

## CHAPTER II

### THEORETICAL BACKGROUND AND LITERATURE REVIEW

#### 2.1 Waste from petroleum industry

Petroleum refineries are responsible for the emission of particulates, carbon monoxide, nitrogen oxides ( $\text{NO}_x$ ), sulfur oxides ( $\text{SO}_x$ ), and carbon dioxide. Catalyst changeovers and cokers release particulates. Volatile organic compounds (VOCs) such as benzene, toluene, and xylene are released from storage, product loading and handling facilities, and oil-water separation systems and as fugitive emissions from flanges, valves, seals, and drains.

Refineries generate polluted wastewaters, containing biochemical oxygen demand (BOD) and chemical oxygen demand (COD) levels of approximately 150-250 mg/l and 300-600 mg/l respectively. Refineries also generate solid wastes and sludges, 80% of which may be considered hazardous because of the presence of toxic organics and heavy metals.

##### 2.1.1 Source of waste

The refining sector generates the predominant share of hazardous wastes in the petroleum industry. Various sources of petroleum refinery wastes are shown in Figure 2.1.

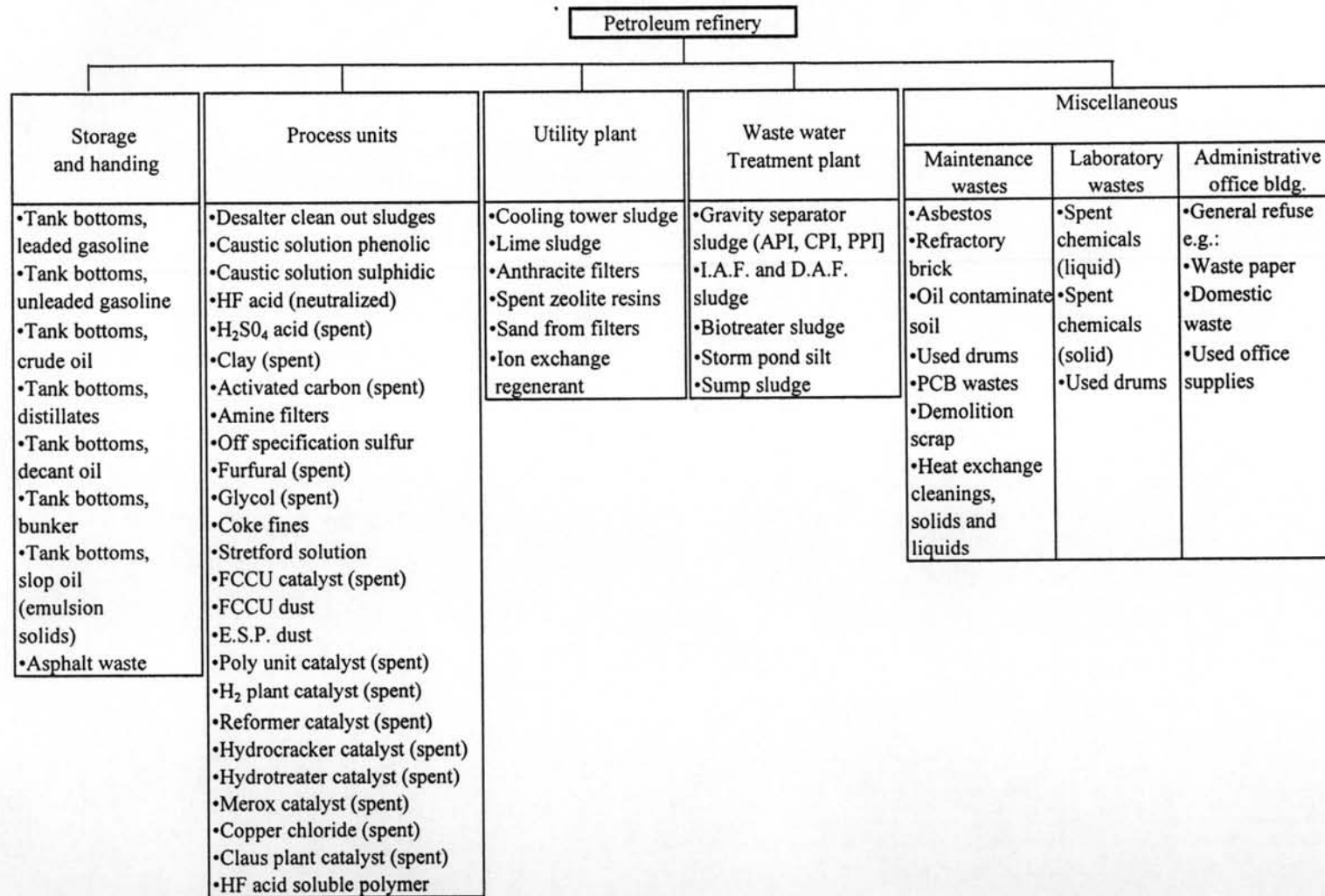


Figure 2.1 Sources of petroleum refinery wastes

### 2.1.2 Waste characteristic

There are usually three types of waste distinguished;

- i) Domestic waste (office and households)
- ii) Special waste (hazardous to health and/or the environment)
- iii) Industrial waste (generate during petroleum activities and which is neither domestic nor special)

The purpose of waste characteristic is to determine whether or not the waste is hazardous. The physical and chemical properties of the waste influence its hazardous characterization is also required to determine and assign the waste stream categories and select options for segregation, minimization, treatment and ultimately disposal. Generally, a waste may be classified as hazardous and consequently falls under the special waste category when it meets one of two conditions;

- i) The waste is specifically regulated by a governmental authority or an internationally recognized organization, or
- ii) The waste possesses one or more of the four hazardous characteristics that are ignitability, corrosivity, reactivity, and toxicity.

