CHAPTER IV
RESULTS AND DISCUSSION

4.1 Preparation of oil sludge extraction

The original oil sludge was a black slurry and slightly viscous. Sludge was sampled after being mixed to be homogenous. Then, the slurry was decanted for 15 minutes to let the solid particle settled down. The upper part of slurry was transferred to the glass plates and air-dried for 5 days under atmospheric conditions to remove the water portion and the excess moisture of the sludge. The hydrocarbons in the air-dried oil sludge were extracted by using n-hexane as a solvent. The mixed solution was filtered by passing through the filter paper (Whatman No.4) to remove any solid particles. The air-dried oil sludge solution was then diluted and injected into GC/MS to analyze for the hydrocarbon contents. Figure 4.1 shows the oil sludge sample before and after air-dried for 5 days and Table 4.1 shows the composition of oil sludge.

(a.) (b.)

Figure 4.1 Oil sludge sample (a.) before drying and (b.) after drying

Table 4.1 Composition of oil sludge

Composition	Oil sludge component (% w/w)
Moisture and volatile matter	35.55
Extracted hydrocarbon	24.69
Non- extracted hydrocarbon	34.25
Residual	5.51

4.2 Enhanced Solubilization of Hydrocarbons in Oil Sludge by Nonionic Surfactant

4.2.1 Determination of Contact Time Required for Solubilization of Oil Sludge by Nonionic Surfactant System

In order to study the effect of nonionic surfactant on the solubilization of petroleum hydrocarbons in the extracted oil from crude oil sludge, it is to determine the amount of time required for the solubilization process in the presence of nonionic surfactant (Tween 80) to reach equilibrium. The surfactant was added into the sets of 4 dam vials containing oil sludge with the MSM. The samples were taken and analyzed for solubilization of hydrocarbons at specific time intervals. The increased solubilization from the control experiment as a result of added surfactant was reported in term of "Enhanced Solubilization" as calculated below:

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

where $\text{solubilization}_{oil+surf}$ = TOC (Total Organic Carbon) of oil sludge and surfactants, $\text{solubilization}_{surf}$ = TOC of surfactants alone and $\text{solubilization}_{control}$ = TOC of oil sludge alone.

In this study, the concentration of surfactant used was 0.1% w/v and the concentration of oil was 1% w/v in vial containing 20 ml MSM. The vials were shaken on the orbital shaker at 150 rpm at room temperature and they were left to stay still for a week. Aqueous phase was filtered and injected into the TOC analyzer.

The results in Figure 4.2 showed that the solubilized hydrocarbon in the aqueous phase required 4 to 5 days to reach the maximum solubilization which was about 660 mg/L. Therefore, it can be concluded that the equilibration time of 4 days is adequate for the solubilization to complete.

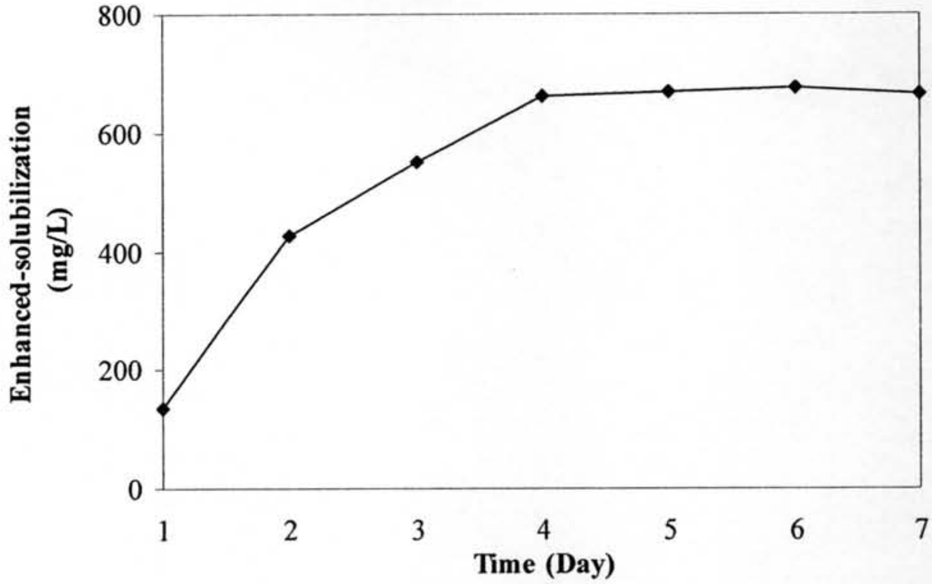


Figure 4.2 Equilibrium Time Required for Solubilization of Oil Sludge by Nonionic Surfactant System.

4.2.2 Effect of Nonionic Surfactants on Solubilization of Hydrocarbons in Oil Sludge

In this part of study, the control experiment was first carried out to quantify the solubility of hydrocarbons in oil sludge in the absence of surfactant. This control experiment revealed that the solubilization of hydrocarbon in oil sludge sample without adding surfactant was relatively low. The net solubilization in the control vial as measured by TOC (Total Organic Carbon) was found to be 35.08 ppm.

In order to improve the solubilization of the hydrocarbon in oil sludge, the subsequent experiments were carried out with addition of surfactants. The amount of extracted oil from oil sludge used was 1% wt/v with varying the surfactant concentration from 0.05 to 5% wt/v in 20 mL of MSM in 4-dam vials and their effect on the solubilization of hydrocarbons in oil sludge was examined by measuring TOC of the filtered solution taken from the vials. The solubilization of hydrocarbon in oil sludge as enhanced by addition of Tween 80 as a function of added surfactant concentration was shown in Figure 4.3. From the results, it showed that the solubilized carbon increased with increasing surfactant concentration. Then the enhanced solubilizations reach its maximum value a specific surfactant concentration. However, Figure 4.4 shows that the surfactant concentration of 0.2% w/v provided the highest ratio between the weight of solubilized carbon and the weight of surfactant compared with the other surfactant concentration. It means that this surfactant concentration was the most suitable to apply in the biodegradation study. Figure 4.3 and Figure 4.4 show the enhanced-solubilization of hydrocarbons at the various concentrations and the weight of solubilized carbon to weight of surfactant ration respectively.

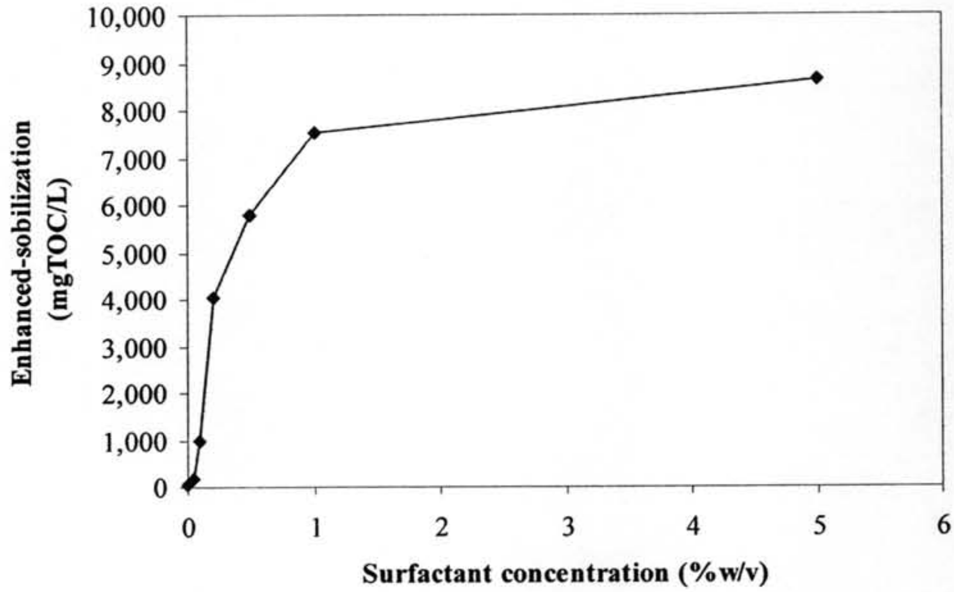


Figure 4.3 Enhanced-solubilization of hydrocarbons at the various concentrations.

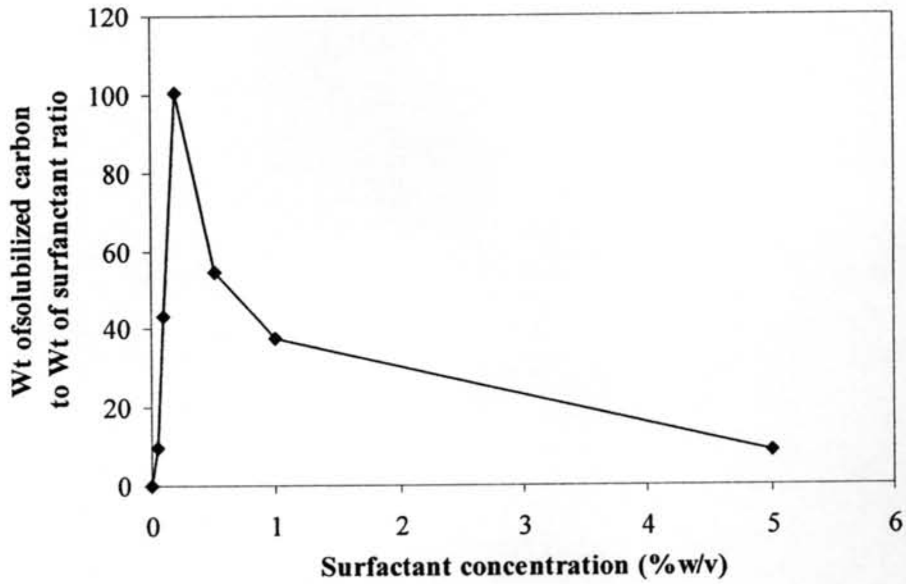


Figure 4.4 Weight of solubilized carbon to weight of surfactant ratio.

4.3 Biodegradation study

4.3.1 Effect of oil loading rate 0.5 kg/m^{3}d and 1.0 kg/m^{3}d in the presence of Tween 80 concentration 0.1% w/v.

In this part, the effect of oil loading was first examined at the amount of oil loading between 1.0 and 0.5 kg/m^{3}d (1% w/v) with the surfactant concentration used at 0.1% w/v and the SBR operation was varied from 1 to 3 cycle per day. The experimental results were quantified by 4 methods which are Chemical Oxygen Demand (COD), Total Organic Carbons (TOC), Total Petroleum Hydrocarbons Extraction (TPH), and Microbial concentrations methods.

4.3.1.1 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (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. COD is expressed in mg/LO_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 value of COD from the experiment at oil loading rate 1 kg/m^{3}d in the present of Tween 80 concentration 0.1 %w/v was first examined. The effluents of

COD values were measured until it was stable. The result showed that after day 11st the COD values began steady at about 579.3 mg/L and the percent COD removal was about 78.5%. Figure 4.5 and Figure 4.6 show the effluent COD values of the oil loading rate 1.0 kg/m^{3}d and the COD removal of the oil loading rate 1.0 kg/m^{3}d respectively.

