CHAPTER IV
RESULTS AND DISCUSSION

4.1 Characterization of Fish Steaming Waste, Diluted Fish Steaming Waste and Mineral Medium with Different Oil Loading Rates

Different nutrient sources such as fish steaming waste (FSW), diluted fish steaming waste (FSW) and mineral medium (MM) were screened and characterized for biosurfactant production in the SBRs. The composition of FSW depends on the type of fish and fish-steaming procedure. FSW used in this work was originated from canned fish industry and collected through filter cloth. It had a distinctly high COD of 33,333 mg/l, total nitrogen (TN) of 4,767 mg/l, total phosphorus (TP) of 457 mg/l, total suspended solids (TSS) of 878 mg/l, total organic carbon (TOC) of 25,107 mg/l, and surface tension of 41 mN/m with a dark brown colour (data shown in Table A1). Characterization of the non-diluted FSW at an oil loading rate of 2 kg/m^3d is presented in Table 4.1. The results show that biosurfactants were not present at significant levels in the FSW feed. However, the initial lower surface tension (41 mN/m) of the FSW feed might be due to colloidal aggregation of proteins and particulate material.

FSW was diluted in a 1:60 proportion with tap water. Diluted FSW had low COD of 377 mg/l, total nitrogen of 79 mg/l, total phosphorus of 8 mg/l, TOC of 466 mg/l, and TSS of 65 mg/l (Table A2). The surface tension and surfactant concentrations were 66 mN/m and below their CMC, respectively which showed that biosurfactants were not presented at measurable levels in the diluted FSW. The results of characterization of diluted FSW feed at oil loading rates (OLRs) of 2 and 8 kg/m^3d are illustrated in Table 4.2. The C:N and C:P ratios were 5 and 55, respectively, thus indicated that the diluted FSW feed contained high amounts of nitrogen but lesser amounts of phosphorus, as compared to mineral medium feed (Table 4.3). However, these ratios in the diluted FSW feed were operated without adding nutrients such as nitrogen and phosphorus, which would have the operation costs.

Table 4.1 Characterization of the non-diluted fish steaming waste feed at an oil loading rate of 2 kg/m^{3}d for the SBR during a cycle

Parameters	Non-diluted FSW at an oil loading rate of 2 kg/m^{3}d
COD (mg/l)	37,011
Total nitrogen (mg/l)	4,744.3
Total phosphorus (mg/l)	454.7
TOC (mg/l)	25,222.1
C:N ratio	5.32
C:P ratio	55.46
Oil concentration (mg/l)	6,000
Total suspended solids (mg/l)	872
Surface tension (mN/m)	40.80
Surfactant concentration (xCMC)	0

Table 4.2 Characterization of the diluted fish steaming waste feed with different oil loading rates for the SBRs during a cycle

Parameters	Oil loading rate (kg/m^{3}d)	
	2	8
COD (mg/l)	4,287	16,018
Total nitrogen (mg/l)	89.5	121.6
Total phosphorus (mg/l)	8.8	12.5
TOC (mg/l)	754.2	1,619.8
C:N ratio	8.4	13.3
C:P ratio	85.9	129.6
Oil concentration (mg/l)	6,000	24,000
Total suspended solids (mg/l)	65	63
Surface tension (mN/m)	65.93	65.93
Surfactant concentration (xCMC)	0	0

Table 4.3 lists the characterization of the influent mineral medium (MM) to the SBRs with four OLRs (1, 2, 6, 10 kg/m^{3}d). $NaNO_3$, K_2HPO_4, and KH_2PO_4 were supplied to maintain C:N and C:P ratios of 16:1 and 14:1, respectively, which are the optimum ratio for rhamnolipid accumulation by *Pseudomonas aeruginosa* (Guerra-Santos *et al.*, 1984). COD, oil concentration, TOC, and TSS of MM were all practically zero (data listed in Table A3). Thus, the culture could only utilize COD and oil from palm oil. No biosurfactant in MM was expressed by surface tension of 67-68 mN/m and surfactant concentrations below their CMC.

Table 4.3 Characterization of the mineral medium (MM) feed with different oil loading rates for the SBRs during a cycle

Parameters	Oil loading rate (kg/m^{3}d)			
	1	2	6	10
COD (mg/l)	1,956	3,913	11,739	19,565
Total nitrogen (mg/l)	9.1	18.2	54.7	91.2
Total phosphorus (mg/l)	10.4	20.8	62.5	104.2
TOC (mg/l)	145.9	291.8	875.5	1,459.1
C:N ratio	16:1	16:1	16:1	16:1
C:P ratio	14:1	14:1	14:1	14:1
Oil concentration (mg/l)	3,000	6,000	18,000	30,000
Total suspended solids (mg/l)	0	0	0	0
Surface tension (mN/m)	67.20	67.60	67.20	68.20
Surfactant concentration (xCMC)	0	0	0	0

4.2 Effect of Nutrient Source on Biosurfactant Production

4.2.1 Non-diluted Fish Steaming Waste Feed

In order to investigate whether non-diluted FSW could be used as a nutrient source for biosurfactant production, effluent COD was measured at a given of oil loading rate (2 kg/m^{3}d). The results are presented in Figure 4.1. The SBR system had reached steady state conditions after 9 day's operation because COD in effluent measurements did not vary noticeably after 9 days. This period provide more than 3 retention times for steady state conditions to be reached in the reactors (Cassidy *et al.*, 2002). An effluent COD quickly reduced to 19,700 mg/l up to day 5, leading to removal efficiencies as high as 47%. After 5 days, the COD removal reduced to 29% and steadily remained to reach steady state period. During this period (days 11-13), an effluent COD was 26,333 mg/l, which was very high, leading to a COD removal as low as 29%. This indicated that palm oil was not efficiently utilized as carbon source for biosurfactant production by the culture due to high COD concentration of 37,011 mg/l in the feed. This resulted in FSW utilization as a nutrient source for the growth and proliferation of the culture, instead of palm oil utilization. Furthermore, the SBR system with non-diluted FSW was difficult to handle at high COD loading because colloidal aggregation of proteins and culture were observed on the top of liquid in the SBR system as shown in Figure 4.2, leading to aeration stop to avoid overflow of the liquid outside the foam collector bottle. Thus, fish steaming waste could not directly serve as a good nutrient source without dilution for biosurfactant production.

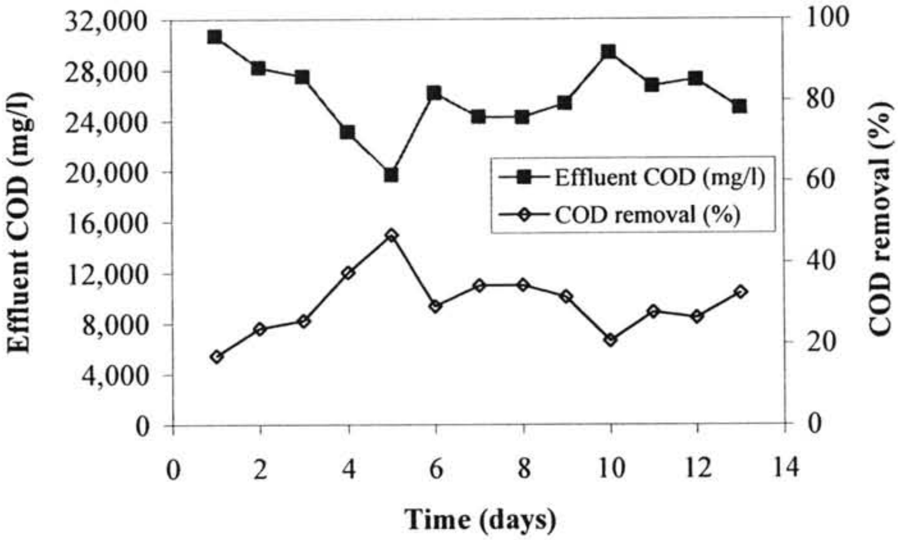

Figure 4.1 Effluent COD and COD removal with time on days 1-13 of operation in the SBR at an oil loading rate of 2 kg/m^3d with non-diluted FSW feed and 1-d cycle.