#### 2.1.2.1 *Oil Sludge*

Oily materials are the primary source of waste for most refineries and are generated when oil coalesces on solids, such as dirt particles. A large portion of the oil material is actually sand or grit covered with a small amount of oil. Oily residues are collected at several points within the refinery, oil/water separators, dissolved air flotation units which are part of the waste water treatment process, heat exchanger cleanings, and tank bottom cleaning (Curran, 1989).

Oily wastes behave just as their definition suggests an oily waste is any solid or liquid substance that, alone or in a solution, can produce a surface film or sheen when it is discharged in clean water. Most oily wastes are derived from petroleum or have characteristics of petroleum products. Waste oil is and oily waste that can not be reused by the ship and it contains only small amounts of water. Any mixture that causes a sludge or emulsion to be deposited beneath the surface oil and chemical pollution of the water is considered to be an oily waste.

Oily wastes frequently present a shipboard pollution problem. Oily wastes derived from lubricating oils are caused by tank cleaning operations,

leakage and drainage from equipment and systems, stripping from contaminated oil-setting tanks, and ballast water from fuel tanks of noncompensated fuel systems during the ship's defueling, refueling, or internal transfer operations.

### 2.1.3 Waste treatment techniques

Treatment and disposal options depend largely on the waste characteristics and regulatory requirements. Available methods are usually limited by ecological, technical or economic factors. Waste treatment aims at reducing waste's toxicity or hazardous properties through chemical, physical, thermal or biological processes.

#### 2.1.3.1 *Incineration*

Controlled incineration is one of the best treatment disposal options because thermally treated wastes are decomposed to non-or less hazardous by-products. Controlled incinerators operate at sufficient temperatures for complete thermal decomposition of hazardous wastes. Non-hazardous and hazardous solids, liquids and gasses can be incinerated. Incineration exposes organic contaminants in soils, sludges, sediments, or other materials to very hot temperature, greater than 1000 °F, in the present of air. Incinerators are usually used to destroy organic wastes, which pose high levels of risk to health and the environment (Niessen, 1978).

#### 2.1.3.2 *Solidification, Stabilization, Encapsulation*

Solidification is the process of reducing the excess water in a waste sludge. Waste management laws generally prohibit waste sludge containing free water to be deposited in the landfills.

Stabilization is the process of chemically changing a hazardous waste material into a less soluble or less soluble or less soluble or less toxic form. Many toxic metals found in waste sludge have their lowest solubility at the higher pH levels and are therefore less likely to leach out. The presence of ferrous iron and sulfur compounds make slag cement an excellent reducing agent that can change toxic metals into less toxic forms.

Solidification/stabilization is a widely used treatment for the management or disposal of a broad range of contaminated media and wastes. The treatment involves mixing a binding reagent into the contaminated media or waste.

The treatment protects human health and the environment by immobilizing contaminants within the treated material. Immobilization within the treated material prevents migration of the contaminants to human, animal and plant receptors. Solidification/stabilization is an effective treatment wide variety of organic and inorganic contaminants present in contaminated soil, sludge and sediment.

The disadvantage of this treatment technique is high concentrations of organic compounds, salts and bentonite have been shown to interfere with the curing process, and therefore limit the application of this treatment technique.

Encapsulation is the process of surrounding waste particles with a layer of material that is very low in permeability. This layer of material inhibits the leaching of the hazardous material.

#### *2.1.3.3 Land treatment*

The use of land treatment under controlled and well-designed and operated conditions should continue to be available to the petroleum and other industries.

Land treatment is a managed technology that involves the controlled application of a waste on the soil surface and incorporation of the waste into the upper soil. 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 technology has been used successfully for the treatment and disposal of petroleum industry wastes.

#### *2.1.3.4 Landfill*

Even today, the disposal to land is often poorly controlled and managed, especially in developing countries. In those countries whose landfill operations are better controlled, the impact of the Directive will still be significant; in the United Kingdom (UK) there are approximately 4000 licensed landfill sites where approximately 85% of controlled waste and 70% of hazardous waste are disposed. The cost of landfill disposal can also be anticipated to increase significantly. As the cost increases, and the differential between landfill and other disposal options such as

incineration decreases, so the easier it becomes to use alternative disposal routes that are more favorably placed in the waste treatment hierarchy.

Landfill has been defined as the engineered deposit of waste onto and into land in such a way that pollution or harm to the environment is prevented and, through restoration, land provided which may be used for another purpose. Prevention of harm to the environment is achieved in a number of ways, but requires effective control of waste degradation processes and effective landfill design, engineering, and management.

#### *2.1.3.5 Biotreatment*

Biological treatment of organic pollutants is a promising field of research, which give reliable, simple and cheap technology over chemical and physical processes. Successful biotreatment of petroleum hydrocarbon contaminated sites remains a challenge and several factors must be fulfilled and optimized to determine the outcome of the biodegradation process such as biomass concentration, population diversity, bacterial growth, metabolic pathway, nature and concentration of pollutants, chemical structure of organic compounds, toxicity of contaminants, and pre-sence of nutrients.

The example of biotreatment is bioremediation. Bioremediation could be a cost-effective clean-up technology to treat oily sludge and sediments containing biodegradable hydrocarbons and indigenous specialized microorganisms. This treatment has 2 processes, which are mineralization and biotransformation. Mineralization is the complete conversion of organic molecules into inorganic substances. Biotransformation is the transformation of a parent compound into other metabolites, which may be more or less toxic than the parent compound. Bioremediation has become a major method employed in the restoration of oil-polluted environments, and attempts to accelerate the natural hydrocarbon degradation rates by overcoming factors that limit bacterial hydrocarbon degrading activities. Moreover bioremediation has been demonstrated to be effective on various types of shoreline. Field studies have showed that bioremediation can be used successfully to clean rocky, cobble and coarse sand shoreline.

