Bioremediation: A boon to hydrocarbon degradation
Sheetal Sonawdekar
Department of Biotechnology and Bioinformatics, Dr D Y Patil University
Belapur, Navi Mumbai 400614 India
sheetal.sonawdekar@gmail.com
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ABSTRACT

The origin of oil and gas industry in India can be traced back to 1867 when oil was struck at Makum near Margherita in Assam. Since then the consumption and demand of different petroleum products has been increasing steadily. As the usage of these products has increased, it has also given rise to certain problems in the environment such as soil contamination, health risks to the habitual organisms etc. some petroleum products have been found to exert carcinogenic and neurotoxic effects. Though several mechanical and chemical methods are followed for the degradation of these products the rate of contamination is quite high and that makes these methods very expensive. In view of this situation bioremediation gives a better solution compared to the currently existing methods. It provides efficacy, safety on long term use, cost and simplicity of administration with promising opportunity for creating better environment.

Keywords: bioremediation, petroleum degradation, petroleum degrading bacteria, in-situ bioremediation, ex-situ bioremediation, biochemical analysis of degradation

1. Introduction

The world depends on different petroleum sources. To meet the demand a vast amount of it is regularly processed, stored, transported and used. The total oil consumption in 2009 was 82,769,370.4 bbl/day out of which India’s share was 2800 bbl/day (source: CIA world factbooks). With such high consumption, oil spills are inevitable.

The amount of natural crude oil seepage was estimated to be 600000 metric tons per year with a range of uncertainty of 200,000 metric tons per year. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations. These oil spills can even cause damage to the sea and shoreline organisms (Tabari and Tabari, 2010) (Rodríguez-Martínez et al, 2006). The other sources of contamination include service stations, garages, scrap yard, waste treatment plants and saw mills etc.

Therefore with the expansion of petroleum industry, one cannot overlook the inevitable spillage and significant contamination occurring during operations and transportation (Snape et al, 2001)(Sivasubramaniam, Seshadri, Shah, 2005). Currently the common methods in use are majorly physical, chemical or mechanical in nature like burying, evaporation, dispersion, and washing etc. however these technologies are quite expensive and may lead to incomplete decomposition of contaminants. So it is the need of the time to look for better methods.
A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. In view of this bioremediation is becoming an increasingly popular technique for cleaning up the contaminated sites. The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry (Medina-Bellver et al, 2005). Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site (Bento et al, 2005) (Williams et al, 2006).

1.1 Historical perspective

The process of bioremediation was invented by George M. Robinson. Throughout the 1960s Robinson pioneered the idea of making custom mixtures of dried bacteria cultures for commercial use. His "bug-brew" recipes subsequently gained acceptance and notoriety after several well publicized demonstrations (Wikipedia contributors, 2012). Later the first commercial in situ bioremediation system was installed in 1972 to cleanup a Sun Oil pipeline spill in Ambler, Pennsylvania (National Research Council, National Academy Press, 1993). In 1979, for the first time Anand Mohan Chakrabarty, an India borne American scientist obtained a strain of Pseudomonas putida that contained the XYL and NAH plasmid as well as a hybrid plasmid derived by recombinating partsb of CAM and OCT (these are incompatible and cannot co-exist as separate plasmids in the same bacterium). This strain could grow rapidly on crude oil because it was capable of metabolizing hydrocarbons more efficiently than any other single plasmid (Wikipedia contributors, 2012). Considering this in mid-1980s emphasis went on bioengineering organisms for bioremediation. But this technology did not produce better results. So by 1990s scientists switched to greater reliance on natural microorganisms and techniques to enhance their performance.