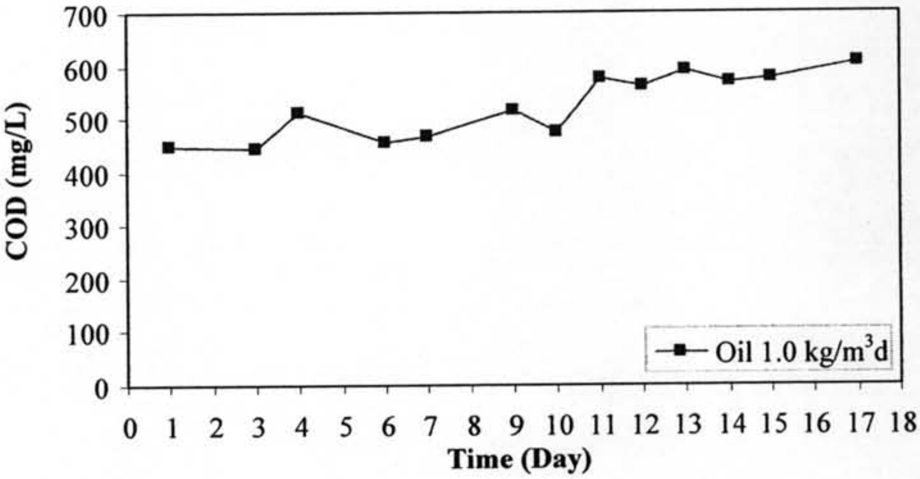


Figure 4.5 Effluent COD values of the oil loading rate 1.0 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

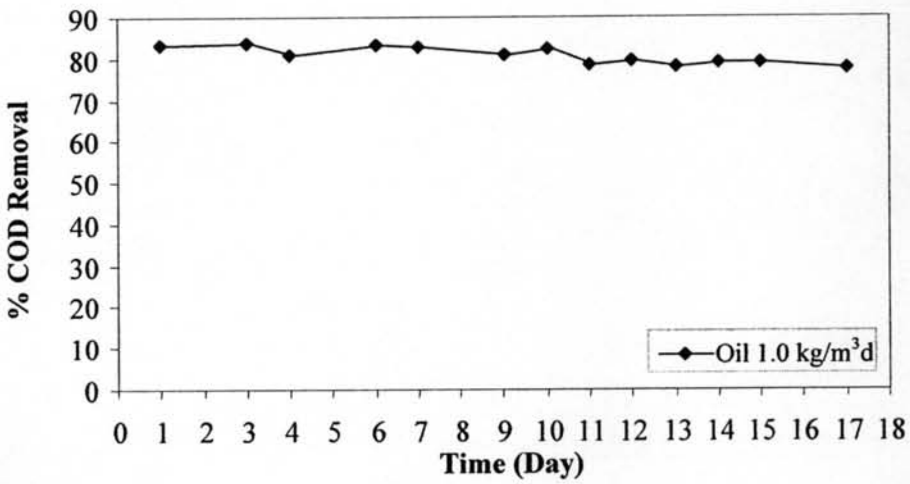


Figure 4.6 COD removal of the oil loading rate 1.0 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

The amount of oil loading was changed from 1.0 kg/m^{3}d to 0.5 kg/m^{3}d but the concentration of extracted oil was still fixed at 1 %w/v and the concentration of surfactant (Tween 80) was also fixed at 0.1 %w/v. The result showed that the COD values of the effluent stabilized after day 13rd at about 1,150 mg/L and the average percentage of the COD removal was 54% as shown in Figure 4.7 and Figure 4.8

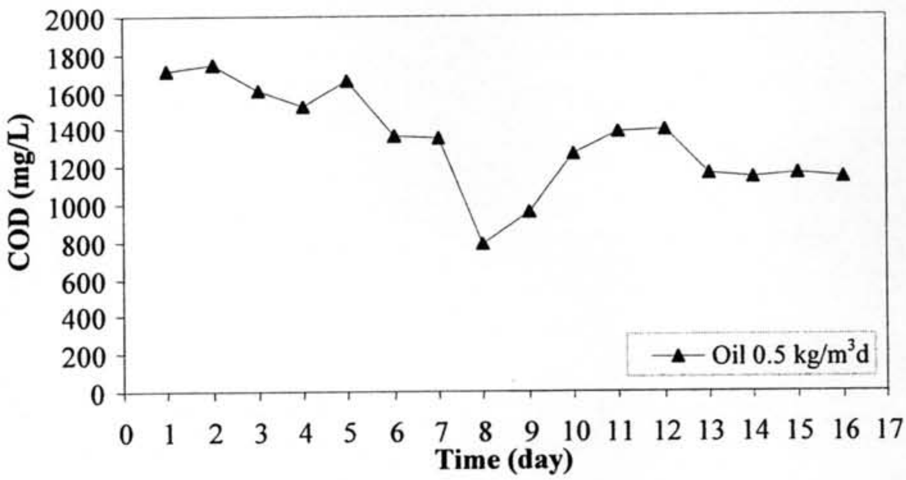


Figure 4.7 Effluent COD values of the oil loading rate 0.5 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

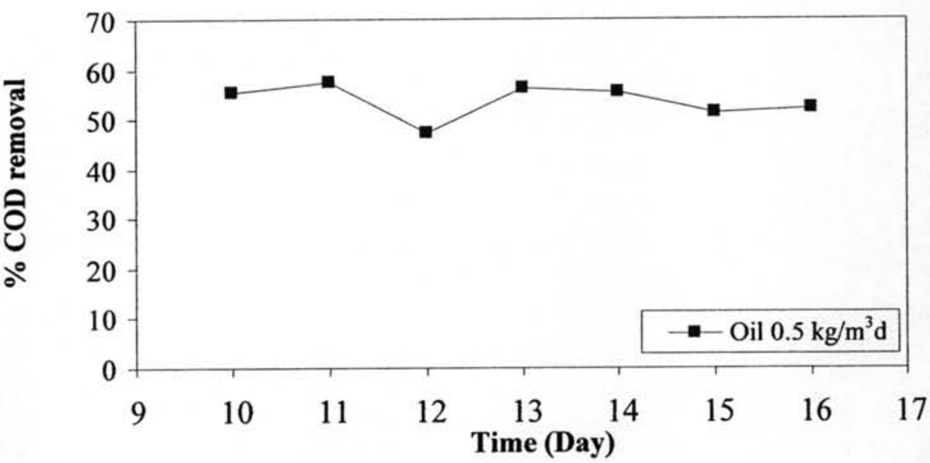


Figure 4.8 Percent COD removal of the oil loading rate 0.5 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

The figures demonstrated that the degradation capability of the microorganism in utilizing hydrocarbon from the extracted oil in the higher organic loading provided higher COD removal to as high as 78.5% whereas the removal efficiency in the lower oil loading decreased to 54%. When the experiment applied low oil loading at 0.5 kg/m^{3}d, the organic substances as carbon source of the microorganism were not enough for the microorganism to utilize. The oil loading in 1.0 kg/m^{3}d was the most appropriate organics loading, so at this oil loading was further used for the biodegradation study.

4.3.1.2 Total Organic Carbon (TOC method)

Total organic carbon (TOC) is the amount of carbon bound in an organic compound. In this study, this method indicated the amount of carbon that it was utilized. The sample from the bioreactor was filtered by passing through the filter paper (Whatman no. 42) and the aqueous phase was injected into the TOC analyzer. Figure 4.9 shows the TOC values of the effluents at the oil loading rate was 1.0 kg/m^{3}d that stable after day 11st with the average values about 426.43 mg/L and the average percentage TOC removal was 72.16% as shown in Figure 4.10.

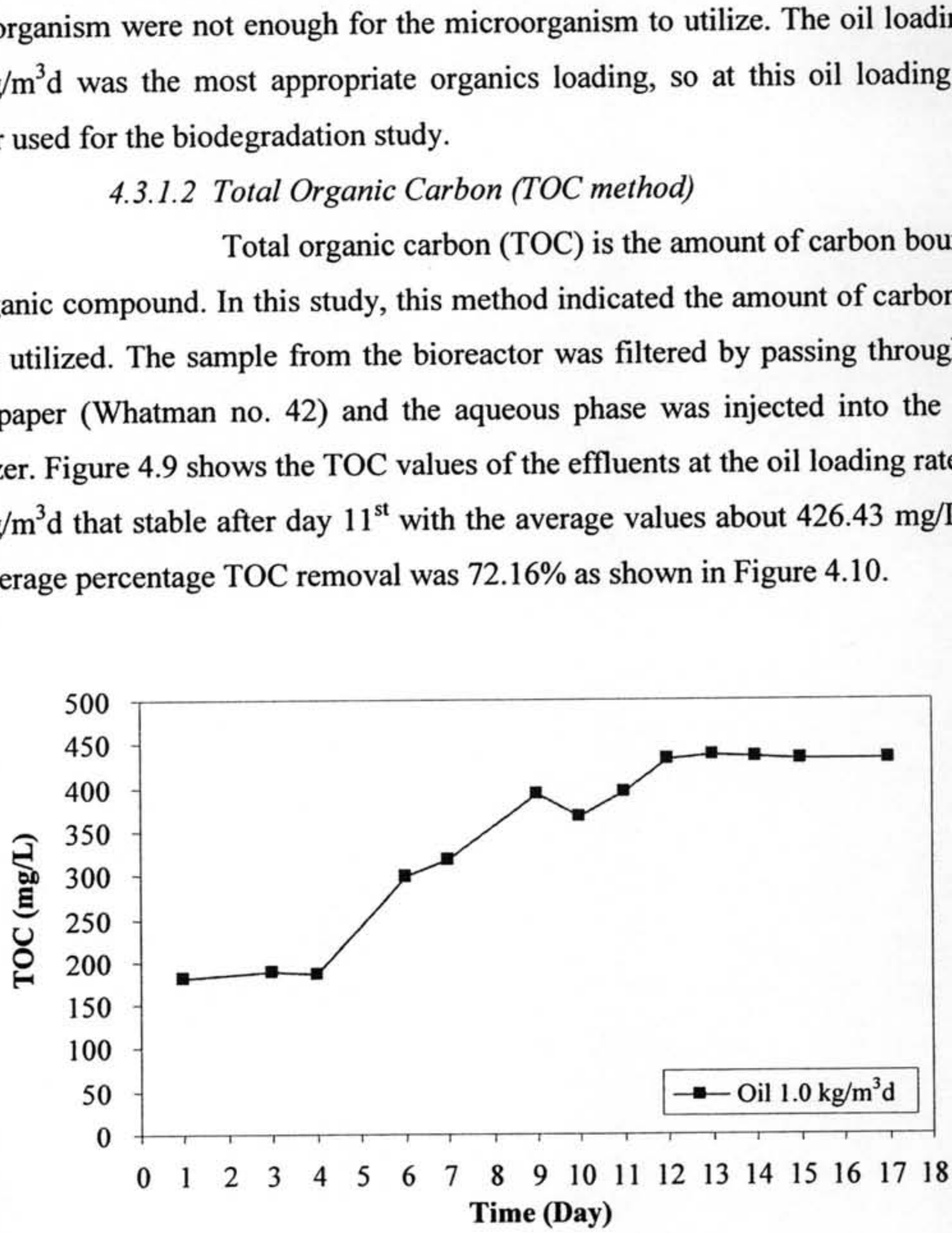


Figure 4.9 Effluents TOC values of the oil loading at 1.0 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

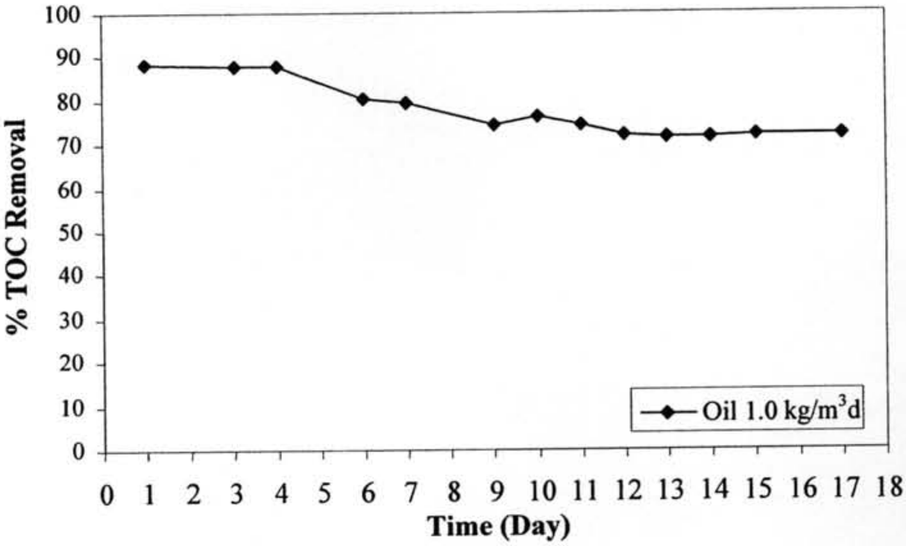


Figure 4.10 Percent TOC removal of the oil loading rate 1.0 kg/m^3d in the presence of Tween 80 0.1% w/v.

