J. BIOL. ENVIRON. SCI.,
2013, 7(19), 39-47

Review

Bioremediation of Petroleum-Contaminated Soil

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Received: 25.01.2013; Accepted: 20.03.2013; Available Online: 27.05.2013

ABSTRACT

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. Bioremediation is one of the most popular remediation technologies in use due to the relatively low cost. It is a rapidly developing field of environmental restoration, utilizing natural microbial activity to reduce the concentration and/or toxicity of various chemical substances such as petroleum products and aliphatic and aromatic hydrocarbons. 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 favorable conditions of oxygen, temperature and nutrients to maximize biological hydrocarbon breakdown. This paper is a short overview of petroleum hydrocarbon biodegradation and bioremediation.

Key Words: Biodegradation, Bioremediation, Petroleum Hydrocarbons, Soil

Petrol ile Kirlenmiş Toprakların Biyoremediasyonu

ÖZET

Petrol ve petrokimyasal ürünlerin sebep olduğu çevresel kirlilik son yıllarda oldukça dikkat çekicidir. Petrol türevlerinin parçalanmasıyla tarıma uygun arazilerde dahil olmak üzere doğal çevrenin kirlenmesi, yeraltı ortamlarında birçok nokta ve alanın da kirlenmesine sebep olmaktadır. Bu durum, çevre ve insan sağlığının büyük miktarda zararlı etkilere maruz kalabileceğini göstermektedir. Petrol ile ilgili kirlilik etkilerinin ve remediasyon bilgilerinin geliştirilmesi, gelişmekte olan ülkeler için çevrenin sürdürülebilir kullanımında önemlidir. Biyoremediasyon, oldukça düşük maliyetli olmasıyla en çok tercih edilen remediasyon tekniğidir. Petrol ürünleri ve alifatik-aromatik hidrokarbonlar gibi çeşitli kimyasal maddelerin doğal mikroorganizmalar kullanılarak konsantrasyonlarının ve/veya toksisitelerinin azaltılması, çevresel restorasyonun hızla geliştiği bir alan olmaktadır. Biyolojik parçalanma, çoğunlukla bakteri ve fungi gibi toprak ve sucul mikroorganizmalar tarafından gerçekleştirilen doğal bir prosestir. Bazı bakteri türlerinin, petrol ürünleri içerisindeki kimyasal maddeleri dönüştürdüğü veya parçaladığı tespit edilmiştir. Petrol kirliliğinde biyoremediasyon metodunun hedefi, maksimum biyolojik hidrokarbon parçalanmasını sağlamak için uygun oksijen, sıcaklık ve nutrient şartlarını sağlamaktır. Bu çalışmada, petrollü hidrokarbonların biyolojik parçalanması ve biyoremediasyonu ile ilgili kısa bir değerlendirme yapılmıştır.

Anahtar kelimeler: Biyolojik Parçalanma, Biyoremediasyon, Petrollü Hidrokarbonlar, Toprak

INTRODUCTION

The pollution of soils and the subsurface environment by petroleum product spills is a major concern in the industrial world (Salanitro, 2001). Petroleum spills may persist in the soil as a source of hazardous hydrocarbons for a long time (e.g., months or years) because of the low solubility and the moderate to low volatility of these compounds. Contamination of this type is common because of storage tank and piping leaks, spills on land surfaces, and improper disposal practices (Ostendorf, 1990; Essaid *et al*., 1995; Wang *et al*., 1998a; Thorn and Aiken, 1998). Many investigations have been carried out on the redistribution and natural attenuation of volatile petroleum hydrocarbon mixtures over time (Eiceman et al., 1986; Mercer and Cohen, 1990; Ostendorf, 1990; Durnford et al., 1991; Johnson and Perrott, 1991; Dean-Ross et al., 1992; Rubin et al., 1994, Chen et al., 1998; MacLeod and Mackay, 1999). These mixtures migrate in the soil profile until a balance is achieved among pressure, gravity and capillary forces. The mixtures are affected during their transport by the volatilization and degradation of the various components of the mixtures. The physical and chemical properties of each component

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of the organic hydrocarbon mixture influence its migration rate and fate. The diversity of the compounds in the organic mixture can lead to a continuing change in the composition of the contaminant mixture (Hayden et al., 1994; Wang et al., 1998b) and, hence, in its behavior in the subsurface environment. The prevailing environmental conditions affect the behavior of contaminants in the soil, influence the partitioning of the organic compounds between the different phases and affect their degradation rate (Imhoff et al., 1997; Abriola and Bradford, 1998; Chen and Wu, 1998). Petroleum hydrocarbons can affect soil ecosystems sufficiently to result in significant losses in soil quality (Amadi *et al*., 1996; Kelly and Tate, 1998; Coyne, 1999). Their negative impact results from their toxicity to biological processes catalyzed by soil microorganisms. Field studies of contaminated soils have demonstrated that elevated loadings of these contaminants can result in diminished microbial biomass, reduced viable bacterial population densities, inhibition of organic matter mineralization and decreased leaf litter decomposition (Chander and Brooks, 1991; Roane and Kellogg, 1996; Testa, 1997).

