CHAPTER III

EXPERIMENTAL

3.1 Materials and Euipment

3.1.1 Nonionic Surfactant

The nonionic surfactant used in this study was Tween 80 (100% solution). It was obtained from Fluka (Milwaukee, WI, USA) and Table 3.1 shows the characteristics of surfactant used in this study.

Table 3.1 The characteristics of surfactant used in this study

Surfactant	Molecular formula	Molecular weight (g/mol)	HLB	CMC(M)
Tween 80	POE(20)sorbitan monooleate	1310	15.0	1.2×10^{-5}

POE: polyoxyethylene, HLB: hydrophilic-lipophilic balance.

3.1.2 Oil Sludge

Oil sludge was kindly provided by Dr. Thawach Chatchupong from PTT Public Company Limited, Thailand. The water portion of the sludge was separated by decantation and the excess moisture was removed by drying the oil sludge at open atmosphere in a petri dish. In this study, Oil in the oil sludge was extracted out of its slurry phase by using n-hexane as a solvent in extraction. The mixed-solution was filtered through the filter paper (Whatman no.4) and then evaporated n-hexane out. The residue oil was the extracted oil from crude oil sludge and it was used in the experiment as a source of carbon from oil sludge.

3.1.3 Media

Mineral salts medium (MSM) used in this study consists of 1.8 g K_2HPO_4, 1.2 g KH_2PO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, and 0.01 g $FeSO_4.7H_2O$ in 1,000 mL distilled water (Ijah and Upke, 1992). The final pH of the

MSM was adjusted to pH 7.4 using 0.1 N NaOH and 10% HCl. MSM was autoclave at 110°C, 15 psi for 15 min before used in the experiment. The medium composition used for culturing the oil sludge degrader was the same as that used in the experiment. The agar medium consisted of 0.1 g of Bacto peptone (Difco), 0.5 g of yeast extract (Difco), 1 g of Glucose (Difco) and 1.8 g of agar (Difco) in 100 mL of MSM.

3.1.4 SBR Set up

The reactor was built by using a glass cylinder tube (borosilicate) as a material having a total working volume of 2.5 liters. The dimensions were an 8 cm. outer diameters and a 60 cm. height. Feed tanks (stainless steel) were connected with pump to feed chemical into the reactor. The air blowers were used for providing oxygen to the microorganisms by connecting into the bottom of reactor. Draining system was operated by using solenoid valve to control volume of the samples flowing into the product tank. In the operation, peristaltic pump, air blower and solenoid valve were controlled by twin timers (OMRON H3CR-F) and relays (OMRON) in a sequence as shown in Figure 3.1.

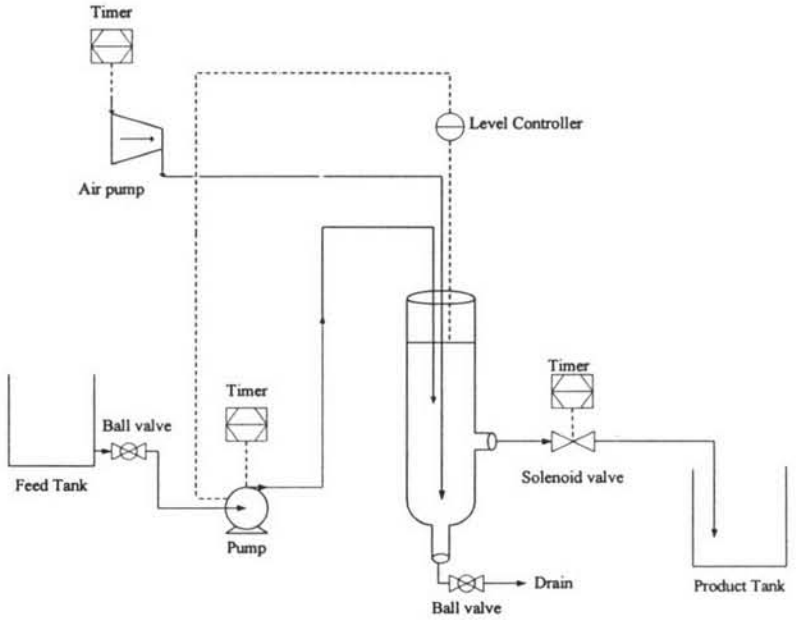

Figure 3.1 Flow diagram shows the planning operation for the SBR process.