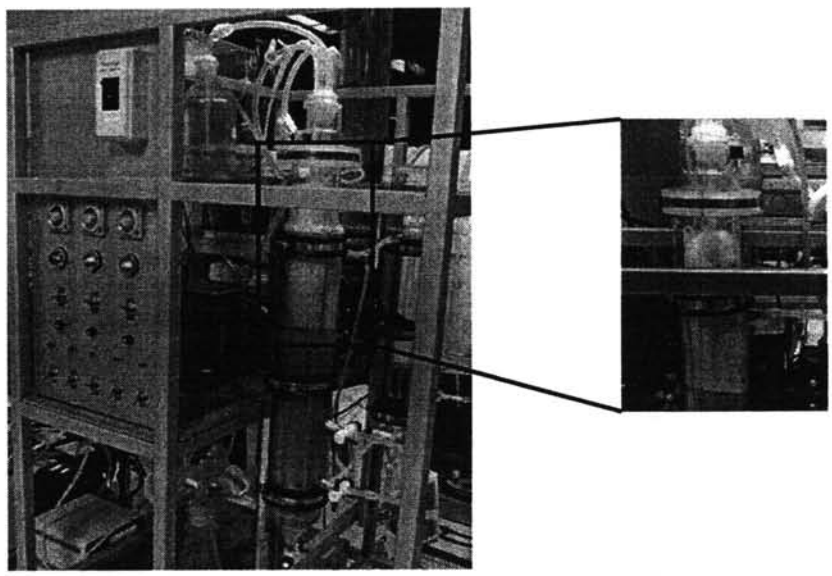

Figure 4.2 Sequencing batch reactor (SBR) with non-diluted FSW feed.

4.2.2 Diluted Fish Steaming Waste Feed

From the above-mentioned problems with non-diluted FSW, FSW was diluted in a 1:60 proportional with tap water and COD of FSW then reduced to a value of 377 mg/l. To investigate whether this diluted FSW could be used as an efficient nutrient source, COD and oil removal, surface tension reduction was measured with effluent TSS and MLSS at two oil loading rate (2, 8 kg/m^{3}d) and 1-d cycle. The results of effluent COD and COD removal are plotted in Figure 4.3 and 4.4, respectively. They are evident that steady state period was reached within 9 days. At an oil loading rate (OLR) of 8 kg/m^{3}d, with every one hydraulic retention time (3 days), the effluent COD fluctuated within the range of 2,850 to 9,387 mg/l, resulting in a COD removal fluctuated until steady state conditions to be reached. This caused by the overload of COD in feed (both nutrient and palm oil) and limitation of the culture for the uptake of palm oil at the high COD feed. Cycle time more than a day might be required to utilize organic substances by the culture at the high oil loading rate. At an OLR of 2 kg/m^{3}d, an effluent COD gradually decreased to approximately 850 mg/l in the first 6 days of operation due to a remaining COD of 26,333 mg/l from last condition (an OLR of 2 kg/m^{3}d with non-diluted FSW). Thereafter, an effluent COD and a COD removal stabilized throughout the rest of 12 days of operation.

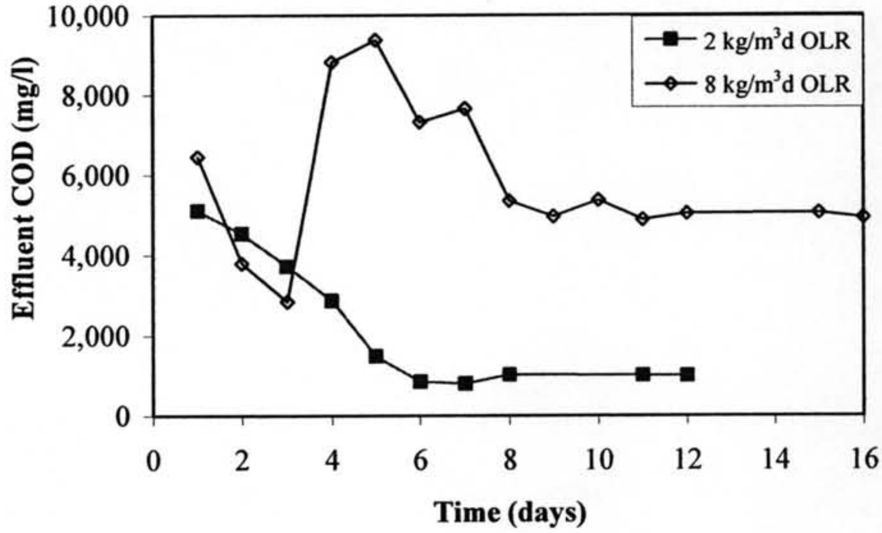

Figure 4.3 Effluent COD with operation time in the SBRs at oil loading rates of 2 (days 1-12) and 8 kg/m^{3}d (days 1-16) with diluted FSW feed and 1-d cycle.

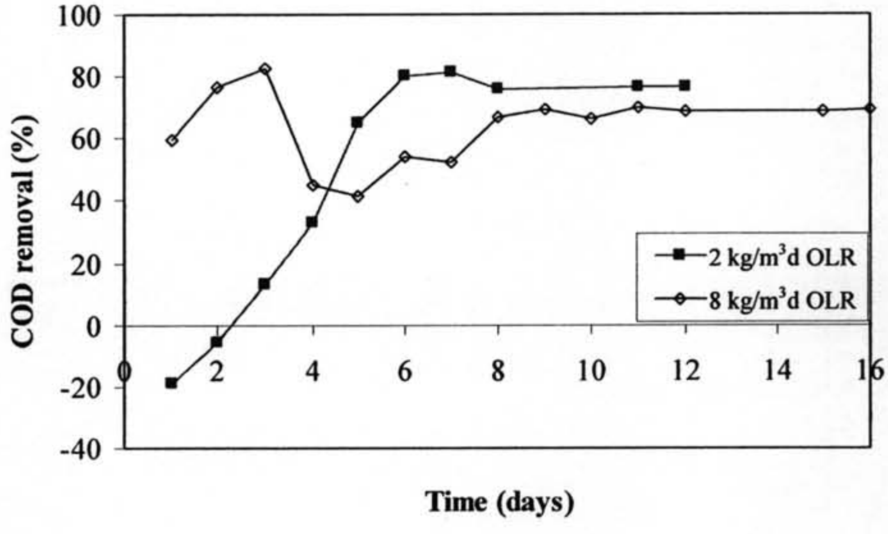

Figure 4.4 COD removal with operation time in the SBRs at oil loading rates of 2 (days 1-12) and 8 kg/m^{3}d (days 1-16) with diluted FSW feed and 1-d cycle.

COD, surface tension, and oil concentration in the effluent during steady state operation are depicted in Figure 4.5. The drop in surface tension to 28.5 and 29.3 mN/m at OLRs of 2 and 8 kg/m^{3}d, respectively are indicative of biosurfactant production. According to Cassidy (2001) and Cassidy *et al.* (2002), although typical metabolic products (e.g., organic acids) present at concentration above 10%, they are not able to reduce surface tension to 30 mN/m. In compare to an oil loading rate of 8 kg/m^{3}d, a higher surface tension reduction of 57% at an OLR of 2 kg/m^{3}d was observed corresponding to higher COD removal and oil removal of 76% and 92%, respectively as shown in Figure 4.6. However, a COD removal as low as 76% at an OLR of 2 kg/m^{3}d is attributed to the overload of COD in feed (4,287 mg/l), which indicated that oil was not efficiently utilized to produce biosurfactants by the culture. This problem of low COD removal could be improved by means of the use of zero organic content in a nutrient source (i.e., mineral medium) in order to utilize only COD and oil from palm oil.

