# CHAPTER II BACKGROUND AND LITERATURE SURVEY

# 2.1 Wastes Generated from Petroleum Refining and Related Processes

# 2.1.1 <u>Types of Wastes</u>

Crude petroleum and many of the products refined from petroleum contain thousands of hydrocarbons and related compounds (Vasudevan and Rajaram, 2001). Wastes generated from refinery are mainly sludges and liquid wastes and these types of wastes are commonly found in refineries around the world. Oil Sludge

Oil sludge may be generated from exploration as well as refinery activities. Separation processes of crude oil from water commonly generates sludge containing heavy oil as well as associated soil particles that cannot be further separated. Storage tank may also accumulate sludge at its bottom part, which requires cleaning regularly. This sludge cannot be just open-dumped since it has great potential to leach and become persistent sources in soil and water environment. The sludge needs either treatment to reduce its potential or to be isolated. Isolation of sludge by storing in small storage tanks nevertheless can cause another difficulty since that tank may occupy spaces.

# Liquid Wastes

Liquid wastes refer to both liquid waste generated from processes in refinery activity and produced water. The quality of final effluent (before discharge) of liquid may vary, depending on waste treatment system applied in each industry.

There are major compositional variations between different crudes and refined oils. Some oils contain toxic hydrocarbons, which may prevent or delay microbial attack; some refined oils have additives, such as lead, which can inhibit microbial degradation of polluting hydrocarbons. Oils contain varying proportions of paraffinic, aromatic, and asphaltic hydrocarbons. Both the rate and extent of biodegradation are dependent upon the relative proportions of these classes of hydrocarbons. Under favorable conditions, microorganisms will degrade 30 to 50% of a crude oil residue, but degradation of such a residue is never complete and does not affect the different main hydrocarbon groups in the same manner (Margesin and Schinner, 2001).

# 2.1.2 Sources of Wastes

These are wastes from nonspecific sources, wastes from specific sources, acutely toxic wastes, and toxic wastes. In addition, there are four characteristics of wastes: ignitability, corrosivity, reactivity, and toxicity. Table 2.1 shows a variety of petroleum wastes commonly found at various locations throughout the world.

**Table 2.1** Types of wastes applied to full-scale refinery (McKetta, 1992)

Oil-water separator sludge Dissolved air floatation sludge Induced air flotation sludge Tank bottom sludge Slop oil emulsion solids Filter clays Biological sludge Cooling tower sludge Lime sludge Coker blowdown sludge

# 2.1.3 Current Treatment Techniques

# Land Disposal

Dewatered sludge may be disposed of in a landfill or specifically designed monofill for sludge. Burial can be the final disposal for dried digested sludge and dewatered raw sludge cake since these pose health problems, the landfill sites are designed to prevent groundwater and surface-water pollution. Many cities bury waste water sludge in their sanitary landfill along with municipal refuse. The basic operations include spreading, compacting, and covering the wastes daily with excavated soil (Mark, 2001). To minimize such spread of contamination, certain categories of waste have been targeted to be banned from land disposal. One of these is liquids that no bulk hazardous waste liquid or and such waste containing free liquids can be placed in landfills. No nonhazardous liquids can be placed in a landfill, which absorbed on material that would release those liquids if compressed or biodegraded, and absorbents used during spill cleanups. A test was developed to determine whether a waste is considered a liquid or contains free liquids. This is the paint filter liquids test. A predetermined amount of waste is put into the paint filter. If any portion of the waste passes through the filter within 5 min, the wastes is considered liquid. It cannot be land filled without further treatment to solidify the Liquid materials must be stabilized before placement in a landfill. waste. Containerized liquids are being regulated as well. Disposal of containerized liquids will be minimized, however, if a bulk liquid is found to contain adsorbents, an owner or operator can containerize the materials and landfill them in compliance with the containerized liquids requirements. This means of disposal can be significantly more expensive than other methods. A refinery generating such waste would be subject to the regulations and added cost of treatment or containerization. However, Ekpo et al. (2000) indicated that landfill sites did not suitable because physico-chemical analysed for temperature, pH, conductivity, dissolved oxygen, biological oxygen demand after 5 days of incubation, sulphate, nitrates, nitrite, trace and major element in the leachate known to impact human health and the environment, indicated high levels of pollution.

