CHAPTER II

LITERATURE SURVEY

2.1 Crude Oil Sludge Generated from Petroleum Industry

2.1.1 Crude Oil Sludge Generation

Pollution and wastes associated with petroleum refining typically includes VOCs (volatile organic compounds), carbon monoxide (CO), sulfur oxides (SO_x), nitrogen oxides (NO_x), particulates, ammonia (NH_3), hydrogen sulfide (H_2S), metals, spent acids, and numerous toxic organic compounds. Sulfur and metals result from the impurities in crude oil. The other wastes represent losses of inputs and final product (Eileen, 1998). The oil refineries are also generating mind boggling quantity of wastes, which may be classified as oily sludge and effluent treatment sludge. The potential problems faced by the end users due to its generation, transportation, handling, storage and end use. Refinery wastes are typically in the form of sludge (including sludge from wastewater treatment), spent process catalysts, filter clay, and incinerator ash. Another important solid waste is the oil sludge in tank bottom, which is come from the storage tank. The accumulation of sludge in crude oil tanks bottom is a problem experienced by all refineries. The settling out of sand, rust and heavy fractions in the crude oil results in a loss of ullage in refinery crude storage tanks and can ultimately cause problems in refining when the sludge of this material is introduced into the plant (Ozten, 2003).

Storage tank are used throughout the refining process to store crude oil and intermediate process feeds for cooling and further processing. Finished petroleum products are also kept in storage tanks before transport off site. Storage tank bottoms are mixtures of iron rust from corrosion, sand, water, and emulsified oil and wax, which accumulate at the bottom of tanks. Liquid tank bottoms (primarily water and oil emulsions) are periodically drawn off to prevent their continued build up. Tank bottom liquids and sludge are also removed during periodic cleaning of tanks for inspection (Eileen, 1998). Oily sludge is composed of oil, water, solids, and their characteristics, such as varied composition, make them highly recalcitrant and very difficult to reutilize (Englert, 1993). Sludge does not behave like

Newtonian fluid but exhibits a variable viscosity with shear rate. Organic precipitation of solids from the liquid oil due to the heavy liquid high-molecular weight compounds present in the residual may be insoluble during blending or storing. They may also be formed from reactions due to moisture, heat and oxidation, but the amounts from these factors are small. When the sludge particles are formed, they are quite small and if they remained so, little trouble would occur. Unfortunately, they increase in size as they adhere to each other, becoming so large as to block filters, strainers, pumps and burner nozzles if drawn into the system. Table 1 shows a proximate analysis of sewage sludge, oil sludge, and API separator sludge. (Prame, 2003)

Table 1 Proximate analysis of sewage sludge, oil sludge, and API separator sludge

Proximate analysis[a]	Sewage sludge	Oil sludge	API separator sludge
Volatile matter (wt.%)	53.50	-	48.10
Ash content (wt.%)	23.48	1.88	38.36
Moisture (wt.%)	11.80	39.15	2.48
Fixed carbon (wt.%)	11.21	-	11.06
Combustible[b]	64.71	79.81	59.16

[a]Wet basis.

[b]Volatile fixed carbon.

2.1.2 Crude Oil Sludge Treatment

The solid wastes or sludge is considered globally as an environmental hazard and the exorbitant cost of labors in terms of manning them for doing the de-sludging operations needs to be addressed. The operations, which involve manual handling of the sludge from the tanks bottom, which are under high exposure limits of toxic gases poses lot of hazards to human health. Hence, sludge treatment usually performed by using land application (bioremediation) or solvent extraction followed

by combustion of the residue or by use for asphalt. These are the conventional treatment for the solid wastes in the petroleum industry.