1.2 Petroleum hydrocarbons

Petroleum and petroleum products are highly complex and varied mixtures. Petroleum is defined as any mixture of natural gas, condensate, and crude oil. Crude oil can consist of thousands of individual compounds with hydrocarbons representing from 50 to 98 percent of the total weight of crude oil. When petroleum compounds such as crude oil are released into the environment, the compounds undergo physical, chemical, and biological changes collectively referred to as weathering. The degree to which various types of petroleum hydrocarbons degrade under these changes depends on the physical and chemical properties of the hydrocarbons. (Gallego et al, 2001) (Hogan, 2010) (Okoh, 2006) (Williams et al, 2006) (Scragg, 2005)

1.3 Types of petroleum hydrocarbons

Petroleum hydrocarbons are generally divided into two groups: aliphatics and aromatics. Aliphatics include alkanes that contain single bonds between carbon atoms and have formulas of C\textsubscript{n}H\textsubscript{2n+2}, alkenes, which contain one or more double bonds between atoms and have formulas of C\textsubscript{n}H\textsubscript{2n}, and cycloalkanes, which contain carbon atoms in cyclic structures. Aromatics have one or more benzene rings as part of their structure. Monoaromatics are aromatics with one benzene ring as part of their structure, polycyclic aromatic hydrocarbons (PAHs) are aromatics with two or more fused benzene rings. Monoaromatics, such as
benzene, toluene, ethylbenzene, and xylenes (BTEX), are some of the most common aromatic compounds in petroleum. Crude oil contains less BTEX than gasoline. On average, crude oil contains approximately 1% PAHs. Typically, crude oil contains high concentrations of aliphatic hydrocarbons and lower concentrations of aromatic hydrocarbons.

Natural gas condensates also are composed primarily of aliphatic hydrocarbons, however, the condensates may contain substantial amounts of BTEX. Several aromatic hydrocarbons are known or suspected human carcinogens, and are classified as priority pollutants regulated by the U.S. Environmental Protection Agency (USEPA).

Cumulative concentrations of petroleum hydrocarbons commonly are referred to as total petroleum hydrocarbons (TPH). Many different analytical techniques including gravimetric, immunoassay, and gas chromatography (GC) have been used to measure TPH in soil and water. None of the techniques measure the entire range of petroleum hydrocarbons. The subsets of hydrocarbons detected by the techniques vary depending on the extraction and analytical methods used (Balba, Awadhi, Daher, 1998) (Scragg, 2005).

2. Principles of bioremediation

By definition, bioremediation is the use of microorganisms to degrade the environmental contaminants into less toxic forms. It involves the use of naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. As mentioned earlier contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. So for bioremediation to be effective it is very important that these organisms should be metabolically active and should carry different enzymes required for the biodegradation. One can run the process aerobically or anaerobically as per the choice of the microorganism (Vidali M., 2001)(Vashishtha S, 2011).

![Figure 1: Main principle of Aerobic degradation of hydrocarbons (Das and Chandran, 2011)](image-url)
The microorganisms act against the contaminants only when they have access to a variety of materials, compounds to help them generate energy and nutrients to build more cells. In a few cases the natural conditions at the contaminated site provide all the essential materials in large enough quantities that bioremediation can occur without human intervention, a process called intrinsic bioremediation. More often, bioremediation requires the construction of engineered systems to supply microbe-stimulating materials—a process called engineered bioremediation. Engineered bioremediation relies on accelerating the desired biodegradation reactions by encouraging the growth of more organisms, as well as by optimizing the environment in which the organisms must carry out the detoxification reactions (figure 1). The suitability of a site for bioremediation depends not only on the contaminant's biodegradability but also on the site's geological and chemical characteristics. For intrinsic bioremediation, the key site characteristics are consistent ground water flow throughout the seasons, the presence of minerals that can prevent pH changes, and high concentrations of oxygen, nitrate, sulfate, or ferric iron. For engineered bioremediation, the key site characteristics are permeability of the subsurface to fluids, uniformity of the subsurface, and relatively low residual concentrations of non aqueous-phase contaminants (Sivasubramaniam, Seshadri, Shah, 2005).

3. Strategies and techniques used for monitoring biodegradation

3.1 Strategies


3.2 In-situ bioremediation of soil

In-situ techniques do not require excavation of the contaminated soils so may be less expensive, create less dust, and cause less release of contaminants than ex-situ techniques. Also, it is possible to treat a large volume of soil at once. In-situ techniques, however, may be slower than ex-situ techniques, may be difficult to manage, and are most effective at sites with permeable (sandy or uncompacted) soil. The main types of in-situ bioremediation are as given below.

