CHAPTER IV
RESULTS AND DISCUSSION

4.1 Determination of the optimum mixing time and surfactant concentration on oil solubilization from solubility parameter of oil (Sp_o)

Due to thermodynamically limited by poor solubility of oil in aqueous phase, nonionic surfactant (Tween 80) was employed to solubilized oil into aqueoue phase. However, there are many parameters and methods to study. And in this research, solubility parameter of oil or Sp_o (ml/g) and enhanced-solubilization (mg/l) were considered. 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 results showed that when the surfactant concentration increases, the Sp_o also increase corresponding with the increasing in solubulized oil in aqueous as shows in Figures 4.1 and 4.2. And from these figures, the equilibration time require for solubilizing oil was 6 days. Therefore, the optimum surfactant concentration for solubilizing the oil was determined at day 6 but there is no optimum surfactant concentration as shows in Figures 4.3 and 4.4. In fact, the optimum surfactant concentration should be shown because the concentration was scan from 0.01–10% w/v but the critical micelle concentration or CMC of Tween 80 is 0.01572% w/v. If the surfactant concentration is lower than CMC, no formation of micelle and so on no solubilization. So the conclusion is the Sp_o is not suitable to determined oil sulubilization because of there are small contact area of oil and aqueous phase, this system is not simulated the practical use with all times mixing in feed tank, the difference in aqueous phase high cannot be detected by cathetometer when the low surfactant concentrations are applied, and after shacking some surfactant solution was trapped in oil phase.

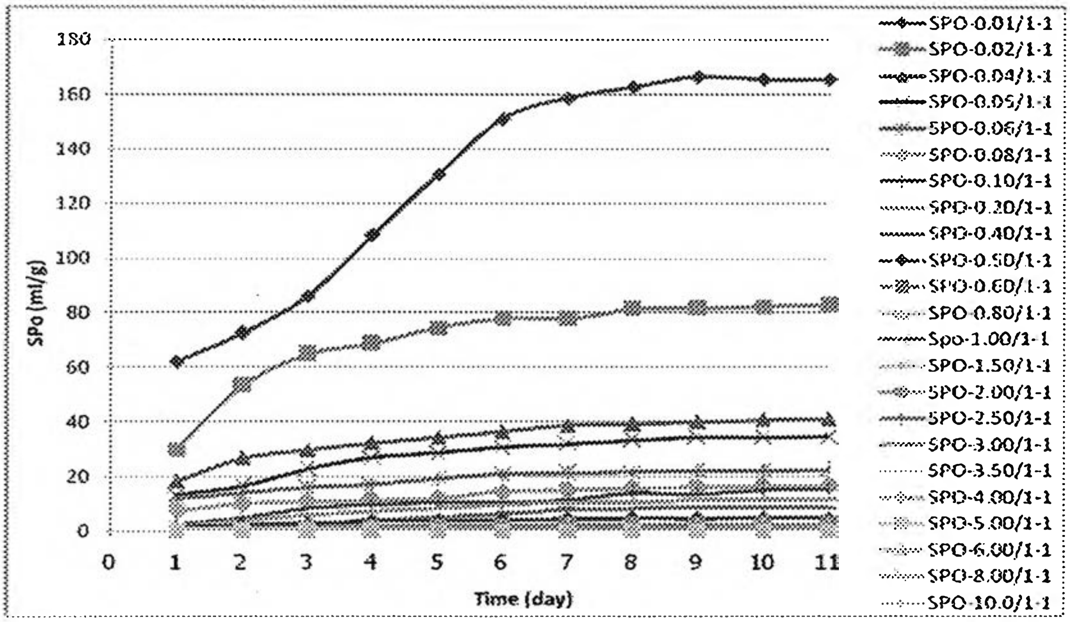

Figure 4.1 Solubility parameter of oil (Sp_o) with various surfactant concentration and time (SPO-0.01/1-1 refers to Sp_o value at surfactant concentration of 0.01% w/v with 1 to 1 oil to surfactant solution volume ratio).

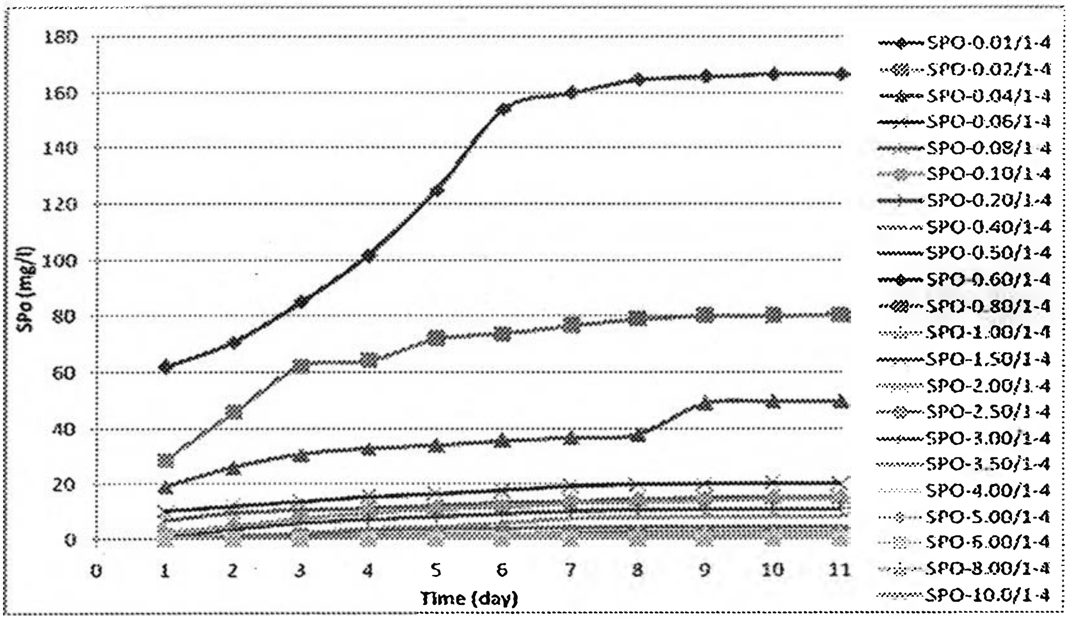

Figure 4.2 Solubility parameter of oil (Sp_o) with various surfactant concentration and time (SPO-0.01/1-4 refers to Sp_o value at surfactant concentration of 0.01% w/v with 1 to 4 oil to surfactant solution volume ratio).

