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KINETICS AND THERMODYNAMIC STUDY OF BIOREMEDIATION OF TERRESTRIAL SPILLS OF LIBYAN FUELS

M.Sc.Thesis

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ الْحَمْدُ لِلَّهِ الَّذِي لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ وَلَهُ الْحَمْدُ فِي الْآخِرَةِ وَهُوَ الْحَكِيمُ الْخَبِيرُ ﴾

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ABSTRACT

The kinetics and thermodynamic study of bioremediation of petroleum hydrocarbons in soil has been presented in this thesis. The soil samples used in this study were collected from the different locations along the side of the oil pipeline from Sarir oil field to Tobruk oil export terminal (Harega). Motor gasoline, kerosene and diesel fuel produced in Sarir refinery have been used as a source of hydrocarbons to contaminate soil samples. A bioremediation treatment that consisted fertilization (addition of nitrogen and phosphorous containing nutrients), tilling (aeration), incubation temperature control was evaluated on the laboratory scale for its effectiveness in cleaning up soil contaminated by petroleum hydrocarbons. Experimental variables included incubation temperature; no treatment; bioremediation treatment, and poisoned evaporations controls. The concentrations of hydrocarbons in soil at different time intervals have been determined for kinetic study. The hydrocarbons were extracted from the soil by using standard technique of extraction of hydrocarbons from soil. The gas chromatographic technique has been used for determination of total hydrocarbon concentration in soil.

The depletion curves were obtained for untreated, treated and poisoned conditions. The degradation of hydrocarbons was faster in case of treated soils and the bioremediation rate is increases as the temperature increases.

The activation energy of bioremediation reaction has been determined as 40.34 kJ mol^{-1}. The other thermodyamic parameters such as enthalpy of activation ($\Delta H^{\#}$), entropy of activation ($\Delta S^{\#}$), and free energy of activation ($\Delta G^{\#}$) at 25 ^{o}C have also been calculated and presented in this thesis.

DEDICATION

To my parents

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I would like to acknowledge thanks to Almighty God, Lord of the Worlds. To whom belongs all that is in the Heavens and the Earth. Through whose mercy all knowledge is attained.

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1. Introduction

Pollution is the introduction of contaminants into an environment that causes harm to human health and other living organisms. Soil pollution comprises the pollution of soil with materials (Atlas and Philip, 2005; Sigh and Lin, 2009), mostly chemicals that are out of place or they are present at concentrations higher than normal, which may have adverse effects on humans or other organisms. It is difficult to define soil pollution exactly because different opinions exist on how to characterize pollution; while some consider the use of pesticides acceptable if their effect does not exceed the intended result, others do not consider any use of pesticides or even chemical fertilizers acceptable. However, soil pollution is also caused by means other than the direct addition of xenobiotic (man-made) chemicals such as agricultural runoff waters, industrial waste materials, acidic precipitates, and radioactive fallout.

Both organic and inorganic contaminants are important in soil. The most prominent chemical groups of organic contaminants are fuel hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated aromatic compounds, detergents, and pesticides. Inorganic species include nitrates, phosphates, and heavy metals such as cadmium, chromium and lead; inorganic acids; and radionuclides (radioactive substances). Among the sources of these

contaminants are agricultural runoffs, acidic precipitates, industrial waste materials, and radioactive fallout. Petroleum hydrocarbons (crude oil) contamination is one of the most significant forms of groundwater and soil contamination (Hinchee and Olfenbuttel, 1991). Each year huge amounts from crude oil are released into the environment due to human activities (Vennila and Kannan, 2011), huge oil spills during transportation and distribution, as well as spills during refining. Soil that is accidentally contaminated by petroleum fuel spills is classified as hazardous waste (Bartha, and Bossert. 1984; Norris *et al*., 1994; Riser-Roberts, 1998).

Cleaning up oil spills is not a small task, there are many methods used to clean up oil spills. An oil spill can be destroyed by fire, but burning often adds to the problems. Smoke, soot, fumes, and particulate matters from the burning oil can pollute the air over large areas, any materials such as chemicals, dust, and harmful gases in air and water are pollutants (Mishra *et al*., 2001).

Soil excavation and groundwater pump and treatment were the first techniques used but in most cases they both proved to be ineffective. Soil excavation was useful in sites that were small and defined but when the contamination was wide spread the technique was not successful. Due to limited solubility of petroleum hydrocarbons and the variation in groundwater flow, groundwater pump and treat had very little impact on

the contamination. Soil vapour extraction and bioventing developed as a result of the known volatile and biodegradable properties of petroleum hydrocarbons. These methods have proved to be more cost-effective than the previous ones mentioned but were still not optimal methods (Hinchee and Olfenbuttel, 1991). Increasing awareness of hydrocarbon contamination led to the investigation of other remediation approaches including bioremediation (Bartha, 1986; Okho, 2006).

1.1. Petroleum Hydrocarbons:

The efficiency of hydrocarbon degradation will depend on the characteristics of contaminated material, and abilities of effectiveness and the microbial population (Van Hamme *et al*., 2003). Petroleum hydrocarbons are naturally occurring chemicals exploited by humans for a wide range of purposes, such as the fueling of vehicles and heating of homes (Committee on *In Situ* Bioremediation *et al.*, 1993). Petroleum hydrocarbons are found in gaseous (natural gas), liquid (crude oil and fuels), or solid (tars and asphalts) forms and are basically mixtures of various classes of hydrocarbon compounds (Lyons, 1996). These chemicals are generally found in minute concentrations almost everywhere in the environment and are generally formed when a fuel is burned (Wetzel *et al.*, 1997; Mackay, 1991).

To ensure the efficacy of the decontamination processes, the concentrations of petroleum hydrocarbons and hydrocarbon-oxidizing bacteria should be monitored. Usually, the growth of oil-degrading cells in the treated soil samples correlates with the activity of induced biodegradation processes. Microbiological culture methods are commonly used to enumerate the hydrocarbon oxidizing bacteria in the soil samples (Piehler *et al*., 1999; Kao *et al*., 2001; Yerushalmi *et al*., 2003; Cunningham *et al*., 2004; Efremenkoa *et al*., 2005) have been performed by using culture independent molecular techniques, little research has focused on microbial communities dominated by photosynthetic organisms in relation to oil degradation. The microbial Petroleum Compounds are considered to be recalcitrant to microbial degradation and persist in ecosystems because of their hydrophobic nature (low water solubility) and low volatility, and thus they pose a significant threat to the environment (Raeid *et al*., 2002).

More importantly, benzene was proved to be a carcinogen, toluene a depressant of human central nervous system, and ethylbenzene a skin irritant; also long term exposure to xylenes has been correlated with aplastic anemia (Sittig). Hence BTEXs (Benzene, toluene, ethylbenzene and xylenes) are often used as indicators groundwater contamination, especially from leaking underground storage tanks (USTs) of soil (Eweis *et al.*, 1998).

Some common findings on the biodegradation studies are as follows:

- Aliphatic hydrocarbons are generally easier to degrade than aromatic compounds.

- Straight-chain aliphatic hydrocarbons are easier to degrade than branched-chain hydrocarbons. The introduction of branching into the hydrocarbon molecule hinders biodegradation.

- Saturated hydrocarbons are more easily degraded than unsaturated-hydrocarbons. The presence of carbon-carbon double or triple bonds hinders degradation.

- Long-chain aliphatic hydrocarbons are more easily degraded than short-chain hydrocarbons. Hydrocarbons with chain lengths of less than 9 carbons are difficult to degrade because of their toxicity to microorganisms. Some specialized microorganisms (methanotrophs) can degrade these short-chain hydrocarbons. The optimal chain length for biodegradation appears to be between 10 and 20 carbons. (Baker and Herson, 1994).

1.1.1. Impact on Human Health

The impact of hydrocarbons on human health depends somewhat on whether exposure was from ingestion, inhalation, or dermal (skin) contact and on whether the exposure was acute (short-term) or chronic (long-term). The acute effects of ingestion may include irritation to the mouth, throat, and stomach, and digestive disorders and/or damage. Small amounts of hydrocarbons can be drawn into the lungs, either from swallowing or vomiting, and may cause respiratory impact such as pulmonary edema or bronchopneumonia. The chronic effects of ingestion may include kidney, liver, or gastointestinal tract damage, or abnormal heart rhythms. Prolonged and/or repeated exposure to aromatics like benzene may cause damage to the blood-producing system and serious blood disorders, including leukemia. The metabolism of aromatic hydrocarbons after ingestion can result in the creation of mutagenic or carcinogenic derivatives, even if the original hydrocarbon is relatively nontoxic (National Research Council, 1993; Okoh and Trejo-Hernandez, 2006).

A number of PAHs have been linked to cancer of the skin, lung, and other sites on the body. There is no epidemiologic evidence for human cancer from intake of PAH contaminated food, however. Most human exposure to PAHs comes from nonpetroleum sources, including cigarette smoke, fossil fuel combustion products, and food.

The acute symptoms of hydrocarbon exposure by inhalation may include irritation of the nose, throat, and lungs, headaches and dizziness, anesthetic effects, and other central nervous system depression effects.

These symptoms can occur at air concentrations of 0.5 mg/1 for 30 minutes (Hastings *et al*., 1984). Epileptic-type seizures may occur months after a high acute exposure to gasoline vapors, and permanent brain damage has been reported. Acute toxic effects are not commonly observed, however, in gas station attendants and auto mechanics.

Chronic effects of inhalation exposure to hydrocarbons containing high concentrations of aromatic compounds, including gasoline, can be weight loss from loss of appetite, muscular weakness and cramps, sporadic electroencephalography irregularities, and possible liver and renal damage.

Exposure of eyes and skin to hydrocarbons may result in irritation, mechanical or chemical damage to eye tissue, or dermatitis. Long-term exposure to vacuum distillates has caused skin cancer in animals. Exposure to petrochemicals, particularly polyaromatic hydrocarbons, increases susceptibility to skin infections, including skin cancer when there is simultaneous exposure to sunlight (Burnham and Bey, 1991; Burnham and Rahman, 1992).

One potential source of hydrocarbon exposure to humans is ingestion of hydrocarbon-contaminated food, particularly seafood. Studies have shown that most organisms cleanse themselves of hydrocarbons within a matter of

weeks after being removed from the source of contamination. This cleansing time, however, depends upon the contaminated organism. The exposure levels of humans to polyaromatic hydrocarbons from crude oil may be lower than those from other, more common sources like grilled food and combustion products, or from naturally occurring sources like coffee, grains, and vegetables (American Petroleum Institute, 1978).

A suggested standard for human exposure to petroleum hydrocarbons varies with the specific hydrocarbon, but ranges between 25 and 430 ppm (National Research Council, 1993). Permitted occupational exposure levels to benzene are on the order 10 ppm, but vary with the prevailing regulations.

1.1.2. Impact on Plant Growth

Hydrocarbons also impact plant growth when released on land. Levels of oil and grease above a few percent in soils (by weight) have shown degradation of plant growth. Levels below a few percent have shown an actual enhancement of some crop growth. Recovery of an exposed site after a one-time hydrocarbon release usually occurs after a few months (Deuel, 1990). A level of 1% oil and grease is recommended as a practical threshold where the hydrocarbons become detrimental to plant life (American Petroleum Institute, 1989b). Airborne hydrocarbons emitted during blowouts can also impact plant growth around the wellhead. Long-term growth rate reductions

have been observed in coniferous forest growth following blowouts at distances as great as 2 km from the wellhead (Baker, 1994).

1.2. Bioremediation:

Bioremediation is an emerging technology by which an organic contaminant is converted into simple molecules such as carbon dioxide and water through biological processes. Bioremediation techniques are very diverse and have been commonly applied in the remediation of groundwater, soil, and sludge contaminated with petroleum compounds. It has advantages of low cost, relatively high performance, and being easy for implementation (Burke *et al*., 2000; Margesin *et al*., 2003; Nweke and Okpokwasili, 2004; Kaplan and KittsY, 2004; Quatrini *et al*., 2008; Chikere *et al.,* 2009).

Different from many other physicochemical techniques, bioremediation can destroy hazardous compounds other than simply transferring them from one phase or location to another. At the sane time, it is natural process that can eliminate the future liability associated with treatment or disposal of contaminants. In situ bioremediation is also viable and straightforward method, often offers the potential to remediate contaminated soil and groundwater without excavation, and can be implemented below and around existing buildings, piping and paved

surfaces (Wainwright, 1999; Kaplan and Kitts, 2004; Ayotamuno *et al*., 2006; Chorom *et al*., 2010).

Efficacy of a bioremediation process is often assessed by the percent reduction in contaminant concentrations (Eweis *et al*., 1998; Shukla *et al*., 2010). Therefore, a decrease of contaminant concentration with the presence of a microbial population that is capable of biodegradation the contaminant in subsurface does not necessarily triggers biodegradation or bioremediation on site (King *et al*., 1998). In addition, microbial strains were usually studied in pure culture; the extensive degradation capabilities of numerous microbial species have been demonstrated in this manner. These capabilities may not occur to the same extent or in the same fashion when the species are active in a mixed microbial community found in nature (Wainwright, 1999). Thereby, an introduction of petroleum hydrocarbon degrading microorganisms is far from the success of bioremediation. Favourable environmental and chemical conditions are important for microbial growth; meanwhile, an effective method is needed to trace the contaminants and to monitor the absorption and metabolism of petroleum hydrocarbons by microorganisms.

There is general interest in studying the diversity of indigenous microorganisms capable of degrading pollutants such as hydrocarbons of crude oil, polycyclic aromatic hydrocarbons, in different environments (Raeid *et al*., 2002). The biodegradation of an oil-contaminated soil can

also be seriously affected by the hydrocarbons structure and by the contamination time, due to weathering processes, which decrease the bioavailability of pollutants to microorganisms. Weathering refers to the result of biological, chemical and physical processes that can affect the type of hydrocarbons that remain in a soil (Loehr *et al*., 2001).

The three basic components of any bioremediation process include:

(i) Microorganisms, (ii) a potentially biodegradable contaminant, and (iii) a bioreactor in which the process can take place. Proper temperature, oxygen, and nutrient levels may need to be provided in the bioreactor. The microbes in the bioreactor use carbon in the organic contaminants as a source of energy, and in doing so, degrade the contaminant (Frick *et al*., 1999).

1.3. Factors Influencing Biodegradation of Petroleum Hydrocarbons

1.3.1. Microorganisms Activities

The number of bacterial cells in the soil is always great, but the individuals are small, rarely more than several microns in length. The bacteria probably account for appreciably less than half of the total microbiological tissue present in soil, (Alexander 1961). The fate of petroleum hydrocarbons in the environment is largely determined by a biotic factor

which influences the weathering (Atlas, 1981; Okoh and Trejo-Hernandez, 2006), method of bioremediation to implement is natural attenuation, where contaminated sites are only monitored for contaminant. Although significant work has been published discussing the bacterial community structure and degradation kinetics associated with bioremediation of environmental contaminants, few have focused on a detailed description of bacterial community dynamics during this process. The occurrence of microorganisms within the community with species ability to metabolize hydrocarbons is related to their adaptation potential, and thus, the rate of biodegradation of such substrates (Spain *et al*., 1980; Ringelberg *et al*., 2001). So the rate of microbial degradation of hydrocarbons in soils is affected by several physico-chemical and biological parameters, the condition for microbial degradation activity (e.g. presence of nutrient, oxygen, pH and temperature) the quality, quantity and bioavailability of the contaminants, and the soil characteristics such as particle size distribution (Obire and Nwaubeta, 2001).

Exposure of soil ecosystems to hydrocarbon pollution may cause a selective inhibition on certain members of the microbial biocenosis, while increasing the relative amounts of those microorganisms able to use hydrocarbons for growth (Bossert and Bartha, 1984; Ekpo and Udofia, 2008). The physical state of petroleum hydrocarbons has a marked effect on their biodegradation. At very low concentrations hydrocarbons are

soluble in water, but most oil spill incidents release petroleum hydrocarbons in concentrations far in excess of the solubility limits (Boylan and Tripp, 1971; Frankenfeld, 1973; Harrison, *et al*., 1975; MeAuHiffe, 1966). Generally, only the fraction of hydrophobic organic contaminants dissolved in the aqueous phase is available for microbiological degradation while the sorbed fraction has a low bioavailability (Allan *et al*., 1997). Moreover, pollutant compounds in a recently contaminated soil are potentially more toxic to the native microorganisms, leading to longer adaptation time (lag phase) before degrading the pollutant and even to an inhibition of the biodegradation process (Trindade, *et al*., 2005; Okoh and Trejo-Hernandez, 2006). In general, microbial communities from contaminated ecosystems can adapt to the presence of pollutants, producing shifts in the metabolic and generic diversity of the community (Wang and Bartha, 1994; Macnaughton *et al*., 1999; Chikere *et al*., 2009). In some cases, the soil microbial biocenosis decreases in species richness in the presence of a selective pollutant (Jensen, 1975). Hollaway *et al*., (1980) detected a higher abundance of hydrocarbon utilizing microorganisms in an active oil field in the Gulf of Mexico compared to an oil-free control, although the diversity of bacterial populations was shown to be unchanged, in pollution of a semiarid soil in Patagonia, Argentina (Pucci *et al*., 2000). In order to accelerate the natural biodegradation of a selected site, the density of hydrocarbon utilisers can

be increased (Margesin and Schinner, 2001; Sigh and Lin, 2009). This method represents an alternative methodology for removing pollutants from the environment. In general, mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil (Mishra *et al*., 2001). The occurrence of microorganisms within the community with species ability to metabolize hydrocarbons is related to their adaptation potential, and thus, the rate of biodegradation of such substrates (Spain *et al*., 1980; Ringelberg *et al*., 2001).

1.3.2. Oxygen Availability

Microorganisms are capable of biodegrading petroleum hydrocarbons under aerobic and anaerobic conditions (Suthersan, 1997). Natural most petroleum-related hydrocarbons are readily biodegraded by aerobic microorganisms (Gibson and Subramanian 1984). In Subsurface environments, the activities of aerobic microorganisms can deplete oxygen levels (Vishvesh *et al*., 2002). The general process of aerobic biodegradation can be described by the following equation:

$$\text{Bacteria} + \text{Organics} + O_2 + \text{Nutrients} \rightarrow CO_2 + H_2O + \text{Biomass} + \text{Byproducts} \quad \ldots(1.1)$$

Aerobic biodegradation of hydrocarbons, such as aliphatics, aromatics and PAHs, involves the incorporation of oxygen by microbial cells to produce oxygenase enzymes (monooxygenases and dioxygenases) to break the hydrocarbon bonds (Suthersan, 1997). Therefore, consistent aeration is beneficial in stimulating the complete biodegradation of petroleum products. Stoichiometric analysis can be used to estimate the theoretical amount of oxygen required aerobically to degrade a given quantity of hydrocarbon, as described in Equation 1.2. Generally, the degradation of 1 mg of a medium length hydrocarbon requires 3 to 4 mg of oxygen (Dragun, 1998).

$$C_xH_y + [x + (y/4)]\ O_2 \rightarrow x\ CO_2 + (y/2)\ H_2O \quad \ldots\ldots\ldots (1.2)$$

The dominance of aerobic technologies is related to historical observations that the initial steps in biodegradation of hydrocarbons by microorganisms involves oxidation of the substrates by oxygenases (Rehm and Rei., 1981) and by the recognition of oxygen as a limiting factor in many natural environments (Alexander, 1980; Baker and Herson, 1990). Studies of the microbial ecology of soil and subsurface habitats have largely focused on aerobic microorganisms (Ghirose and Balkwill, 1983). Many pure cultures of aerobic bacterial strains capable of degrading pollutants have been isolated from these habitats; there have

been only few examples of pure cultures of anaerobic bacteria, which degrade the organic compounds of petroleum hydrocarbons. Anaerobic bioremediation of hydrocarbon-contaminated soils and groundwater can be promoted under denitrifying conditions. Although aromatic hydrocarbons readily degrade under aerobic conditions, the use of nitrate as an electron acceptor may offer advantages over the use of oxygen (Boopathy, 2004). Aerobic conditions and appropriate microorganisms are necessary for an optimal rate of bioremediation of soils contaminated with petroleum hydrocarbons. In soils, the oxygen content depends on microbial activity, soil texture, water content and depth. Low oxygen content in soils has been shown to limit bioremediation of soils contaminated with petroleum hydrocarbons and in a laboratory experiment; mineralization of hydrocarbons from soil was severely limited when the oxygen content was below 10%. Tillage is a mechanical manipulation of soil to improve soil conditions. It alters physical and chemical properties of soil in such a way that it stimulates microbial activity. Tillage redistributes carbon, nitrogen and water and reduces spatial distribution within the soil (Vasudevan and Rajaram, 2001). Generally, petroleum biodegradation rate is low in anaerobic sediments because molecular oxygen is required by most microorganisms for the initial step in hydrocarbon metabolism (Atlas, 1995). Sims and Overcash, (1983) reported that 45% and 55% of pyrene and PAH compounds were

degraded under soil gas oxygen concentrations between 2% and 21%. No statistically significant mineralization was found to occur at 0% oxygen. In a laboratory bioremediation study of hydrocarbon contaminated groundwater and sediment, Heterotrophic microorganisms utilise O_2 as the terminal electron acceptor in aerobic respiration, but also as substrate in oxygenase catalysed reactions. As O_2 is consumed under formation of CO_2, differences in the partial pressure of the two soil gases compared to atmospheric (Irving *et al*., 2002). The main gas transport processes in soil are advection and diffusion. It is reported that advective transport accounts for about 10% of the total oxygen transported through the vadose zone. Reaeration is thus mainly caused by diffusion of O_2 from atmospheric air into soil and CO_2 from soil to air. When only diffusion governs gas transport, microbial O_2 consumption in biodegradation processes results in vertical gradients of O_2 of a few meters in thickness in the soil. In unsaturated noncontaminated soils this mechanism is normally sufficient to prevent O_2 deficiencies and toxic CO_2 excess. In oil-contaminated soils it is well known that O_2 supply often limits biodegradation, although it is known that even low O_2 levels are able to sustain aerobic respiration (Anne *et al*., 2003).

1.3.3. Moisture

Water is important to the general health of plants and microbes (Eweis *et al*., 1998), water is not only a major component of living organisms, it also serves as a transport medium to carry nutrients to biota and carry wastes away. If the moisture content of the soil is low, there will be a loss of microbial activity and dehydration of plants. Too much moisture results in limited gas exchange and the creation of anoxic zones where degradation is dominated by anaerobic microorganisms which may help degrade organic contaminants (Frick *et al*., 1999). For each microorganism there is an optimal moisture content for it to grow, microbes are limited to soluble materials that are transported across their cell membranes into the interior of the cell, the moisture solubilizes the substrate and allows the substrate to enter the cell. For hydrocarbon contaminated soils, moisture level below 50% appear to inhibit degradation of hydrocarbon by the microbial (Cookson, 1995; Sigh and Lin, 2009). Moisture level is a limiting factor for microbial growth and activity in unsaturated subsurface inadequate supply of water can severely restrict biodegradation in surface soil. However, excessive water will displace air from pores in soil, which inhibits gas exchange and results in anaerobic zones and elimination of aerobic processes (Eweis *et al.*, 1998). Moisture level considerations are also important for microbial enzymatic activity and proper operation of bioventing processes.

Experience with bioremediation sites has shown that the optimum moisture level for enzymatic reactions is soil saturation or field capacity (Dick and Tabatabai, 1999). In general, enzymatic reaction rates increase with increased moisture, although enzymatic reactions have been shown to decrease when specific metal ions were mobilized with increased soil moisture (Acosta-Martinez and Tabatabai, 2001). However, in a bioventing system, the presence of saturated soils with water would limit airflow, permeability, or conductivity, through the soil bed and would impact oxygen distribution. Operation of the system with unsaturated soil would improve bioreactor efficiency. The optimal level of moisture depends on many factors and is considered to be soil and contamination specific. Alexander (1977) reported that the optimal soil moisture for microbial activity in bioventing systems is considered to be between 50% and 75% of the soil moisture holding capacity. Huddleston *et al*., (1986) indicated that a wide range of soil water holding capacity (25-85%) had little effect on biodegradation in soil. In any case, too little water will reduce enzymatic activity and reduce the region where solubilization and biodegradation can occur. Biosurfactant production is related to soil moisture. Atlas (1981) found biosurfactants hydrocarbons degrading bacteria at the soil/water interface.

1.3.4. Temperature

Temperature is a strong environmental variable responsible for growth and activity of the microbes to mineralize the organic component in contaminated soil. Temperature of both air and soil affects the rate of the biological degradation processes in the soil, as well as the soil moisture (JRB Associates, Inc., 1984). Microorganism's growth at temperature between 0 to 110 °C according to the temperature at which bacteria are growing optimally, one can characterise different groups:

• Psychrophilic organisms have a growth temperature optimum at 15 °C or lower.

• Mesophilic organisms grow best at temperatures between 20 and 45°C.

• Thermotolerant organisms still grow at temperatures above 45°C.

• Thermophilic organisms grow best at temperatures between 45 and 55°C.

• Extreme thermophilic organisms grow optimally between 55 and 80°C.

• Hypothermophilic organisms grow optimally above 80°C.