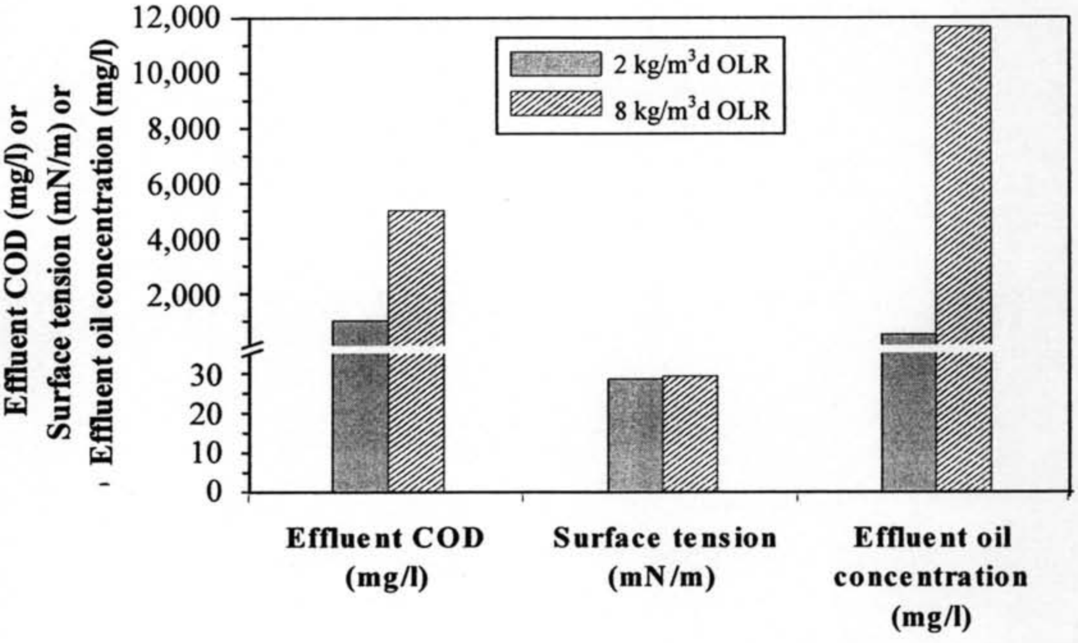

Figure 4.5 Effluent COD, surface tension, and effluent oil concentration during steady state operation in the SBRs at oil loading rates of 2 (days 8-16) and 8 (days 12-16) kg/m^{3}d with diluted FSW feed and 1-d cycle.

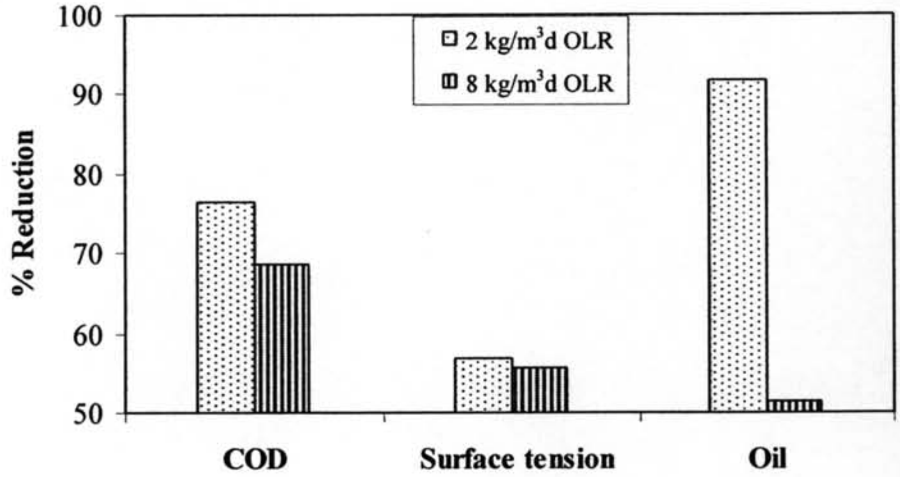

Figure 4.6 COD, surface tension, and oil reduction during steady state operation in the SBRs at oil loading rates of 2 (days 8-16) and 8 kg/m^3d (days 12-16) with diluted FSW feed and 1-d cycle.

The results presented in Figure 4.7 show that diluted FSW was a good nutrient source for growth and multiplication of the culture. The total suspended solids (TSS) increased from an initial feed of 63-65 mg/l to 349 mg/l and 5,498 mg/l at OLRs of 2 and 8 kg/m^3d, respectively at steady state period of operation. The mixed liquor suspended solids (MLSS) also increased to steady state value of 747 mg/l and 5,667 mg/l at OLRs of 2 and 8 kg/m^3d, respectively, which were much higher than those of feed. The high effluent TSS relative to MLSS indicated high cells wash-out for both the OLRs. However, fast-growing microorganisms (i.e., *Pseudomonas aeruginosa* SP4) could compensate the net cells loss. In 2006, Paisanjit reported the maximum growth of *Pseudomonas aeruginosa* SP 4 at 22 h. Higher MLSS in the case of an OLR of 8 kg/m^3d are attributed to higher levels of nitrogen and phosphorous, which preferred faster cell proliferation relative to an OLR of 2 kg/m^3d. Although the higher nitrogen concentrations enhanced the bacterial growth, an overproduction of rhamnolipids took place under nitrogen limiting conditions in the medium (Dubey and Juwarkar, 2001; Benincasa *et al.*, 2002; Zulfiqar *et al.*, 2006).

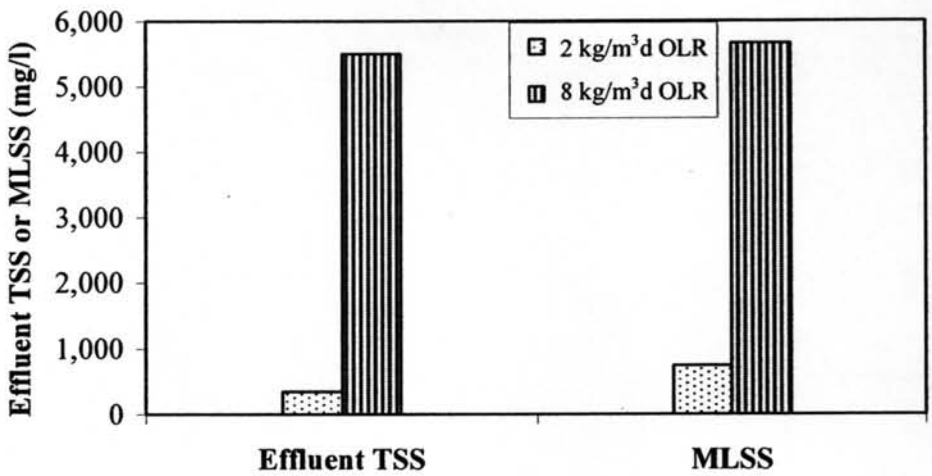

Figure 4.7 Effluent TSS and MLSS during steady state operation in the SBRs at oil loading rates of 2 (days 9-11) and 8 (days 13-15) kg/m^{3}d with diluted FSW feed and 1-d cycle.

4.2.3 <u>Comparison of Diluted Fish Steaming and Mineral Medium Feed</u>

Diluted FSW and mineral medium (MM) were evaluated as possible nutrient sources for biosurfactant production by *Pseudomonas aeruginosa* SP 4 at an OLR of 2 kg/m^{3}d. Figure 4.8 and 4.9 represent the effluent COD and COD removal, respectively at a given of OLR (2 kg/m^{3}d) with diluted FSW and mineral medium. The time required to reach steady state COD levels was considerably shorter with mineral medium (1 day) compared with diluted FSW (6 days). The effluent COD with mineral medium decreased from an initial feed of 3,913 mg/l to 517 mg/l within the first day of operation, where it remained stable throughout 13 days of operation.

