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Bioremediation of Polluted Environment: A review of microbiological aspects

Mahak Lalwani*[1], Vikram Kumar Yadav[2]

[1]Biotech Scholar, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-303002, Rajasthan, India
[2]Assistant Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-303002, Rajasthan, India

ABSTRACT

Microbial Bioremediation uses microorganism metabolism to remove pollutants it uses relatively lowcost,low-technology techniques, which generally have a high public acceptance and can often be carried out on site.This technology includes biostimulation (stimulating viable native microbial population), bioaugmentation (artificial introduction of viable population), bioaccumulation (live cells), biosorption (dead microbial biomass), phytoremediation (plants) and rhizoremediation (plant and microbe interaction) are also used for bioremediation of contaminated environmental sites. Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. Contamination of the natural environment with oil derivatives causes soil, including arable land, to degrade, while the occurrence of many spots and areas of contamination may result in underground environments. This has been shown to have harmful effects on the environment and human beings at large. Improving our knowledge of the effects and remediation of oil-related pollution therefore is important for the future of developing countries with respect to the sustainable use of the environment. Biodegradation is a natural process carried out by soil and aquatic microorganisms, mostly bacteria and fungi. Certain bacterial strains have a demonstrated ability to break down or transform the chemical substances present in petroleum products. The goal of oil-spill bioremediation methods is to provide favourable conditions of oxygen, temperature and nutrients to maximize biological hydrocarbon breakdown. This paper is a short overview of petroleum hydrocarbon biodegradation and bioremediation.

Keywords: - Bioremediation, bioaugmentation, biodegradation, microorganisms, biostimulation.

INTRODUCTION

***Corresponding Author:**
Mahak Lalwani
Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-303002, Rajasthan, India
E.Mail: mahak.lalwani307@gmail.com
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The use of microorganism metabolism for the removal of pollutants is termed as Bioremediation .Relatively it uses lowcost, low-technology techniques, which is usually having a high public acceptance and often it can be carried out on site. This technology includes, bioaugmentation (artificial introduction of viable population), biostimulation (stimulating viable native microbial population), bioaccumulation (live cells), biosorption (dead microbial biomass),phytoremediation (plants) and rhizoremediation (plant and microbe interaction).It is an emerging technology in which the living organisms are being used to control or remediate polluted soils. It basically includes elimination, transformation of substances that cause contamination by using the biological processes.

Intensive industrialization is responsible for generating hazardous wastes which comprises inorganics, organics, heavy metals and munitions that are needed to be tackled in a safe manner. Natural as well recombinant microorganisms are used in bioremediation to break down hazardous and toxic substances by aerobic and anaerobic means. They can be applies on site(in situ) or off site(ex situ).

Most bioremediation systems are run under aerobic conditions, but to run a system under the anaerobic conditions may allow microorganisms to degrade otherwise recalcitrant molecules. Bioremediation is the innovatively most effective technology to come along in which biological systems are used for treatment of contaminants. Although, this recent and novel technology is an approach which is multidisciplinary as on microbiology, its central thrust depends. For the bioremediation process, recent advances in the molecular biology field for microorganisms have been applied to produce novel strains with properties desirable for bioremediation. These include the adaptation or construction of catabolic pathways; redirection of carbon flux for preventing the formation of harmful intermediates; modification of catabolic enzyme specificity and affinity; genetic stability improvement of catabolic activities; increasing the pollutants bioavailability; and enhancement of the monitoring, control, yield, and efficiency of processes.

Certain bacterial strains have the ability of breaking down or transforming the chemical substances present in petroleum products. The oil-spill bioremediation methods provide favorable conditions of oxygen, nutrients and temperature maximize biological hydrocarbon breakdown.

Under heavy metals stress some microorganisms may develop resistance against the elevated levels of the toxic metals and evolve various strategies for resistance against the metal stress. Therefore, microorganisms that are metal resistant including bacteria can be exploited as agents of bioremediation. Generally, the higher concentration of these metals above threshold levels has deleterious impact on the functional activities of microbial communities in soils. Otherwise, microorganisms exposed to the higher concentrations of toxic heavy metals may develop resistance against the elevated levels of these metals [1]. In addition, microorganisms inhabiting in metal polluted soils have evolved various strategies to resist themselves against metal stress [2]. Such metal resistant microorganisms can be used as successful bioremediation agents [3].

