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Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons

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ABSTRACT

In the whole environment, one of the most vital contaminations is caused by the petrochemical industries, products, effluents which is released from the petrochemical industries, road accidents, ship accidents etc. These petroleum products contain the carcinogenic and mutagenic compounds which are known as poly aromatic hydrocarbons. In the present picture of world a major environmental pollution of soil and water is due to hydrocarbon contamination resulting by the petrochemical industries, refineries, human activities etc. The better understanding of the mechanisms and factors which affect biodegradation is of great ecological significance, since the choice of bioremediation strategy depends on it. For successful implementation of bioremediation technologies on contaminated areas must be depends upon the characteristics of the contaminated site and a complex system of many factors that affect the petroleum hydrocarbons biodegradation processes The main factors which limit the overall biodegradation rate can be grouped as: soil characteristics, contaminant characteristics, bioavailability, microorganism's number, catabolism evolution etc. In order to adopt and implement some effective bioremediation strategy it is extremely important to consider and understand those limiting factors. The present study explains some factors which can the rate of biodegradation process.

Keywords: Polyaromatic hydrocarbons; factors; biodegradation; bioremediation etc.

INTRODUCTION

The intent of the present review is to present a broad and updated overview of the microbial ecology of hydrocarbon degradation, emphasizing both environmental and biological factors which are involved in determining the rate at which an extent to which hydrocarbons are removed from the environment by biodegradation. Aspects of biodegradation of petroleum and individual hydrocarbons in marine, freshwater and soil ecosystems are presented.

In the modern industrial age of technologies various aspects of human life changes. The quality of life on earth is highly related with its environment. It is very important for life to sustain 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.

The development of human society all over history has lead to rising disruption of the natural equilibrium and the rate of different types of pollution. The planet depends on oil, and the use of oil as fuel has lead to intensive economic development worldwide. The great need for this power source has led to the gradual exhaustion of normal oil capital. on the other hand, mankind will witness the results of oil utilization for centuries after its termination. Ecological pollution with petroleum and petrochemical products has been recognized as a significant and serious problem¹.

Today, the most common environment problem is caused due to petroleum products. If these products are exposed to the environment they can be hazardous to the surroundings as well as to life forms. Millions of liters of petroleum enter into the environment, from both natural and anthropogenic sources every year.

It has been estimated that global production of crude oil more than twelve million metric tons annually and about 1.7 to 8.8 million metric tons of petroleum hydrocarbon escapes into the terrestrial and marine environment.

In the whole environment the release of hydrocarbons whether it is accidentally or due to human activities is a main cause of water and soil pollution. The 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 accidental oil spills can even cause damage to the sea and shoreline organisms³⁸. The other sources of contamination due to hydrocarbons include service stations, garages, scrap yard, waste treatment plants and saw mills etc.

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^{14, 31,32,42,53}.

1.1 Poly Aromatic Hydrocarbons

The sites contaminated by automobile or related with petrochemical compounds contain a variety of aliphatic, aromatic and polyaromatic hydrocarbons (PAHs). Among them, PAHs pose more threat to the environment and human health¹⁸. The PAHs consists of two or more than two fused aromatic rings in linear, angular and cluster arrangements. The PAHs are the pollutants which are present in air, soil and sediments. PAHs enter into the environment from various sources and their derivatives are results of incomplete combustion of organic materials in the environment. They arise from natural combustion like; forest fires and volcanic eruptions, activities related to chemical and petro-chemical industries, agriculture wastes, sludge wastes etc.

PAHs are widely found in high concentrations at many industrial sites, particularly those associated with petroleum, gas production and wood preserving industries. Due to their toxicity, carcinogenicity and mutagenicity these PAHs have gained much concern^{50,17}. More than 100 different PAHs are ubiquitously distributed in environment; many of them possess mutagenic, carcinogenic, teratogenic properties⁵⁸. Among them, 16 PAHs have been identified as priority pollutants and seven of them classified as probable human carcinogens by U.S environmental protection agency & European Union³⁵.

In recent years the major sources of PAHs pollution include industrial production, transportation, refuse burning, gasification and plastic waste incineration. The fate of PAHs in nature is related to environment due to their toxic, mutagenic and carcinogenic properties (phenanthrene is known to be a human skin photosensitizer and mild allergen, Benzo (pyrene) is carcinogenic for humans). PAHs may get absorbed to organic-rich soils/ sediments and get accumulate in fish and other aquatic organisms. From there they might be transferred to humans through food chains.

1.2 Bioremediation

There are several methods to treat the contamination caused by petroleum and petroleum products such as physical, chemical and biological treatment. Biological degradation appears to be the main process responsible for the removal of PAHs in soil^{45,54}. Microorganisms, such as bacteria and fungi may transform PAHs to other less harmful organic compounds or to inorganic end products such as carbon dioxide and water^{15,5}.

The biological methods like natural attenuation, biostimulation, bioaugmentation and bioremediation are efficient and adequate methods to clean up soil with petroleum hydrocarbons as contaminants because these methods do not adversely affect the site. The bioremediation is one of the economical methods compared to the other methods like incineration and washing of the soil etc Bioremediation is the one of the useful and inexpensive method to achieve the optimum biodegradation condition, in which by the use of microorganisms hydrocarbons degraded which is the sources of carbon and energy for the microorganisms.

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. 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.

