CHAPTER III
EXPERIMENTAL

3.1 Materials and Equipments

3.1.1 Chemicals:

The nonionic surfactant used in this study was polyethylene sorbitan monoleate or Tween 80 (100% solution). It was obtained from Fluka, Milwaukee, WI, USA. Mineral salts such as laboratory reagent grade ammonium hydrogen carbonate (NH_4HCO_3), analytical reagent grade di-Potassium hydrogen orthophosphate (K_2HPO_4), analytical reagent grade magnesium chloride ($MgCl_2 \cdot 6H_2O$), laboratory reagent grade magnesium chloride ($MgCl_2 \cdot 6H_2O$), laboratory reagent grade iron (II) sulphate ($FeSO_4 \cdot 7H_2O$), analytical reagent grade copper (II) sulphate ($CuSO_4 \cdot 5H_2O$), analytical reagent grade cobalt (II) chloride ($CoCl_2 \cdot 5H_2O$), and laboratory reagent grade sodium hydrogen carbonate ($NaHCO_3$) were obtained from UNIVAR, Australia. Analytical reagent grade dichloromethane (CH_2Cl_2) was obtained from Lab-Scan, Thailand. Moreover, distilled palm oil was supplied from Morakot Industrial Public Company Limited, Thailand.

3.1.2 Apparatus for setting up an upflow anaerobic sludge blanket (UASB) Reactor :

An UASB reactor was constructed from borosilicate glass with a 16-liter working volume. Water jacket system was used to provide heat to the reactor with hot water form heater bath (Memmert). Peristaltic pump (Master Flex Model Easy-load II) was used to carry hot water to water jacket system and also carried the influent to the reactor. Moreover, effluent pH was measured by pH electrode (Cole-palmer KH-27012-27).

3.2 Methodology

3.2.1 Bacteria and cultivation

Bacterial sludge was collected from wastewater treatment plant, Suksomboon palm oil Co. Ltd., Chonburi, Thailand. The pH and total suspended solids (TSS) of the sludge were 4.30 and 187.67 g/l, respectively. The sludge was boiled for 15 minutes to inhibit bioactivity of hydrogen consumers. And finally, 20 g/l of total suspended solid (TSS) was applied to the reactor.

Mineral salts medium (MSM) used in this study consists of 5,240 mg NH_4HCO_3, 125 mg K_2HPO_4, 15 mg $MgCl_2 \cdot 6H_2O$, 25 mg $FeSO_4 \cdot 7H_2O$, 5 mg $NaHCO_3$, 0.125 mg $CoCl_2 \cdot 5H_2O$, and 6720 mg $CuSO_4 \cdot 5H_2O$ in 1000 ml distilled water (Lin and Chang, 2004).

3.2.2 Feed preparation

Synthetic oil wastewater was simulated from distilled palm oil and water with 2% of oil v/v. Then, it was mixed with nonionic surfactant and MSM in stirred feed tank. After that they were fed in to the reactor as an influent.

3.2.3 Effect of time and surfactant concentration on oil solubilization

The oil solubilization was considered in terms of solubility parameter of oil or Sp_o (mg/l) and enhanced-solubilization (mg/l). Sp_o is defined as a volume of oil solubilized in aqueous phase per weight of surfactant in aqueous phase. The system is composing of surfactant solution and oil with total volume of 10 ml. And the surfactant concentration was varied from 0.01 to 10% w/v with 50% and 20% v/v of oil or 1/1 and 1/4 oil to surfactant solution volume ratio. The vials were shacked slowly 10 times for creating contact area between oil and surfactant solution and kept in water bath at 37°C. The aqueous phase high was measured every day by cathetometer. The increasing in aqueous phase high shows that oil is solubilized into aqueous phase. So the aqueous phase height was measured every day and convert to Sp_o. The enhanced-solubilization study was conducted by varying the amount of surfactant in the range of 0.01 to 5% w/v with the fixed concentration of oil at 2% w/v with total

volume of 10 ml in vial 6 dam. Two control were performed by the first one was only oil with distilled water and the other was only surfactant. The vials were shaken in heater bath at the temperature of 37°C from 1 day to 10 days. After that the lower part was sampling and determined by TOC analyzer (Shimadzu, 500A). The effects of surfactant concentration and time on oil solubilization were considered in terms of enhanced-solubilization as shown below.

Enhanced Solubilization = (solubilization$_{oil+surf}$ -solubilization$_{surf}$)- solubilization$_{control}$

where

solubilization$_{oil+surf}$ = TOC of oil sludge and surfactants

solubilization$_{surf}$ = TOC of surfactants alone

solubilization$_{control}$ = TOC of oil sludge alone

3.2.4 UASB operation

The UASB reactor was constructed from borosilicate glass with a 16-liter working volume. Temperature inside the reactor was controlled at 37°C by water jacket system with heater bath. The flow diagram of UASB operation is shown in Figure 3.1. Synthetic oily wastewater and nonionic surfactant (Tween 80) were mixed with the MSM by using stirred feed tank. The feed was pumped into the bottom of the reactor, and passed through the flocculants of microorganisms. The oil was digested by the microorganisms, and gaseous by-products were produced. A three-phase separator was used to prevent an outflow of flocculants, collected the gaseous by-products, and collected the overflow liquid effluent to the product tank. Then the gaseous by-products were measured the gas production rate and determined the gas content by gas meter and GC, respectively. And the liquid effluent was determined the COD value, oil content, pH, and VFAs.

