CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Wastewater from petroleum industry

2.1.1 Waste Characteristics

There are three types of wastewaters distinguished: domestic wastewater, agricultural wastewater, and industrial wastewater. Industrial wastewaters are considered to be the most difficult to handle in terms of low treatment efficiency because of they have a large variety in composition and contain several hardly biodegradable organic compounds and some cases can have toxic compounds. Normally, a waste can be divided as hazardous and consequently falls under the special waste category when it meets one of two conditions;

1) The waste is specifically regulated by a governmental authority or an internationally recognized organization, or

2) The waste possesses one or more of the four hazardous characteristics that are corrosivity, ignitability, reactivity, and toxicity.

2.1.2 Petroleum Wastewater

Petroleum refineries are responsible for the emission of particulates, carbon monoxide, nitrogen oxides, sulfur oxides, carbon dioxide. Volatile organic compounds (VOCs) (e.g. benzene, toluene, and xylene) are released from storage, product loading and handing facilities, and oil-water separation systems and as fugitive emissions from flanges, valves, seals, and drains.

The wastewater from refineries contains both organic and inorganic compounds and their biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are about 150-250 mg/l and 300-600 mg/l respectively. Solid wastes and sludge are generated, 80% of which can be considered hazardous because of the presence of toxic organics and heavy metals.

2.1.3 Biodiesel Wastewater

Biodiesel was produced form spent cooking oil by reacting with ethanol and NaOH was used as the catalyst. This biodiesel production process is known as tranesterification reaction. After complete reaction, the mixture was washed with water for 5 times. Hence, there were five waste streams which their biochemical oxygen demand (BOD), chemical oxygen demand (COD), and pH were shown in table 2.1. In biodiesel production process, wastewater consists of soap occurring from reaction between sodium hydroxide and fatty acid or oil, glycerin dissolving in methyl ester layer, sodium hydroxide, and methanol left over.

Table 2.1 Characteristics of biodiesel wastewaters from Bangchak Petroleum Public Company Limited

Washing step	Volume (litres)	COD (mg/L)	BOD (mg/L)	pH
1	1,600	277,992	232,191	-
2	1,600	48,649	34,265	10
3	1,600	18,774	12,585	9.8
4	1,600	5,225	3,588	8.4
5	1,600	465	300	~7

2.1.4 <u>Treatment Techniques for Petroleum Wastewater</u>

Choosing treatment techniques for wastewater depends largely on the wastewater characteristics and regulatory requirements. There are several wastewater techniques available as follows:

2.1.4.1 *Incineration*

Incineration, which is one of the oldest waste treatment techniques, uses high temperatures (greater than 1,000°F) to burn waste materials such as non-hazardous and hazardous solids, liquid, and gases. Organic contaminants in soils, sludge, sediments or other materials are decomposed to non-hazardous or less-hazardous by products in the presence of excess air. This technique is usually used to wipe out organic wastes, which pose high levels of risk to health and the environment (Niessen, 1978). It becomes uneconomical if the waste contains a large fraction of water and so most wastewaters should not be treated by incineration.

2.1.4.2 *Solidification, Stabilization, and Encapsulation*

Solidification is the technique to decrease the excess water in wastewater which stabilization is one of the chemical techniques changing from hazardous waste materials to non- or less toxic forms. Almost toxic metals found in wastewater have low solubility at high pH, therefore, they are 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 non- or less toxic forms.

Both of techniques are extensively used for the management or disposal of a broad range of contaminated media and wastes. The treatments deal with mixing a binding reagent into a contaminated media or waste. Immobilizing contaminants within the treated material prevents migration of the contaminants to human, animal, and plant receptors. These techniques are an effective and efficient treatment widely used for a diversity of organic and inorganic contaminants present in contaminated soil, sludge, and sediment. The disadvantage of these techniques is feasible economically for only highly concentrated wastes. High concentrations of organic compounds, salts, and bentonite have been shown to interfere with the treatment process, and so limit the application of these treatment techniques.

Another technique is encapsulation. This technique is the process of surrounding waste particles with a layer of material which is very low in permeability. And it inhibits the leaching of the hazardous materials. It is feasible economically for solid or semi-solid wastes.