2.1.2.1 Solidification, Stabilization and Encapsulation

Solidification, Stabilization and Encapsulation are the processes produce dry solid (either as a monolith or a dry granular solid similar to coarse soil). Wastes treated by these techniques are stored and not destroyed but the concentrations or mobilities of constituents in treated waste must be different in the original waste. Cement-based and pozzolanic (e.g. flyash) processes, as well as stabilization processes, have been applied in the oil industry to solidify and/or stabilize wastes. These processes are effective for stabilization of metals in the wastes because, at the high pH of the cement mixture, most metal compounds are converted into non-biologically available insoluble metal hydroxides. However, 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. Hydrocarbons and salts do not interact with the cement matrix and are physically rather than chemically bound within the matrix (Wiles, 1987).

2.1.2.2 Landfill

Landfills are generally constructed and monitored facilities designed to accommodate burial of large volumes of wastes. However, some landfill may be little more than open dumps. A landfill may be constructed in a manner that makes it an appropriate disposal site for certain toxic wastes. Landfilled materials should not be capable of reacting to generate excess heat or noxious gases. Special systems may need to be installed to collect generated methane. The operator should remember that landfilled wastes are not destroyed, but are actually in long-term storage (Willson and Sikora, 1983).

2.1.2.3 Burial

Due to its simplicity, burial of wastes in small pits at drilling and production sites has been a popular means of waste disposal in the past. However, with current awareness of pollutant migration pathways, the risks associated with burial of wastes should be carefully considered. In general, wastes with high oil, salt, or biologically available metal content, industrial chemicals, and other materials with harmful components that could migrate from the pit to

contaminate usable water resources should not be buried. Burial may be the best method of disposal for inert unrecyclable materials and it is also a logical choice for wastes that have been stabilized, since the stabilization process will retard migration of the constituents of the waste. The mechanism of the burial is to reduce concentrations in the waste, via dilution with soil, as it is being disposed (Smith, 1979).

2.1.2.4 Incineration

Incineration uses combustion to convert wastes into less bulky materials. Incineration can refer to the practice of open burning of wastes in pits, although the degree of combustion achieved in commercial incinerators will be difficult to achieve in open burning. This is because commercial incinerators can control the residence time, temperature and turbulence within the incineration chamber to optimize combustion. These incinerators are often equipped with air pollution control devices to remove incomplete combustion products, remove particulate emissions, and reduce SO_x and NO_x emissions. Incinerators are usually used to destroy organic wastes, which pose high levels of risk to health and the environment (Niessen and Marcel, 1978).

2.1.2.5 Biotreatment

Biotreatment is the process that converts or degraded many organic compounds in the petroleum wastes into carbon dioxide and waters. The most important factors for control of biological degradation of hydrocarbons are:

1. An adequate supply of hydrocarbon degrading bacteria
2. Availability of sufficient oxygen (and mixing) for cell metabolism
3. Availability and balance of nutrients and micro-nutrients necessary for optimum bacterial metabolism
4. Moisture control
5. Temperature and pH
6. Salinity

The concentration and type of compounds to be degraded may have a significant impact on the biodegradation process. Some compounds may be readily degraded at low concentrations but inhibit degradation at higher concentrations (for example, some hydrocarbons compounds may be toxic to

degrading organisms at high concentrations). High levels of asphaltenes and/or polynuclear aromatic hydrocarbons (PAH) in a waste may make biological treatment an unlikely option for rapid removal of the hydrocarbon fraction. Conversely, high concentrations of alkanes in a waste may make it a good candidate for biological treatment. In general, the order of the biodegradability of petroleum hydrocarbon fractions follows the relationship: saturates > aromatics > polars > aspathenes. The example of biotreatment is the biological treatment in tanks. It is the aerobic biological reaction that occurs during composting processes may be accomplished at an accelerated rate using an open or closed vessel or impoundment. The process is typically operated as a batch or semi-continuous process. Nutrients are added to slurry of water and waste, and oxygen (for aerobic degradation) is provided by air sparging and/or intensive mechanical mixing of the reactor contents. The mechanical mixing also provides a high degree of contact between microorganisms and the waste component to be biodegraded. A source of microbes capable of degrading the organic constituents of the waste may also be required to accelerate start-up of the system. Operating conditions (temperature, pH, oxygen transport and mixing, and nutrient concentrations may be easily monitored and controlled in tank based bioreactor systems. This control makes optimization of biological processes possible; hence bioreactors generally require less space than land-based biological treatment processes. A disadvantage of bioreactors is that the maintenance and capital investment required is high relative to other forms of biological treatment.