# Incineration

Incineration, perhaps the oldest waste treatment technology, uses very high temperatures to burn waste materials. Incineration exposes organic contaminants in soils, sludges, sediments or other materials to very hot temperatures, greater than 1,000°F, in the presence of air. These conditions result in the combustion (burning) and destruction of organic wastes. A secondary combustion chamber (afterburner) may be used to help ensure that unburned organics do not enter the flue gases. Flue gases are then quickly cooled to below 350°F to minimize the possibility of organics (like dioxin) reforming in stack emissions. Gases are then

treated in air pollution control equipment to remove particulates and acids before release through the stack.

#### Land Treatment

Land treatment relies on the dynamic physical, chemical, and biological processes occurring in the soil. As a result, the constituents in the applied wastes are degraded, immobilized, or transformed to environmentally acceptable components. Land treatment is a managed technology that involves the controlled application of a waste on the soil surface and the incorporation of the waste into the upper soil. It is not the indiscriminate dumping of waste on land, and differs significantly from landfilling, waste piles, and surface impoundments. These methods store wastes in man-made or natural excavations and use a combination of liners and leachate collection systems to control the migration of the waste of resultant by-products

# 2.2 Microbial Degradation of Hydrocarbon

Biodegradation or microbial degradation refers to the natural process whereby bacteria or other microorganisms alter and break down organic molecules into other substances, such as fatty acids and carbon dioxide. Microbial attacks on petroleum have been shown to occur toward n- and branched alkanes or toward several aromatic compounds; the high molecular weight aromatics, resins, and asphaltenes generally being considered to be recalcitrant to biodegradation (Sugiura *et al.*, 1997). Microorganisms require energy to maintain by themselves. They must carry out oxidations to obtain sufficient energy for their essential functions. Different microorganisms utilize different metabolic processes to derive their energy, and based on what they use for an energy source, these organisms can be classified as autotrophic or heterotrophic.

Autotrophic bacteria are able to live in a strictly inorganic environment, utilizing carbon dioxide or carbonates as a sole source of carbon. The chemosynthetic autotrophs (also called lithotrophs) obtain their energy by oxidation of an inorganic substrate (e.g. iron, sulfur, ammonia and nitrite). Included with the chemototrophs are the hydrogen bacteria, which oxidize ferrous salts to ferric hydroxide. Those organisms that can oxidize hydrogen can often similarly utilize carbon compounds.

Heterotrophic aerobes employ respiration to oxidize organic compounds as a source of carbon and energy. The heterotrophic bacteria require a more complex source of carbon than  $CO_2$  and obtain their energy by the degradation of organic matter. These organisms are the most active in degradation of petroleum hydrocarbons (Samiullah, 1990). Individual components of petroleum and oganisms capable of degrading them are given in Table 2.2.

Fuel Component/Hydrocarbon	Microorganism
n-Alkanes (C1 to C4) gaseous	Mycobacterium ketoglutamicum
n-Alkanes (C3 to C16)	Mycobacterium rhodochrous
n-Alkanes (C8 to C16)	Mycobacterium fortitum, M.smegmatis
n-Alkanes (C12 to C16)	Mycobacterium marinum, M. tuberculosis,
	Corynebacterium
n-Alkanes (C5 to C16)	Arthrobacter, Acinetobacter, Peudomonas putida,
	yeasts
n-Alkanes (C11 to C19)	Prototheca zopfii, Pseudomonas sp.
alkanes (straight chain)	Pseudomonas putida
Alkenes (C6 to C12)	Pseudomonas oleovorans
Anthracene	Flavobacterium, Beijierinckia sp., Cunninghamella
	elegans,
	Pseudomonas/Alccaligenes sp., Acinetobacter sp.,
	Arthrobacter sp.
Aromatics	Pesudomonas sp.
Benzene	Pseudomonas putida, P. rhodochrous, P.

**Table 2.2** Fuel components/hydrocarbons and microorganisms capable ofbiodegradation/biotransforming (modified from Riser-Roberts, 1992)

# 2.2.1 General Pathway of Microbial Hydrocarbon Metabolism

The eventual mineralization of organic compounds may be attributed almost entirely to biodegradation processes. Although higher organisms can metabolize compounds, microbial degradation plays the most significant role in environmental systems. The majority of biodegradation reactions can be categorized as oxidative, reductive, hydrolytic or conjugative (Samiullah, 1990). Table 2.3 presents examples of these reactions.



# Table 2.3 Examples of biodegradation reactions

## Table 2.3(continued)



# 2.2.2 Factors Affecting Biodegradation

Biodegradation can be affected by physical, chemical, biological, and environmental factors, such as solubility, dispersion and diffusion, sorption, volatility, viscosity, density, chemical structure, environmental factors, toxicity, concentration, and biological factors (Samiullah, 1990).