2.1.4.3 *Land Treatment*

This technique should be employed for the treatment of wastewaters from the petroleum and other industries under controlled, well-designed, and operated conditions. Land treatment involves the controlled application of a waste on the soil surface and incorporation of the waste into the upper soil. This technique depends on the dynamic physical, chemical, and biological processes occurring in the soil. Therefore, the constituents in the applied wastes are degraded, immobilized, or transformed to environmentally acceptable components. This technique has been used effectively for the treatment and disposal of petroleum industry wastes provided that a large area is available. A major drawback of this technique possibly leads to soil contamination.

2.1.4.4 *Biotreatment*

Biotreatment of organic pollutants is a promising field of research, giving reliable, simple, and cheap technology over chemical and physical processes. Successful biological treatment of hydrocarbon containing wastewaters remains a challenge and several factors must be fulfilled and optimized to determine the outcome of the biodegradation process for example, biomass concentration, population diversity, bacterial growth, metabolic pathway, nature and concentration of pollutants, chemical structure of organic compounds, toxicity of contaminants, and presence of nutrients (Morris and Michael, 1993).

Bioremediation is one of the examples of biotreatment. It can be a cost effective and clean-up technology for treating oily sludge and sediments containing biodegradable hydrocarbons and indigenous specialized microorganisms. There are 2 processes for treatment: mineralization and biotransformation. The first process is the complete conversion of organic molecules into inorganic substances. The second process is the transformation of a parent compound into other metabolites, and may be more or less toxic than the parent compound. This treatment has become a major method employed in the restoration of oily polluted environments, and attempts to accelerate the natural hydrocarbon degradation rates by overcoming factors which limit bacterial hydrocarbon degrading activities.

2.2 Biodegradation of Hydrocarbons

Petroleum hydrocarbons are a mixture of aliphatic, aromatic, and polycyclic hydrocarbons ranging from short C_3 to much longer carbon chains. Even though the biodegradation of petroleum hydrocarbons can be completed in anaerobic condition (Cooker, 1995) but aerobic biodegradation seems to be more effective and efficient because the presence of oxygen is very significant factor to the success of the high treatment efficiency (Pritchard *et al.*, 1992). The biodegradation of petroleum hydrocarbons is a complex process that relies on the nature and amount of oil or hydrocarbons present in the wastewater. Petroleum hydrocarbons can be degraded naturally provided that the system has sufficient amount of oxygen and nutrients. However, the natural biodegradation is usually too slow to prevent the spread of contamination (Hinchee *et al.*, 1991). The order of the biodegradability of petroleum hydrocarbon fractions is as follows: saturates > aromatics > polars > aspathenes.

2.2.1 Types of Microbial Degradation

The principal types of microbial degradation used for the biological treatment of wastewater can be divided with respect to their metabolic functions as aerobic and anaerobic degradation. Figure 2.1 shows the possible pathways the microbial utilization of hydrocarbons (Gibson, 1984).