2.2 Biodegradation of Hydrocarbon Compounds

Biodegradation or microbial degradation refers to the natural process, which bacteria or other microorganisms alter and break down organic molecules into other substances, such as fatty acids and carbon dioxide. This process is used for cleaning up environments and often used in sewage treatment (Watanabe, 2001). 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 organisms utilize different metabolic processes to derive their energy, and based on what they use for an energy source. Bacteria are microorganisms, which are found virtually everywhere: in soil, water, plant, animals and rocks. The average size of these single celled organisms is one micron. Their microscopic size means that bacteria can easily move compounds in and out of their cells. This makes them perfect processing units. After death, all organisms decompose to their base elements: water, carbon nitrogen, phosphate and trace elements. This process is called biodegradation or microbial degradation, and is carried out by bacteria. There are many kinds of bacteria that can biodegrade organic compounds. Each bacterium has different properties in degrading organic compounds.

2.2. Physical and Chemical Factors Affecting the Degradation of Hydrocarbons Chemical Composition of Hydrocarbons

Petroleum hydrocarbons can be divided into four classes: saturates, aromatics, asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) (Colwell and Walker, 1977). 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, 1994). Biodegradation rates have been shown to be the highest for saturates, followed by the light aromatic, with high-molecular-weight aromatics and polar compounds exhibiting extremely low rates of degradation (Foght and Westlake, 1982).

2.2.1.1 Physical State of the Hydrocarbons

The 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. The large masses (or plates) of mousses establish unfavorably low surface-to-volume ratios, inhibiting biodegradation (Davis 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).

2.2.1.2 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 and the higher molecular-weight aromatic hydrocarbons, such as naphthalene and phenanthrene, are related to aqueous solubility rather than total substrate concentration (Thomas *et al.*, 1986). High concentration of hydrocarbons, can be associated with heavy, undispersed oil slicks in water, causing inhibition of biodegradation by nutrient or oxygen limitation or though toxic effects exerted by volatile hydrocarbons. Fusey and Oudot (1984) reported that contamination of seashore sediments with crude oil above threshold concentration prevented biodegradation of oil because of oxygen and/or nutrient limitation. The high concentration of oil have negative effects on biodegradation rates including the low-energy environments such as beach, harbors and small lakes or ponds, in which the oil is relatively protected from dispersion by wind and wave action.

2.2.1.3 Temperature

Temperature influences petroleum biodegradation by its effect on the physical nature and chemical composition of the oil, rate of hydrocarbon metabolism by microorganisms, and composition of the microbial community (Atlas, 1981). 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 decrease with decreasing temperature. This is believed to be a result of decreased rates of enzymatic activity. Higher temperature increases the rates of hydrocarbon metabolism to a maximum, typically in the range of 30 to 40°C, above which membrane toxicity of hydrocarbons is increased (Bossert and Bartha, 1984).

2.2.1.4 Oxygen

The initial step in the catabolism of aliphatic, cyclic, and aromatic hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases, for which molecular oxygen is required (Perry, 1984). Aerobic conditions are necessary for the route of microbial oxidation of hydrocarbons in the

environment. The appropriate microorganisms are important for an optimal rate of the bioremediation of petroleum hydrocarbons contaminated in the environment. In soil, the oxygen content depends on microbial activity, soil texture, water content and depth. Low oxygen content in soils has been shown to limit bioremediation of soils contaminated with petroleum hydrocarbons. 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).