PRINCIPLE OF BIOREMEDIATION:

Bioremediation is defined as the process whereby wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities [4]. For effective bioremediation, microorganisms must convert the pollutants into harmless products by enzymatically attacking the pollutants. As bioremediation can be effective only where conditions of the environment permit microbial growth and activity, its application usually involves the manipulation of parameters of the environment to allow microorganism's growth and degradation to proceed at a faster rate. The techniques of Bioremediation are typically more economical than the traditional methods such as incineration, and few pollutants can be treated on site, therefore reducing the exposure risks for clean-up personnel, or potentially broader exposure as a result of transportation accidents. As bioremediation is based on natural attenuation the public accepts it more than other technologies. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions may allow microbial organisms to degrade otherwise recalcitrant molecules [5].

BIOREMEDIATION STRATEGIES:

IN-SITU BIOREMEDIATION

In situ bioremediation includes application of biological treatment to the cleaning of chemicals that are hazardous and exists in the subsurface. The control and optimization of microbial transformations of organic contaminants need the integration of many scientific and engineering disciplines.

BIOSPARGING

Biosparging includes the involvement of the injection of air under pressure beneath the water table for increasing groundwater oxygen concentrations and enhancing the biological degradation rate of contaminants by bacteria occurring naturally.

The mixing in the saturated zone is increased by biosparging and thereby the contact between soil and groundwater is increased. The low cost and ease of installing air injection points of small diameter permits considerable flexibility in the construction and design of the system (Fig-1).

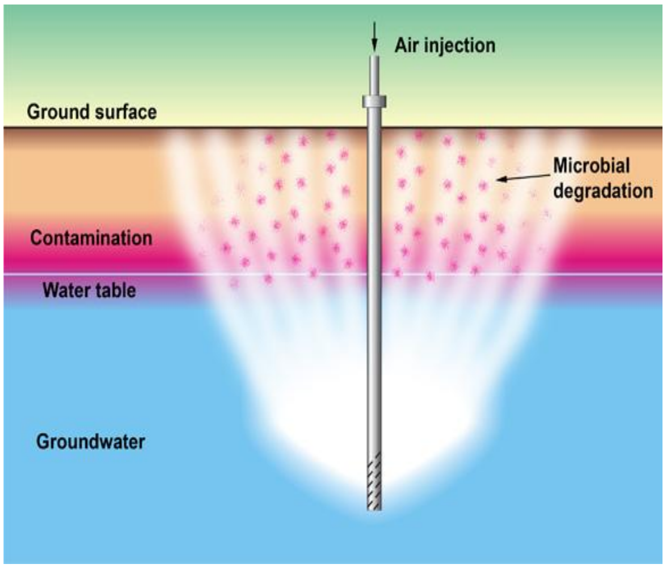

Fig-1: Enhancement of microbial degradation of contaminants by Biosparging

BIOVENTING

Bioventing is an encouraging and reassuring new technology that within the soil stimulates the natural biodegradation (in-situ) of aerobically degradable compounds by supplying oxygen to existing soil microorganisms. In contrast to soil-vapor extraction (SVE), low air-flow rates are used by bioventing to provide only sufficient oxygen for sustaining microbial activity. Most commonly oxygen is supplied via air injection directly into residual contamination in the soil by means of wells. Fuel residuals that are adsorbed are biodegraded, and as vapors move steadily through biologically active soil so volatile compounds are also biodegraded [6].

BIOAUGMENTATION

Bioaugmentation (Fig-2) is the treatment of contaminated soil or water by introducing a group of natural microbial strains or a genetically engineered variant. It is usually used to restart activated sludge bioreactors in municipal wastewater treatment. Most cultures available consist of a consortium based on research of Microbial cultures, consisting all necessary microorganisms. Bioaugmentation is basically used to increase the performance of the biological treatment plant by increasing the microorganism's number with the desired characteristics that are involved directly in biodegradation and breakdown process of the chemical compounds present in the wastewater. Most cultures available consist of a consortium based on research of Microbial cultures, consisting all necessary microorganisms. It is used to ensure the complete degradation of the contaminants by the *in situ* microorganisms at sites where groundwater and soil are contaminated with chlorinated ethenes ,such as trichloroethylene and tetrachloroethylene to chloride and ethylene, which are non-toxic[7]. This system is difficult to monitor.

