BIOREMEDIATION OF OIL SPILL
Pratima Sharma*, Jyoti Singh, Shipra Dwivedi, Mahendra Kumar
Department of Biochemical Engineering and Food Technology,
Harcourt Butler Technological Institute, Kanpur-208002, India
*corresponding author E-mail: pratima1197@gmail.com

Abstract: At present, petroleum hydrocarbon is one of the most serious environmental problem. Oil spill occurring accidently in the ocean during transportation or other working places like refineries causes great threat to the flora and fauna existing nearby. Modern methods of remediation such as incineration, land filling, burning, etc. are expensive as well as their by-products produced are often toxic. Bioremediation stands as a substitute for petroleum hydrocarbon treatment in terms of efficiency, long term safety and environment friendly. It utilises the biological agents such as microorganisms and plants for complete degradation of complex organic compounds into simpler organic and inorganic compounds. Modern technique of biotechnology exploits the microorganisms metabolically degrading capabilities to degrade the hydrocarbon at a faster rate through genetic engineering to tolerate the external environmental parameters. However, there are only few achievements and still we have to go far away in this regard.

Keywords: Bioremediation; bioaugmentation, polycyclic aromatic hydrocarbon, biostimulation.

1. Introduction

Today one of the major environmental problem is liquid petroleum hydrocarbon contamination. This mainly results from the activities related to petroleum refineries, accidental releases of petroleum products during shipping, transportation, storage, mishandling and massive explosion during wars. 600,000 metric tonnes per year with a range of uncertainty of 200,000 metric tons per year of natural crude oil seepage was estimated by researchers [1]. On ground oil spills posses three types of hazards. These are fire, ground water pollution and air pollution. Oily sludge (generated by petroleum refineries) contamination is major environmental concerns because it contains hazardous hydrocarbon and its constituents are toxic and carcinogenic in nature. It is also poorly biodegradable in nature.

The main cause of air, water and soil pollution is the release of hydrocarbons in the environment whether accidently or due to human activities [2]. Soil and air contamination with petroleum hydrocarbons causes wide damage of ecosystem by accumulation of pollutants in animal and plant tissues and may cause death or mutations [3]. Petroleum is lighter than water so its floats on it posing a great threat for flora and fauna of fire hazard. The contaminated soil, groundwater or waste water contain a mixture of contaminants types including salts, organics, alcohol, phenols, acids, radionucliods, heavy metals, PAHs, and trace element like cadmium, mercury, copper, chromium, lead etc. at widely varying concentrations [4].

Spilt oil penetrates into the feathers of birds and the fur of mammals, reducing its insulating ability, and making them more vulnerable to temperature fluctuations and much less buoyant in the water. Cleanup and recovery from an oil spill is difficult and depends upon many factors, including the type of oil spilled, the temperature of the water (affecting evaporation and biodegradation), and the types of shorelines and beaches involved. Spills may take weeks, months or even years to clean up.

Bioremediation is a scientific approach for removing pollutants from soil and water surfaces by using microorganisms and their metabolic activity power. In-situ bioremediation is carried out where contamination is huge. A few species of bacteria, fungi, and plants are capable of chelating heavy metals and different
types of chemical from soil and water. Such species are incorporated for purpose of cleaning water bodies and lands. Scientists are designing microbes through genetic engineering that can function in same way. The chemical load, pH, concentration of breakdown is also taken into consideration for determining the efficiency of microbes during bioremediation.

2. Bioremediation methods for oil spills

Different techniques are applied for the elimination of toxic substances from the soil either in-situ or ex-situ. The application of techniques depends on the nature and the intensity of the pollution. The microbes breakdown the chemicals with the help of the enzymes secreted by them. Thus, the soil or water becomes clear when the chemicals are taken up by them.

2.1. Using microbial species

Pseudomonas is a potent bacterium that is capable of degrading hydrocarbons from petrol and diesel, thereby reducing the impact of oil spills. P. alcaligenes is capable of breaking down polycyclic aromatic hydrocarbons while P. mendocina and P. putida can remove toluene. P. veronii can degrade large number of aromatic organic compounds. These oil based compounds are eaten up by the bacteria as they utilize them as substrates for carrying out metabolism. These microorganisms occur in abundance in water bodies and soil and are effective in cleansing oil spills. With an increase in density of these microorganisms, the process of bioremediation is also accentuated. Other bacteria that help in bioremediation are Achromobacter, Flavobacterium, Acinetobacter etc.