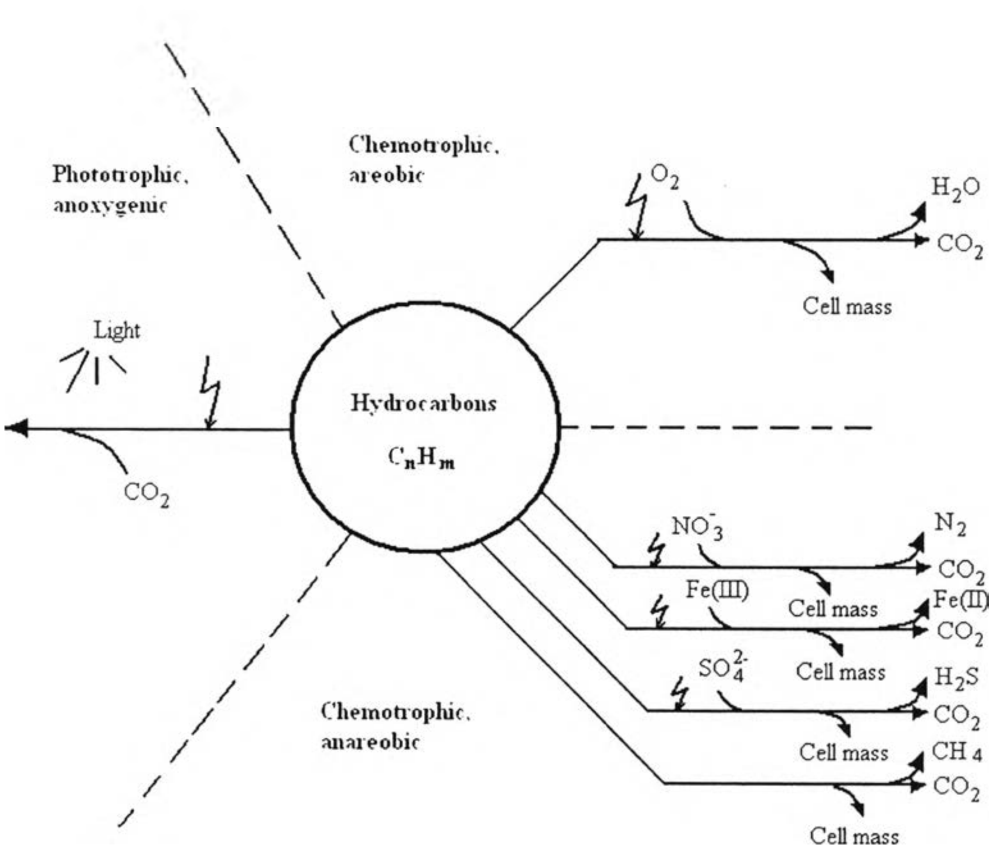

Figure 2.1 Pathways of Microbial utilization of hydrocarbons (Gibson, 1984).

2.2.1.1 *Anaerobic Degradation*

Anaerobic degradation is the treatment technique using nitrate, Fe (III), sulfate and carbon dioxide as electron acceptors. This often occurs in any habitat, where the oxygen consumption rate exceeds its supply rate and is a common phenomenon in many natural aquatic environments receiving a high leading of organic materials. Under anaerobic conditions, the microbial degradation of oxidized aromatic compounds such as benzoate and for halogenated aromatic compounds has been shown to occur under anaerobic conditions. In generally, the anaerobic treatment process is applied for highly organic contains wastewaters.

2.2.1.2 *Aerobic Degradation*

Aerobic degradation is a treatment process, involving the oxidation-reduction 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, and actonomycetes, use the oxygen during the chemical degradation. The aerobic bacteria require oxygen to decompose organic compound into carbon dioxide and other inorganic compounds (Nora and Juan, 2001). Aerobic degradation occurs via more efficient and rapid metabolic pathway than anaerobic reaction. Moreover, the most site decontaminations involving refined oils and fuels all diluted organic wastewaters are treated aerobically to meet the effluent standards.

2.2.2 Factors Affecting Biodegradation Performance

2.2.2.1 *Physical and Chemical Factors*

2.2.2.1.1 Chemical Composition of the Hydrocarbons

The varieties in chemical composition are attributed to different degrees of biodegradation (Ahsan *et al.*, 1996). The normal the structure of a compound, the faster rate of biodegradation. Simple alkanes are depleted prior to isoalkanes which, in turn, are depleted prior to cycloalkanes. In the same compound class, lower molecular weight compounds are depleted prior to high molecular weight compounds (Magoon and Claypool, 1981). Hydrocarbons vary in their susceptibility to microbial attack and have been ranked in the order of reducing susceptibility: n-alkanes > branched alkanes > low molecular-weight aromatics > cyclic alkanes (Perry, 1984). The biodegradation of PAHs is highly relied on the number of aromatic rings present in the molecule and the rate of biodegradation reduces with increasing number of aromatic rings (Huang *et al.*, 2004).

2.2.2.1.2 Physical State of the Hydrocarbons

Organic compounds differ widely in their solubility, from infinitely miscible polar compounds, such as methanol, to extremely low solubility nonpolar compounds, such as high molecular weight polynuclear aromatic hydrocarbons. Compounds with greater aqueous solubility are normally more available to catabolic enzymes. The aqueous solubility of a hydrocarbon molecule reduced as its carbon atoms number increases (Sugiura *et al.*, 1997).

The microbial degradation or biodegradation of petroleum hydrocarbons has still been limited by the low solubility of hydrocarbons and their bioavailability to degrading microorganisms. Janiyani *et al.*, (1993) reported that increase in soluble chemical oxygen demand with increase in surfactant concentration and contact time resulted in the enhancement in the microbial degradation performance. The contaminant concentration will be effect on the number of organism present. It has been showed that higher concentrations of pyrene were related to higher counts of microorganism (Chung and Alexander, 1999). It has been shown that the same compounds in different crude oil samples were degraded to different degrees by the same organism (Sugiura *et al.*, 1997).