When the oil loading was changed to 0.5 kg/m^3d, the microorganism had ability to degraded carbon about 869.4 mg/L after day 11^{st}. Figure 4.11 shows average TOC effluent of the organic loading at 0.5 kg/m^3d and Figure 4.12 shows the percent TOC removal at the same organic loading. When compare both organic loading between 0.5 and 1.0 kg/m^3d, the results are quite similar to the COD removal that at the oil loading 1.0 kg/m^3d had higher the percent TOC removal than the other one and supported the reason that the oil loading at 1.0 kg/m^3d was the most suitable to further use in the biodegradation study.

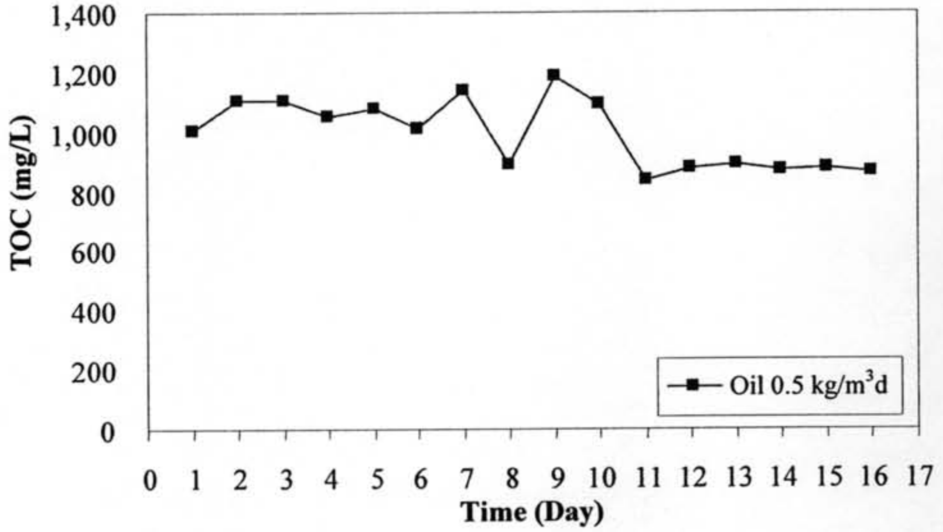


Figure 4.11 Effluents TOC values of the oil loading at 0.5 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

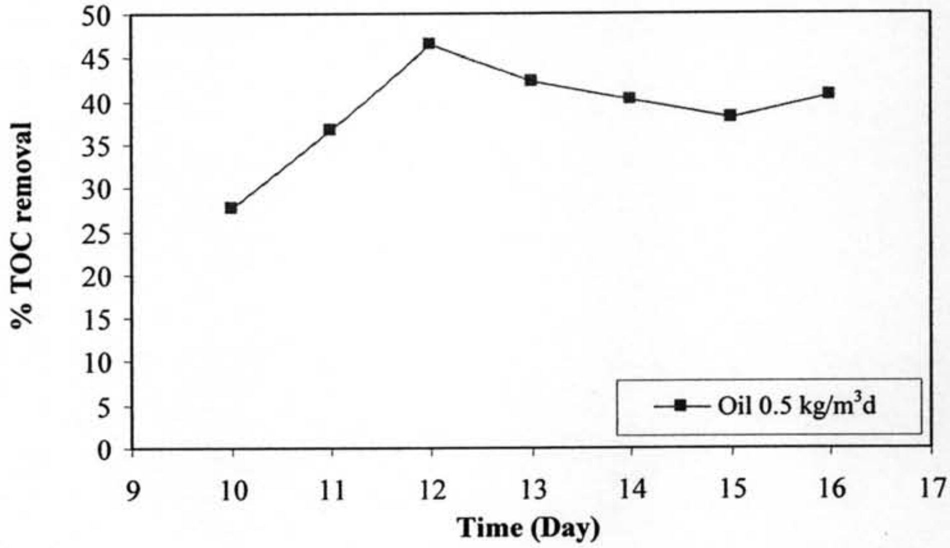


Figure 4.12 Percent TOC removal of the oil loading rate 0.5 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

4.3.1.4 Total Petroleum Hydrocarbon Extraction (TPH)

The biodegradation of extracted oil with oil loading rate 1 kg/m^{3}d (1%w/v) in the presence of Tween 80 (0.1%w/v) was first examined. The results showed that the biodegradation of oil sludge was stable after 7 days with and the average percent TPH removal was about 68.85% as shown in Figure 4.13 and Figure 4.14. From Figure 4.13, it can be seen that the ability to utilize carbon source of mixed cultures was only about 734.67 mg/L per day. After the system was stable, the amount of oil loading was changed to 0.5 kg/m^3d but the concentration of extracted oil was still fixed at 1 %w/v and the concentration of surfactant (Tween 80) was also fixed at 0.1 %w/v. It was shown that the biodegradation of oil loading rate at 1.0 kg/m^3d was higher than oil loading rate at 0.5 kg/m^3d. The oil loading 1.0 kg/m^3d in the present of Tween 80 in MSM showed more than 68% TPH removal efficiency compared to the oil loading 0.5 kg/m^3d which only about 60% TPH removal as shown in Figure 4.16. The hydrocarbons were utilized by the microorganisms cultivated inside the reactors as a carbon source for their growth but it still had amount of undegraded hydrocarbons remained in the system which were likely to be high molecular weight polycyclic aromatic hydrocarbons (PAHs). These components are very stable in the nature and require a long time to be degraded so the degrading microorganisms can not degrade these compounds in the short time.

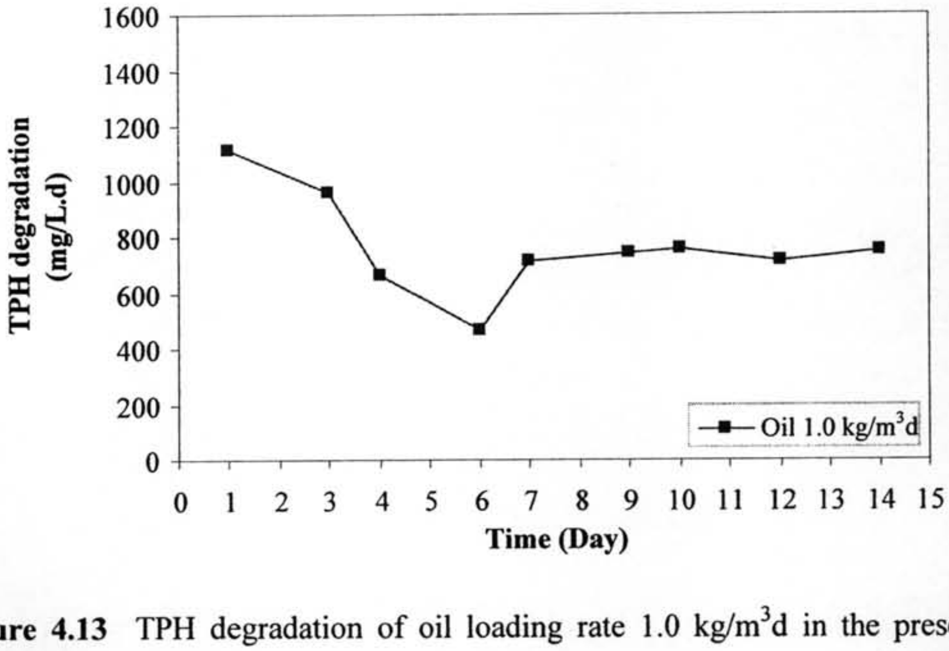


Figure 4.13 TPH degradation of oil loading rate 1.0 kg/m^3d in the presence of Tween 80 0.1% w/v.

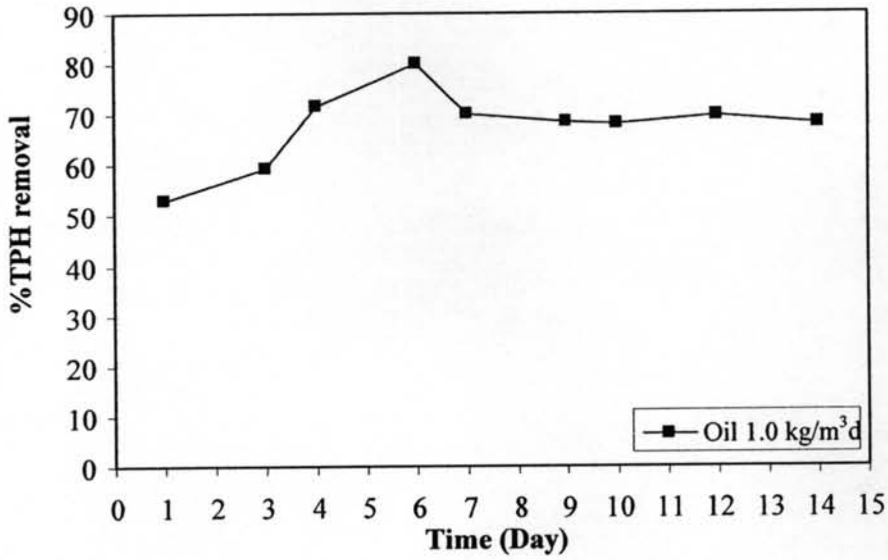


Figure 4.14 Percent TPH removal of oil loading 1.0 kg/m^3d in the presence of Tween 80 0.1% w/v.

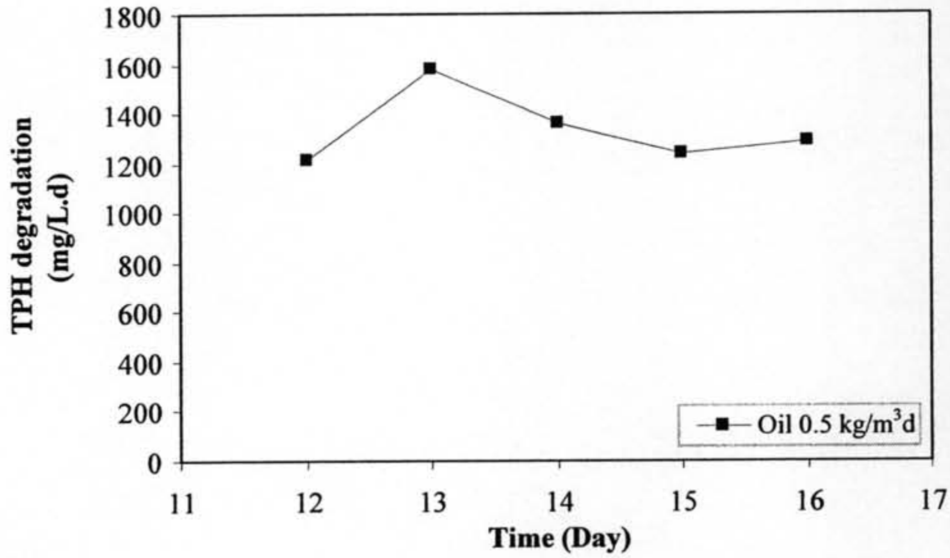


Figure 4.15 TPH degradation of oil loading rate 0.5 kg/m^3d in the presence of Tween 80 0.1% w/v.

