

CHAPTER 2

LITERATURE REVIEW

Accidental contamination of soil with petroleum hydrocarbons is a risk inherent in oil production and shipping. About 8.8 million metric tons of oil are spilled on land each year (Rhykerd *et al.*, 1999). Oil spilled on soil is an environmental concern because contaminated soils may be unsuitable for agricultural, industrial or recreational use and are potential sources for surface and ground water contamination. Clean-up technologies, that involve excavation and incineration or burial in a landfill are costly. Onsite remediation technologies offer an economical alternative that is effective at removing pollutants from soil (Rhykerd *et al.*, 1999).

Biodegradation of hydrocarbons by natural population of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbons pollutants are eliminated from the environment (Leahy *et al.*, 1990). Biodegradation strategies aim to alleviate constraints on microbial metabolism, either by providing electron acceptors (such as oxygen), the addition of essential nutrients (such as biologically available nitrogen or phosphorus), or adjusting soil pH. When this is done with appropriate care, hydrocarbon biodegradation is stimulated without any adverse environmental impacts. Land treatment of hydrocarbon contaminated soils has been demonstrated to be a safe and cost-effective treatment method for hydrocarbon waste products, due to its simplicity of design and operation, and its relatively high treatment efficiency (Semple *et al.*, 2001 and Christopher *et al.*, 2004).

Biopile technology involves forming petroleum-contaminated soils into piles or cells above ground and stimulating aerobic microbial activity within the soils through aeration (Li *et al.*, 2004 and Jorgensen *et al.*, 2000). Microbial activity can be enhanced by adding moisture and nutrients such as nitrogen and phosphorous. The aerobic microbial activity degrades the petroleum-based constituents absorbed into soil particles, thus reducing the concentrations of these contaminants (Von Fahnstock *et al.*, 1996 and Li *et al.*, 2004).

2.1 Factors affecting bioremediation of petroleum products

Petroleum biodegradation in soil ecosystems is related to the movement and distribution of the oil in soil environment, and the ability of microbes to degrade the contaminant. Factors affecting the rate of hydrocarbon biodegradation in soils include: 1)the type and amount of hydrocarbons present, 2)soil temperature, 3)nutrients, 4)soil pH, 5)aeration, 6)available water, 7)the type and the population of the hydrocarbons microbial and the degrading microorganisms present or those introduced into the contaminated soil, and 8)the mobility of the contaminate. The manipulation and optimization of these factors is needed to neutralize the effects of Leibig's Law of the Minimum (Cookson, 1995). This law states that the rate of biological processes (such as growth or metabolism) is limited by the factor present at its minimum level.

2.1.1 Effect of petroleum hydrocarbon composition and concentration

Crude oil, the source material of nearly all petroleum products, contains a wide variety of elements combined in various forms. The principal constituents are carbon and hydrogen, which in their combined form are hydrocarbons. In the refining process, petroleum products are strongly enriched with hydrocarbons, leaving crude-based inorganic materials and other types of organic compounds containing sulfur, nitrogen, and oxygen in the residual material. Petroleum and petroleum products such as gasoline, fuels oils (kerosene) and diesel fuels are complex mixtures of organic compounds.

Petroleum hydrocarbons are organic compounds comprised of carbon and hydrogen atoms arranged in varying structural configurations. In a broad sense, they are divided into two families, aliphatics (fatty) and aromatics (fragrant). Aliphatic hydrocarbons are straight or branch chains of carbon atoms with sufficient hydrogen to satisfy the valence requirements of the carbon. They have the empirical formula C_nH_m . Aliphatics are further divided into three main classes, alkanes, alkenes and cycloalkanes. Alkynes, another type of aliphatic structure, are not commonly found in petroleum hydrocarbon. Aromatic hydrocarbons have one or more benzene rings as structural components. Aromatic hydrocarbons are further divided into monoaromatic compounds (benzene), diaromatic compounds (naphthalene) and polynuclear aromatic hydrocarbons (PAHs).

