Sagban, F.O.T. and Baskaya, H.S. 2013. Bioremediation of petroleum contaminated soil. J. of Biological Environ. Sci., 7(19): 39-47.
- Dubey, R.C. 2004. A Text Book of Biotechnology. 3rd Edition, S. Chand & Company Ltd. New Delhi, India, pp. 365-375.
- El-Naggar, A.Y., Kamel, M.M., Hassan, Y.M., Youseff, K.A., Al-Adly, A.A. and Ismail, N.A. 2012. Gas chromatography assessment of bioremediation of hydro-pollutants using *Bacillus amyloliquefaciens*. Archives of App. Sci. Res., 4(4): 1593-1599.
- Erdogan, E.E. and Karaca, A. 2011. Bioremediation of crude oil polluted soils. Asian J. of Biotechnol., 3: 206-213.
- Farhadian, M., Vachelard, C., Duchez, D. and Larroche, C. 2008. In situ bioremediation of monoaromatic pollutants in groundwater: A review, Bioresour. Technol., 99: 5296-5308.
- Hamme, V.J.D., Singh, A. and Ward, O.P. 2003. Recent advances in petroleum microbiology. Microbiol. Mol. Biol. Rev., 67: 503.
- Hejazi, R.F., Husain. T. and Khan, F.I. 2003. Land farming operation of oil sludge in arid region - human health risk assessment. J. of Hazard. Materi., 99(B): 287-302.
- Ho, J. and Rashid, M. 2008. Application of EZ-Enzyme in bioremediation of oily sludge. Turnal Teknologi., 48(F): 21-32. http://www.Petroleum,nic.in
- http://www.teriin.org/index.php?option=com_casestudy&task=details &sid=6

- Igwo-Ezikpe, M.N., Gbenle, O.G., Ilori, M.O., Okpuzor, J. and Osuntoki, A.A. 2010. High molecular weight polycyclic aromatic hydrocarbons biodegradation by bacteria isolated from contaminated soils in Nigeria. Res. J. of Environ. Sci., 4: 127-137.
- Jain, P.K. and Bajpai, V. 2012. Biotechnology of bioremediation A review. Int. J. of Environ. Sci., 3(1).
- Jain, P.K., Gupta, V.K., Gaur, R.K., Lowry, M., Jaroli, D.P. and Chauhan, U.K. 2011a. Bioremediation of petroleum oil contaminated soil and water. Res. J. of Environ. Toxicology, 5(1): 1-26.
- Jain, P.K., Gupta, V.K., Pathak, H., Lowry, M. and Jaroli, D.P. 2010a. Characterization of 2T engine oil degrading indigenous bacteria, collected from high altitude Mussoorie region of India. World J. of Microbiol. and Biotechnol., 26: 1419-1426.
- Jain, P.K., Lowry, M., Gupta, V.K., Bajpai, V., Sharma, S., Joshi, N. and Gaur, R.K. 2010b. *Enterobacter* sp. as an engine oil degrader. Biospectra, 5(1): 115-120.
- Kaplery, A. and Purohit, H.J. 2009. Genomic tools in bioremediation. Indian Microbiol., 49: 108-113.
- Karamalidis, A.K. and Voudrias, E.A. 2001. Stabilization/solidification of oil refinery sludge: Immobilization of heavy metals. 7th Conference of Environ. Sci. and Technol. Ermonpolis, Syros Island, Greece.
- Kosaric, N. 2001. Biosurfactant for soil bioremediation. Food Technol. Biotechnol., 39(4): 295-304.
- Kumari, M. and Abraham, J. 2011. Biodegradation of diesel oil using yeast *Rhodosporidium toruloides*. Res. J. of Environ. Toxicol., 5(6): 369-377.
- Lal, B., Mishra, S., Ramesh, K.C. and Jyot, J. 2000. Oilzapper: An effective bioremediation tool. In: Proceedings of the International Seminar on Oily Sludge Management, pp. 1-12.
- Laskova, T., Zabukas, V. and Vaitiekunas, P. 2007. Influence of meteorological conditions on volatile organic compound spread in the atmospheric boundary layer. Journal of Environ. Engg. Landscape Manag., 15(3): 135-143.
- Lewis, Ceri., Chris, Pook. and Tamara, Galloway 2008. Reproductive toxicity of the water accommodated fraction (WAF) of crude oil in the polychaetes *Arenicola marina* (L.) and *Nereis virens* (Sars), Aquatic Toxicol., 90(1): 73-81.