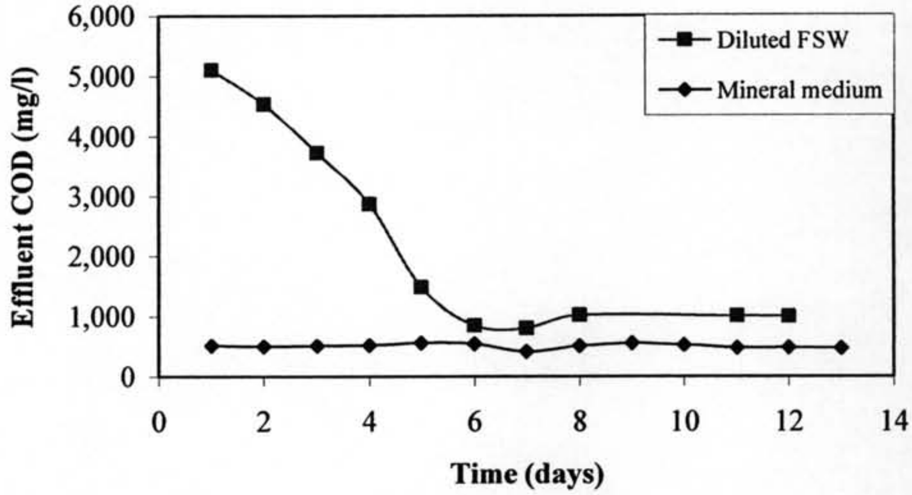

Figure 4.8 Effluent COD with operation time in the SBRs at an oil loading rate of 2 kg/m^3d with diluted FSW (days 1-12) and mineral medium (days 1-13) feed, and 1-d cycle.

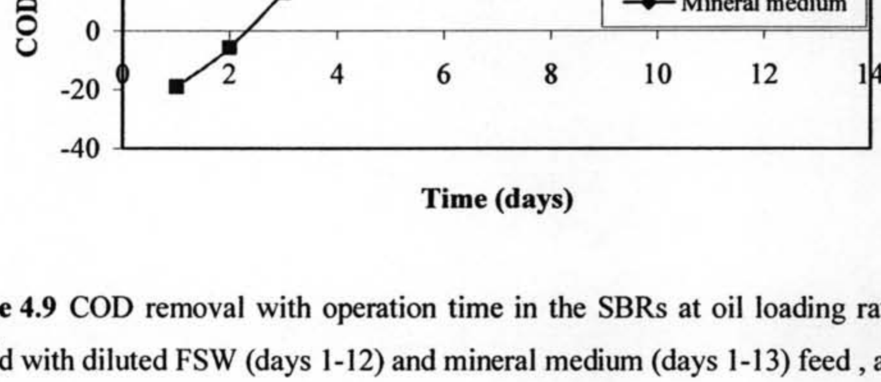

Figure 4.9 COD removal with operation time in the SBRs at oil loading rate of 2 kg/m^3d with diluted FSW (days 1-12) and mineral medium (days 1-13) feed , and 1-d cycle.

The effluent COD and oil concentrations, and surface tension with mineral medium were respectively less than 477 and 363 mg/l, and 28.1 mN/m with diluted FSW of 1,012 mg/L COD, 500 mg/L oil and 28.5 mg/L surface tension (Figure 4.10). Among two nutrient sources tested, a surface tension was efficiently reduced by 58% with mineral medium, corresponding to a COD removal of 88% and an oil removal of 94%, while diluted FSW gave the surface tension reduction of 57%, corresponding to a COD removal of 76% and an oil removal of 92% (Figure 4.11). With diluted FSW, although the SBR was able to achieve high oil removal, but a low COD removal obtained was 76% due to a high influent COD 377 mg/l in diluted FSW as compared to zero value of COD in mineral medium. This also resulted in the higher extent of oil removal from the mineral medium. Interestingly, a similar surface tension reduction was obtained with both nutrient sources, showing that diluted FSW has similar potential for biosurfactant production to mineral medium.

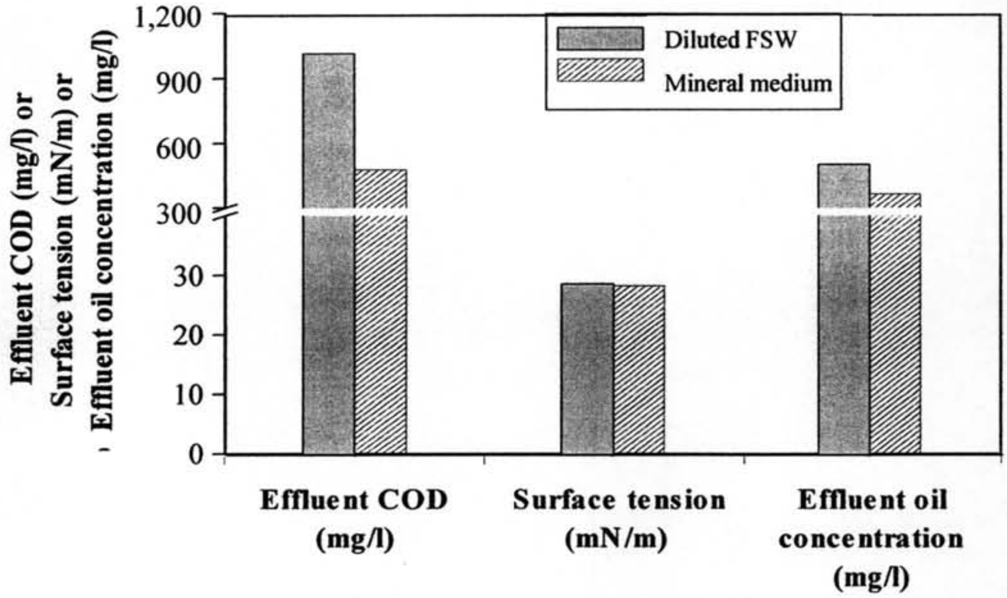

Figure 4.10 Effluent COD, surface tension, and effluent oil concentration during steady state operation in the SBRs with diluted FSW (days 8-12) and mineral medium (days 11-13) for oil loading rate of 2 kg/m^3d and 1-d cycle.

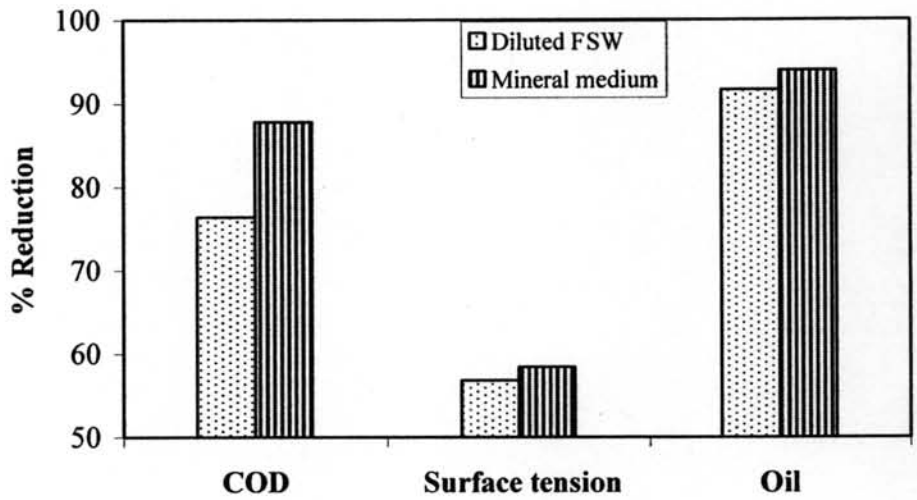

Figure 4.11 COD, surface tension, and oil reduction during steady state operation in the SBRs at an oil loading rate of 2 kg/m^{3}d with diluted FSW (days 8-12) and mineral medium (days 11-13), and 1-d cycle.