1.3 Factors

The cleaning up of petroleum hydrocarbons in the soil environment is a real world problem. Better understanding of the mechanisms and factors which affect biodegradation is of great ecological significance, since the choice of bioremediation strategy depends on it. Microbial degradation processes aid the elimination of spilled oil from the environment, together with various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. Even if the optimal conditions for microbial degradation are provided, the extent of hydrocarbon removal is strongly affected by its bioavailability and stages of weathering. As a consequence, some fractions of hydrocarbons remain undegraded. This residual fraction of hydrocarbon in soil can represent an acceptable end point for bioremediation if¹ hydrocarbon biodegradation is too slow to allow further bioremediation, in which case other technologies must be applied;² those concentrations are unable to release from the soil and pose adverse effects to the environment and human health, like those presented in the given case studies

Various studies have addressed the successful applications of remediation of hydrocarbon contaminated soil and water. It was carried out integrated study of contamination caused by hydrocarbon and PAHs and its treatment with bioremediation method³⁵. By inoculating an enzymatic microbial compound it was measured concentration of hydrocarbon and PAHs. Some eco-toxicological and microbiological tests were performed and reductions of pollutants were obtained while toxicological and phytotoxicity tests evidenced a great improvement of the soil.

The maximum biodegradation occurred when conditions are favourable for microorganisms. It is important to know the characteristics of the contaminated site before beginning the treatments. The basic information such as residual oil concentration, population density of hydrocarbon degrading microorganisms and the biodegradation potential, environmental factors such as pH, temperature etc. are some of the key factors to be considered for bioremediation^{12,19,41}.

Degradation of PAHs in situ is often slow, and research over the last decades has shown that these compounds very often are persistent. This persistence may be due to several factors such as nutrients, bioavailability of PAHs (sorption to particles), temperature, oxygen, and presence of PAH-degrading microorganisms.

A full factorial design can be used to test every possible combination of different environmental factors. Successful implementation of bioremediation technologies on contaminated areas depends on the characteristics of the contaminated site and a complex system of many factors that affect the petroleum hydrocarbons biodegradation processes¹⁹. The main factors which limit the overall biodegradation rate can be grouped as: soil characteristics, contaminant characteristics, bioavailability, microorganism's number and catabolism evolution¹. In order to adopt and implement some bioremediation strategy it is extremely important to consider and understand those limiting factors. For the effective bioremediation, information regarding the physicochemical properties and the indigenous microbial community of the automobile contaminated soil are essential⁵².

A large part of the oil resources in our planet is constituted by heavy oil, i.e., oil reservoirs that have suffered biodegradation at a certain extent. There are many factors contributing to the oil degradation, including physical-chemical factors, such as the environmental pH, organic matter content, temperature, and the oil chemical composition, as well as biological factors, such as the microbial distribution in the environment, physiological and metabolic.

Many published studies have investigated the efficacy of bioremediation on a bench scale and under ideal laboratory conditions, such as a circum-neutral pH and mesophilic temperatures. However, it is apparent that environmental factors that vary from site to site (such as soil pH, nutrient availability and the bioavailability of the contaminant) can influence the process of bioremediation by inhibiting growth of the pollutant-degrading microorganisms.

There are a variety of factors like pH, temperature, oxygen, nutrient availability etc., which can affect the rate of degradation of poly aromatic hydrocarbons^{56,57} studied the effect of temperature, salinity and oil concentration on biodegradation of crude oil, the biosurfactant activity and naphthalene degradation by a newly identified strain *Bacillus cereus* 28BN was investigated the metabolic capability of 15 bacterial isolates isolated from oil contaminated site by using enrichment culture technique which were able to degrade aromatic and polyaromatic fractions^{48,26}.

Various factors, including the additional presence of carbon sources, pH, moisture content and size of inoculums, influenced the degradation² Because of the toxicity and carcinogenicity of PAHs it is more important to reduce its effect. By the manipulation in the factors pH of the soil microbial manipulation the rate of degradation of Polyaromatic Hydrocarbons can be enhanced³⁶.

However, determination of the optimization values for the degradation process of abiotic factors [(C/N/P/), the nitrogen source, the iron source, the iron concentration, the pH and the carbon source] of PAHs (naphthalene, phenanthrene and anthracene) were carried out by isolating bacterial consortium belonging to C2PL05⁴⁴.

Table 1.1:- Factor affecting microbial degradation

Limiting factors	Effect
Weathering	Aggregation, spreading, dispersion, adsorption.
Water potential	Evaporations, photo oxidation.
Temperature	Osmotic and matrix forces, exclusion of water from hydrophobic aggregates
Oxidant	Influence on evaporation and degradation rates.
Mineral nutrients	O ₂ required to initiate oxidation, NO ₃ ⁻ or SO ₄ ²⁻ to sustain, PHC biodegradation.
Reaction	N,P, Fe may be limiting Low pH may be limiting
Microorganisms	PHC degraders may be absent or low in numbers

Table 1.2: Optimal conditions for microbial growth and hydrocarbon biodegradation

Parameter	Microbial growth	HC biodegradation
Water holding capacity	25 -28	40-80
pH	5.5-8.8	6.5-8.0
Temperature (°C)	10-45	20-30
Oxygen(air-filled pore space)	10%	10-40%
C: N :P	100:10:1(0.5)	100:10:1(0.5)
Contaminants	Not too toxic	HC 5–10% of dry weight of soil
Heavy metals	<2000 ppm	<700 ppm

FACTORS AFFECTING BIODEGRADATION OF POLYAROMATIC HYDROCARBONS

The main environmental factors that could affect the feasibility of bioremediation are discussed.

2.1 Temperature

Temperature has a considerable effect on the ability of the *in situ* microorganisms to degrade PAHs and, in general, most contaminated sites will not be at the optimum temperature for bioremediation during every season of the year. The solubility of PAHs increases with temperature, which ultimately, increases the bioavailability of the PAH molecules. In addition, oxygen solubility decreases with increasing temperature, which reduces the metabolic activity of aerobic microorganisms. Biodegradation of PAHs occurs over a wide temperature range; however, most studies tend to focus on mesophilic temperatures rather than the efficiency of transformations at very low or high temperatures. However, it is apparent that

microorganisms have adapted to metabolize PAHs at extreme temperatures. For example naphthalene and phenanthrene degradation was reported from crude oil in seawater at temperatures as low as 0^o C. In comparison, the laccase and manganese peroxidase enzymes of ligninolytic fungi were reported to have a temperature optimum of ~50 °C and >75 °C respectively in spent-mushroom compost during the degradation of PAHs. Over 90% degradation of the contaminating PAHs was occurred at these temperatures.

