CHAPTER III
METHODOLOGY

3.1 Materials and Equipment

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 Bangchak 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 for 5 days 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.

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.

3.1.4 Bacteria and Cultivation

Oil sludge degrader was cultivated in the 2.5 Liters glass tube bioreactors. 50 ml of the fresh oil sludge solution was taken into the rectors. At first, the addition of nutrient consists of 0.1 g of Bacto peptone (Difco), 0.5 g of yeast extract (Difco) and 1 g of Glucose (Difco) in 100 mL of MSM which required to build up biomass. Then, the nutrient was fed with the oil sludge and surfactant solution to make the microorganisms used 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 cultivated at 1 L.

3.2 Methodology

3.2.1 Effect of Time On Solubilization of Hydrocarbon 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 5 % 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 of the vials were shaking at 150 rpm for a week. Then amount of hydrocarbons in the aqueous phase was determined by TOC analyzer.

3.2.2 Biodegradation

The medium is always prepared fresh and 1 L of medium is added directly to the feed tank, followed by the addition of oil sludge and nonionic surfactant (Tween 80) in the unit of weight by volume basis. Then the solution will be agitated by stirrer at room temperature for 4 days to reach the complete solubilization before using it as feed. Firstly, the conditions in the biodegradation study were studied at the oil loading of 50 mL per day and also studied the difference amount of oil loading at 0.5 and 1 kg/m^3d with the surfactant concentration was 0.1%

w/v. Both conditions were fixed the ratio of extracted oil at 1% w/v. Table 3.2 shows the conditions of oil loading in the biodegradation study. Then the effect of number of cycle per day was studied by varying the number of cycle per day from 1 to 3 cycles per day by using the amount of oil loading at 1 kg/m^3d and the surfactant concentration at 0.1% w/v. Table 3.3 shows the condition in the SBR operation.

Table 3.2 The condition in the biodegradation study

Extracted Oil from oil sludge	Tween 80	Total volume (with MSM)	Oil loading rate
0.5 gram	0.05 gram	1,000 mL	0.5 kg/m^3d
1.0 gram	0.10 gram	1,000 mL	1.0 kg/m^3d

Fixed condition with: Oil, 1% w/v + Tween 80, 0.1% w/v
Fill and draw 50 mL, Reaction time = 1 day

Table 3.3 The condition in SBR operation

Cycle period of reactor during sequence

Number of cycle	Filling (min)	Aeration (hr)	Settling (min)	Withdrawal (min)
1	15	23	30	15
2	15	11	30	15
3	15	7	30	15

Fixed condition with: Oil, 1% w/v + Tween 80, 0.1% w/v
Fill and draw 50 mL

The dispersing power of surfactant was characterized by determining the total organic carbon of the aqueous phase. The sludge samples were filtered through filter paper (Whatman 42, size 11.5 cm.) and filtrates were injected into a

TOC analyzer (Shimadzu, 500A) and also use COD reactor (HACH, 45600) to analyze for Chemical Oxygen Demand (COD). The surfactant as added at various concentrations of surfactants for evaluating their effect on the solubilization of hydrocarbons from oil sludge. The growth of the microorganism as a result of biodegradation of petroleum components can be measured by dry weight cell method. The growth of the microorganism as a result of biodegradation of surfactants can also be measured by optical density at 600 nm using spectrophotometer (UV-VIS Spectrophotometer, Shimadsu).