3.2.1 Intrinsic bioremediation

Also known as natural attenuation, this type of bioremediation occurs naturally in contaminated soil or water. This natural bioremediation is the work of microorganisms and is seen in petroleum contamination sites, such as old gas stations with leaky underground oil tanks. Application of this technique requires close monitoring of contaminant degradation to ensure that environmental and human health are protected.

Intrinsic bioremediation is an option when the naturally occurring rate of contaminant biodegradation is faster than the rate of contaminant migration. These relative rates depend on the type and concentration of contaminant, the microbial community, and the subsurface hydrogeochemical conditions. The ability of native microbes to metabolize the contaminant must be demonstrated either in field tests or in laboratory tests performed on site-specific samples. In addition, analyzing the fate of the contaminants and other reactants and products indicative of biodegradation must continually monitor the effectiveness of intrinsic bioremediation. (Biobasics, Canadian Biotechnology Secretariat, 2006)
In intrinsic bioremediation the rate-controlling step is frequently the influx of oxygen. When natural oxygen supplies become depleted, the microbes may not be able to act quickly enough to contain the contamination. Lack of a sufficiently large microbial population can also limit the cleanup rate. The microbial population may be small because of a lack of nutrients, limited availability of contaminants resulting from sorption to solid materials or other physical phenomena, or an inhibitory condition such as low pH or the presence of a toxic material.

3.2.2 Engineered bioremediation

Engineered bioremediation may be chosen over intrinsic bioremediation because of time and liability. Where an impending property transfer or potential impact of contamination on the local community dictates the need for rapid pollutant removal, engineered bioremediation may be a more appropriate remedy than intrinsic bioremediation. Because engineered bioremediation accelerates biodegradation reaction rates, it requires less time than intrinsic bioremediation. The shorter time requirements reduce the liability for costs required to maintain and monitor the site. Since many petroleum hydrocarbons require oxygen for their degradation, the technological emphasis of engineered bioremediation systems in use today has been placed on oxygen supply. Bioremediation systems for soil above the water table usually consist of a set of vacuum pumps to supply air (containing oxygen) and infiltration galleries, trenches, or dry wells to supply moisture (and sometimes specific nutrients). Bioremediation systems for ground water and soil below the water table usually consist of either a set of injection and recovery wells used to circulate oxygen and nutrients dissolved in water or a set of compressors for injecting air. Emerging applications of engineered bioremediation, such as for degradation of chlorinated solvents, will not necessarily be controlled by oxygen. Hence, the supply of other stimulatory materials may require new technological approaches even though the ultimate goal, high biodegradation rates, remains the same.

3.2.3 Biostimulation

Nutrients and oxygen - in a liquid or gas form - are added to contaminated water or soil to encourage the growth and activity of bacteria already existing in the soil or water. The disappearance of contaminants is monitored to ensure that remediation occurs. (Bento et al, 2005) (Trindade, et al) (Biobasics, Canadian Biotechnology Secretariat, 2006) The goal of aerobic in-situ bioremediation is to supply oxygen and nutrients to the microorganisms in the soil. Aerobic in-situ techniques can vary in the way they supply oxygen to the organisms that degrade the contaminants. Two such methods are bioventing and injection of hydrogen peroxide. Oxygen can be provided by pumping air into the soil above the water table (bioventing) or by delivering the oxygen in liquid form as hydrogen peroxide. In-situ bioremediation may not work well in clays or in highly layered subsurface environments because oxygen cannot be evenly distributed throughout the treatment area. In-situ remediation often requires years to reach cleanup goals, depending mainly on how biodegradable specific contaminants are. Less time may be required with easily degraded contaminants.

3.2.4 Bioventing

Bioventing systems deliver air from the atmosphere into the soil above the water table through injection wells placed in the ground where the contamination exists. The number, location, and depth of the wells depend on many geological factors and engineering
considerations. An air blower may be used to push or pull air into the soil through the injection wells. Air flows through the soil and the oxygen in it is used by the microorganisms. Nutrients may be pumped into the soil through the injection wells. Nitrogen and phosphorous may be added to increase the growth rate of the microorganisms.