Various environmental factors were investigated in the water soluble fraction of Kuwait crude oil. Results were indicate almost all PAHs were degraded at 15^oC and at an oxygen level of 4ppm on the other hand at 40^oC most of PAHs degraded optimally at 0ppm oxygen level⁵⁹.

The microorganisms which degrade hydrocarbons are most active in specified temperature ranges that govern the production of enzymes. The three classes and their optimum temperature ranges are Psychrophiles (below 20^oC), mesophiles (15^oC-45^oC), and thermophiles (above 50^oC). Most oil degrading microorganisms are active in the mesothermic range of 20^oC to 35^oC and provide optimum degradation rates at these temperatures. Temperature will determine to a certain extent the types of organisms that will be present for degradation. In general, degradation rates will be slower in colder waters than in warmer climates. At lower temperatures, the oil viscosity increases, the volatility of the lower chain hydrocarbons decreases and solubility increases, making the oil more toxic and less appealing to degrading microbes. Seawater ranges from -2^oC to 35^oC, with over 90% of the oceans having a temperature below 5^oC. Biodegradation has been observed in this entire temperature range. One experiment showed that a temperature drop from 25^oC to 5^oC caused a tenfold decrease in response rates⁶⁰. Degradation has been observed in Arctic ice and frozen tundra, but at negligible rates⁶¹. Heat is released during degradation processes, but a fire by spontaneous combustion is not possible. Temperatures do not exceed 71^oC, the limiting maximum temperature for microbial survival. Higher temperatures would be required for most products to ignite, but the chance remains small due to the volatilization of these molecules.

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^oC - 40^oC temperature in the mesophilic range or sometimes 60^oC 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^o-10^oC. 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 bioremediation³⁰.

2.2 Soil Characteristics

Soil characteristics are especially important for successful hydrocarbon biodegradation, some of the main limiting factors are: soil texture, permeability, pH, water holding capacity, soil temperature, nutrient content and oxygen content. Soil texture affects permeability, water content and the bulk density of soil. Soil with low permeability (such as clays) hinders transportation and the distribution of water, nutrients and oxygen. To enable the bioremediation of such soil, it should be mixed with amendments or bulking materials (straw, sawdust etc.), as the bioremediation processes rely on microbial activity, and microorganisms require oxygen inorganic nutrients, water and optimal temperature and pH to support cell growth and sustain biodegradation^{62, 63}.

2.3 pH

Many sites contaminated with PAHs are not at the optimal pH for bioremediation. For example, retired gasworks sites often contain significant quantities of demolition waste such as concrete and brick. Leaching of this material will increase the pH of the native soil and/or made ground of the site, resulting in less favorable conditions for microbial metabolism. In addition, the oxidation and leaching of coal create an acidic environment by the release and oxidation of sulfides. As the pH of contaminated sites can often be linked to the pollutant, the indigenous microorganisms at the sites will not have the capacity to

transform PAHs under acidic or alkaline conditions. It was studied the effect of pH on the ability of sodium phosphate (Na_3PO_4) in degrading polycyclic aromatic hydrocarbons (PAHs) in contaminated soil. Results obtained from Gas Chromatography/Mass Spectroscopy (GC/MS) analyses indicated that 23% of 2-methylnaphthalene was degraded as the overall highest polycyclic aromatic hydrocarbon at pH 2.0 using 2 g Na_3PO_4 , while acenaphthene (1.7%) was the least overall degraded polycyclic aromatic hydrocarbon at pH 2.0 using 4 g Na_3PO_4 powder. An increase in PAHs degraded trend was observed using 4 g Na_3PO_4 as the pH was increased from 2.0 to 4.0.

Therefore, it is common practice to adjust the pH at these sites, for example by the addition of lime.⁽⁶⁴⁾ Phenanthrene degradation in liquid culture has been investigated at a range of pH values (pH 5.5–7.5) with *Burkholderia cocovenenas*, an organism isolated from a petroleum-contaminated soil⁶⁵. Although bacterial growth was not significantly affected by the pH, phenanthrene removal was only 40% at pH 5.5 after 16 days, whereas at circum-neutral pH values, phenanthrene removal was 80%. *Sphingomonas paucimobilis* (strain BA 2) was however, more sensitive to the pH of growth media, with the degradation of the PAHs phenanthrene and anthracene significantly inhibited at pH 5.2 relative to pH 7⁶⁶.

PAH degradation has been recorded in an acidic soil (pH 2) contaminated by coal spoil by the indigenous microorganisms, with the concentrations of naphthalene, phenanthrene and anthracene reduced over a 28-day period⁶⁷. Naphthalene concentrations were reduced by 50% in soils downstream of a nearby coal pile, with phenanthrene and anthracene reduced by between 10 and 20%. It was found that some of the environmental isolates were able to both reduce the pH of the liquid media from 9 to 6.5 within 24 h, and also utilize naphthalene as a sole source of carbon. In contrast, the naphthalene degrading microorganisms *Pseudomonas fredrikbergensis* (DSM 13 022) and *Pseudomonas fluorescens* (DSM6506), were severely inhibited by the elevated pH. It suggests that *in situ* microorganisms at a contaminated site may be not only tolerant of the site conditions, but may have the potential to metabolize PAHs in sub-optimal conditions (in this case, high 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. Although there are certain bacteria that are alkaliphiles found in alkaline lakes at pH 7.5–10. 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.