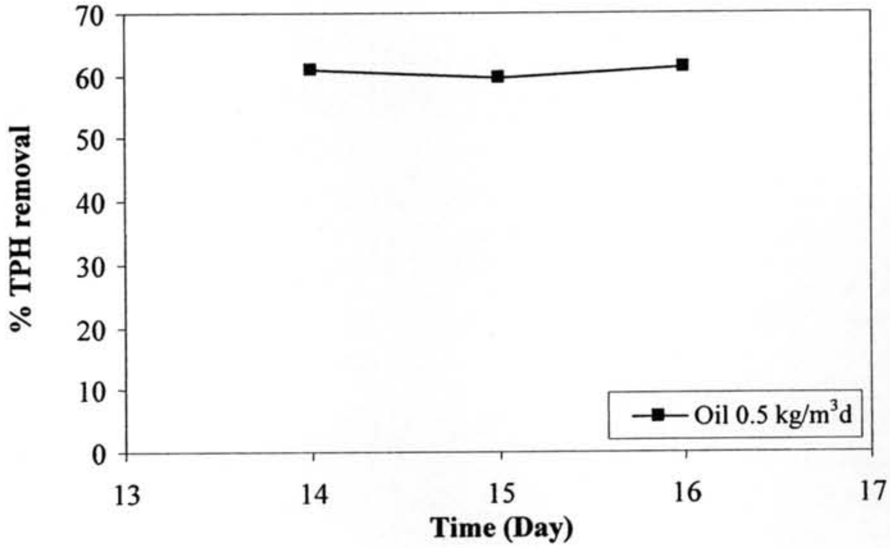


Figure 4.16 Percent TPH removal of oil loading 0.5 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

4.3.1.4 Microbial Growth

As the oil sludge was found to contain microorganisms capable of growing on and degrading hydrocarbon present in the oil sludge, it is important to first examine the growth of the microorganism. In this part of the study, the microbial concentration analysis was performed in order to quantify the growth of the microorganisms inside the reactors. After the oil was loaded into the reactor, the microorganism would utilize the hydrocarbon in the extracted oil as carbon source for their growth. Figure 4.17 shows that after 10 days, the growth of microorganism would become constant about 1,120 mg dried weight/L per day. After the COD value was constant, the microbial concentration was varied to the oil loading rate at 1 and 0.5. The results showed that the growth of microorganisms at 0.5 kg/m^{3}d was constant about 1,220 mg dried weight/L but at 1.0 kg/m^{3}d oil loading rate was increased and then become constant about 4,840 mg dried weight/L. The results showed that the growth was quite proportional to the oil loading but too low oil loading was not enough for the microorganism used to degrade the hydrocarbon as a carbon source. The higher the growth rate, the higher the crude oil degradation which resulted in an increase of acid metabolic by-product. This is the reason why the microbial concentration of 0.5 kg/m^{3}d of oil loading was relatively small when compared to 1.0 kg/m^{3}d oil loading.

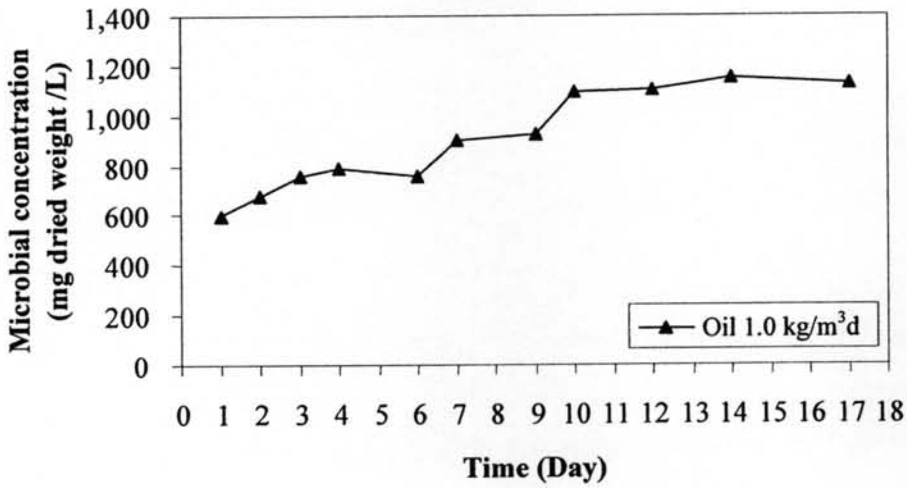


Figure 4.17 Growth of the indigenous microorganisms at oil loading 1.0 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

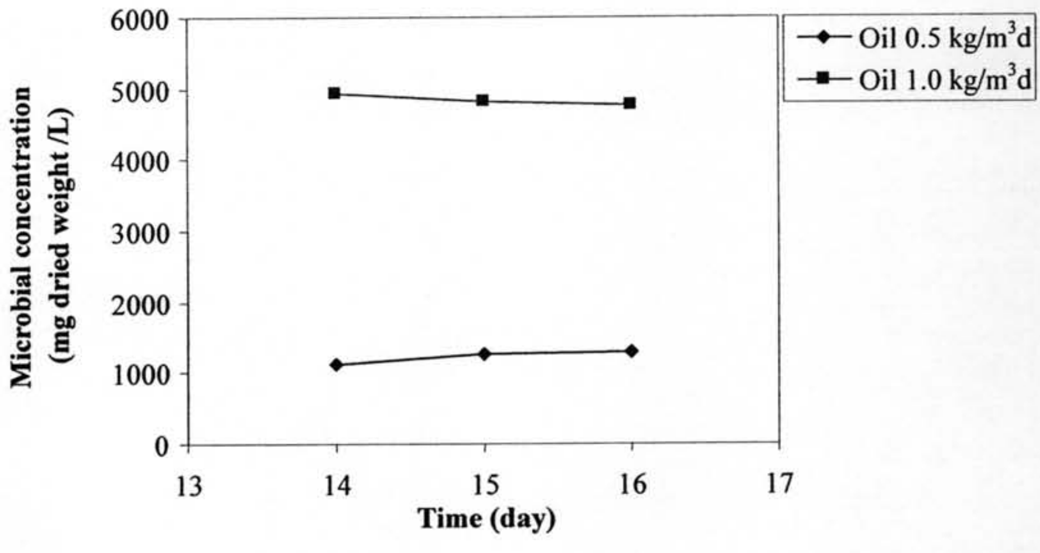


Figure 4.18 Growth of the indigenous microorganisms at oil loading 0.5 and 1.0 kg/m^{3}d in the presence of Tween 80 0.1% w/v.

4.3.2 Effect of Number of Cycle per Day of the Sequencing Batch Reactor Operation in the presence of Tween 80 concentration 0.1% w/v.

In this part of the study, the effect of number of cycle per day was examined by varying the number of cycle per day from 1 to 3 cycles per day. The amount of oil loading was used at 1.0 kg/m^{3}d with fixed the oil concentration at 1% w/v and the concentration of surfactant was used at 0.1% w/v. The results were quantified by 4 methods which the same as used in the previous part.

4.3.2.1 Chemical Oxygen Demand (COD)

From the effect of oil loading rate, the oil loading rate 1.0 kg/m^{3}d gave the highest percent removal when compare to other oil loading rate. The used operation of the SBR at the pervious part was 1 cycle per day so in this part the SBR operation was varied to 2 and 3 cycles per day. When compare the COD values of 2 and 3 cycles per day, the COD values of the influent and the effluent of both conditions were stabilized after 4 days. For 2 cycles per day, the COD values of influent and effluent after stable were 5,091.25 mg/L and 1,305 mg/L, respectively and the average percentage of the COD removal of 2 cycles per day was 74.37%. For 3 cycles per day, the COD values of influent and effluent after stable were 3,675 mg/L and 1,416.67 mg/L, respectively and the average percentage of the COD removal of 3 cycles per day was 61.40%. Figure 4.19 shows the COD influent and effluent of the SBR operation at 2 and 3 cycles per day and Figure 4.20 shows the percent COD removal of the SBR operation at 2 and 3 cycles per day.

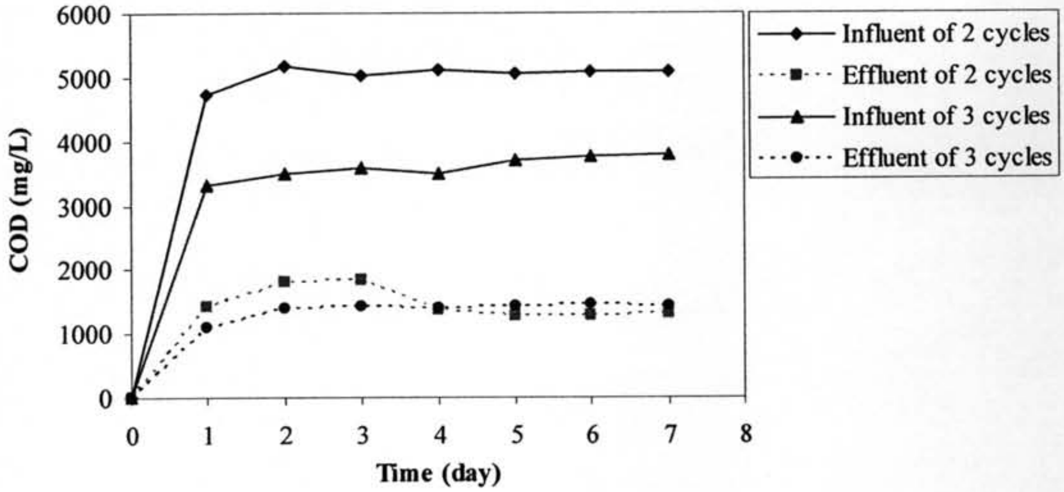


Figure 4.19 COD influent and effluent of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.1 %w/v.

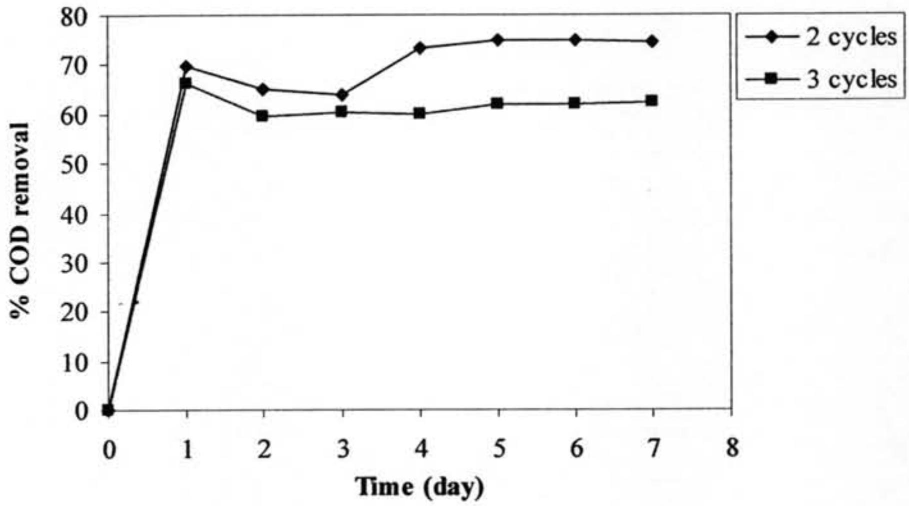


Figure 4.20 Percent COD removal of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.1 %w/v.

When compare the percent COD removal of the SBR operation at 2 and 3 cycles per day to the percent of SBR operation at 1 cycle per day, the results showed that at 1 cycle per day provided the highest percent COD removal to as high as 78.5%. When the reaction period time of the SBR operation decreased, the hydrocarbon was utilized by the microorganism in the short time (for 2 cycles, the aeration time was 11 hours and for 3 cycles, the aeration time was 7 hours). Some hydrocarbon compounds especially PAHs with more than 2 rings; for example phenanthrene, pyrene, and chrysene, are very stable in the nature and require a long time to be degraded by the degrading microorganisms. It can be seen that the percent COD removal decrease with decreasing the aeration time of the SBR operation.

4.3.2.2 Total Organic Carbon (TOC)

Figure 4.21 and Figure 4.22 show the TOC values of 2 conditions and the percent TOC removal which had the same trend as the COD results. The SBR operation at 2 cycles per day gave 61.62% removal and at 3 cycles per day provided the percent TOC removal at 59.92% but at 1 cycle of the SBR operation provided the highest percent TOC removal as high as 72.16%.