1. Physical and Chemical Factors

#### Solubility

Organic compounds differ widely in their solubility, from infinitely miscible polar compounds, such as methanol, to extremely low solubility nonpolar compounds, such as high molecular weight polynuclear aromatic hydrocarbons. The availability of a compound to an organism will dictate its biodegrability. Compounds with greater aqueous solubilities are generally more available to catabolic enzymes, of a hydrocarbon molecule decreases as the number of its carbon atoms increases (Sugiura *et al.*, 1997). Janiyani *et al.* (1993) showed that increase in soluble chemical oxygen demand with increase in concentration of surfactant and contact time was observed indicating enhancement in solubility of hydrocarbons in the aqueous phase. The concentration of a contaminant will affect the number of organism present. It has been noted that higher concentrations of pyrene were related to higher counts of microorganism (Chung and Alexander, 1999). It has been shown that the same compounds in different crude oil samples were degraded to different degrees by the same organism. The most simplest interpretation of the observation is that the bioavailability of these alkenes in different crude oil samples was different (Sugiura *et al.*, 1997).

# **Dispersion and Diffusion**

Diffusion contaminants spread as they move with the groundwater (Mackey *et al.*, 1985). This dispersion results from molecular diffusion and mechanical mixing, and causes a net flux of the solutes from a zone of high concentration to a zone of lower concentration. This movement causes the concentrations to diminish with increasing distance from the source and the plume to become more uniform, such as oxygen diffusion which is required for microbial hydrocarbon biodegradation processes (Hueseman and Truex, 1996). This is an important consideration when deciding whether dispersants should be added to oil spill. However, dispersants may contain chemicals that are inhibitory to microorganisms. Without toxicity, how ever, dispersion can enhance petroleum biodegradation (Atlas, 1981).

# Sorption

Sorption is perhaps the most important single factor affecting the behavior of organic chemicals in the soil environment. Adsorption to soil constituents will affect the rate of volatilization, diffusion, or leaching, as well as the availability of chemicals to microbial or chemical degradation. Compounds that sorb strongly onto solids are retarded in their movement. The main subsurface solids responsible for adsorption of organic chemicals are solid organic matter (strong associations with hydrophobic organic compounds), such as oil sludge consisting of hydrophobic substances and substances resistant to biodegradation (Samiullah, 1990).

# <u>Volatility</u>

The surface sheen residue emanating from an emulsified oil slick after evaporation of the light boiling components gradually becomes enriched in high molecular constituent concomitant with a change in chemical composition. A film of crude oil will, therefore, adsorb a considerable amount of solar energy, leading to transformation and degradation of the film through photo-induced oxidation (Dutta and Harayama, 2000).

# Viscosity

Viscosity and surface-wetting properties affect the transport of an organic liquid phase. Viscosity of polluting oils is an important property that determines, in part, the spreading and dispersion of the hydrocarbon mixture and, thus, the surface area available for microbial attack. For example, the viscosity of the heat-treated Maya and Shengli samples was much higher than that of the heat-treated Dubai and Arabian light crude (Sugiura *et al.*, 1997).

# Density

Density differences of only 1 percent can influence fluid movement in the subsurface (Mackey et al., 1985). The specific gravities of hydrocarbons (gasoline and other petroleum distillates may be as low as 0.7 and halogenated hydrocarbons, are almost without exception, significantly more dense than water (Samiullah, 1990). Density determines where in the aquifer the contaminant will most likely be concentrated. Low-density hydrocarbons have a tendency to float on water and may be found in the upper portions of an aquifer. High-density hydrocarbons would sink to the lower portions of the aquifer. It is important to recognize that migration of dense organic liquid is largely uncoupled from the hydraulic gradient that drives advective transport and that the movement may have a dominant vertical component, even in horizontally flowing aquifers. An organic liquid contaminant, such as gasoline, which is immiscible with and less dense than groundwater, would migrate vertically to and then float on the water table, spreading out in the downgradient direction (Mackey et al., 1985). If the organic liquid contains a contaminant slight soluble in water, e.g., benzene, a plume would form in the saturated zone. A complex pattern of overlapping plumes can develop when many contaminants are involved. The crude oil with higher API gravity being more susceptible to biodegradation (Sugiura *et al.*, 1997).