Results presented in Figure 4.12 show that mineral medium was found to be the best nutrient source for growth and proliferation of the culture, as the steady state MLSS of 928 mg/l was achieved, because of suitable C:N and C:P ratios of 16:1 and 14:1, respectively. For maximum biosurfactant production, nutrient source for the growth of culture should has optimum carbon, nitrogen, phosphorus and iron concentrations. In 1984, Guerra-Santos *et al.* revealed that the maximum rhamnolipid production was reached after nitrogen limitation at a C:N ratio of 16:1 to 18:1 and no surfactant production below a C:N ratio of 11:1, where the culture was not nitrogen limited. They also showed a C:P ratio below 16:1 and a C:Fe ratio of 72,400:1 provided the maximum rhamnolipid production. Dilution of FSW reduced its COD, nitrogen, phosphorus and proteins of FSW by sixty times. However, although diluted FSW was rich in other mineral contents and nitrogen, it was deficient in phosphorus. This leaded to lower proliferation of the culture in diluted FSW as compared to mineral medium. Nutritional components of diluted FSW were not efficiently utilized for the culture build-up and biosurfactant production. Hence, inorganic phosphorus sources, i.e. KH_2PO_4 and K_2HPO_4, were added to the diluted FSW to yield the maximum biosurfactant production.

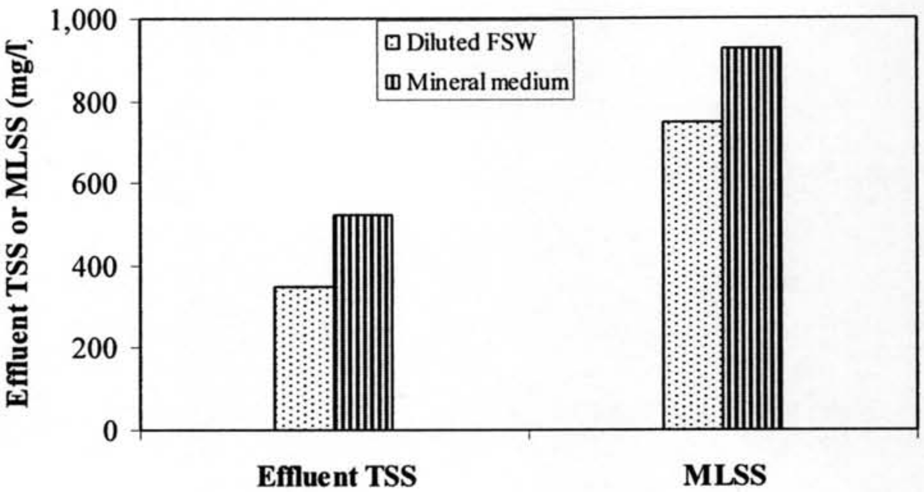

Figure 4.12 Effluent TSS and MLSS during steady state operation in the SBRs at an oil loading rate of 2 kg/m^{3}d with diluted FSW (days 9-11) and mineral medium (days 11-13), and 1-d cycle.

4.3 Effect of Oil Loading Rate with Mineral Medium on Biosurfactant Production

Because the SBR system with mineral medium was easier to handle as compared to diluted FSW, the effect of OLR on biosurfactant production was investigated with four OLRs (i.e., 1, 2, 6, and 10 kg/m^{3}d) and 1-d cycle by using mineral medium as a nutrient source. Suitable OLRs were selected on the basis of reduction of surface tension of mineral medium, and COD and oil removal in the effluent. The results of effluent COD and COD removal during operation with four OLRs are presented in Figure 4.13 and 4.14. The effluent COD and COD removal fluctuated considerably at high OLRs of 6 and 10 kg/m^{3}d throughout the first 9 days of operation and the effluent COD subsequently maintained at stable levels of approximately 4,775 and 9,688 mg/l at OLRs of 6 and 10 kg/m^{3}d, respectively, which were considerably high. These can be attributed to the overload of COD in feed (both nutrient and palm oil) and the uptake of palm oil at the high COD feed by the culture was limited. Therefore, the cycle time required to utilize organic substances was longer than a day at the high oil loading rate. In contrast, the low effluent COD of 514 and 517 mg/l, and the COD removal as high as 74% and 88% at low OLRs of 1 and 2 kg/m^{3}d, respectively were obtained within the first day after operation which later on remained stable until the last day of operation. These demonstrated that most of organic carbons were quickly utilized within 1 day at low OLR in feed.

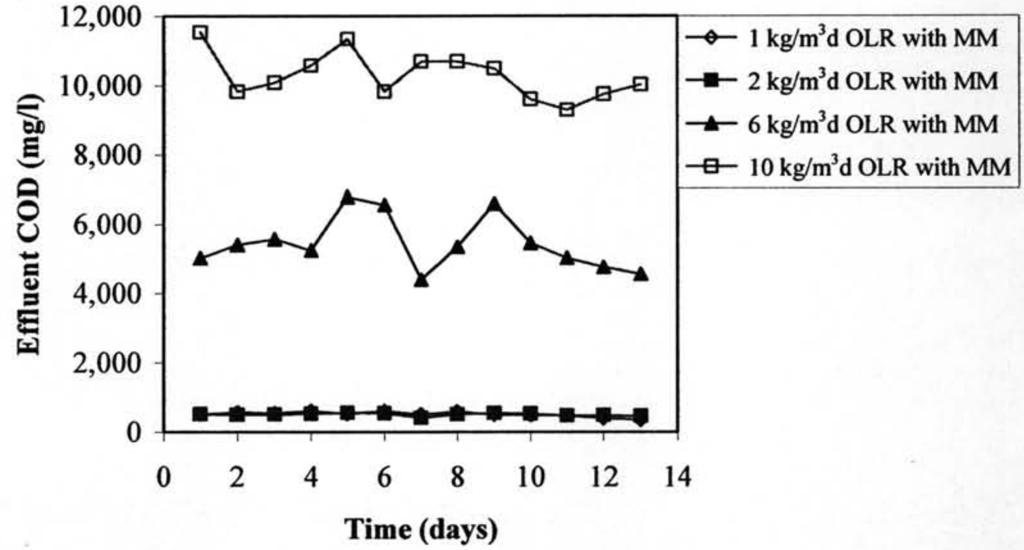

Figure 4.13 Effluent COD with time on days 1-13 of operation in the SBRs with mineral medium (MM) feed, and 1-d cycle for four oil loading rates (1, 2, 6, 10 kg/m^{3}d).

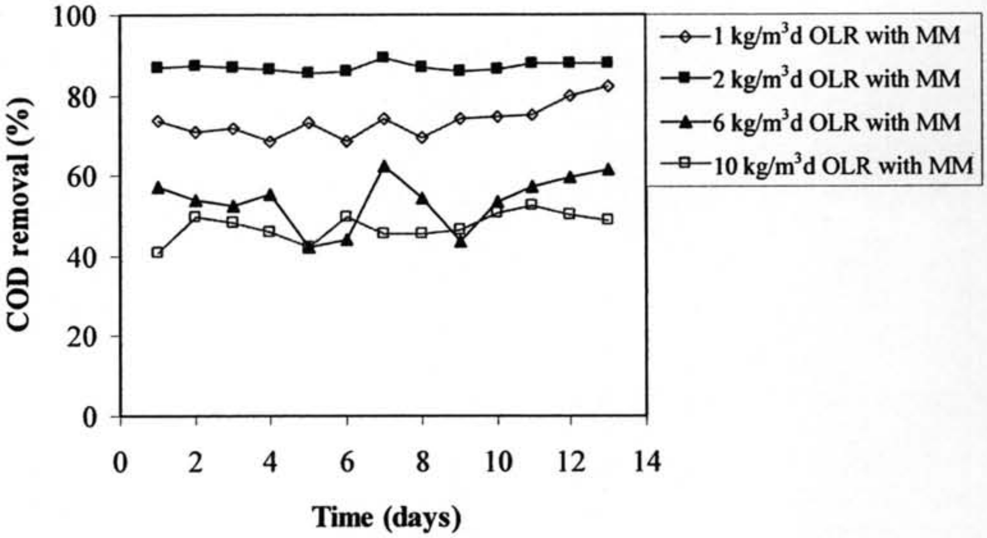

Figure 4.14 COD removal with time on days 1-13 of operation in the SBRs with mineral medium (MM) feed, and 1-d cycle for four oil loading rates (1, 2, 6, 10 kg/m^{3}d).