## 2.2 Biodegradation of Hydrocarbon Compounds

Sludge and liquid waste from oil and gas industry contains mainly petroleum hydrocarbon. Petroleum hydrocarbon is mixture of aliphatic, aromatic, polycyclic hydrocarbon ranging from short  $C_3$  to much longer carbon chains. Although biodegradation of petroleum hydrocarbon can be performed in anaerobic condition (Cookson, 1995), however aerobic biodegradation seems to be more effective. Thus in order to biotreat oil sludge the presence or supply of oxygen is very important factor to the success of the treatment (Pritchard *et al.*, 1992). Biodegradation of petroleum hydrocarbon is a complex process that depends on the nature and amount of oil or hydrocarbon present. In nature, biodegradation of a crude oil typically involves a succession of species within the consortia of microbes present. The indigenous populations of microorganisms, which are ubiquitous in soil and groundwater and self adapted to hard conditions, actually grow by using the carbon from the pollutants as energy source and cells building blocks. This break down the contaminants into carbon dioxide and water as end products (Scrag, 1999).

Many microorganisms possess the enzymatic capability to degrade petroleum hydrocarbons. Some microorganisms degrade alkanes (normal, branched and cyclic paraffins), others aromatics, and others both paraffinic and aromatic hydrocarbons (Atlas, 1981; Leahy and Colwell, 1990; Atlas and Bartha, 1992). Often the normal alkanes in the range  $C_{10}$  to  $C_{26}$  are view as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene and xylene, which are among the toxic compounds found in petroleum, are also very readily biodegraded by many marine microorganisms. More complex structures are more resistant to biodegradation, meaning that fewer microorganisms can degrade those structures. The greater the complexity of the hydrocarbon structure, i.e. the higher the number of methyl branched substituents or condensed aromatic rings, the slower the rates of degradation and the greater the likelihood of accumulating partially-oxidized intermediary metabolites.

Many petroleum hydrocarbons biodegrade if indigenous soil bacteria have adequate oxygen and nutrients. Because of oxygen limitations, however, natural biodegradation is frequently too slow to prevent the spread of contamination

(Hinchee *et al.*, 1991). In general, the order of the biodegradability of petroleum hydrocarbon fractions follows the relationship: saturates > aromatics > polars > aspathenes.

## 2.2.1 Factors affecting biodegradation

### 2.2.1.1 *Physical and Chemical Factors*

#### a.) Chemical Composition of the oil or Hydrocarbons

The differences in chemical composition are thus attributed to different degrees of biodegradation (Ahsan *et al.*, 1996). The simpler the structure of a compound the more easily it is biodegraded. Normal alkanes are depleted prior to isoalkanes which in turn are depleted prior to cycloalkanes. Within the same compound class, lower molecular weight compounds are depleted prior to higher molecular weight compounds (Magoon and Claypool, 1981). Hydrocarbons differ in their susceptibility to microbial attack and have been (in the past) ranked in the order of decreasing susceptibility: *n*-alkanes > branched alkanes > low molecular-weight aromatics > cyclic alkanes (Perry, 1984). The biodegradation of PAHs is highly dependent on the number of aromatic rings present in the molecule, the rate of biodegradation decreasing with increasing number of aromatic rings (Huang *et al.*, 2004).

#### b.) Physical State of the Hydrocarbons

Oil spilled in water tends to spread and form a slick. As a result of wind and wave action, oil-in-water or water-in-oil emulsions may form (Cooney, 1984). Dispersion of hydrocarbons in the water column in the form of oil-in-water emulsions increases the surface area of the oil and thus its availability for microbial attack. However, large masses (or plates) of mousse establish unfavorably low surface to volume ratios, inhibiting biodegradation (David and Gibbs, 1975). Tarballs, which are large aggregates of weathered and undegraded oil, also restrict access by microorganisms because of their limited surface area (Colwell *et al.*, 1978).

The formation of emulsions through the microbial production and release of biosurfactants is an important process in the uptake of hydrocarbons by bacteria and fungi (Singer *et al.*, 1984). Artificial dispersants have been studied as



a means of increasing the surface area and hence the biodegradability of oil slicks. The effectiveness of dispersants in enhancing the biodegradation of oil has been shown to be extremely variable and to be dependent on the chemical formulation of the dispersant, its concentration, and the dispersant/oil application ratio.

c.) Concentration of the Oil or Hydrocarbons

The rate of uptake and mineralization of many organic compounds by microbial populations in the aquatic environment are proportional to the concentration of the compound. The rates of mineralization of the higher-molecular-weight aromatic hydrocarbons, such as naphthalene and phenanthrene, are related to aqueous solubilities rather than total substrate concentration (Thomas *et al.*, 1986).

High concentrations of hydrocarbons can be associated with heavy, undispersed oil slicks in water, causing inhibition of biodegradation by nutrient or oxygen limitation or through toxic effects exerted by volatile hydrocarbons. Fusey and Oudot (1984) reported that contamination of seashore sediments with crude oil above a threshold concentration prevented biodegradation of the oil because of oxygen and/or nutrient limitation.

d.) Viscosity

Viscosity and surface-wetting properties affect the transport of an organic liquid phase. Koopman *et al.* (2002) suggested that the large variation in viscosity of the Liaohe oils can be explained by mixing, to various extents, of heavily biodegraded oils with less degraded oils as reflected in the co-occurrence of n-alkanes. Moreover viscosity of polluting oils is an important property that determines, in part, the spreading and dispersion of hydrocarbon mixture and, thus, the surface area available for microbial attack.

e.) pH

pH is a selective environmental factor affecting microbial diversity and activity, controlling enzyme and activity, transport process and nutrient solubility (Wong *et al.*, 2002). Most bacteria and fungi favor a pH near neutrality, with fungi being more tolerant of acidic conditions. Extremes in pH have negative influence on the ability of microbial populations to degrade hydrocarbons.

f.) Toxicity

Polycyclic aromatic hydrocarbon may enhance the toxicity of the metals (e.g. Cd, Cu and Zn). These metals are very toxic for the microorganisms because they can penetrate into the perforated microbial cells easily causing changes in the membrane structure and might alter the permeability of the membranes (Gogolev and Wilke, 1997). The changes in the microorganisms will affect to the biodegradation making the ability of microbial degradation decreases.