Oil contamination in soil results in an imbalance in the carbon-nitrogen ratio at the spill site because crude oil is essentially a mixture of carbon and hydrogen. This causes a nitrogen deficiency in oil-soaked soil, which retards the growth of bacteria and the utilization of carbon sources. Furthermore, large concentrations of biodegradable organics in the top layer deplete oxygen reserves in the soil and slow down the rates of oxygen diffusion into deeper layers.

Many remediation technologies have been developed to treat soil contaminated by petroleum pollutants. A particular contaminated site may require a combination of procedures to allow the optimum remediation for the prevailing conditions. Biological, physical, and chemical technologies may be used in conjunction with one another to reduce the contamination to a safe and acceptable level (Reddy et al., 1999; RAAG, 2000; Khan *et al*., 2004). Although many technologies are available for the treatment of contaminated sites, the selection depends on contaminant and site characteristics, regulatory requirements, costs, and time constraints (Riser-Roberts, 1998; Reddy et al., 1999)

Mechanical (e.g., skimming) and chemical (e.g., surfactants and dispersants) methods have limited effectiveness and can be expensive. Several researchers have studied the use of microorganisms to decompose petroleum products and have shown this to be a promising technological alternative (Grishchenkov et al., 2000; Bielicka et al., 2002; Gogoi et al., 2003; Townsend et al., 2004; Lakha et al., 2005). Bioremediation works well for remediating soils contaminated with petroleum hydrocarbons (Flathman et al., 1994). A large number of microorganisms (bacteria, fungi and some algae) that are capable of using petroleum hydrocarbons as the sole source of carbon and energy have been described (Das and Chandran 2011). Microbiological activity is affected by a number of environmental factors, including energy sources, donors and acceptors of electrons, nutrients, pH, temperature and inhibition by the substratum or metabolites. These parameters influence how quickly microorganisms adapt to the available substratum (Boopathy, 2000). This paper is a short overview of the petroleum hydrocarbon degradation and bioremediation assays.

AN ALTERNATIVE REMEDIATION TECHNOLOGY FOR PETROLEUM HYDROCARBONS: BIOREMEDIATION

Petroleum production, refining, transportation and use contribute to environmental pollution. The contamination of soil with petroleum hydrocarbons causes a significant decline in its quality, and such soils become unusable. Petroleum and petroleum products are complex mixtures consisting of thousands of compounds that are usually grouped into four fractions: aliphatics, aromatics, nitrogen–oxygen–sulfur (NSO) compounds and asphaltenes. According to Perry (1984), the susceptibility of hydrocarbons to microbial attack is ranked in the following order: n-alkanes>isoalkanes>low-molecular-weight aromatics>naphthenes.

Microbial degradation of crude oil often occurs by attacks on alkanes or light aromatic fractions, while the high-molecular-weight aromatics, resins and asphalthenes are considered recalcitrant (Lal and Khanna, 1996). Once released into the environment, petroleum products are subject to physical, chemical, and biological processes that further change their composition, toxicity, availability, and distribution (partitioning) within the environment (Figure 1).

To control the environmental risks caused by petroleum products, bioremediation as an environmentally friendly technology has been established and applied, especially biostimulation and bioaugmentation of the easy-to-degrade petroleum hydrocarbons. The primary mechanism for the elimination of hydrocarbons from contaminated sites is biodegradation by natural populations of microorganisms.

To maximize the process in bioremediation technologies, two main approaches have been explored: biostimulation, in which nutrients are added to stimulate the intrinsic hydrocarbon degraders, and bioaugmentation, in which microbial strains with specific degrading abilities are added to work cooperatively with normal indigenous soil microorganisms (Alvarez and Illman, 2006).