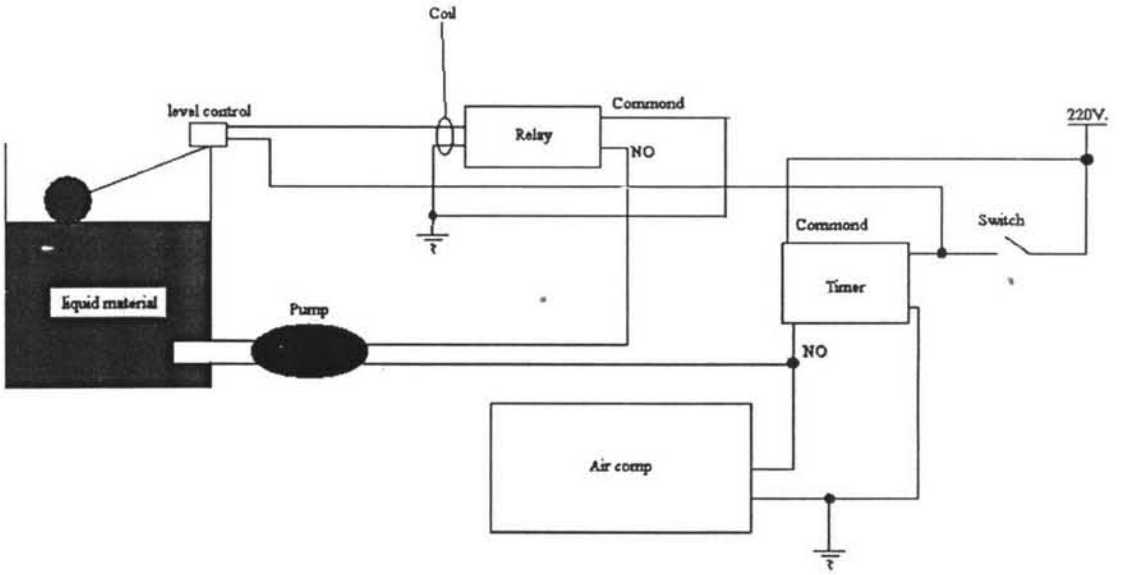

Figure 3.2 Electrical Flow Diagram of SBR Process.