Ruttanapol (2004) studied the effects of added nonionic surfactant on the solubility and biodegradation of the hydrocarbons in an oil sludge sample. This research used three nonionic surfactants: Brij 30, Triton X-100, and Tween 80. The results showed that the solubility of the hydrocarbons was significantly enhanced by the addition of these surfactants. Brij 30 provided the highest enhancing effect on the solubilization than Triton X-100 and Tween 80. From the solubilization, the optimum surfactant concentration of polyoxyethylene sorbitan monoleate (Tween 80) for the oil extracted from crude oil sludge was found to be 0.1% w/v (Attavavuthichai, 2006). Therefore, the solubility and biodegradation depend on the types and concentrations of surfactant and the nature of hydrocarbons.

2.2.2.1.3 Concentration of the Hydrocarbons

The rate of uptake and mineralization of organic compounds by microbial populations in the aquatic environment are proportional to the concentration of the compounds. The rates of mineralization of high molecular weight aromatic hydrocarbons, for example naphthalene and phenanthrene, are governed by their aqueous solubility rather than the total substrate concentration (Thomas *et al.*, 1986). High concentrations of hydrocarbons can be related with heavy, undispersed oil slicks in the water, occurring inhibition of biodegradation by nutrient or oxygen limitation or through toxic effects exerted by volatile hydrocarbons. Fusey and Oudot (1984) reported that the contamination of seashore sediments with a high crude oil concentration above a thredhold concentration prevented the biodegradation of the oil because of the oxygen and/or nutrient limitation.

2.2.2.1.4 Viscosity

Both viscosity and surface-wetting properties relate to the transport of an organic liquid phase. Koopmans *et al.* (2002) reported that the large variation in viscosity of the Liaohe oils was found to affect directly the microbial degradation rate. The higher the viscosity the lower degrade the oil dispersion and spreading, resulting in lowering the surface area available for microbial attack.

2.2.2.1.5 pH

pH is one of the affecting factors, influencing on the microbial diversity and activity, controlling enzyme and activity, transport process and nutrient solubility (Wong *et al.*, 2002). Besides, extremes in pH have negative affect the ability of microbial populations to degrade hydrocarbons.

2.2.2.1.6 Toxicity

All heavy metals are very toxic to microorganisms because they can penetrate into the perforated microbial cells and may change the permeability of the membranes (Gogolev and Wilke, 1997).

2.2.2.2 *Environmental Factors*

2.2.2.2.1 Temperature

At a high temperature, the growth of filamentous bacteria becomes more performable and temperature affects the rate of microbial growth. The optimum temperature for aerobic degradation is around 37°C while the anaerobic have two optimum temperature of 37 and 55 °C. Moreover, temperature has an effect on bioavailability and solubility of less soluble hydrophobic substances such as aliphatic and aromatic hydrocarbons. Increasing temperature causes decreasing viscosity, thereby affecting the degree of distribution, and increasing diffusion rates of organic compounds. With decreasing temperature, the viscosity of the oil increases, the volatilization of toxic short chain alkanes decreases, and their water solubility decreases, delaying the onset of biodegradation (Atlas and Bartha, 1972).

2.2.2.2.2 Oxygen

If indigenous soil bacteria have adequate amounts of oxygen and nutrients, most petroleum hydrocarbons can be biodegraded. Natural biodegradation is always too slow to prevent the spread of contamination because of the oxygen limitations (Hinchee *et al.*, 1991). One of the important limiting factors in bioremediation soils contaminated with hydrocarbons is short of oxygen to support microbial metabolism (Atlas, 1986). The oxygen content depends on microbial activity, soil texture, water content and depth. Besides, the availability of oxygen in the soils is dependent on the rate of microbial oxygen consumption; and the kind of soil. The presence of utilizable substrates in high concentration can lead to oxygen depletion (Bossert and Bartha, 1984).

2.2.2.2.3 Nutrients

Nitrogen and phosphorous are basically required for the effective removal of organics by microorganisms in biological processes. Deficiency in the nutrient supply, especially nitrogen generally results in bulking of activated sludge in the activated sludge process. Biodegrading microorganisms usually produce significant amounts of extracellular polysaccharides (ECP) under the nitrogen deficit condition (Aquino and Stuckey, 2003). The supplementation of these nutrients could stimulate the oil degradation in soils immediately or require shorter time of incubation.