Many microorganisms possess the enzymatic capability to degrade petroleum hydrocarbons. Some microorganisms degrade alkanes, others aromatics, and others both paraffinic and aromatic hydrocarbons. Often the normal alkanes in the range C_{10} to C_{26} are viewed as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene and xylene, which are among the toxic compounds found in petroleum, are also very readily biodegraded by many marine microorganisms. More complex structures are more resistant to biodegradation, meaning that fewer microorganisms can degrade those structures and the rates of biodegradation are lower than biodegradation rates of the simpler hydrocarbon structures found in petroleum. The greater the complexity of the hydrocarbon structure, i.e., the higher the number of methyl branched substituents or condensed aromatic rings, the slower the rates of degradation [5].

3. Chemical method of degradation

In this process, microorganisms, like bacteria and fungi (fungi used for assisting bioremediation is called mycoremediation) are once again employed but they show chemical action on the petroleum products. The hydrocarbons are rapidly oxidized by the enzyme oxygenase in presence of oxygen. This reaction forms the basis of bioremediation for oil spills. Bacteria and fungi are capable of hydroxylating the aromatic rings to form diols. These compounds are subsequently degraded and taken up by the microorganisms for aiding in proper functioning of the tricarboxylic acid cycle (TCA). Interestingly, fungi and bacteria form intermediates with differing stereochemistries. Fungi, like mammalian enzyme systems, form trans-diols, whereas bacteria almost always form cis-diols (many trans-diols are potent carcinogens whereas cis-diols are not biologically active). Since bacteria are the dominant hydrocarbon degraders in the marine environment, the biodegradation of aromatic hydrocarbons results in detoxification and does not produce potential carcinogens. The complete biodegradation (mineralization) of hydrocarbons produces the non-toxic end products carbon dioxide and water, as well as cell biomass (largely protein) which can be safely assimilated into the food web.
3.1. Using biostimulators or accelerators

Biostimulators are added to soil and water to accelerate the rate of degradation. They are used in case of huge contamination. Nutrients are added to increase the growth rate of oil-eating bacteria, by keeping the temperature, salinity, pH and other factors into account. The accelerating agents are sometimes chemical compounds that form a nutrient rich emulsion with water. Bacteria and fungi grow fast in such an environment, thereby eliminating the hydrocarbons rapidly. Various tests are done in the soil and water before mixing the biostimulating agents. This process is also carried out for decontaminating wells and ponds as they are often polluted with hydrocarbons and heavy metals.

4. Need for bioremediation

It is particularly important to address oil polluted waters as soon as possible as the contamination can have the potential to damage fishery resources and affect the health of those animals and humans that consume contaminated fish [6].

Besides the varying rates of biodegradation, researchers have consistently documented a lag time after oil is spilled before indigenous microbes begin to break down the oil molecules. This lag time is related to the initial toxicity of the volatile fractions of the oil, which evaporate in the first few days of a spill. Microbial populations must begin to use oil and expand their population before measurable degradation takes place, a period usually lasting several days. This fact becomes very important when considering the appropriateness of bioremediation as a quick or first response technique [7].

4.1. Strategies for removal of petroleum hydrocarbons from environment

Remediation refers to the removing, degrading or transforming harmful contaminants to harmless or less harmful substances. Currently accepted methods for removal of pollutants are incineration, land filling and safe disposal. But these methods become expensive while handling with large amounts of contaminants. Methods like pyrolysis, gasification incineration and land filling are efficient, but after treatment produces some by-products that causes negative impacts on environment and public health [8]. Thermal and chemical methods for hydrocarbon removal are expensive and limited in effectiveness and also causes recontamination by secondary contaminants.