2.2.1.2 *Environmental Factors*

a.) Temperature

Temperature affects all biological reactions. The growth of filamentous bacteria is favored at high temperature. In fact, the temperature-dependent filamentous growth can be interpreted well by the kinetic selection theory. Temperature is also of utmost importance because of its effect on the rate of microbial growth. However, ever since microbiologists first grew microbes in pure culture, it has been appreciated that growth of particular microorganism is favored only over a restricted range of temperatures (Hua, 2004).

Bioavailability and solubility of less soluble hydrophobic substances such as aliphatic and aromatic hydrocarbons are temperature dependent. A temperature increase affects decrease in viscosity, thereby affecting the degree of distribution, and increase in diffusion rates of organic compounds. At low temperatures, the viscosity of the oil increases, the volatilization of toxic short chain alkanes is reduced, and their water solubility is increased, delaying the onset of biodegradation (Atlas and Bartha, 1972). Rate of degradation are observed to decreasing temperature. Higher temperature increases the rate of hydrocarbon metabolism to maximum, typically in the range of 30 to 40 °C, above which membrane toxicity of hydrocarbons is increased (Bossert and Bartha, 1984).

b.) Oxygen

Many petroleum hydrocarbons biodegrade if indigenous soil bacteria have adequate oxygen and nutrients. Because of oxygen limitations, however, natural biodegradation is frequently too slow to prevent the spread of contamination (Hinchee *et al.*, 1991). One important limiting factor in bioremediation soils contaminated with hydrocarbons is the lack of oxygen to

support microbial metabolism (Atlas, 1986). Soil venting is promising method for overcoming this limitation for supplying oxygen. Wilson and Ward (1986) discuss the potential for using air as an oxygen carrier to stimulate hydrocarbon biodegradation in the vadous zone. They point out that this could potentially be 1000 times more efficient for oxygen transfer than using water as a carrier. In soils, the oxygen content depends on microbial activity, soil texture, water content and depth. The availability of oxygen in soils is dependent on rates of microbial oxygen consumption, the type of soil, whether the soil is waterlogged, and the presence of utilizable substrates which can lead to oxygen depletion (Bossert and Bartha, 1984).

c.) Nutrients

Biological processes require nitrogen and phosphorous for effective removal of organics by microorganisms. Deficiency in the nutrient supply, especially nitrogen commonly results in bulking of activated sludge in waste water treatment facilities using the activated sludge process. In the absence of sufficient nitrogen, biodegrading microorganisms often produce significant amounts extracellular polysaccharides (ECP) (Aquino and Stuckey, 2003). The application of these nutrients could stimulate oil degradation in soils immediately or require longer time of incubation.

d.) Salinity

Kerr and Capone (1988) observe a relationship between the naphthalene mineralization rate and salinity in sediments of the Hudson river that was dependent upon the ambient salinity regime, with estuarine sites exhibiting a lack of inhibition of mineralization over a wider range of salinities than was the case for the less saline upstream site. Ward and Brock (1978) showed that the rate of hydrocarbon metabolism decreased with increasing salinity in the range 3.3 to 28.4‰ and attributed the results to a general reduction in microbial metabolic rates.

e.) Pressure

The importance of pressure as a variable in the biodegradation of hydrocarbons is probably confined to the deep-sea environment. Schwarz *et al.* (1974) studied the degradation of tetradecane, hexadecane, and a mixed hydrocarbon substrate by a mixed culture of deep-sea sediment bacteria was monitored at 1 atm and 495 or 500 atm. At 4 C, 94% of the hexadecane was utilized

only after a 40-week incubation under condition of high pressure, compare with 8 weeks at 1 atm. Colwell and Walker (1977) have suggested that oil or petroleum hydrocarbons, which reach the deep-ocean environment, will be degraded very slowly by microbial populations.

#### 2.2.1.3 Biological factor

Hydrocarbons in the environment are biodegraded primarily by bacteria and fungi. Although ubiquitous in terrestrial (Atlas *et al.*, 1980) and aquatic (Buckey *et al.*, 1976) ecosystems. Individual organisms can metabolize only a limited range of hydrocarbon substrate (Britton and Bartha, 1984), so that assemblages of mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil, freshwater, and marine environment. The ability to degrade and/or utilize hydrocarbon substrates is exhibited by a wide variety of bacterial and fungal genera. Based on the number of published reports, the most important hydrocarbon-degrading bacteria in both marine and soil environments are *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Nocardia*, and *Pseudomonas* spp. and the coryneforms.

The extent to which bacteria, yeasts, and filamentous fungi participate in the biodegradation of hydrocarbons has been the subject of only limited study, but appears to be a function of ecosystem and local environment conditions.

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 microorganisms, 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 and oil droplet; growth does not absent appear to occur within oil droplets in the absence of entrained water. Availability of increased surface area should accelerate the biodegradation (Atlas, 1981). Sugira *et al.* (1997) showed that the outer membrane permeability may be one of the factors to determined 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 molecular weight compounds on the saturated fraction were less susceptible to biodegradation.

### 2.2.2 Types of microbial degradation

Petroleum hydrocarbons are very complex mixtures, consisting of various organic compounds. For microorganisms to biodegrade petroleum completely or attack even simpler refined oils, thousands of different compounds may be involved, which can be metabolized. The chemical nature of these petroleum components varies from the simple n-paraffin, monoalicyclic, and monoaromatic compounds, too much more complex branched chains and condensed ring structures.

#### 2.2.2.1 *Aerobic Degradation*

Aerobic degradation is the process involving the oxidation-reductions in which molecular oxygen serves as the electron acceptor while the organic component of the contaminating substance functions as the electron donor or energy source in heterotrophic metabolism. Microorganisms, such as bacteria, fungi or actinomycetes, require the oxygen during the chemical degradation. The aerobic bacteria use oxygen to decompose organic compound into carbon dioxide and other inorganic compounds. Aerobic biodegradation of many classes of aromatic compounds is common and proceed through the key intermediate (Nora and Juan, 2001). Aerobic biodegradation occurs via more efficient and rapid metabolic pathway than anaerobic reaction. Therefore, most site decontaminations involving refined oils and fuels are conducted under aerobic condition.