Most of the vegetative cells only survive temperatures below 70°C (Müller, 2000).

In general the rate of microbial degradation or transformation doubles for every 10 °C increase in temperature up to 80°C (Eweis *et al*., 1998; Wright *et al*., 1997). In an experiment involving oil bioremediation in salt marsh mesocosms, degradation of applied hydrocarbons averaged

72% during summer compared to 56% during winter, even though the winter exposure was 42 days longer (Wright *et al*., 1997).

Little is known about hydrocarbon biodegradation processes and rates in cold environments. It has been assumed that in Antarctica, temperatures are too low for effective biodegradation (Morita, 1992; Nedwell, 1999). However, the metabolism of cold-adapted microorganisms is prepared to function optimally at low temperature (Margesin, 2000; Okoh and Trejo-Hernandez, 2006). Such organisms have been shown to be useful tools for bioremediation process in cold environments like in Alpine, Arctic and Antarctic areas (Gounot, 1991; Margesin and Schinner, 1999; Delille *et al*., 1997; Sigh and Lin, 2009). However, many questions raised about the feasibility of bioremediation in cold regions are still unanswered. Some bioremediation experiments have been conducted on Antarctic soils (Kerry, 1993; Delille, 2000; Ferguson *et al*., 2003; Delille *et al*., 2004) demonstrated that the hydrocarbon degradation rate was reduced when the temperature decreased from 20 to 5°C when the microbial population was in the log phase of growth. In contrast, the degradation rate was temperature independent when the population was in the stationary phase. The effects of temperature are interactive with other factors, such as the quality of the hydrocarbon mixture and the composition of the microbial community. Hydrocarbon biodegradation can occur at the low temperatures (< 5°C) that characterize most of the

ecosystems which are likely to be contaminated by oil spills. Temperature often is not the major limiting factor for hydrocarbon degradation in the environment except as it relates to other factors (Atlas, 1981; Okoh and Trejo-Hernandez, 2006).

1.3.5. Nutrient Requirements for Effective Biodegradation of Hydrocarbons

Inorganic nutrients [nitrogen (N), and phosphorus (P) are needed to support microbial cell growth and for the production of metabolic enzymes required for biodegradation (Suthersan, 1997; USEPA, 1995; Ghasimi *et al*., 2009). If these nutrients are not available in sufficient quantities in the contaminated aquifer, then nutrients may need to be added to enhance the biodegradation processes. In the absence inorganic nutrients, biodegradation continues in the subsurface due to the natural recycling of elements. However, nutrient limited biodegradation proceeds at a slower rate (Suthersan, 1997). The most critical inorganic nutrients to the bioremediation of hydrocarbons are nitrogen and phosphorus (Venosa *et al*., 1996; Lee *et al*., 1997; Onwurah, 1999; Sigh and Lin, 2009). Both N and P are critical in cell growth and account for 14 and 3 % (N and P respectively) of the dry weight of a typical microbial cell (Suthersan, 1997; Liebeg and Cutright, 1999). N and P are also the nutrients most often in limited supply in contaminated soil and

groundwater. The recommended carbon: nitrogen: phosphorus mole ratios for enhanced biodegradation are in the range of 100: 10: 1 to 100: 1: 0.5 (USEPA, 1995; Liebeg and Cutright, 1999). Biostimulation consists of adding nutrients and/or electron acceptors that limit bacterial growth to the contaminated zone. Using contaminants as substrate for energy and growth, microorganisms convert the contaminants into harmless products, principally CO_2; in addition, soil fertility may affect microbial community. A number of studies have found that addition of ammonium nitrate, ammonium sulfate, and urea suppressed soil microbial respiration. However, increased soil microbial respiration by the addition of urea and ammonium nitrate was also noted in short term incubations (Dzantor, 1999; Margesin and Schinner, 2001; Ausma *et al*., 2002). The tracking of various components of the microbial community is important in predicting the overall response of the ecosystem. The enumeration of these populations provides a direct measure of microbial response (Irving *et al*., 2002). Many studies have indicated that bioremediation with fertilizers, such as nitrogen and phosphorus, increases oil degradation. In a mesocosm experiment, addition of phosphorus and nitrogen significantly increased oil degradation, but nitrogen alone did not. In a laboratory study on the effects of nitrogen and phosphorus levels on petroleum bioremediation, in previous study at 10 °C, degradation rates of diesel fuel were not affected by fertility

treatments. At 20°C, the rates were increased by the addition of P, but unaffected by N. These results suggest that phosphorus may limit microbial degradation of oil in some cases. Effectiveness was determined to depend primarily on the amount of nitrogen fertilizer delivered to the sediment, in a laboratory bioremediation study of crude oil; in addition at 3% oil in the soil, addition of P, without N, generally did not enhance biodegradation when addition of N, without P, approximately tripled the quantity of oil degraded. At 6 and 9 % oil concentrations, CO_2 evolution increased by adding P and N together in comparison to adding N alone, and total petroleum hydrocarbon (TPH) biodegradation increased by 30 % in 60 days. These results suggest that nitrogen may be more important in microbial degradation of oil than phosphorus, but phosphorus can increase oil degradation once nitrogen limitations are relieved.

1.3.6. Acidity and Alkalinity (pH)

It is important to remember, that the pH is a logarithmic function, thus a change of one pH unit represents a ten-fold change in hydrogen ion concentration. Natural environments mostly have a pH between 5 and 9, so organisms with pH optima within this range are very common. Fungi like it a little more acidic and generally grow best at a pH of 3.5 – 4. Only a few organisms manage to grow at pH values below 3 (e.g. Sulfolobus pH 1.5) or above 11. Organisms growing at acidic pH are

called acidophiles and those growing at high pH are called alkaliphiles. Since most bacteria require neutral pH for growth the number of bacteria e.g. in food can be reduced by lowering the pH (Müller, 2000).

1.4. Kinetics of Bioremediation

Bioremediation of polycyclic aromatic hydrocarbons (PAHs) - Polycyclic aromatic hydrocarbons (PAHs) are important environmental contaminants because of their known or suspected carcinogenicity, and because of their extensive occurrence are environmental pollutants. Significant interest in the potential for bioremediation of PAH contaminated sites has resulted in considerable progress toward understanding biodegradation of PAHs and degradation pathways (Evans *et al*., 1965; Gibson and Subramanin, 1984; Bossert and Bartha, 1986; Guha *et al*., 1999). Biodegradation kinetics in PAH-contaminated environments is complicated by the possibility of substrate interactions. Few studies have investigated the kinetics of growth and substrate depletion in systems containing mixtures of PAHs. An early study on the impacts of PAHs on the rates of disappearance of other PAHs in sediments revealed that interactions may result from enzyme inductions that occur due to pre-exposure of a culture to a PAH compound (Bauer and Capone, 1988a). The study of (Keck *et al*., 1989), on PAH biodegradation in prepared mixtures and in complex wastes showed both positive and negative substrate interactions. The half-lives of PAHs in sole-substrate systems were

shorter than those in PAH mixtures, indicating competitive inhibition. However, when the PAH mixtures were present in matrices containing other degradable hydrocarbons, cometabolic interactions overrode the competitive inhibition effects. A study that combined PAHs with polar creosote-related compounds (Millette *et al*., 1995) showed that phenanthrene biodegradation may be inhibited by the presence of more soluble and degradable compounds. In a study using six different bacterial strains and mixtures of six PAHs, (Bouchez *et al*., 1995) observed a variety of substrate interaction effects depending on the choice of bacterial strain and the PAH mixture. Kelly and Cerniglia (1995) observed that mineralization rates of individual PAHs were reduced by the presence of other PAH compounds. Furthermore, they inferred that the initial oxidation step of PAH biodegradation is not compound-specific, as degradation was observed for a range of different PAHs. Another recent study (stringfellow and Atken, 1995), reported competitive inhibition of phenanthrene uptake by naphthalene, methylnaphthalene, and fluorene, providing compelling evidence of common enzyme systems for biodegradation of a number of PAHs. (Shuttleworth and Cerniglia, 1996) also observed inhibition of phenanthrene degradation in the presence of naphthalene for three different microbial strains. This brief overview of the literature reveals that substrate interactions for PAH mixtures have been observed and there is no simple rule for predicting these interactions. Hence, simple models that do not account for the combined effects of inhibition due

to the presence of preferred substrates and enhanced degradation due to the presence of multiple growth substrates may not be suitable for predicting biodegradation rates of PAHs in contaminated environments.

1.4.1. Theory of Multisubstrate Monod Kinetic Modelling

Substrate interactions may result from the dual effects of (i) competitive metabolism in which one substrate inhibits the utilization of another because of competition for the active binding site of an enzyme and (ii) the fortuitous growth of biomass due to the presence of multiple substrates. The first will negatively impact a substrate's biodegradation rate and the second will enhance it. The multisubstrate Monod kinetic model has the capability to capture both these interaction effects.

In a multisubstrate system in which all the substrates can serve as primary substrates for growth, biomass growth is due to the utilization of all the compounds:

$$\mu_T = \sum_{i=1}^{n} \mu_i \quad \text{.....(1.3)}$$

Where μ_T is the total specific growth rate and μ_i is the specific growth rate on substrate i, and the summation is taken over the n substrates. The specific growth rate, μ_i, is related to the concentrations of the substrates through the multisubstrate Monod growth relationship:

$$\mu_i = (\mu_{\max i}\, C_i)/\left(K_{si} + \sum_{j=1}^{n}\left(K_{si}/K_{sj}\right)C_j\right) \ldots\ldots\ldots\ldots (1.4)$$

where $\mu_{max,i}$ is the maximum specific growth rate from substrate i, C_i is the concentration of substrate i, K_{Si} is the half-saturation constant for substrate i, and K_{Sj} is the half-saturation constant for each substrate j. Eq. (1.4) assumes that all the components in the mixture share a common rate-limiting enzyme reaction pathway. This relationship is analogous to the theoretical multisubstrate enzyme kinetic expressions derived by (Segal, 1975) and presented by (Yoon *et al*., 1977; Machado and Grady, 1989; Chang *et al*., 1993; Costa and Malcata, 1994; Stringfellow and Aitken, 1995). Eq. (1.4) is different from the substrate interaction models discussed by others, in which the summation in the denominator is replaced with $C_i + K_i$, where K_i is an empirical interaction parameter (Klecka and Maier, 1988; Oh *et al*., 1994). The relationship in Eq. (1.4) is fully predictive in the sense that the parameters can be determined from independent measurement in sole substrate systems.

The use of this model as a predictive tool implies an assumption that the microbial community in the multisubstrate system is comparable to that in the sole-substrate systems with respect to physiological state. This assumption is valid, even in a mixed culture system, provided that all the substrates are

utilized by a common enzyme system. For PAHs, this is a fairly good assumption in light of the findings of Kelly and Cerniglia (1995) and Stringfellow and Aitken (1995) discussed above. Hence, despite the complexities present in a mixed culture system, this relatively simple predictive model may be useful to describe biodegradation kinetics of PAH mixtures in the field or in engineered systems where mixed cultures exist.

The mathematical implication of the summation term in the denominator of Eq. (1.4) is that μ_i in the multisubstrate case is less than the specific growth rate that would be predicted by sole substrate Monod kinetics. However, since the total biomass growth rate in the multisubstrate case is larger than what would occur if there was only one substrate Eq. (1.3), the actual substrate depletion rate can be enhanced. These two competing effects are captured in the substrate depletion rate equation:

$$\frac{dC_i}{dt} = -\frac{\mu_i\, X}{Y_i} \qquad \text{..... (1.5)}$$

where X_i is the biomass concentration and Y_i is the biomass yield coefficient from growth on substrate i. The n different substrate depletion relations Eq. (1.5) combined with an expression for biomass growth rate

$$\frac{dX}{dt} = \mu_T X \qquad \text{..... (1.6)}$$

represent a system of differential equations coupled through the dependence of each μ_i on the concentrations of all the substrates. Depending on which substrates are present, their initial concentrations, the initial biomass concentration, and their respective affinity constants and maximum specific growth rates, the biodegradation rates for the individual compounds in a mixture may be enhanced or reduced relative to the comparable sole-substrate case.

1.4.2. Bioremediation Kinetics of Multisubstrates

A multisubstrate system contains the possibility of both positive and negative interactions between the substrates. First, the presence of multiple carbon sources may result in faster biomass growth compared to that in a sole-substrate system. Because the rate of substrate depletion is a function of the biomass concentration, the biodegradation rate for each compound may be increased if common populations are responsible for their degradation. Second, if common enzymes are involved, the presence of multiple substrates may result in competition, thereby inhibiting degradation. The net result of these two effects could be an increase in the rate of biodegradation as a result of enhanced biomass growth, a decrease in biodegradation rate from competitive inhibition, or no noticeable effect because the two effects balance and cancel each other out.

Previous studies have found evidence of enhanced biodegradation rates as well as decreased biodegradation rates for PAHs present in mixtures (Bauer and Capone, 1988b; keck *et al*.,1989; Mannisto *et al*., 1996; Bouchez *et al*., 1997; Beckles, 1998; Millette *et al*., 1998; McNally *et al*., 1999). These studies provided valuable insights regarding the significance for potential substrate interactions in the biodegradation of mixtures of PAHs, but when examined collectively, no clear pattern to the substrate interactions was found. Because of the range of substrate and biomass conditions represented, it certainly is possible that biomass-enhanced biodegradation was dominant in some cases whereas multisubstrate inhibitions were dominant in other cases. The absence of routine measurement of biomass growth and the differences in initial substrate concentrations and experimental designs make it difficult to infer underlying mechanisms.

Only a few studies have investigated kinetics of PAH multisubstrate biodegradation in aqueous systems, which eliminate confounding effects deriving from kinetically limiting sorption to and from soils and sediments. Even fewer studies have used enzyme kinetic modeling, rather than empirically inferring inhibitory effects, to investigate these kinetics. Seminal work in this area was performed by (Stringfellow and Aitken,1995), who studied biodegradation kinetics of phenanthrene (PHN) in binary mixtures with naphthalene (NPH), 1-methylnaphthalene (1MN), 2-methylnaphthalene (2-MN), and fluorene (FLR). Those authors observed competitive inhibition

of PHN degradation, suggesting that these PAHs probably share a common enzyme system within each of the two species tested. Despite the inhibition effect, enhanced oxygen uptake was observed in some of their binary experiments, implying enhanced biomass growth as a result of the presence of the second substrate. (Guha *et al*., 1999) studied substrate interactions in binary and ternary mixtures of NPH, PHN, and pyrene (PYR) using a PAH-degrading consortium. That work demonstrated the importance of substrate interactions in simple mixtures and demonstrated the feasibility of using predictive multisubstrate modeling, laying the groundwork for its use in more complex systems (Guha *et al*., 1998; Lotfabad and Gray, 2002) measured the biodegradation kinetics of PAH mixtures with a mixed culture and examined the data using a multisubstrate model. Competitive inhibition explained biodegradation kinetics in creosote-contaminated soils but not for the PAHs added to pristine soils.

1.5. Thermodynamic of Bioremediation

The thermodynamic studies of bioremediation of hydrocarbons have not been found in literature. Although some studies of bioremediation of some individual organic compounds have been reported (Van Trump and Coates, 2009). This study describes 2,6-anthrahydroquinone disulfonate (AH_2DS) as a model thermodynamically ‘targeting’ electron donor capable of selectively

stimulating respiratory processes relevant to the bioremediation of perchlorate. Pure cultures of *Dechloromonas aromatica*, *Dechloromonas agitata* and *Azospira suillum*, as well as uncharacterized microbial consortia, were capable of stoichiometrically reducing perchlorate to chloride upon oxidation of AH_2DS to the corresponding quinone 2,6-anthraquinone disulfonate (AQDS). No degradation of the anthraquinone structure was observed, and no organism tested grew by this metabolism. Thermodynamic calculations suggest that AH_2DS oxidation should support nitrate and perchlorate reduction, whereas sulfate reduction and methanogenesis are predicted to be unfavorable (Trump and Coates, 2009). Mixed community microcosms oxidizing AH_2DS reduced nitrate and perchlorate, whereas sulfate reduction never occurred. In contrast, microcosms amended with acetate respired nitrate, perchlorate and sulfate, as would be predicted by thermodynamic calculation. The results suggest that the thermodynamic properties of hydroquinones allow for targeted stimulation of only a subset of potential respiratory processes (Van Trump and Coates, 2009). This observation could help improve enhanced *in situ* bioremediation of perchlorate by negating many of the detrimental aspects of biofouling.

1.6. Soil Contamination

Beginning with the industrial revolution, a number of organic compounds have been synthesized. The use of these chemicals through direct and indirect applications generated chemical wastes in every economic sector. Unlike, naturally occurring organic compounds that are readily degraded, these synthetic chemical are resistant to degradation. The environmental protection agency (EPA) estimates that only about10% of all wastes are disposed of safely (Chaudhry and Chapalamadugu, 1991). There are many methods of treatment available to sites that have been contaminated. Some of these treatment methods are ex-situ while other in-situ. Ex-situ processes require removing the soil or water from the site and transporting the waste to a treatment facility. In-situ processes take place with minimal disruption of the contaminated area.

Petroleum hydrocarbons are commonly classified as environmental contaminants, though they are not usually classified as hazardous wastes (Abu Bakar *et al*., 2003; Vennila and Kannan, 2011). Many petroleum products are used in modern society, including those that are fundamental to our lives (i.e; transportation fuels, heating and power-generating fuels, etc). The volume of crude oil or petroleum products that is used today dwarfs all other chemicals of environmental and health concern (Zhu *et al*., 2004). Due to the numbers of facilities, individuals, and processes and the various ways the products are stored and handled, environmental contamination is potentially widespread.

Aromatic compounds are among the most prevalent and persistent pollutants in the environment (Viñas *et al*., 2005; Seo *et al*., 2009). Aromatics derived from industrial activities often have functional groups such as alkyls, halogens and nitro groups.

Naturally occurring soil is a complex and changing mixture of living organisms, decaying organic matter, air, water, and relatively stable rock and mineral fragments such as clay and sand. Although there are many different types of soils in the state of California, specific local areas contain relatively few of these varieties. Each type may exist for a few square yards or for many square miles with the amount of variation in a single soil being quite limited. Hence, it is not feasible to exactly pinpoint the origin of a particular naturally occurring soil sample, but rather to relate it to areas of occurrence (Brady and Weil, 1996; Moayedi *et al*., 2012).

Soil samples may also contain debris from human habitation or industrial operations. The latter type of debris; e.g., paint droplets, cinders, chemicals or fibers, if sufficiently varied and unique, can be most valuable in individualizing a specimen. Soil samples containing such unusual features can be excellent and unexpected physical evidence. Consequently, all soil samples should be submitted in anticipation that this rare occurrence may actually happen (Brady and Weil, 1996).

Not only do the character and composition of soils vary laterally, but also with depth. Unless a crime is committed which involves the digging of a

grave, most samples for soil comparison will be from the top surface. Although the color and texture of soils visually do not appear to vary along the ground, the chemical composition can change sufficiently in a short distance, so that it may be significant in localizing the source of the soil sample. Therefore, sufficient samples should be submitted in order to establish the normal distribution of soil of a particular type in and about a crime scene (Brady and Weil, 1996; Moayedi *et al*., 2012).

1.6.1. Causes of Contamination

Organic contaminants may enter the soil through many different pathways. Accidental spills, poorly designed hazardous waste facilities (Pavlostathis and Mathavan, 1992), and leaking underground storage tanks (LUSTs; Atlas and Cerniglia, 1995) are all routes by which organic contaminants may enter soils. The magnitude of the problem is difficult to assess. One estimate is that there are >250 000 LUSTs at service stations across the United States (Atlas and Cerniglia, 1995).

Manufactured gas plants (MGPs) are often singled out as a category of contaminated sites (Peters and Luthy, 1993; Yeom *et al*., 1996; Ramaswami and Luthy, 1997,). These sites were usually in operation for several decades, and upon their decommissioning, soil contamination with cod tar and petroleum residues is identified. It is suspected that there are over 1000 contaminated MGP sites in the United States alone (Peters and Luthy, 1993).

Wood preserving facilities are also likely sites of contamination, after many years of using chemicals to treat lumber (Deschênes *et al*., 1996), and Mueller *et al*., (1989) estimate the number of active and inactive such sites in the United States to be 700.

Soils at industrial sites may be contaminated with a variety of pollutants and the date or duration of the contamination is usually uncertain. Due to long life span of such sites, it is reasonable to believe that much of the contamination is cumulative, the result of many years of site use.

1.6.2. Types of Contamination

A rich literature exists concerning pesticide, inorganic compound, and metal contamination of soils, however this thesis examines hydrocarbons as contaminants, i.e. as materials that are present where they are not wanted. The main source of hydrocarbon contamination in soil is petroleum oils.

Cod tar , exists as a nonaqueous phase liquid (NAPL) that is denser than water and contains high concentrations of polycyclic aromatic hydrocarbons (PAHs; Mueller *et al*., 1989; Peters and Luthy, 1993; Ramaswami and Luthy, 1997). PAHs are compounds made of conjoined aromatic rings and include representatives varying from 2- member Rings such as naphthalene, to 4, 5, and 6-member rings such as pyrene and chrysene 4 rings (Liu *et al*., 1992; Song *et al*., 1990). PAHs are of particular concern in the environment because many are known to be, or suspected to be, carcinogenic or teratogenic

(Cerniglia, 1992; Kawahara *et al*., 1995; Liu *et al*., 1992; van Schooten *et al*., 1995).

Petroleum contains a range of compounds classified among four proximate categones: asphaltenes, paraffins, aromatics, and resins (Petrakis *et al*., 1980; Launen *et al*., 1995). Included within these categories are aliphatic compounds: straight and branched chain alkanes, cycloalkanes, and alkenes; aromatics: monoaromatics and PAHs and resins or NSO-containing compounds (McGill *et al*., 1981). The asphaltenes are high molecular weight, are soluble in toluene and precipitated in pentane, and may be highly condensed aromatic and cyclic polymers containing N, S, and O (McGill *et al*., 1981).

The conditions under which these contaminants exist in soil further compound their hazard because they occur as a multiphase problem: the solid phase of the soil, soil solution, and soil vapour are all contaminated. If present as a free phase, the contaminant NAPL will modify the normal attributes of the soil (Pollard *et al*., 1994), and therefore modify the predicted sorption behaviour (bioavailability) of the contaminants. Specific contaminant compounds that are highly hydrophobic may sorb to the NAPL, and be inaccessible to microorganisms capable of metabolizing them.

1.6.3. Bioavailability of Contamination

Microbial access to contaminants (an aspect of bioavailability) may be the major limitation to successful bioremediation. The potential substrate, the contaminant, may be made unavailable by a number of mechanisms: it may be physically sorbed to native soil constituents (Deschênes *et al*., 1996; Murphy *et al*., 1990; Pavlostathis and Mathavan, 1992), it may be sorbed in the NAPL component of the contamination (McGroddy *et al*., 1996; Yeom *et al*., 1996), or it may be protected within small pores in aggregates from which microorganisms are excluded by size (Ramaswarni and Luthy, 1997). Several opinions of how microbes metabolize substrates exist: the substrate may have to be in aqueous solution, the microbe may be able to colonize the surface of a non-aqueous phase to access the substrate, or proximity may be a factor.

Sorption of the contaminant to soil constituents may be described mathematically. Models that are commonly used to describe types of sorption include the Langmuir model and the Freundlich model. If the mechanism of sorption is known to be partitioning, such as may occur when hydrophobic compounds interact with a free NAPL or SOM, the partition coefficient (K_d) relates the solution concentration (C) of a compound to the fraction sorbed (S) to a solid surface (Scow and Hutson, 1992):

$$S = K_d C \quad \text{……..……} \quad (1.7)$$

The K_d may be normalized to the soil organic carbon content (f_{oc}) and a new constant, K_{oc} is derived (Xing *et al*., 1994a; Ding *et al*., 2002).

$$K_{oc} = \frac{K_d}{f_{0c}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1.8)$$

The K_{ow} is th e ratio of the solubility of a compound in octanol to its solubility in water. It can be used to estimate the degree to which a hydrophobic organic compound will partition into organic matter or a contaminant NAPL in soil. The K_{oc} may be calculated from the K_{ow} by solving linear equations (Xing *et al*., 1994a; Ding *et al*., 2002):

$$logK_{oc} = a + b\ logK_{ow} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1.9)$$

In Eq. 2.3, a and b are empirial constants.

The normalization of K_d to K_{oc} is useful because K_{oc} is compound specific, whereas K_d is soil-and-compound specific. A single organic contaminant may have different partition coefficients in different soils (Murphy *et al*., 1990), a phenomenon that was observed in studying contaminants in field-contaminated soils (Pavlostathis and Mathavan, 1992). The values of K_d for hydrophobic organic contaminants vary as the soil organic C contents Vary. Another implication of this normalization is that the contaminant is being sorbed to soil organic matter components. Xing *et al*., (1994a) sorbed benzene, toluene, and xylene to a range of biopolymers and humic acid in pure systems. As these and similar compounds are present in soil, it is likely that organic chemicals will sorb to such materials. Xing *et al*., (1994b) reported that K_{oc} decreased with increasing mass ratio [(O+N)/C] of non-protein organic sorbents. They proposed the term "polarity index" (PI) for this

mass ratio, recognizing it ignores polarity that may arise from configuration and structure. The advantage of K_{oc} as a chemical-specific variable, may be illusory if the native organic phase varies substantially among soils and sediments; or if the native organic phase is dominated by CO-contaminants or NAPLs that become sorbent phases. Consequently, the assumption of uniformity of sorbents in Eq. 1.2 may lead to errors in predictions of sorption by soils and in retardation coefficients used in transport and fate models.