2.2.1.5 Nutrients

The availability of nitrogen and phosphorous limits the microbial degradation of petroleum hydrocarbons contaminated in aquatic environments. Rates of nutrient replenishment generally are not sufficient to support rapid biodegradation of large quantities of oil. The adjustment of carbon/nitrogen phosphorous ratio by the addition of nitrogen and phosphorous in the form of fertilizer stimulates the biodegradation of hydrocarbons contaminated in aquatic environments (Atlas and Bartha, 1972).

2.2.1.6 Salinity

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.

2.2.1.7 Pressure

The importance of pressure as a variable in the biodegradation of hydrocarbons is probably confined to the deep-sea environment. 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.

2.2.1.8 pH

Most bacteria and fungi favor a pH near neutrality, with fungi being more tolerant of acidic conditions (Atlas, 1988). Extremes in pH have negative influence on the ability of microbial populations to degrade hydrocarbons. Verstracte *et al.* (1976) reported that a near doubling of rates of biodegradation of gasoline in acidic (pH 4.5) soil by adjusting the pH to 7.4. The rate of biodegradation is dropped significantly.

2.2.1.9 Toxicity

Metals are very toxic for the microorganisms because they can penetrate into the perforated microbial cells more 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.

2.2.2 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 enszymes 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.

2.2.3 Types of Microbial Degradation

Petroleum hydrocarbons are very complex mixtures, consisting of various organic compounds. Because of leakage of underground storage tanks and pipelines, spills at production wells, refineries, distribution terminals, improper

disposal and accidents during transport even wastes generated from the petroleum industry, these compounds have become the most frequently encountered environmental pollutants. In all chemotropic reactions, a part of the hydrocarbon is oxidized for energy conservation (catabolism) and another part is assimilated into cell mass. In the long established aerobic oxidation of hydrocarbons (upper right), oxygen is not only the terminal electron acceptor, but is also needed for substrate activation (oxygenate reactions). The anaerobic pathways involve novel hydrocarbon activation mechanisms that differ completely from the aerobic mechanisms. Jagged arrows indicate hydrocarbon activation (Friedrich and Ralf, 2001). Figure 2.1 below shows the experimentally verified possibilities for the microbial utilization of hydrocarbons (Gibson, 1984).
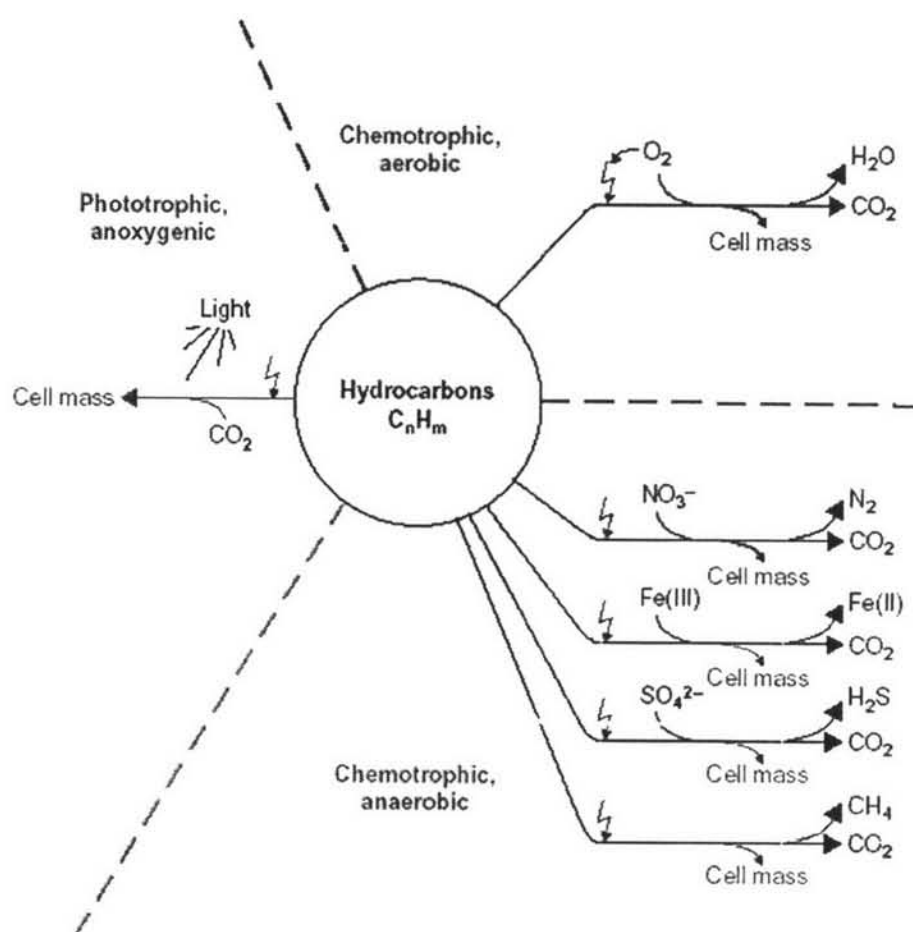
Figure 2.1 Microbial utilization of hydrocarbons.