The results of surface tension reduction, and COD and oil removal during steady state operation in the SBRs are respectively plotted in Figure 4.15, 4.16, and 4.17. The results clearly demonstrate that the optimum OLR of 2 kg/m^{3}d provided the lowest surface tension of 28.1 mN/m and the highest surface tension reduction of 58%, corresponding to the highest COD removal of 88% and the highest oil removal of 94%. At metabolic products (e.g., organic acids) concentrations above 10%, surface tension obtained was not below 30 mN/m (Cassidy, 2001; Cassidy *et al.*, 2002). These results indicated the production of biosurfactant in the SBRs, resulting in the surface tension reduction. Biosurfactant production is associated with the uptake of hydrophobic carbon source (i.e., palm oil) by the culture, as reflected by the COD and oil removal. The culture produced biosurfactants to emulsify water-immiscible carbon source for well assimilation. COD and oil removals were respectively above 50% and 72% for all OLRs tested. A maximum reduction in surface tension was observed at an OLR of 2 kg/m^{3}d, followed by OLRs of 6, 10 and 1 kg/m^{3}d. Although the high COD and oil removal observed at an OLR of 1 kg/m^{3}d were respectively 79% and 85%, but the surface tension reduction was 53% which was relatively low in comparison to other OLSs. This indicated that it was deficient in carbon source, corresponding to its lowest MLSS value (364 mg/l) as shown in Figure 4.18. MLSS were collected throughout the aeration step, indicated the microbial concentration in the reactor. At the highest OLR (10 kg/m^{3}d), a low surface tension reduction (56%) corresponded to the lowest COD removal (50%) and oil removal (72%), indicating an inhibitory effect on the bacterium activity due to the limitation of palm oil solubility as well as the difficulty of nutrient transport (Santa Anna *et* al., 2002; Rashedi *et al.*, 2005). Additionally, numerous reports have manifested the overproduction of biosurfactants when *P. aeruginosa* reaches the stationary phase of growth owing to nitrogen limiting conditions (Dubey and Juwarkar, 2001; Rahman *et al.*, 2002; Nitschke *et al.*, 2005a; Soberón-Chávez *et al.*, 2005; Raza *et al.*, 2006).

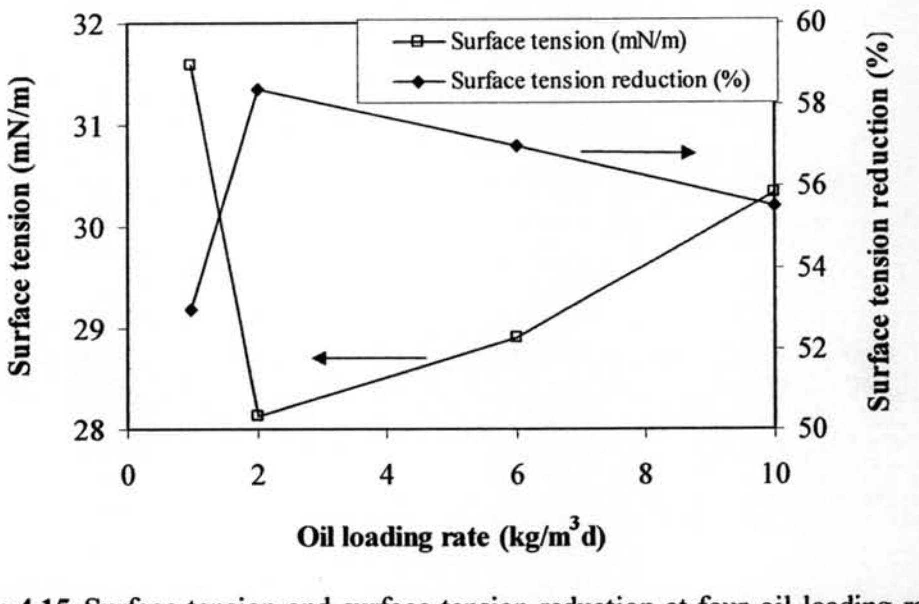

Figure 4.15 Surface tension and surface tension reduction at four oil loading rates (1, 2, 6, 10 kg/m^{3}d) during steady state operation (days 11-13) in the SBRs for mineral medium (MM) feed and 1-d cycle.

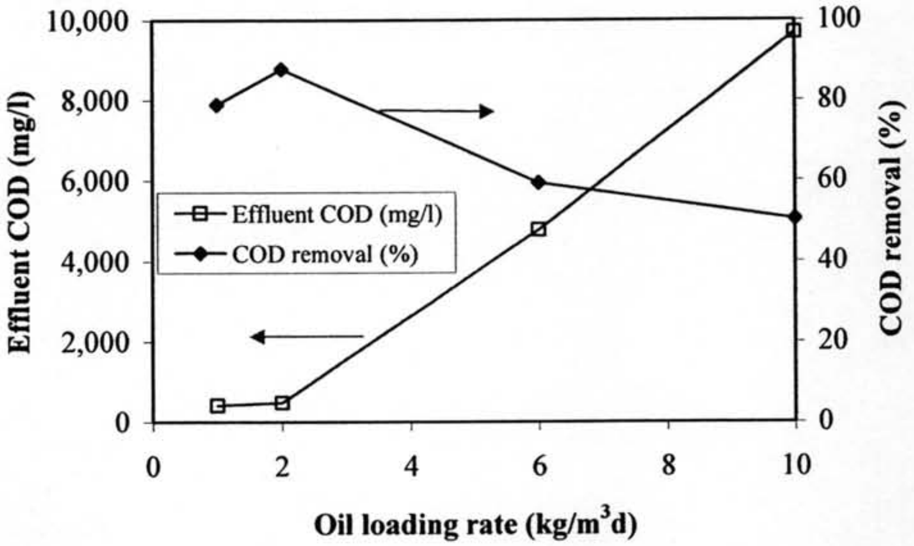

Figure 4.16 Effluent COD and COD removal at four oil loading rates (1, 2, 6, 10 kg/m^{3}d) during steady state operation (days 11-13) in the SBRs for mineral medium (MM) feed and 1-d cycle.

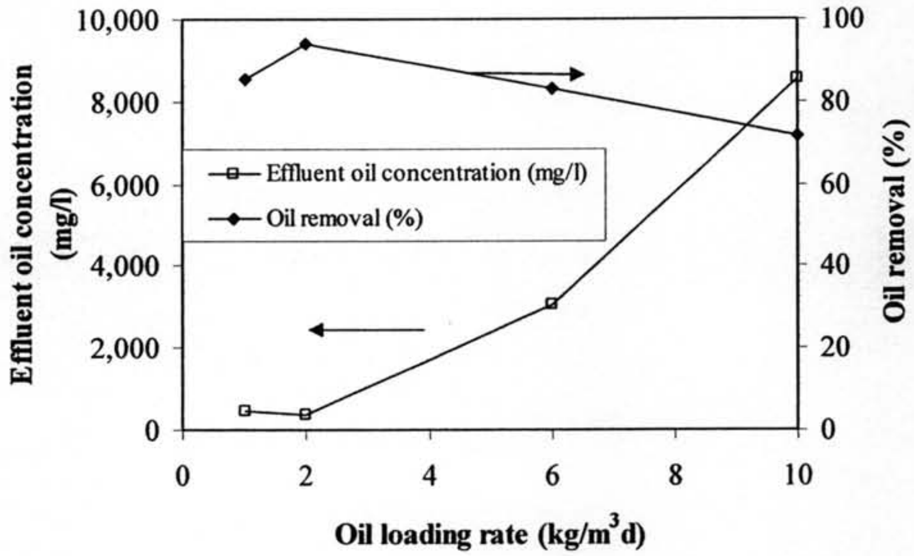

Figure 4.17 Effluent oil concentration and oil removal at four oil loading rates (1, 2, 6, 10 kg/m^{3}d) during steady state operation (days 11-13) in the SBRs for mineral medium (MM) feed and 1-d cycle.

In this study, the lowest surface tension of 28.1 mN/m by *P. aeruginosa* SP 4 was higher than 31.8 mN/m by *P. aeruginosa* LBI with palm oil as a carbon source (Nitschke, 2005b).