Suehara *et al.* (2005) studied the biological treatment of the wastewater discharged from a biodiesel fuel production plant with the alkali-catalyzed transesterification. The biodiesel wastewater which has a high pH and high hexane-extracted oil and low nitrogen concentrations, was found to inhibit the growth of microorganisms. The treatment efficiency was very low because the composition of such wastewater is not suitable for the microbial growth. So, the pH was adjusted to 6.8 and various nutrients such as a nitrogen source (ammonium sulfate, ammonium chloride or urea), yeast extract, KH_2PO_4, and $MgSO_4.7H_2O$ were added to the wastewater. In these studies, the optimal initial concentration of yeast extract was 1 g/l and the optimal C/N ratio was between 17 and 68 when using urea as a nitrogen source.

2.2.2.2.4 Salinity

Ward and Brock (1978) showed the rate of hydrocarbon metabolism reduced with increasing salinity in the range 3.3 to 28.4% and attributed the results to a general reduction in microbial metabolic rates.

2.2.2.2.5 Pressure

Pressure has an important effect on the biodegradation of hydrocarbons which is possibly margined 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.

2.2.2.3 *Biological Factors*

Both bacteria and fungi are ubiquitous in terrestrial (Atlas *et al.*, 1980) and aquatic (Bucker *et al.*, 1976), they can biodegrade hydrocarbons in the environment. Each microorganism can metabolize only a limited range of hydrocarbons (Bossert and Bartha, 1984), thus the 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.

Increasing persistence of chemicals may result from different types of biological interactions: 1) the biocide properties of 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. Inhabitably microbial degradation may ultimately affect the mobility of a chemical in soil (Atlas, 1981). If a microorganism is prevented from utilizing a simple carbon sources, it is thus totally made dependent on the utilization of toxic compounds from polluted environments (Samanta *et al.*, 2001). Hydrocarbon-degrading microorganisms act principally at the oil-water interface and they can grow over the entire surface of oil droplet. The availability of increased surface area should accelerate the biodegradation rate (Atlas, 1981). Besides, the outer membrane permeability may be one of the factors to determine the biodegradability which the proportion of cycloalkanes to linear alkanes on the saturated fraction slowly increases as the number of carbon atom increases (Sugiura *et al.*, 1997). It may be one of the reasons why high molecular weight compounds on the saturated fraction are less susceptible to biodegradation.

Suehara *et al.* (2005) studied the microbiological treatment of biodiesel wastewater using an oil degradable yeast, *Rhodotorula mucilagninosa*. It was found the inhibition of microbial growth by the raw biodiesel wastewater and the critical point of the inhibition was about 1.7 g/*l* based on the solid content of the aqueous phase obtained by the hexane extraction of the raw biodiesel wastewater. So, the measurement of the solid content of the biodiesel wastewater is very important for the efficient biological treatment of the biodiesel wastewater.

Ruttanapol (2004) studied surfactant enhanced biodegradation of oil sludge from the petroleum industry. The main objective of this research was to study the effect of nonionic surfactant (e.g. Brij 30, Triton X-100, and Tween 80) on the solubility and biodegradation of hydrocarbons by indigenous bacteria consortia and *P. aeruginosa*. In the presence of Brij 30 or Tween 80, the biodegradation of the hydrocarbons by either indigenous bacteria consortia or *P. aeruginosa* considerably increased. When Triton X-100 was added that the degrading microorganisms could utilize this surfactant as a source of carbon for their growth, leading to a low extent of hydrocarbons degradation. Therefore, the addition of surfactant can increase the solubility, leading to the enhancement of biodegradation.

2.3 Bioreactors for Wastewater Treatment

Bioreactors are defined as a vessel in which biological reactions are carried out by microorganisms or enzymes contained within the reactor itself. Aerobic bioreactors are primarily used in wastewater treatment to acceptably low levels. Aerobic biological treatment is versatile and cost effective when the concentration of pollutants in wastewater is low. Bioreactors can be classified into 2 types: aerobic reactors and anaerobic reactors which can be operated as batch, continuous, and sequencing batch.