Murphy *et al*., (1990) examined the interactions of three organic chemicals: carbazole, dibenzothiophene, and anthracene with aquifer sediments. They concluded that the organic compounds had sorbed to mineral-bound humic materials. Pyrene has been observed to bind more strongly to sediments than its log K_{ow} (Sims and Overcash,1983) predicts (McGroddy *et al*., 1996). The authors hypothesize that this is due to interactions between the pyrene, the soot and diesel of the PAH carrier matrix and organic matter.

Crude petroleum usually contains hydrocarbons classified as alkanes, cycloalkanes, aromatics, polycyclic aromatics, asphaltines, and resins. Besides, small amounts of alkenes are often generated through refining processes and end up in refined petroleum products. Variations in chain length or branching and in ring condensations or interclass combinations, as well as the presence of oxygen, nitrogen, or sulfur, result in a wide variety of petroleum hydrocarbons (Eweis *et al.*, 1998). Aromatics compounds in the petroleum hydrocarbons are among the most recalcitrant substances from

petroleum products. They are more stable than other cyclic compounds owing to the sharing of delocalized electrons by the pi bonds (Eweis *et al.*, 1998). Benzene, toluene, ethylbenzene and xylenes, which are frequently referred to as BTEX compounds, are monoaromatics that have high water-solubility and relatively low sediment-water coefficient (Rooney-Varga, *et al*., 1999). Light non-aqueous phase liquids (LNAPLs) in petroleum products are hydrocarbons with low boiling point, e.g., gasoline that consists of a number of light distillates with a boiling-point range of -12 to 200 ºC (Eweis *et al.*, 1998). Theoretically, gasoline could contain more than 1,200 different hydrocarbon compounds, of which about 230 hydrocarbons in the carbon number range of C_3 to C_{12} have been identified (Fan *et al*., 1994). Major constituents of gasoline are aliphatic hydrocarbons, alicyclic hydrocarbons, aromatic hydrocarbons and other additives. The aromatic hydrocarbons include benzene, toluene, ethylbenzene, m-, o- and p-xylene, trimethylbenzene, and other benzene forms (Steffan *et al*., 1997). BTEX compounds could make up over 40 percent of gasoline's composition (Cookson *et al*., 1995). Another example of LNAPLs is condensates from natural gas that have a similar com position with gasoline containing hydrocarbons ranging from C_5 to C_{10} (Clifton, 1999).

Salanitro (2003) found review of the literature on the biodegradability of representative hydrocarbons in petroleum (PHCs) indicates that microbes (bacteria and fungi) isolated from soil, sediments, and biosolids can readily

metabolize compounds of chain lengths up to C_{30}-C_{44} including n-alkanes, branched alkanes with few alkyl groups, and 1-to 3-ring alkylated or nonalkylated aromatics. In general,highly branched alkanes, cycloalkanes, 4-to 6-ring condensed aromatics, and alkylated thiophenes and dibenzothiophenes are partially metabolized or are completely recalcitrant. The extent of microbial degradation of crude oils, oily wastes, and fuels in soils depends on the distribution of PHC structures, concentration, presence of a nonaqueous phase liquid (NAPL), degree of evaporative loss (weathering), and sequestration (nonbioavailability).

Declines in bulk PHC in laboratory and field experiments with crude oil or refined oil products in soils are the result of volatilization and biodegradation. Studies on vapor losses of PHC from oily soils indicate that previously reported rates of decline attributed primarily to biodegradation have been overestimated. Bioremediated soils, however, are characterized by low leaching (aqueous) potential, reduced toxicity (soil species bioassays), and the persistence of a readily extractable, nonbioavailable residual phase PHC fraction.

1.7. Brief Description of Thermal Crude Oil Processing (Conventional Refining)

Refining crude oil principally involves separation of molecules by boiling point, along with some breaking and making of bonds (molecular rearrangement - chemical reactions). In order to begin the refining process, crude oil is first water washed to remove water soluble components. The washed crude is then sent to a furnace for preheating. The extent of energy expended in the preheating step depends on the crude viscosity. More viscous crudes require more heat in order to flow. The preheated crude is then sent to the distillation tower, where the energy expended to distill it depends on the compounds contained in the oil. Significant energy input for heat to conduct a distillation, cooling, compression, etc is required for refining to occur. Chemical reactions can be exothermic (giving off heat), but the vast majority are endothermic (requiring heat). Heat management and energy conservation are optimized to maximize product yield, meet product specifications and produce the highest economic return. Within narrow limits, refineries can alter processing configurations to adjust for changing feedstocks and product demand, while still meeting product specifications. Figure (2.1) shows a very simplified schematic of a refinery streams.

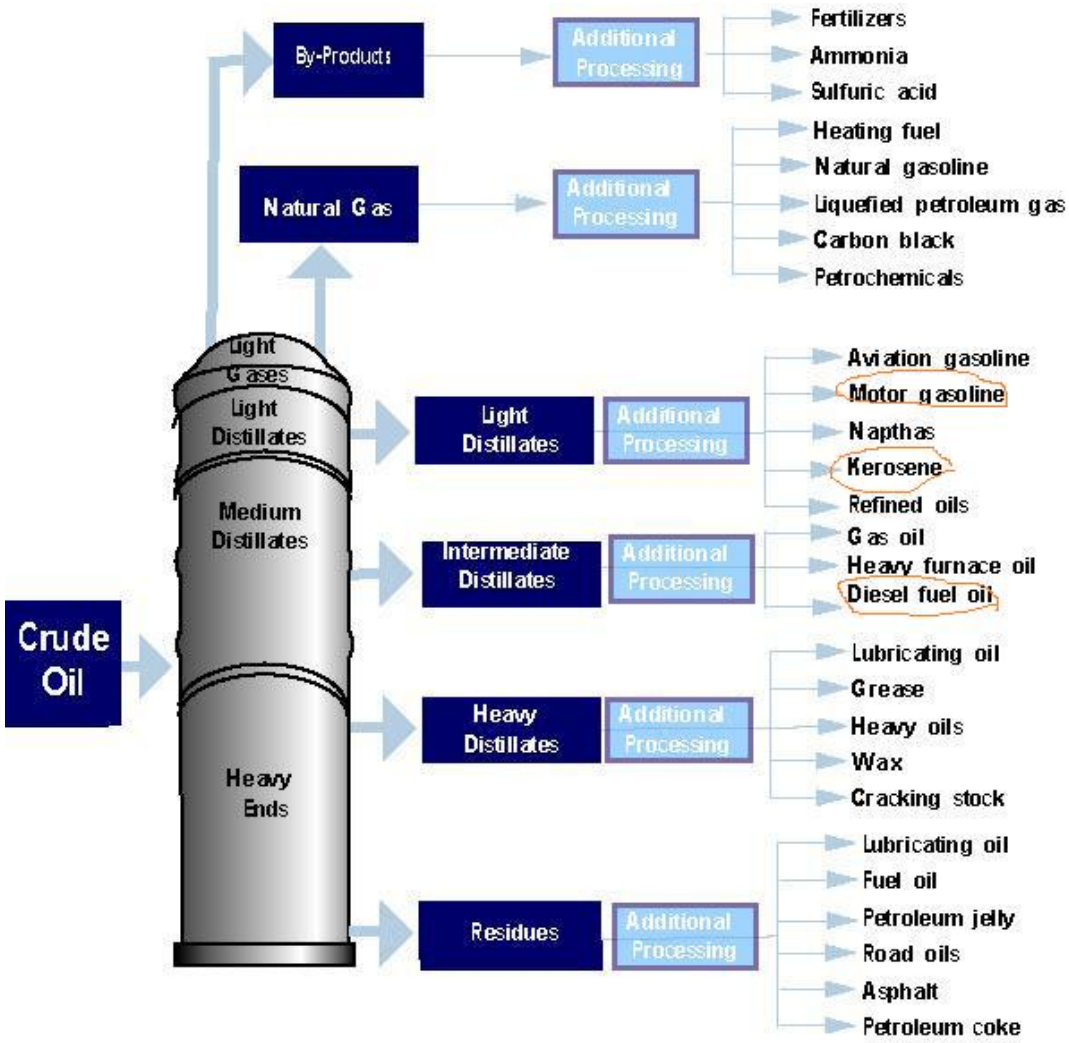


Figure 1.1: Simplified Schematic of Refinery Showing Principal Products

1.8. Bioremediation

Bioremediation is one of process or the treatment of contaminated soil and groundwater. Biodegradation can be defined as the biologically catalyzed reduction in complexity of chemicals. Certain microorganisms have the capability to degrade contaminants in the environment. Not every contaminate can be remediated through the use of microorganisms. However, there are a number of contaminants that can be degraded using this method. The

contaminants that may be degraded via bioremediation can be subdivided into five main categories (Hickey and Smith, 1996):

- Organic solvents
- Polyaromatic hydrocarbon (PAH) (creosote oily wastes)
- Halogenated aromatic hydrocarbons
- Pesticides
- Munitions wastes

1.8.1. Bioremediation Techniques:

There are several bioremediation techniques that may be applied. Some examples (Baker and Herson, 1994):

- *Bioaugmentation* - The addition of bacterial cultures to a contaminated medium; frequently used as an ex-situ process.
- *Biofilters* - The use of microbial stripping columns. Employed to treat air emissions.
- *Biostimulation* - The stimulation of the indigenous microbial populations in soils and/or ground water. This process may be done in situ and ex situ.
- *Bioreactors* - The use of biological processes in a contained area or reactor. This method is used to treat slurries or liquids.

- *Bioventing* - The process of drawing oxygen through the soil in such a way to stimulate microbial growth and activity.
- *Composting* - Aerobic, thermophillic process that mixes contaminated soil with a bulking agent. Done using static piles, aerated piles or continuously fed reactors.
- *Landfarming* - Solid phase treatment system for contaminated soil. May be done as an in situ process or in a soil treatment cell.

Independent of the method of bioremediation there are several criteria that must be satisfied before biodegradation will take place in an environment (Alexander, 1994; Sigh and Lin, 2009).

1.9. Metabolism of Petroleum Hydrocarbons

Bioremediation of petroleum-contaminated sites is based on the metabolism of petroleum hydrocarbons by microorganisms. This leads to the destruction of contaminants.

A variety of microorganisms were found capable of degrading petroleum hydrocarbons by aerobic, anaerobic or fermentation metabolisms (Canter and Knox, 1985), utilizing the hydrocarbons as their growth substrates (Wainwright, 1999).

1.9.1. Electron Donors and Accepters

Degradation of petroleum contaminants is usually conducted through a redox reaction during which an electron donor becomes oxidized after releasing electrons while an electron acceptor becomes reduced after receiving electrons. Bacteria obtain chemical energy through such a reaction to support their living (Eweis *et al.*, 1998). Petroleum hydrocarbons, mostly aliphatic or aromatic compounds that contain functional groups like OH, and Cl, usually function as electron donors; oxygen often acts as terminal electron acceptor or directly reacts with the petroleum hydrocarbon molecules during the microbial metabolism (Wainwright, 1999). Oxygen can be replaced as an electron acceptor by organic or inorganic compounds such as metal ions, nitrate, sulfate and carbon dioxide (Alexander, 1999; Abbassi and Shquirat, 2007). However, energy released from these compounds is much smaller than that obtained from oxygen, and they cannot react directly to oxidize the contaminants (Wainwright, 1999).

1.9.2. Aerobic Respiration

In aerobic respiration, the substrate molecules, e.g. petroleum hydrocarbons, are broken down by enzyme-mediated (usually oxygenase-mediated) reactions in microbial cells. Most aerobic bacteria decompose organic compounds into carbon dioxide, H_2O and other inorganic compounds with the consumption of oxygen (Freeze and Cherry, 1979).

Serving as an external electron acceptor in this process, oxygen is the most efficient electron acceptor in biodegradation (Eweis *et al.*, 1998). Aerobic biodegradation occurs via more efficient and rapid metabolic pathways than anaerobic one (Zitrides, 1983).

1.9.3. Anaerobic Respiration

In anaerobic respiration, petroleum hydrocarbons are broken down by enzyme-mediated reactions in which oxidative compounds other than oxygen serve as external electron acceptors. According to the free energy yielded per reaction for oxidizing the same substrate, when oxygen is depleted, the next best electron acceptor will be Fe^{3+}, followed by NO_3^- and NO_2^-. Sulfate and carbon dioxide come next. Nitrate and sulfate are the most commonly used electron acceptors because of their abundance in nature. The Fe^{3+} oxides may not be accessible to microorganisms because of their insolubility (Eweis *et al.*, 1998).

1.9.4. Fermentation

In fermentation metabolism, organic compounds are used as both electron donors and electron acceptors. Within the same organic molecule some atoms may become oxidized while the others may be reduced. During this metabolism process, organic substrates are not completely oxidized. Because

of the low efficiency in energy production, fermentation is seldom utilized for bioremediation (Eweis *et al.*, 1998).

1.10. Degradation Pathways

There are several degradation pathways for petroleum hydrocarbons. The purpose of this section is to provide an overview of the common pathways of degradation. Straight chain alkanes are degraded primarily through the oxidation of the terminal methyl group, followed by cleavage of the molecules between the second and the third carbon in the chain. The initial reaction in the degradation of the straight chain alkanes involves the direct addition of oxygen to the terminal carbon. This forms an alcohol that can subsequently oxidize to a corresponding aldehyde and finally forms a fatty acid. From the fatty acid a two carbon long fragment is cleaved (Walsh, 1999).

Aromatic hydrocarbons are found mainly in light petroleum products; however, they may be present in small amounts in any petroleum product. Aromatic hydrocarbons are also widely used in industrial solvents. Aromatic hydrocarbons, in general, are very soluble in water and have low boiling points due to their small molecular size. These compounds are also very volatile. There are a large number of different pathways that are used by bacteria to degrade aromatic compounds. To aid in the understanding of the

degradation of aromatic compounds, the degradation of benzene follows (Walsh, 1999).

Benzene is first converted to catechol or protacatechuate. The aromatic nucleus is subsequently opened by one of two pathways as shown in figure (1.2): the orthocleavage or the metacleavage. Orthocleavage: The aromatic ring of catechol or protocatechuate is opened as a result of the introduction of molecular oxygen into the hydroxyl groups. Acetyl-CoA and succinate are formed as a result of the cleavage. These products can then be further oxidized by the Krebs cycle and the electron transport system. Meta-cleavage: Once again the aromatic ring is opened by the introduction of molecular oxygen. In this case, the cleavage occurs between a hydroxylated carbon and the adjacent unsubstituted carbon. Acetaldehyde and pyruvate, which can be broken down by the Krebs cycle and electron transport are the products of the ring cleavage (Baker and Herson, 1994).

The above processes are aerobic in nature. Aerobic degradation is the most common; however, anaerobic degradation can occur. Anaerobic degradation will occur under denitrifying conditions, sulfate-reducing conditions, and methanogenic conditions. The initial step in anaerobic degradation is dissimilar to the aerobic degradation path. The first stage of degradation in an anaerobic system is the hydrogenation of the benzene ring, thus destabilizing the ring. Cleavage through hydration reaction yield aliphatic hydrocarbons that can be further metabolized to the Krebs cycle intermediates as described

above. In anaerobic degradation, water acts as the oxygen source for metabolic reactions (Walsh, 1999).

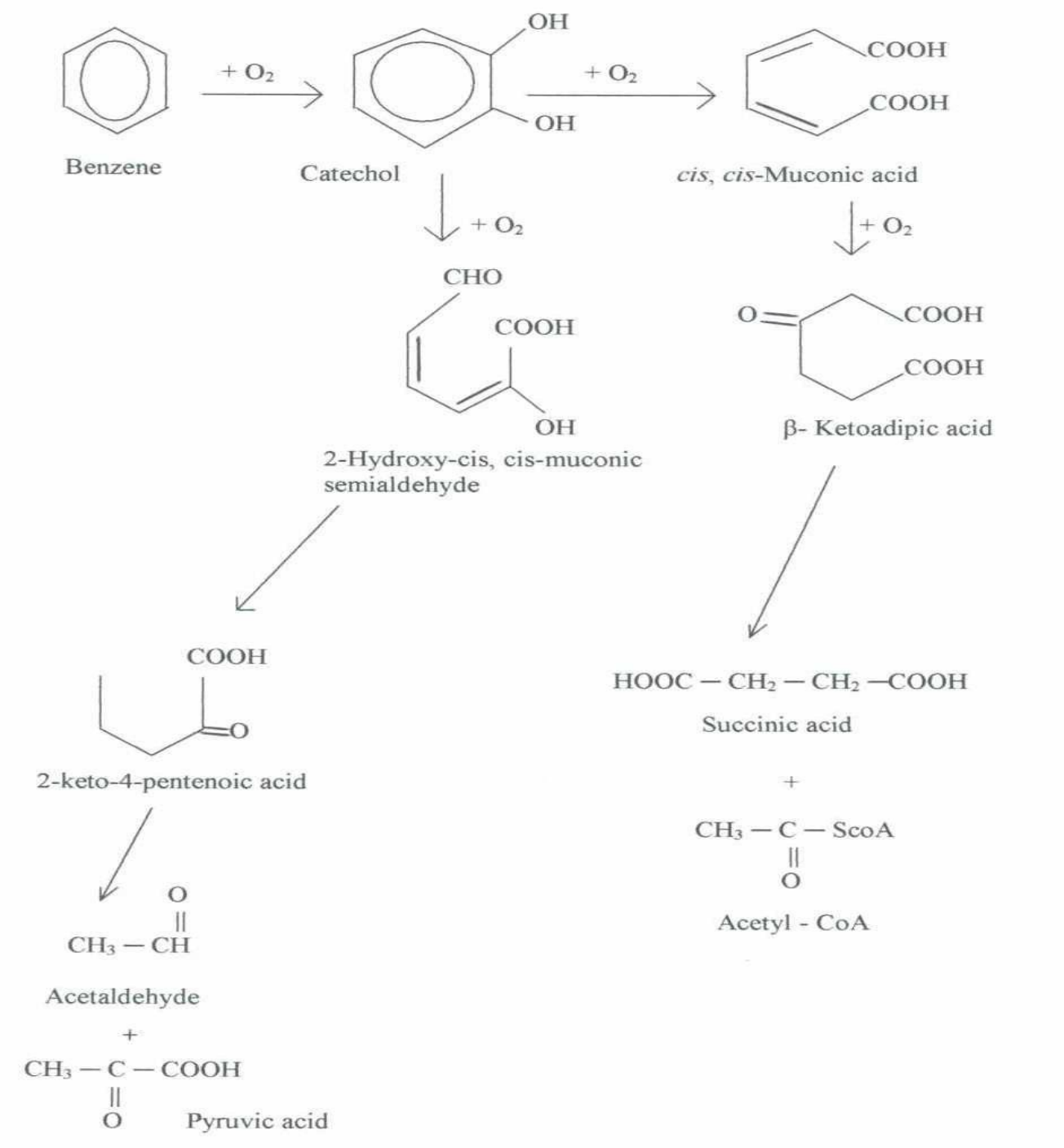


Figure1.2: Microbial metabolism of the aromatic ring by meta or ortho cleavage as shown for benzene (Atlas and Bartha 1992).

1.10.1. Polyaromatic Hydrocarbons (PAHs)

Polyaromatic hydrocarbons are generated and released from the incomplete combustion of organic material, including automobile exhaust. Petroleum related activities are reported to account for more than 70% of the artificially generated sources of PAHs. The degradation of these compounds are dependent upon the complexity of the PAH chemical structure. The ease of degradation is dependent upon the following (Cookson, 1995):

- Solubility of the PAH
- Number of Fused Rings
- Number of Substitutions
- Type of Substitutions
- Position of Substitutions
- Nature of Atoms in Heterocyclic Compounds

Polyaromatic hydrocarbons contain two or more fused aromatic rings, they are found in trace amounts in heavy petroleum products. PAHs are present as contamination in the form of naphthalene, phenanthrene, pyrene and benzopyrene. Degradation of two and three ring compounds, such as naphthalene and anthracene, has been shown to occur among aerobic bacteria. Biodegradation of the higher ring structures is dependent upon the molecule's solubility in water (Cookson, 1995; Walsh, 1999).

1.10.1.1. Anaerobic degradation

Degradation of PAHs by anaerobic organisms has not been very successful. However, some degradation has been achieved under denitrifying, sulfate reducing, and methanogenic conditions. Napthalene and anthracene was found to be slightly degraded anaerobically under denitrifying conditions. Under sulfate reducing or methanogenic conditions the degradation rate is independent of the nitrate concentrations, but is dependent upon the soil to water ration. However, Naphthol that contains a hydroxyl group substitution was found to be anaerobically degraded by denitrifying conditions, sulfate reducing conditions, and methanogenic conditions (Cookson, 1995; Walsh, 1999).

1.10.1.2 Aerobic degradation

As the number of fused rings increases the degree of degradation decreases, one methyl addition significantly decreases the degree of degradation the influence of alkyl substitutes is less predictable, the effect of the methyl addition varies with the position in which it is substituted in the ring, another way of reducing the degradation is to increase the degree of saturation through the addition of hydrogen atoms and the removal of double bonds between the carbons providing another valence bond. (Cookson, 1995) For unsubstituted PAHs degradation occurs readily in the presence of soil bacteria. The rate of degradation of unsubstituted PAHs appears to be related

to the solubility in water of these compounds. There is little information on the degradation of PAHs with more than three rings (Cookson, 1995; Walsh, 1999).

Eukaryotic organisms have also shown an ability to degrade PAHs under aerobic conditions. The mechanism in this situation involves a reaction sequence called NIH shift in the initial stages of transformation. An example of an eukaryotic organism that is capable of degrading PAHs is the white-rot fungi (Baker and Herson, 1994; Walsh, 1999).

1.10.2. Halogenated Aliphatic and Aromatic Hydrocarbons:

1.10.2.1. Halogenated Aliphatic Hydrocarbons:

Halogenated hydrocarbons are widely used an industrial solvents and degreasers. The definition of a halogenated hydrocarbon is a compound that has one or more of the hydrogen molecules have been replaced with a halogen.

Trichlorethene (TCE) is the most common halogenated aliphatic hydrocarbon contaminant in groundwater and soil. These compounds can be degraded under both aerobic and anaerobic conditions. Many water and soil chemical properties will influence the stability of the halogenated aliphatic hydrocarbons. These compounds undergo abiotic transformations in the environment. These transformations include substitution and

dehydrohalogenation of a haloaliphatic compound in water and soil. Dehydrohalogenation results in the removal of the halogen to form an alkene.

1.10.2.1.1. Anaerobic degradation

Anaerobically a process called reductive chlorination takes place. In reductive chlorination halogen atoms are removed sequentially from the molecule and replaced with hydrogen atoms. In reactions such as this, the halogenated hydrocarbon is not used a C source but rather as an electron acceptor. This indicates that in order for reductive chlorination to occur an sample carbon source must be present to allow for microbial growth.

Dehalogenation is dependent upon the oxidation-reduction (redox) potential of the molecule. Redox potential is determined by the strength of the halogen-carbon bond. The higher the bond strength, the less likely the halogen will be removed. The bond strength in turn is dependent upon the type and the number of halogen atom present and the degree of saturation of the halogenated molecule. As the degree of saturation decreases the bond strength increases and the molecule becomes more difficult to degrade. This indicates that alkanes are more susceptible to reductive dehalogenation than alkenes and alkynes (Baker and Herson, 1994; Walsh, 1999).

1.10.2.1.2. Aerobic degradation

It was believed that aerobic degradation did not occur in the case of Halogenated Aliphatic Hydrocarbons. Degradation has been shown to occur in soils that have been exposed to methane or natural gas. It was therefore determined that a group of organisms known a methanotrophs. Methanotrophs have been isolated and in the presence of aromatic compounds can degrade TCE. The understanding of the degradation pathway for aerobic degradation is incomplete. The initial stage involves the oxidation of the molecule. It is believed that the initial oxidation is carried out in the same manner as in the degradation of aliphatic and aromatic hydrocarbons (Baker and Herson, 1994).

1.10.2.2. Halogenated aromatic compounds

Sources of Halogenated Aromatic Compounds are vast and varied. Many of these compounds are produced from commercial use and as chemical intermediates during the synthesis of chemicals. Potential releases are associated with the industrial operations dealing with pharmaceutical, pesticide formulation, dyes, rubber, solvents, cleaners, etc (Cookson, 1995). Halogenated Aromatic Compounds include toluene and phenol. It is an immense group or chemical related to benzene. Due to the diversity of this group a thorough overview of the microorganisms and metabolic pathways involved is not possible (Walsh, 1999).