2.2.3.1 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.

2.2.3.2 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, elthylbenzenes, and possibly benzene.

2.3 Surfactant

2.3.1 Type of Surfactant

Surfactants are amphiphilic compounds (Containing hydrophobic and hydrophilic) that reduce the free energy of the system by replacing the bulk molecule of higher energy at an interface. They contain a hydrophobic portion with the little affinity for 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 and 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 users, as in enhanced oil removal applications. In this application, surfactants increase the solubility of petroleum components (Falatko, 1991). Typical desirable properties include solubility enhancement, surface tension reduction, and low critical micelle concentrations. Surfactants concentrate at interfaces (solid–liquid, liquid–liquid or vapor–liquid). An interfacial boundary exists between two immiscible phases. The hydrophobic portion concentrates at the surface while the hydrophilic portion is oriented towards the solution. The effectiveness of a surfactant is determined by its ability to lower the surface tension, which is a measure of the surface free energy per unit area required to bring a molecule from the bulk phase to the surface (Rosen, 1978). The surface tension correlates with the concentration of the surface-active compound until the critical micelle concentration (CMC) is reached as shown in Figure 2.2. Efficient surfactants have a low critical micelle concentration (i.e. less surfactant is necessary to decrease the surface tension). The CMC is defined as the minimum concentration necessary to initiate micelle formation (Becher, 1965). In practice, the CMC is also the maximum concentration of surfactant monomers in water and is influenced by pH, temperature and ionic strength.
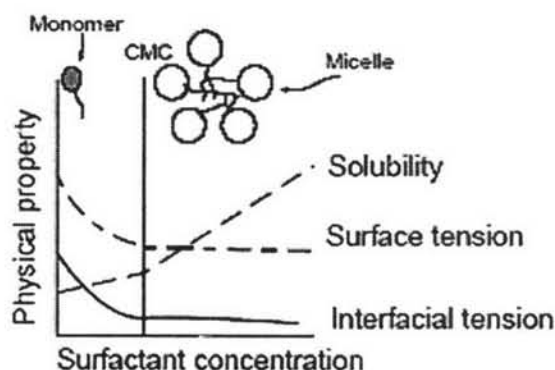

Figure 2.2 Schematic diagrams of the variation of surface tension, interfacial and contaminant solubility with surfactant concentration.

The four basic classes of surfactants are defined as follows:

2.3.1.1 Anionic Surfactants

Anionic surfactants are the surfactants that carry a negative charge on the active portion of the molecule. 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.