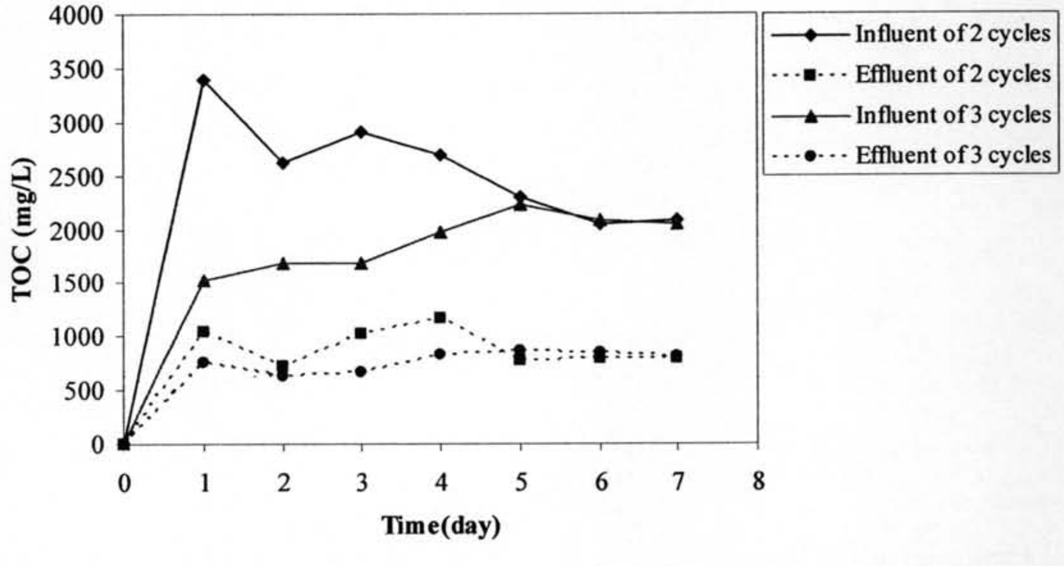


Figure 4.21 TOC influent and effluent of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.1 %w/v.

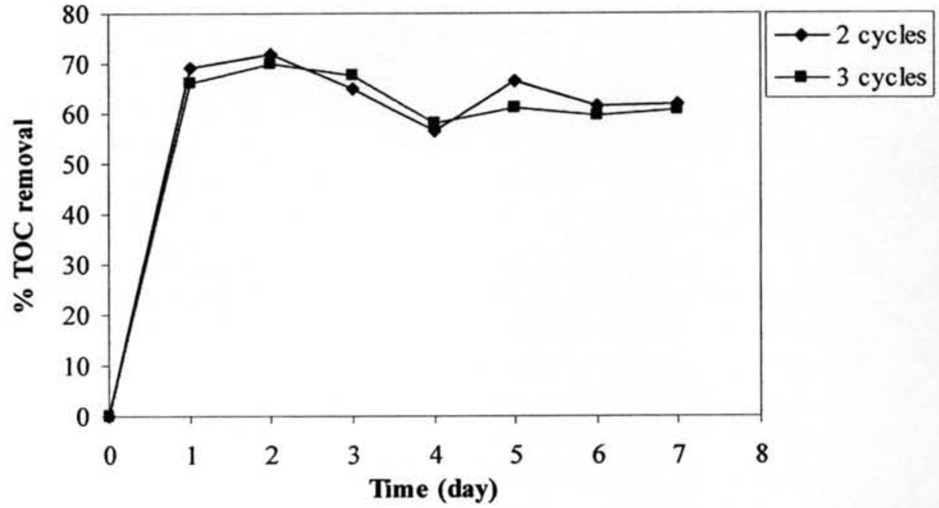


Figure 4.22 Percent TOC removal of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.1 %w/v.

4.3.2.3 Total Petroleum Hydrocarbon (TPH)

For the TPH extraction, it was shown that the biodegradation efficiency increased with increasing the number of cycles per day. The SBR operation with 3 cycles per day gave more than 60 % TPH removal efficiency compared to the SBR operation with 2 cycles per day as shown in Figure 4.23 and 4.24. When increasing the number of cycles per day to 3 cycles per day, it showed that the ability to utilize carbon source of mixed cultures was about 1,870 mg/L per day. The percent TPH removal increased from 47 to 60 % when increased the number of cycles per day from 2 to 3 cycles per day respectively. However, when compared to 1 cycle per day of the SBR operation, both conditions still had the percent TPH removal efficiency lower than that of 1 cycle per day which showed the percent TPH removal as high as 68 %.

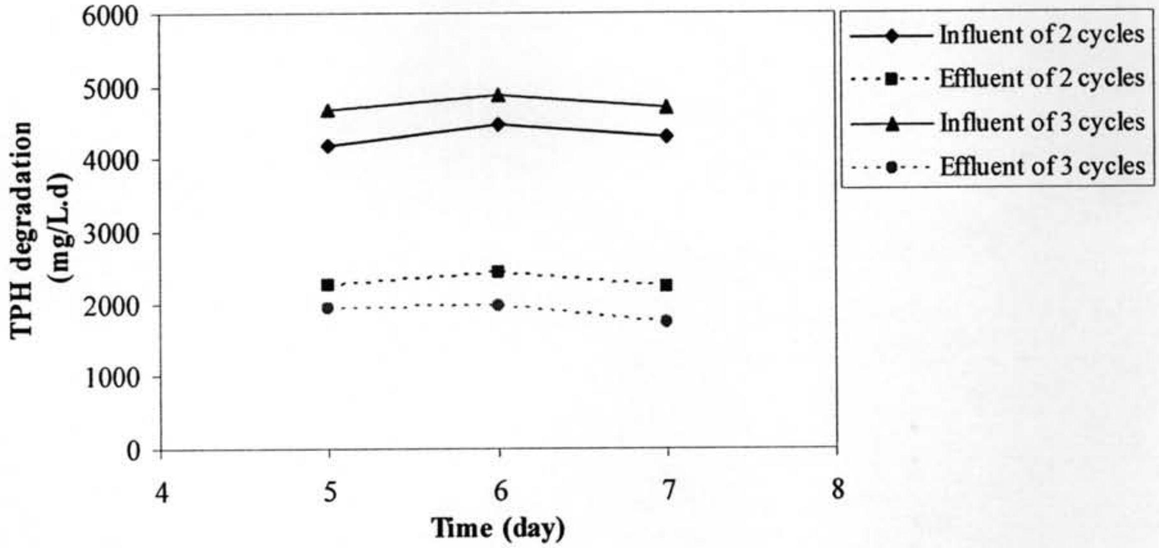


Figure 4.23 TPH degradation of influent and effluent of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.1 %w/v.

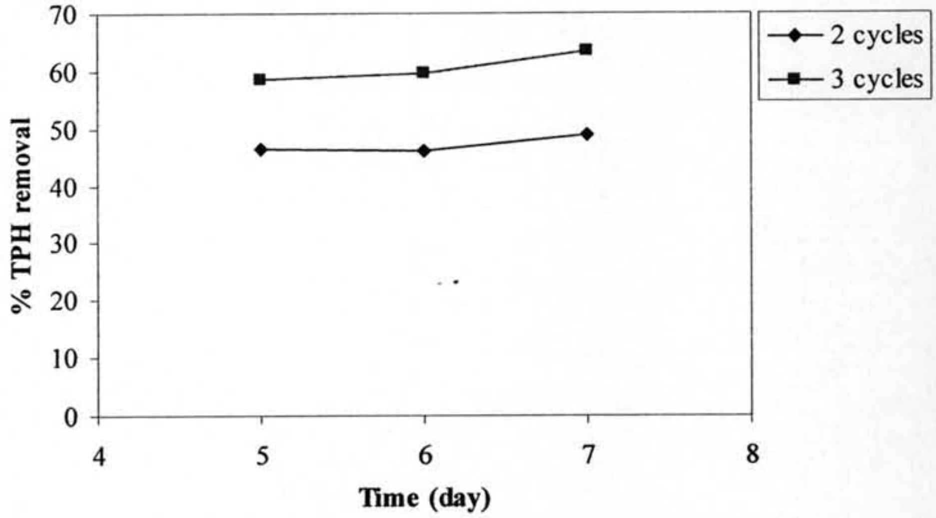


Figure 4.24 Percent TPH removal of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.1 %w/v.

The results showed the corresponding between COD, TOC, and TPH extraction as shown in Figure 4.25 and for COD and TOC gave the same trend that the system was optimized at the number of SBR operation with 1 cycle per day. This condition provided the most appropriate quantity of oil loading rate and provided the appropriate time for the microorganism to utilize the carbon, nutrients and other minerals for their growth. Too short time the microorganism can not degrade the hydrocarbon completely. COD showed the highest percent removal compared to TPH and TOC. It was because COD included the mineral salts in the organic loading and the microorganisms could utilize this mineral for their living. The results showed that at 1 cycle per day represents the 70% removal in all measurement and gradually decreased when the number of cycle increased.

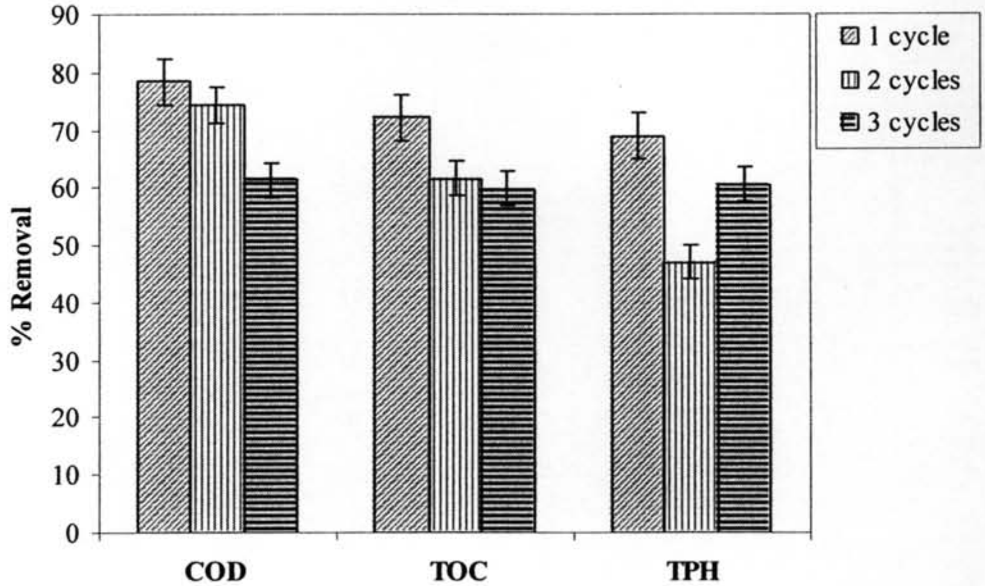


Figure 4.25 Percent removal of TPH, COD and TOC at the various number of cycle of SBR operation in the presence of Tween 80 concentration 0.1% w/v.

4.3.2.4 Microbial Growth

After the SBR operation was changed to 2 and 3 cycles per day, the growth of the microorganisms was measured after the system maintain steady which observed from the COD effluent that shown the system was stable after 4^{th} day. Figure 4.26 comparing the growth of microorganism of both operation times, the result shown that at 2 cycles per day was higher microbial concentration of microorganisms inside the bioreactor than 3 cycles per day. There have been a number of studied focused on monitoring the biodegradation of PAHs. Phenanthrene, fluorine and fluoranthene have been shown to be biodegraded by a mixed culture of bacteria (Weissenfle *et al.*, 1990) but these PAHs were strongly bound to the soil and unavailable for biodegradation and they required long time to degrade.