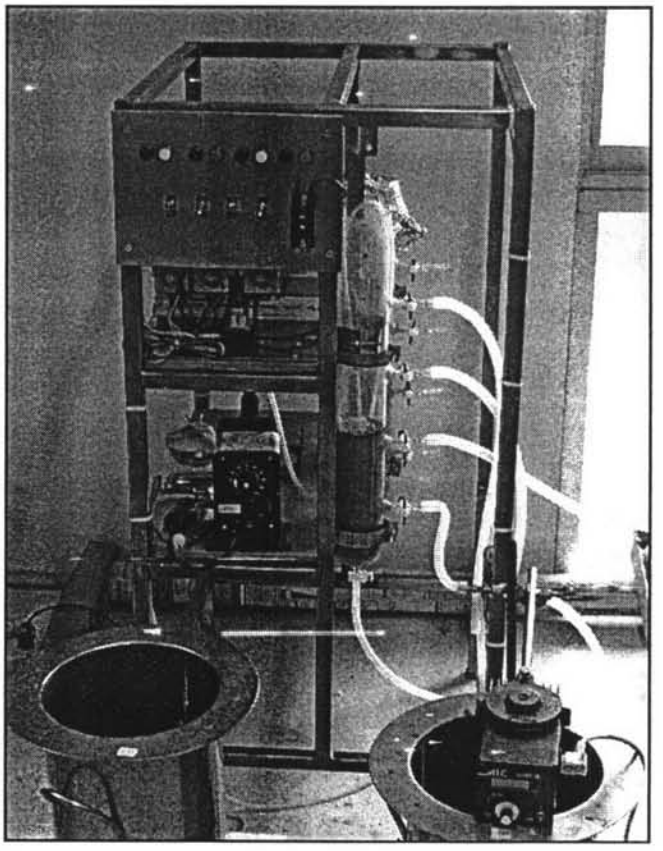

Figure 3.3 Sequencing Batch Reactors.

3.1.5 Cultivation of the Oil Sludge Degrader

Oil sludge degrader was cultivated in the 2.5 Liters glass tube bioreactors. 50 ml of the fresh oil sludge solution was fed into the rectors. At first, the addition of glucose, yeast extracted and peptone was required to build up biomass. Then, the nutrient was fed along with the oil sludge and surfactant solution so that the microorganisms could use the oil sludge as a carbon source. Finally, no addition of glucose, yeast extracted and peptone was required because the microorganism was already using the oil sludge as a carbon source and then the biodegradation study started. The total effective volume of oil sludge degrader was 1 L.

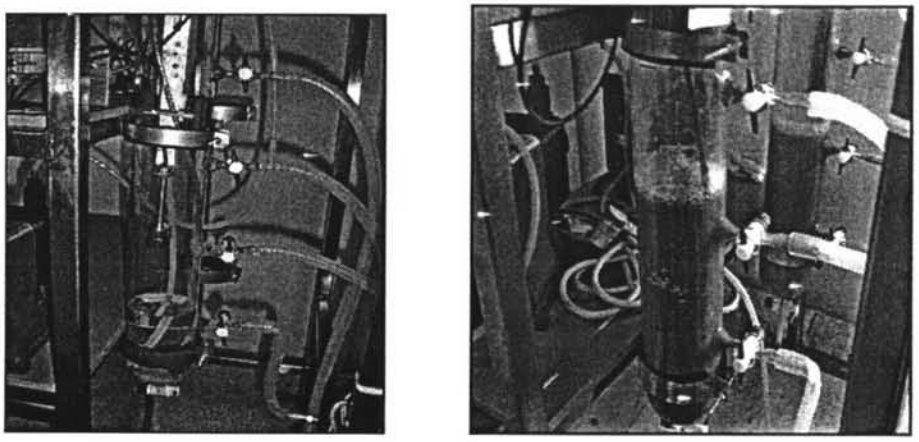

Figure 3.4 Cultivation of the Oil Sludge Degrader.

Table 3.2 The conditions in SBR Operation

Cycle period of reactor during sequence

Phase	Cycle period
Filling (min)	15
Aeration (hr)	23
Settling (min)	30
Withdrawal (min)	15

3.2 Method

3.2.1 Effect of Time On Solubilization of Hydrocarbons in Oil Sludge by Nonionic Surfactant

The solubilization study was conducted by varying the amount of surfactant in the range of 0.05 % w/v to 7 % w/v with the fixed concentration of extracted oil from oil sludge 1% w/v in 20 ml of autoclaved MSM in vial 6 dam. Two control were performed by the first one was only extracted oil with MSM and the other was only surfactant with MSM. All vials were shaking at 150 rpm for 3 days and left to stay still for 30 days and the observation in their phase behaviors were conducted. After 30^{th} day, the amount of hydrocarbons in the aqueous phase was determined by TOC analyzer.