# Chemical Structure

The structure, concentration, and toxicity of a chemical are important in determining whether it is accumulated in the environment and the environment impact of the accumulation. The chemical will accumulate if its structure prevents mineralization or biodegradation by organisms. This may be due to its insolubility or to a novel chemical structure to which microorganisms have not been exposed during evolutionary history. Such compounds are termed xenobiotic. This name refers to compounds of anthropogenic (man made) origin, as well as to compounds that may occur naturally but exceed normal levels in the environment. Various laboratory culture techniques applied to samples from nature can be used to select or develop bacteria with the ability to biodegrade many of these chemicals. The degree of biodegradation of crude oil components differed according to the crude oil. Generally, the larger and more complex the structure of a hydrocarbon, the more slowly it is oxidized. The saturated fraction, e.g., n-alkanes, are highly degraded, while asphaltenes and aromatics are often resistant to microbial attack (Sugiura et al., 1997; Dutta and Harayama, 2000). Although n-alkanes are probably metabolized more rapidly than napthenes or other aromatics, the reactions appear to be slower with increasing chain length, possibly because of differences in water solubility, and because the n-alkanes with shorter chains are more easily used as a source of carbon and energy by microorganisms than those with longer chain lengths (Sugiura et al., 1997). The degree of substitution affects biodegradation. Compounds that pissess amine, methoxy, and sulfonate groups, ether linkages, halogens, branched carbon chains, and substitutions at the meta position of benzene rings are generally persistent (Samiullah, 1990). The biodegradability of the aromatic compounds was lower with increasing size of their alkyl side chain (Samiullah, 1990; Sugiura et al., 1997).

# Toxicity

Crude oils are mixtures of tremendous complexity, containing hundreds of hydrocarbon and nonhydrocarbon components, many of them still unidentified and some of these very toxic toward microorganisms, such as polycyclic aromatic hydrocarbon may enhance the toxicity of the metals (e.g. Cd, Cu and Zn) because they can penetrate into the perforated microbial cells more easily this could cause changes in the membrane structure and might alter the permeability of the membranes (Gogolev and Wike, 1997).

# 2. Environmental Factors

The fate of petroleum hydrocarbons in the environment is largely determined by abiotic factors which influence the weathering that mean the chemically and biologically induced changes in the composition of a polluting petroleum hydrocarbon mixture, including biodegradation of the oil. Factors that influence rates of microbial growth and enzymatic activities affect the rates of petroleum hydrocarbon biodegradation. The persistence of petroleum pollutants depends on the quantity and quality of the hydrocarbon mixture and on the properties of the affected ecosystem. In one environment petroleum hydrocarbons can persist indefinitely, whereas under another set of conditions the same hydrocarbons can be completely biodegraded within a relatively few hours or days (Atlas, 1981). Metabolism by indigenous microflora is influenced by environmental factors, such as temperature, nutrients, oxygen, salinity and pressure.

# Temperature

Temperature plays a significant role in controlling the nature and the extent of microbial hydrocarbon metabolism, which is of special significance for in situ bioremediation. Bioavailability and solubility of less soluble hydrophobic substances such as aliphatic and polyaromatic hydrocarbons, are temperature dependent. A temparature increase affects a decrease in viscosity, thereby affecting the degree of distribution, and an increase in diffusion rates of organic compounds. Therefore, higher reaction rates due to smaller boundary layers are expected at elevated temperatures. The increased volatilization and solubility of some hydrocarbons at elevated temperature affects toxicity and allows biotransformations with high substrate concentrations (Margesin and Schinner, 2001).

# Nutrients

The available concentration of nitrogen and phosphorus severely limit the extent of hydrocarbon degradation after most major oil spills. Rates of nutrient replenishment generally are inadequate to support rapid biodegradation of large quantities of oil. The addition of nitrogen and phosphorus containing fertilizers can be used to stimulate microbial hydrocarbon degradation (Atlas, 1981).

# Oxygen

As with nutrients, there has been controversy over whether oxygen is absolutely required for hydrocarbon biodegradation or whether hydrocarbons are subject to anaerobic degradation (Atlas, 1981). 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. A 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% (Huesemann and Truex, 1996; Rhykerd *et al.*, 1999; Vasudevan and Rajaram, 2001).

#### Salinity and Pressure

These factors are specific features of particular ecosystems such as saline lakes or deep seas, where the hydrostatic pressure is notably high (Atlas, 1981). A combination of high pressure, low temperatures and high salinity in the deep ocean results on low microbial activity (Margesin and Schinner, 2001).