1.10.2.2.1. Anaerobic degradation

Anaerobic degradation was found to occur in a variety of environments. Once again the process proceeds through reductive chlorination. Highly substituted compounds are more easily dehalogenated than monohalogenated compounds. Typically the degradation of these compounds is performed by a group of organisms rather than a single strain. Thus far, the majority of studies have focused on the use of methanogenic enrichment for degradative purposes. As for sulfur reducing agents, there is limited information. However, due to the lack of thermodynamic barriers to the degradation of Chlorinated Aromatic Hydrocarbons (Baker and Herson, 1994).

1.10.2.2.2. Aerobic degradation

One requirement for microbial degradation of any compound is the need to induce the production of enzymes. However, not all halogenated aromatic compounds will generate enzymes. Most halogenated aromatic compounds are degraded by cometabolism. The relatively nonspecific nature of the enzyme that transform benzoate to catechol . In some cases this degradation is not complete (Walsh, 1999).

Degradation of Halogenated Aromatic Compounds proceeds through many of the same pathways as nonhalogenated compounds. For example, the first stage is to convert the compound to a chlorcatechol type substance, this followed by the aromatic nucleus being broken down. The next stage is the

dechlorination of the ring cleavage products. Halogenated polyaromatic compounds, such as chlorinated biphenyls, are generally degraded by the cleavage of a nonsubstituted ring, followed by the degradation of the resulting chlorobenzene (Cookson, 1995). The susceptibility of the Halogenated Aromatic Hydrocarbons is dependent on the nature of the halogen substitution, the number of substitutes and the placement of the substitutes. The susceptibility is decreased as the number of substitutions increases. However, some highly chlorinated compounds such as pentachlorophenal have been shown to be susceptible to aerobic degradation (Baker and Herson, 1994).

1.11. Methanotrophic Treatment Technology

Methanotrophic Treatment Technology (MTT) is based on the use of methanotrophs (bacteria that derive energy from the oxidation of methane to methanol) to biodegrade chlorinated hydrocarbon. The organisms that are responsible for the degradation of compounds such a TCE do not derive energy from the transformation of the chemical, but instead the conversion is brought about by cometabolism with enzyme or cofactors produced by the microorganisms for other purposes. To do this, the methanotrophs use the enzyme methane monoxygenase to catalyze the oxidation of methane to methanol. This enzyme is not very specific, it will oxidize TCE to an unstable epoxide that will undergo decomposition to yield a variety of products

including carbon monoxide, glyoxylic acid and a range of chlorinated acids (Hickey and Smith, 1996; Walsh, 1999).

Chapter Two
Experimental

2.1. Location of Study

The Sarir- Tobruk oil transport pipeline was selected for this study as show in figure 3.1. Sarir to Tabruk is a pipeline of 34 inch diameter and 513.6 km long. This pipeline is used for transporting Sarir oil from Sarir field to Harega oil export terminal which is located at Tobruk city. The soil samples have been collected from ten locations along this pipeline. The soil samples were mixed together and used for further study.

Fig.2.1: Elsarrir-Tobruk Pipeline map

2.2. Method of Collection of Soil Samples

Soil samples were collected randomly from ten sites along the Sarrir-Tobruk pipeline from a depth of 10 cm. The soil samples (about 50 kg each) were collected in a sterilized container and stored at room temperature.

2.3. Preparation of Sample

All soil samples which were collected were mixed together. The soil was analyzed and its pH was measured at room temperature. The characteristics of soil is given in the following table:

Table 2.1: Characteristics of soil samples

Test	**Result**
Granular hierarchy	Medium 100%-55%
	Fine 55%-5.21%
Qualitative weight	18300 Kg/m^3
permeability	$4.42\times10^{3-}$ cm/sec
Average specific gravity	2.61
pH	7.00
Holding Capacity	8%

2.4. Materials Used

2.4.1. Reagents Used

- Dicholoromethan - B.D.H., Analar grade
- Ammonium nitrate (NH_4NO_3) - B.D.H. Reagent grade
- Dipotassium hydrogen phosphate (K_2HPO_4) - B.D.H. Reagent grade
- Mercuric chloride ($HgCl_2$) - B.D.H. Reagent grade
- Product oil:
    - ✓ Motor gasoline
    - ✓ Demestics kerosene
    - ✓ Diesel fuel
- Distillation water

2.4.2.Glass ware

- Separator funnel (100 ml) (Figure 2.2)
- Beakers (25,50 ml)
- Funnel
- Cylinder(50 ml)

Fig.2.2: Separator funnel.

2.4.3. Apparatus

- Balance
- Incubator show in Figure 2.3
- Soxhlet extraction
- Rotary evaporator
- GC

Figure 2.3. Incubator

2.5. Sources of Hydrocarbons Used in this Study

Kerosine, diesel fuel and gasoline were selected for use in this study. All fuel products were received from Sarrir refinery. The fuel products were initially characterized and the results are shown in Tables 2.2 – 2.4.

Table 2.2: Analysis of kerosene used in biodegradation

Test Description	Result	Test Method
Specific gravity at 15/15 ^{o}C	0.7069	ASTM D1298
Total acidity, mgKOH/g	0.003	ASTM D3242
Total sulphur, Wt.%	0.015	ASTM D1266
Mercaptan sulphur, wt.%	0.0001	ASTM D1219
Distillation Initial boiling point, ^{o}C 10% volume recovered at ^{o}C 20% volume recovered at ^{o}C 50% volume recovered at ^{o}C 90% volume recovered at ^{o}C Final boiling point, ^{o}C	 151 171 178 198 233 250	ASTM D86
Freezing point, ^{o}C	-53	ASTMD2386
Viscosity at -20 ^{o}C, cSt	4.3	ASTM D445
Calorific value, kcal/kg	10987	ASTM D240
Smoke point, mm	28	IP 57

Table 2.3: Analysis of diesel fuel used in biodegradation study

Test Description	Result	Test Method
Specific gravity at 15/15 ^{o}C	0.8323	ASTM D1298
Kinematic viscosity at 37.8 ^{o}C	3.476	ASTM D445
Flash point, ^{o}C	63	ASTM D93
Pour point, ^{o}C	0	ASTM D97
Total sulphur, wt.%	0.27	ASTM D129
Total acidity, mgKOH/g	0.053	ASTM D3242
Distillation Initial boiling point, ^{o}C 10% volume recovered at ^{o}C 20% volume recovered at ^{o}C 30% volume recovered at ^{o}C 40% volume recovered at ^{o}C 50% volume recovered at ^{o}C 60% volume recovered at ^{o}C 70% volume recovered at ^{o}C 80% volume recovered at ^{o}C 90% volume recovered at ^{o}C Final boiling point, ^{o}C	202 260 275 285 294 302 311 321 335 353 365	ASTM D86
Gross calorific value, kcal/kg	10945	ASTMD240

Table 2.4: Analysis of motor gasoline used in biodegradation

Test Description	Result	Test Method
Specific gravity at 15/15 ^{o}C	0.7548	ASTM D1298
Total sulphur, mg/kg	64.3	ASTM D4294
Reid Vapour Pressure (Rvp), kPa	62.99	ASTM D323
Distillation Volume Recovered at 70 ^{o}C, Vol.% Volume Recovered at 100 ^{o}C, Vol.% Volume Recovered at 150 ^{o}C, Vol.% Final Boiling Point, ^{o}C Residue, Vol.%	 35.7 62 92.7 172.7 0.5	ASTM D86
RON	95.0	ASTM D2699
Benzene Content, Vol.%	4.8	ASTM D4420

2.6. Chemical Characterization of Kerosine and Diesel

2.6.1. Chemical Characterization of Kerosine

For class separation, kerosene was separated into saturates, olefins and aromatics by using standard procedure [ASTM 1319]. A kerosene sample (0.75 mL) was introduced into a glass adsorption column packed with silica gel. A small layer of the silica gel contains a mixture of fluorescent dyes. When all the sample has been adsorbed on the gel, isopropyl alcohol was added to desorb the sample and force it down to column through air pressure. The hydrocarbons were separated according to their adsorption affinities into aromatics, olefins and saturates. The fluorescent dyes were also separated selectively, with the hydrocarbon types and made the boundaries of the aromatics, olefins, and saturate zones visible under violet light.

Table 2.5: Compositional analysis of kerosene

Class Composition	**Result (Vol.%)**	**Carbon Range**
Olefins	87.7	$C_8 - C_{15}$
Aromatics	12.3	

2.6.2. Chemical Characterization of Diesel

Diesel fuel was separated into aromatics and non-aromatics by using standard procedure described in ASTM D2549 method. A weighed amount (10g) of sample was charged to the top of a glass chromatographic column packed with activated bauxite and silica gel. Normal pentane (150 mL) was added to the column to elute the non aromatics. When all of the non-aromatics were eluted, the aromatic fraction was first eluted by additions of diethyl ether (100 mL), then chloroform (100 mL) and in the last by ethyl alcohol (175 mL). The solvents were completely removed by evaporation, and the residues were weighed and calculated as aromatics and non-aromatics fractions of the sample.

Table 2.6: Compositional analysis of diesel fuel

Class Composition	**Result (Wt. %)**	**Carbon Range**
Non-aromatics	81.8	$C_{10} - C_{25}$
Aromatics	18.2	

2.7. Experimental Set Up

The mixed soil samples which were collected from the locations defined earlier were divided into three parts and used separately in the following experiments:

- **Untreated Samples (US) -** These experiments will allow degradation of hydrocarbons in soil due to microbes present in soil itself.

- **Treated Samples (TS) -** These experiments will allow degradation of hydrocarbons in soil due to microbes present in soil and having better conditions for biodegradation. The samples contain some nutrients (nitrogen and phosphorous containing compounds), moisture and aeration.

- **Poisoned Samples (PS) -** These experiments do not allow and degradation due to microbes since all microbes have been killed by adding poison (mercuric chloride). Any decrease in concentration of hydrocarbons is only due to evaporation.

2.8. Preparation and Incubation of Hydrocarbon Contaminated Samples

Collected soil samples were partially but not completely air dried to allow sieving (2 mm diameter openings) for uniform consistency, but without damaging their biological activity. The sieved soils were packed into glass columns (outer diameter, 25 mm ; length, 250 mm) at the bulk density of cores collected from the field. The resulting columns were 22 mm in diameter, and 150 mm in length (Fig 2.2). The lower ends of the columns were closed with a plug and a closable drain spout (Fig 2.4). After packing, water was added to the top of the column to adjust the moisture content of the soil to 50% of its holding capacity. Lime ($CaCO_3$) was added to semidry soil prior to column packing to adjust the pH to 7.5.

Nitrogen (NH_4NO_3) and phosphorous (K_2HPO_4) fertilizers were added to keep N and P concentration in soil maintained for biodegradation study. They were dissolved in water that was used for adjusting moisture content. Soil columns were contained with hydrocarbons (kerosene, diesel & gasoline) on top of the columns and allowing them to infiltrate by gravity flow.

The maximal application rate (100 mg per g of soil) was chosen so that it would not result in either hydrocarbon or water flowing out from the soil column. The evaporation of water during incubation was compensated for by weighing the prepared soil columns and distilled water to compensate for any weight loss during incubation. tilling of the soil columns was performed by inserting the stainless steel wire into the soil column 15 times. This treatment was much less effective in aerring the soil than conventional tilling in the fields. 2% mercuric chloride was used as biologically inactive poisoned control to differentiate losses from biodegradative losses.

Fig.2.4: Soil columns

2.9. Analytical Method

For each point of analysis, hydrocarbon in the soil of 10 g was extracted. Jet fuel, diesel fuel and gasoline were Soxhelet extracted for 6 hours by using dicholomethan ((Helaleh *et al*., 2001; Singh and Lin, 2009). Anhydrous sodium sulphate was added to the extraction of fuels to adsorb water. Extracts of hydrocarbons were brought to volume by removing solvent using rotary evaporator (fig 2.5). The hydrocarbon samples were analyzed by gas chromatography model 439 equipped with flame ionization detector. The column used was 25 meter long capillary column packed with Cp-Cil 5CB WCOT. Nitrogen was used as carrier gas. The flow rate of carrier gas was adjusted at 1 mL/min. The temperature of the detector and injection port was maintained 300 ^{o}C.

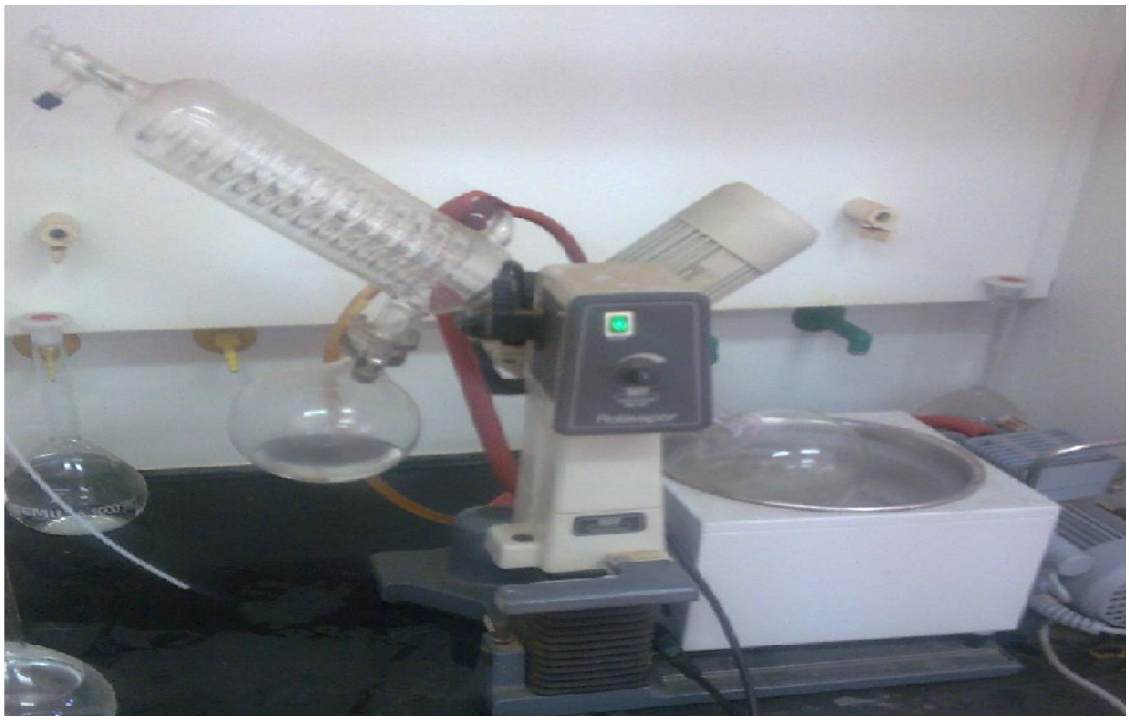


Fig. 2.5: Rotary evaporator

A gas chromatographic (GC) as shown figure 3.6 method for the determination of petroleum hydrocarbons in soil was evaluated (Delft et al., 1994). The extraction was carried out using minimal amounts of acetone and heptane, prior to a clean up with silica gel. The extraction procedure was tested by means of standard solutions of petroleum products and soil samples. The clean up procedure did not have any significant effect on the amounts of petroleum hydrocarbons present and hydrocarbons of natural origin were removed effectively. The recovery of the extraction and clean up procedure for petroleum products in soil was greater than 90%. The standard deviation for the repeatability was estimated to be less than 10% based on multiple analyses of homogenized soil samples. The detection limit for soil was determined to be 10 mg/kg dry matter. Comparing the GC method with the widely used infrared spectrometry (IR) method in combination with a Soxhlet-extraction using Freon-113, the results obtained are equivalent.

Fig. 2.6. Gas chromatographic

Chapter Three
Results & discussion

3.1. Introduction

A bioremediation study of degradation of hydrocarbons in soil was studied. The soil samples were collected from the sides of pipeline of Sarir to Tobruk oil export terminal (Hareiga Terminal). A bioremediation treatment that consisted fertilization (adjustment of nitrogen and phosphorous containing nutrients), tilling (aeration), temperature control was evaluated on the laboratory scale for its effectiveness in cleaning up soil contaminated by petroleum hydrocarbons. Experiments were performed at different incubation temperature, no treatment, bioremediation treatment, and poisoned evaporation control conditions. In this study degradation of hydrocarbons of fuels were measured. Remaining hydrocarbon concentrations in soil were determined quantitatively at different time intervals by using gas chromatographic technique. Nitrogen containing (ammonium nitrate, NH_4NO_3) and phosphorous containing (potassium dihydrogen phosphate, KH_2PO_4) fertilizers were added to soil to keep their required concentrations. They were mixed in soil samples that were used for bioremediation treated study. The soil was contaminated with hydrocarbons of fuels. Daily tilling to provide oxygen was performed by inserting the stainless steel wire into the soil and tilling eight times in each incubation sample. 100 ppm mercuric chloride was used as biologically inactive poisoned control to differentiate losses from biodegradative losses.

Total area of chromatographic peaks represents concentration of total hydrocarbons. Depletion curves were obtained for the prescribed experimental variables. The hydrocarbons concentrations under a variety of incubation conditions (untreated, bioremediation treated, poisoned) are given in Table 3.1and depletion of fuel hydrocarbon concentration is under defined incubation conditions are shown in Fig. 3.1.

Table 3.1: The degradation of hydrocarbons under various incubation conditions

Time (Weeks)	Untreated Soil Conditions		Bioremediation Treated Soil Conditions		Poisoned Soil Conditions	
	Total Peak Area	Concentration of Total Hydrocarbons (in g/kg of soil)	Total Peak Area	Concentration of Total Hydrocarbons (in g/kg of soil)	Total Peak Area	Concentration of Total Hydrocarbons (in g/kg of soil)
0	87854131	10.00	87854131	10.00	87854131	10.00
1	78336102	8.92	71337554	8.12	83598185	9.52
2	69752929	7.94	52976041	6.03	78216249	8.90
3	62009993	7.06	47177668	5.37	71878113	8.18
4	53721834	6.11	35405215	4.03	65433019	7.45
5	44190628	5.03	26180531	2.98	6193716	7.05
6	42169983	4.87	19591471	2.23	61322183	6.98

Total Hydrocarbons] = 2 g/200g of soil ; [Amount of Soil]= 200 g ; [NH_4NO_3] in case of Bioremediation treated soil = 6.00 mg ; [KH_2PO_4] = Only in case of bioremediation treated soil = 26.00 mg ; Incubation Temperature, 30 ^oC ; Moisture, 50% of holding capacity

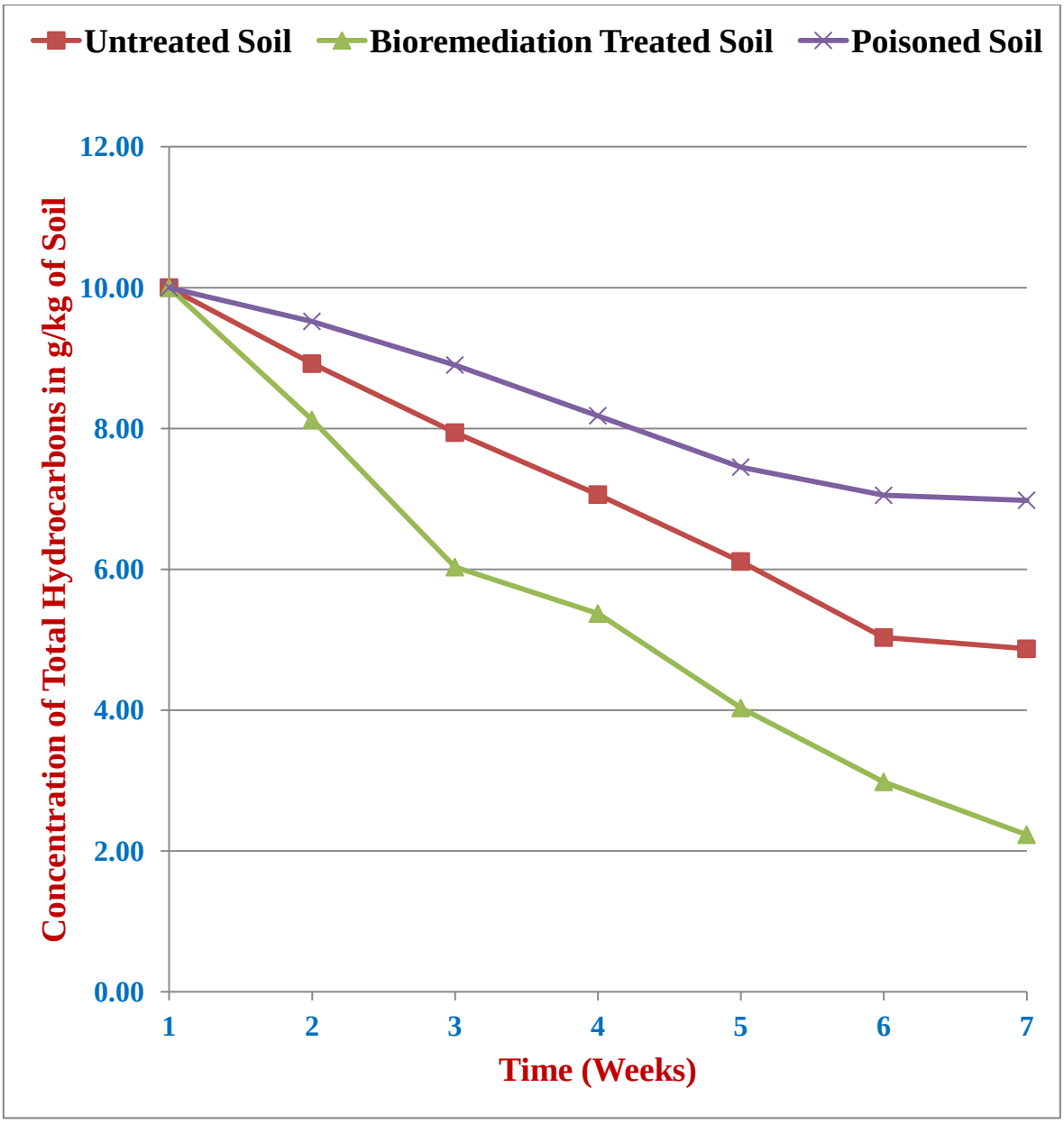


Fig. 3.1: Depletion Curve of Hydrocarbons at Different Incubation Conditions

Incubation Conditions:

[Total Hydrocarbons] = 2 g/200g of soil ; [Amount of Soil] = 200 g ; [NH_4NO_3] in case of Bioremediation treated soil = 6.00 mg ; [KH_2PO_4] = Only in case of bioremediation treated soil = 26.00 mg ; Incubation Temperature, 30 ^oC ; Moisture, 50% of holding capacity

3.2. Kinetics of Bioremediation

In order to determine order of reaction of degradation of hydrocarbons under the incubation conditions, experiments have designed to determine the concentration of hydrocarbons at different time intervals. The gas chromatographic technique is used for determination of concentration of hydrocarbons. It has been established that the hydrocarbon concentration is directly proportional to the total area of the GC peaks. The experiments have been designed to contaminate soil with different initial concentrations of hydrocarbons and keeping all other variables such as amount of nutrients, incubation temperature, moisture content constant. Three sets of experiments have been performed by using different initial total hydrocarbon concentrations. In all these three sets only initial concentration of hydrocarbons were changed and all other variables kept constant. The concentrations of hydrocarbons were determined at different time intervals. The results are presented in Table 3.2. The log of concentration of hydrocarbons determined at different time intervals were plotted against time which gave fairly good straight lines shown in Fig.3.2. This confirms first order dependence of rate of bioremediation as described below:

$$-\frac{d[Hydrocarbons]}{dt} = k_1[Hydrocarbons] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

$$or, \quad -\frac{d[Hydrocarbons]}{[Hydrocarbons]} = k_1 dt \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.2)$$

(Abbassi and Shquirat, 2007)

Where, k_1 is first order rate constant.

$$-ln[Hydrocarbons] = k_1 t + constant$$

$$-2.303\ log[Hydrocarbons] = k_1 t + constant$$

$$log[Hydrocarbons] = k_{obsd} t + constant \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

where

$$k_{obsd} = {}^{k_1}/_{2.303} \qquad or \qquad k_1 = -2.303\ k_{obsd}$$

When t = 0 ; [Hydrocarbons] = $[Hydrocarbons]_o$

Under these conditions, equation (3.3) becomes

$$log[Hydrocarbons] = k_{obsd} t + [Hydrocarbons]_0 \ldots\ldots\ldots\ldots\ldots\ldots (3.4)$$

The above equation is a form of straight line which suggests that the plot of log [Hydrocarbon] versus time should be a straight line for the first order bioremediation reaction. The same observations have been observed in experiments of degradation of hydrocarbons in soil under bioremediation incubation conditions (Fig. 3.2). This confirms first order dependence of rate of reaction. The slope of the plot will give k_{obsd} (or $– k_1/2.303$) and intercept will give initial hydrocarbon concentration ($[Hydrocarbons]_o$). The values of first order rate constant (k_1) were calculated in these experiments and presented in Table 3.2. The negative sign is simply an indicative of decreasing order.