2.3.1 Aerobic Reactors

The aim of aerobic reactors is to reduce for wastewater treatment measurement the organic concentration lower than the permitted level and so the treated effluent can be discharged into a nearly receiving waterway without causing any adverse effects to the environment. The process is needed excess oxygen supply. These systems have a rapid metabolism making the aerobic processes have short resident time.

Suna *et al.* (2008) studied the aerobic and anaerobic degradation of municipal solid waste in bioreactor landfills. They found aerobic reactor had higher organic, nitrogen, phosphorus, and alkali metal removal efficiencies than the anaerobic one in a thermo-insulated room at a constant temperature of 32°C. Moreover, stabilization time considerably reduced when using aerobic processes with leachate recirculation compared to the anaerobic system with the same recirculation scheme.

2.3.2 Anaerobic Reactors

Anaerobic reactors are generally employed to treat hardly concentrated organic wastewaters, The process does not require aeration and biogas containing methane and carbon dioxide is produced and can be used as a fuel substitute in many industries. However, the effluent from anaerobic reactors still contains a very high concentration of organic compounds and so it is required a subsequent aerobic treatment to meet the effluent standards.

Suna *et al.* (2008) studied the comparison of aerobic and anaerobic degradation of municipal solid waste in bioreactor landfills. They reported that the anaerobic bioreactor landfill shows the highest levels of all pollutional parameters, with high concentrations of COD, TOC, BOD, ammonia, phosphorus and alkali metals in the leachate. Moreover, concentrations of Ca and Mg in the anaerobic reactor were significantly greater than those of the aerobic reactor.

2.3.3 Batch Reactors

For batch reactors, a wastewater is added at the beginning of the operation but no product is withdrawn until the end of the operation. They are simple, require least support equipment, and well suited to treat small amounts of wastewaters. These batch reactors are not popular used for wastewater treatment since they have low treatment efficiency and they are not practical for large quantities of wastewaters.

2.3.4 Continuous Reactors

Continuous reactors are usually used in the large-scale wastewater treatment systems, which wastewaters are fed continuously with a constant feed flow rate. Hence the environmental conditions in the bioreactors are relatively consistent, leading to high treatment efficiency as compared with batch reactors. There are several types of aerobic continuous reactors system for wastewater treatment such as activated sludge, tricking filter, rotation biological contactor, and sequencing batch reactor.

2.3.5 Sequencing Batch Reactors

The sequencing batch reactor (SBR) has been increasingly gained more and more popular in wastewater treatment because of its simplicity in operation, high treatment performance, and low treatment cost. This process is generally divided into 4 phases: filling, aeration, settling, and decanting. A wastewater is fed into the reactor during the filling step. For the reaction step, the system works as a batch reactor with continuous aeration. During setting step, the aeration was stopped to allow the microbial cells to settle. For the final step (decanting), the clear solution is withdrawn form the tank. A next cycle will be repeated (Andrea *et al.*, 2004).

Attavavuthichai (2006) used sequencing batch reactors (SBR) to study the surfactant-enhanced biodegradation of crude oil sludge. Two units of sequencing batch reactors were designed and constructed for this study. When the oil loading rate increased, the oil removal efficiency decreased. A maximum oil removal of 90% was achieved at the lowest oil loading rate of 4.0 kg/m^3 per day and an optimum surfactant concentration of 0.1%..

Comchumpoo (2007) studied surfactant-enhanced biodegradation of crude oil sludge in sequencing batch reactors at different surfactant concentrations, oil loading rates, and number of cycles per day. In this experiment, Tween 80, as a nonionic surfactant was used to enhance the solubilization of the crude oil sludge. The optimum surfactant concentration was 0.2%. At a surfactant concentration of 0.1% w/v, the optimum oil loading was 1.0 kg/m^3 provided the highest removal efficiency. When the number of cycles per day increased, the oil removal efficiency decreased. At a surfactant concentration of 0.2% and 1 cycle per day, the highest removal efficiency provided was found to be as high as 80%.

Celis *et al.* (2008) studied biodegradation of agricultural herbicides in sequencing batch reactors under aerobic and anaerobic conditions. They investigated the biodegradability of the herbicides isoproturon and 2,4-dichlorophenoxyacetic acid (2,4-D) in sequencing batch reactors. They found that the biodegradation using anaerobic was better than using aerobic conditions.