3.2.5 Effect of COD loading rate on biodegradation and biogas production

To determine the effect of COD loading rate on biohydrogen production, influent with 22,000 mg COD/l (2% v/v of oil) with the optimum surfactant concentration from the solubilization study was fed into the reactor by varying COD

loading rate from 10 to 80 kg COD/m³d. The feed flow rate and hydraulic retention time which are corresponding to the oil loading rate are shown in Table 3.1. The products properties such as pH, biogas production rate, biogas content, and effluent COD were determined until the system reach steady state and at this point these properties are relatively unchanged.
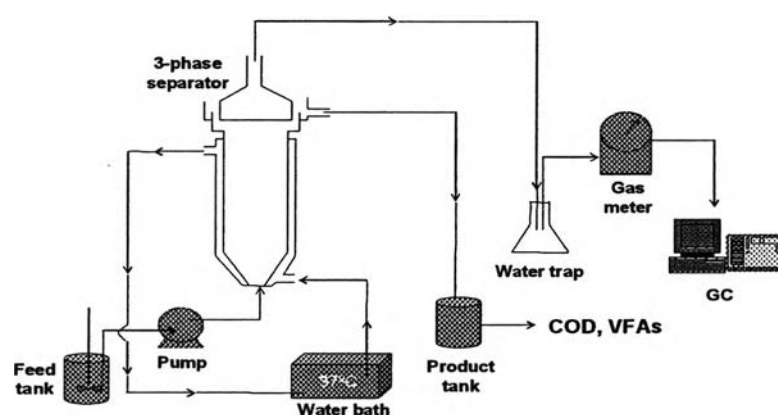

Figure 3.1 Flow diagram of UASB operation.

Table 3.1 COD loading rate, flow rate, and hydraulic retention time (HRT) for determining effect of oil load rate

COD loading rate (kg COD/ m³d)	Flow rate (l/hr)	HRT (hr)
10	0.30	53.33
20	0.60	26.67
30	0.90	17.78
40	1.20	13.33
60	1.80	8.89
80	2.40	6.67

For the biodegradation of oil, suspended solids were first separated by centrifuging at 12,000xg (Hermile, Z383K) for 15 minutes. After that COD value of the effluent liquid phase was measured by COD reactor (HACH 45600) and spectro-

photometer (HACH DR/2000) and the percentage of COD removal could then be calculated. Moreover, 5 ml of liquid phase was extracted by 5 ml of dichloromethane for 3 times. After dichloromethane was evaporated for a day at ambient condition, the remaining oil was determined in terms of oil content (% v/v) and percentage of oil removal. The pH was also determined by pH electrode (Cole-palmer KH-27012-27). The biogas production rate was measured by gas meter, and biogas content was determined by gas chromatograph or GC (AutoSystem GC, Perkin-Elmer) using thermal conductivity detector (TCD) and a packed column (stainless-steel 10'x 1/8' x .085" HayeSep D 100/120 mesh (Altech) packed column. Injector and detector temperature were kept at 60, 35, and 150°C, respectively. Argon was used as the carrier gas at pressure of 345 kPa. Moreover, amounts of VFAs in mg as acetic acid per liter were determined after the distillation of the effluent by titrating with 0.1 M NaOH using phenolphthalein as an indicator (Greenberg *et al.*, 1992). VFAs and alcohol distribution was analyzed by GC (PR2100, Perichrom) equipped with a flame ionization detector and a 50 m x 0.32 ID, 0.25 μm film thickness DB-WAXetr (J &W scientific) capillary column in the splitless mode with helium at a pressure of 22 kPa as carrier gas, hydrogen at 50 kPa as combustion gas and air zero at 50 kPa as a combustion-supporting gas. The column temperature program was started at 60°C, heated to 125°C at a ramping rate of 10°C/min, held for 2 min, then heated to 180°C at a ramping rate of 15°C/min, and held for 15 min. The temperature of both injector and conductor was 250°C. Lastly, the growth of the microorganism as a result of biodegradation of organic compounds can be measured by mixed liquor suspended solids or MLSS (APHA, 1995). At day 25 the liquid and solid components were drained out from the reactor then stirred with stirrer until the system was homogeneous. A 5 ml welled-mixed sample was collected from mid point of the container but not from vortex which is a point middepth and midway between wall and vortex. The sample was filtered through a glass fiber filter then washed with distilled water and dried in oven at 103-105°C for 1 hour. The dried residue sample was determined in g of suspended solids per litre as MLSS.

3.2.6 Effect of surfactant on biodegradation and biogas production

To determined the effect of surfactant on biodegradation and biogas production, the UASB reactor was also operated at 37°C and uncontrolled pH. The effect of surfactant was determined at the optimum COD loading rate with the optimum surfactant concentration and without surfactant. COD removal, oil removal, specific biogas production rate, biogas content, pH, VFAs concentration, VFAs and alcohol composition, and MLSS were also the main parameters observed in this section.