Table 3.2: Hydrocarbon concentrations at different time intervals during bioremediation treatment

Time (Weeks)	Set 1 [Initial Total Hydrocarbons] = 5.0 g/kg of Soil			Set 2 [Initial Total Hydrocarbons] = 7.5 g/kg of Soil			Set 3 [Initial Total Hydrocarbons] = 10.0 g/kg of Soil		
	Peak Area	[Total HC]	Log [Total HC]	Peak Area	[Total HC]	Log [Total HC]	Peak Area	[Total HC]	Log [Total HC]
0	43927065	5.00	0.70	65890598	7.50	0.88	87854131	10.00	1.00
1	34175257	3.89	0.59	51746083	5.89	0.77	71337554	8.12	0.91
2	26531947	3.02	0.48	47177668	5.37	0.73	52976041	6.03	0.78
3	20557867	2.34	0.37	32681737	3.72	0.57	47177668	5.37	0.73
4	15638035	1.78	0.25	25301989	2.88	0.46	35405215	4.03	0.60
5	12123870	1.38	0.14	19679325	2.24	0.35	22051387	2.98	0.47
6	9400392	1.07	0.03	15638035	1.78	0.25	19327909	2.23	0.35
	$k = 2.55 \times 10^{-5}$ min.$^{-1}$			**$k = 2.40 \times 10^{-5}$ min.$^{-1}$**			**$k = 2.44 \times 10^{-5}$ min.$^{-1}$**		

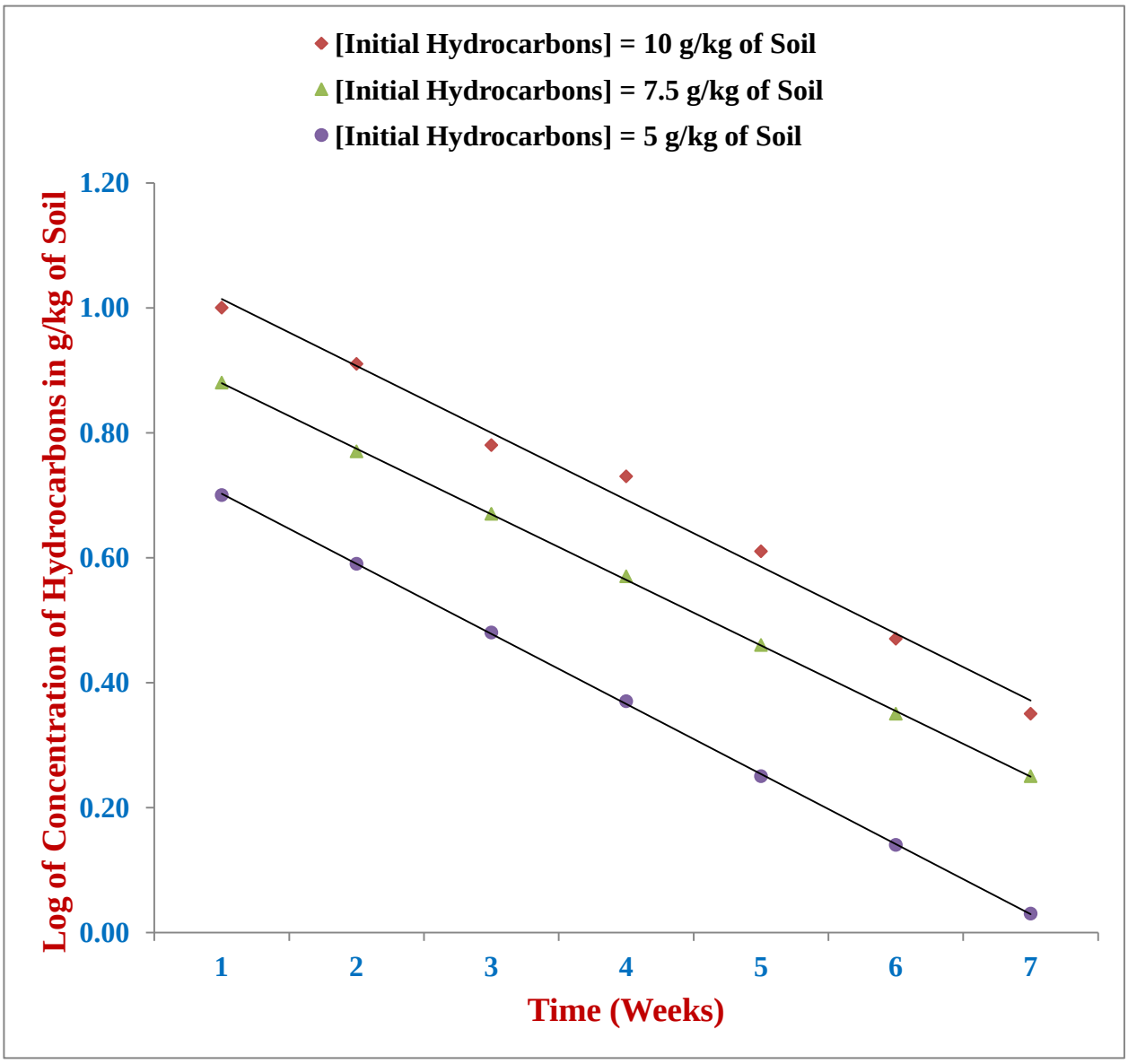


Fig. 3.2: First order dependence of decomposition of hydrocarbons under bioremediation conditions.

[Amount of Soil] = 200 g ; [NH_4NO_3] = 6.00 mg; [KH_2PO_4] = 26.00 mg ; Moisture, half of the holding capacity ; Incubation Temperature, 30 ^oC

3.3. Effect of Incubation Temperature on Rate of Bioremediation

The effect of temperature on the rate of bioremediation of hydrocarbons in soil has been studied by changing the incubation temperature keeping all other variables constant. It has been observed that the temperature has pronounced effect on the rate of bioremediation of hydrocarbons. The concentrations of hydrocarbons have been determined by chromatographic techniques at different time intervals. The results are reported in Tables 3.3 to 3.7 and plotted in Figs. 3.3 to 3.7 as shown below:

Table 3.3: The bioremediation of hydrocarbons at 25 ^{o}C incubation temperature

Time (Weeks)	Total Peak Area (Chromatographic Determination)	Concentration of Total Hydrocarbons in g/kg of soil	Log of Total Hydrocarbon Concentration
0	87854131	10.00	1.00
1	73094637	8.32	0.92
2	60795058	6.92	0.84
3	49374022	5.62	0.75
4	41115733	4.68	0.67
5	37513714	4.27	0.63
6	27146926	3.09	0.49

Amount of hydrocarbons added to soil = 2g ; Amount of soil = 200 g Amount of NH_4NO_3 Added to soil = 6.00 mg ; Amount of KH_2PO_4 added to soil = 26.00 mg

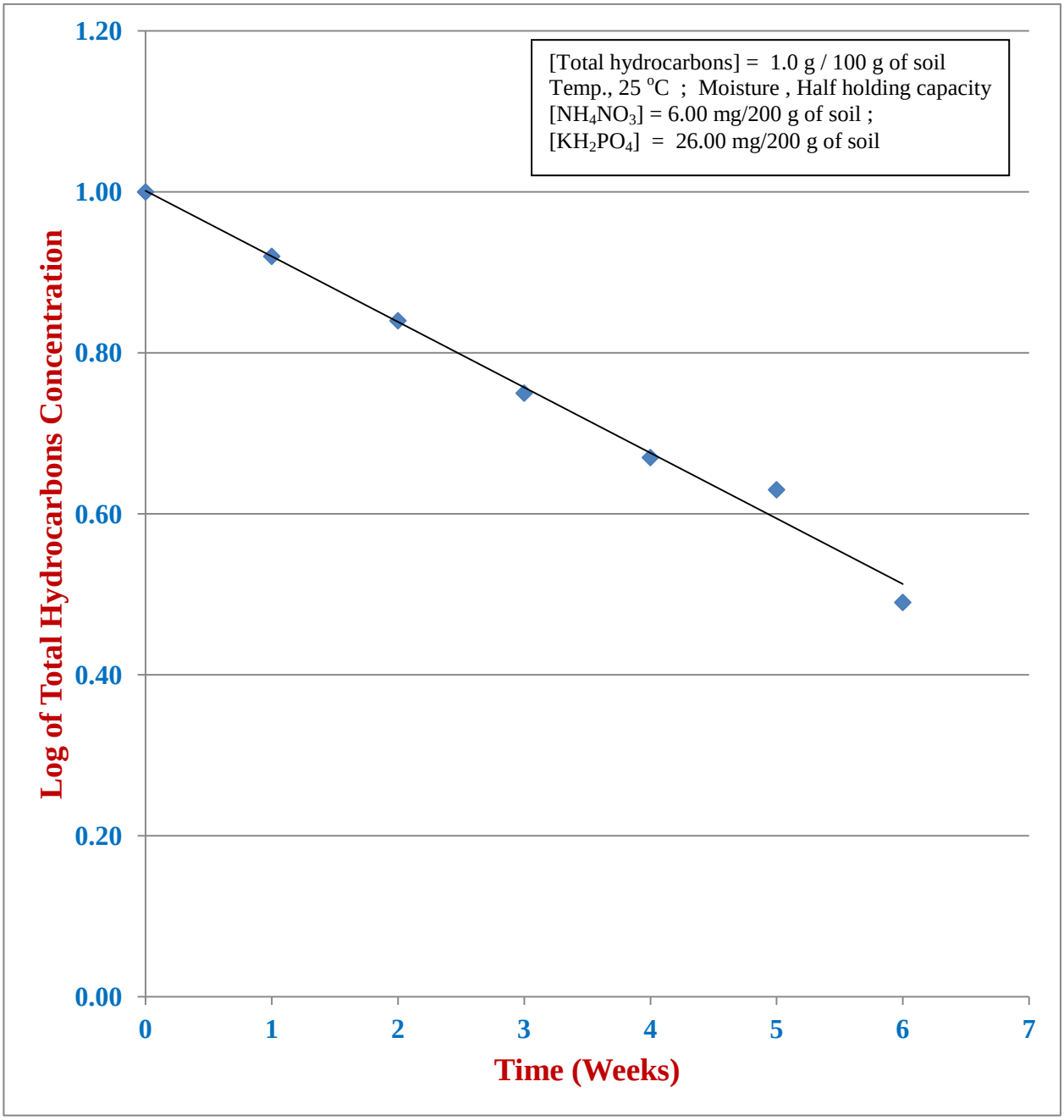


Fig. 3.3: First order dependence of bioremediation rate of hydrocarbons at 25 °C incubation temperature

Table 3.4: First order dependence of bioremediation of hydrocarbons at 30 ^{o}C Incubation Temperature

Time (Weeks)	Total Peak Area (Chromatographic Determination)	Concentration of Total Hydrocarbons in g/kg of soil	Log of Total Hydrocarbon Concentration
0	87854131	10.00	1.00
1	71337554	8.12	0.91
2	52976041	6.03	0.78
3	47177668	5.37	0.73
4	35405215	4.03	0.60
5	22051387	2.98	0.47
6	19327909	2.23	0.35

[Total Hydrocarbons] = 2g in 200 g of Soil ; [Amount of Soil] = 200 g ; [NH_4NO_3] = 6.00mg; [KH_2PO_4] = 26.00 mg ; Moisture, half of the holding capacity

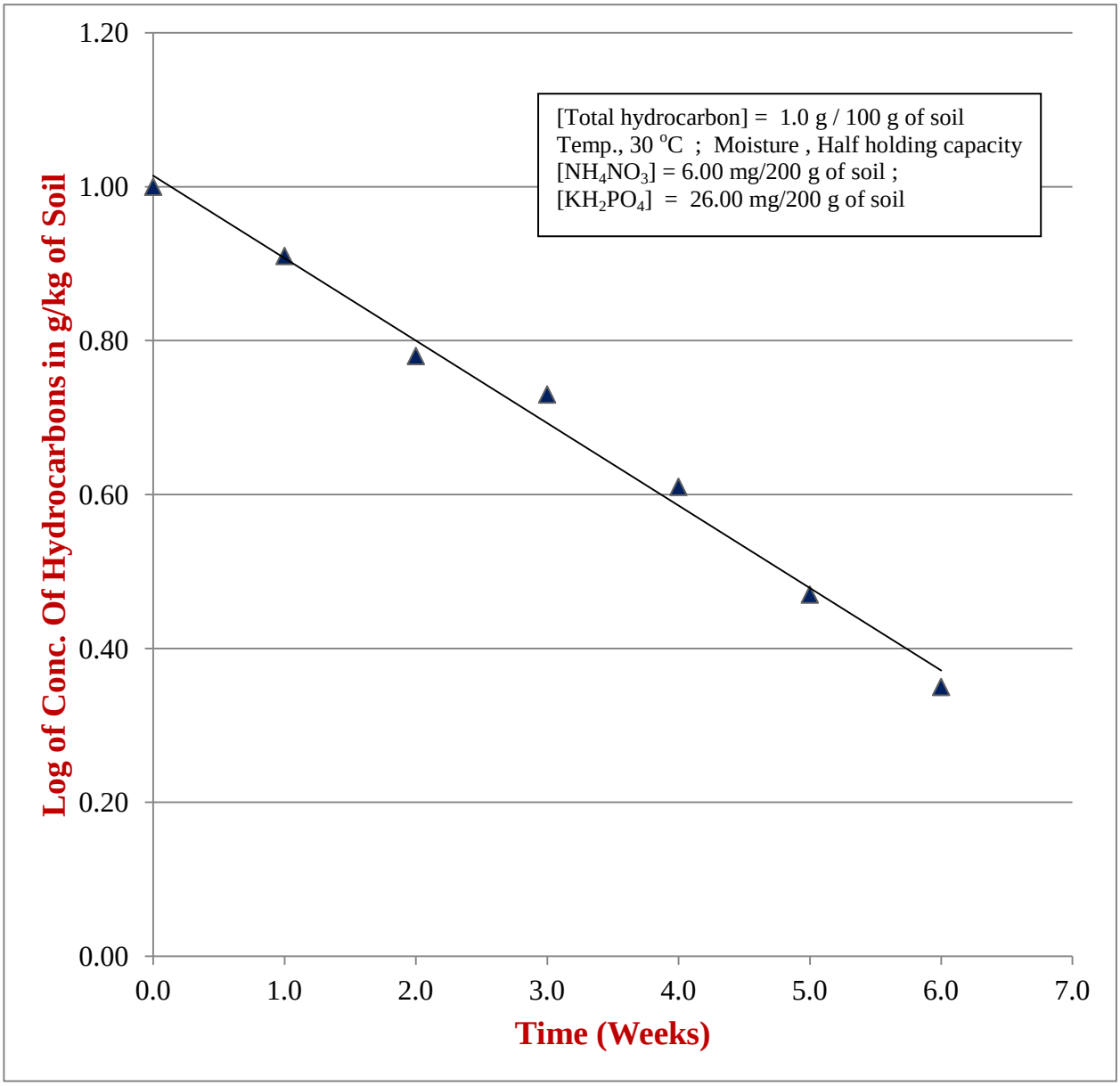


Fig. 3.4: First order dependence of bioremediation rate of hydrocarbons at 30 °C incubation temperature

Table 3.5: First order dependence of bioremediation of hydrocarbons at 35 ^{o}C Incubation Temperature

Time (Weeks)	Total Peak Area (Chromatographic Determination)	Concentration of Total Hydrocarbons in g/kg of soil	Log of Total Hydrocarbon Concentration
0	87854131	10.00	1.00
1	66681285	7.59	0.88
2	46123419	5.25	0.72
3	36635173	4.17	0.62
4	26531947	3.02	0.48
5	20118596	2.29	0.36
6	14583786	1.66	0.22

[Total Hydrocarbons] = 2g in 200 g of Soil ; [Amount of Soil] = 200 g ; [NH_4NO_3] = 6.00mg; [KH_2PO_4] = 26.00 mg ; Moisture, half of the holding capacity

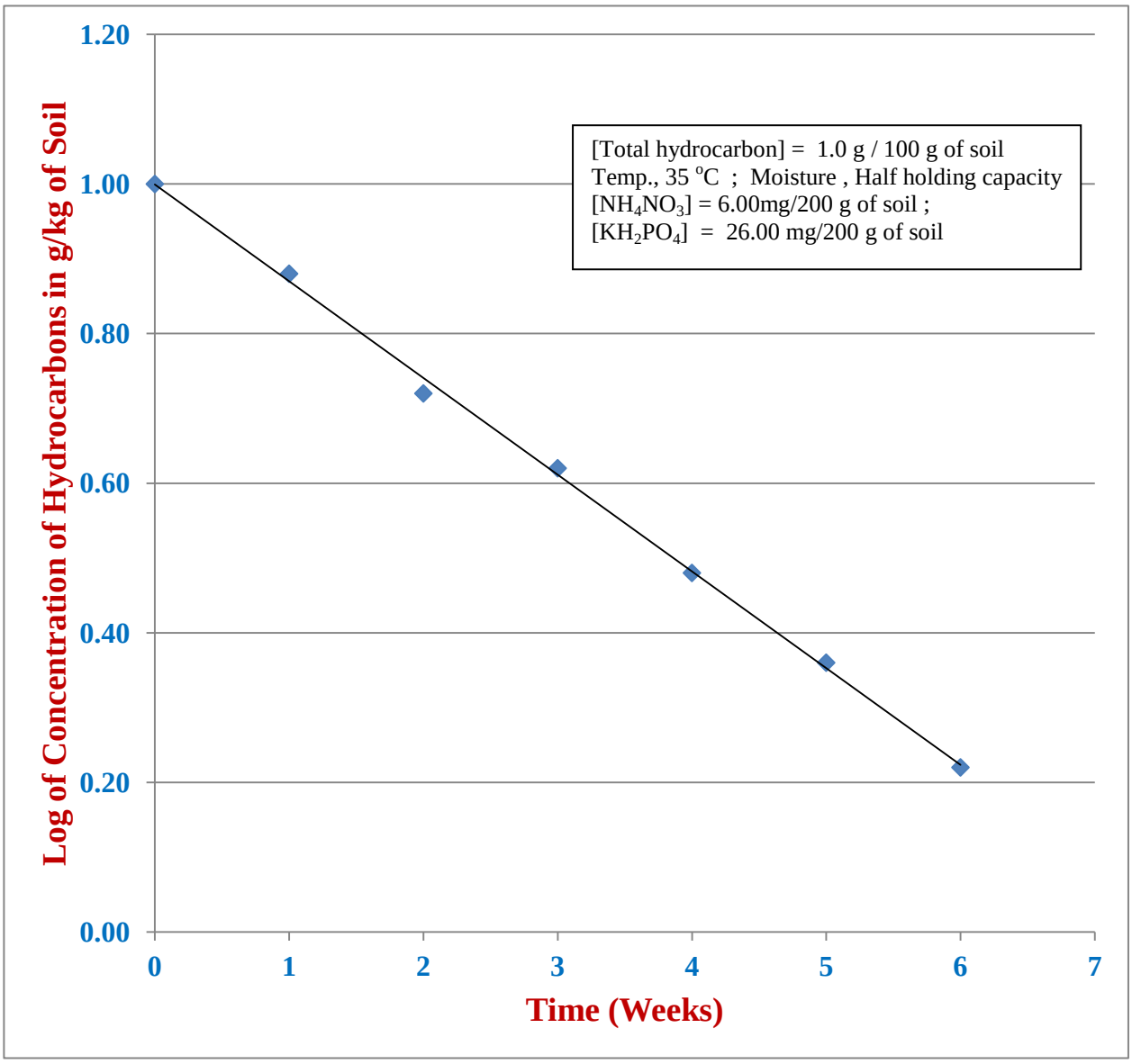


Fig. 3.5: First order dependence of bioremediation rate of hydrocarbons at 35 °C incubation temperature

Table 3.6: First order dependence of bioremediation of hydrocarbons at 40 ^{o}C Incubation Temperature

Time (Weeks)	Total Peak Area (Chromatographic Determination)	Concentration of Total Hydrocarbons in g/kg of soil	Log of Total Hydrocarbon Concentration
0	87854131	10.00	1.00
1	62200725	7.08	0.85
2	42082129	4.79	0.68
3	27146926	3.09	0.49
4	17482972	1.99	0.30
5	10542496	1.20	0.08

[Total Hydrocarbons] = 2g in 200 g of Soil ; [Amount of Soil] = 200 g ; [NH_4NO_3] = 6.00 mg; [KH_2PO_4] = 26.00 mg ; Moisture, half of the holding capacity

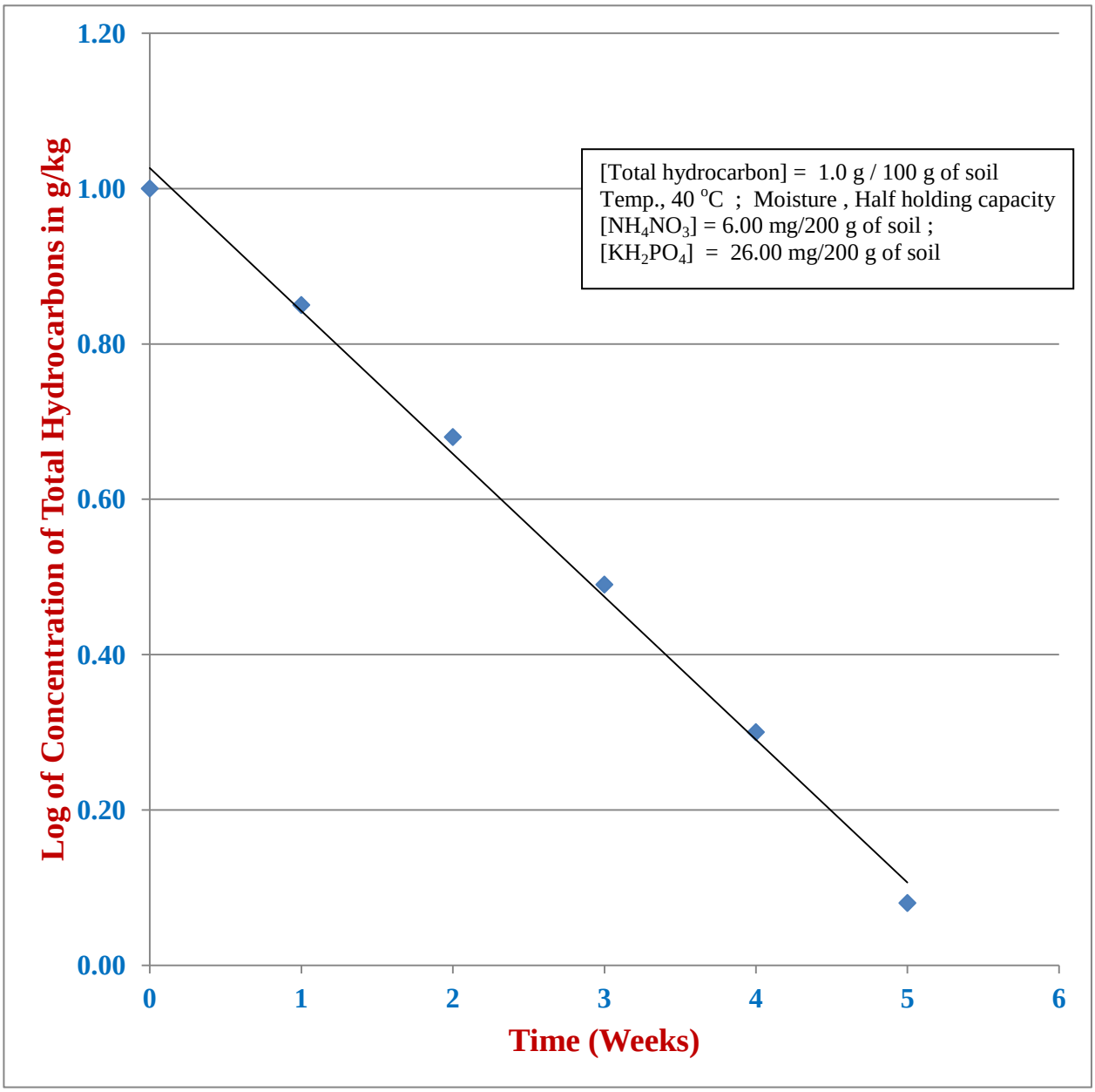


Fig. 3.6: First order dependence of bioremediation rate of hydrocarbons at 40 °C incubation temperature

Table 3.7: First order dependence of bioremediation of hydrocarbons at 45 ^{o}C Incubation Temperature

Time (Weeks)	Total Peak Area (Chromatographic Determination)	Concentration of Total Hydrocarbons in g/kg of soil	Log of Total Hydrocarbon Concentration
0	87854131	10.00	1.00
1	56753769	6.46	0.81
2	3384569	3.80	0.58
3	19679325	2.24	0.35
4	11596745	1.32	0.12

[Total Hydrocarbons] = 2g in 200 g of Soil ; [Amount of Soil] = 200 g ; [NH_4NO_3] = 6.00 mg; [KH_2PO_4] = 26.00 mg ; Moisture, half of the holding capacity