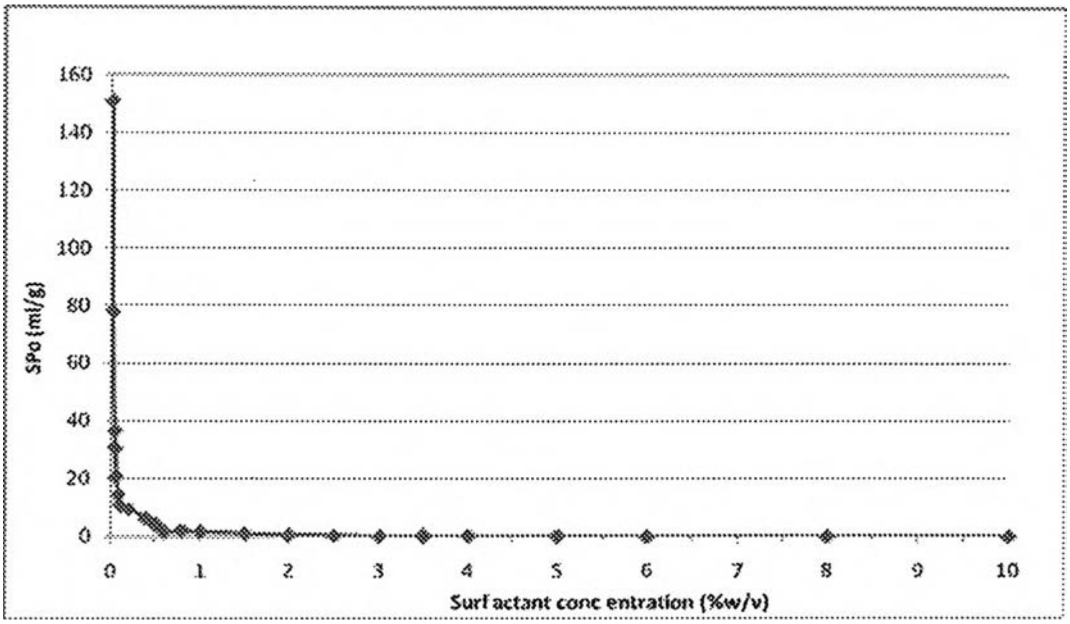

Figure 4.3 Solubility parameter of oil (Sp_o) with various surfactant concentration at day 6 with 1 to 1 oil to surfactant solution volume ratio.

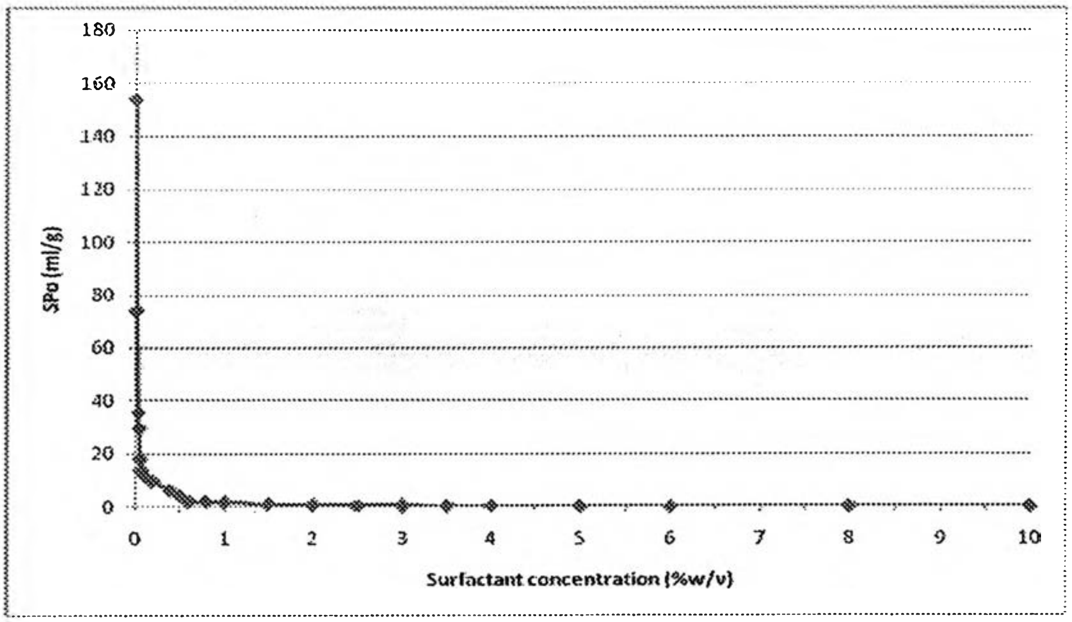

Figure 4.4 Solubility parameter of oil (Sp_o) with various surfactant concentration at day 6 with 1 to 4 oil to surfactant solution volume ratio.

4.2 Determination the optimum mixing time and surfactant concentration on oil solubilization from enhanced-solubilization

The effect of mixing time and surfactant concentration on oil solubilization was considered in terms of enhanced-solubilization. In this study, the surfactant concentration was varied from 0.05 to 5% w/v with 2% v/v oil loading (critical micelle concentration or CMC of Tween 80 is 0.01572% w/v). And the samples were left in shaker bath at 37°C for 10 days and aqueous phase was separated and injected into TOC analyzer every day. The results showed that to solubilize oil in aqueous phase required 4 days to reach equilibrium as shown in Figure 4.5.

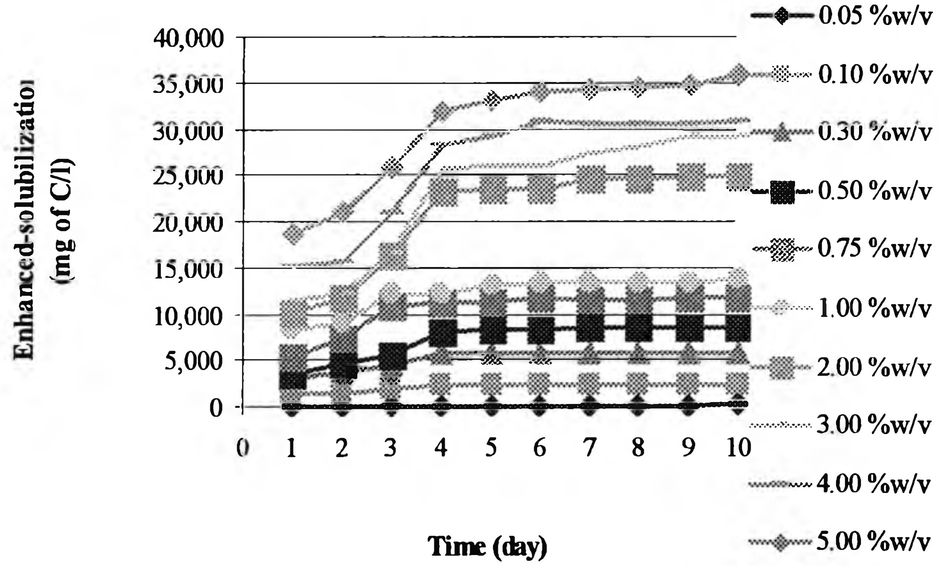

Figure 4.5 Equilibrium time required to solubilize oil in aqueous by Tween 80.

And for optimum surfactant concentration, the enhanced-solubilization was determined at day 4 as shown in Figure 4.6. The results clearly demonstrated a proportional relation between enhanced-solubilization and surfactant concentration. Therefore, Figure 4.7 shows that at surfactant concentration of 0.10% w/v (which is equivalent to a weight ratio of oil to surfactant of 18.8:1) provided the highest ratio between the weight of solubilized carbon and the weight of surfactant compared with the others. It means that this surfactant concentration was the most suitable to solubilize oil.