2.4 Oxygen

Though it is now well established that bioremediation of organic contaminants such as PAHs can proceed under both aerobic and anaerobic conditions, most work has tended to concentrate upon the dynamics of aerobic metabolism of PAHs. This is in part due to the ease of study and culture of aerobic microorganisms relative to anaerobic microorganisms. During aerobic PAH metabolism, oxygen is integral to the action of mono- and dioxygenase enzymes in the initial oxidation of the aromatic ring⁶⁸. Ways in which to maintain adequate oxygen levels for aerobic metabolism for *in situ* treatments are discussed below and include hydrogen peroxide for sub-surface contamination. For surface contamination, simple soil tilling and/or mixing, for example using compost turners, can aerate contaminated material well enough to allow PAH transformation to proceed. There is still debate as to whether the benefits of anaerobic bioremediation are outweighed by the negatives, with the aeration of contaminated anaerobic aquifers successfully used to stimulate aerobic microbial communities resulting in significant reductions in PAH concentrations in groundwater. This has been accomplished using hydrogen peroxide, sodium nitrate⁶⁹ and perchlorate⁷⁰. In addition, the aerobic biodegradation of hydrocarbons has been reported to be up to an order of magnitude higher relative to anaerobic biodegradation⁷¹. However, it has also been reported that rates of anaerobic PAH degradation under denitrifying conditions were comparable to those under aerobic conditions⁷². Though it appears that the future of anaerobic bioremediation is promising, there are several drawbacks to the promotion of anaerobic bioremediation.

Not all environments contain an active population of anaerobes that are able to degrade PAHs. This has been shown in creosote-contaminated sediment, where limited biodegradation of PAHs was seen under denitrifying, sulfate-reducing and methanogenic conditions,⁵⁹ even though there was an actively respiring anaerobic community present in the sediment. Similar results were found when investigating the potential for PAH degradation in sediment samples from San Diego Bay, California⁷³. Metabolism of PAHs in these sediments that had had low levels of previous exposure to PAHs only occurred after a long lag period, and was promoted when they were ‘spiked’ with PAH-contaminated sediments that contained an active community of PAH-degraders. This suggests that the dominant *in situ* microbial community did not consist of PAH-degrading microorganisms and that bioremediation was limited by low numbers of PAH-degrading microorganisms rather than adverse environmental conditions⁷³. Another potential disadvantage of the promotion of *in situ* anaerobic bioremediation is that the geochemistry of the subsurface will be altered by the imposition of reducing conditions. As an environment is driven anaerobic, all residual oxygen is depleted, and electron acceptors such as nitrate, ferric iron and sulfate are reduced during respiration⁷⁴. This results in the mobilization of ferrous iron, and therefore the release of phosphate and iron (III)–phosphate complexes. Both of these are toxic to the environment; iron (II) is rapidly oxidized when exposed to oxygen, causing an orange precipitate in freshwater frequently associated with acid mine drainage⁷⁵ and excess phosphate in freshwaters can cause eutrophication. In addition, there is often a concomitant increase in pH, which can result in the solubilization of carbonate minerals and the release of trace metals⁷⁶. Respiration will also produce potentially potent greenhouse gases such as H₂S, CH₄ and N₂O⁷⁷. It is clear that more research is needed to fully understand the implications associated with the promotion of anaerobic bioremediation. The discovery of a wide diversity of pollutant-transforming anaerobes is a significant step forward in understanding the processes involved in bioremediation, and the design and application of anaerobic remediation both *in situ* and *ex situ* to the contaminated site.

Dissolved molecular oxygen is required for respiration of the microbe and is used throughout the subsequent degradation pathway. Requirements for oxygen uptake are significant. It usually takes 3 to 4 ml of dissolved oxygen to oxidize 1 ml of hydrocarbons to carbon dioxide and water⁴⁹. This relatively high demand is due to the high concentration of hydrogen and carbon in the oil, but very low concentrations of oxygen. Surface waters, such as oceans, harbors, and lakes, essentially have an infinite supply of oxygen due to the air/water interface and the wind and wave action. But, oxygen becomes the rate limiting effect as depth increases. At sufficient depths and in deep water sediments, degradation of the hydrocarbons can turn anaerobic when the oxygen supply is depleted. Thus, oil that is dispersed and sinks to the deep oceans and is covered by sediments will take much longer to degrade. Oxygen replenishment can be hindered by large, thick pools of oil on water surfaces due to the blanketing of the air/water interface. This problem is most likely to occur in marshes, harbors, and inlets that rely on the flushing process provided by tidal movements. Mechanical removal is required to increase the air interface boundary necessary for oxygen replenishment. For soil environments, the availability of oxygen is dependent on the type of soil, amount of moisture, and the rate of microbial degradation that has occurred. Oxygen is plentiful on beach surfaces and sub surfaces where wave and tidal mechanisms contribute to re-aeration and replenishment. Oxygen content will be high also near jetties, pier structures, and retaining walls subject to crashing waves. Oxygen will be limited in fine grain soils, beach fronts with little or no tidal movements, and as soil depths increase. Oxygen has been shown to be the rate limiting step of degradation of hydrocarbons in deep soils and groundwater. To alleviate these 3 problems, the soil can be mechanically tilled to provide aeration, or, for deeper soils and groundwater, oxygen can be supplied by pumping it into a series of buried perforated pipes, by soil venting, air sparging, injection of hydrogen peroxide³ and by aerating the water to provide the necessary amounts of 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 bacteria⁷. 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, hydrocarbon degradation rates in soil, fresh water, and marine environments⁹.