3.2.5 Injection of hydrogen peroxide

This process delivers oxygen to stimulate the activity of naturally occurring microorganisms by circulating hydrogen peroxide through contaminated soils to speed the bioremediation of organic contaminants. Since it involves putting a chemical (hydrogen peroxide) into the ground (which may eventually seep into the groundwater), this process is used only at sites where the groundwater is already contaminated. A system of pipes or a sprinkler system is typically used to deliver hydrogen peroxide to shallow contaminated soils. Injection wells are used for deeper contaminated soils.

3.2.6 Bioaugmentation

Microorganisms that can clean up a particular contaminant are added to the contaminated soil or water. Bioaugmentation is more commonly and successfully used on contaminants removed from the original site, such as in municipal wastewater treatment facilities. To date, this method has not been very successful when done at the site of the contamination because it is difficult to control site conditions for the optimal growth of the microorganisms added. Scientists have yet to completely understand all the mechanisms involved in bioremediation, and organisms introduced into a foreign environment may have a hard time surviving. (Margesin and Schinner, 2001) (Trindade et al) (Biobasics, Canadian Biotechnology Secretariat, 2006)

3.2.7 In-Situ bioremediation of groundwater

In-situ groundwater bioremediation is a technology that encourages growth and reproduction of indigenous microorganisms to enhance biodegradation of organic constituents in the saturated zone. In-situ groundwater bioremediation can effectively degrade organic constituents, which are dissolved in groundwater and adsorbed onto the aquifer matrix. (Mishra et al, 2001)

In-situ bioremediation of groundwater can be combined with other saturated zone remedial technologies (e.g., air sparging) and vadose zone remedial operations (e.g., soil vapor extraction, bioventing).

3.2.8 System design

In-situ bioremediation can be implemented in a number of treatment modes, including: Aerobic (oxygen respiration), anoxic (nitrate respiration), anaerobic (non-oxygen respiration), and co-metabolic. The aerobic mode has been proven most effective in reducing contaminant levels of aliphatic (e.g., hexane) and aromatic petroleum hydrocarbons (e.g., benzene, naphthalene) typically present in gasoline and diesel fuel. In the aerobic treatment mode, groundwater is oxygenated by one of three methods: Direct sparging of air or oxygen through an injection well, saturation of water with air or oxygen prior to re-injection, or addition of hydrogen peroxide directly into an injection well or into reinjected water. Whichever method of oxygenation is used, it is important to ensure that oxygen is being distributed throughout the area of contamination. Anoxic, anaerobic, and co-metabolic modes
are sometimes used for remediation of other compounds, such as chlorinated solvents, but are generally slower than aerobic respiration in breaking down petroleum hydrocarbons.

The key parameters that determine the effectiveness of In-situ groundwater bioremediation are:

1. Hydraulic conductivity of the aquifer, which controls the distribution of electron acceptors and nutrients in the subsurface,
2. Biodegradability of the petroleum constituents, which determines both the rate and degree to which constituents will be degraded by microorganisms, and
3. Location of petroleum contamination in the subsurface. Contaminants must be dissolved in groundwater or adsorbed onto more permeable sediments within the aquifer.

3.3 Application

In-situ groundwater bioremediation can be effective for the full range of petroleum hydrocarbons. While there is some notable exceptions like the short-chain, low molecular weight, more water-soluble constituents are degraded more rapidly and to lower residual levels than are long-chain, high molecular weight, less soluble constituents. Recoverable free product should be removed from the subsurface prior to operation of the in-situ groundwater bioremediation system. This will mitigate the major source of contaminants as well as reduce the potential for smearing or spreading high concentrations of contaminants.

3.3.1 Ex-situ bioremediation

These techniques involve the excavation or removal of contaminated soil from ground. Ex-situ bioremediation can be divided into two main types: Solid phase and Slurry phase ex-situ bioremediation which can be further divided as follows (Pavel and Gavrilescu, 2008) (Vidali, 2001) (Rao, 2005):

3.3.2 Landfarming

It is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically turned over until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative.

3.3.3 Composting

This is a technique that involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting.

3.3.4 Biopiles

This technique is a hybrid of landfarming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface
contamination with petroleum hydrocarbons they are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms.