Technologies used in soil remediation such as mechanical, burying, evaporation, dispersion and washing are expensive and lead to incomplete decomposition of contaminants. In recent years microbial degradation of pollutants is a sustainable way to clean up the contaminated environment [9]. Petroleum and hydrocarbon contamination can be degraded effectively by using microorganism activity. The effects of environmental parameters on microbial degradation of hydrocarbons, the elucidation of metabolic pathways, genetic basis for hydrocarbon dissimilation by microorganisms and the effects of hydrocarbon contamination on microbial communities have been the areas of interest and the subject of several reviews [10], [11].

Bioremediation is principally based on complete degradation of organic contaminants into carbon dioxide, water and other inorganic compounds with the help of biological agents like microorganisms and plants that transform complex organic compounds into simpler compounds by exploiting their diverse metabolic capabilities for the removal and degradation of many environmental pollutants [12]. Plants have potential cellular mechanism for detoxification of heavy metals and petroleum hydrocarbons and this process of using plants in removal of contaminants from environment is called phytoremediation [13].
Microbial degradation of hydrocarbons is accomplished by a wide range of microorganisms, particularly indigenous bacteria present in soil. These microorganisms can degrade a wide range of constituents present in oily sludge [14],[15],[16]. A large number of pseudomonas strains capable of degrading PAHs (Polycyclic Aromatic Hydrocarbons) have been isolated from soil and aquifers [17],[18],[19]. Other microorganisms include Acinetobacter sp., Flavobacter sp., Streptococcus sp., Bacillus sp., Alcaligenes sp., Morexella sp., etc [20],[21],[22],[23]. Other organisms such as fungi are also capable of degrading hydrocarbons to a certain extent but they lack longer growth period as compared to bacteria.

Petroleum degradation can occur aerobically and anaerobically by bacteria [24]. In aerobic process of degradation requires oxygenase enzymes which incorporate molecular oxygen into reduced substrate. Anaerobic biodegradation of surface oil is mediated by sulphate reducing bacteria or other anaerobes using a variety of other electron acceptors as oxidants [25].

Bacteria have developed two general strategies for enhancing contact with water insoluble hydrocarbons: specific adhesion mechanism and production of extracellular emulsifying agents. In some cases, emulsifier production is induced by growth on hydrocarbons [26].

There are several different bioremediation techniques. The underlying idea is to accelerate the rates of natural hydrocarbon biodegradation by overcoming the rate-limiting factors. Several techniques can lead to the results striven for. Indigenous populations of microbial bacteria can be stimulated through the addition of nutrients or other materials. Exogenous microbial populations can be introduced in the contaminated environment. The addition of extra bacteria is known as bio augmentation. If necessary, genetically altered bacteria can be used. Once the bacteria are chosen, the engineer must carefully meet their nutritional needs by choosing the correct mix of fertilizer [27]. Furthermore, the contaminated media can be manipulated by, for example, aeration or temperature control. Two of these concepts shall be observed in more detail: seeding with microbial cultures and environmental modification.

a. Seeding with microbial cultures

One approach often considered for the bioremediation of petroleum pollutants after an oil spill is the addition of microorganisms (seeding) that are able to degrade hydrocarbons. Most microorganisms considered for seeding are obtained by enrichment cultures from previously contaminated sites. However, because hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats, adding seed cultures has proven less promising for treating oil spills than adding fertilizers and ensuring adequate aeration. Most tests have indicated that seed cultures are likely to be of little benefit over the naturally occurring microorganisms at a contaminated site for the biodegradation of the bulk of petroleum contaminants.

b. Environmental modification

Hydrocarbon biodegradation in marine environments is often limited by abiotic environmental factors such as molecular oxygen, phosphate and nitrogen (ammonium, nitrate and organic nitrogen) concentrations. Rates of petroleum biodegradation are negligible in anaerobic sediments because molecular oxygen is required by most microorganisms for the initial step in hydrocarbon metabolism. Oxygen, however, is not limiting in well aerated (high energy) marine environments. Usually, marine waters have very low concentrations of nitrogen, phosphorus and various mineral nutrients that are needed for the incorporation into cellular biomass, and the availability of these within the area of hydrocarbon degradation is critical.
5. Role of Genetically Modified Microorganisms in Bioremediation

During past 20 years, genetic engineering has shown a drastic improvement degradation of hazardous wastes by enhancing the degradative capabilities of microorganisms encompassing a wide range of chemical contaminants [28]. These GEMs (Genetically Engineered Microorganisms) have potential benefit for removal of petroleum hydrocarbons from soil, ground water and activated sludge environment.