2.5 Nutrient availability

In addition to a readily degradable carbon source, microorganisms require mineral nutrients such as nitrogen, phosphate and potassium (N, P and K) for cellular metabolism and therefore successful growth. In contaminated sites, where organic carbon levels are often high due to the nature of the pollutant, available nutrients can become rapidly depleted during microbial metabolism⁶¹. Therefore it is common practice to supplement contaminated land with nutrients, generally nitrogen and phosphates to stimulate the *in situ* microbial community and therefore enhance bioremediation^{78,79}. The amounts of N and P required for optimal microbial growth and hence bioremediation have been previously estimated from the ratio of C:N:P in microbial biomass (between 100:15:3⁸⁰ and 120:10:1⁸¹). However, a recent study has shown that optimal microbial growth and creosote biodegradation occurred in soil with a much higher C:N ratio (25:1) than those predicted from the ratio in microbial biomass, with lower C:N ratios (5:1) causing no enhancement in microbial growth⁸⁰. The level of nutrients required for PAH transformation are generally thought to be similar to those required for other organic pollutants such as petroleum compounds. However, little work has been done regarding the most favorable nutrient levels required for the optimal degradation of PAHs, and further work in this area would benefit future bioremediation trials. It is worth noting that fungi are able to effectively recycle nutrients (specifically nitrogen), and that excessively high nutrient loadings may in fact inhibit microbial metabolism. In addition, the high molecular weight PAH-oxidizing ligninolytic enzymes of the white-rot fungi are produced under nutrient deficient (often low nitrogen) conditions⁸². It therefore appears imperative that the nutrient status of the site is established prior to the supplementation of the site with additional nutrients. Even though microbial metabolism may be temporarily increased, the long-term inhibition of functionally important organisms may result in the failure of the bioremediation of high molecular weight PAHs (such as benzo[*a*]pyrene). Nutrients are required to support the biological activity, and hence bioremediation. Microorganisms commonly require carbon, nitrogen and phosphorus 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. Various studies have been conducting to evaluate the factors which enhance the remediation process. It was investigated the degradation of phenanthrene by natural micro flora present in seawater samples from Guayanilla bay by the addition of KNO₃ as a source of inorganic nitrogen which was resulted 10 fold increase in the phenanthrene degradation⁵⁴.

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^{38,21}.

The types and quantities of nutrients present in the system play a much more important role in limiting the rate of hydrocarbon degradation. Many studies have shown that an inadequate supply of nutrients may result in a slower rate of degradation for hydrocarbons (Roberts, 1992). The capacity of the microorganism to grow in a given system depends on the organism's ability to utilize any available nutrient. Aerobic microorganisms utilize various types of nutrients including nitrogen, phosphorus, and trace amounts of potassium, calcium, sulfur, magnesium, iron, and manganese. Nitrogen and phosphorus are vital nutrients especially since oil contains very little of either⁶. The lack of either nutrient will retard natural degradation rates. Seawater is often deficient in these nutrients because non-oil degrading microorganisms consume them as well as the oil consuming species. Also, phosphorus precipitates as calcium phosphate in the presence of seawater⁴⁸. Concentrations of nitrogen compounds in seawater range from .1 to 1 mg/l, and phosphorus ranges from 0 to .07 mg/l, depending on seasonal temperatures⁶. To compensate for the lack of nutrients, fertilizers have been applied. Amending the soil in this manner improves the nutritional status of microorganisms and encourages growth. The amount required to degrade a certain volume of oil has not yet been thoroughly understood, though the subject is being researched²⁰.

2.6 Microorganisms Number and Catabolism Evolution

The ability of the soil's microbial community to degrade hydrocarbons depends on the microbe's number and its catabolic activity. Microorganisms can be isolated from almost all environmental conditions. Soil microflora contains numbers of different microorganisms including bacteria, algae, fungi, protozoa and actinomycetes, which have a diverse capacity for attacking hydrocarbons. The main factors which affect the rate of microbial decomposition of hydrocarbons are: the availability of the contaminants to the microorganisms that have the catabolic ability to degrade them; the numbers of degrading microorganisms present in the soil; the activity of degrading microorganisms, and the molecular structure of the contaminant⁴². The soil microorganisms number is usually in the range 10^4 to 10^7 CFU, for successful biodegradation this number should not be lower than 10^3 per gram of soil. Microorganism numbers lower than 10^3 CFU per gram of soil indicate the presence of toxic concentrations of organic or inorganic contaminants^{27,33}. The activity of soil microflora can be controlled by the factors discussed above - pH, temperature, nutrients, oxygen etc. For successful biodegradation, it is also necessary that the microorganisms can develop catabolic activity, by the following activities: induction of specific enzymes, development of new metabolic capabilities through genetic changes, and selective enrichment of organisms able to transform the target contaminant^{27,33}.