Figure 4.18 plotted the results of effluent TSS and MLSS during steady state operation in the SBRs at four OLRs. The results show that increasing OLR from 1 to 10 kg/m^{3}d fostered the microbial growth as indicated by increasing MLSS in the SBRs. MLSS increased from 364 to 8,217 mg/l as OLR increased from 1 to 10 kg/m^{3}d. This was attributed to the higher nitrogen, phosphorous, and oil concentration in the case of higher OLR, which favoured faster culture growth and multiplication as compared to the lesser OLR. The effluent TSS also increased from 271 at an OLR of 1 kg/m^{3}d to 8,133 mg/l at an OLR of 10 kg/m^{3}d.

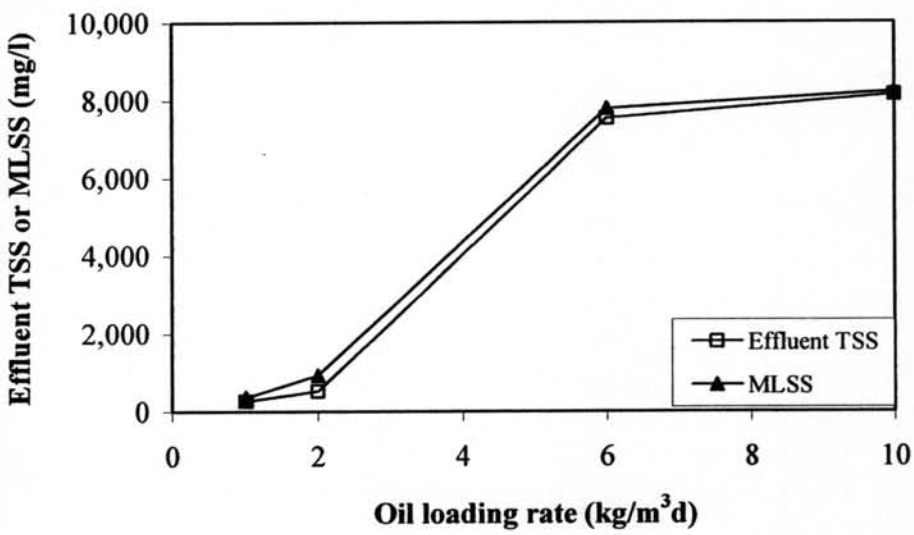

Figure 4.18 Effluent TSS and MLSS at four oil loading rates (1, 2, 6, 10 kg/m^{3}d) during steady state operation (days 11-13) in the SBRs for mineral medium (MM) feed and 1-d cycle.

For all OLRs tested, the effluent TSSs were high as compared to MLSS of each OLR tested, which is indicative of high cells wash-out. Nevertheless, the net cells loss was compensated by fast-growing microorganisms (i.e., *Pseudomonas* *aeruginosa* SP4). Accordingly, the maximum growth of *Pseudomonas aeruginosa* SP 4 at 22 h was reported by Paisanjit (2006). The lowest cell wash-out was obtained at an OLR of 2 kg/m^{3}d due to the highest difference between MLSS and effluent TSS (403 mg/l). This further confirmed that the optimum biosurfactant production was attained at an OLR of 2 kg/m^{3}d.

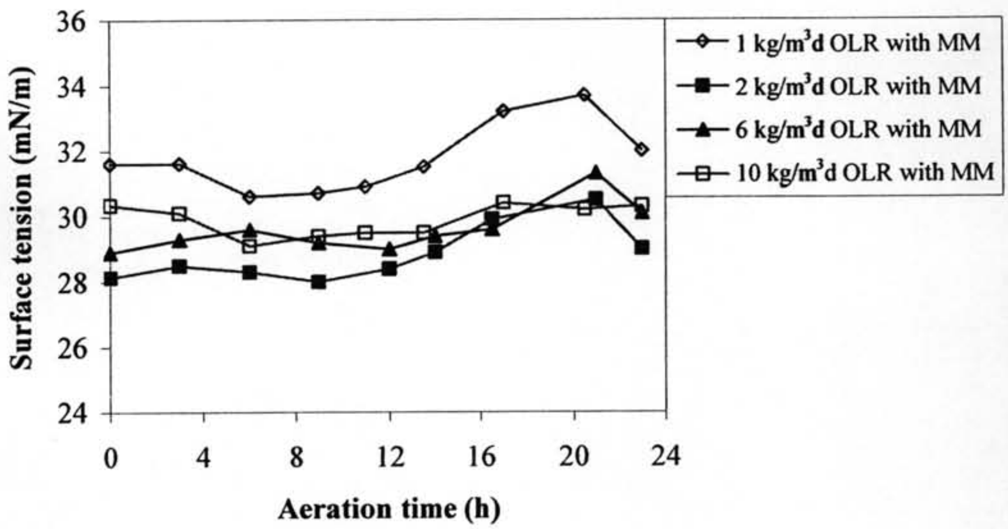

Figure 4.19 Surface tension with aeration time during a steady state cycle (day 13) of the SBRs at four oil loading rates (1, 2, 6, 8 kg/m^{3}d), mineral medium (MM) feed, and 1-d cycle.

Figure 4.19 represents the results of surface tension during a steady state cycle at four OLRs (1, 2, 6, 10 kg/m^{3}d). Similar trends were observed at all OLRs tested. The surface tension curves show a short lag surface tension reduction of 3 h, which can be attributed to the time taken for the culture to acclimatize to a carbon source (i.e., palm oil). However, the surface tension in effluent was minimum in the range of 6 to 10 h of the aeration period, which indicated that rates of biosurfactant production exceeded rates of degradation. Thereafter, the surface tension was maximum at 21 h of the aeration period, which implied that rates of biodegradation of biosurfactant exceeded rates of production. Biosurfactant are biodegradable

(Cassidy *et* al., 2000 and 2002; Cassidy, 2001; Cassidy and Hudak, 2001). According to Mohan *et al.* (2006), the degradation process for rhamnolipids is manifested with a huge peak, which is due to the readily biodegradable fraction, followed by a long tail corresponds to the slowly biodegradable.

4.4 Effect of Cycle Time with Mineral Medium on Biosurfactant Production

After optimizing the oil loading rate, the effect of cycle time on biosurfactant production was studied. The effluent COD and COD removal with 1-d cycle and 3-d cycle are shown in Figure 4.20. The SBRs with1-d cycle and 3-day cycle produced noticeably similar trends. After the first day of operation for both the cycle time, the SBRs reached the COD removal of more than 87%, where it maintained almost constant upto the end of operation. This indicated that most of organic carbons were efficiently utilized as carbon source at low OLR for biosurfactant production within 1 day as mentioned previously.

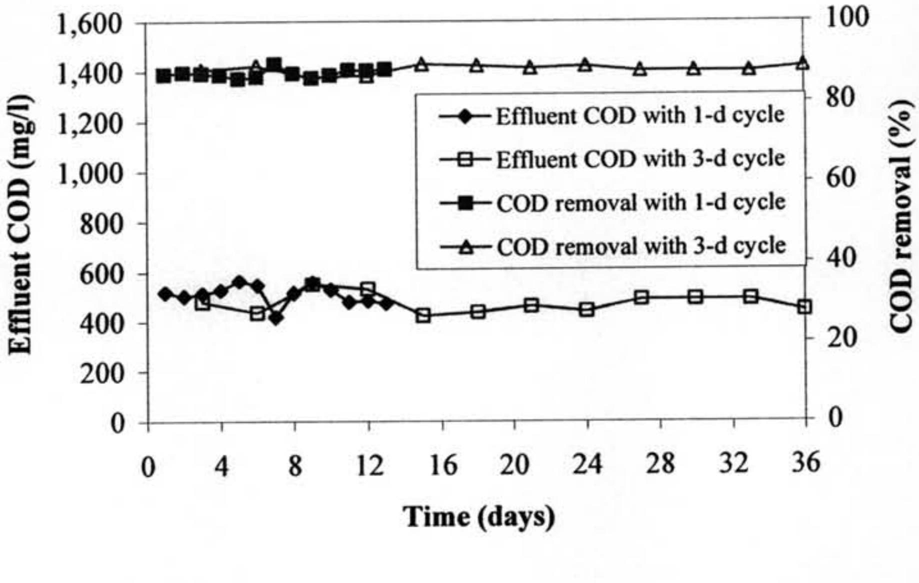

Figure 4.20 Effluent COD and COD removal with operation time in the SBRs for 1-d cycle (days 1-13) and 3-d cycle (days 3-36) at an oil loading rate of 2 kg/m^3d-cycle with mineral medium (MM) feed.