The way to manipulate the degrading capabilities of microorganisms through genetic engineering, e.g. rate limiting steps in metabolic pathways can be manipulated genetically to increase the degrading rates or completely new metabolic pathway constituting gene can be incorporated into bacterial strain to degrade wide range of chemicals [29]. Genes specifying enzyme for catabolism of particular substrate are inserted into the bacterial plasmid. Plasmids have been implicated for the catabolism of octane, naphthalene, camphor and toluene as well as other compounds [30],[31],[32],[33].

The first GEM was released in the contaminated soil environment by the University of Tennessee in collaboration with Oak Ridge National Laboratory for remediation purposes [34]. The purpose of the research was to develop a GEM capable of sensing and responding to it through a easily detectable signal, such as bioluminescence.

5.1. Physical, chemical and environmental factors affecting the biodegradation of hydrocarbons

Bioremediation of contaminated systems requires the knowledge of characteristics of the site and parameters that affects the biodegradation of pollutants [35]. The overall ratio of hydrocarbons biodegradation in soil is limited by various parameters [36].

i. Temperature: Temperature plays an important role in petroleum hydrocarbon biodegradation by its direct effect on the chemistry of the pollutants and its effect on physiology and diversity of microorganisms. Ambient temperature of an environment affects both the property of spilled oil and microorganisms activity [37]. At low temperature, viscosity of oil increases while toxic low molecular weight hydrocarbons is reduced, delaying the onset of biodegradation.

Biodegradation also decreases with decrease in temperature. Temperature also affects the solubility of hydrocarbon [38]. Degradation rate is high at the temperature ranging between 30-40°C in soil environment, 20-30°C in some fresh water environment and 15-20°C in marine environments [39],[40]. Hydrocarbon biodegradation have also been reported [41],[42],[43].

Hydrocarbon degraders comprise less than 0.1% of microbial community in unpolluted environment but can constitute upto 100% of cultivable microorganisms in hydrocarbon polluted environment. The studies on effects of temperature of consortium of toluene-degrading bacteria was found that their consortium grew best at 30°C [44]. The studies on effects of temperature on bacteria from benzene contaminated aquifer, Alaska using glutamate as carbon source was found that overall microbial metabolic rates were higher at 25°C than 10°C [45].

ii. Nutrients: It was reported that the C/N ratio approximately 2-200 is optimum for fertiliser application. In principle, problem can be solved by using low C:N and C:P ratios nitrogen and phosphorus compound [46]. Nitrogen and Phosphorus are necessary for cellular metabolism and can be found in low concentrations in many soils, including Arctic soils [47],[48],[49].
Biodegradability is inherently influenced by composition of oil pollutants. For example, kerosene (consists of almost exclusively medium chain alkanes) is totally biodegradable. Similarly, crude oil is also biodegradable quantitatively, but heavy asphatic-naphthenic crude oil, only about 11% biodegradable within a reasonable time period, even if the conditions are favourable.

It has been reported that the petroleum biodegradation is mostly enhanced in presence of a consortium bacteria species compared to monospecies activities [50]. Percentage of degradation by mixed bacterial consortium decreased from 78-52% as crude oil concentration increased from 1-10% [51].

It was reported that excess of nutrients concentration can inhibit biodegradation activity and others also reported the negative effect of a high NPK levels on hydrocarbon biodegradation and more especially on aromatics [52],[53],[54],[55].

iii. Effects of chemical composition of petroleum hydrocarbon:

Petroleum hydrocarbons can be divided into four classes- saturates, aromatics (phenols, fatty acids, ketones, esters and porphyrins) and resins (pyridines, quinoles, carbazoles, sulfoxides and amides). Hydrocarbons differ in their susceptibility to microbial attack and ranked in following order of decreasing susceptibility: n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes [56].

iv. Solubility: Biodegradability of hydrocarbons in soil dependent on their water solubility because bacteria in the unsaturated soil occur mainly in the interstitial water of soil. Therefore solubility of chemical will determine its concentration in soil.

v. Bioavailability: It is a key factor governing the efficient degradation of pollutants and can be defined as amount of substance that is physicochemically accessible to microorganisms. Chemotaxis of motile organisms towards or away from chemicals and also intracellular accumulation of aromatic molecules via various transport mechanism are also important [57]. Also reported that uptake of hydrocarbons occurs by attachment and then incorporation into cytoplasmic membrane [58].