Hydrocarbons are a rich source of carbon and energy that microbes need for growth. Before the carbon is available for use, larger hydrocarbon molecules must be broken down into simpler molecules suitable for use by other microbes. More than 70 microbial genera are known to contain organisms that can degrade hydrocarbon molecules. Hydrocarbons released in the environment are biodegraded primarily by bacteria and fungi. These microbes are ubiquitous in soil, fresh water, and sea water environments. Of the many bacteria found to degrade hydrocarbons, the most important found in marine and soil environments are *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Nocardia*, and *Pseudomonas*²⁰. Among the fungi listed, *Aureobasidium*, *Candida*, *Rhodotorula*, and *Sporobolomyces* are common in marine environments and *Trichoderma* and *Mortierella* are most common in soil environments²⁰. In marine environments, bacteria are considered to be the predominant hydrocarbon organism. Both bacteria and fungi contribute to hydrocarbon degradation in soils, with percent contributions ranging from 50-50 to 80-20 in favor of bacteria. Even less is known about the comparative roles of bacteria, fungi, and yeasts in freshwater, as are the roles of protozoa and algae in overall degradation rates. The microorganisms which use hydrocarbons as a food source can readily be found in vast quantities near places subjected to oil pollution, such as natural oil seeps, shipping lanes, harbors, oil fields, fuel terminals, and similar facilities. Relatively few hydrocarbon utilizers live in virgin soil or in the vast open sea. Few to none reside in petroleum as it emerges from the deep underground, as shown by the vast amount of oil that still remains underground even after millions of years. The population of microorganisms will vary from sample to sample, depending on the location at which it was taken. Oil polluted harbors can contain 1EIOA3 to 1EIOA6 microbes/ml. In terms of percentages, water not polluted by hydrocarbons typically have one percent of the population made up of hydrocarbon degrading bacteria, whereas in polluted areas they can constitute ten percent or more of the total microbial population¹⁵. Thus cleanup of long standing military facilities such as fuel depots and harbors may be easier than that of an isolated accident using bioremediation technology due to an increase of hydrocarbon utilizing microorganisms.

2.7 Consortium of Microorganisms

A consortium may be defined as a mixture of different hydrocarbons utilizing microorganism. Hydrocarbons range in size from single to many carbon molecules. The microorganisms produce enzymes (groups of proteins that mediate or promote the transformation of the hydrocarbon into a simpler compound) that attack the hydrocarbon molecule.

Some microorganisms can produce enzymes which are capable of attacking almost any size or type of hydrocarbon; others can only produce enzymes that attack and breakdown one specific type or size molecule. Once the hydrocarbon is broken, other enzymes may be required to further break the remaining hydrocarbon. Lack of a specific enzyme to attack the remaining molecule further may provide a barrier to

the complete degradation and stop the process, until one is introduced to the system. This complex series of steps by which degradation occurs is called a metabolic pathway. No single species of microorganism is capable of degrading the many different hydrocarbon components in oil products, thus many different enzymes and metabolic pathways are required to degrade the significant number of compounds contained in petroleum and related products. When a petroleum spill occurs, certain microorganisms in the system will exhibit rapid growth due to the vast availability of easily degradable hydrocarbons. These fast growing species may hinder other species by depleting oxygen or nutrients in the system. When those easily degradable hydrocarbons are depleted, the microorganisms may lack the enzyme necessary to degrade the other hydrocarbons available, and die off. Other microorganisms capable of utilizing the remaining hydrocarbons will then exhibit growth and flourish in the system. Thus, the cycle continues, as species flourish and recede as the hydrocarbons they excel at degrading become available, then are depleted.

Microorganisms that readily degrade the hydrocarbons found in petroleum products can usually be found near the surfaces of soil and water. The main reason for this is the availability of oxygen, moisture, and the food source (the hydrocarbon) near the surface. Although some microbes are anaerobic (not growing in the presence of molecular oxygen), the vast majority of microbes that degrade hydrocarbons are aerobic (those that utilize molecular oxygen).

2.8 Bioavailability

Bioavailability can be defined as the effect of physicochemical and microbiological factors on the rate and extent of biodegradation⁸³ and is believed to be one of the most important factors in bioremediation. PAH compounds have a low bioavailability, and are classed as hydrophobic organic contaminants.⁽⁸⁴⁾ These are chemicals with low water solubility that are resistant to biological, chemical and photolytic breakdown⁸⁴. The larger the molecular weight of the PAH, the lower its solubility, which in turn reduces the accessibility of the PAH for metabolism by the microbial cell^{85,86}. In addition, PAHs can undergo rapid sorption to mineral surfaces (i.e. clays) and organic matter (i.e. humic and fulvic acids) in the soil matrix. The longer that the PAH is in contact with soil, the more irreversible the sorption, and the lower is the chemical and biological extractability of the contaminant⁸⁷. This phenomenon is known as ‘ageing’ of the contaminant. Therefore the bioavailability of a pollutant is linked to its persistence in a given environment.

Release of PAHs from the surface of minerals and organic matter can be achieved by the use of surface-active agents (also known as surfactants or detergents). These are compounds that contain both a hydrophobic and hydrophilic moiety, thus providing a ‘bridge’ between the hydrophobic PAH molecule and the hydrophilic microbial cell. Some microorganisms can produce surfactants (bio surfactants) that can enhance the desorption of PAHs from the soil matrix^{88,89}. These are potentially more effective than using synthetic surfactants, as they are thought to be less toxic to the *in situ* microbial community and do not produce micelles, which can encapsulate contaminant PAHs and prevent microbial access⁸⁹. The bioavailability of PAHs in soil can be assessed using both chemical and biological methods; though it is questionable which type of test(s) is most representative of the bioavailability of hydrophobic organic contaminants in soil, as both approaches have inherent limitations⁹⁰. There are many biological techniques to assess the bioavailability of PAHs in soil. These can be based upon the monitoring of biological function, such as microbial respiration rates (mineralization) of ¹⁴C-labelled contaminants, the bioluminescence of microorganisms such as *lux* microorganisms and/or *lux*-tagged pollutants that are in contact with a contaminated material⁹¹ and by assessing the degree of dermal diffusion and gastrointestinal sorption of PAHs in earthworms and hence the bioavailability and ecotoxicity of PAHs in the soil⁷⁴. In addition, bioavailability can be measured by monitoring changes in the expression of genes that code for PAH degradation using molecular probes, and also by the extraction of soil pollutants using a simulated mouth and gut digestive fluid such as saliva, in order to demonstrate the risk that the contaminant will pose if ingested⁽⁹²⁾. Biological assays are often supported by performing a chemical assay of the bioavailability of the contaminant in the soil, which physically extracts the contaminant from

the soil matrix using a chemical solvent. Organic solvents have been traditionally used to extract organic contaminants during harsh extraction processes (such as Soxhlet extraction), although this does not demonstrate the true bioavailability of the contaminant, but the total contaminant concentration in the soil. However, a more representative approach was used by Hatzinger and Alexander,⁸⁶ who extracted the contaminants with mild organic solvents (such as methanol) to represent the bioavailable proportion in the soil. As microorganisms can mostly only access those contaminants that are in the aqueous phase, water-based solvents are also being used to more accurately predict the bioavailable fraction of organic contamination in soil. One such compound is hydroxypropyl- β -cyclodextrin⁹⁰ (HPCD), which can encapsulate hydrophobic contaminants. In addition, HPCD does not appear to inhibit *lux*-type microorganisms, allowing for a combined biological and chemical assessment of bioavailability.