- Liu, T., Hou, J., Zuo, Y., Bi, S. and Jing, J. 2011. Isolation and characterization of a biosurfactant producing bacterium from Daqing oil-contaminated site. Afr. J. of Microb. Res., 5(21): 3509-3514.
- Liu, W., Luo, Y., Teng, Y., Li, Z. and Ma, Q.L. 2010. Bioremediation of oily sludge contaminated soil by stimulating indigenous microbes. Env. Geochem. and Health, 32(1): 23-29.
- Loick, N., Hobbs, P.J., Hale, M.D.C. and Jones, D.L. 2009. Bioremediation of poly-aromatic hydrocarbon (PAH) contaminated soil by composting. Critical Reviews in Environ. Sci. and Technol., 39: 271-332.
- Low, Y.S., Abdullah, N. and Vikineswary, S. 2008. Biodegradation of polycyclic aromatic hydrocarbons by immobilized *Pycnoporus* sanguineus on ecomat. J. of App. Sci., 8: 4330-4337.
- Luqueno, F.F., Encinas, C.V., Marsh, R., Suarez, C.M., Nunez, E.V. and Dendooven, L. 2011. Microbial communities to mitigate contamination of PAH's in soil - possibilities and challenges - a review. Env. Sci. Res., 18: 12-30.
- Machin-Ramirez, C.A.I., Okoh, D.K., Morales, M., Mayolo-Deloisa, R. and Quintero, T.H. 2008. Slurry-phase biodegradation of weathered oily sludge waste. Chemosphere, 70: 737-744.
- Mac Naughton, S.J., Stephen, J.R., Venosa, A.D., Davis, G.A, Chang, Y.J. and White, D.C. 1999. Microbial population changes during bioremediation of an experimental oil spill. Appl. Environ. Microbiol., 65: 3566.
- Mahmoud, S. 2004. Novel technology for sustainable petroleum oil

sludge management: Bio-neutralization by indigenous fungalbacterial co-cultures. A Masters Degree Thesis, Department of Building, Civil and Environmental Engineering, Concordia University, Montreal, Quebec, Canada.

- Maier, R.M. 2003. Biosurfactant: Evolution and diversity in bacteria. Adv. Appl. Microbiol., 52: 101-121.
- Mancera-López, M.E., Rodríguez-Casasola, M.T., Ríos-Leal, E. Esparza-García, F., Chávez-Gómez, B., Rodríguez-Vázquez, R. and Barrera-Cortés, J. 2007. Fungi and bacteria isolated from two highly polluted soils for hydrocarbon degradation. Acta Chim. Slov., 54: 201-209.
- Mandal, A.K., Sarma, P.M., Singh, B., Jeyaseelan, C.P., Channashettar, V.A., Lal, B. and Dutta, J. 2012. Bioremediation: An environment friendly sustainable biotechnological solution for remediation of petroleum hydrocarbon contaminated waste. J. of Sci. and Tech., 2: 1-12.
- Marin, J.A., Hernandez, T. and Garcia, C. 2004. Bioremediation of oil refinery sludge by landfarming in semiarid conditions: Influence on soil microbial activity. Environmental Research, 98: 185-195.
- Medina-Bellver, J., Marin, P., Delgado, A., Rodriguez-sanches, E.R., Ramos, J.L. and Marques, S. 2005. Evidence for *in situ* crude oil biodegradation after the Prestige oil spill, Environ. Microbiol., 7(6): 773-779.
- Medjor, O.W., Egharevba, F., Akpoveta, O.V., Ize-Iyamu, O.K. and Jatto, E.O. 2012. Kinetic studies of bioremediation of hydrocarbon contaminated ground water. Res. J. of Chemical Sci., 2(1): 38-44.
- Micky, V. 2006. Microbial bioremediation of polycyclic aromatic hydrocarbons (PAHs) in oily sludge wastes. J. of Biochem., Microbio. and Biotechnol., 1(1): 1-12.
- Mishra, S., Jyoti, J., Kuhad, R.C. and Lai, B. 2001. In situ bioremediation potential of an oil sludge-degrading bacterial consortium. Curr. Microbiol., 43: 328-335.