Hydrocarbons differ in their susceptibility to microbial attack and have generally been ranked in the following order of decreasing susceptibility: aliphatic

hydrocarbon 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 hydrocarbon are more easily degraded than unsaturated hydrocarbons (the presence of carbon-carbon double or triple bonds hinders degradation), long-chain aliphatic hydrocarbon 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 microorganism (methanotrophs) can degrade these short-chain hydrocarbons. The optimal chain length for biodegradation appears to be between 10 and 20 carbon (Katherine *et al.*, 1994). Microbial biodegradation can be affected by the degree that the contaminate has spread, the compositional heterogeneity, and by a low-energy environment (Leahy *et al.*, 1990).

2.1.2 Effect of soil temperature

Temperature influences petroleum biodegradation through its effect upon the physical and chemical composition of the oil, the rate of the hydrocarbons metabolism by the microorganism, and the composition of the microbial community (Leahy *et al.*, 1990). Vidali (2001) notes that the optimum temperature conditions required for microbial activity, especially for oil degradation, is in the range between 25°C-30°C.

The rule of thumb that has been used classically is that the microbial activity doubles with each 10⁰C increase in temperature, up to an optimal temperature for that species. Soil temperature can also have a profound effect on both the soil matrix and the physicochemical state of the contaminants (Katherine *et al.*, 1994).

2.1.3 Effect of soil pH

The soil pH may influence the biodegradation process, because soil microorganisms require a specific pH range to survive. Most bacteria function in a pH range between 5 and 9 with the optimum being slightly above 7. A shift in pH may result in a shift in the microbial population because each species will exhibit optimal growth at a specific pH (Von Fahnstock *et al.*, 1996). Furthermore, Katherine *et al.*, 1994 stated biodegradation of contaminants is typically fastest at neutral or near-neutral pH values.

If soil sampling and analysis indicates that pH is not of the optimal range, amendments can be introduced during the initial soil preparation. If the pH soil is too acidic, lime may be added to increase the pH. If the soil pH is too basic, sulfur, ammonium sulfate, or aluminum sulfate may be added to decrease the pH (Von Fahnstock *et al.*, 1996).

2.1.4 Effect of nutrients

Microorganisms must get all their nutrients necessary for growth from the environment. The nutrients availability, especially that of nitrogen and phosphorous,

appears to be the most common limiting factor (Van Hamme *et al.*, 2003). The microbial requirements for nutrients are approximately the same as the composition of their cells. The chemical structure of bacteria can be expressed as $C_5H_7O_2N$ with only minor, but important, tracers of other atoms. Carbon is usually supplied by organic carbon. Nitrogen and phosphorous are naturally found in soil, but for highly contaminated sites their concentrations may be too low. The available nitrogen and phosphorous at a site are compared with the microbial need for mineralization of the available organic compounds (Cookson, 1995). Vidali (2001) notes the optimum value of ratio C:N:P for an oil degradation is 100:10:1.

Biopiles work to degrade contaminants by means of the microorganisms in the pile that use the contaminants as a source of carbon and energy. The organisms need a supply of carbon so as to build biomass. The contaminants and natural organic compounds in the soil typically provide an adequate amount of carbon, but the availability of other essential nutrients, such as nitrogen and phosphorous, may be insufficient when compared to the quantity of carbon. According to U.S. EPA, (1995) typically, the C:N:P ratio is brought to within the range of 100:10:1 to 100:10:0.5 (Von Fahnstock *et al.*, 1996).

2.1.5 Effect of soil moisture content

The soil must contain enough moisture to encourage growth of the hydrocarbon-degrading microorganisms, but not so much as to reduce soil permeability. Water is essential for biological processes because it not only provides

the transport medium for chemicals that supply energy and nutrients to the microorganisms but also enables the metabolic processes to proceed. However, excessive moisture will fill the pores in the soil pile and reduce soil permeability, making it difficult to aerate the biopile. Nevertheless, microorganisms will effectively degrade hydrocarbons over a wide range of moisture content (Von Fahnstock *et al.*, 1996).