### 3.3.5 Bioreactors

This is a type of slurry phase treatment. Slurry reactors or aqueous reactors are used for treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor is a containment vessel and apparatus 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 bioremediation by bioreactors is better than in situ bioremediation due to better contact between microorganisms and contaminants.

### 3.3.6 Phytoremediation

A new and rapidly developing form of bioremediation is phytoremediation, which uses green plants and their associated biota to destroy, remove, contain, or otherwise detoxify environmental contaminants. Four types of destruction and removal reactions can occur in phytoremediation. (Fulekar, 2005) (Rittmann and Mccarty, 2005)

1. Phytovolatilization is an enhancement of the volatilization process from the soil or through the plant's roots or shoots. Enhanced volatilization can occur via plant transpiration of volatile compounds or transformation of contaminants to more volatile forms.

2. Phytodegradation involves uptake by the plant and subsequent metabolism by plant enzymes to form benign products.

3. Phytoextraction involves uptake by the plant and absorption of the contaminant into plant tissue, which subsequently is harvested. Hydrophobic contaminants are most susceptible to photoextraction.

4. Rhizosphere degradation to benign products is catalyzed by plant enzymes excreted by the roots or by the microorganisms found in the rhizosphere.

In addition, phytoremediation can sequester contaminants; a process called phytostabilization. The benefit of phytostabilization is that it makes the contaminants less bioavailable to humans and other receptors. Three forms of phytostabilization are Humification, Lignification, Aging. The distinctions among the various stabilization, destruction, and removal mechanisms often are not sharp. Phytoremediation is still in its early stages of research and development. Nonetheless, it has promise for the decontamination of petroleum hydrocarbons, a range of chlorinated aliphatics and aromatics (including PCBs), and pesticides.

### 3.3.7 Petroleum hydrocarbon degrading bacteria

Hydrocarbon degrading microorganisms are ubiquitous in nature but are found at relatively higher densities in petroleum contaminated sites. Arctic environments, estuaries, oceans and marine sediments, deep sea, thermal rents, etc. are some of the sites explored for isolating
hydrocarbon degrading microorganisms. Hydrocarbon degrading bacteria (aerobic or anaerobic) and fungi (lignolytic) are widely distributed in these habitats. Similarly, hydrocarbon degrading cyanobacteria have been reported but there can be some exceptions to behavior of cyanobacteria. Some hydrocarbon degrading microorganisms are as listed in table 1. (Adebusoye et al, 2006) (Gallego et al, 2001) (Oboh et al, 2006) (Vidali, 2001).

**Table 1:** Different types of petroleum hydrocarbon degrading bacteria (Desai and Vyas, 2006, Microbewiki, 2011)

<table>
<thead>
<tr>
<th>Crude oil component</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturates</td>
<td>Arthrobacter sp., Acinetobacter sp., Candida sp.,</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas sp., Rhodococcus sp., Streptomyces sp.,</td>
</tr>
<tr>
<td></td>
<td>Bacillus sp., Aspergillus japonicus</td>
</tr>
<tr>
<td>Monocyclic aromatic hydrocarbons</td>
<td>Pseudomonas sp., Bacillus sp., B. stereothermophilus,</td>
</tr>
<tr>
<td></td>
<td>Vibrio sp., Nocardia sp., Corynebacterium sp., Achromobacter sp.</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td>Arthrobacter sp, Bacillus sp., Burkholderia cepacia.,</td>
</tr>
<tr>
<td></td>
<td>Pseudomonassp., Mycobacterium sp., Xanthomonas sp.,</td>
</tr>
<tr>
<td></td>
<td>Phanerochaete chrysosporium, Anabena sp., Alcaligenes</td>
</tr>
<tr>
<td>Resins</td>
<td>Pseudomonas sp., Members of Vibrionaceae., Enterobacteriaceae, Moraxella sp.</td>
</tr>
</tbody>
</table>

3.4 Techniques


3.4.1 Respirometry

The rate of carbon dioxide production and oxygen consumption is measured in a soil with a soil respirometer or an electrolytic respirometry apparatus. The technique appears to provide accurate estimates of microbial activity in soils with low organic matter content.