- Mnif, S., Chamkha, M. and Sayadi, S. 2009. Isolation and characterization of *Halomonas* sp. strain C2SS100, a hydrocarbon degrading bacterium under hypersaline conditions. J. of App. Microbiol., 107: 785-794.
- Mukherjee, A.K. and Das, K. 2005. Correlation between diverse cyclic lipopeptides production and regulation of growth and substrate utilization by *Bacillus subtilis* strains in a particular habitat. FEMS Microbiol. Ecol., 54: 479-489.
- Mukred, A.M., Hamid, A.A., Hamzah, A. and Yusoff, W.M.W. 2008. Development of three bacteria consortium for the bioremediation of crude petroleum-oil in contaminated water. Online J. of Biolo. Sci., 8(4): 73.
- Nataraj, S.K., Sridhar, S., Shaikha, I.N., Reddy, D.S. and Aminabhavi, T.M. 2007. Membrane-based microfiltration/electrodialysis hybrid process for the treatment of paper industry waste water. Separation and Purification Tech., 7: 185-195.
- Oteyza de, T.G., Grimalt, J.O., Lliros, M. and Esteve, I. 2006. Microcosm experiments of oil degradation by microbial mats. Sci. Total Environ., 357: 12-24.
- Pathak, H., Jain, P.K., Jaroli, D.P. and Lowry, M. 2008. Technical note: Degradation of phenanthrene and anthracene by *Pseudomonas* strain isolated from coastal area. Biorem. J., 12: 111-116.
- Paulauskiene, T., Zabukas, V. and Vaitiekunas, P. 2009. Investigation of volatile organic compound (VOC) emission in oil terminal storage tank. Park. J. of Environ. Engnr. Landscape Manag., 17(2): 81-89.
- Piskonen, R. and Itävaara, M. 2004. Evaluation of chemical pretreatment of contaminated soil for improved PAH bioremediation. Appl. Microbiol. Biotechnol., 65: 627.

- Plaza, G.A., Pacwa-Plociniczak, M., Piotrowska-Seget, Z. and Cameotra, S.S. 2011. Environmental applications of biosurfactants: Recent Advances. Int. J. of Mol. Sci., 12: 633-654.
- Prescott, L.M., Harley, J.P. and Klein, D.A. 2002. Microbiology, 5th Edition, McGraw-Hill, New York, 1014.
- Qing, D.S., Jian, Z., Long, G.Z., Jain, D., Li, W., gina, V. and Kalyuzhnyi, S. 2007. Bioremediation of oil sludge in Shegli Oilfield. Water Air Soil Pollution, 185: 177-184.
- Radetski, C.M., Mater, L., Sperb, R.M., Madureira, L.A.S., Rosin, A.P. and Correa, A.X.R. 2006. Proposal of a sequential treatment methodology for the safe reuse of oil sludge-contaminated soil. J. of Hazard. Mat., 136(B): 967-971.
- Selivanokaya, S.Y., Gumerova, R.K. and Galitskaye, P.Y. 2013. Assessing the efficiency of methods for bioremediation of oil produced wastes. Contemporary Problems of Ecology, 6(5): 542-548.
- Sim, L. and Ward, O.P. 1997. Production characterization of a biosurfactant isolated from *Pseudomonas aeruginosa* UW-1. J. of Ind. Microbiol. Biotechnol., 19: 232.
- Singh, H. 2006. Mycoremediation: Fungal Bioremediation. Wiley-Interscience, New York.
- Singh, A., Mullin, B. and Ward, O. 2001. Reactor-based process for the biological treatment of petroleum wastes. Petrotech, Petrozyme Technologies, Inc.
- Sharma, S. 2012. Bioremediation: Features, strategies and application. Asian J. of Pharmacy and Life Sci., 2(2): 202-213.
- Shie, J., Lin, J., Chang, C., Wu, C., Lee, D., Chang, C. and Chen, Y. 2004. Oxidative thermal treatment of oil sludge at low heating rates. Energy Fuel, 18: 1272-1281.
- Shiohara, K., Diehl, S.V. and Borazjani, H. 2001. Use of commercial surfactants for enhanced biodegradation of organic wood-preservative contaminated process water. In: Proceedings of the 2001 Mississipi Water Resources Conference, Raymond, Mississipi, USA.
- Soccol, C.R., Vandenberghe, L.P.S., Woiciechowski, A.L., Thomaz-Soccol, V., Correia, C.T. and Pandey, A. 2003. Bioremediation-An important alternative for soil and industrial wastes clean-up. Indian J. of Experimental Biol., 41: 1030-1045.