Introduction of non-ionic surfactants, influences the alkane degradation rate [59]. But the use of surfactants in oil contamination may have stimulatory, inhibitory or neutral effect on bacterial degradation of the oil components [60]. In contrast to chemical dispersants, which cause ecocological damage after application on spilled oil in marine ecosystems, biosurfactants from soil or freshwater microorganisms are less toxic and partially biodegradable [61],[62].

Other methods for increasing bioavailability include physical disruption of soil aggregates using sonication increases biodegradation rate effectively in land farm experiment [63]. Weathering or aging of contamination also affect the bioavailability by physically trapping, hindering and slowing desorption from the soil [64].

vi. Physiochemical properties of soil:

This property of soil is determined by composition of soil, organic matter and fraction of soil as soil vary with respect to geology, hydrology, climate, fertility and other physical attributes [65].

vii. Oxygen: This parameter determines bacterial pattern of dissimilatory and energy yielding process. Microbial utilization of aliphatic, cyclic and aromatic hydrocarbons by bacteria and fungi requires molecular oxygen as electron sink [56],[67]. Further degradation of partially oxidised intermediates may be supported by nitrate or sulphate reduction in absence of molecular oxygen. Little or no hydrocarbon metabolism occurs in strictly anoxic sediments [68].
6. Recent applications of bio-remediation techniques and their effectiveness

i. Amoco Cadiz: In the case of the Amoco Cadiz spill, which contaminated large stretches of the Brittany shoreline in France in March 1978, natural biodegradation was found to occur rapidly. While it might have been predicted that the microbial populations in that region would be adapted to petroleum hydrocarbon degradation, since they had frequently been exposed to releases from ballast water tanks, it had not been predicted that the rates of low-molecular-weight hydrocarbon degradation would be as faster as chemical evaporation and dissolution. Until that spill, it had been accepted that biodegradation occurred only after a significant lag period, typically of the order of 2-4 weeks, and that chemical and physical weathering of the oil always preceded biological weathering. Besides mechanical recovery, four different bioremediation products have been applied to the beaches. They only lead to limited and inconclusive results. Some changes in oil content were found in the experiments, but it remained unclear, if the removal was physically or biologically mediated [69].

ii. Exxon Valdez: The Exxon Valdez oil spillage in March, 1989 created the largest spill ever with more than 2,000 km of oiled shoreline. The cleanup efforts included removing bulk oil, manual pickup of oil with sorbent pads, shore washing with hot, warm, and cold water, mechanical tilling, removal or oiled sediments, and bioremediation [70].

Regarding the last method, both techniques, seeding with microbial cultures and environmental modification were considered as bioremediation methods.

A) Seeding With Microbial Cultures

In the initial effort to identify cultures that might be applied to the clean-up effort in Prince William Sound, products from 10 companies were selected for laboratory phase testing by EPA. Some products delayed biodegradation. Most natural biodegradation, when it occurred, started after a 3-5 day lag period and reached significant levels after 20-30 days. Of the products tested, two were selected for further field testing in Prince William Sound on shorelines impacted by the spill. In the field trials, four small plots were used to assess the effectiveness of seeding. These field trials failed to demonstrate enhanced oil biodegradation by these products. There were no significant differences between the four plots during a 27-day trial period. It must be noted, however, that the oil was already highly degraded by the time these field trials were conducted, and that environmental variability makes it difficult to observe statistically significant differences between experimental and reference sites when relatively few samples are collected and analyzed.