Microorganisms used for bioremediation are usually grouped as indigenous and exogenous microbes. The addition of nutrients increases the activity of native microorganisms however bioremediation is boosted with the addition of exogenous bacteria. Native microbes need a long time to domesticate and therefore show low growth rates and low metabolic activity, which make decontamination slow and ineffective. Therefore, the application of bioremediation using indigenous microbes is restricted. Nevertheless, the application of hydrocarbon-degrading bacteria in oil-contaminated sites does not guarantee the removal of all components of crude oil because some components still remain difficult to degrade, such as alkanes with shorter and longer chains (<C_{10} and C_{20}–C_{40}) and polycyclic aromatic hydrocarbons (PAHs) (Yuste et al., 2000).

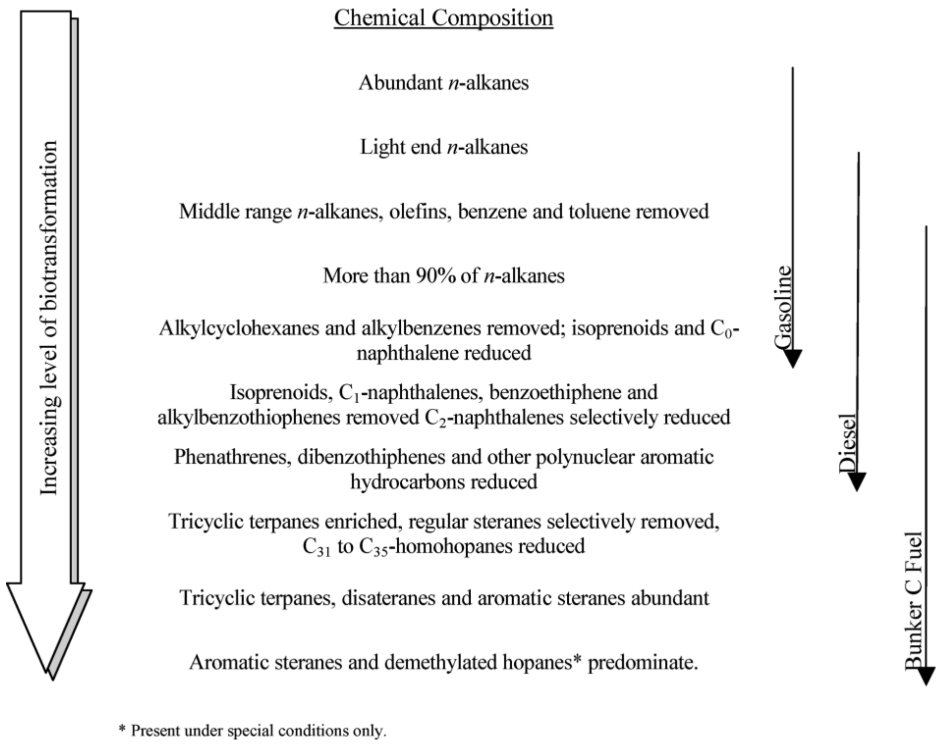

Figure. 1 General petroleum hydrocarbon degradation pattern (Brassington et al., 2007)

Biodegradation of petroleum hydrocarbon oil (14,000 mg/kg) were investigated in six biopile batches, differing in the remediation strategy: bioaugmentation (selected consortium and kitchen waste were introduced), biostimulation (added with rhamnolipid, high-level, or low-level nutrient), and bioaugmentation plus biostimulation (added both with rhamnolipid and bacterial consortia). After the 140-day operation, the kitchen waste (KW) and the low-level nutrient (NEL) batches achieved the highest total petroleum hydrocarbon degradation efficiency (>80%). The result of the hydrocarbon analysis revealed that the bioaugmentation approaches were the most effective ones in removing aromatic components (64% and 68%), and KW and NEL were the only two approaches that could remove the polar component with positive efficiency, 11% and 21%, respectively (Liu et al., 2011). Similarly, Turgay et al., (2007) evaluated the effects of biostimulation (powdered gyttja and its humic-fulvic extract-HFA) and bioaugmentation (a commercial bioremediation product) on hydrocarbon degradation in crude oil contaminated soil. Based on a 60 days incubation period, the results showed that biostimulation was an effective treatment by its 51-56% of oil removal while bioaugmentation with a commercial bioremediation product gave an 50% of oil degradation. Control treatment that was only applied with nutrient showed a performance of 46%. The result of study by Turgay et al., (2009) also revealed that biostimulation of polluted soil with friendly organic materials such as humic substances may be a beneficial way as much as bioaugmentation.

Sorkhoh et al., (1995) observed a sequential change of the composition of the oil-degrading bacteria over a period of time in sand samples that were contaminated with oil. Venkateswaran and Harayama (1995) reported similar observations in sequential enrichments in media containing residual crude oil.