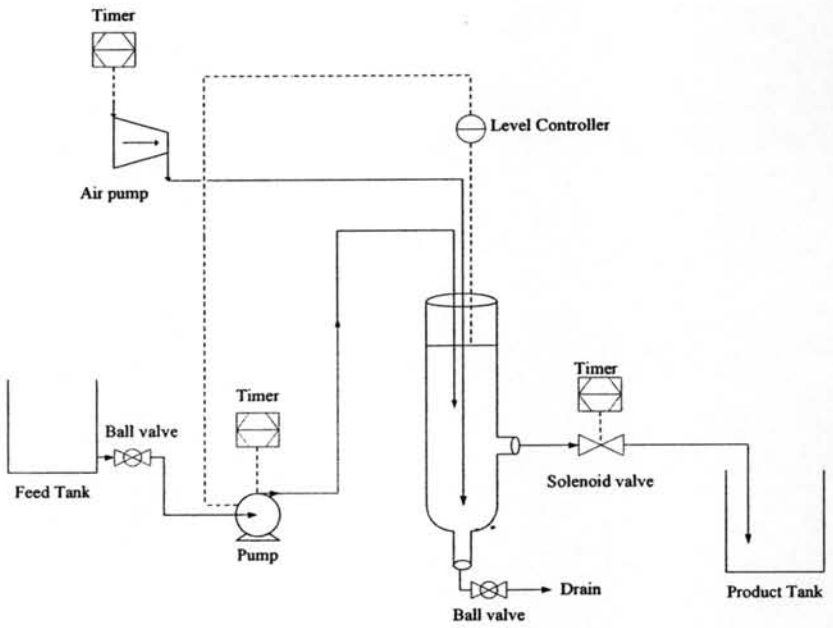

Figure 3.1 Flow diagram shows the planning operation for the SBR process.

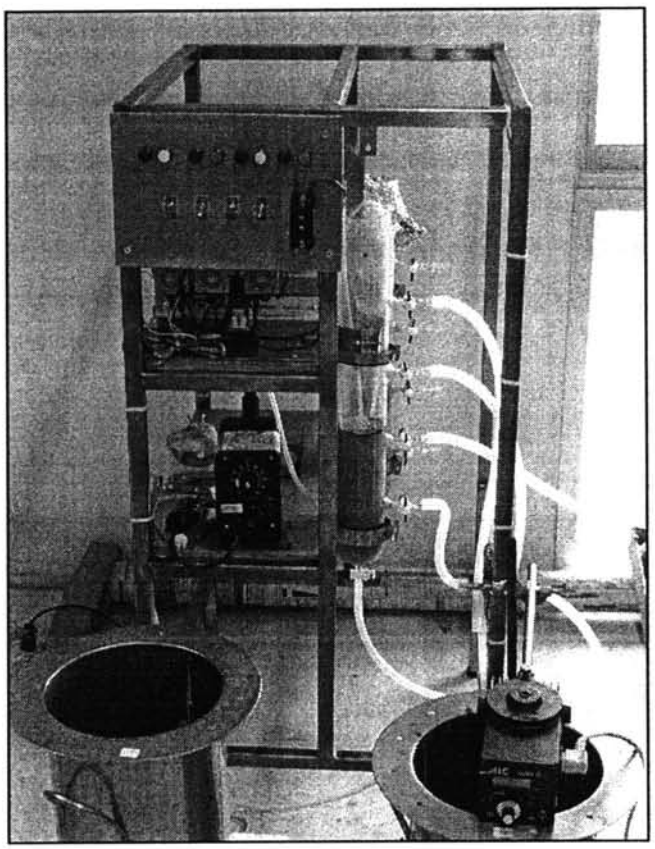

Figure 3.2 Sequencing Batch Reactors.

3.2.3 Determination of total petroleum hydrocarbons (TPH) in oil sludge by Oil extraction

Following the 30 mL of sample solution from the bioreactor, 20 mL of dichloromethane (DCM) was added to flasks and mixed for 15 minutes by sonicator (Crest, 575D) into obtain total petroleum hydrocarbons extract. The solution was separated into 2 phases. The upper layer 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. 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 including the lower oil layer from the first step were filtered into a round-bottom flask through sodium sulfate to remove residual water. The majority of the solvent was removed under vacuum with an Evapotec Rotary Evaporator (Heidolph, VV2011) and allowed to dry 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 injected into the GC/MS.