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.

Even if the optimal conditions for hydrocarbon biodegradation are provided at the field, it has been shown that a residual fraction of hydrocarbon remains undegraded. Mainly, after its arrival in the soil, an organic contaminant may be lost by biodegradation, leaching or volatilization, or it may accumulate within the soil biota or be sequestered and complex within the soil's mineral and organic matter fractions. The rate at which hydrocarbon-degrading microorganisms can convert chemicals depends on the rate of transfer to the cell and the rate of uptake and metabolism by the microorganisms. It is controlled by a number of physical-chemical processes such as sorption/desorption, diffusion, and dissolution^{4,8}. The mass transfer of a contaminant determines microbial bioavailability. The term "bioavailability" refers to the fraction of chemicals in soil that can be utilized or transformed by living organisms. The bioavailability of a compound is defined as the ratio of mass transfer and soil biota intrinsic activities. Most soil contaminants show biphasic behaviour, whereby in the initial phase of hydrocarbon biodegradation, the rate of removal is high and removal is primarily limited by microbial degradation kinetics. In the second phase, the rate of hydrocarbon removal is low and removal is generally limited by slow desorption. Altogether, less bioavailable fraction of hydrocarbon contamination is formed by hydrocarbons which desorb slowly in the second phase of bioremediation²². The biodegradation of an oil-contaminated soil can also be seriously affected by the contamination time, due to weathering processes, which decrease the bioavailability of pollutants to microorganisms. Weathering refers to the results of biological, chemical and physical processes that can affect the type of hydrocarbons that remain in a soil^{22,24,42}. Those processes enhance the sorption of hydrophobic organic contaminants to the soil matrix, decreasing the rate and extent of biodegradation. Moreover, a weathered oil-contaminated soil normally contains a recalcitrant fraction of compounds composed basically of high molecular weight hydrocarbons, which cannot be degraded by indigenous microorganisms^{22,24,3}. In contrast, a recently oil-contaminated soil contains a higher amount of saturated and aliphatic compounds, which are the most susceptible to microbial degradation. However, the pollutant compounds in a recently contaminated soil are potentially more toxic to the native microorganisms, leading to a longer adaptation time (lag phase) before degradation of the pollutant and even to an inhibition of the biodegradation process^{22,27,33}.

As was mentioned above, sequestration and weathering of organic contaminants in the soil reduces the bioavailability of organic compounds and results in non-degraded residues in the soil. Contaminants that have been weathered and sequestered in soil are not available for biodegradation in soil, even though freshly added compounds are still biodegradable¹. Sorption is a major factor preventing the complete bioremediation of hydrocarbons in soil. Slow sorption leads to the hydrocarbon fraction becoming resistant to desorption and increases its persistence within the soil organic matrix. The following hypotheses have been proposed as a explanation for weathering:¹ weathering results in a slow diffusion of the hydrocarbon fraction in the solid fraction of the organic matter in the soil; ² the contaminant slowly diffuses through the soil and becomes sorbed and trapped in the soil nano-and micropores^{42,46}.

2.9 Contaminant Characteristics

Petroleum hydrocarbons contain a complex mixture of compounds; all the components of petroleum do not degrade at the same rate. The rate by which microorganisms degrade hydrocarbons depends upon

their chemical structure and concentration. Petroleum hydrocarbons can be categorized into four fractions: saturates aromatics, resins and asphaltene. The various petroleum fractions, n-alkanes of intermediate length (C₁₀-C₂₅) are the preferred substrates for microorganisms and tend to be the most readily degradable, whereas shorter chain compounds are rather more toxic. Longer chain alkanes (C₂₅-C₄₀) are hydrophobic solids and consequently are difficult to degrade due to their poor water solubility and bioavailability, and branched chain alkanes and cycloalkanes are also degraded more slowly than the corresponding normal alkanes. Highly condensed aromatic and cycloparaffinic structures, tars, bitumen and asphaltic materials, have the highest boiling points and exhibit the greatest resistance to biodegradation. It has been suggested that the residual material from oil degradation is analogous to, and can even be regarded as, humic material^{3,22,4,45}.

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. 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^{10,12}. In addition to this high concentration may disturb C: N: P ratio and create oxygen limitations.