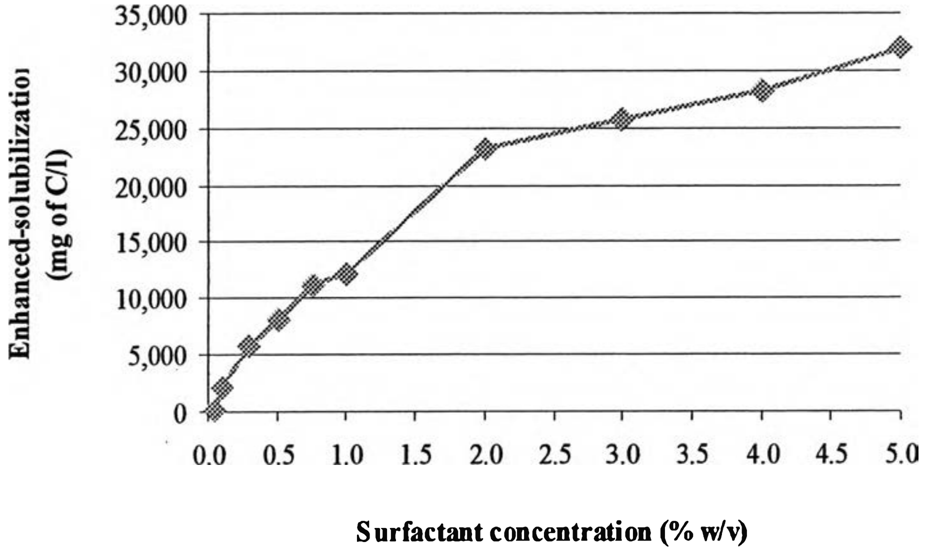

Figure 4.6 Enhanced-solibilization of oil at the various surfactant concentration.

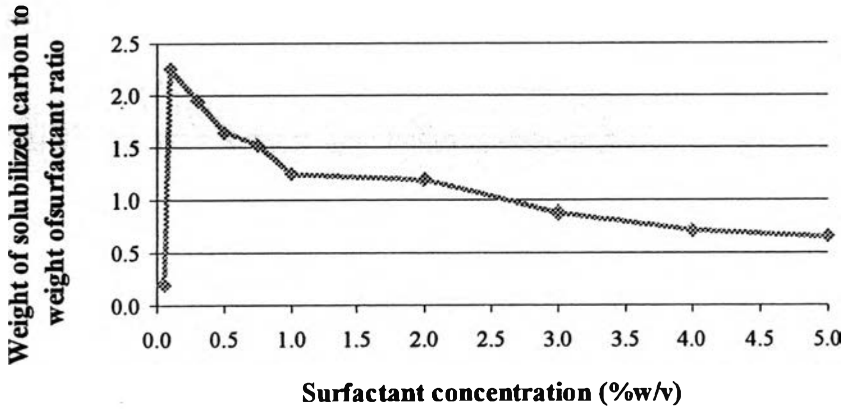

Figure 4.7 Weight of solubilized carbon to weight of surfactant with various surfactant concentration.

4.3 Effect of COD loading rate on biodegradation and biogas production

An upflow anaerobic sludge blanker (UASB) reactor was constructed for treating an oily wastewater simulated from distilled palm oil and water, and for producing biohydrogen. A mixed bacterial culture was used to treat the oily wastewater at the optimum temperature of 37°C (Chittibabu *et al.*, 2006*)* and uncontrolled pH. Moreover, the optimum surfactant concentration (0.10% w/v which is equivalent to a weight ratio of oil to surfactant of 18.8:1) from solubilization study was used to enhance oil solubilization and thus biodegradation, as well as biohydrogen production. The COD loading rate was varied from 10–80 kg COD/m^{3}d to determine its effect on biodegradation and biogas production.

4.3.1 Chemical Oxygen Demand (COD) removal

COD is defined as the quantity of a specified oxidant that reacts with a sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence and COD is expressed in mg/l O_2. In this experiment, the COD method was conducted to determine the degradation capability of the microbe in utilizing organic substances as their nutrients. The influent COD value was controlled at 22,000 mg/l. The effluent COD value was determined every day until the system reach steady state and at this point the effluent COD value was relatively unchanged. The results showed that at day 20 the COD value began steady. After the system reached a steady state, COD value of five more days were collected and then used to determined COD removal as an averaged value as show in Table 4.1. Figures 4.8 and 4.9 show the effluent COD values and COD removal at different COD loading rate, respectively. The results showed that when the operation time increases, the effluent COD value decreases until the system reaches steady state as shows in Figure 4.8, and corresponding with the increasing of COD removal as shows in Figure 4.9. Moreover, when COD loading increases, the effluent COD value increases, corresponding with the decreasing of the COD removal with increasing COD loading, as shown in Figures 4.8 and 4.9, respectively. Because of the high COD loading rate, the system has low hydraulic retention time (HRT) which means that microorganisms have a short time for digesting the organic compounds. On the other hand, at low COD loading rate, longer time was provided for biodegradation.

Table 4.1 The averaged effluent COD value and % COD removal at various COD loading rate with influent COD 22,000 mg/l

COD loading rate (kg COD/m^{3}d)	Average effluent COD value at equilibrium (mg/l)	Average percentage of COD removal at equilibrium
10	6,819	69.01
20	6,859	68.83
30	6,896	68.66
40	7,458	66.10
60	11,192	49.13
80	13,041	40.72

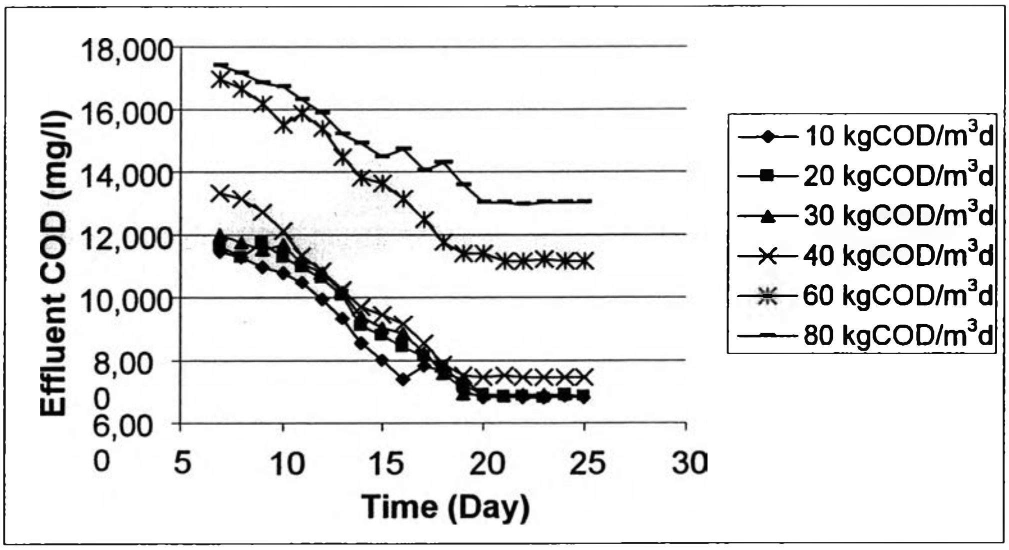

Figure 4.8 Effluent COD values with various COD loading rate and operation time.