3.4.2 Gas Chromatography (GC)

This technique identifies and quantifies contaminants by separating a complex organic mixture into its components. The sample extract is injected into a heated chamber and then is carried through column by an inert gas (mobile phase). The analytes pass through absorbent material (stationary phase). From the chromatogram and the information contained in the report, numerous components can be accurately identified and quantified. This technique can be used alone or in combination with Mass Spectrosbcopy (MS) or Flame Ionization Analysis (FIA) of separated analytes (Mittal A, 2009) (Sharma A, 2009).

3.4.3 Luminescence techniques

Reporter gene cassettes consisted of luciferase genes and contaminant-specific promoter sequences are inserted into a bacterial host. Their expression is induced by contaminant of
interest. Measurements of light emitted by such systems reflect bacterial metabolic activity in real time. This technique has limited application in multi-species oil-degrading microbial communities and under field conditions.

3.4.4 Fluorescence analysis

During fluorescence analysis of a compound, excitation and emission spectra are recorded. These spectra can be manipulated and used for the identification of the compound. This technique is used for oil fingerprinting and has become a part of a basic and routine analytical procedures in analysis following oil spills in coastal waters. Although valuable as a rapid means of discrimination between some suspect sources this method’s discriminating capacity is severely limited (Oil Degradation Monitoring at Metamicrobe.com).

3.5 Use of internal petroleum biomarkers

This technique measures the relative increase in the internal biomarker concentration in the source oil during weathering and biodegradation. This method relies on Gas Chromatography/Flame Ionization Detection (GC/FID) and GC/MS to determine total oil or specific analyte concentration. Several assumptions are made in applying this technique: (1) the source of contamination has been identified and is primarily a single source, (2) the biomarker is neither formed nor degraded as a result of microbial activity, (3) the extraction efficiency of the chemical marker is the same as the rest of other oil components. This approach allows effective degradation monitoring under variable conditions.

3.6 Determination of Total Petrol Hydrocarbons (TPH) by Infrared Spectrometry (IR) and Gas Chromatography (TPH-IR and TPH-GC)

The sample of oil is solvent-extracted and total mass of dissolved hydrocarbons is subsequently quantified by comparing the infrared absorption of the extraction liquid against that of control hydrocarbon mixture. This method is characterized by low technical complexity and quick quantification of oil contaminants (Mittal A, 2009) (Sharma A, 2009).

4. Factors influencing bioremediation of hydrocarbons

The main factors that influence the microbial degradation of hydrocarbons are temperature, pH, moisture level of soil, salinity of environment and chemical composition and concentration of the contaminant hydrocarbons (Desai and Vyas, 2006, Jain et al, 2011, Saleh et al, 2003).

4.1 Temperature

It is an important factor to govern the metabolic activity of the degrading microorganisms as well as physical and chemical nature of hydrocarbons. At the mesophilic and thermophilic range of temperatures it is been found that the enzyme activity of microorganisms increases which helps in increasing the rate of hydrocarbon degradation. Therefore majorly the 30-40°C temperature in the mesophilic range or sometimes 60°C temperatures in thermophilic range is used. Though it is been also observed that some hydrocarbons like, diesel can be degraded at lower temperatures i.e. between 0-10°C (figure.2). But at low temperatures the viscosity of oil increases which suppresses the spreading of oil on surface which makes the degradation difficult. Moreover in the mesophilic range more variety of organisms can be
available for degradation so mesophilic or thermophilic temperatures are the better choice for bioremediation. (Obuekwe et al, 2001).

4.2 pH

The choice of pH depends on the microorganisms to be used for the degradation. Fungal strains are found to carry biodegradation at lower temperatures. Even when indigenous microbial consortium inclusive of fungi, yeast and several bacteria is used they are found to survive at pH 2. Though there are certain bacteria that are alkaliphiles found in alkaline lakes at pH 7.5-10.

4.3 Salinity

Many isolates are capable of growing at salinity comparable to sea water. The significant hydrocarbon degradation was observed with 0.1-2M NaCl where maximum was at 0.4M i.e. almost equivalent to natural sea water. Though at higher salinity level, the degradation rate was found to decrease.