Furthermore, Leahy *et al.*, 1990 stated moisture content of soil affects the availability of contaminants, the transfer of gases, the effective toxicity level of contaminants, the movement and growth stage of microorganisms, as well as species distribution.

However excess moisture can pool at selected spots within the treatment system leading to development of localized anoxic zones and reduced biodegradation (Katherine *et al.*, 1994).

2.1.6 Microbial population

In the environment, one the principal processes responsible for the passive mitigation of petroleum contamination is biodegradation of the petroleum hydrocarbons by naturally occurring microorganisms. These microorganisms, mainly bacteria, are found in almost all hydrocarbon affected soils. They use the hydrocarbons as an alternative food source, mostly for biomass carbon and energy production. This process involves the breakdown of hydrocarbon into smaller units and finally the incorporation into cell material (biomass) or breakdown into carbon

dioxide, resulting in cellular biochemical energy production. The overall result of this process in nature is: 1)reduction in petroleum hydrocarbon concentration, 2)reduction in the overall toxicity of the area, and 3)reduction in the mobility of the remaining hydrocarbon in the environment. This process is used industrially to treat petroleum waste and restored contaminated areas. Known as bioremediation, it manages the growing conditions of the bacteria in waste treatment processes to maximize biodegradation (Schroeder *et al.*, 2000).

There is no one species of microorganisms will completely degrade any particular oil. The degradation of both crude and refined oils seems to involve a consortium of microorganisms, including both eukaryotic and prokaryotic forms (Balba *et al.*, 1998 and Leahy *et al.*, 1990). The most common genera known to be responsible for oil degradation comprise mainly *Nocardia*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Micrococcus*, *Arthrobacter*, *Corynebacterium*, *Achromobacter*, *Rhodococcus*, *Alcaligenes*, *Mycobacterium*, *Bacillus*, *Aspergillus*, *Mucor*, *Fusarium*, *Penicillium*, *Rhodotorula*, *Candida* and *Sporobolomyces* (Balba *et al.*, 1998).

Hydrocarbon degradation has been studied extensively: several reviews have been published on the microbial metabolism of straight-chain and branched alkanes, cyclic alkanes, aromatic hydrocarbons and halogenated hydrocarbons. In general, alkanes terminally oxidized to the corresponding alcohol, aldehyde and fatty acid. Fatty acids derived from alkanes are then further oxidized to acetate and propionate (odd-chain alkanes) by inducible β -oxidation systems. Different microorganisms

exhibit different hydrocarbon specificities, example some grow on alkanes of six to 10 carbons in chain length, whereas others grow on long-chain alkanes. Some of the oxygenases are encoded on plasmids and others on chromosomal genes (Rosenberg *et al.*, 1996).

Enumeration of petroleum degrading microorganisms is important both to determine the potential for the removal of oil via microbial degradation and to assess the amount of oil pollution that has occurred. It is then possible that the populations of petroleum-degrading microorganisms can be matched to the concentration of polluting oil present at the site (Walker *et al.*, 1976).

Initial soil analyses of the total heterotrophic microbial counts and specific hydrogen degrading microbial counts in the contaminated soil can provide useful information on soil biological activities, and the extent to which the indigenous microbial population has acclimated to the site condition. The results will also indicate whether the soil contains a healthy indigenous microbial population capable of supporting bioremediation. In addition to the initial microbial assessment of the contaminated soil monitoring microbial populations during the soil bioremediation is a useful tool for following the changes and for discerning the microbes active in hydrocarbon degradation (Balba *et al.*, 1998).

Typical plate counts for total soil organisms are between 10,000 and 10,000,000 (Katherine *et al.*, 1994).

2.1.7 Effect of soil void volume

Soil is a mixture of differently sized organic and inorganic materials. Soils can be described by the dry-weight fraction of material able to pass through differently sized sieves. The size composition influences the amount of open space between the soil particles (the porosity). Porosity is important in transporting gases and liquids through environments (Katherine *et al.*, 1994).