- Soleimani, M., Akbar, S. and Hajabbasi, M.A., 2011. Enhancing phytoremediation efficiency in response to environment pollution stress. In: Plants and Environment, Dr. Hemanth Vasanthaiah (Ed.), ISBN: 978-953-307-779-6.
- Sonawdekar, S. 2012. Bioremediation: A boon to hydrocarbon degradation. Int. J. of Environ. Sci., 2(4): 2408-2424.
- Sood, N. and Banwari, L. 2009. Isolation of a novel yeast strain Candida digboiensis TERI ASN6 capable of degrading petroleum hydrocarbons in acidic conditions. J. of Environ. Manag., 90: 1728-1736.
- Srinivasarao-Naik, B., Mishra, I.M. and Bhattacharya, S.D. 2011. Biodegradation of total petroleum hydrocarbons from oily sludge. Bioremed. J., 15(3): 140-147.
- Strong, P.J. and Burgess, J.E. 2008. Treatment methods for winerelated ad distillery wastewaters: A review. Biorem. J., 12: 70-87.

- Tabassum, N., Jha, R. and Kumari, A. 2010. Isolation of engine oil degrading microorganisms. Industrial Microbiol., 11(2): 86-88.
- Taiwo, E.A. and Otolorin, J.A. 2009. Oil recovery from petroleum sludge by solvent extraction. Petrol. Sci. and Technol., 27(8): 836-844.
- TERA 2008. Peer Consultation on Relationship Between PAC Profile and Toxicity of Petroleum Substances (API Report), http:// www .tera .org/ peer/ API/ API Welcome.htm, accessed 28 Oct 2009 and "Report of the Peer Consultation on Relationship between PAC Profile and Toxicity of Petroleum Substances Volume I" (TERA peer review) http://www.tera.org/peer/API/PAC MEET-ING REPORT Final.pdf, accessed, 28 Oct 2009.
- Thapa, B., Kumar, A. and Ghimire, A. 2012. A review on bioremediation of petroleum hydrocarbon contaminants in soils. Kathmandu University J. of Sci., Eng. and Technol., 8(1): 164-170.
- Tyagi, M., Manuela, M., Fonseca, D., Carla, C.C. and Larvalno, R. 2011. Bioaugmentation and biostimulation strategies to improve the effective bioremediation process. Biodegradation, 22: 231-241.
- Ubani, O., Atagana, H.I. and Thantsha, M.S. 2013. Biological degradation of oil sludge: A review of the current state of development. African J. of Biotechnol., 12(47): 6544-6567.
- Udotong, I.R., Udotong, I.J., Inam, E. and Kim, K. 2011. Bioconversion of crude oil production into soil conditioner using sawdust as organic amendment. Geosyst. Engr. 14(2): 51-58.
- US-EPA 1997. EPA's contaminated sediment management strategy. Office of Water and Solid Waste. US-EPA, Washington DC.
- Van Hamme, J.D., Singh, A. and Ward, O.P. 2003. Recent advances in petroleum microbiology. Microbiol. Mol. Rev., 67: 649.
- Vidali, M. 2001. Bioremediation. An overview. Pure Appl. Chem., 73(7): 1163.
- Vinas, M., Sabate, J., Espuny, M.J. and Solanas, A.M. 2005. Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil. Appl. Environ. Microbiol., 71: 7008.
- Walker, J.D., Colwell, R.R., Vaituzis, Z. and Meyer, S.A. 1975. Petroleum-degrading achlorophyllous alga *Prototheca zopfii*. Nature, 254: 423-424.
- Wang, J., Yin, J., Ge, L., Shao, J. and Zheng, J. 2010. Characterization of oil sludge from two oil fields in China. Energy Fuel, 24: 973-978.
- Wang, J., Zhang, Z.Z., Su, Y.M., He, W., He, F. and Song, H.G. 2008. Phytoremediation of petroleum polluted soil. Petroleum Sci., 5(2): 167.
- Ward, W., Singh, A. and van Hamme, J. 2003. Accelerated biodegradation of petroleum hydrocarbon waste. J. Ind. Microbiol. Biotechnol., 30: 260.
- Wu, T. and Crapper, M. 2009. Simulation of biopile processes using a hydraulics approach. J. of Hazardous Material, 171(1-3): 1103-1111.
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