# 3. **Biological Factors**

Increased persistence of chemicals may result from several types of biological interactions: 1) the biocidal properties of the chemicals to soil microorganisms may preclude their biodegradation, 2) direct inhibition of the adaptive enzymes of effective soil microorgranisms, and 3) inhibition of the proliferation processes of effective microorganisms. Inhibition of microbial degradation may ultimately affect mobility of a chemical in soil (Atlas, 1981). If a microorganism is prevented from utilizing a simple carbon sources and is thus made totally dependent upon the utilization of toxic compounds, the efficiency of the microorganism in removing toxic compounds from polluted environments will be greatly enhanced (Samanta *et al.*, 2001). Hydrocarbon-degrading microorganisms act mainly at the oil-water interface. Hydrocarbon-degrading microorganisms can be observed growing over the entire surface of an oil droplet; growth does not appear to occur within oil droplets in the absence of entrained water. Availability of increased surface area should accelerate biodegradation (Atlas, 1981). Sugiura *et al.* (1997) showed that the outer membrane permeability may be one of the major factors to determine biodegradability which the proportion of cycloalkanes to linear alkanes on the saturated fraction gradually increases as the number of carbon atom increases. This may be one of the reasons why the higher molecular weight compounds on the saturated fraction were less susceptible to biodegradation.

# 2.3 Use of Surfactants for Enhancing Microbial Degradation of Hydrocarbon

Surfactants are surface-active compounds capable of reducing surface and interfacial tension at the interfaces between liquids, solids and gases, thereby allowing them to mix or disperse readily as emulsions in water or other liquids. Surfactants are amphiphilic molecules consisting of a hydrophilic and a hydrophobic domain. The non-polar, hydrophobic part is frequently a hydrocarbon chain. The polar component appears in many variations (Garcia et al., 2001). The enormous market demand for surfactants is currently met by numerous synthetic, mainly petroleum-based, chemical surfactants. These compounds are usually toxic to the environment and non-biodegradable. They may bio-accumulate and their production, processes and by-products can be environmentally hazardous. Tightening environmental regulations and increasing awareness for the need to protect the ecosystem have effectively resulted in an increasing interest in biosurfactants as possible alternatives to chemical surfactants. Biosurfactants are amphiphilic compounds of microbial origin with considerable potential in commercial applications within various industries. They have advantages over their chemical counterparts in biodegradability and effectiveness at extreme temperature or pH and in having lower toxicity (Banat et al., 2000).

# 1. Synthetic Surfactants

Chemical surfactants have the potential to improve crude oil biodegradation in complex microbial systems and surfactant selection should consider factors such as molecular structure, hydrophile-liophile, and surfactant concentration (Hamme and Ward, 1999). The most commonly used surfactants are non-ionic surfactants such as ethoxylates, ethylene and propylene oxide co-polymers and sorbitan esters (Banat *et al.*, 2000). Janiyani *et al.* (1993) showed that treatment of oil sludge with the synthetic surfactant polyoxyethylene sorbitan monooleate indicated the increase of hydrocarbons in the aqueous phase. Increase in soluble chemical oxygen demand with increase in concentration of surfactant and contact time was observed indicating enhancement in solubility of hydrocarbons in the aqueous phase. Bruheim *et al.* (1999) indicated that there was synergism between the mixtures of nonionic and anionic surfactants on bacteria oxidation of acetate and alkanes in crude oil. In another study, a commercial nonionic surfactant, Igepal CO-630 was found to result in an increased total petroleum hydrocarbon removal by mixed bacterial cultures (Hamme and Ward, 2001).

# 2. Biosurfactants

Microorganism can also produce surfactant biologically, or so called "biosurfactant". Microbial compounds, which exhibit particularly high surface activity and emulsifying activity, are classified as biosurfactants. Biosurfactants are structurally diverse compounds, mainly produced by hydrocarbon-utilizing microorganisms that exhibit surface activity. Biosurfactants can be produced using relatively simple and inexpensive procedures and substrates. Some structural types of surfactants are produced using biological systems and cannot easily be synthesized by chemical processes. These molecules can be tailor-made to suit different applications by changing the growth substrate or growth conditions. Biosurfactants are both biodegradable, which is a positive ecological aspect or less toxic than chemical surfactants. They occur naturally in soil, which makes them acceptable from a social and ecological point of view. There are many potentially useful biosurfactants, including both ionic and non-ionic surfactants that range from short fatty acids to large polymers. This wide range results in a broad spectrum of potential industrial applications. The largest possible market for biosurfactant is the oil industry, both for petroleum production and for incorporation into oil formulations. Other application, related to the oil industries includes oil spill bioremediation/dispersion, both inland and at sea, remove/mobilization of oil sludge from storage tanks and enhanced oil recovery. The second largest market for biosurfactants is emulsion polymerization for paints, paper coatings and industrial coatings (Banat *et al.*, 2000).