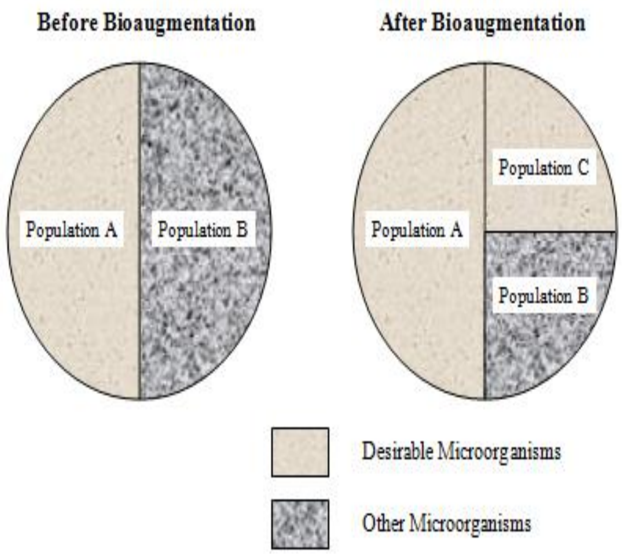

Fig-2: Bioaugmentation

EX-SITU BIOREMEDIATION

Ex-situ bioremediation is a biological process to enhance the degradation of organic contaminants by the indigenous microbial population in which excavated soil is placed in a lined above-ground treatment area and aerated following processing to enhance the degradation of organic contaminants by the indigenous microbial population. Specific micro-organisms,under aerobic condition, can utilize organic contaminants such as petroleum hydrocarbon mixtures, polycyclic aromatic hydrocarbons (PAH), cresols,phenols and some pesticides as carbon and energy source and ultimately degrade them to carbon dioxide and water.

BIOPILING

Biopile treatment is a full-scale technology in which placed on a treatment area the excavated soils are blended with soil amendments and using forced aeration they are bioremediated. Reduction of contaminants to carbon dioxide and water takes place. A treatment bed, irrigation/nutrient system, an aeration system and a leach ate collection system is included in the basic biopile system. Heat, moisture, oxygen, pH and nutrients are controlled for the enhancement of biodegradation. The irrigation/nutrient system is buried beneath the soil for passing of air and nutrients either by positive pressure or vacuum. Piles of soil (soil piles) can be up to 20 feet high and can be covered with plastic for controlling runoff, evaporation and volatilization, and for promoting solar heating. If volatile organic compounds (VOCs) in the soil volatilize into the stream of air, the air going out from the soil may be treated for removing or destroying VOCs before they are released into the atmosphere. The time of Treatment is typically 3 to 6 months [8].

BIOREACTORS

Slurry reactors or aqueous reactors are used for *ex situ* treatment which includes treatment of contaminated soil and water pumped up from a contaminated plume. An engineered containment system is used for the bioremediation in reactors which includes the processing of contaminated solid material (sediment, sludge, soil) or water. A slurry bioreactor can be defined as an apparatus and containment vessel used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor (Fig-3) [9].