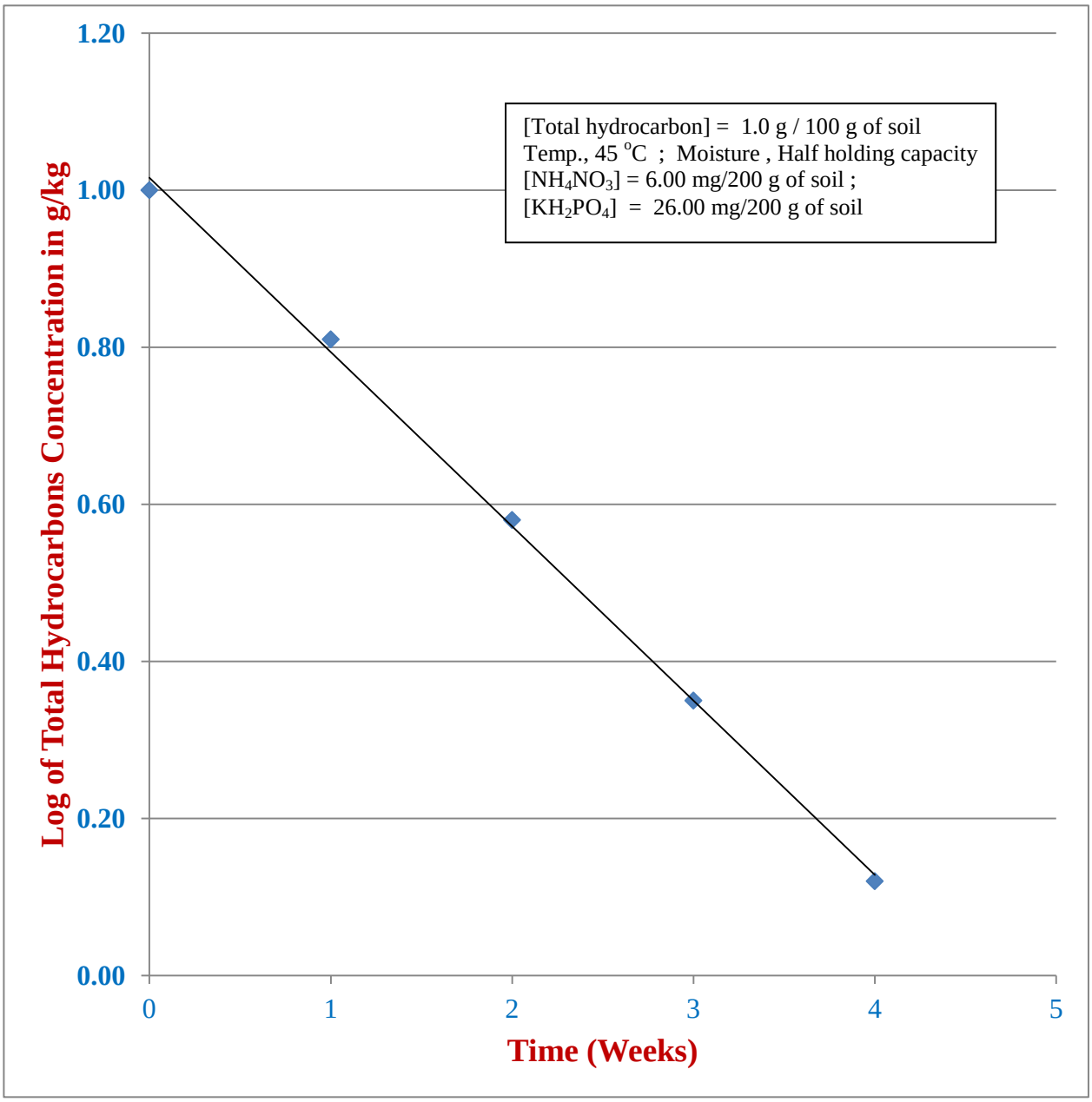


Fig. 3.7: First order dependence of bioremediation rate of hydrocarbons at 45 °C incubation temperature

3.4. Calculation of Activation Energy and Enthalpy of Reaction

The activation energy of bioremediation of hydrocarbons were determined by performing bioremediation reaction at five different temperatures ranging from 25^{o}C to 45^{o}C and keeping all other variables such as concentrations of nutrients, initial concentration of hydrocarbons, method of tilling and moisture content during the reaction. The hydrocarbon concentrations were determined at different time intervals every week for six weeks. The results are presented in Tables 3.3 to 3.7. The plots of log of concentration of total hydrocarbons versus time were plotted which gave straight lines. The first order rate constants were calculated from the slopes of these lines and tabulated in Table 3.8.

Table 3.8: Variation of First Order Rate Constants on Incubation Temperature

Incubation Temperature in ^{o}C	**First Order Rate Constant (k_1) in min^{-1}**
25	1.85 x 10^{-5}
30	2.44 x 10^{-5}
35	2.95 x 10^{-5}
40	4.20 x 10^{-5}
45	5.07 x 10^{-5}

[Total Hydrocarbons] = 2g in 200 g of Soil ; [Amount of Soil] = 200 g ; [NH_4NO_3] = 6.00 mg; [KH_2PO_4] = 26.00 mg ; Moisture, half of the holding capacity

The activation energy has been calculated by using Arrehenius equation (Chang, 2000) shown below:

$$k_1 = A\, e^{-E_a/RT} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

Where, k is first order rate constant

- A is Arrehenius Costant
- E_a is energy of activation in J
- R is gas constant J K^{-1} mol^{-1}
- T is temperature in Kelvins

Taking logarithm of equation (3.5) we get,

$$ln k_1 = \ln A - {E_a}/{RT}$$

or

$$2.303 \log k_1 = 2.303 \log A - {E_a}/{RT}$$

or

$$log k_1 = \log A - \frac{E_a}{2{,}303RT} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.6)$$

The plot of log k versus 1/T should be a straight line and the activation energy can be calculated from the slope of this line. The results of bioremediation experiments at five different temperatures are shown in Table 3.9. The plot of log k versus 1/T is also a straight line (Fig. 3.8). The activation energy has been calculated from the slope of this straight line which gave 40.34 kJ.

Table 3.9.: The rate constants of bioremediation reaction at different temperatures

Temperature (^oC)	**1/T (T in Kelvin)**	k_1 **(min.$^{-1}$)**	**5 + log** k_1
25	3.3557×10^{-3}	1.85×10^{-5}	0.2672
30	3.3003×10^{-3}	2.44×10^{-5}	0.3874
35	3.2467×10^{-3}	2.95×10^{-5}	0.4698
40	3.1949×10^{-3}	4.20×10^{-5}	0.6232
45	3.1447×10^{-3}	5.07×10^{-5}	0.7050

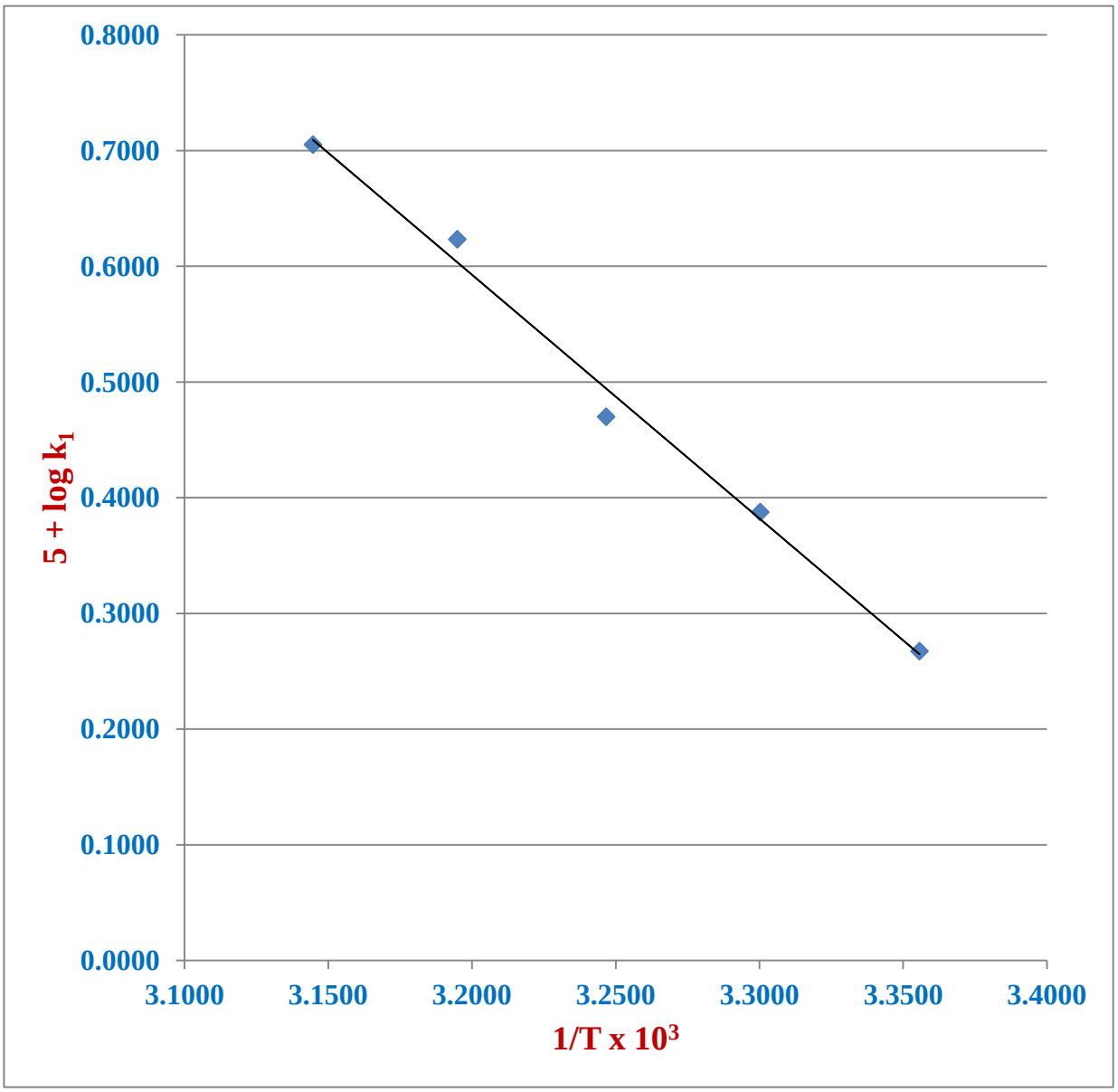


Fig. 3.8: The plot between log k_1 versus 1/T

From the Eyring equation (Chang, 2000)

$$k = \frac{k_B T}{h} \cdot e^{\left(-\Delta H^{\#}/RT\right)} \cdot e^{(\Delta S^{\#}/R)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.7)$$

Where, k is the rate constant;

k_B is Boltzmann's constant;

h is Planck's constant;

T is the temperature in Kelvin;

R is the gas constant

$\Delta H^{\#}$ and $\Delta S^{\#}$ are the enthalpy and entropy of activation, respectively.

Taking natural log of eqn. (3.7), becomes,

$$lnk = ln(K_B/h) + lnT - (\Delta H^{\#}/RT) + (\Delta H^{\#}/R) \ldots\ldots (3.8)$$

Differentiating eqn. (3.8) with respect to T,

$$dlnk/dT = \frac{1}{T} + \frac{\Delta H^{\#}}{RT^2} = \frac{(RT+\Delta H^{\#})}{RT^2} \ldots\ldots\ldots\ldots\ldots (3.9)$$

and

$$d(1/T)/dT = -\frac{1}{T^2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.10)$$

Dividing eqn. (3.9) by eqn. (3.10),

$$\frac{dlnk}{dT}.\frac{dT}{d(1/T)} = \left[\frac{(RT + \Delta H^{\#})}{RT^2}\right].\left[-\frac{T^2}{1}\right]$$

Therefore,

$$\frac{dlnk}{d(1/T)} = \frac{(-RT + \Delta H^{\#})}{R} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.11)$$

On the other hand the Arrhenius equation is given in eqn. (3.5) as

$$k = A.e^{(-E_a/RT)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

$$lnk = lnA - \frac{E_a}{RT} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.12)$$

Differentiating above eqn. with respect to (1/T),

$$dlnk/d(1/T) = -E_a/R \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.13)$$

Comparing eqns. (3.11) and (3.13), we get,

$$-\frac{E_a}{R} = -\frac{(RT + \Delta H^{\#})}{R}$$

Or,

$$E_a = \Delta H^{\#} + RT \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.14)$$

Using eqn. (3.14) and energy of activation calculated earlier as 40.34 kJ mol^{-1}, enthalpy of activation ($\Delta H^{\#}$) has been calculated which gave value as 37.86 kJ mol^{-1} at 25ºC.

3.5. Calculation of Entropy of Activation ($\Delta S^{\#}$) and Free Energy of Activation ($\Delta G^{\#}$)

Recalling Eyring's equation

$$k = \frac{k_B T}{h} . e^{\left(-\Delta H^{\#}/_{RT}\right) . e^{\left(\Delta S^{\#}/R\right)}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.7)$$

Or,

$$k/T = K_B/h \, . e^{\left(-\Delta H^{\#}/RT\right)} . e^{\left(\Delta S^{\#}/R\right)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.15)$$

Taking natural log of above equation, we get,

$$ln(k/T) = \ln (K_B/h) - \Delta H^{\#}/RT + \Delta S^{\#}/R \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.16)$$

or,

$$2.303 log(k/T) = 2.303 \log (K_B/h) - \Delta H^{\#}/RT + \Delta S^{\#}/R$$

$$log(k/T) = -\Delta H^{\#}/2.303RT + [log(K_B/h) + \Delta S^{\#}/R] \ldots\ldots (3.17)$$

The above equation suggests that log (k/T) versus 1/T is an equation of straight line and the intercept of this line is [log (k_B/h) + $\Delta S^{\#}$/(2.303 R)]. From intercept of this equation entropy of activation ($\Delta S^{\#}$) can be calculated. The experimental data of log (k/T) and 1/T are summarized in Table 3.10.

Table-3.10: Data of log(k/T) and 1/T for calculation of $\Delta S^{\#}$ and $\Delta G^{\#}$

Temperature (T) (^{o}K)	Rate Constant (k) (min^{-1})	1/T	log (k/T)
298	1.85×10^{-5}	0.003355705	-7.207044536
303	2.44×10^{-5}	0.00330033	-7.094052802
308	2.95×10^{-5}	0.003246753	-7.018728701
313	4.20×10^{-5}	0.003194888	-6.872295047
318	5.07×10^{-5}	0.003144654	-6.797419161

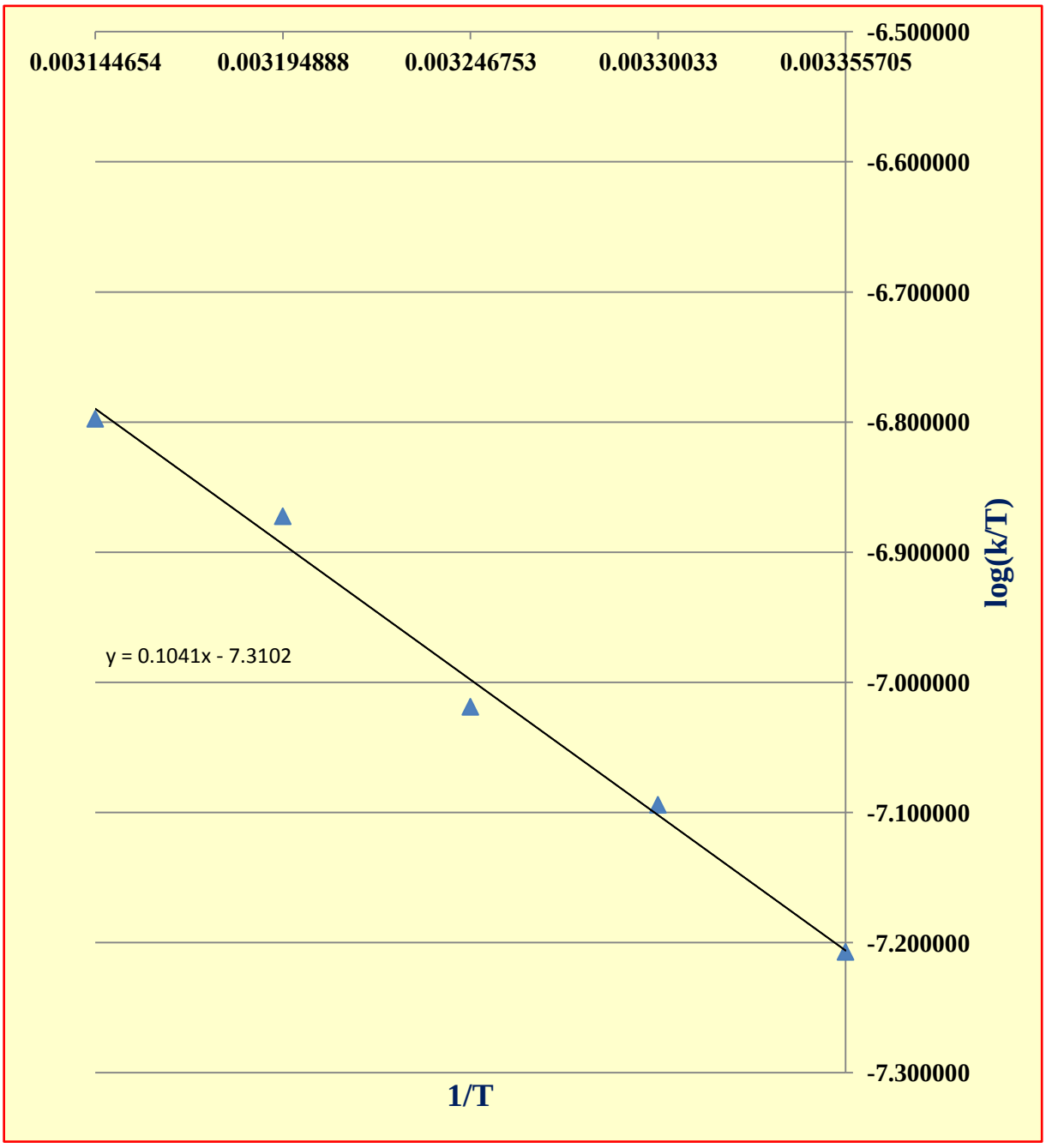


Fig. 3.9: The Plot Between log(k/T) versus T

The intercept of the plot was found to be – 7.310 and the entropy of activation has been calculated as below.

$$log(K_B/h) + \Delta S^{\#}/2.303R = -7.310$$

$$log\left(\frac{1{,}381 \times 10^{-23}}{6.62608 \times 10^{-34}}\right) + \frac{\Delta S^{\#}}{2.303 \times 8.314} = -7.310$$

$$\frac{\Delta S^{\#}}{2.303 \times 8.314} = -7.310 - log\left(\frac{1{,}381 \times 10^{-23}}{6.62608 \times 10^{-34}}\right)$$

$$\Delta S^{\#} = -337.54 \text{ J K}^{-1} \text{ mol}^{-1}$$

Or, $$\Delta S^{\#} = -0.3375 \text{ kJ K}^{-1} \text{ mol}^{-1}$$

Therefore, the value of entropy of activation is - 0.3375 kJ $K^{-1}mol^{-1}$.

The free energy of activation ($\Delta G^{\#}$) has been calculated by using following equation,

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.18)$$

Substituting the value of $\Delta H^{\#}$ = 37860 J K^{-1}; $\Delta S^{\#}$ = - 337.54 J K^{-1} mol^{-1} ; and T =298, the value of $\Delta G^{\#}$ was calculated to be 138.45 kJ at 25ºC.

Therefore, the thermodynamic parameters calculated for bioremediation reaction of hydrocarbons are summarized in Table 3.11.

Table 3.11: Thermodynamic parameters of reaction of bioremediation of petroleum hydrocarbons

Thermodynamic Parameter	Calculated Value
Energy of activation (ΔE_a)	40.34kJ
Enthalpy of activation ($\Delta H^{\#}$)	37.86kJ mol^{-1}
Entropy of activation ($\Delta S^{\#}$)	- 0.3375kJ K^{-1} mol^{-1}
Free energy of activation ($\Delta G^{\#}$)	138.45 kJ

3. 6. Comparative Rate of Bioremediation of Different Type of Fuels

For determination of comparative rate of bioremediation of different fuels, motor gasoline, kerosene and diesel fuel were selected. The properties and chemical composition of these fuels are described in experimental section. The bioremediation study of these fuels were performed at the same incubation conditions. Experiments have been designed to determine total hydrocarbon concentrations at different time intervals. Table 3.12 represents the half-lives of depletion of concentration of total hydrocarbons under various incubation conditions. The half-life is simply the time needed to reduce the total hydrocarbon concentrations in soil to 50% of the initial amount. If a 50% reduction is not achieved within the time period of the experiment.

Table 3.12: Half-life of depletion of total hydrocarbons at different incubation conditions

Incubation Temperature (^oC)	Motor Gasoline	Kerosine	Diesel
	Half Live in Weeks		
25	3.0	4.0	5.0
30	2.8	3.2	4.0
35	2.0	3.0	3.5
40	1.8	2.1	3.0
45	1.0	1.5	2.8

Amount of hydrocarbons added to soil = 2g ; Amount of soil = 200 g;Amount of NH_4NO_3 Added to soil = 6.00 mg ; Amount of KH_2PO_4 added to soil = 26.00 mg

Above table indicates that the incubation temperature has great influence on the degradation of hydrocarbons under bioremediation conditions. The bioremediation rate is increases as the temperature is increases. In other words it takes lesser time to reduce half of the total hydrocarbon concentration as temperature is increases. Moreover, as the molecular weight of hydrocarbons decreases the rate of bioremediation increases. The half lives of bioremediation are in the order of motor gasoline < kerosene < diesel.

Conclusions and Recommendations

CONCLUSIONS

1. The degradation of hydrocarbons was faster in case of bioremediation treated soils. The half-lives of degradation of hydrocarbons at different incubation temperatures have been calculated. The bioremediation rate is increases as the temperature increases.

2. The order of reaction of degradation of hydrocarbons were determined by examining the hydrocarbon concentration at different time intervals and keeping all other variables such as incubation temperature, moisture content, concentration of nutrients and duration of tilling constant. First order dependence of bioremediation rate on total hydrocarbon concentration was observed. The rate constants have also been calculated.

3. The activation energy of bioremediation reaction has been determined by studying the degradation of hydrocarbons at different incubation temperature and keeping all other variables constant. The activation energy of bioremediation of petroleum hydrocarbons was found to be 40.34 kJ mol^{-1}.

4. The other thermodynamic parameters have also been calculated and found to be as follows:

    - Enthalpy of activation ($\Delta H^{\#}$) = 37.86 k J mol^{-1} at 25 ^{o}C
    - Entropy of activation ($\Delta S^{\#}$) = - 0.3375kJ K^{-1} mol^{-1}at 25 ^{o}C
    - Free energy of activation ($\Delta G^{\#}$) = 138.45 kJ at 25 ^{o}C

5. This study confirms that bioremediation technique can be successfully applicable for cleaning of hydrocarbon contaminated soil.

RECOMMENDATIONS

1- Continuing work on the study of contamination in the soil (reapplication of this research to the remaining distance not included in our research in this study).

2- Study clean soil bioremediation technology to other oil products are not used in our study or the study of bioremediation of crude oil.

3- The use of other types of nutrients as well as the study of types of specialized microorganisms in the bioremediation process.

References

***References*==========**

Abbassi, B. E, and Shquirat, W. D. (2007), "Kinetics of Indigenous Isolated Bacteria Used for Ex-Situ Bioremediation of Petroleum Contaminated soil", American-Eurasian J. Agric. Environ. Sci., Vol. 2(6), pp.761- 766.

Abu Baker, S., Farinazleen, M.G.; Abd Rahman, R. N. Z., and Mahiran, B. (2003), "Bioremediation of Petroleum Hydrocarbon Pollution", Ind. J. Biotechnol. Vol. 2(3), pp.411-425.

Acosta, M. V. and Tabatabai, M. A. (2001), "Arylamidase Activity in Soils Effect of Trace Elements and Relationships to Soil Properties and Activities to Amidohydrolases", Soil Biology and Biochemistry. Vol. 33, pp.17-23.

Alexander, M. (1961), "Introduction to Soil Microbiology", by John Wiley & Sons, 19 .

Alexander, M. (1977), "Introduction to Soil Microbiology", (2nd ed), John Wiley and Sons, Inc. New York, NY., pp.467.

Alexander, M. (1980), "Biodegradation of Chemicals of Environmental Concern", Science Vol. 211, pp.132–133.

***References*==========**

Alexander, M. (1994), "Biodegradation and Bioremediation", Academic Press, Inc. USA, pp. 1-260.

Alexander, M. (1999), "Biodegradation and Bioremediation", Academic Press, San Diego, pp. 200-202, 271-279, 282-283, 287.

Allan, K. A., Herbert, B. E., Morris, P. J. and Mc. Donald, T.J. (1997), "Biodegradation of Petroleum in Two Soils: Implications for Hydrocarbon Bioavailability", In Situ On-Site Bioremediat, Vol. 5, pp.629–634.

Anne, G. R., Braathen, H., Marion, B., Bjarne, E. and Per, K. (2003), "In Situ Biodegradation of Petroleum Hydrocarbons in Frozen Arctic Soils", Cold Regions Sci. Tech., Vol. 37 , pp.97– 120.

Atlas, R. M. (1981), "Microbial Degradation of Petroleum Hydrocarbons: An environmental perspective", Microbiol. Rev. vol.45, pp.180-209.