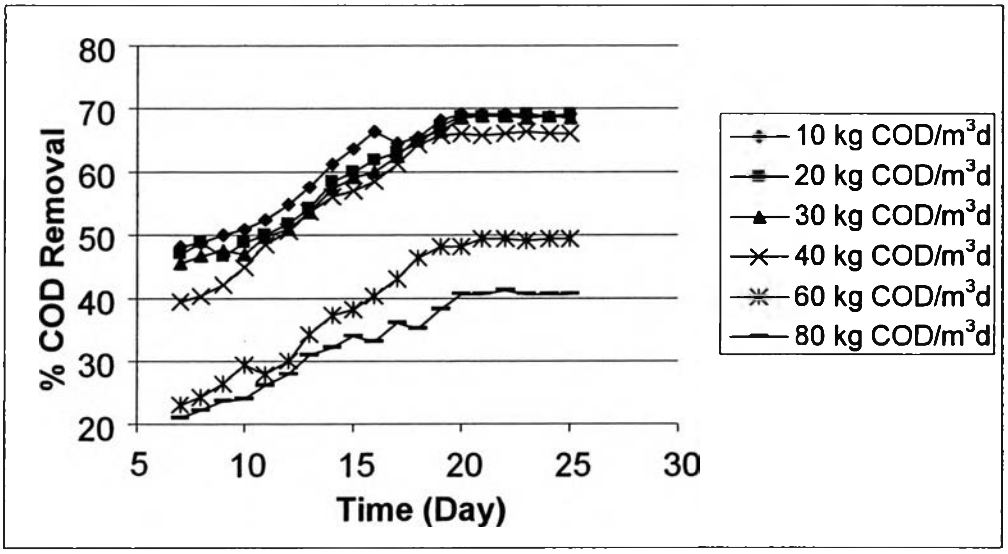

Figure 4.9 Percentage of COD removal with various COD loading rate and operation time.

4.3.2 Oil removal

In this part, oil removal was determined in terms of % oil removal. This method was also conducted to determine the degradation capability of the microbe in utilizing organic substances as their nutrients. Oil was extracted from the effluent by dichloromethane and then dichloromethane was evaporated at ambient condition for a day. The remaining oil was weighted and converted in terms of oil content in % v/v and after that compared with the 2% v/v from influent. The oil removal was determined every day until the system reach steady state and at this point the oil removal value was relatively unchanged. The results showed that at day 20 the oil removal began steady. After the system reached steady state, the percentage of oil removal of five more days were determined as an averaged value as show in Table 4.2. Effluent oil content and percentage of oil removal at different COD loading rate were shown in Figures 4.10 and 4.11, respectively. The results showed that the effluent oil content decreases with the increasing of operation time until the system reaches steady state and that the oil removal increases with the increasing of operation time. Moreover, when COD loading increases, the effluent oil content increases, corresponding with the decreasing of the oil removal with increasing COD loading as shown in Figures 4.10 and 4.11, respectively. Because of the high COD loading rate, the system has low hydraulic retention time (HRT) which means that microorganisms have a short time for digesting the organic compounds. On the other hand, at low COD loading rate, longer time was provided for biodegradation.

Table 4.2 The averaged effluent oil content (% v/v) and % oil removal at various COD loading rate with influent oil content of 2% v/v

COD loading rate (kg COD/m^{3}d)	Average oil content in effluent (% v/v)	Average percentage of oil removal at equilibrium
10	0.1962	90.19
20	0.2013	89.94
30	0.2215	88.93
40	0.2630	86.85
60	0.3086	84.57
80	0.4233	78.84

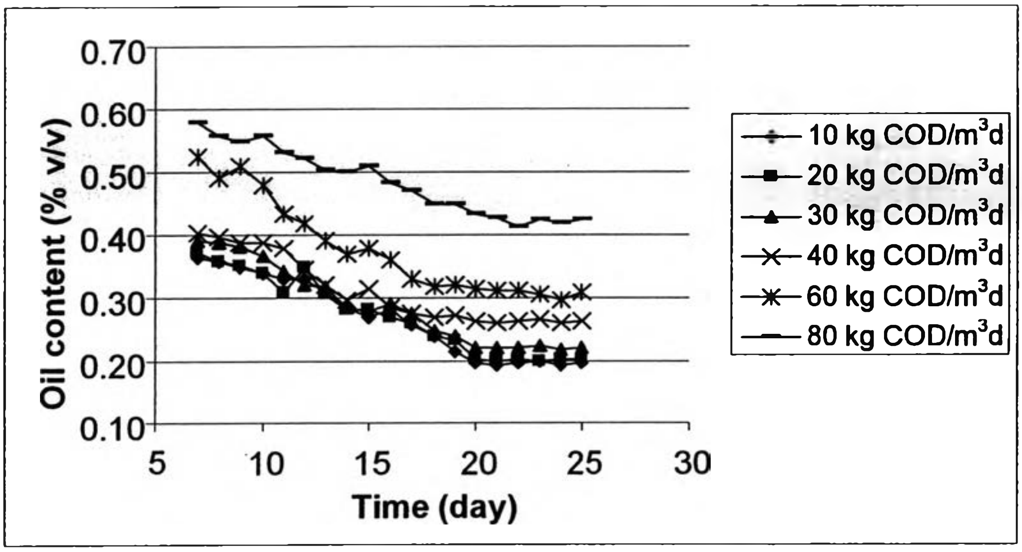

Figure 4.10 Effluent oil content with various COD loading rate and operation time.

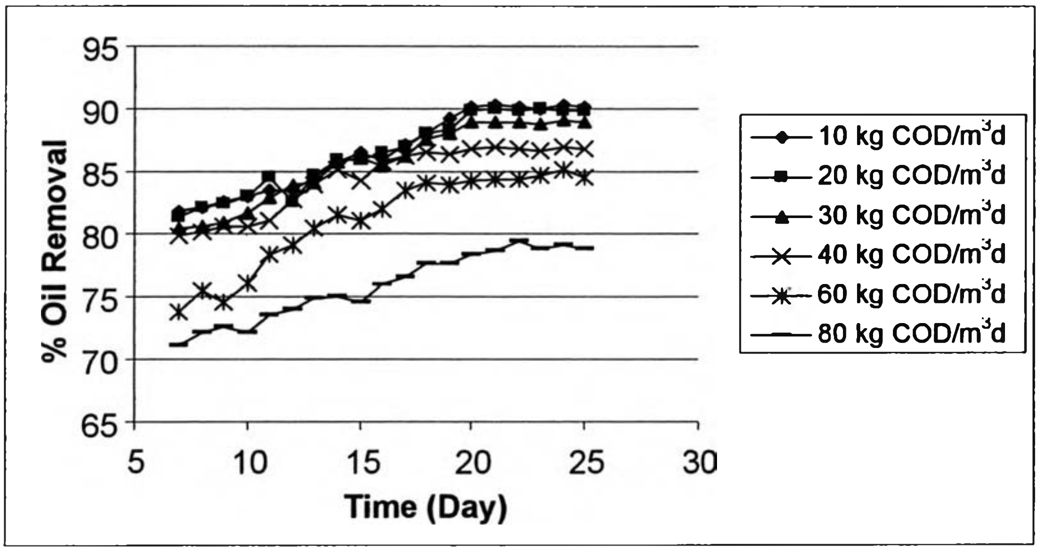

Figure 4.11 Percentage of oil removal with various COD loading rate and operation time.