Margesin and Schinner, (1998) studied the effect of SDS in different concentration on biodegradation of diesel oil. They found that at low SDS concentrations. (50-100 $mg.l^{-1}$) significantly enhanced oil biodegradation by a psychrotrophic inoculum in liquid culture, whereas higher SDS concentrations (500-1000 $mg\ l^{-1}$) inhibited hydrocarbon biodegradation and Figure 2.3 shows the characteristics of anionic surfactant
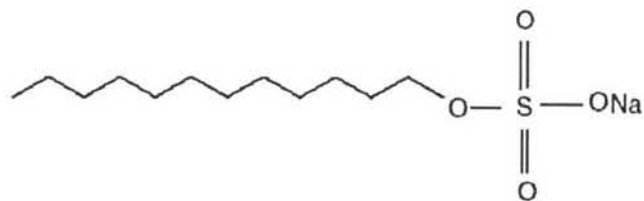
Chemical structure of sodium dodecyl sulfate (SDS)
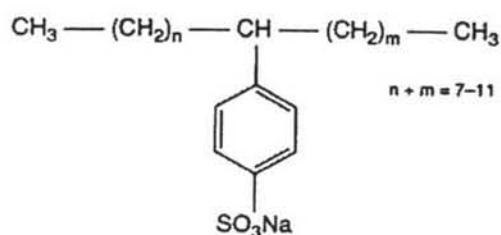
Chemical structure of linear alkyl benzene sulfonate (LAS)

Figure 2.3 The characteristics of anionic surfactant.

2.3.1.2 Cationic Surfactants

Cationic surfactants are the surfactant carrying a positive on the active portion of the molecule. There are two important categories of cationic

surfactants, which differ in the nature of the nitrogen-containing group. The first consists of the alkyl nitrogen compounds containing at least one long-chain alkyl group and one or more amine hydrogen, and quaternary ammonium compounds in which all amine hydrogen have been replaced by organic radical substitutions. The amine substitutions may be long-or short-chain alkyls, alkylaryls or aryls. The counterion may be halide, sulfate or acetate for example, the quaternary ammonium halides ($R_4N^+Cl^-$). The second category contains heterocyclic materials typified by the pyridinum, morpholinium and imidazolinium derivatives as shown in the figure 6, in which R is the surfactant –length hydrophobic groups and R' is a short chain alkyl or hydroxyalkyl chain. The importance of the cationic surfactants is the unique properties that can kill or inhibit the growth of many microorganisms. Figure 2.4 shows the typical structures of cationic surfactants.

Alklytrimethylammonium bromide

Alkylquinolinium chloride
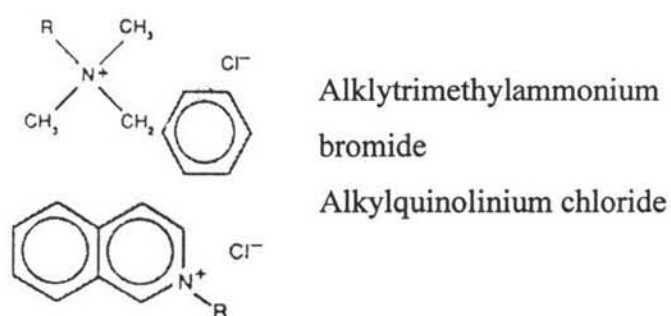
Figure 2.4 Typical structures of cationic surfactants.

2.3.1.3 Nonionic Surfactants

Nonionic surfactants are the surfactants that carry no electrical charge, their water solubility being derived from the presence of polar functionalities capable of significant hydrogen bonding interaction with water. 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. Nonionic surfactants, which are the most numerous and the most important in terms of techniques are the polyoxyethylenes (POE). An interesting characteristic of the POE surfactants is that they exhibit an inverse temperature-solubility relationship. As the solution

temperature is increased, their solubility in water decreases. 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 ($-OCH_2CH_2O-$) or polyol groups.

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.

Patrick et al. (1995) investigate the effect of a non-ionic surfactant, Triton X-100, on biodegradation of Creosote-contaminated soil. They found that surfactant addition can be considered as a possible method to enhance bioremediation of contaminated soils with PAH compounds, showing increase in contaminant availability to the microorganisms when compared with samples without surfactant.