The permeability and absorptive properties of the soil depends upon the soil type. A high clay content in the soil will adversely affect biodegradation processes in the biopile. Thus, high clay content will reduce the permeability of the soil and increase the difficulty of adequately and uniformly aerating pile. This may decrease the availability of the organic contaminants and decrease the biodegradation rate. This is because clay strongly adsorbs organic contaminants, reducing the biological availability of contaminants. The void volume of soil to be treated should be higher than 25% (Von Fahnstock *et al.*, 1996).

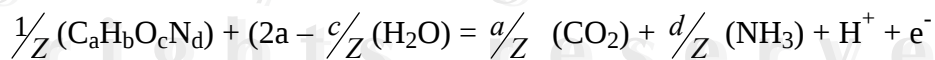
Movement of air in the biopile occurs primarily through the gas-filled pores. The amount and configuration of soil porosity is an important determinant of the gas permeability of the soil. Soils with connected porosity generally are more amenable to treatment in a biopile. The soil permeability or porosity can be increased by adding bulking agents or by soil shredding.

2.1.8 Oxygen as electron acceptor and air flow rate

Aerobic conditions are necessary for optimal rates of bioremediation of soils contaminated with petroleum hydrocarbons. In soils, the O₂ content depends on microbial activity, soil texture, water content, and depth. A low O₂ 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 O₂ content was below 10%. At 30 cm depth soil O₂ content was shown to vary from 15% in a dry soil to 5% in a moist soil (Rhykerd *et al.*, 1999). Viable aerobic bioactivity is typically found in contaminated sites where the oxygen levels in the soil are above 2%. When the oxygen content of the soil air falls below 2%, aerobic bioactivity becomes dormant or absent. At this point the soil air is infused instead by carbon dioxide and methane (Suthan, 1997).

Since aerobic remediation of electron acceptors (oxygen) is calculated based on oxidation and reduction, and since the redox reactions consists of two parts, an oxidation reaction and a reduction reaction, the electron flow must be balanced. After the electron flow is balanced, the two half reactions can be combined for a mass balance.

Half Reaction of Electron Donor ~ H_D



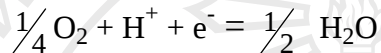
Where:

$Z = 4a + b - 2c - 3d$; a , b , c and d represent the average number of atoms for

C, H, O and N, respectively, in the organic contaminant.

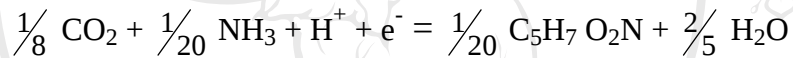
Half Reaction of Electron Acceptor $\sim H_A$

Aerobic : when oxygen is the electron acceptor

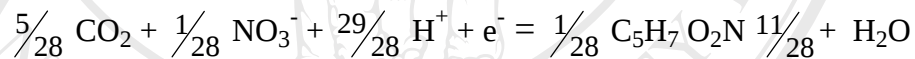


Cell Synthesis Equation $\sim C_S$

When ammonia is the nitrogen source:



When nitrate is the nitrogen source:



The overall reaction can be given in general terms by:



Where:

H_D = half reaction for the organic compound oxidation, electron donor

H_A = half reaction for the electron acceptor

C_S = reaction that provides nutrient requirements for biomass synthesis

The cell synthesis reaction equates the nutrient demand to the amount of biomass that will be produced. During the degradation of the organic compound, a portion of that energy yields cellular growth. A factor is included in the reaction for

distribution of this energy between biomass synthesis and other needs. These factors are represented by:

f_e = fraction of organic oxidized for energy

f_s = fraction associated with conversion to microbial cells

where :

$$f_e + f_s = 1$$

for aerobic systems, the f_s factor for energy distribution is found to range between 0.12 and 0.6 Table 2.1.

Table 2.1 Factors for energy distribution for biomass synthesis in redox reaction

Electron acceptor	f_s values for equation
O ₂	0.12 – 0.60
N O ₃	0.1 – 0.5
S O ₄	0.04 – 0.2
CO ₂	0.04 – 0.2

Note: The approximate composition of cellular structure is C₅H₇O₂N.