Aerobic granules had compact and strong microbial structure, excellent settleability, high biomass retention and ability to handle high strength wastewater. It has been believed that aerobic granulation is independent on

characteristics on substrate, e.g. aerobic granules can grow on acetate, ethanol, glucose, phenol and synthetic wastewater, while the application of aerobic granules to remove nitrogen and phosphorus were also reported (Yang *et al.*, 2003). These seem to indicate that aerobic granulation technology would have great potential in biological treatment of municipal and industrial wastewater.

#### 2.2.2.2 Anaerobic Degradation

Anaerobic degradation is the treatment processes using nitrate, Fe (III), sulfate and carbon dioxide as electron acceptors. There is usually occur in any habitat, where the oxygen consumption rate exceeds its supply rate and is a common phenomenon in many natural aquatic environments receiving organic material. This process is an alternative for the bioremediation of hydrocarbon-contaminated sites because petroleum can be biodegraded without the presence of molecular oxygen, include toluene, xylene, ethylbenzenes, and possibly benzene.

The microbial degradation of oxidized aromatic compounds such as benzoate and for halogenated aromatic compounds has been shown to occur under anaerobic conditions.

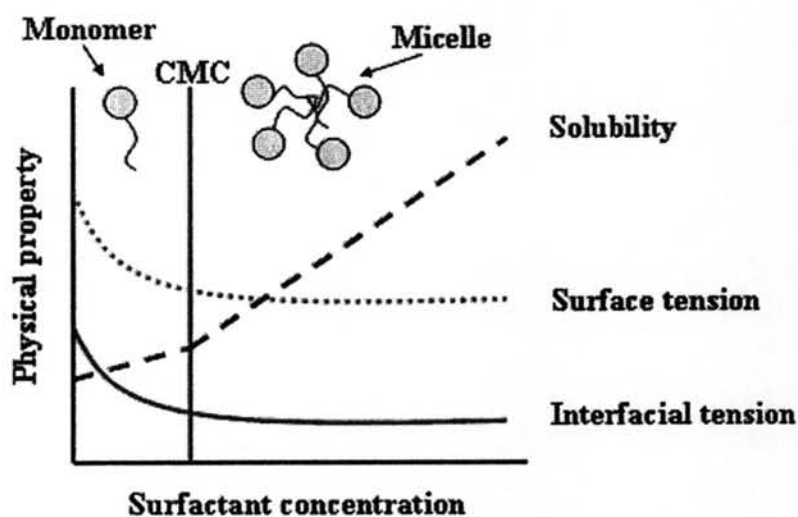
### 2.3 Surfactant enhance biodegradation

#### 2.3.1 Background of surfactant

Surfactants or surface active agents are amphipathic molecules of a polar/hydrophilic head and a nonpolar/hydrophobic tail. When added to water, a surfactant molecule may dissolve as a monomer, adsorb to an interface with its hydrophobic end pointing away from water, or aggregate with other surfactant molecules into clusters called micelles. The critical micelle concentration (CMC) corresponds to the surfactant concentration at which micelles are formed. Surfactants can reduce the free energy of the system by replacing the bulk molecules of higher energy at the interface. They contain a hydrophobic portion with little affinity for the bulk medium and a hydrophilic group that is attracted to the bulk medium. Surfactant monomer will form spheroid or lamellar structures with organic pseudo-phase interiors. This coincides will lower surface and inter facial tension as shown in the Figure 2.2 the minimum concentration at which this occurs is called the critical

micelle concentration (Rosen, 1978), salinity, hydrocarbon chain length and surfactant type (hydrophile-lipophile HLB) will influence the concentration. Generally, surfactants have been used as adhesives, flocculating, wetting and foaming agents, de-emulsifiers and penetrants (Mulligan and Gibbs, 1993). They are used for these applications based on their abilities to lower surface tensions, increase solubility, detergency power, wetting ability and foaming capacity. The petroleum industry has traditionally been the major user, as in enhanced oil removal applications. In this application, surfactants increase the solubility of petroleum components (Falatko, 1991) or lower the interfacial tension to enhance mobility of the petroleum.

The main factors that should be considered when selecting surfactants include effectiveness, cost, public and regulatory perception, biodegradability and degradation products, toxicity to humans, animals and plants and ability to recycle. The first consideration is that the surfactants are efficient in removing the contaminant. This can be established by previous experience or by laboratory studies prior to the field-scale demonstrations.



**Figure 2.2** Schematic diagram of the variation of surface tension, interfacial and contaminant solubility with surfactant concentration.

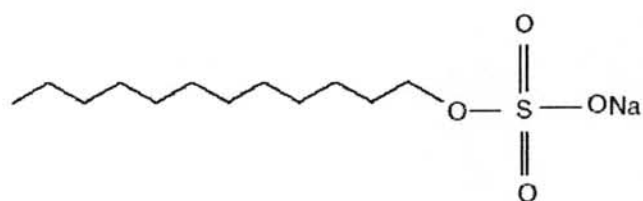
Surfactants can generally be classified into 4 types: anionic, cationic, nonionic and amphoteric surfactant.

#### 2.3.1.1 Anionic surfactants

The surface-active portion of the molecule bears a negative charge. The major subgroups of this class are the alkali carboxylates or soaps, sulfates, sulfonates and to a lesser degree phosphates. The variety of anionic materials available arises primarily from the many types of hydrophobic group that can be modified by the addition of the proper anionic species.