4.3.3 Biogas production

Biogas production along the fermentation process in an UASB reactor was determined by gas meter and its composition was determined by GC with TCD. In this section, biogas production was determined in terms of specific biogas production rate in m^3 biogas/m^{3}d. After 20 day the system reach steady state, corresponding with COD and oil removal study. So in this section the biogas production was measured and determined at equilibrium. From Table 4.3 and Figure 4.12, at 20 kg COD/m^{3}d loading shows the highest specific biogas production at 2.21 m^{3}biogas/m^{3}d. From Figure 4.12, at low COD loading rate (10-20 kg COD/m^{3}d), specific biogas production rate increased with the increasing COD loading rate. After that at high COD loading rate (30-80 kg COD/m^{3}d) the specific biogas production rate was drop because influent flow rate is too high. Biogas content at different COD loading rate is shown in Figure 4.13 and the results showed that the highest composition was CO_2 follow by CH_4, and H_2, respectively (Yang *et al.*, 2007). The large amounts of CO_2 were produced because the type of feed which was distilled palm oil. The high number of carbon atoms in distilled palm oil provided large amount of CO_2 produced compared to glucose which has lower carbon atom in its structure. Therefore, H_2 production rate, CH_4 production rate, COD removal and oil removal were used as criteria for determining the optimum COD loading rate. With this criterion, the optimum COD loading rate was found to be 20 kg COD/m^3d as shown in Figure 4.14, 4.15, 4.16, and 4.17. At the optimum COD loading rate, 68.9% of COD removal and 89.9% of oil removal were presented. Moreover, 10.1% v/v of H_2 and 28.1% v/v of CH_4 were found. Therefore, the biogas yield in m^3/kg COD fed and m^3/kg COD removed were reported in Table 4.4. It was shown that the biogas yield in m^3/kg COD fed was highest at 20 kg COD/m^3d loading, corresponded with the specific biogas production rate in Table 4.3. However, the biogas yield in m^3/kg COD removed increased with COD loading rate because the higher COD loading rate the lower COD removal as shown in Table 4.1. So to remove the same amounts of COD from the influent, the system required the longer time for the higher COD loading rate. With the longer time requirement for high COD loading rate then the higher biogas accumulation in the system.

Table 4.3 Specific biogas production rate and content with various COD loading rate

COD loading rate (kg COD/m^3d)	Specific biogas production rate (m^3 biogas/m^3d)	Biogas content (% v/v)			Specific H_2 production rate (m^3 H_2/m^3d)	Specific CH_4 production rate (m^3 CH_4/m^3d)	Specific CO_2 production rate (m^3 CO_2/m^3d)
		H_2	CH_4	CO_2			
10	2.03	9.1	30.1	60.8	0.18	0.61	1.24
20	2.21	10.1	28.1	61.9	0.22	0.62	1.37
30	2.15	10.1	25.8	64.1	0.22	0.55	1.37
40	2.12	8.3	20.0	71.7	0.18	0.42	1.52
60	1.81	7.8	17.5	74.7	0.14	0.32	1.35
80	1.77	6.6	14.9	78.5	0.12	0.26	1.39

Table 4.4 Biogas yield with various COD loading rate

COD loading rate (kg COD/m^{3}d)	Biogas yield (m^3/kg COD fed)				Biogas yield (m^3/kg COD removed)			
	Biogas	H_2	CH_4	CO_2	Biogas	H_2	CH_4	CO_2
10	0.092	0.008	0.028	0.056	0.134	0.012	0.040	0.081
20	0.100	0.010	0.028	0.062	0.146	0.015	0.041	0.090
30	0.098	0.010	0.025	0.062	0.142	0.014	0.037	0.091
40	0.096	0.008	0.019	0.069	0.146	0.012	0.029	0.105
60	0.082	0.006	0.014	0.061	0.167	0.013	0.029	0.125
80	0.080	0.005	0.012	0.063	0.198	0.013	0.029	0.155

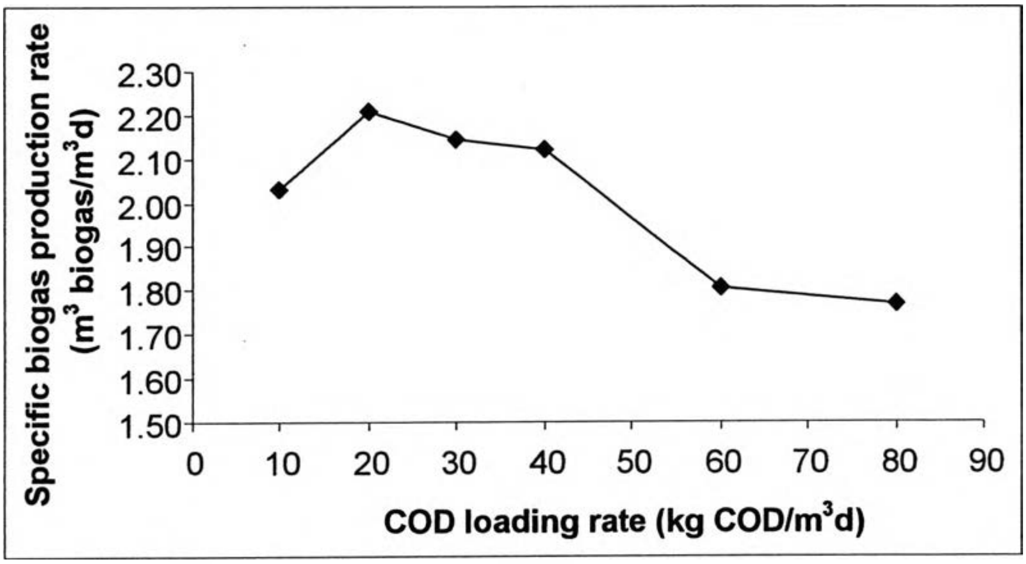

Figure 4.12 Effect of COD loading on biogas production.

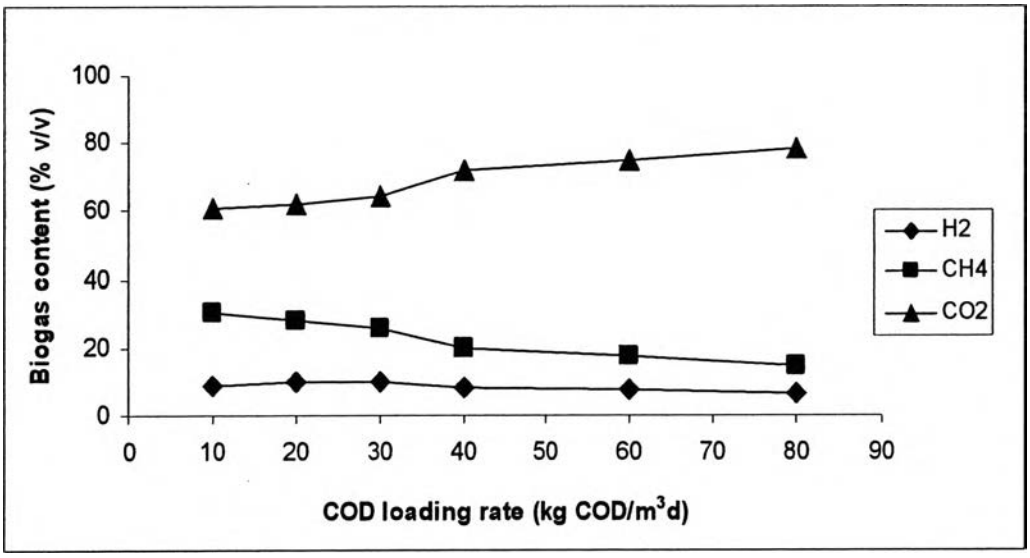

Figure 4.13 Biogas content with various COD loading rate.