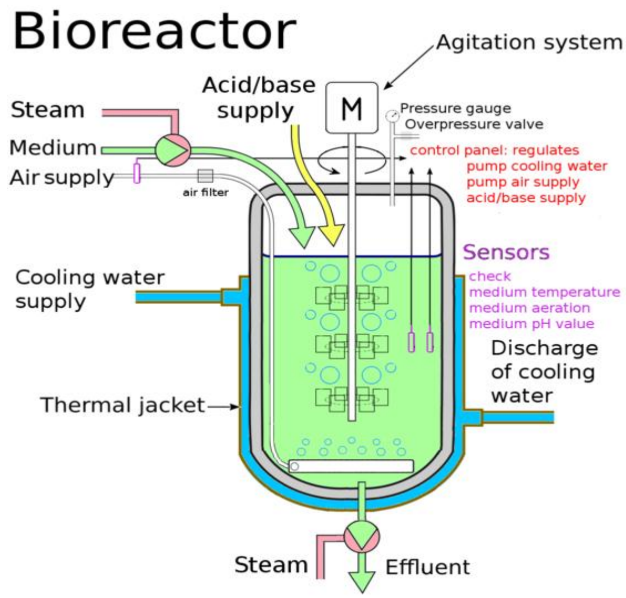

Fig-3: Bioreactor

BIOREMEDIATION: AN ALTERNATING REMEDIATION FOR THE PETROLEUM HYDROCARBONS

Production of petroleum, refining, transportation and its usage contribute to pollution of environment. Soils become unusable due to the contamination of soil with petroleum hydrocarbons which causes a significant decline in its quality. Petroleum and products of petroleum are complex mixtures which consist of thousands of compounds that are generally grouped into four fractions: aromatics, aliphatics, nitrogen–oxygen–sulfur (NSO) compounds and asphaltenes. According to Perry [18], the hydrocarbons susceptibility to microbial attack is ranked in the succeeding order: n-alkanes>isoalkanes>low-molecular-weight aromatics>naphthenes.

Table 1 : Developmental methods applied in bioremediation [10,11]

Technique	Examples	Benefits	Applications	Refrences
In Situ	Biosparging Bioventing Bioaugmentation	Most efficient Non Invasive Relative passive Naturally attenuated process, treat soil and water	Biodegradative abilities of indigenous microorganisms. Presence of metals and inorganic compounds Environmental parameters Biodegradability of pollutants. Chemical solubility Geological Factors. Distribution of pollutants	[7,12,13]
Ex situ	Land farming (Solid-phase treatment system) Composting (Anaerobic,converts solid organic wastes into humus-like material) Biopiles	Cost efficient ,Simple, Inexpensive ,self-heating Low cost Rapid reaction rate, Inexpensive, self heating Can be done on site	Surface application, aerobic process,application of organic materials to natural soils followed by irrigation and tilling. To make plants healthier good alternative to land filling or incinerating practical and convenient. Surface application, agricultural to municipal waste.	[14,15]
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic. Optimized environmental parameters Enhances mass transfer. Effective use of inoculants and surfactant.	Bioaugmentat Toxicity of Amendments. Toxic concentrations of contaminants.	[16]
Precipitation or Flocculation	Non-directed physico-chemical complex -ation reaction between dissolved contaminants and charged cellular components (dead Biomass).	Cost-effective	Removal of heavy Metals	[17]
Microfiltration	Microfiltration membranes are used at a constant pressure.	Remove dissolved solids rapidly.	Waste water treatment; recovery and reuse of more than 90% of original waste water.	-
Electrodialysis	Uses cation and anion exchange membrane pairs.	Withstand high temperature and can be reused.	Removal of dissolved solids Efficiently.	-

Microbial degradation of microbes of crude oil often takes place by attacks on light aromatic fractions or alkanes, while the high-molecular-weight aromatics, asphalthenes and resins are considered recalcitrant [19]. Once released into the environment, the products of petroleum are subject to physical, chemical and biological processes that further within the environment change their composition, toxicity, availability, and distribution (partitioning.

bioremediation technology which is environmentally friendly has been established and applied for controlling the environmental risks which is caused by petroleum products, especially biostimulation and bioaugmentation of the easy-to-degrade hydrocarbons of petroleum. Biodegradation by natural populations of microorganisms is the primary mechanism for eliminating the hydrocarbons from contaminated sites.

For maximizing the process in bioremediation technologies, two important approaches have been explored: biostimulation, in which there is addition of nutrients for the stimulation of the intrinsic hydrocarbon degraders, and bioaugmentation, which includes the addition of microbial strains with degrading abilities for cooperative working with normal indigenous microorganisms of soil [20].

Microorganisms used for bioremediation are generally grouped as indigenous and exogenous microorganisms. Activity of native microorganisms Increases by the addition of nutrients however bioremediation is boosted with the addition of exogenous bacteria. Native microbes require a long time for domestication and therefore show low growth rates and also low metabolic activity, due to which decontamination is slow and ineffective. Therefore, the application of bioremediation employing indigenous microbes is restricted. Nevertheless, some components still remain difficult to degrade as the application of hydrocarbon-degrading bacteria in oil-contaminated sites does not guarantee the removal of all components of crude oil , such as alkanes with shorter and longer chains (<C10 and C20–C40) and polycyclic aromatic hydrocarbons (PAHs) [21].