3.2.2 Effect of Agitation Speed on Solubilization of Hydrocarbons in Oil Sludge by Nonionic Surfactant

Extracted oil from oil sludge 10 g and 1 L of MSM was mixed in feed tank. The required amount of surfactant was added on a weight by volume basis. Two controls were performed in the feed tank. The first control received oil sludge but no surfactant whereas the second control received only surfactant. The solution in the tank was mixed by stirrer at room temperature. The agitation speed was varied at 60, 120 and 200 rpm. The dispersing power of surfactant was characterized by determining the total organic carbon of the aqueous phase. The sludge sample was filtered through filter paper (Whatman 42, size 11.5 cm.) and filtrate was injected into a TOC analyzer (Sjimadzu, 500A). The samples were also analyzed for Chemical Oxygen Demand (COD) by COD reactor (HACH, 45600) for evaluating their effect on the solubilization of nonionic surfactant. The estimation of hydrocarbons in oil sludge was performed by the method of oil extraction as described in the next section.

3.2.3 Determination of Total Petroleum Hydrocarbons (TPH) in Oil Sludge by Oil Extraction

Following incubation, 20 mL of dichloromethane (DCM) was added to flasks which were mixing 15 minutes by sonicator (Crest, 575D) into obtain total petroleum hydrocarbons extract. The aqueous and solvent phase was transferred to four 50 mL centrifuge tubes, and flasks rinse three more times to bring the total volume of DCM used to 50 mL. The tubes were centrifuged at 12,000 x g (Hermile, Z383K) to break oil in water emulsions. The upper layer was discarded and the lower oil containing phase was filtered into a round-bottom flask through sodium sulfate to remove residual water. The majorities of the solvent was removed under vacuum with an Evapotec Rotary Evaporator (Heidolph, VV2011) and allow drying to a constant weight on a fume hood prior to a gravimetrical measurement of the TPH extract. Asphaltenes were precipitated by adding 5 mL of n-hexane to the TPH extract, mixing with a glass rod. The contents of the beaker were then filtered through Whatman GF/A glass microfiber filter (Whatman International Ltd., Maidstone, England). The concentrated residue of hydrocarbons was diluted to 10 mL with n-hexane and it was injected into the GC/MS (Fison) with GC-8000 series and MS-FD 800.

3.2.4 Biodegradation

A 1 liter medium was always prepared fresh and the 10 grams of extracted oil from oil sludge was mixed with the MSM and nonionic surfactant in the unit of weight by volume basis (0.1% w/v). Then, the mixed solution was mixed by stirrer at 200 rpm at room temperature for 2 days to reach the complete solubilization before using it as a feed. The conditions in the biodegradation study were studied at the loading of 50 ml per day and also varied the amount of oil and surfactant. The oil loading was varied from 1 to 10 g/L.d and the amount of nonionic surfactant with the increased amount of extracted oil from oil sludge was also varied. For every condition, the ratio was fixed 1% w/v of oil and 0.1% w/v of nonionic surfactant, but difference amounts were made by varying the amount of MSM. Table 3.3 shows the conditions used in the biodegradation study.

Table 3.3 The conditions used in biodegradation study

Extracted oil from oil sludge (g)	Tween 80 (g)	Total volume with MSM (L)	Oil loading rate (g/Ld)
1.0	0.1	1	1
2.0	0.2	1	2
4.0	0.4	1	4
6.0	0.6	1	6
8.0	0.8	1	8
10.0	1.0	1	10

Fixed condition with: Oil, 1 %w/v + Tween 80, 0.1% w/v

Fill 50 ml, Draw 50 ml, Reaction time = 1 day

The extent of the biodegradation of the nonvolatile fractions of oil sludge was determined by comparing to control flask (no surfactant) at a time zero and autoclaved control. The dispersing power of surfactant was characterized by determining the total organic carbon of the aqueous phase. The sludge samples was filtered through filter paper (Whatman 42, size 11.5 cm.) and filtrates was injected into a TOC analyzer (Shimadzu, 500A) and also used COD reactor (HACH, 45600) to analyze for Chemical Oxygen Demand (COD). The TOC and COD values were used in the calculation of mass balance in the SBR process. The growth of the microorganisms as a result of the biodegradation of petroleum components were also measured by using dry weight cell method.