2.10 Toxicity of end-products

The principle of bioremediation is to remove or detoxify a contaminant from a given environment using microorganisms. Most commercial bioremediation trials tend to monitor the success of the treatment by the degree of removal of the parent contaminant and do not consider the possibility of the biological production of more toxic breakdown metabolites. However, it is important to ensure that the contaminated material is suitably detoxified at the end of the treatment^{93,94}. A recent study using a bioreactor to treat PAH contaminated gasworks soil monitored both the removal of PAHs and the accumulation of oxy-PAHs, such as PAH-ketones, quinones and coumarins⁹⁴. These compounds are formed during the microbial metabolism of PAHs and can also be formed from chemical oxidation and photo transformation of PAHs⁹⁵. Such transformation products can be equally toxic, if not more toxic, to human health when compared with the parent PAH,⁶⁰ with many of the oxy-PAHs formed during the treatment of PAH contaminated soils more persistent than the parent compounds⁹⁴. In this study, Lundstedt and colleagues investigated that although there were no new oxy-PAHs formed during the bioremediation of an aged gasworks soil, the concentrations of 1-acenaphthenone and 4-oxapyrene-5-one increased in the soil by 30% and 60% respectively over 30 days of bioslurry treatment. In addition, they showed that some oxy-PAHs actually increased in concentration during treatment, and were subsequently more persistent to microbial degradation than their corresponding parent PAH compound. As oxy-PAHs are more toxic than the parent PAHs, this study highlights the importance of monitoring the metabolites of bioremediation, specifically for toxic dead-end products, and assessing the toxicity of the material both before and after treatment.

However, it is important to understand the relevance of ecotoxicity tests to the overall toxicity of the remediated land. Many of these tests monitor for 'acute' toxicity of compounds (via organism death), whereas it would be more representative, particularly when assessing the carcinogenic and mutagenic PAHs, to consider the 'chronic' toxicity of these soils, such as monitoring for organism DNA damage and the occurrence of DNA adducts⁶⁰.

2.11 Moisture

Moisture is required for all biological processes to help transport nutrients, foods, and waste products in and out of the microorganisms. For oceans, lakes, and other surface water environments, this poses no immediate problems. For soil environments, some moisture must be present for degradation to occur. Too much water can impede the re aeration of the soil, and the process may turn anaerobic. The optimum ratio of moisture will depend on the climate and soil type. Ratios range from 30-90% in one study and 12-32% in others and the aerobic biodegradation of hydrocarbons in soils is greatest in ranges of 50-70% of the soil water holding capacity^{20,36} whereas waves and tidal actions are useful in supplying aerated sea water to beaches and marshes, rainfall is useful in the biodegradation of inland soils by supplying moisture and useful dissolved oxygen to the microbes.

2.12 Organic matter

The presence of naturally occurring organic matter can have different effects on soils, such as water retention, soil temperature, and the ability of microorganisms to degrade pollutants³⁶. The role it plays in sorption processes can affect the availability of nutrients for the microorganisms. Contaminants targeted for biodegradation can be resistant to enzymatic attack by sorption onto soil particles. The bound material becomes unavailable for the attack. Addition of organic material can slow the natural rate of biodegradation due to increased competition, but long term it can increase infiltration and permeability and porosity¹⁵. The addition of the material can be helpful in low moisture retaining soil environments.

2.13 Oil surface and concentration

Since the majority of the biodegradation of hydrocarbons occurs at or near the air/water interface in water environments and the air/soil interface in soil environments, the amount of oil surface area exposed at these interfaces will affect the rate of degradation. The greater the area exposed, the faster the product will degrade. Concentration of the hydrocarbon is related to oil surface area. Thick rafts, blankets, or pools of oil or other petroleum products constitute a high concentration/low surface area situation. The available sites that can be attacked are reduced. The oil acts as a blanket, hindering the replenishment of nutrients or oxygen to the microbes. Thus, at high concentrations those compounds most readily degraded will be attacked, leaving the more resistant components behind. These in turn combine to form even more resistant compounds, such as tar balls that have limited moisture contact and surface area. Concentrations of hydrocarbons in the range of 1 to 100 µg/ml of water or 1 to 100 µg/g of soil (dry weight) are not generally considered toxic to common bacteria or fungi³⁶.

Thus, dispersants and emulsifiers used at sea on heavy concentrations of oil increase the oil's ability to spread and volatilize, which in turn allows for an overall increase in the rate of degradation, pending there are no adverse toxic effects of the type of dispersant used. Other means of removing heavy concentrations of oil and products should first be employed prior to remediation, such as using skimmers, vacuum trucks, adsorbents, and earth moving equipment.

2.14 Salinity

Microorganisms are typically well adapted to cope with the wide range of salinities common to the world's ocean³². There is little evidence to suggest they are affected by other than hyper saline environments, such as saltwater from oil wells. Estuaries may present a special case because salinity values will vary in levels as compared to levels in the ocean⁴⁹. Thus, if microbes are to be added to the environments, it must be known if they are compatible with the saline levels present in the system.

CONCLUSION

In the present scenario major environmental pollution of soil and water is due to hydrocarbon contamination resulting by the petrochemical industries activities. It can be caused by accidental liberation of petroleum industries discharge in the environment or it can also be caused by human activity. Hydrocarbon compounds are known for their carcinogenic and neurotoxic behavior.

Many published studies have investigated the efficacy of bioremediation on a bench scale and under ideal laboratory conditions, such as a circum-neutral pH and mesophilic temperatures. However, it is apparent that environmental factors that vary from site to site (such as soil pH, nutrient availability and the bioavailability of the contaminant etc) can influence the process of bioremediation by inhibiting growth of the pollutant-degrading microorganisms.

These factors play significant role in the biodegradation process of polyaromatic hydrocarbon and contaminated soils, such as physical conditions, nutrition, the ratios of various structural hydrocarbons present, the bioavailability of the substrate and the diversity of the bacterial communities involved, pH, water holding capacity of the soil, microorganisms. There are some standard conditions on which these factors play their role like water holding capacity of the soil for the microbial growth is 25-28 and for hydrocarbon degradation is 40-80. The range of pH is from 5.5-8.8 for microbial growth and for hydrocarbon degradation is 6.5-8.0. The favorable conditions of temperature for the growth of microorganisms vary from 10-45 °C and for the degradation of contaminants are from 20-30 °C.

Therefore, this mini review touches upon various aspects of environmental factors which affect the biodegradation of polyaromatic hydrocarbons.

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