Many land farming techniques: mineral fertilization, cropping systems, organic amendments etc have been proposed and tested for improving the natural tendency of soil microorganisms for decomposing hydrocarbons from crude oil. [22]. An oil spill in the environment results into an adaptive process, and if the quick addition of metabolically active hydrocarbon-utilizing microorganisms takes place, the wasting of the long period before the indigenous population can respond would be considerably reduced. The need for seeding with complementary hydrocarbon-degrading bacteria arises from the rationale which is indigenous microbial populations may not be capable of degradation of a broad range of potential substrates present in a complex mixture, for example crude oil [23]. Most crude oil components can b degraded by natural microbial community involving a variety of microorganisms that can degrade, alone or together, but in a single batch culture owing to unfavorable physiological conditions, some of their degrading ability would not be expressed conditions [24]. Some organic acids which prevent the growth of the bacteria are formed when mixed cultures are grown. Because of the nutrient stress and competition the growth of the organism cannot be regulated in a mixed culture system. For the degradation of crude oil, the ability of the designed bacterial consortium and individual bacteria with wide crude oil degrading capacity has been employed. in a mixed culture microorganisms may have a varied relationship to hydrocarbon substrates such as (a) direct interacting directly with soluble hydrocarbons, (b) assimilating the dispersed (emulsified) hydrocarbons and (c) by the hydrophobic cell surface, attachment to the hydrocarbon drop [25]. Mixed cultures not only have wide substrate specificity but also in a system of co-oxidation and commensalism, degradation can be achieved. An advantage to the usage of mixed cultures is a wider degradation capacity, synergic effect and co-metabolism [26,27]. Additionally, the consortium members should preferably be belonging to various taxonomic groups that have developed various adaptation and survival mechanisms.

THE DEGRADATION OF PETROLEUM-HYDROCARBONS BY PREDOMINANT MICROORGANISMS

The most major contribution to the mineralization of oil pollutants is made by the bacteria and fungi [28]. The Gram-negative bacteria species of the alpha proteobacteria group are most commonly encountered, such as species of *Sphingomonas*, *Pseudomonas*,*Moraxella*, *Alcaligenes*,*Acinetobacter*, and *Proteus*. Low G+C Gram-positives are the other important groups, such as *Micrococcus* and *Bacillus*, and the high G+C Gram-positives, specifically the actinomycetes [29-31]. Species of *Pseudomonas* are often isolated from hydrocarbon-degrading cultures and hydrocarbon-contaminated sites. Members of this genus have a wide affinity for hydrocarbons and can degrade selected alkanes, alycyclics, aromatics and thiophenes [24,32]. Among the most recalcitrant components of crude oil are the polycyclic aromatic hydrocarbons (PAHs) [33]. Degraders of The isolated crude oil belong to the genera *Micrococcus*, *Bacillus*, *Corynebacterium* ,*Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Alcaligenes*, , *Moraxella*, *Aeromonas*, *Vibrio* and *Acinetobacter*. The flora indicates the normal heterotrophic bacteria existing in the soil, and native genera seem to be the crude oil utilizers. Many other workers also reported on the above genera as hydrocarbon-degrading microorganisms [34-36]. In general, after 20 days of incubation the bacterial consortium shows the maximum percentage (78%) of degradation of crude oil. [23] Using a semicontinuous crude oil–fed reactor using a four member consortium approximately 60% of degradation of crude oil was reported.

Table 1 summarizes information on some bacterial and fungal strains that are commercially available used for petroleum hydrocarbon bioremediation. The capacity of bioremediation of bacteria has been studied more extensively because they are (1) easier to culture,, (2) capable of metabolizing chlorinated organics, (3) more amenable to molecular biology techniques and (4) capable of mineralizing these chemicals and using them as carbon energy sources. Some aromatic contaminants although capable of metabolizing, fungi need a primary growth substrate, such as cellulose or glucose to co-oxidize these compounds. However, mixed cultures with bacteria are needed for complete mineralization of the organic contaminant because fungi cannot further metabolize the products of co-oxidation [12].

BIODEGRADATION OF PETROLEUM-HYDROCARBONS AFFECTED BY THE ENVIRONMENTAL FACTORS

On the quantity and quality of the hydrocarbon mixture, the persistence of petroleum pollutants depends and also on the properties of the affected ecosystem. In one environment, the persistence of petroleum hydrocarbons can almost be indefinitely, whereas under another set of conditions, the same hydrocarbons can be biodegraded completely within a few hours or days. With regard to natural degradation rates, these typically have been found to be limited and low by environmental factors [37]. Factors of environment affecting oil biodegradation include temperature, oxygen,nutrients, salinity and pH.