Comparing the surface tension, and effluent COD and oil concentration obtained with 1-d cycle and 3-d cycle during steady state operation are shown in Figure 4.21. Increasing cycle time from 1 to 3 days did not significantly affect COD removal. Nevertheless, the oil removal with the 3-d cycle operation was higher than that with the 1-d cycle operation. Interestingly, a lesser surface tension reduction was attained with the 3-d cycle operation (Figure 4.22). These presumably caused by deficient in carbon source with 3-d cycle, as evident from a lesser MLSS of 539 mg/l as compared to 1-d cycle. The extent of oil concentration in the influent (0.6% w/v) with 3-d cycle was equal to that with 1-d cycle, but an OLR with 3-d cycle was lower, i.e. 0.67 kg/m^3d than that provided with 1-d cycle as listed in Table 3.4. Therefore, optimum results were obtained at the OLR of 2 kg/m^3d under operating at 1-d cycle, which gave the highest surface tension reduction of 58%, corresponding to a COD removal of 88% and an oil removal of 94%.

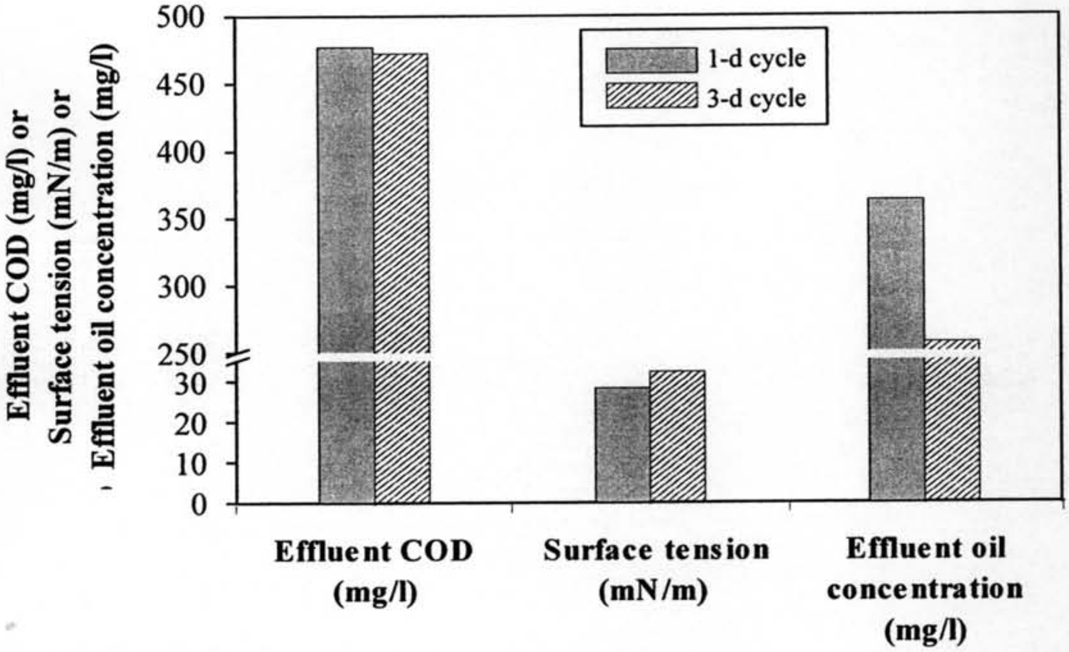

Figure 4.21 Effluent COD, surface tension, and effluent oil concentration during steady state operation in the SBRs at an oil loading rate of 2 kg/m^3d-cycle with 1-d cycle (days 11-13) and 3-d cycle (days 30-39), and mineral medium (MM) feed.

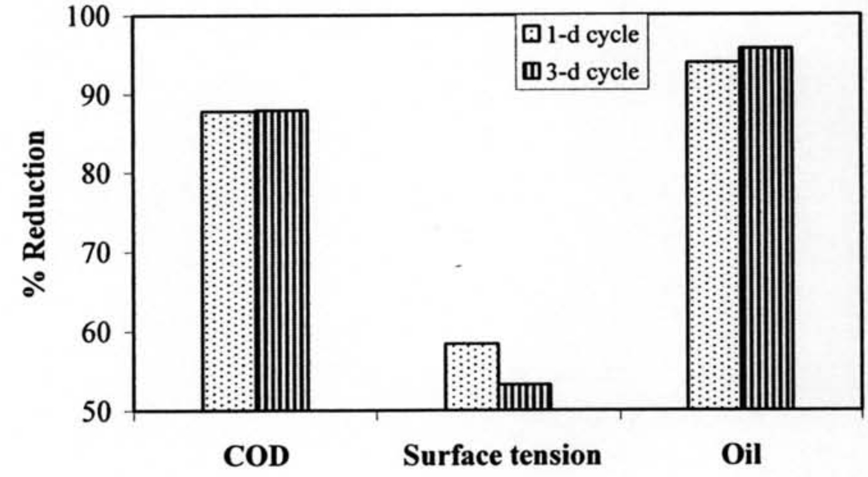

Figure 4.22 COD, surface tension, and oil reduction during steady state operation in the SBRs at an oil loading rate of 2 kg/m^{3}d-cycle with 1-d cycle (days 11-13) and 3-d cycle (days 30-39), and mineral medium (MM) feed.

As shown in Figure 4.23, the growth and multiplication of the culture with 1-d cycle were better compared to 3-d cycle, which were reflected from a higher MLSS of 928 mg/l in the SBR with 1-d cycle. Probably, this was attributed to sufficient in carbon source for uptake with 1-d cycle. A higher difference between MLSS and effluent TSS (403 mg/l) with 1-d cycle, as compared to 3-d cycle, leads to lower cell wash-out with 1-d cycle which supported the optimum biosurfactant production with 1-d cycle.

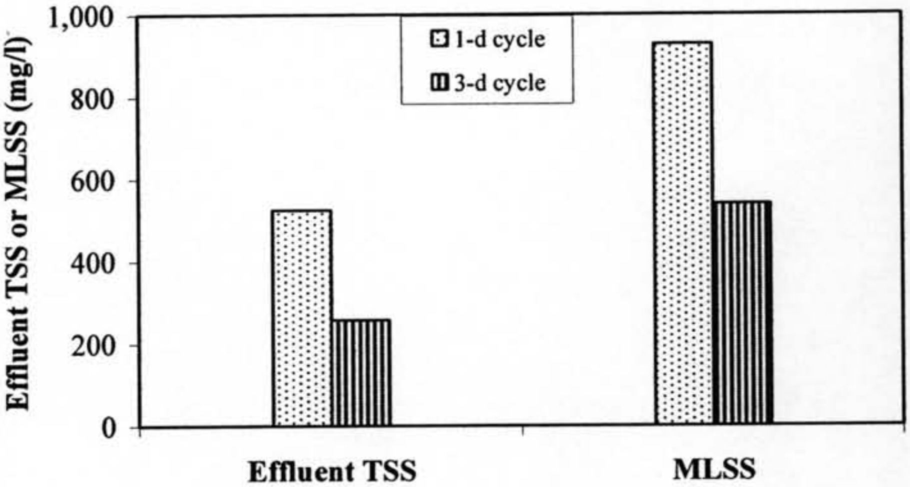

Figure 4.23 Effluent TSS and MLSS during steady state operation in the SBRs at an oil loading rate of 2 kg/m^{3}d-cycle with 1-d cycle (days 11-13) and 3-d cycle (days 30-36), and mineral medium (MM) feed.