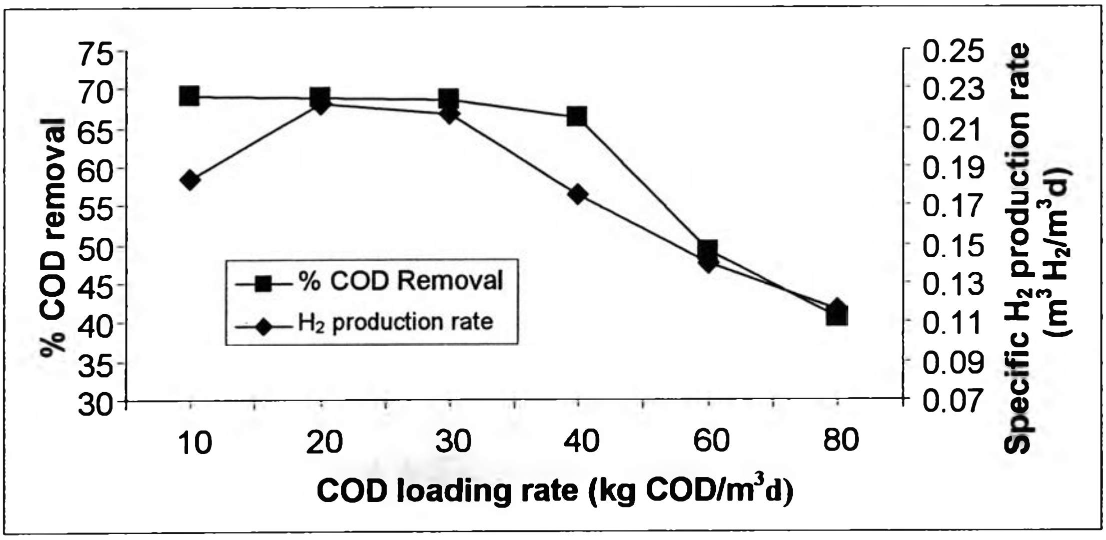

Figure 4.14 Percentage of COD removal and specific H_2 production rate with various COD loading rate.

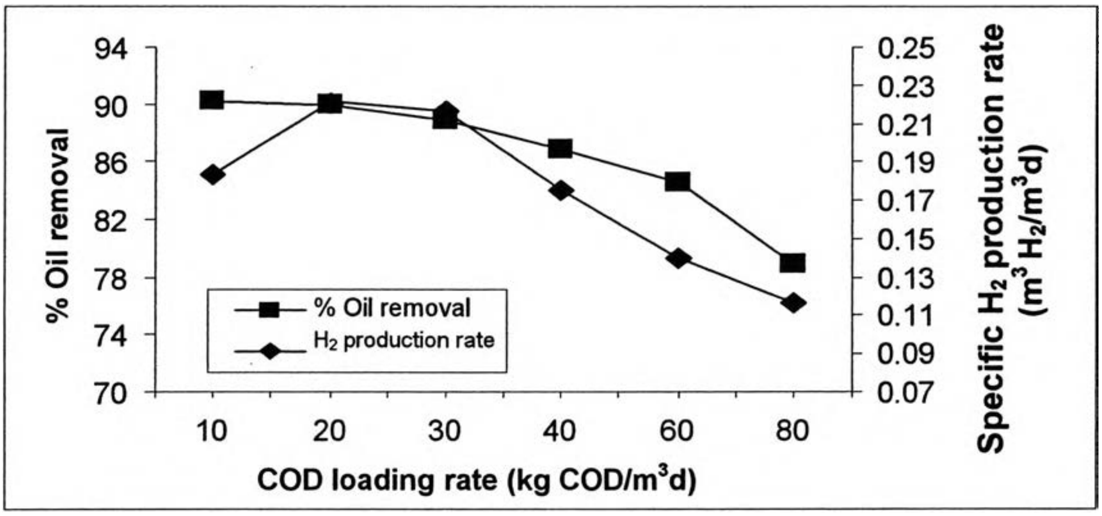

Figure 4.15 Percentage of oil removal and specific H_2 production rate with various COD loading rate.

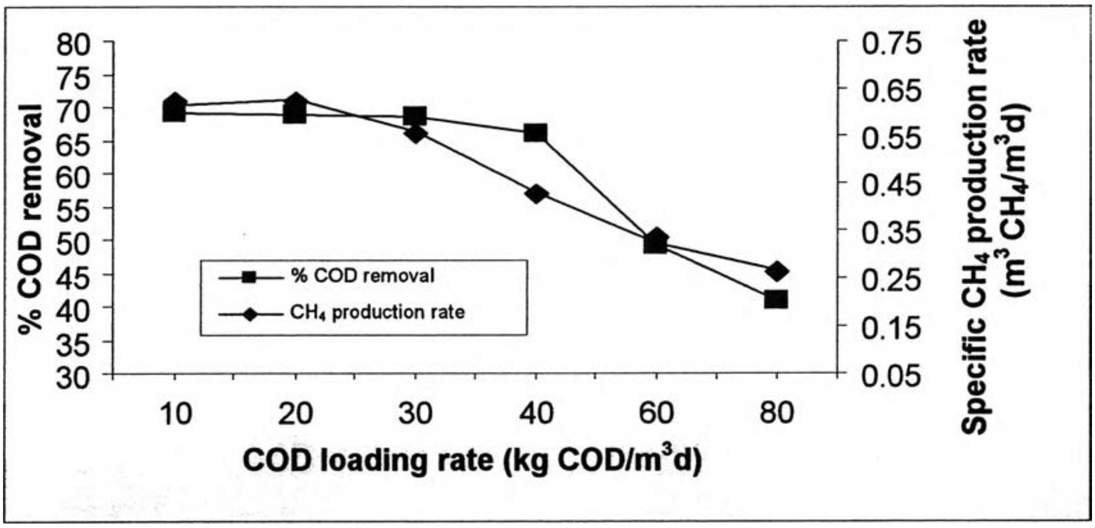

Figure 4.16 Percentage of COD removal and specific CH_4 production rate with various COD loading rate.

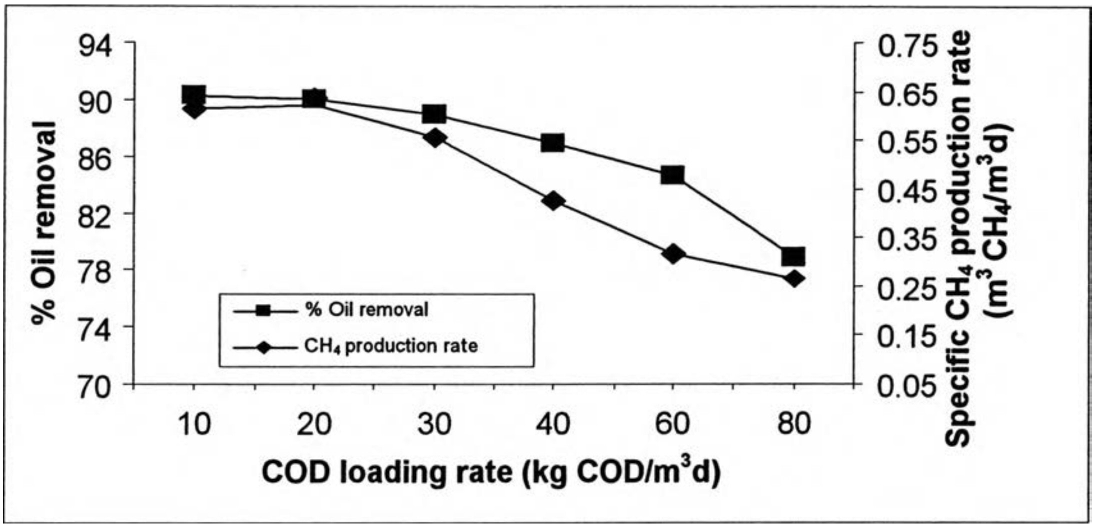

Figure 4.17 Percentage of oil removal and specific CH_4 production rate with various COD loading rate.