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) as shown in figure 2.5. It is important that amphoterics 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:

1. Imidazoline Derivatives ($RCONHCH_2CH_2NR'R''$), R is the fatty acid residue, R' and R'' can be several functionalities such as quaternary ammonium/carboxylic acids or amine/carboxylic acids,

2. Betaine and sulfobetaines (($CH_3)_3N^+CH_2COO^-$)
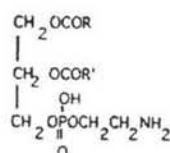
3. Phosphatides or lecithins

Figure 2.5 The structure of Phosphatides.

2.3.2 Use of Surfactants for Enhancing Biodegradation of Hydrocarbon Compounds

Surfactants are amphipathic molecules with both hydrophilic and hydrophobic (generally hydrocarbon) moieties that partition preferentially at the interface between fluid phases with different degrees of polarity and hydrogen bonding such as oil/water interfaces or air/water interfaces. These properties render surfactant capable of reducing surface and interfacial tension and forming microemulsion where hydrocarbons can solubilize in water or where water can solubilize in hydrocarbons (Greek, 1991). Disposal of oil sludge poses an environmental pollution problem due to the presence of high molecular weight hydrocarbons, which are hydrophobic and hence non-biodegradable in the nature. Hydrocarbons present in the oil sludge are insoluble in the aqueous phase and are not available to hydrophilic microorganisms when subjected to biodegradation. It requires special mechanisms to facilitate the uptake of hydrocarbons by microorganisms. Low molecular weight hydrocarbons such as alkanes are partially soluble in water and are utilized easily by microorganisms (Fish et al., 1982). However, high molecular weight hydrocarbons require to be solubilized in the aqueous phase by physical means such as addition of surface-active agents (surfactant) incorporation of organic solvent, and co-metabolism. The growth of microorganism on low molecular weight alkanes induces the production of bioemulsifiers, which facilitate their utilization by microorganisms. Surfactants have also been reported to be produced by microorganisms on carbohydrate substrates. The enhanced biodegradation of polycyclic aromatic hydrocarbons (PAHs) may be enhanced by the use of nonionic surfactant (In et al., 2001). The PAHs solubility was linearly proportional to the surfactant concentration when above the critical micelle concentration (CMC), and increased as the hydrophile-lipophile balance (HLB) value decreased.

Rhamann et al. (2003) showed that the enhancement of biodegradation was achieved through bioaugmentation and biostimulation. Their purpose was to investigate possible methods to enhance the rate of biodegradation of oil sludge from crude oil tank bottom. The sludge samples were treated with

bacterial consortium, rhamnolipid biosurfactant and nitrogen, phosphorous and potassium (NPK) solution. Maximum n-alkane degradation occurred in 10 % sludge contaminated samples.

2.4 Bioreactors for Biodegradation

The bioreactor is the center of all biochemical processing. It can be defined as a vessel in which biological reaction 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. Bioreactor can be divided into 5 categories, which are 1) batch reactors 2) continuous reactors 3) aerobic reactors 4) anaerobic reactors and 5) sequencing batch reactors

2.4.1 Batch Reactors

Batch reactor systems are generally simpler and require the minimal support equipments in the operations. Feed normally wastewater is sent to the reactor and the process allowed proceeding to completion. Batch reactor is used when the resident time is required for the decontamination reaction. The disadvantages of batch reactors are the labor intensive and requiring the presence of the storage facilities to temporarily store the incoming material, which will be used in the processing. An intermediate mode of operation between the batch and the continuous processes is called semi-batch process, in which the material is continuously fed to a semi-batch operated reactor. Once the detoxification process is complete, the reactor is emptied and the process is started again. Bethany et al. (2003) did the experiment about the biodegradation of haloacetic acid by bacterial enrichment culture used the two 1-liter batch reactors, which were inoculated with