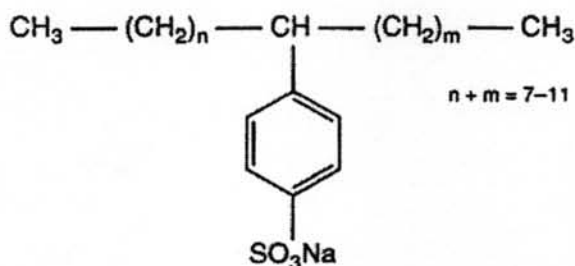
The intense adsorption of anionic surfactants onto biological systems was studied by Ewing (1962). Ewing passed natural and synthetic sewage through sand filled columns, developing a biological slime growth on the sand grains. Addition of surfactant resulted in its adsorption by biological slime. It is noteworthy that adsorption is almost negligible with the sand alone. A similar idea was invoked by Ewing to explain an anomalous rise in surfactant concentration occasionally observed after an initial drop during biodegradation: the bacteria are said to have died and released surfactant back into solution.

The solubilization of kerosene by anionic surfactant micelles in soil-water system enabling the transfer of the organic compound from soil-sorbed phase to the aqueous phase. Biodegradation of surfactant and kerosene depended on their free aqueous-phase concentration (Laha and Luthy, 1991).



Chemical structure of sodium dodecyl sulfate (SDS)





Chemical structure of linear alkyl benzene sulfonate (LAS)

**Figure 2.3** The example of anionic surfactant.

### 2.3.1.2 Cationic surfactants

The surface-active portion of the molecule bears a positive charge, thus adsorbs strongly onto most solid surfaces (which are usually negatively charged), and can impart special characteristics to the substrate. This adsorption also makes possible the formation of emulsions that break in contact with negatively charged substrates, allowing deposition of active phase on substrate. The disadvantages of cationic are more expensive than anionics or nonionics, slow poor detergency and poor suspending power for carbon. The importance of the cationic surfactants is the unique properties that can kill or inhibit the growth of many microorganisms. For example,  $\text{RNH}_3^+\text{Cl}^-$  (salt of long chain amine),  $\text{RN}(\text{CH}_3)_3^+\text{Cl}^-$  (quaternary ammonium chloride).

Anionic, cationic and nonionic surfactants are particularly used for soil washing and flushing. They contain both hydrophobic and hydrophilic portion, making them ideal for solubilization of hydrophobic compounds. Numerous studies have indicated that pretreatment of soil with surfactant washing enhanced solubilization of hydrophobic compound (Mulligan *et al.*, 2001).

### 2.3.1.3 Nonionic surfactants

The surface-active portion bears on apparent ionic charge. Generally available as 100 % active material free of electrolyte. It can be made resistant to hard water, polyvalent metallic cations, electrolyte at high concentration; soluble in water and organic solvents, including hydrocarbons. The most important advantages of nonionic surfactants include a significantly lower sensitivity to the

presence of electrolytes in the system, a lessened effect of solution pH, and the synthetic flexibility of being able to design the required degree of solubility into the molecule by controlling the size of the hydrophilic group. Polyoxyethylenes (POE) nonionics are generally excellent dispersing agents for carbon. Ethylene oxide derivatives show inverse temperature effect on solubility in water, may become insoluble in water on heating. The general formula of the POE surfactants is  $RX(CH_2CH_2O)_nH$ , where R is a typical surfactant hydrophobic group and X is O, N or another functionality capable of linking the polyoxyethylene chain to the hydroprobe. The example of nonionic surfactant is a polyoxyethylene (-OCH<sub>2</sub>CH<sub>2</sub>O-) or polyol groups.

Doong and Lei (2003) report that surfactants with short chain POE chain have a high capacity for enhancing the solubilization of PAHs. This suggests that surfactant with high hydrophobic property (low POE chain or HLB number) is suitable for enhancement of PAH solubility.

Putchá and Domach (1993) studied the biodegradation of PAH and effect of micelles of the nonionic surfactant, Triton-X-100, has on the biodegradation process. They found that complete bacteria degradation of petroleum hydrocarbons in 65 hours. The presence of surfactant Triton-X-100 retarded biodegradation. Micelles protected PAH against copper quenching and also suppressed biodegradation.

Mueller (1990) test the effect of Tween 80 on the solubility and biodegradation of fluoranthene by *Pseudomonas paucimobilis*. He found that the concentration of surfactant up to 2 g/L enhanced the biodegradation of fluoranthene without any toxic effect.

In *et al.* (2001) used three nonionic surfactants, which are Brij 30, Tween 80 and Triton X-100 in enhancing biodegradation of polycyclic aromatic hydrocarbons in soil slurry. They indicated that Brij 30 was the most biodegradable surfactant tested when compare to Tween 80 and Triton X-100.

#### 2.3.1.4 Amphoteric surfactants

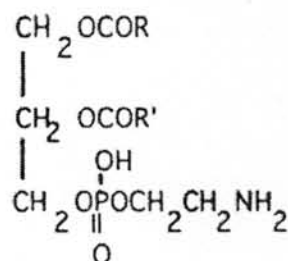
Amphoteric surfactants are the surfactant species that can be either cationic or anionic depending on the pH of the solution, including also those which are zwitterionic (possessing permanent charges of each type). It is important

that amphoteric often show synergism when employed with other classes of surfactants. Their amphoteric nature can make them useful in applications requiring biological contact. The general chemical natures of this material are:

a) Imidazoline Derivatives ( $\text{RCONHCH}_2\text{CH}_2\text{NR}'\text{R}''$ ), R is the fatty acid residue, R' and R'' can be several functionalities such as quaternary ammonium/carboxylic acids, amine/carboxylic acids, amine/sulfonic acids or quaternary ammonium/sulfonic acids.

b) Betaine and sulfobetaines ( $((\text{CH}_3)_3\text{N}^+\text{CH}_2\text{COO}^-)$ )

c) Phosphatides or lecithins



**Figure 2.4** The structure of Phosphatides.

### 2.3.2 Use of Surfactants for Enhancing Biodegradation of Hydrocarbon Compounds

The hydrocarbons present in oil sludges are insoluble in the aqueous phase and hence are not available to hydrophilic microorganisms when subjected to biodegradation. It requires special mechanisms to facilitate the uptake of hydrocarbons such as alkanes are partially soluble in water and are utilized easily by microorganisms. However, high molecular weight hydrocarbons (dominated by solid alkanes) require to be solubilized in the aqueous phase by physical means, addition of surface active agents. Degradation of high molecular weight hydrocarbons has been studied in oil-aqueous environments, in organic solvent, or by the addition of microbial surfactants. Bacteria cultures have been developed which secrete the biosurfactant in oil media thus forming the stable emulsion. Surfactants, including biosurfactants, can increase concentration of hydrophobic compounds in solution and enhance the accessibility of these compounds to microorganisms. Both enhancement

and inhibition of biodegradation due to the effects of surfactants are obtained. Surfactants not only stimulated biodegradation rate, but also affected desorption of hydrophobic organic compounds onto soils.