Atlas, R. M. (1995), " Bioremediation", C & EN Special Report. ACS Products services, American Chemical Society, Washington, DC.

Atlas, R. M. and Bartha, R. (1992), " Hydrocarbon Biodegradation and Oil Spill", Bioremediation.In: Advances in Microbial Ecology. Marshall K. C. Vol. 12. Plenum. New York,.USA, pp. 287-338.

***References*==========**

Atlas, R. M. and Cernigiia, C. E. (1995), "Bioremediation of petroleum pollutants, Bioscience, Vol. 45, pp.332-338.

Atlas, R. M. and Philp, J. (2005), "Bioremediation: Applied Microbial Solutions for Real-World Environmental Cleanup", ASM Press, Washington D.C., pp.1-292.

Ausma, S., Edwards, G. C., Fitzgerald, H. C. R. and Halfpenny-M, L. (2002), "Volatile Hydrocarbon Emissions from a Diesel Fuel Contaminated Soil Bioremediation Facility", Air Waste Manag. Association, Vol. 52, pp.769-780.

Ayotamuno, M. J., Kogbara, R. B., Ogaji, S. O. T. and Pobert, S.D. (2006), "Bioremediation of a Crude Oil Polluted Agricultural Soil Polluted at Port Harcourt", Nigeria. Appl. Energy, Vol. 83, pp.1249-1257.

Baker, H. A. and Herson, D.S. (1990), "In Situ Bioremediation of Contaminated Aquifers and Subsurface Soils", Geomicrobiol. J. Vol. 8, pp.133–146.

Baker, K. and Herson, D. (1994), "Bioremediation", McGraw-Hill, Inc. New York.

***References*==========**

Baker, R.J., Baehr, A.L. and Lahvis, M.A. (2000), " Estimation of Hydrocarbon Biodegradation Rates in Gasoline Contaminated Sediment from Measured Respiration Rates", J. Contam. Hydrol. Vol.41, pp.175– 192.

Bartha, R. (1984), "the Fate of Petroleum in Soil Ecosystems", In: Atlas, R. M. (ed.), Petroleum Microbiology. Macmillan Publishing Co, New York, pp.435–473.

Bartha, R. (1986), "Biotechnology of Petroleum Pollutant Biodegradation", Microb Ecol. vol.12, pp.155-172.

Bartha, R. and Bossert, I. (1984), "the Treatment and Disposal of Petroleum Refinery Wastes", In R. M. Atlas (ed.), Petroleum microbiology. Macmillan, New York. pp.553-577.

Bauer, J. E. and Capone, D.G. (1988a), "Nonlinear Parameter Estimation", M.A. Reading, Academic Press.

Bauer, J. E. and Capone, D.G. (1988b), "Effects of Co-occurring Aromatic Hydrocarbons on Degradation of Individual Polycyclic Aromatic", Environ. Toxicol. Chem. Vol. 25, pp.1755.

Beckles, D. M., Ward, C. H. and Hughes, J. B. (1998), " Effect of Mixtures of Polycyclic Aromatic Hydrocarbons and Sediments on Fluoranthene Biodegradation Patterns", Environ. Toxicol. Chem. Vol. 17, pp.1246–1251.

Boopathy, R. (2004), "Anaerobic Biodegradation of no. 2 Diesel Fuel in Soil: a Soil Column Study", Bioresource Tech. Vol. 94, pp.143–151.

Bossert, I. and Bartha, R. (1984), "The Fate of Petroleum in Soil Ecosystems", In: Atlas, R.M. (ed.), Petroleum Microbiology", Macmillan Publishing Co, New York. pp.435–473.

Bossert, I., and Bartha, R. (1986), "Structure- Biodegradability Relationships of Polycyclic Aromatic Hydrocarbons in Soil", Bull. Environ. Contam. Toxicol. Vol. 37, pp.490 – 495.

Bouchez, M., Blanche, D. and Vandecasteele, J. P. (1995), "Degradation of Polycyclic Aromatic Hydrocarbons by Pure strains and by Defined Strain Associations: Inhibition Phenomena and Cometabolism", Appl. Microbiol. Biotechnol. Vol. 43, pp.156-164.

Bouchez, M., Blanchet, D., Besnaiou, B., Leveau, J. Y. and Vandecasteele, J. P. (1997), "Kinetic Studies of Biodegradation of Insoluble Compounds by

Continuous Determination of Oxygen Consumption", J. Appl. Microbiol. Vol. 82, pp.310–316.

Boylan, D. B. and Tripp, B. W. (1971), "Determination of Hydrocarbons in Seawater Extracts of Crude Oil and Crude Oil Fractions", Nature (London) Vol. 230, pp.44-47.

Brady, N. C. and Weil, R. R. (1996), "The Nature and Properties of Soils. Prentice Hall: Upper Saddle River, New Jersey petroleum pollutant biodegradation", Microb. Ecol. vol.12, pp.155-172.

Burke, G., Singh, B. R. and Theodore, L. (2000), "Handbook of Environmental Management and Technology", John Wiley & Sons, Inc. pp.597.

Burnham, K. and Bey M. (1991), "Effects of Crude Oil and Ultraviolet Radiation on Immunity within Mouse Skin", J Toxicol Environ Health P.T. A. vol. 34 (1), pp. 83-93.

Burnham, K. and Rahman, M. (1992), "Effects of Petrochemicals and Ultraviolet Rediation on Epidermal lA Expression in Vitro", vol. 35 (3), pp. 175-185.

***References*==========**

Canter, L.W. and Knox, R.C. (1985), "Ground Water Pollution Control", Lewis Publishers, Chelsea, MI. pp.526.

Cerniglia, C. E. (1992), "Biodegradation of Polycyclic Aromatic Hydrocarbons", Biodegradation, vol.3, pp.35 1-368.

Chang, R. (2000), "Phsical Chemistry for the Chemical and Biological Sciences", University Sciences Books.

Chang, M. K., Voice, T.C. and Criddle, C.S. (1993), "Kinetics of Competitive Inhibition and Cometabolism in the Biodegradation of Benzene, Toluene, and p-xylene by Two Pseudomonas Isolates", Biotechnol. Bioeng. Vol. 41, pp.1057-1065.

Chaudhry, G. R. and Chapalamadugu, S. (1991), "Biodegradation of Halogenated Organic Compounds", Microb. Rev. Vol. 55, pp.59-79.

Chikere, C. B., Okpokwasili, G. C. and Chikere, B. O. (2009), "Bacterial Diversity in a Tropical Crude Oil-Polluted Soil Undergoing Bioremediation", African J. of Biotechnol. Vol. 8 (11), pp. 2535-2540.

***References*==========**

Chorom, M., Sharifi, H. S. and Motamedi, H. (2010), "Bioremediation of a Crude Oil - Polluted Soil by Application of Fertilizers", Iran. J. Environ. Health. Sci. Eng., Vol. 7, No. 4, pp. 319-326.

Clifton Associates Ltd., TransGas Ltd, (1999). "Environmental Site Investigation", Cantuar Field Scrubber Site, Cantuar, Saskatchewan. File R2789.

Committee on In Situ Bioremediation, (1993), "Water Science and Technology Board, Commission on Engineering and Technical Systems, and National Research Council", In Situ Bioremediation: When Does It Work? National Academy Press: Washington, D.C.

Cookson , J. T. Jr. (1995), "Bioremediation Engineering: Design and Application", McGraw Hill, Inc. New York.

Costa, R. M. and Malcata, F.X. (1994), "Multisubstrate Michaelis Menten Kinetics: Explicit Dependence of Substrate Concentration on Time for Batch Reactors", Bioprocess Eng. Vol. 10, pp.155-159.

Cunningham, C. J., Ivshina, I. B., Lozinsky, V. I., Kuyukina, M. S. and Philp, J. C. (2004), "Bioremediation of Diesel-Contaminated Soil by

Microorganisms Immobilised in Polyvinyl Alcohol", International Biodeterioration and Biodegradation Vol. 54, pp.167–174.

Delille, D. (2000), "Response of Antarctic Soil Assemblages to Contamination by Diesel Fuel and Crude oil", Microb. Ecol. Vol. 40, pp. 159-168.

Delille, D., Basse`res, A. and Dessommes, A. (1997), "Seasonal Variation of Bacteria in Sea Ice Contaminated by Diesel Fuel and Dispersed Crude Oil", Microb. Ecol. Vol. 33, pp.97–105.

Delillea, D., Coulona, F. and Pelletierb, E. (2004), "Effects of Temperature Warming During a Bioremediation Study of Natural and Nutrient-Amended Hydrocarbon-Contaminated Sub-Antarctic Soils", Cold Regions Sci. and Tech. Vol. 40, pp.61– 70.

Deschênes L.; Lafrance P.; Villeneuve J.-P. and Samson R., (1996). "Adding Sodium Dodecyl Sulfate and Pseudomonas areuginosa UaG 2 Biosurfactants Inhibits Polycyclic Aromatic Hydrocarbon Biodegradation in a Weathered Creosote Contaminated Soil". Appl. Microbiol. Biotechnol. Vol. 46, pp.638-646.

Deuel, L. E. (1991), "Evaluation of Lmiting Contituents Suggested for Land Disposal of Exploration and Production Wastes", Chevron Research and Technology Company, CH2M Hill, US, pp 38-40.

Dick, W. A. and Tabatabai, M. A. (1999), "Use of Immobilized Enzymes for Bioremediation", In American Society of Agronomy Inc. (ed.), "Bioremediation of Contaminated Soils", American Society of Agronomy Inc., Madison, Wisconsin.

Ding, G., Novak, J. M., Amarasiriwardena, D., Hunt P. G., and Xing, B. (2002), " Soil Organic Matter Characteristics as Affected by Tillage Management", Soil Sci. Soc. am. J., vol. 66, pp.421–429.

Dragun, J. (1998), "The soil Chemistry of Hazardous Materials", Amherst Scientific Publishers, Amherst MA.

Dzantor, E. K. (1999), "Bioremediation of Contaminated Soils - What it is and How to do it", University of Agriculture and Natural Resources. pp.1007-1019.

Efremenkoa, E. N., Azizova, R. E., Raevaa, A. A., Abbasovb, V. M. and Varfolomeyev, S. D. (2005), "An Approach to the Rapid Control of Oil Spill Bioremediation by Bioluminescent Method of Intracellular ATP Determination", International Biodeterioration & Biodegradation, Vol. 56, pp.94–100.

Ekpo, M. A. and Udofia, U. S. (2008), " Rate of biodegradation of crude oil by microorganisms isolated from oil sludge environment", Afr. J. of Biotechnol. Vol. 7 (24), pp. 4495-4499.

Evans, W. C., Fernley, H. N. and Griffiths, W.E. (1965), "Oxidative Metabolism of Phenenthrene and Anthracene by Soil Pseudomonads", J. Biochem. Vol. 95, pp. 819-831.

Eweis, J. B., Ergas, S. J., Chang, D, P. Y. and Schroeder, E. D. (1998), "Bioremediation Principles", Published by McGraw-Hill Companies", Inc.,pp. 86, 105, 109, 111-113.

Fan, C., Krishnamurthy, S. and Chen, C. T. (1994), "Soil Contaminated with Petroleum Constituents. A Critical Review of Analytical Approaches for Petroleum Contaminated Soil.

Ferguson, S. H., Franzman, P. D., Snape, I., Revill, A. T., Trefy, M. G. and Zappia, L. R. (2003), "Effects of Temperature on Mineralisation of Petroleum in Contaminated Antarctic Terrestrial Sediments", Chemosphere, Vol. 52, pp.975–987.

Frankenfeld, J. W. (1973), "Factors Governing the Fate of Oil at Sea, Variations in The amounts and Types of Dissolved or Dispersed materials During the Weathering process", In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C. pp.485-495.

Freeze, R. A. and Cherry, J. A. (1979). "Groundwater". Prentice-Hall, Inc., Englewood Cliffs,NJ. pp.227.

Frick , C. M., Farrell, R. E. and Germida, J. J. (1999), "Assessment of Phytoremediation as an In-Situ Technique for Cleaning Oil-Contaminated Sites", Prepared by: Department of Soil Science University of Saskatchewan Saskatoon, SK Canada Submitted to: Petroleum Technology Alliance of Canada (PTAC) Calgary, AB.

Ghasimi, S. M. D., Idris, A., Chuah, T. G. and Tey, B. T. (2009), "The Effect of C:N:P Ratio, Volatile Fatty Acids and Na^+ Levels on the Performance of an Anaerobic Treatment of Fresh Leachate from Municipal Solid Waste Transfer Station", African J. of Biotechnol. Vol. 8 (18), pp.4572-4581.

Ghirose, W. C. and Balkwill, D.L. (1983), "Enumeration and Morphological Characterization of Bacteria Indigenous to Subsurface Environments", Dev. Ind. Microbiol. Vol. 24, pp.213–224, Marcel Dekker, New York, New York.

Gibson, D. T. and Subramanian, V. (1984), "Microbial Degradation of Aromatic Hydrocarbons", pp.181–252. In D. T. Gibson (ed), Microbial Degradation of Organic Compounds, Marcel Dekker, Inc., New York, N.Y.

Gounot, A. M. (1991), "Bacterial Life at Low Temperature : Physiological Aspects and Biotechnological Implications", J. Appl. Bacteriol. Vol. 71, pp.386– 397.

Guha, S., Catherine, A. P. and Jaffe, P. R. (1999), "Multisubstrate Biodegradation Kinetics of Naphthalene, Phenanthrene, and Pyrene Mixtures", Biotechnol. and Bioengineering, Vol. 65(9), pp.491-499.

Guha, S. S., Jaffe, P. R. and Peters, C. A., (1998), "Bioavailability of Mixtures of PAHs Partitioned into the Micellar Phase of a Nonionic Surfactant", Environ. Sci. Technol. 32:2317–2324.

Harrison, W., Winnik, M. A., Kwong, P. T. Y. and Mackay, D. (1975), "Crude Oil Spills. Disappearance of Aromatic and Aliphatic Components from Small Sea-Surface Slicks", Environ. Sci. Technol. Vol. 9, pp.231-234.

Hichee, R. E. and Olfenbuttel R. F. (1991), "Application and Investigations for Hydrocarbons and Contaminated sites Remediation", Butterworth-Heinemann. Columbus. pp.14-28.

Hickey, R. F. and Smith, G. (1996), "Biotechnology in Industrial Waste Treatment and Bioremediation". Lewis Publishers, New York.

Hollaway, S. L., Faw, G. M. and Sizemore, R. K. (1980), " The Bacterial Community Composition of an Active Oil Field in the Northwestern Gulf of Mexico", Mar. Pollut. Bull. Vol. 11, pp. 153-156.

Huddleston, R. L., Bleckman, C. A. and Wolfe, J. R. (1986), "Land Treatment - Biological Degradation Processes", In Loehr, R. C. and Malina, J. F. Jr. (ed.), Water Resources Symposium Number 13. Center for Research in Water Resources.

Irving, A., Mendelssohn, Q. L. and Charles, B. H. (2002), "An Investigation of the Potential Toxicity of Bioremediation Agents in Wetland Mesocosms,

Development of Bioremediation for Oil Spill Cleanup in Coastal Wetland", Coastal Marine Institute, OCS Study

Jensen, V. (1975), "Bacterial Nora of Soil after Application of Oily Waste", Oikos, Vol. 26, pp.152–158.

JRB Associates, Inc. (1984), Summary report: Remedial response at hazardous waste sites. prepared for municipal environment research laboratory.

Kao, C. M., Chen, S. C., Liu, J. K. and Wang, Y. S. (2001), "Application of Microbial Enumeration Technique to Evaluate the Occurrence of Natural Bioremediation", Water Resources Vol. 35, pp.1951–1960.

Kaplan, C. W. and Kitts, C. L. (2004), "Bacterial Succession in a Petroleum Land Treatment Unit", Appl. Environ. Microbiol. Vol. 70, pp1777-1786.

Kawahara, F., Ky Davila, B., Al-Abed, S. R., Vesper, S. J., Ireland, J. C. and Rock, S. (1995), "Polynuclear Aromatic Hydrocarbon (PAH) Release from Soil During Treatment with Fenton's Reagent", Chemosphere. Vol. 31, pp.4131-4142.

Keck, J., Sims, R. C., Coover, M., Park, K. and Symons, B. (1989), "Evidence for Cooxidation of Polynuclear Aromatic Hydrocarbons in Soil", Water Res. Vol. 23, pp.1467- 1476.

Kelley, I. and Cerniglia, C. E. (1995), "Degradation of a Mixture of High Molecular Weight Polycyclic Aromatic Hydrocarbons by a Mycobacterium Strain PYR-1", J. Soil Contamination 4: 77-91.

Kerry E., (1993). "Bioremediation of Experimental Petroleum Spills on Mineral Soils in the Vesfold Hills", Antarctica. Polar Biol. Vol. 13, pp.163– 170.

King, R. B., Gilbert, M. L. and John, K. S. (1998), "Practical Environmental Bioremediation: Field Guide", Lewis Publisher, New York,.

Klecka, G. M. and Maier, W. J. (1988), "Kinetics of Microbial Growth on Mixtures of Pentachlorophenol and Chlorinated Aromatic Compounds", Biotechnol. Bioeng. Vol. 31, pp.328-335.

Launen, L., Pinto, L., Wiebe, C., Kiehlmann, E. and Moore, M. (1995), "The Oxidation of Pyrene and Benzo [a] Pyrene by Non-Basidiornycete Soil Fungi", Can. J. Microbiol. Vol. 41, pp.477-488.

Lee, K. Tremblay, G. H. and Gauthier, J., Cobanli, S. E., Griffin, M. (1997), "International Oil Spill Conference, American Petroleum Institute", Washington DC, pp.697-705.

Liebeg, E. W. and Cutright, J. C. (1999), "The Investigation of Enhanced Bioremediation through the Addition of Macro and Micro Nutrients in a PAH Contaminated Soil". International Biodeterioration and Biodegradation, Vol. 44, pp.55-64.

Liu, D., Maguire, R. J., Pacepavicius, G. J. and Nagy, E. (1992), "Microbiai Degradation of Polycyclic Aromatic Hydrocarbons and Polycyclic Aromatic Nitrogen Heterocyclics", Environ. Tolucol. Water Quai. Vol. 7, pp.355-372.

Loehr, R. C., McMillen, S. J. and Webster, M.T. (2001), "Predictions of Biotreatability and Actual Results: Soils with Petroleum Hydrocarbons", Pract. Period. Hazard., Toxic, Radioact. Waste Manage. Vol. 5, pp.78–87.

Lotfabad, S. K. and Gray, M. R. (2002), "Kinetics of Biodegradation Mixtures of Polycyclic Aromatic Hydrocarbons", Appl. Microbiol. Biotechnol. Vol. 60, pp.361–366.

Lyons, W. C. (1996), "Standard Handbook of Petroleum and Natural Gas Engineering", Gulf Publishing Company, Houston.

Machado, R. J. and Grady, Jr. C. P. L. (1989), "Dual Substrateremoval by an Anexic Bacterial Culture", Biotechnol. Bioeng. Vol. 33, pp.327-337.

Mackay, D. (1991), "Multimedia Environmental Models: the Fugacity Approach", Lewis Publishers, Inc.: Chelsea, Michigan.

Macnaughton, S. J., Stephen, J. R., Venosa, A. D., Davis, G. A., Chang, Y. J. and White, D. C. (1999), "Microbial Population Changes During Bioremediation of an Experimental Oil Spill", Applied and Environmental Microbiology Vol. 65, pp.3566–3574.

Mannisto, M. K., Melin, E. S., Puhakka, J. A. and Ferguson, J.F. (1996), "Biodegradation of PAH Mixtures by a Marine Sediment Enrichment", Polycyclic Aromatic Hydrocarbons Vol. 11, pp.27–34.

Margesin, R. (2000), "Potential of Cold-Adapted Microorganisms for Bioremediation of Oil-Polluted Alpine soils", Int. Biodeterior. Biodegrad. Vol. 46, pp.3 –10.

Margesin, R., Labbe, D., Schinner, F., Greer, C. W. and Whyte, L.G. (2003), "Characterization of Hydrocarbon Degrading Microbial Populations in Contaminated and Pristine Alpine soils", Appl. Environ. Microbiol. Vol. 69, pp.3085-3092.

Margesi, R. and Schinner, F. (1999), "Biological Decontamination of Oil Spills in Cold Environments", J. Chemical Technol. Biotechnol. Vol. 74, pp.381-389.

Margesin, R. and Schinner, F. (2001), "Bioremediation (natural attenuation and biostimulation) of Diesel-Oil-Contaminated Soil in an Alpine Glacier Skiing Area", Appl. and Environ. Microbiol. Vol. 67(7), pp.3127–3133.

McGill, W. B., Rowell, M. J. and Westlake, D. W. S. (1981), "Biochemistry, Ecology, and Microbiology of Petroleum Cornponents in Soil", In: E.A Paul and J.N. Ladd (Eds.), Soil Biochemistry, Marcel Dekker New York. Vol (3), pp.229-296.

McGroddy, S. E., Farrington, J. W. and Gschwend, P. M. (1996), "Cornparison of the in Situ and Desorption Sediment-Water Partitionhg of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls", Environ. Sci. Technol. Vol. 30, pp.172- 177.

McNally, D. L., Mihelcic, J. R. and Lueking, D. R. (1999), "Biodegradation of Mixtures of Polycyclic Aromatic Hydrocarbons under Aerobic and Nitrate-Reducing Conditions", Chemosphere Vol. 38, pp.1313–1321.

MeAuHiffe, C. (1966), "Solubility in Water of Paraffin, Cycloparaffin, Olefin, Acetylene, Cycloolefin, and Aromatic Hydrocarbons", J. Phys. Chem. Vol. 70, pp.1267-1275.

Millette, D., Barker, J. F., Comeau, Y., Butler, B. J., Frind, E. O., Clement, B. and Samson, R. (1995), "Substrate Interaction during Aerobic Biodegradation of Creosote-Related Compounds: A Factorial Batch Experiment", Environ. Sci. Technol., Vol. 29, pp.1944-1952.

Millette, D., Butler, B., Frind, E. O., Comeau, Y., Samon, R. and Comeau, Y. (1998), "Substrate Interaction During Aerobic Biodegradation of Creosote-Related Compounds in Columns of Sandy Aquifer Material", J. Contam. Hydrol. Vol. 29, pp.165–183.

Mishra, S., Jyot, J., Kuhad, R. C. and Lal, B. (2001), "Evaluation Ofinoculum Addition to Stimulate in Situ Bioremediation of Oily-Sludge-Contaminated Soil", Appl. and Environ. Microbiol. Vol. 67, pp. 1675–1681.

Moayedi, H., Huat, B. B. K., Kazemian, S. and Daneshmand, S. (2012), " Stabilization of Organic Soil Using Sodium Silicate System Grout", International J. of Physical Scien. Vol. 7(9), pp. 1395 - 1402, 23.

Morita, R. Y. (1992), "Low Temperature Environments", In: Lederberg, J. (ed.), Encyclopaedia of Microbiology. Academic Press, New York, pp.625– 637.

Mueller, J. G., Chapman, P. J., and Pritchard, P. H. (1989), "Creosote-Contaminated Sites", Environ. Sci. Technol. Vol. 23, pp.1197-1201.

Müller, R. (2000), "Environmental Microbiology-Degradation of Environmental Pollutants Lecture for Students of Chemical and Civil Engineering and for students of the master program Environmental Engineering", WS 99/2000 pp.5Song.

Murphy , E. M., Zachara, J. M. and Smith, S. C. (1990), "Influence of Mineral-Bound Humic Substances on the Sorption of Hydrophobic Organic Compounds", Environ. Sci. Technol. Vol. 24, pp.1507- 1516.

National Research Council (NRC), (1993), "The Current Practice of Bioremediation", In: In Situ Bioremediation. When does it work? Publishers, National Academic Press, Washinton, DC, USA, pp. 47-62.

Nedwell, D. B. (1999), "Effect of Low Temperature on Microbial Growth: Lowered Affinity for Substrates Limits Growth at Low Temperature", FEMS Microbiol. Ecol. Vol. 30, pp.101–111.

Norris, R. D. and Robert , S. K. (1994), "Environmental Research Laboratory", Handbook of Bioremediation, Published by Lewis Publishers.

Nweke, C. O. and Okpokwasili, G. C. (2004), "Effects of Bioremediation Treatments on the Bacterial Populations of Soil at Different Depths", Nig.J. Microbiol. Vol. 18, pp.363-372.

Obire, O., Nwaubeta, O. (2001), "Biodegradation of Refined Petroleum Hydrocarbons in Soil", J. of Appl. Sci. & Environ. Management, Vol. 5(1), pp. 43

Oh, Y. S., Shareefdeen, Z., Baltzis, B. C. and Bartha, R. (1994), "Interactions between Benzene, Toluene, and p-Xylene (BTX) during their Biodegradation", Biotechnol. Bioeng. Vol. 44, pp.533-538.

Okoh, A. I. and Trejo-Hernandez, M. R. (2006), " Remediation of petroleum hydrocarbon polluted systems: Exploiting the bioremediation strategies", African Journal of Biotechnology Vol. 5 (25), pp. 2520-2525

Onwurah, I. (1999), "Role of Diazotrophic Bacteria in the Bioremediation of Crude Oil-Polluted Soil", J. of Chemical Technol. and Biotechnol. Vol. 74, pp.957-964.