To improve the natural tendency of soil microorganisms to decompose hydrocarbons from crude oil, many landfarming techniques have been proposed and tested: mineral fertilization, organic amendments, cropping systems, etc. (Sims and Sims, 1999). An oil spill in the environment leads to an adaptive process, and if metabolically active hydrocarbon-utilizing microorganisms can be added quickly, the long period wasted before the indigenous population can respond would be reduced considerably. The necessity for seeding with complementary hydrocarbon-degrading bacteria arises from the rationale that indigenous microbial populations may not be capable of degrading a wide range of potential substrates in a complex mixture such as crude oil (Chhatre et al., 1996). It seems that a natural microbial community includes a variety of microorganisms that can degrade, alone or together, most crude oil components, but that some of their degrading ability would not be expressed in a single batch culture owing to unfavorable physiological conditions (Vankateswaran et al. 1995). When mixed cultures are grown in crude oil medium, some organic acids, which prevent the growth of the bacteria, are formed. In a mixed culture system, the growth of the organism cannot be regulated because of nutrient stress and competition. The ability of the designed bacterial consortium and individual bacteria with wide crude oil degrading capacity has been employed for the degradation of crude oil. Microorganisms in mixed culture may have a different relationship to hydrocarbon substrates such as (a) direct interaction with soluble hydrocarbons, (b) assimilation of dispersed (emulsified) hydrocarbons and (c) attachment to the hydrocarbon drop by the hydrophobic cell surface (Gojgic-Cvijovic et al., 2012). Mixed cultures not only have broad substrate specificity but also degradation can be achieved in a system of co-oxidation and commensalism. An advantage to the use of mixed cultures is a broader degradation capacity, synergic effect and co-metabolism (Mishra et al., 2001; Rahman et al., 2003). Additionally, the consortium members should preferably belong to different taxonomic groups that have developed different adaptation and survival mechanisms.

PREDOMINANT MICROORGANISMS RESPONSIBLE FOR THE DEGRADATION OF PETROLEUM-HYDROCARBONS

Bacteria and fungi make the most major contribution to the mineralization of oil pollutants (Abed et al., 2001). The bacteria most commonly encountered are the Gram-negative species of the alpha proteobacteria group, such as species of *Pseudomonas*, *Sphingomonas*, *Moraxella*, *Acinetobacter*, *Alcaligenes*, and *Proteus*. Other important groups are the low G+C Gram-positives, such as *Bacillus* and *Micrococcus*, and the high G+C Gram-positives, particularly the actinomycetes (Amund, 2000; Wackett and Hershberger, 2001; Parales et al., 2003). *Pseudomonas* species are often isolated from hydrocarbon-contaminated sites and hydrocarbon-degrading cultures. Members of this genus have a broad affinity for hydrocarbons and can degrade selected alkanes, alycyclics, thiophenes and aromatics (Vankateswaran et al., 1995; Allen et al., 1997). Polycyclic aromatic hydrocarbons (PAHs) are among the most recalcitrant components of crude oil (Kanaly and Harayama, 2000). The isolated crude oil degraders belong to the genera *Micrococcus*, *Corynebacterium*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Moraxella*, *Aeromonas*, *Acinetobacter* and *Vibrio*. The flora reflects the normal heterotrophic bacteria present in soil, and native genera seem to be crude oil utilizers. Several other workers also reported on the above genera as hydrocarbon-degrading microorganisms (Atlas, 1981; Leahy and Colwell, 1990; Banat et al., 2000). In general, a bacterial consortium shows the maximum percentage (78%) of

degradation of crude oil after 20 days of incubation. Chhatre et al., (1996) reported approximately 60% of degradation of crude oil using a semicontinuous crude oil–fed reactor using a four member consortium.
Table 1 summarizes information on some commercially available bacterial and fungal strains used for petroleum hydrocarbon bioremediation. The bioremediation capacity of bacteria has been investigated more extensively because they are (1) easier to culture, (2) more amenable to molecular biology techniques, (3) capable of metabolizing chlorinated organics, and (4) capable of mineralizing these chemicals and using them as carbon energy sources (Bouwer and Zehnder, 1993). Although capable of metabolizing some aromatic contaminants, fungi require a primary growth substrate, such as glucose or cellulose to co-oxidize these compounds. However, because fungi cannot further metabolize the products of co-oxidation, mixed cultures with bacteria are required for complete mineralization of the organic contaminant (Bouwer and Zehnder, 1993).