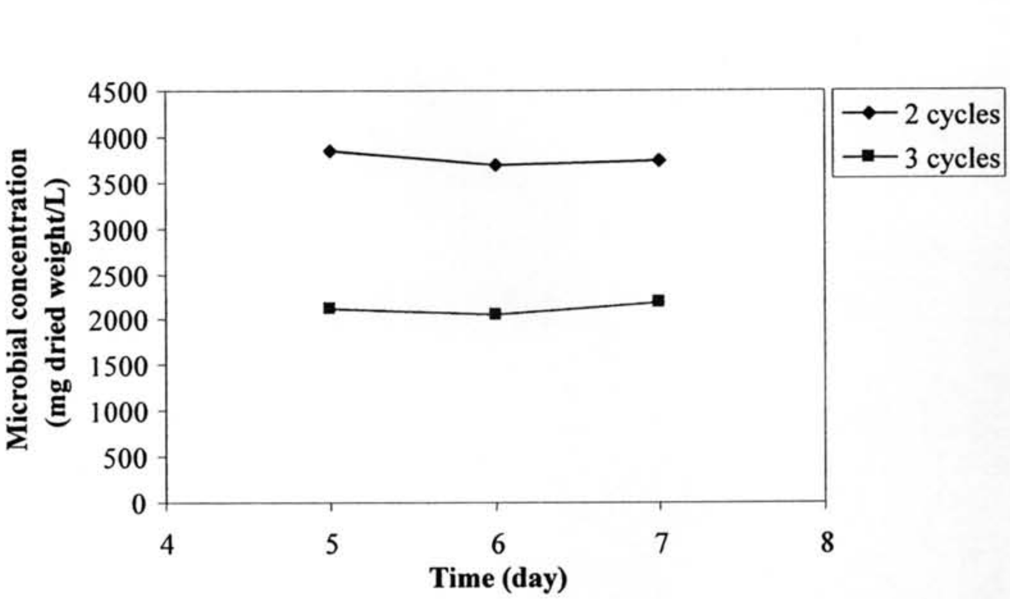


Figure 4.26 Growth of the indigenous microorganisms of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.1% w/v.

4.4 Biodegradation of Hydrocarbon in Oil Sludge by Using Nonionic Surfactant Concentration at 0.2% w/v

In this study, the nonionic surfactant concentration was changed to 0.2% w/v in order to improve the solubilization of the hydrocarbon in oil sludge but the amount of extracted oil from oil sludge was fixed at 1% w/v. The oil loading rate was used at 1 kg/m^{3}d with varying the number of cycle of the SBR operation from 1 to 3 cycles per day. The results were also quantified by 4 methods which are COD, TOC, TPH extraction and Microbial concentration method.

4.4.1 Chemical Oxygen Demand (COD)

The biodegradation of hydrocarbon in extracted oil in higher number of cycles of the SBR operation provided the high COD effluent, the COD effluent increased with increasing the number of cycles per day. Initially, the COD influent and effluent of 1, 2 and 3 cycles per day were stable after day 4th. The capability of degrading organic in higher number of cycles per day showed lower COD removal, for 3 cycles per day, providing about 56% COD removal and the percentage gradually increased to 76% and 80% when the cycles of SBR was changed to 2 and 3 cycles per day respectively. Figure 4.27 represents the average COD influent and effluent in the various numbers of cycles per day of the SBR operation and Figure 4.28 shows the percent COD removal in the various numbers of cycles per day.

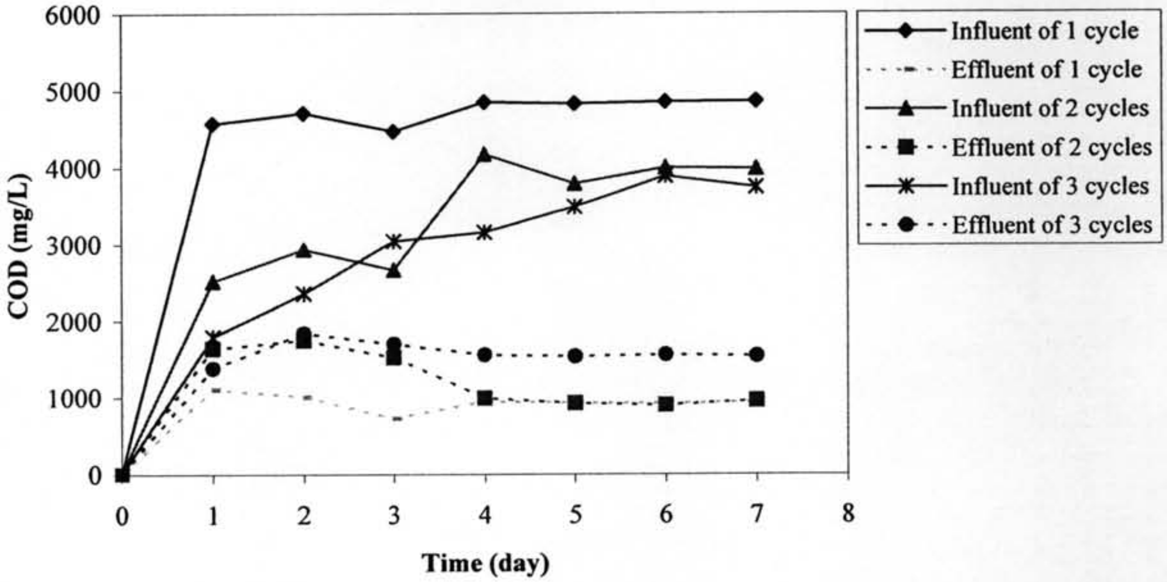


Figure 4.27 COD influent and effluent of the SBR operation at various number of cycles per day in the presence of Tween 80 concentration 0.2 %w/v.

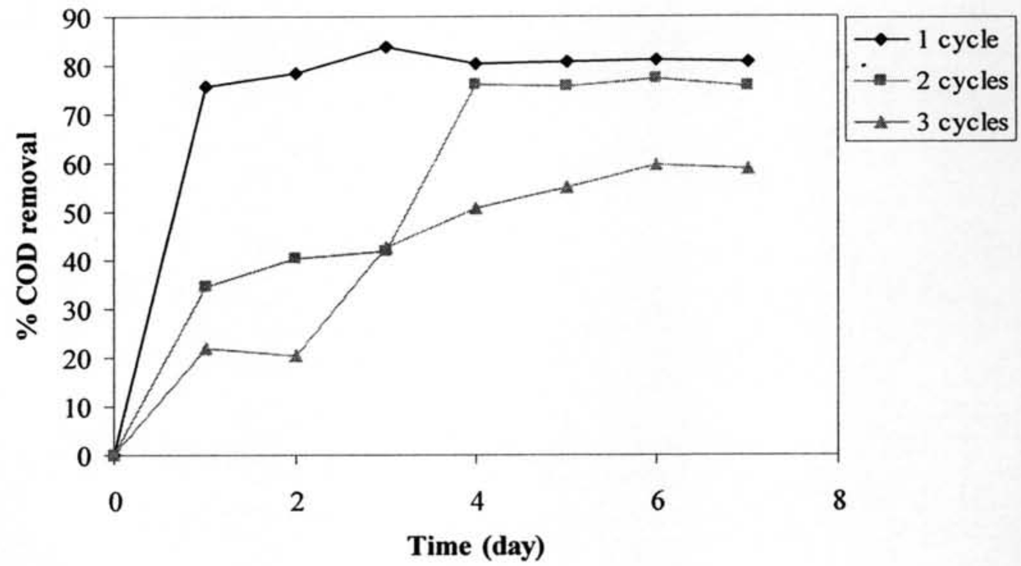


Figure 4.28 Percent COD removal of the SBR operation at various numbers of cycles per day in the presence of Tween 80 concentration 0.2% w/v.

4.4.2 Total Organic Carbon (TOC)

After the biodegradation, the microorganisms had the highest capability to degrade carbon at 1 cycle per day. Figure 4.29 shows the average TOC influent and effluent of various cycles per day of SBR operation while Figure 4.30 shows the percent TOC removal of the SBR operation at various numbers of cycles per day. The microbes could utilize the carbon source at about 890, 630 and 1,300 mg/L with 1, 2 and 3 cycles per day of the SBR operation respectively but the percent of TOC removal of 1 cycle per day was the highest percentage about 71%. Most of hydrocarbons were long chain alkane and the polycyclic aromatic hydrocarbon more than 2 rings such as phenanthrene, pyrene or chysene, these compounds forced the microorganisms to required long time in the biodegradation process. For 1 cycle per day, reaction time was 23 hours per day so the capability to degrade was the highest when compared to 2 and 3 cycles per day which had the reaction time only 11 and 7 hours respectively. Not surprisingly that why the microorganisms could utilize organic carbon better at longer reaction time.

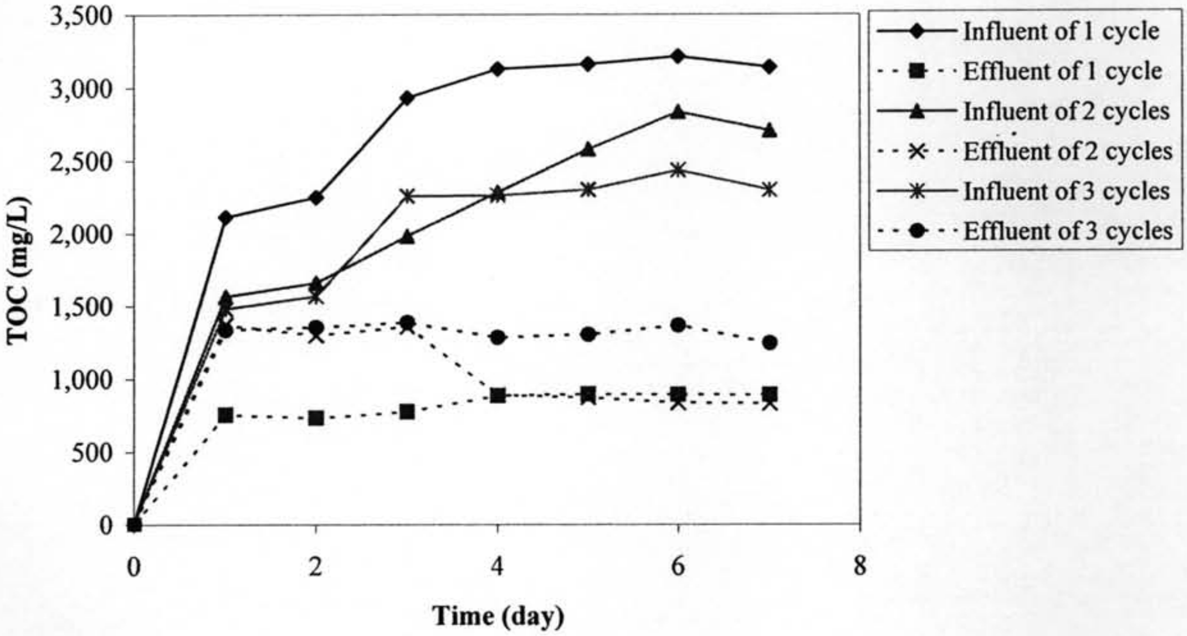


Figure 4.29 TOC influent and effluent of the SBR operation at various number of cycles per day in the presence of Tween 80 concentration 0.2 %w/v.

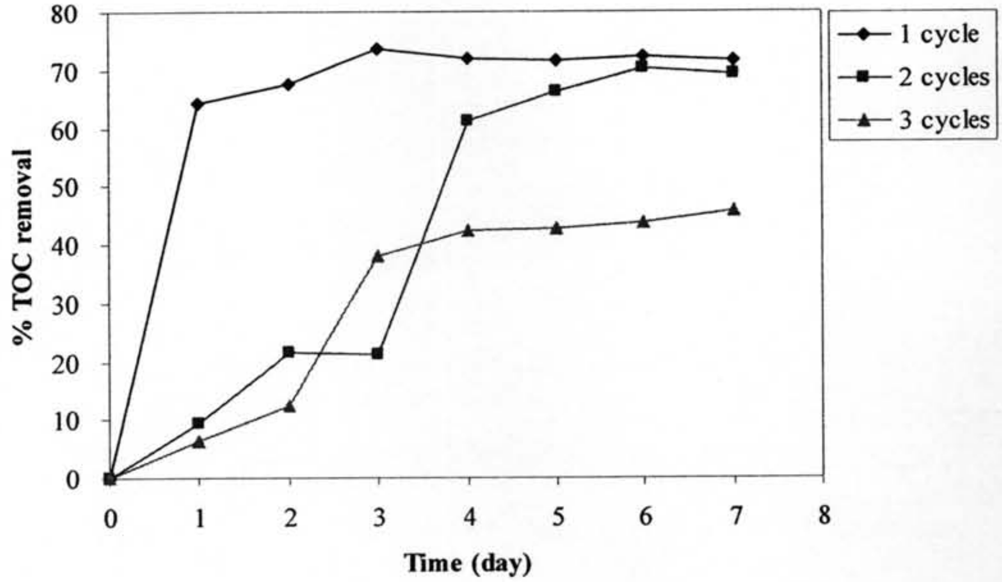


Figure 4.30 Percent TOC removal of the SBR operation at various numbers of cycles per day in the presence of Tween 80 concentration 0.2% w/v.