Pavlostathis , S. G. and Mathavan, G. N. (1992). "Desorption Kinetics of Selected Volatile Organic Compounds from Field Contaminated Soils", Environ. Sci. Technol. Vol. 26, pp.532-538.

Peters, C. A. and Luthy, R. G. (1993), "Cod Tar Dissolution in Water-Miscible Solvents: Experhental Evaluation", Environ. Sci. Technol. Vol. 27, pp.283 1-2843.

Petrakis, L., Jewell, D. M. and Benusa, W. F. (1980), Analytical Chemistry of Petroleum. An o v e ~ e w of Practices in Petroleum Industry Laboratories with Emphasis on Biodegradation", Adv. Chem Ser. Vol. 185, pp.25-53.

Piehler, M. F., Swistak, J. G., Pinckney, J. L. and Paerl, H. W. (1999), "Stimulation of Diesel Fuel Biodegradation Byindigenous Nitrogen. Xing Bacterial Consortia", Microbial Ecology, Vol. 38, pp.69–78.

Pollard, S. J. T., Hrudey, S. E. and Fedorak, P. M. (1994), "Bioremediation of Petroleum and Creosote-Contaminates Soils: a Review of Constraints", Waste Mgmt. Res. Vol. 12, pp.173-194.

Pucci, O. H., Bak, M. A., Peressutti, S. R., Klein, S.R., HFartig, C., Alvarez , H. M. and WFunsche, L. (2000), "In Nuence of Crude Oil Contamination on the Bacterial Community of Semiarid Soils of Patagonia (Argentina)", Acta Biotechnolo. Vol. 20, pp.129–146, 29–73.

Quatrini, P., Scaglione, G., De Pasquale, C., Reila, S. and Puglia, A. M. (2008), "Isolation of Gram-positive n-alkane Degraders from a Hydrocarbon Contaminated Mediterranean Shoreline", J. Appl. Microbiol. Vol.104, pp.251-259.

Raeid, M. M., Abed, N. M. D., Safi, J. K., Dirk B., Y. E. (2002), "Microbial Diversity of a Heavily Polluted Microbial Mat and Its Community Changes following Degradation of Petroleum Compounds appl. Environ. Microbial", Vol. 68(4), pp. 1674–1683.

Ramaswarni, A. and Luthy, R. G. (1997), "Mass Transfer and Bioavailability of PAH Compounds in Coal Tar NAPL-Slurry Systems. Mode1 development", Environ. Sci. Technol. Vol. 31, pp.2260-2267.

Rehm, H. J. and Rei, T. (1981), "Mechanism and Occurrence of Microbial Oxidation of Long Chain Alkanes", Adv. Biochem. Eng. Vol. 19, pp.175–215.

Ringelberg, D. B., Talley, J. W., Perkins, E. J., Tucker, S. G., Luthy, R. G., Bouwer, E. J. and Fredrickson, H. L. (2001), "Succession of Phenotypic, Genotypic, and Metabolic Community Characteristics During in Vitro Bioslurry Treatment of Polycyclic Aromatic Hydrocarbon-Contaminated Sediments", Appl. and Environ. Microbiolo. Vol. 67, pp.1542–1550.

Riser-Roberts, E. (1998), "Remediation of Petroleum Contaminated Soils: Biological, Physical, and Chemical Processes", Lewis Publishers CRC Press LLC, Boca Raton.

Rooney-Varga, J. N., Anderson, R. T., Fraga, J. L., Ringelberg, D. and Lovley, D. R. (1999), "Microbial Communities Associated with Anaerobic Benzene Degradation in a Petroleum-Contaminated Aquifer", Appl. and environ. Microbial. Vol. 65(7), pp.3056-3063.

Salanitro, J. P. (2003), Crude Oil Hydrocarbon Bioremediation and Soil Ecotoxicity Assessment", Environ. Sci. Technol., Vol. 31, pp.1769.

Scow, K. M. and Hutson, J. (1992), "Effect of Diffusion and Sorption on the Kinetics of Biodegradation: Theoretical Considerations", Soil Sci. Soc. Am J. Vol. 56, pp.119-127.

Segal, I. H. (1975), " Enzyme Kinetics", New York: Johm Wiley & Sons Inc.

Seo, J., Keum, Y., Li, Q. (2009), " Bacterial Degradation of Aromatic Compounds", Int J Environ Res Public Health. Vol. 6(1), pp.278–309.

Shukla, K. P., Singh, N. K., Sharma, S. (2010), "Bioremediation: Developments, Current Practices and Perspectives", Genetic Engineering and Biotechnol, J, vol.2010, pp.GEBJ-3.

Shuttlewort, K. L. and Cerniglia, C. E. (1996), "Bacterial Degradation of Low Concentrations of Phenanthrene and Inhibition by Naphthalene", Microb. Ecol. Vol. 31, pp.305-317.

Sims, R. C. and Overcash, M. R. (1983), "Fate of Polynuclear Aromatic Compounds in Soil Plant Systems", Residue Reviews. Vol. 88, pp.1- 68.

Singh, C. and Lin, J. (2009), "Evaluation of Nutrient Addition to Diesel Biodegradation in Contaminated Soils", African J. of Biotechnolo. Vol. 8(14), pp. 3286-3293.

Song , Hm-G. W., ang , X. and Bartha, R. (1990), "Bioremediation Potential of Terrestrial Fuel Spills", Appl. Environ. Microbiol. Vol. 56, pp.652-656.

Spain, J. C., Pritchard, P. H. and Bourquin, A. W. (1980), "Effects of Adaptation on Biodegradation Rates in Sediment Water Cores from Estuarine and Freshwater Environments", Appl. and Environ. Microbiolo. Vol. 40, pp.726–734.

Steffan, R. J., McClay, K., Vainberg, S., Condee, C. W. and Zhang, D. (1997), "Biodegradation of the Gasoline Oxygenates Methyl tert-Butyl Ether, Ethyl tert-Butyl Ether, and tert-Amyl Methyl Ether by Propane-Oxidizing Bacteria", 167Appl. and environ. Microbial. Vol. 63(11), pp.4216-4222.

Stringfellow, W. T. and Aitken, M. D. (1995), "Competitive Metabolism of Naphthalene, Methylnaphthalenes, and Fluorine by Phenanthrene-Degradating Pseudomonads", Appl. Environ. Microbiol. Vol. 61, pp.357-362.

Suthersan, S. S. (1997), "Remediation Engineering Design Concepts", CRC Press Inc, Boca Raton, FL.

Trindade, P. V. O., Sobral, L. G., Rizzo, A. C. L., Leite, S. G. F. and Soriano, A. U. (2005), "Bioremediation of a Weathered and a Recently Oil-Contaminated Soils from Brazil", a Comparison Study Chemosphere Vol.58, pp.515–522.

USEP (1995), "How to evaluate Alternative Clean-up Technologies for Underground Storage Tank Sites", EPA pp.510-B-95-007.

Van Hamme, D., Singh, A. and Ward, O. P. (2003), "Recent Advances in Petroleum Microbiology", Microbiology and Molecular Biology Reviews. Vol. 67(4), pp.503-549.

van Schooten, F. J., Maas, L. M., Moonen, E. J. C., Kleinjans, J. C. S. and van der, O. R. (1995), "DNA Dosirnetry in Biological Indicator Species Living on PAHcontaminated Soils and Sediments", Ecotoxicol. Environ. Safety. Vol. 30, pp.171-179.

Van Trump, J. I. and Coates, J. D. (2009), The ISME J.vol. 3, pp.466–476.

Vasudevan, N. and Rajaram, P. (2001), "Bioremediation of Oil Sludge-Contaminated Soil", Environment International Vol.26, pp.409- 411.

Vennila, R. and Kannan, V. (2011), "Bioremediation of Petroleum Refinery Effluent by Planococcus Halophilus", African J. of Biotechnol. Vol. 10(44), pp.8829-8833.

Venosa, A. D., Suidan, M. T., Wrenn, B. A., Strohmeier, K. L., Haines, J. R., Eberhart, B. L., King, D. W. and Holder, E. (1996), "Bioremediation of Experimental Oil Spill on the Shoreline of Delaware Bay", Environ.l Sci. and Technol. Vol. 30, pp.1764-1775.

Vinas, M., Sabate, J., Espuny, M. J. and Solanas, A. M. (2005), "Bacterial Community Dynamics and Polycyclic Aromatic Hydrocarbon Degradation During Bioremediation of Heavily Creosote-Contaminated Soil", Appl. Environ. Microbiol. Vol. 71, pp.7008.

Vishvesh, K., Bhupathiraju, P. K., Hoi-Ying, N. H., Mark, E. C., Paul, F. D., Alexis, S. T., James, R. H., Mark, H. and Lisa, A. C. (2002), "Assessment of in-Situ Bioremediation at a Refinery Waste-Contaminated Site and an Aviation Gasoline Contaminated Site", Biodegradation, vol.13, pp.79–90.

Wainwright, M. (1999), "An Introduction to Environmental Biotechnology", Kluwer Academic Publishers. pp.38-39.

Walsh, J. B. (1999), "A Feasibility Study of Bioremediation in a Highly Organic Soil", Environ. Engineering.

Wang, X. and Bartha, R. (1994), "Effects of Bioremediation on Toxicity, Mutagenesis, and Microbiota in Hydrocarbon-Polluted Soils", In: Wise, D.L., Trantolo, D.J. (eds.), Remediation of Hazardous Waste Contaminated Soils. M. Dekker Inc, New York, pp.175–197.

Wetzel, S. C., Banks, M. K. and Schwab, A. P. (1997), "Rhizosphere Effects on the Degradation of Pyrene and Anthracene in Soil", Phytoremediation of soil and water contaminants.

Wright, A. L., Weaver, R. W. and Webb J. W. (1997), "Oil Bioremediation in Salt Marsh Mesocosms as Influenced by N and P Fertilization, Flooding, and Season", Water, Air and Soil Pollution. Vol. 95, pp.179-191.

Xing, B., McGill, W.B. and Dudas, M.J. (1994a), "Cross-Correlation of Polarity Curves to Predict Partition Coefficient of Nonionic Organic Contaminants", Environ. Sci. Technol.vol. 28, pp.1929–1933.

Xing, B., McGill, W.B. and Dudas, M.J. (1994b), "Sorption of benzene, toluene, and Oxylene by collagen compared with non-protein organic sorbents", Can. J. Soil Sci. Vol.74, pp.465-469.

Yeom, T., Ghosh, M. M., Cox, C. D. and Ahn, K. H. (1996), "Dissolution of Pofycyclic Aromatic Hydrocarbons from Weathered Contaminated Soil", Water Sci.Technol. vol.34, pp.3 3 5-342.

Yerushalmi, L., Rocheleau, S., Cimpoia, R., Sarrazin, M., Sunahara, G., Peisajovich, A., Leclair, G. and Guiot, S. R. (2003), "Enhanced Concentrations of Total Petroleum Hydrocarbons", J. Agric. Environ. Sci. vol. 7, pp.37-51.

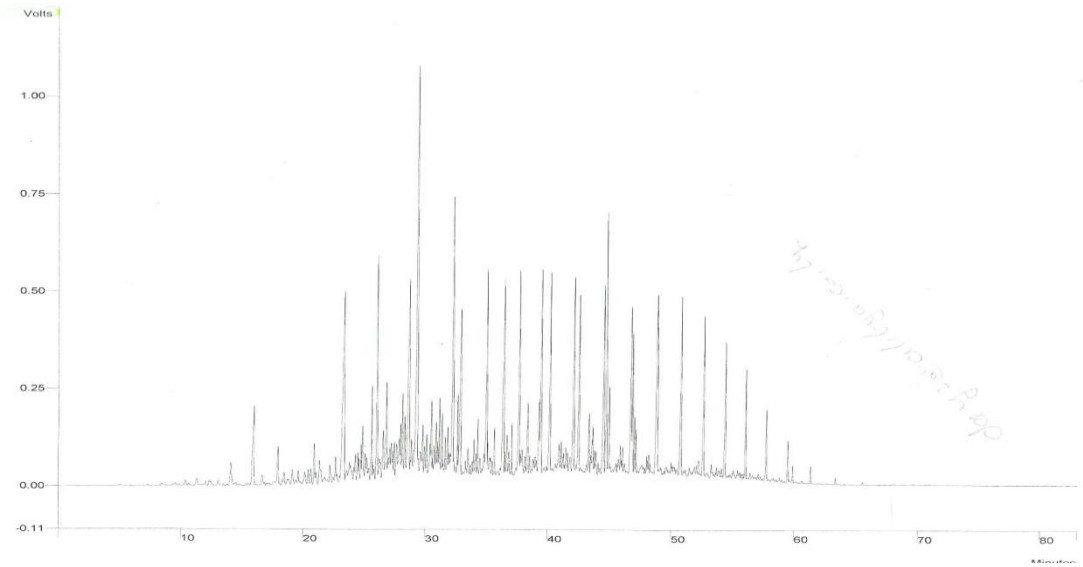
Yoon, H., Klinzing, G. and Blanch, H. W. (1977), "Competition for Mixed Substrates by Microbial Populations", Biotechnol. Bioeng. Vol.19, pp.1193-1210.

Zhu, X., Venosa, A. D. and Suidan, M.T. (2004), "Literature Review on the Use of Commercial Bioremediation Agents for Cleanup of Oil-Contaminated Estuarine Environments", EPA/600/R-04/075.

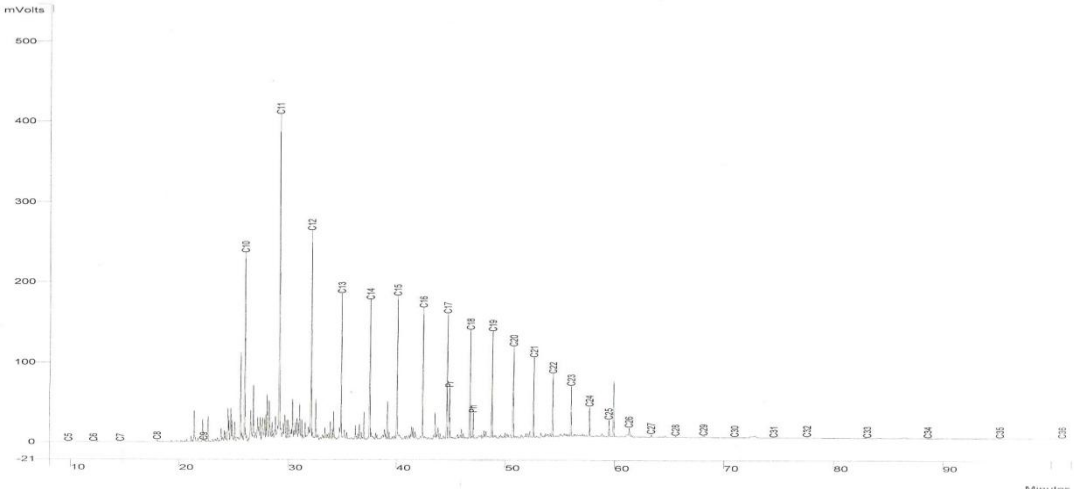
Zitrides, T. (1983), "Biodecontamination of Spill Site", Pollution Engineering, vol.15, pp.25-27.

Appendixe

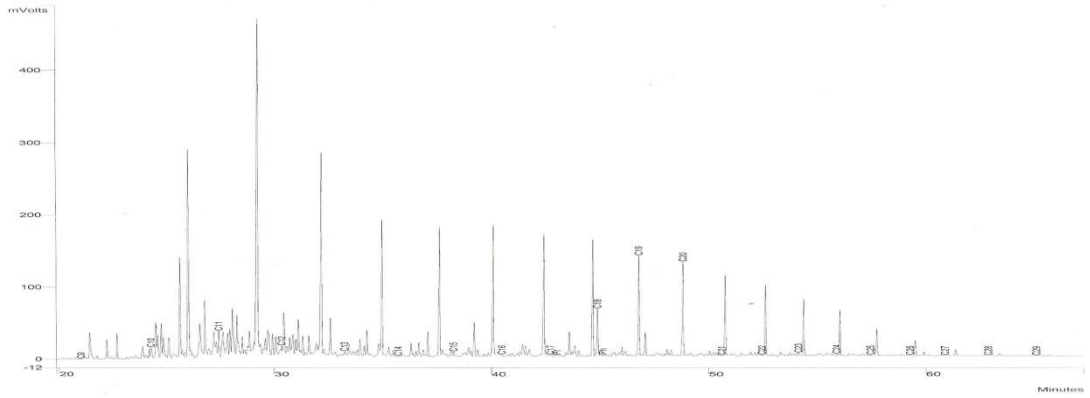
0 week


Volts
1.00
0.75
0.50
0.25
0.00
-0.11
10
20
30
40
50
60
70
80

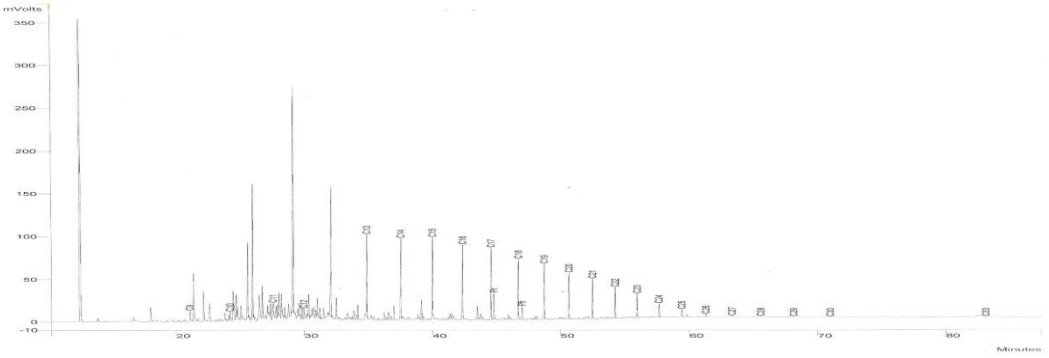
1 week


mVolts
500
400
300
200
100
0
-21
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
Pr
Ph
C18
C19
C20
C21
C22
C23
C24
C25
C26
C27
C28
C29
C30
C31
C32
C33
C34
C35
C36
10
20
30
40
50
60
70
80
90

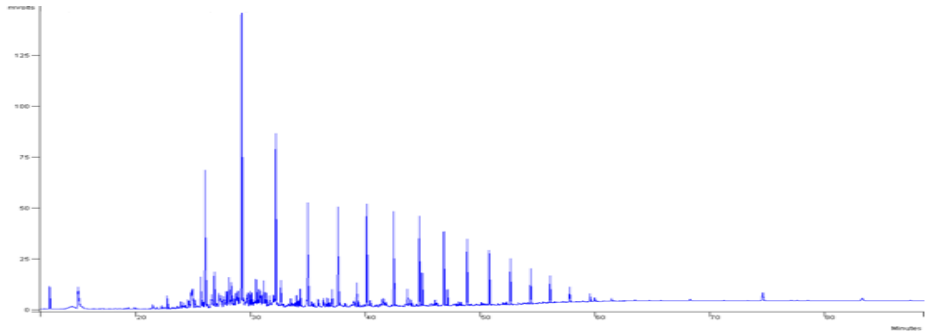
2 week



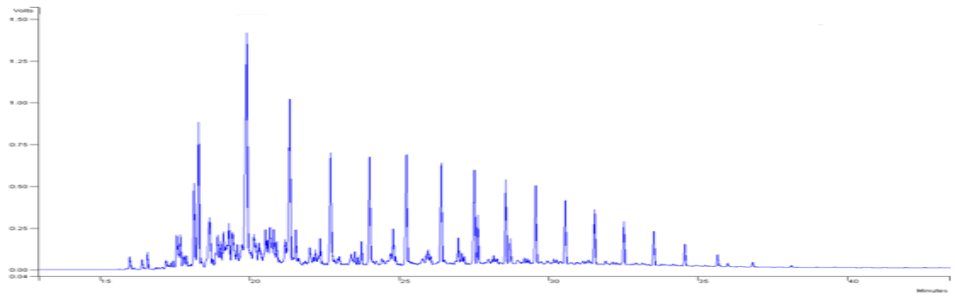
3 week



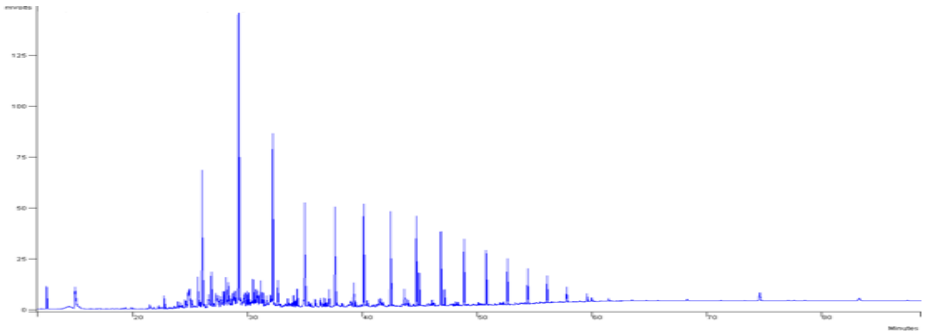
4 week



5 week



6 week



الملـــــــــخص

قد تم عرض ودراسة الحركية والديناميكية الحرارية لمعالجة المواد الهيدروكربونية بيولوجيا في التربة في هذه الأطروحة. عينات التربة تم التحصل عليها من مواقع مختلفة على امتداد خط الأنابيب من حقل السرير إلى ميناء التصدير في مدينة طبرق (الحريقة)، والمشتقات النفطية المستخدمة في هذه الدراسة وقود المحركات (Motor gasoline)، الكيروسين(kerosene) و الديزل (diesel) المنتجة في مصفاة السرير حيث تم استخدامها كمصدر لتلويث عينات التربة. وقد تم تقسيم عينات التربة إلى ثلاث مجموعات المجوعة الأولى (المعالجة) وهي تحتوي على سماد لتنشيط البكتيريا (اضافة النيتروجين والفوسفور) والجموعة الثانية (الغير معالجة) وهي عينة لاتحتوي على أي شئ والمجوعة الثالثة (المسممة) وهي التي تم تسميمها بي100 جزء في المليون من كلوريد الزئبق للقضاء على أي نمو بكتيري فيها للتحكم في عملية التبخير كما تم تهوية جميع عينات التربة باستخدام سلك مقاوم للصدأ وذلك بتحريك التربة ثماني مرات بصورة يومية وكما حُفظ على درجة الحرارة المطلوبة للدراسة باستخدام الحضانات. وقد تم قياس تركيز الهيدروكربون في التربة في فترات زمنية مختلفة لدراسة حركية المعالجة البيولوجية للمواد الهيدروكربونية. وكما استخدمت الطريقة القياسية لاستخلاص المشتقات النفطية من التربة واستخدم ايضاَ تقنية الكروماتوغرافي الغاز لقياس التركيز الكلي للهيدروكربون في التربة.

استخدمت (NH_4NO_3) كمصدر للنيتروجين، كما أضيفت (KH_2PO_4) للحصول على الفوسفور وتم خلطهما في عينات التربة المستخدمة في الدراسة والملوثة بمشتقات الوقود الهيدروكربونية لتنشيط البكتيريا.

وجد أن انخفاض في كمية الهيدروكربون وكان الاسرع في العينات المعالجة وقد تم حساب نصف عمر تدهور الهيدروكربون (degradation) عند درجات حرارة مختلفة (25، 30، 35، 40 و45) لكل مجموعة من مجموعات التربة (المعالجة، الغير معالجة والمسممة) حيث وجد أن درجة الحرارة

له تأثير كبير على تدهور الهيدروكربون degradation ووجد أن المعالجة الحيوية تزيد بزيادة درجة الحرارة.

رتبة تفاعل تدهور الهيدروكربون (degradation) تم حسابه بقياس تركيز الهيدروكربون في أوقات مختلفة وإبقاء جميع المتغيرات الأخرى (درجة الحرارة، الرطوبة، تركيز السماد، ومدة التهوية) ثابتة. وقد اعطيت منحنيات لوغاريتم تركيز الهيدروكربون مقابل الزمن خطوط مستقيمة تدل على ان حركية المعالجة الحيوية تندرج تحت الرتبة الاولى وكما تم حساب ثابت معدل سرعة التفاعل لها.

طاقة التنشيط E_a للمعالجة الحيوية تم حسابها عند درجات حرارة مختلفة حيث وجد أنها تساوي ($40.34\ kJ\ mol^{-1}$)، كما تم حساب ثابت معدل سرعة التفاعل من ميل المنحنيات للوغاريتم تركيز الهيدروكربون الكلي مقابل درجات الحرارة المختلفة.

عوامل الديناميكية الحرارية مثل $\Delta H^{\#}$، $\Delta S^{\#}$ و $\Delta G^{\#}$ تم حسابها عند درجة حرارة 25 درجة مئوية وكانت على التوالي ($37.86\ kJ\ mol^{-1}$، $0.3375 kJ\ K^{-1}\ mol^{-1}$ - و$138.45\ kJ$).