There are two mechanisms by which surfactants can enhance the removal of organic compounds in soils. The first and most important mechanism involves solubilization of contaminants in surfactant micelles. The second mechanism involves the mobilization of the contaminants from the soils; this depends on the tendency of surfactants to reduce the interfacial tensions and capillary forces trapping contaminant in the soil (Rosen, 1989).

Bioremediation is an attractive alternative for the treatment of such wastes. However, the presence of surfactants will complicate the biological process due to the interactions between surfactant, hydrophobic organic compounds (HOCs) and microorganisms. A number of researchers indicated surfactant enhancement of the microbial degradation of organic contaminants. Such enhancement could be due to increased solubility and hence the increased bioavailability of HOCs to the microorganisms, the increased hydrophobicity of the cell membrane, or reducing interfacial tensions, thus promoting more contact area between HOCs and microorganisms.

Several mechanisms existed for the surfactant enhanced biodegradation.

a) The enhancement by surfactant could be due to the promoted microbial growth, since surfactant can be utilized as a readily available carbon and energy source.

b) Surfactant enhancement could also be the result of co-metabolism between surfactants and contaminants, since the concentration of some of the contaminants could be below the threshold to sustain significant microbial growth.

c) The presence of surfactant will not only stimulate bacterial growth, but also induces enzymes (proteins) that may be needed to the further break down contaminants.

d) Surfactant enhancement could also be the enhanced solubility and/or the greater bioavailability of HOCs to the microorganisms.

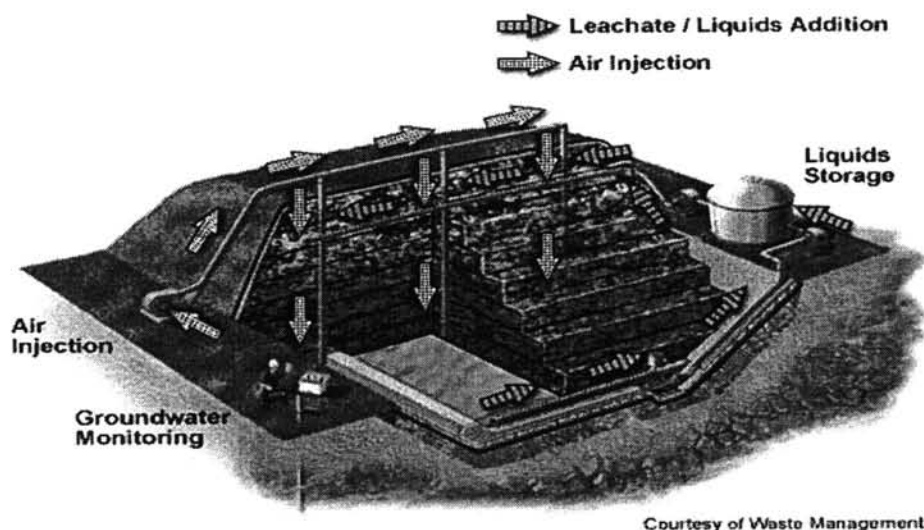
## 2.4 Bioreactor for Biodegradation

The bioreactor is the center of all biochemical processing. It can be defined as a vessel in which biological reactions are carried out by microorganisms or enzymes contained within the reactor itself. In hazardous, municipal, or industrial waste treatment, bioreactors are used primarily to reduce the concentration of contaminants in incoming wastewater to acceptably low levels. In particular, biological treatment appears to be especially versatile and cost effective when the concentration of pollutants in the wastewater is relatively low and the volumes to treat are large, thus the other alternative treatments are unattractive (i.e. incineration or stabilization). A number of measurements indicating the level of concentration of pollutants have been devised and are commonly used in industrial practice.

The bioreactor landfill is generally defined as a municipal solid-waste landfill, operated to transform and more quickly stabilize the readily and moderately decomposable organic constituents of the waste stream by enhancing microbiological processes. Bioreactor can be divided into 5 categories, which are 1) aerobic reactors 2) anaerobic reactors 3) batch reactors 4) continuous reactors and 5) sequencing batch reactors

### 2.4.1 Aerobic reactors

The objective of aerobic bioreactor is to minimize landfill gas generation overall by introducing oxygen into the waste mass. From figure 2.5 shows a cut-away view of an aerobic bioreactor. Leachate is removed from the bottom layer of the landfill and piped to a liquids storage tank. From the tank, the leachate is piped across the top layer, where it is released to filter down through the landfill to be collected again. A blower forces air into the waste mass through vertical or horizontal wells located in the top layer of the landfill. Groundwater monitoring occurs at wells situated around the perimeter of the landfill.



**Figure 2.5** Path way of aerobic bioreactor.

Moreover, aerobic reactors are the reactors containing suspended biomass almost require the use of an air-sparging or bubbling system to provide the microorganisms with oxygen. One of the main drawbacks of oxygen as a key substrate is its low solubility in water as opposed to most other substrates, which have much higher saturation concentration. Mostly, the majority of existing biological treatment plants is aerobic processes. The reasons for the preference in the aerobic processes are the greater range of wastewater that can be treated, easier control and greater stability of the process, and more significant degree of removal of BOD, nitrogen and phosphorous. The aerobic treatment systems have a quick metabolism making the aerobic processes have short resident time.