B) Environmental Modification

Additionally, EPA carried out a comprehensive, large-scale project applying different fertilizers to the contaminated shorelines in Prince William Sound. Its objective was to demonstrate the enhancement of biodegradation through the addition of nitrogen and phosphorus in the form of three different types of fertilizers: Inipol EAP22™, an oleophilic fertilizer formulation, and Customblen™, a granular slow-release fertilizer. Oleophilic means literally oil loving.

Inipol™ contains surfactants as well as nutrients, and is designed to stick to oil on rocky substrates, providing nutrients at the oil/air interface where microbial degradation takes place. Several monitoring programs measured the effectiveness of these fertilizers in reducing oil contamination and evaluated potential environmental impacts as, for example, nutrient enrichment in adjacent waters and toxicity to marine organisms.

The most controversial aspect of bioremediation applications in Prince William Sound centred on the 2-butoxy-ethanol component in Inipol™ and
its potential toxicity to wildlife and cleanup workers. This was addressed by following worker safety guidelines during application of Inipol™, and by using wildlife deterrents during the first 24 h when toxicity is of most concern.

Nevertheless, Inipol™ turned out to produce very dramatic results in field tests, stimulating biodegradation so that the surfaces of the oil-blackened rocks on the shoreline turned white and appeared to be free of surface oil within 10 days after treatment[71]. The striking visual results strongly supported the idea that oil degradation in Prince William Sound was nutrient limited and that fertilizer application was a useful bioremediation strategy. Because of its success, Inipol™ was approved for shoreline treatment and used as a major part of the clean-up effort. Additionally, Customblen has been applied. In approximately 2-3 weeks, oil on the surface of cobble shorelines treated with Inipol™ and Customblen was degraded so that these shorelines were visibly cleaner than non-bioremediated shorelines. Tests demonstrated that fertilizer application sustained higher numbers of oil-degrading microorganisms in oiled shorelines and that rates of biodegradation were enhanced, as evidenced by the chemical changes detected in recovered oil from treated and untreated reference sites [72].

iii. Mega Borg: Bioremediation of the open water Mega Borg spill off the Texas coast in June 1990 consisted of applying a seed culture produced by the Alpha Corporation. This spill was also treated with dispersants and some burning of the oil occurred. The Texas General Land Office reported that the use of the Alpha culture on the Mega Borg spill was effective at removing significant amounts of oil. There was, however, no systematic or independent monitoring for effectiveness. In contrast, the study demonstrated the potential problems with the application of bioremediation problems at sea.

iv. Apex Barges: Biotreatment with the Alpha culture was also used in a spillage from the Apex Barges after an accident at Galveston Bay in Texas in July 1990. Here again, the Texas General Land Office reported that the bioremediation was effective. Independent observations, however, indicated that treated oil changed in physical appearance and may have emulsified as a result of addition of the Alpha product. Chemical analyses on samples from impacted and reference sites failed to demonstrate that treatment with the Alpha product enhanced rates of petroleum biodegradation. No significant differences in C18/phytane ratios that would indicate biodegradation enhancement were detected between Alpha-treated and untreated sites. Thus, scientifically valid conclusions cannot be reached substantiating the effectiveness of seeding of open water or coastal spills. Clearly designed and extensive experiments, with appropriate controls, will be needed if the efficacy of seeding open water oil spills is ever to be resolved.

7. Conclusion

Bioremediation is proven to be an alternative, sustainable and ecofriendly treatment for the removal petroleum hydrocarbon contaminated environment. It is very efficient and cost effective method in comparison to other methods used commercially for pollutant removal. Microorganisms feed on the organic pollutants and degrade it completely without producing any harmful residues in the environment. However due to its slow process of degradation it is not generally preferred. Research is being performed on the use of genetically engineered microorganisms to degrade the contaminants at a faster rate. But it is not yet successful for application as in laboratory because microorganisms need favourable conditions for growth and to perform their activity normally. The physical and chemical parameters of environment affects the
activity of microorganisms thus slowing down the degradation process.

Acknowledgement

We are very grateful to our guides Mr. Brajesh Singh, H.O.D. (Department of Biochemical Engineering and Food Technology, H.B.T.I., Kanpur-208002) and Mr. Mahendra Kumar (Faculty member) for their guidance and support in completion of this paper.

8. References