Biodegradation of petroleum is influenced by temperature effect on the chemical composition and physical nature of the oil, hydrocarbon metabolism rate by microorganisms, and composition of the community of microorganisms [34,39].

Table 1. Available bacterial and fungal strains used in bioremediation [38].

Name	**Description**
HYDROBAC	Bacterial preparation specifc for petroleum hydrocarbon materials
Pseudomonas, Rhodococcus, Arthrobacter	Biosurfactant-producing bacteria
P. oleovorans	Naphthalene-degrading bacteria
Acinetobacter calcoaceticus MM5	Bacterial species
Pseudomonas fluorescens 2a	Bacterial species
Candida sp.	Fungus
Candida tropicalis VSB-637 and *Mycococcus lactis*	Bacterial and fungal species
Acinetobacter oleovorum subsp. *paraphinicum* VSB-576 and *Candida guilliermondii* subsp. *paraphinicum* VSB-638 (pair)a	Bacterial and fungal species
Trichoderma sp. AP-5	Fungus
Rhodococcus erythropolis	Bacterial species
Bacillus sp.	Petroleum-degrading bacterium
BB-232	Petroleum-degrading bacterium
Pseudomonas putida, and *Geotrichum candidum*	Mixed bacteria/fungi culture
Pseudomonas alkaligenes or *Alcaligenes sp.* ER-RL3 Bacterial species NCIMB 40464 Anonymous 1993b *Pseudomonas sp.* ER-RL4 NCIMB 40465 *Gluconobacter sp.* ER-RT NCIMB 40466 *Acinetobacter calcoaceticus* ER-RLD NCIMB 40506 *Acinetobacter calcoaceticus* ER-RL	Bacterial species

Due to Higher temperatures there is maximum increment in the rates of hydrocarbon metabolism, typically in the range of 30 to 40°C, above which there is increase in the membrane toxicity of hydrocarbons [40,41]. Under aerobic and anaerobic conditions, bioremediation can take place. Conversion of many organic contaminants to water, carbon dioxide and other chemicals (i.e., sulfates, nitrates etc.) by microorganisms takes place under aerobic condition. As shown in experiments, aerobic degradation is much quicker than anaerobic degradation [42].

In limiting the biodegradation rate in soil, nutrients such as phosphorus, nitrogen,and iron play a much more critical role than oxygen. Nutrients amendment in a high dose can accelerate the initial oil degradation rate can be accelerated by nutrients amendment in high dose, and due to this the treatment period may be shorten to clean up the contaminated environments [43]. Nutrient supplementation stimulates bioremediation by increasing biomass of microorganisms as suggested by the previous studies [44-48]. The supply of carbon dramatically increases, and the availability of phosphorus and nitrogen generally becomes the limiting factor for oil degradation when a major oil spill occurs in the environment [35,41]. Salinity and ph are the other important factors that affect the biodegradation of petroleum hydrocarbons. The effects of salinity on the microbial degradation of hydrocarbons are addressed by few published studies. A neutral pH is favored by Most heterotrophic bacteria and fungi, with fungi being more tolerant of acidic conditions [49] observed an optimal pH of 7.8, ranging in 5.0 to 7.8, for the oily sludge mineralization in soil [50].

CONCLUSION

Soil contamination with oil derivatives is oftenly observed in cities, around industrial facilities and in places where crude oil and earth gas drilling occur.

Bioremediation is one of the most popular remediation technology for soils contaminated with petroleum hydrocarbons. Benefits of this technique are high treatment efficiency, low cost, in site and ex site application, and compatibility with other techniques and relatively quick action. The biodegradation of petroleum hydrocarbons biodegradation depends on the presence specific microbial population. Further studies should be carried out for the identification of new bacterial strains that can metabolize a broad range of the hydrocarbons present in crude oil, especially the components that are highly persistent.

Environmental conditions and the composition of the hydrocarbons affect the composition of the microbial population. A review of the available literature indicated that for successful bioremediation, microorganisms need an environment with a temperature of –2 to 60°C and a pH of 5.5-10.

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