Woo and Park (1999) reported that out of range of bioremediation technologies, treatment in a slurry bioreactor is considered to be one of the fastest bioremediation methods since substrates can be efficiently transported to the microbial population. However, soil treatment using vertical slurry bioreactor is a relatively expensive method for bioremediation. Moreover, slurry bioreactors have been limited to the treatment of fine soils.

### 2.4.2 Anaerobic reactor

In anaerobic bioreactors, a key objective is to enhance the generation of landfill gas (i.e., methane), by minimizing oxygen infiltration, over a shorter period of year. Anaerobic reactors are the reactors that use the closed system in order to eliminate oxygen from the system and another reason is to control the odors associated with anaerobic fermentation. An anaerobic reactor must be installed an appropriate vent or collection system to remove the gases produced during an aerobiosis.

Boopathy (2003) study the biodegradation of diesel fuel contaminated in soil under anaerobic conditions using soil slurry reactors with the various groups of anaerobic bacteria. The various anaerobic groups included sulfate, nitrate reducing, methanogenic, and mixed electron acceptor conditions. The results indicated enhanced biodegradation of diesel fuel under all conditions compare to no electron-supplemented reactor. However, the rate of diesel degradation was the highest under mixed electron acceptor conditions followed in order by sulfate-, nitrate-reducing, and methanogenic conditions.

### 2.4.3 Batch reactors

In a batch system, all of the substrate is added at the beginning of the batch but no product is withdrawn until the end of the batch. The waste is charged to the reactor and the process is allowed to proceed to completion. In addition, batch systems are generally simpler, require minimal support equipment, and well suited to treat small amounts of waste. Batch reactors are also used, when the residence time required for the decontamination reaction is exceedingly large and solids are treated. However, batch processes are rather labor-intensive, and require the presence of storage facilities to temporarily store the incoming waste material while the treatment process is proceeding in the reactor.

Batch and fed-batch modes are preferred as they have the advantage of avoiding excessive substrate feed which can inhibit microorganism growth. Since product is also withdrawn at the end of the batch, sterilized conditions can be maintained during process operation. The (fed-) batch modes of operation suffer

from certain disadvantages as they are personnel intensive due to the need to sterilize the equipment after every batch. Furthermore, there are difficulties in operation and control such as maintaining the specific growth conditions for the microorganism in the face of variations in medium properties, the batch-to-batch system variability (Shuler and Kargi, 1992), and the inherent nonlinear dynamic nature of fed-batch bioreactors.

#### 2.4.4 Continuous reactors

Continuous reactors are widely used in the large-scale wastewater treatment systems, which are operated in a continuous mode. A wastewater stream or feed is continuously fed into the plant and the clarified stream is continuously removed. The waste generated in the operation are inevitably occurred. An important concept associated with continuous reactors is the resident time, defined as the average amount of time spent by a fluid element in the reactor.

Nora *et al.* (2001) studied the phenol biodegradation using a repeated batch culture of *Candida tropicalis* in a multistage bubble column. They found that the growth rate of *Candida tropicalis* is affected by phenol. Beside, when the yeast is aerobically cultivated in a medium containing phenol, using a bubble column, the yeast cell floatation phenomenon occurs making the continuous operation of this type of reactor difficult. Therefore, a system of phenol degradation, which recycles the biomass separated by floatation, was devised. In order to reduce the substrate toxicity observed at high phenol concentrations, the bubble column used in the biodegradation studied was fed in a semi-batch mode.

#### 2.4.5 Sequencing batch reactors

The sequencing batch reactor (SBR) is a widely used process for biological removal of nutrient (nitrogen and phosphorus) from wastewater. It is based on the metabolism of specialized bacteria, which under alternate anaerobic/aerobic conditions uptake phosphorus and performs denitrification. The SBR process is noted for its operational flexibility, which requires a deep understanding of its features (Artan and Orhon, 2005). The SBR process is normally operated on a five phases: filling, aeration, settling, idling and sludge discharge but in



aerobic degradation in SBR, idling phase is often not a part of the operation. During the fill step, the slurry is fed into the reactor, in the reaction step the system works as a batch reactor, performing the biodegradation process, the final step consists of the extraction of a fraction of treated slurry from the reactor and the replacement with an equal volume of untreated slurry (Andrea *et al.*, 2004). Moreover, the settling time is likely to exert a selection pressure on the sludge particles, i.e. only particle that can settle down within the given settling time would be retained in the reactor, otherwise they would be washed out of the system (Qin *et al.*, 2004).

Andrea *et al.* (2004) studied the applicability of sediment slurry sequencing batch reactors (SBR) to treat Venice lagoon sediments contaminated by polycyclic aromatic hydrocarbons (PAHs). The total PAH efficiency removal close to 55% was achieved for long (98 days), middle (70 days) and short (35 days) HRT of the SBR; moreover, although the addition of lactose (external carbon source) in the SBR has increased the biological activity. The results show that the investigated biological treatment system can be pursued, with respect to the dredged sediment used.

Meltem (2005) investigated the effect of pure cultures on the enhancement of biological phosphorus removal capability of a sequencing batch reactor (SBR) inoculated initially with a mixed culture. Pure cultures of *Acinetobacter lwoffii*, *A. lwoffii*-*Pseudomonas aeruginosa* mixture and *P. aeruginosa* were added into the first, second and third reactors, respectively. The results showed that the addition of *A. lwoffii* to the mixed culture in the first reactor significantly enhanced the biological phosphorus removal rate. Complete removal ( $E = 100\%$ ) of  $20 \text{ mg l}^{-1} \text{ PO}_4\text{-P}$  was achieved within 35 days of operation. Corresponding removal efficiencies obtained using *A. lwoffii*-*P. aeruginosa* mixture (second reactor) and *P. aeruginosa* alone (third reactor) were 25% and 20%, respectively.