biomass from aeration tank. Özer, (2004) studied the stability of aerobic benzoate-degrading capability of *pseudomonas aeruginosa* 142 in a batch reactor. *P. aeruginosa* 142 was grown on benzoate as sole carbon source in chemostats under fully aerobic condition and tested for the aerobic ability to degrade benzoate at maximum oxygen uptake rate (OUR) after exposure to anaerobic conditions in the absence of substrate. He found that aerobically grown *P. aeruginosa* 142 lost around 75% initial max OUR in 8 h of anaerobic exposure. The 75% loss of activity was due to the aerobic ring-cleaving enzymes while the remainder was due to cytochrome oxidases.

2.4.1.1 Aerobic Reactors

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.

Xiangchun *et al.* (2004) using an airlift inner loop bioreactor packed with honeycomb-like ceramic as the carrier adding with bacteria *Achromobacter sp.* to study the biodegradation of 2,4-dichlorophenol and phenol under fed-batch reactor and continuous operations. The result showed that the pure strain could be easily immobilized on the carrier and proliferated using 2,4-DCP as the carbon source and the increasing of phenol loading rate inhibited the biodegradation of 2,4-DCP.

Gallego *et al.* (2003) worked on biodegradation of phenolic compound by using pure and mixed indigenous cultures in aerobic reactor. Biodegradation of a mixture of persistent compounds: 2-chlorophenol, phenol, m-cresol, by used in both batch and continuous flow fluidized bed aerobic reactors. The results showed that the biodegradation in batch reactor is more efficiency than in

the continuous reactor and the degradation in the mixed of bacteria were similar to those observed in isolated bacteria. This study demonstrated that the presence of the other microorganisms does not affect to the process of individual degradation.

2.4.1.2 Anaerobic Reactor

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 accepter 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.

2.4.2 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 wasted 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.

2.4.3 Sequencing Batch Reactors

Sequencing batch reactor (SBR) is a typical cyclic process. Operation of SBR normally consists of four steps: fill, react, settle and idle (Carucci *et al.*, 1995). The most important operating factors influencing the SBR removal efficiency are the hydraulic retention time (HRT), the volume of slurry replaced at the end of each cycle, the solids concentration and the mixing speed. The HRT and the volume of slurry replaced per cycle can be adjusted to face different contaminant loads to the reactor.

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.

Ong *et al.* (2003) studied the evaluation of the effect of Copper and the efficiency of the powered activated carbon (PAC) and activated rice husk (ARH) in reducing the toxic effect of copper on the activated sludge microorganisms. The SBR reactor system were operated with FILL, REACT, SETTLE, DRAW and IDLE modes in ratio of 0.5: 3.5: 1: 0.75: 0.25 for a cycle time of 6 hours. The efficiency of copper and COD removal is 90 and 85% respectively.

Tomei *et al.* (2004) reported the investigation of the biodegradation kinetics of 4-nitrophenol (4NP) was investigated in lab-scale sequencing batch reactor fed with the compound as the sole carbon source. The experimental results showed that the complete 4NP removal can be easily achieved with acclimatized biomass, even if inhibition kinetics is observed; furthermore, an improvement in the removal kinetics is obtained if the substrate concentration peak, reached in the reactor at the end of the filling time, is maintained to quite a low value.

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 mg l^{-1} PO_4–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. The COD removal efficiency was 90% in all reactors. The biodegradation of phenol and 2,4,6-trichlorophenol (2,4,6-TCP) by immobilized white-rot fungal cultures was studied in pinewood chip and foam glass bead-packed trickling reactors, which were operated in sequencing batch format. Removal efficiency increased over time and elevated influent phenol and 2,4,6-TCP (800 and 85 mg l^{-1}) concentrations were removed by greater than 98% in 24–30 hours batch cycles (Ehlers and Rose, 2005).