ENVIRONMENTAL FACTORS AFFECTING THE BIODEGRADATION OF PETROLEUM-HYDROCARBONS

The persistence of petroleum pollutants depends on the quantity and quality of the hydrocarbon mixture and on the properties of the affected ecosystem. In one environment, petroleum hydrocarbons can persist almost indefinitely, whereas under another set of conditions, the same hydrocarbons can be completely biodegraded within a few hours or days. With regard to rates of natural degradation, these typically have been found to be low and limited by environmental factors (Atlas, 1995). Environmental factors affecting oil biodegradation include temperature, nutrients, oxygen, pH, and salinity.

Table 1. Available bacterial and fungal strains used in bioremediation (Korda et al., 1997).

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-RLX	Bacterial species

Temperature influences petroleum biodegradation by its effect on the physical nature and chemical composition of the oil, rate of hydrocarbon metabolism by microorganisms, and composition of the microbial community (Atlas, 1981; Alexander, 1999). Higher temperatures increase the rates of hydrocarbon metabolism to a maximum, typically in the range of 30 to 40°C, above which the membrane toxicity of hydrocarbons is increased (Bossert and Bartha, 1984; Zhu et al., 2001).

Bioremediation can take place under aerobic and anaerobic conditions. Under aerobic conditions, microorganisms will convert many organic contaminants to carbon dioxide, water and other chemicals (i.e., nitrates, sulfates, etc.). Aerobic degradation is much faster than anaerobic degradation, as has been shown in experiments (Grishchenkov et al., 2000).

Nutrients such as nitrogen, phosphorus, and iron play a much more critical role than oxygen in limiting the rate of biodegradation in soil. Nutrients amendment in a high dose can accelerate the initial oil degradation rate, and this may shorten the treatment period to clean up the contaminated environments (Oh et al., 2001). Previous studies suggest nutrient supplementation stimulates bioremediation by increasing microbial biomass (Sanchez et al., 2000; Margesin and Schinner, 2001; Duncan et al., 2003; Maki et al., 2003; Sarkar et al., 2005). When a major oil spill occurs in the environment, the supply of carbon is dramatically increased, and the availability of nitrogen and phosphorus generally becomes the limiting factor for oil degradation (Leahy and Colwell, 1990; Zhu et al., 2001; Venosa and Zhu, 2003).

Other important factors affecting the biodegradation of petroleum hydrocarbons include pH and salinity. There are few published studies that address the effects of salinity on the microbial degradation of hydrocarbons (Leahy and Colwell, 1990). Most heterotrophic bacteria and fungi favor a neutral pH, with fungi being more tolerant of acidic conditions (Das and Chandran, 2011). Dibble and Bartha (1979) observed an optimal pH of 7.8, in the range 5.0 to 7.8, for the mineralization of oily sludge in soil.

CONCLUSIONS

Eight million tons of petroleum is spilled into the environment every year worldwide. Oil contamination is a severe threat for our environment and therefore invites general concern. Consequently, the remediation of oil-polluted sites has become an important issue worldwide. The contamination of soil with oil derivatives is very often observed in cities, around industrial facilities and in areas where crude oil and earth gas drilling occur.
Bioremediation is one of the most popular remediation technology for soils contaminated with petroleum hydrocarbons. This technique has the benefits of high treatment efficiency, low cost, relatively quick action, in site and ex site application, and compatibility with other techniques. The biodegradation of petroleum hydrocarbons depends on the specific microbial population present. Further studies should be carried out to identify new bacterial strains that can metabolize a broad range of the hydrocarbons contained in crude oil, especially the highly persistent components.

The composition of the microbial population is affected by the environmental conditions and the composition of the hydrocarbons. A review of the available literature indicated that microorganisms require an environment with a temperature of –2 to 60°C and a pH of 5.5-10 for successful bioremediation. Other factors that can inhibit the success of microorganisms in bioremediation are a lack of oxygen, moisture, or mineral nutrients and the presence of harmful concentrations of hydrocarbons.

Future research should be directed toward the improvement of existing techniques and the devising of innovative methods of bioremediation. Attention should be paid to the factors governing the bioavailability of organic contaminants and the methods used to increase availability and microorganism activity; monitor bioremediation; study the ecology and fate of introduced microorganisms; initiate the transfer of laboratory findings to the field; understand fertilizer action; discover new and more efficient fertilizers and application techniques; understand the coupling of major biochemical cycles, such as nitrogen and phosphorus, with bioremediation cases; and understand the effects of other environmental factors. Most importantly, all of the above issues should be investigated in the field under realistic conditions.

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