4.3.4 pH determination

pH or per hydron or per hydrogen, and also power of hydrogen is defined as the number arises from a measure of the activity of hydrogen ion or their equivalent. In this part, pH was determined every day until the system reach steady state and at this point the effluent pH was relatively unchanged. The results showed that at day 20 the pH began steady. At steady state the final pH was measured and it was found that the final pH decrease from 5.13 at 10 kg COD/m^{3}d loading to 4.29 at 40 kg COD/m^{3}d because of the formation of Volatile Fatty Acid or VFAs during the fermentation process (Morimoto *et al.,* 2004). But when COD loading rate was higher than 40 kg COD/m^{3}d, the final pH was increased because of the reactor was flooding and the microorganisms were washed out of the reactor because feed flow rate was too high.

4.3.5 VFAs determination

In anaerobic fermentation process, not only biogas was generated as a by-product but soluble metabolites were also produced. Figure 4.20 shows the VFAs concentration in mg as acetic acid per liter at different COD loading rate. The results showed proportional relation between VFAs concentration and COD loading rate at 10–40 kg COD/m^{3}d loading. But at 60 and 80 kg COD/m^{3}d loading VFAs concentration was decrease corresponding with the final pH in Figure 4.19. Moreover, Chromatographic analysis was used to determine the distribution of VFAs and alcohol in % v/v. It was found that acetic acid and butyric acid were found to be the major soluble metabolites (Zhang *et al.*, 2006) as shown in Table 4.4. The large amount of propionic acid as shown in Table 4.5 can be used to explain why small amounts of H_2 were detected in this research. The production of propionic acid was not favor for biohydrogen production because the consumption of H_2 during the production of propionic acid (Zhang *et al.*, 2006) as shown below.

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$$

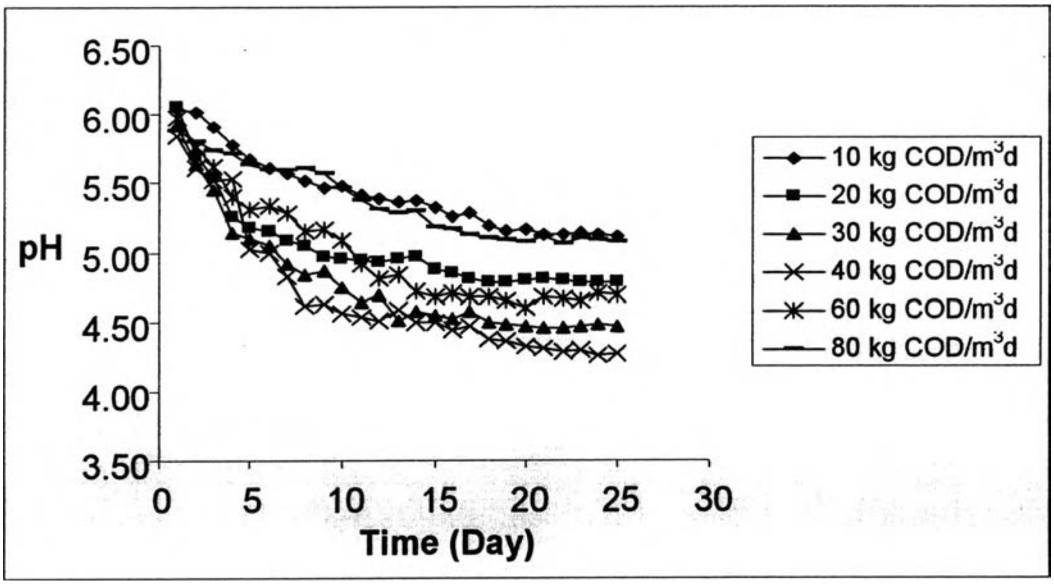

Figure 4.18 Effluent pH with various COD loading rate and operation time.

Table 4.5 The distribution of VFAs and ethanol concentration in % v/v from the effluent at various COD loading rate.

COD loading rate (kg COD/m^3d)	Final pH	Concentration (% v/v)				
		Acetic acid	Butyric acid	Propionic acid	Valeric acid	Ethanol
10	5.11	32.9	37.1	25.8	4.2	0
20	4.78	43.8	32.5	18.6	3.4	1.7
30	4.47	39.5	34.8	22.6	2.2	0.9
40	4.27	35.8	37.2	21.6	5.4	0
60	4.67	33.2	36.9	24.1	4.4	1.4
80	5.08	32.8	38.7	25.7	2.8	0
20 without surfactant	4.80	39.9	32.6	22.8	3.3	1.4

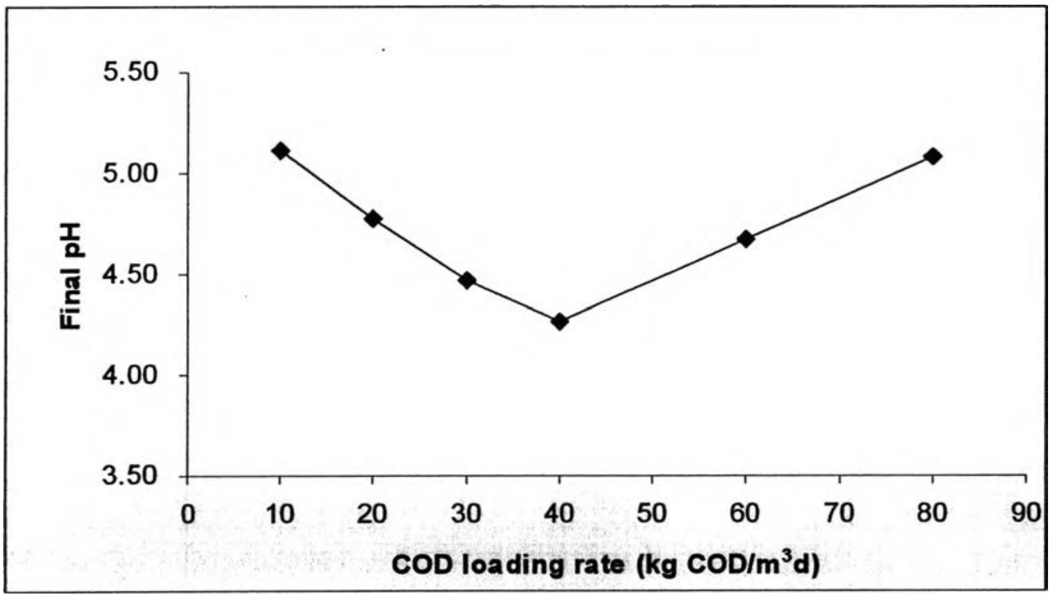

Figure 4.19 Final pH with various COD loading rate.