Source: Cookson, 1995.

2.1.9 Factor of abiotic processes of hydrocarbon removal from soil

In addition to biodegradation of hydrocarbon contaminants, there are numerous abiotic mechanisms, such as sequestration and toxic material. These processes result in the deterioration of air quality and inaccessibility of hydrocarbons to microbial degradation.

2.1.9.1 Sequestration of recalcitrant hydrocarbons

The sequestration model represents a common explanation of residual hydrocarbon persistence in soil. Certain fractions of hydrocarbons are “locked up” in soil nanopores within the soil aggregates, making them inaccessible to microorganisms. Because many bacteria have diameters greater than 1000 nm and no free-living organism has a dimension less than 100 nm, a molecule within these smaller nanopores is not available for metabolism, so long as it is retained within the pores and does not diffuse into the larger pores inhabited by microorganism (Martin, 1999).

2.1.9.2 Toxic materials

A toxic material is one that inhibits the rate at which microbiological activity occurs. Whether or not a material is toxic depends on its concentration, the characteristics of the material, the ability of the microorganisms to adapt to the toxic material and the amount of time they are exposed to it (Katherine *et al.*, 1994).

High concentration of metal content will retard the bioremediation process such as arsenic and mercury have no nutrient value and may reduce biological activity when present at low concentrations (Von Fahnestock *et al.*, 1996). Vidali 2001 stated the total content of heavy metal for condition required for microbial activity in bioremediation should be less than 2000 ppm.

2.2 Biodegradation rate

The ultimate goal of land-treatment is the biodegradation of hydrocarbons. For solid-phase treatment, polynuclear aromatic hydrocarbons (PAHs) usually control the clean up time because of their low rate of degradation. The affinity of a chemical contaminant for the soil matrix and its rates of desorption controls the availability of the compound as a microbial substrate. The rate of desorption is dependent on the soil-water ratio (Cookson, 1995).

The biodegradation reaction in the piles can be described by first order kinetic (Li *et al.*, 2003 and Laleh *et al.*, 2003) :

$$\frac{dH}{dt} = -bH \quad (1)$$

where: H is the hydrogen concentration and b is the degradation rate.

The solution of H is thus:

$$H = H_0 \exp(-bt) \quad (2)$$

Where H_0 is the initial oil content. The logarithm of the equation is:

$$\ln(H) = \ln(H_0) - bt \quad (3)$$

In order to get b or degradation rate thus a weighted regression method may be conducted where b indicate slope of line liner regression.

2.3 Fang Petroleum Refinery Thailand

The Fang District is a large area of about 829 square kilometers situated on the plateau near mountains within 152 kilometers of Chiang Mai Province. Fang has a long

history and has many ancient places as well as a culture which epitomizes Northern Thai civilization.

Petroleum exploration began in Thailand with the operations of the first Thai train system. In 1921, HRH, Prince Kumpaengphet Akrayothin, then the Director of the Railway Authority of Thailand, established a survey for petroleum in the Fang district; and, even though only natural gas traces were located, it was the beginning of petroleum activities within Thailand. In the following years, the Department of Mineral Resources became responsible for petroleum exploration and during 1949-1952 crude oil fields were discovered and named “Chaiprakarn”.



Figure 2.1 Fang Petroleum Refinery, Fang District, Thailand
Source : website <http://www.npdc.mi.th>

It was at this time that the Thai government decided that petroleum activities be transferred to the Defense Energy Department. In 1956, a refinery capable of producing

1,000 barrels of oil per day was constructed. This was first oil refinery plant constructed in Thailand. The construction of the refinery began on 31 December 1958 and was built by the Refining Associated Corporation of the United States. Its construction was completed on 27 December 1959 (website http://www1.mod.go.th/opsd/dedweb/english_information.htm).

The refining units consists of topping unit, vacuum unit, thermal cracking unit, gasoline treating unit, lube oil treating unit and gasoline blending unit. And product from refined oil included medium diesel 8%, lubricants (various grades) 27%, crack gasoline 8% and heavy stove oil 57%. Only 200000 reserved barrels of crude oil were found from the Chai Prakarn source. The refinery refined 1000 barrels per day because crude oil was insufficient (website <http://www.npdc.mi.th>).