4.4.3 Total Petroleum Hydrocarbon

The TPH degradation efficiency of hydrocarbon in extracted oil was measured after the system become stable. The average TPH influent and effluent are shown in Figure 4.31. Initially, the maximum influent TPH was observed in the SBR operation at 3 cycles per day but the effluent TPH degradation of this operation was higher than other cycle numbers of SBR operations. When the cycle of the SBR operation increased, the TPH degradations were gradually decreased. The lowest of number of the SBR operation (1 cycle per day) shows more than 87% TPH removal efficiency compared to 2 cycles per day (85% TPH removal) and 3 cycles per day (83% TPH removal). The degradation is never complete and does not affect the different hydrocarbon families in the same manner (Walker *et al.*, 1976). For example, n-alkanes are metabolized more rapidly than either naphthelenes or aromatics, although reaction seems to be slower with increasing chain length, probably because of differences in water solubility (Albaiges and Cuberes, 1980). Figure 4.31 and Figure 4.32 show the TPH degradation of influent and effluent and the percent TPH removal of the SBR operation at various numbers of cycles per day in the presence of Tween 80 concentration 0.2% w/v.

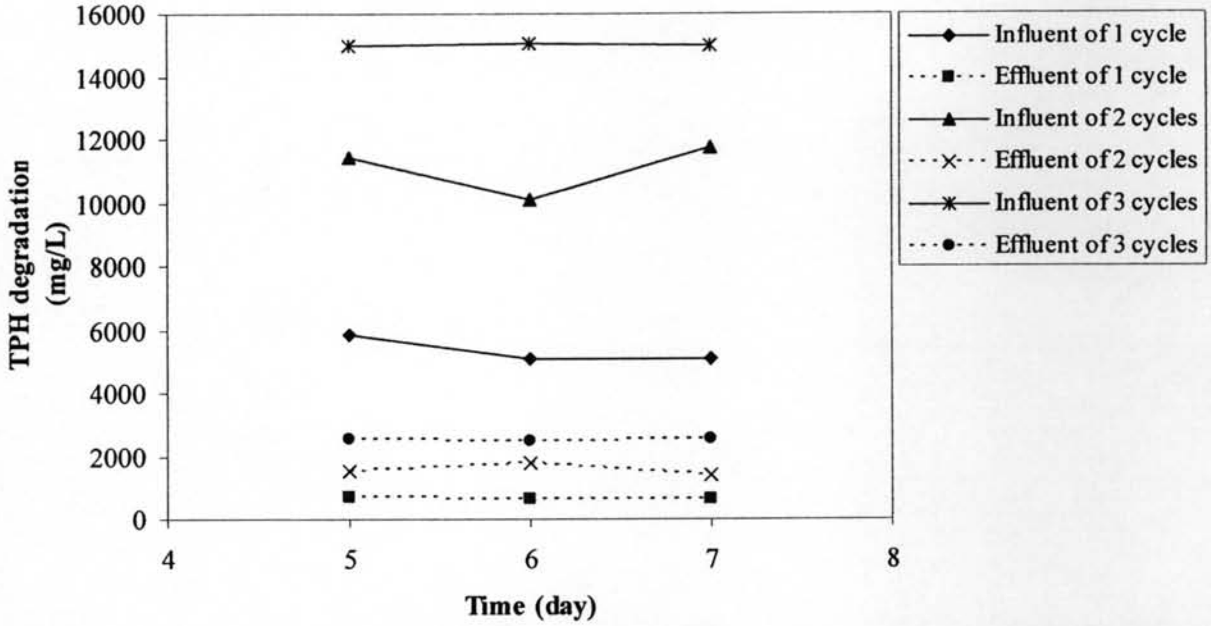


Figure 4.31 TPH influent and effluent of the SBR operation at various number of cycles per day in the presence of Tween 80 concentration 0.2 %w/v.

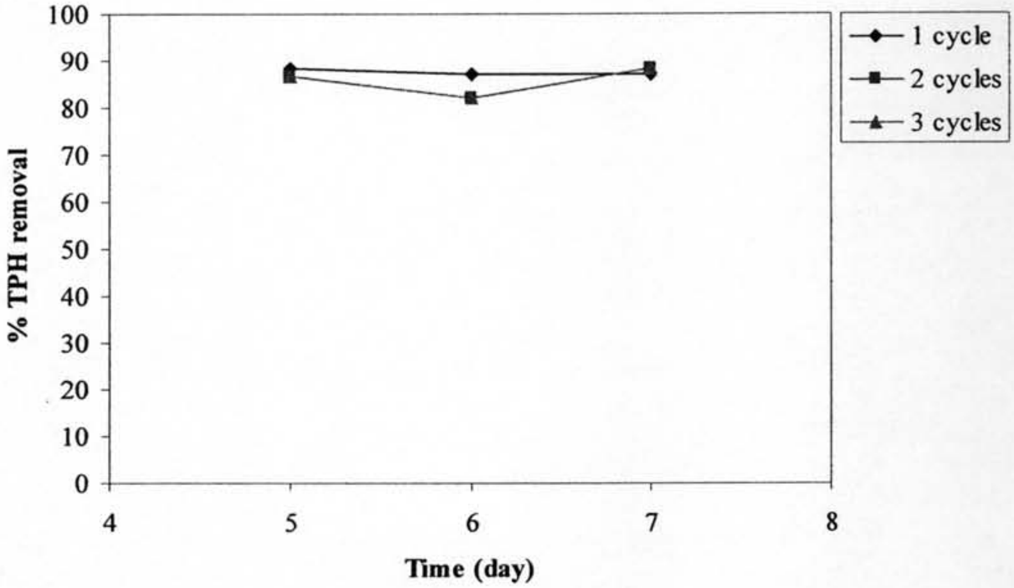


Figure 4.32 Percent TPH removal of the SBR operation at various numbers of cycles per day in the presence of Tween 80 concentration 0.2% w/v.

All of the results of COD, TOC and TPH extraction correspond reasonably well as shown in Figure 4.33. They showed the same trend that the system was optimized at 1 cycle per day of the SBR operation which provided the highest percent removal when compared to other SBR operations. This condition provided the most appropriate quantity of nutrients or the amount of carbon and other minerals in the aqueous phase for the microorganisms. Addition of Tween 80 at increasing concentration (0.2% w/v) showed a gradual increased in chemical oxygen demand when compared to the system using lower surfactant concentration (0.1% w/v).

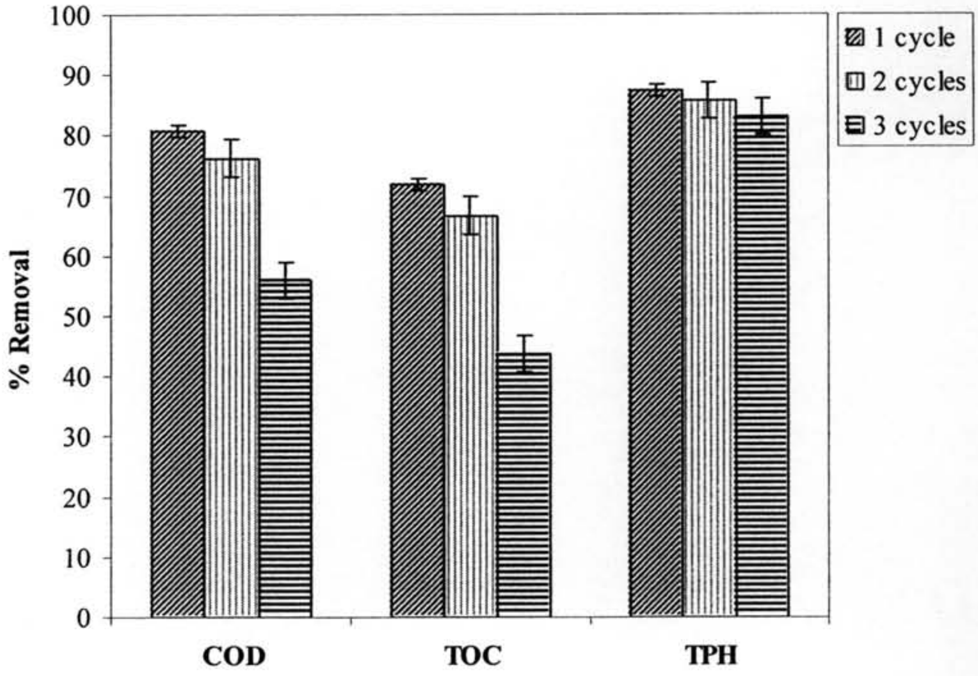


Figure 4.33 Percent removal of TPH, COD and TOC at the various number of cycle of SBR operation in the presence of Tween 80 concentration 0.2% w/v.

4.4.4 Microbial Growth

Figure 4.34 illustrates the growth of microorganisms of various SBR operation times which shows that the growth of microorganism reached stationary phase after 5 days. It can be seen that 3 cycles per day of the SBR operation provided the highest growth of microorganisms. However, as microorganisms utilize the hydrocarbon as carbon source and energy source, if hydrocarbons loading to the reactor is low and time for degradation is short, the microbial population can decrease due to the limited carbon source for their growth. The period time was also very important to the biodegradation, especially for the polyaromatic hydrocarbon compounds which required a long time to degrade completely.

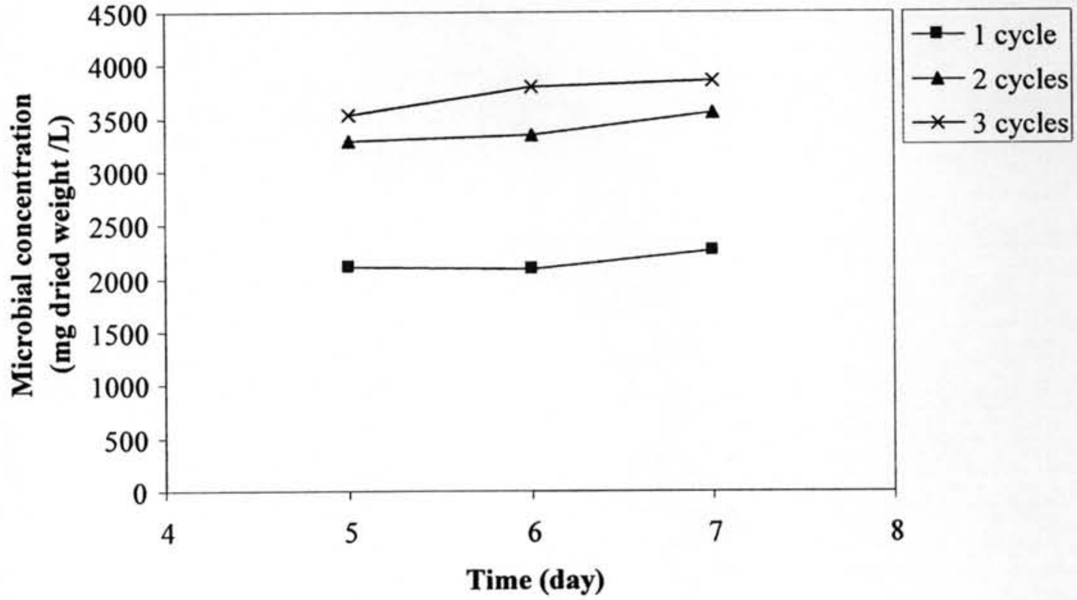


Figure 4.34 Growth of the indigenous microorganisms of the SBR operation at 2 and 3 cycles per day in the presence of Tween 80 concentration 0.2% w/v.