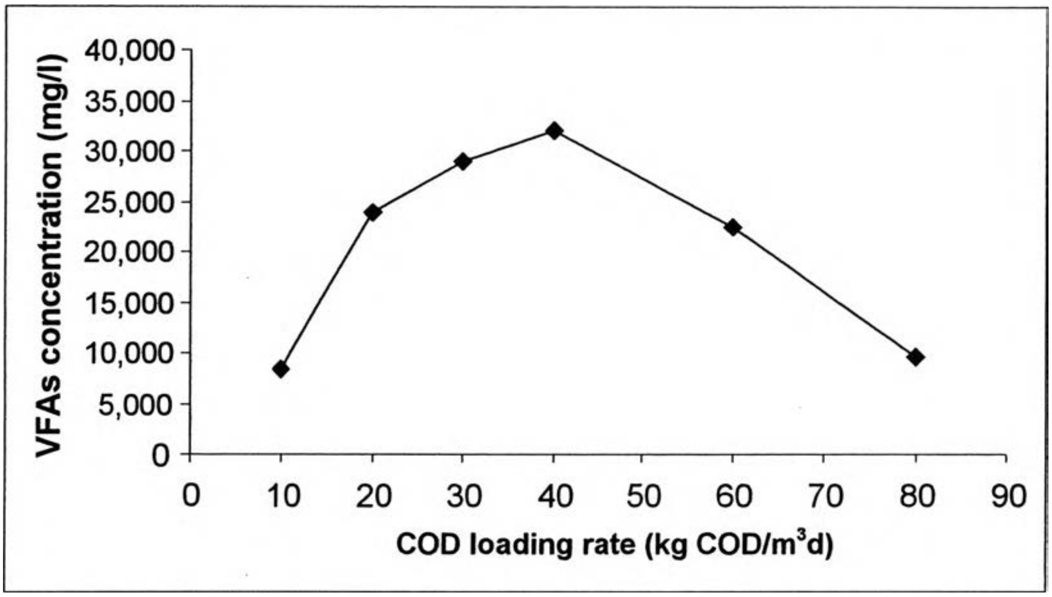

Figure 4.20 VFAs concentration with various COD loading rate at steady state.

4.3.6 Microbial growth

Under the UASB operation for treating oily wastewater, wastewater sludge was applied to the reactor with a concentration of 20 g total suspended solids (TSS) per liter. The microbial growth is the parameter for determining the capability of microorganism for growing and degrading the organic compounds presence in the reactor. So it is important to examine the microbial growth. In this part of the study, the microbial growth was examined in terms of mixed liquor suspended solids (MLSS) in g of suspended solids per liter at the steady state of the operation (at day 25). Figure 4.21 shows the effect of COD loading rate on MLSS. The results showed that at low COD loading rate, MLSS was increased because of the growth of micro-organisms. On the other hand, at high COD loading rate the decreasing of MLSS was found because the reactor was folding and the microorganisms were washed out of the reactor because feed flow rate was too high.

After determining the final pH, VFAs concentration, and MLSS with various COD loading rate, it was found that these parameters were related. At COD loading rate of 10-40 kg COD/m^{3}d, the final pH was decreased due to the formation of VFAs corresponding with the increasing of MLSS in the low COD loading rate range. But when COD loading rate was higher than 40 kg COD/m^{3}d, the final pH, was increased corresponding with the decreasing of VFAs concentration and MLSS as shown in Figure 4.19, 4.20, and 4.21, respectively. Because the higher COD loading rate the larger amounts of VFAs were produced until the microbial limitation. It is means that the microbial can be survived at some VFAs concentration because the VFAs in form of free VFAs can easily diffuse to the microbial cells and also damage the microbial cells. So at high COD loading rate and also high VFAs concentration which were higher than the microbial threshold for surviving, the MLSS was dropped. Another reason was with the high COD loading rate the microbial cells were easily washed out from the reactor because feed flow rate was too high.

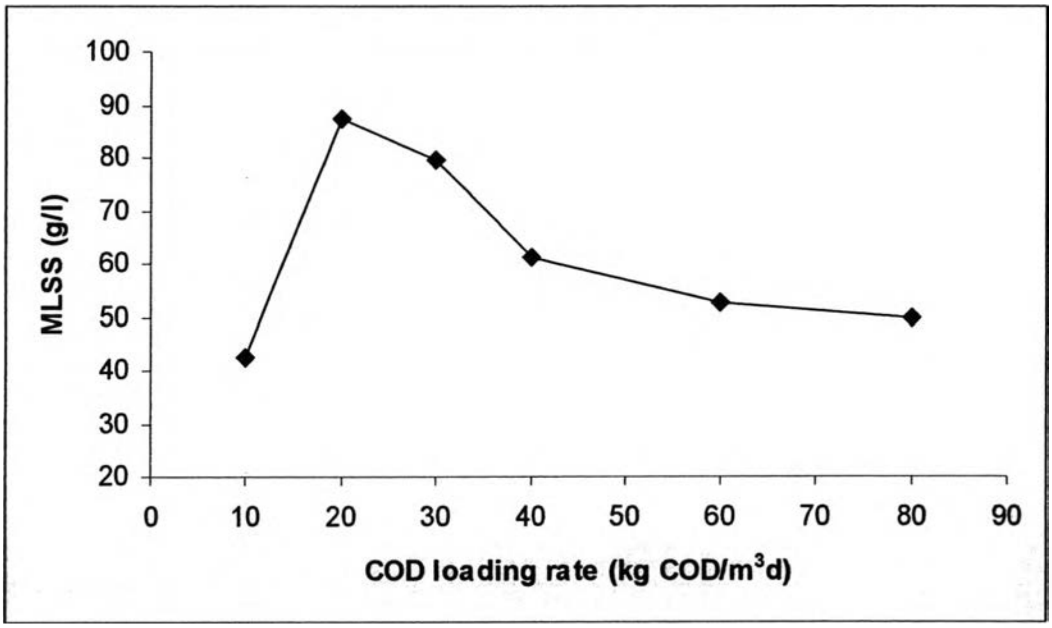

Figure 4.21 MLSS with various COD loading rate at steady state.

4.4 Effect of surfactant on biodegradation and biogas production

In this section, the UASB reactor was also operated at 37°C and uncontrolled pH. The effect of surfactant was determined at the optimum COD loading rate (20 kg COD /m^{3}d) with 0.10% w/v of surfactant (which is equivalent to a weight ratio of oil to surfactant of 18.8:1) and without surfactant. Surfactant is an amphipathic agent which is proposed to enhance oil solubilization, and thus the biodegradation, as well as the biohydrogen production. COD removal, oil removal, specific biogas production rate, biogas content, biogas yield, pH, VFAs concentration, and MLSS were also the main parameters observed in this section. The results are shown in Table 4.5 and they show that with the addition of surfactant, the COD removal percentage, oil removal percentage, specific biogas production rate, and specific H_2 production rate were higher than without surfactant. Therefore, the increase these parameters confirms that the surfactant can enhance oil biodegradation and biogas production,

Table 4.6 Parameters observed for studying effect of surfactant on biodegradation and biogas production.

Parameters	20 kg COD /m^{3}d with 0.10% w/v of surfactant (wt. ratio of oil to surfactant of 18.8:1)	20 kg COD /m^{3}d without surfactant
Effluent COD (mg COD/l)*	6,858.50	12,702.43
COD removal COD (%)*	68.9	42.3
Effluent oil concentration (% v/v)*	0.2013	0.8198
Oil removal (%)*	89.9	59.0
Specific biogas production rate (m^3 biogas/m^{3}d)**	2.21	1.44
Specific H_2 production rate (m^3 H_2/m^{3}d)**	0.22	0.14
Specific CH_4 production rate (m^3 CH_4/m^{3}d)**	0.62	0.40
Biogas yield (m^3 biogas/kg COD fed)**	0.100	0.065
Biogas yield (m^3 biogas/kg COD removed)**	0.146	0.1549
H_2 content**	10.1	9.9
CH_4 content**	28.1	27.6
Final pH*	4.80	4.97
VFAs concentration (mg/l)**	23,807	12,054
MLSS (g/l)**	87.34	51.46

*The averaged value at steady state (day 20-25)

**The value conducted at steady state (day 25)