2.4 History oil production and development in Thailand

In Thailand, the search for hydrocarbons began in 1921 in the Fang Basin of northern Thailand after oil seeps had been reported (website <http://www.ccop.or.th>). Thailand contains 583 million barrels of proven oil reserves. In 2003, Thailand produced 259,000 barrels per day (bbl/d) of oil, an increase of about 49,000 bbl/d from the previous year. Of that production, only about 96,000 bbl/d was crude oil. Oil consumption in 2003 was 851,000 bbl/d, up from 843,000 bbl/d in 2002. The oil industry in Thailand is dominated by PTT, formerly the Petroleum Authority of Thailand. Thai Oil, the country's largest refiner, is also controlled by PTT. Thailand has four oil refineries, a combined capacity of 703,100 bbl/d. The three main refineries are Shell Co. of Thailand

Ltd. (275,000 bbl/d) located in Rayong, Thai Oil Co. Ltd, in Sriracha (192,850 bbl/d), and Esso Standard Thailand Ltd. (173,500 bbl/d), also located in Sriracha (website <http://www.eia.doe.gov/ema/cabs/thailand.html>).

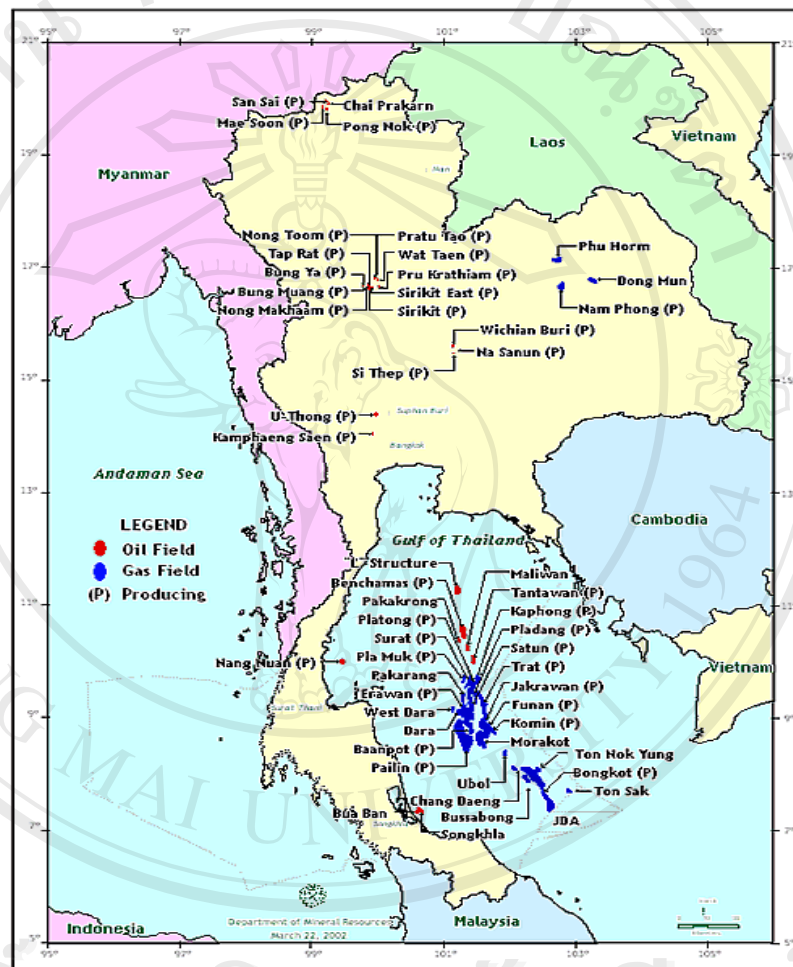


Figure 2.2 Map of petroleum field in Thailand

Source: (website <http://www.ccop.or.th>)