4.4 Oxygen

It is one of the basic requirements for the biodegradation. But again the concentration of oxygen used will highly depend on the choice of microorganism used. For aerobic bacteria, stoichiometrically 3.1mg/ml of oxygen is required for the degradation of 1mg/ml hydrocarbons without taking into consideration the total mass of bacteria. So it may vary with increasing or decreasing mass of bacteria (Curtis and Lammey, 1998). Even anaerobic biodegradation has proved its importance. Many different types of bacteria are tested and found to be useful in degrading hydrocarbons like benzene, alkanes, toluenes etc.

Figure 2: Hydrocarbon degradation rates in soil, fresh water, and marine environments (Das and Chandran, 2011)
4.5 Nutrients

Nutrients are required to support the biological activity, and hence bioremediation. Microorganisms commonly require carbon, nitrogen and phosphorous for the degradation of hydrocarbons. The amount of various nutrients and ratio of particularly, nutrients like C, N and P is quite conceivable regarding the success of the bioremediation process. The organic carbon content in hydrocarbon contaminated site is found to be very high attributed to constant input of hydrocarbons. Because of the high carbon content of oil and the low level of other nutrients essential for microbial growth, the rate and extent of degradation are, in general, limited by the low availability of nitrogen and phosphorus. Consequently, growth of hydrocarbon-degrading bacteria and hydrocarbon degradation can be strongly enhanced by fertilization with inorganic N and P. In majority of the treatments the C:N:P ratio is maintained as 120:10:1. Though it may vary as per the need of the microorganisms. Other than these some more nutrients may be added as and when required. (Leys et al, 2005, Ro¨Ling et al, 2002)

4.6 Chemical composition of petroleum

Petroleum hydrocarbons can be considered of four different types for degradation: (1) saturates, (2) aromatics, (3) asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and (4) resins (pyridines, quinolines, carbazoles, sulfoxides, and amides). In general, hydrocarbons have been ranked in the following order of decreasing susceptibility to biodegradation: n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes, with high molecular weight aromatics and polar compounds being extremely recalcitrant. Some studies so support this. However under some conditions (composition of microbial community, abiotic factors), the order of biodegradation can vary greatly. (Atlas and Bragg, 2009) (Das and Chandran, 2011) (Obuekwe et al, 2001) (National Research Council, National Academy Press, 1993)

4.7 Physical State of hydrocarbons

The bioavailability of hydrocarbons is also dependent on physical state, hydrophobicity, sorption onto soil particles, volatilization and solubility of hydrocarbons greatly affects the extent of biodegradation.

4.8 Hydrocarbon concentration

The rate of microbial uptake and biodegradation of hydrocarbons is dependent on the solvent solubility of the hydrocarbons. Hydrocarbons that are highly soluble in water their degradation rates are almost proportional to the concentration but it differs for less aqueous hydrocarbons. Though it needs to be considered that high solubility may become detrimental to the degrading organisms. Usually oil loading greater than 5% leads to decrease in the microbial activity (Del’arco and De, 2001, El-Tarabily, 2002). In addition to this high concentration may disturb C: N: P ratio and create oxygen limitations.

5. Summary
The quality of life on earth is highly related with its environment. For life to sustain it is very important that the environment should remain clean and healthy. In recent times due to the increase in industrialization and utilization of petroleum related products not to forget negligence using them, the environment is getting damaged. To clean up especially the subsurface hydrocarbon concentration is a worldwide problem. And to solve this we need to have the better understanding of the bioremediation techniques. If the challenges of bioremediation, particularly of in situ techniques, can be overcome, bioremediation has potential to provide a low cost, non-intrusive, natural method to render toxic substances in soil less harmful or harmless over time. Currently, research is being conducted to improve and overcome limitations that hinder bioremediation of petroleum hydrocarbons. On a broader scope, much research has been and continues to be developed enhance understanding of the essence of microbial behavior as microbes interact with various toxic contaminants. Additional research continues to evaluate conditions for successful introduction of exogenic and genetically engineered microbes into a contaminated environment, and how to translate success in the laboratory to success in the field. More research related to co-metabolism of certain microorganisms and plants i.e. amalgamation of microbial degradation and phytoremediation represents a research frontier with broad implications. As these new techniques are brought into commercial practice, the importance of sound methods for evaluating bioremediation will increase. And that will help to create a better and cleaner environment.

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