There are several mechanisms exist for the surfactant enhanced biodegradation as follws: (1) Surfactant enhancement could also be the enhanced solubility and/ or the greater bioavailability of HOCs to the microorganisms. (2) The presence of surfactant will not only stimulate bacterial growth, but also induces enzymes that may be needed to further break down contaminants. (3) The enhancement by surfactant could be due to the promoted microbial growth since the surfactant can be utilized as a readily available carbon and energy source. (4) Surfactant enhancement could also result in co-metabolism between surfactant and contaminants, since the concentration of some of the contaminant could be below the threshold to sustain significant microbial growth. However, these reasons are an advantage to the surfactant-enhanced biodegradation of oil sludge because microorganisms focused on the biodegradation of oil sludge not from surfactant. If the surfactant was degraded more than hydrocarbons, the hydrocarbons would not be solubilized into the aqueous phase but it would separate as an oil phase on the top of solution. This effect might occur in some kind of surfactant but not for Tween 80. Tween 80 was very suitable surfactant to aid the biodegradation of oil sludge

Finally, as the hydrocarbons loading was provided as food for the microorganisms inside the bioreactors, hence, the amount of food per the number of microorganisms (or F/M ratio) was necessary to calculate. This ratio shows whether the amount of food to the microbes proper or not. If the ratio was close to 0.1, it expressed the suitable organic loading to the microorganisms. Figure 4.35 shows the F/M ratio in each the SBR operation cycle which showed that the F/M ratio decreased with increasing number of cycle per day of the SBR operation. At 1 cycle per day, the average F/M ratio was about 0.091 which was close to 0.1 F/M ratio than the others. This indicates that this operation cycle was the most appropriate for the system studied.

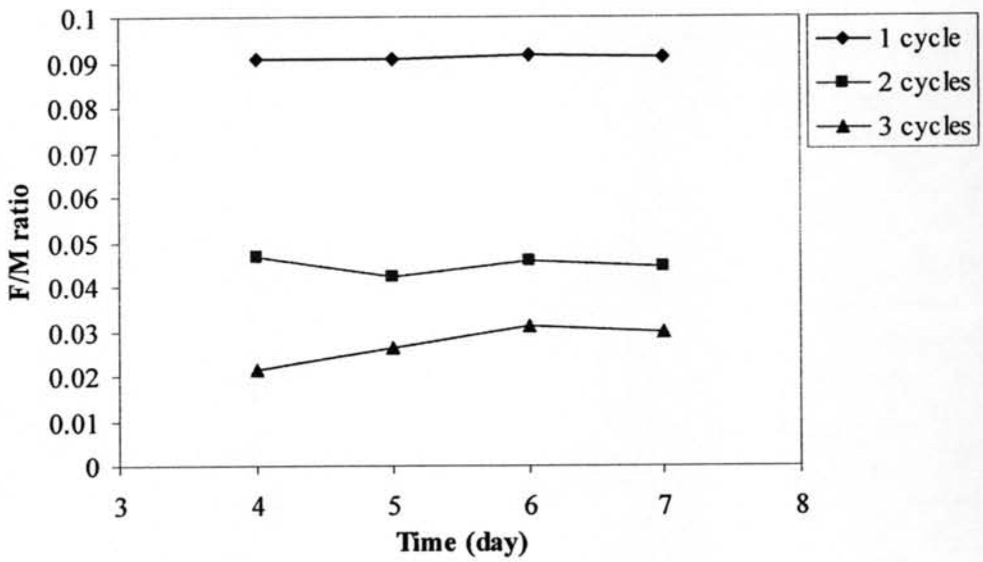


Figure 4.35 F/M ratio of the various SBR operation in the presence of Tween 80 concentration 0.2%w/v.

4.4.5 Yield of Bacteria and Yield of TPH Degradation

In the previous sections, the degradation of hydrocarbon in the sludge oil by the microorganisms was reported as the TPH degradation during the course of biodegradation. It is also important to observe the yield in which the hydrocarbons were degraded biologically and the yield in which the microorganisms utilize hydrocarbon for their growth. Figure 4.36 shows the yield of bacteria and yield of TPH degradation at various cycles of SBR operation per day in the presence of Tween 80 at the concentration of 0.2% w/v. The results showed that when the cycle of the SBR operation increased, the yield of bacteria decreased and the yield of TPH degradation increased. The highest yield of bacteria was observed at 1 cycle per day of the SBR operation which was about 0.46 mg microbial concentration/ mg degraded TPH. The highest yield of TPH degradation was observed at 3 cycles per day of the SBR operation which was about 3.60 mg degraded TPH/ mg microbial concentration.

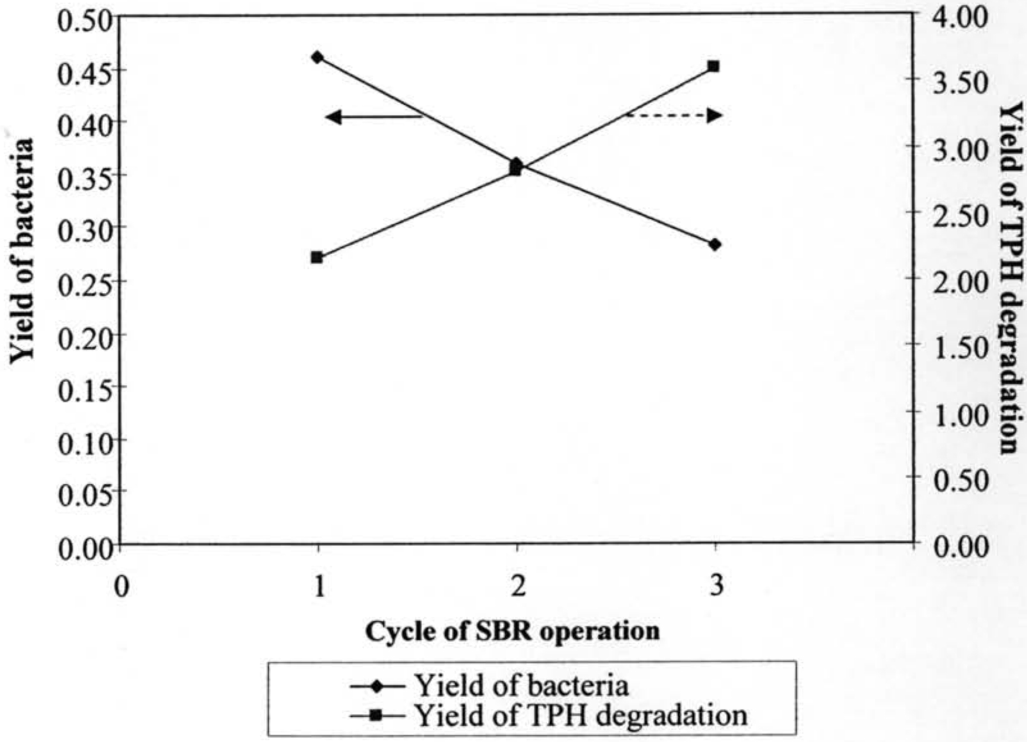


Figure 4.36 Yield of bacteria and yield of TPH degradation at various cycles of SBR operation per day in the presence of Tween 80 concentration 0.2% w/v.

4.4.6 GC/MS Analysis of The Eenhanced Biodegradation of Hydrocarbons in Oil Sludge by Nonionic Surfactant

In this section, the optimum conditions for the biodegradation were chosen which consist of the oil loading rate 1.0 kg/m^{3}d, 1% w/v oil loading, nonionic surfactant (Tween80) at the concentration of 0.2% w/v and the SBR operation of 1 cycle per day. These conditions were chosen to analyze for the hydrocarbon contents before and after the biodegradation by GC/MS. Figure 4.37 and Figure 4.38 show the identification peaks of the hydrocarbon compounds in the oil sludge before and after degradation as analyzed by GC/MS.

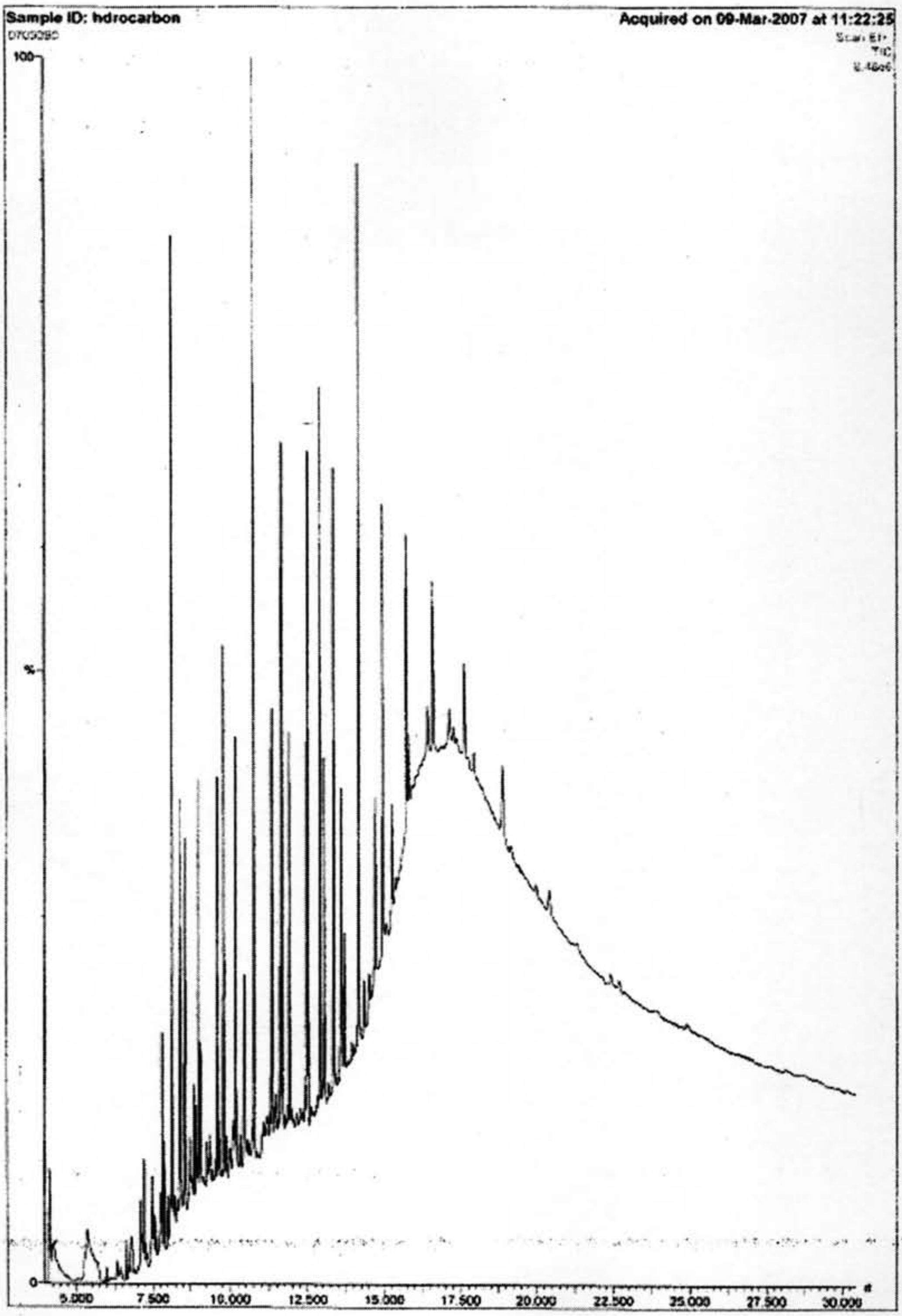


Figure 4.37 Identification peaks of the hydrocarbons in the oil sludge before degradation analyzed by GC/MS.

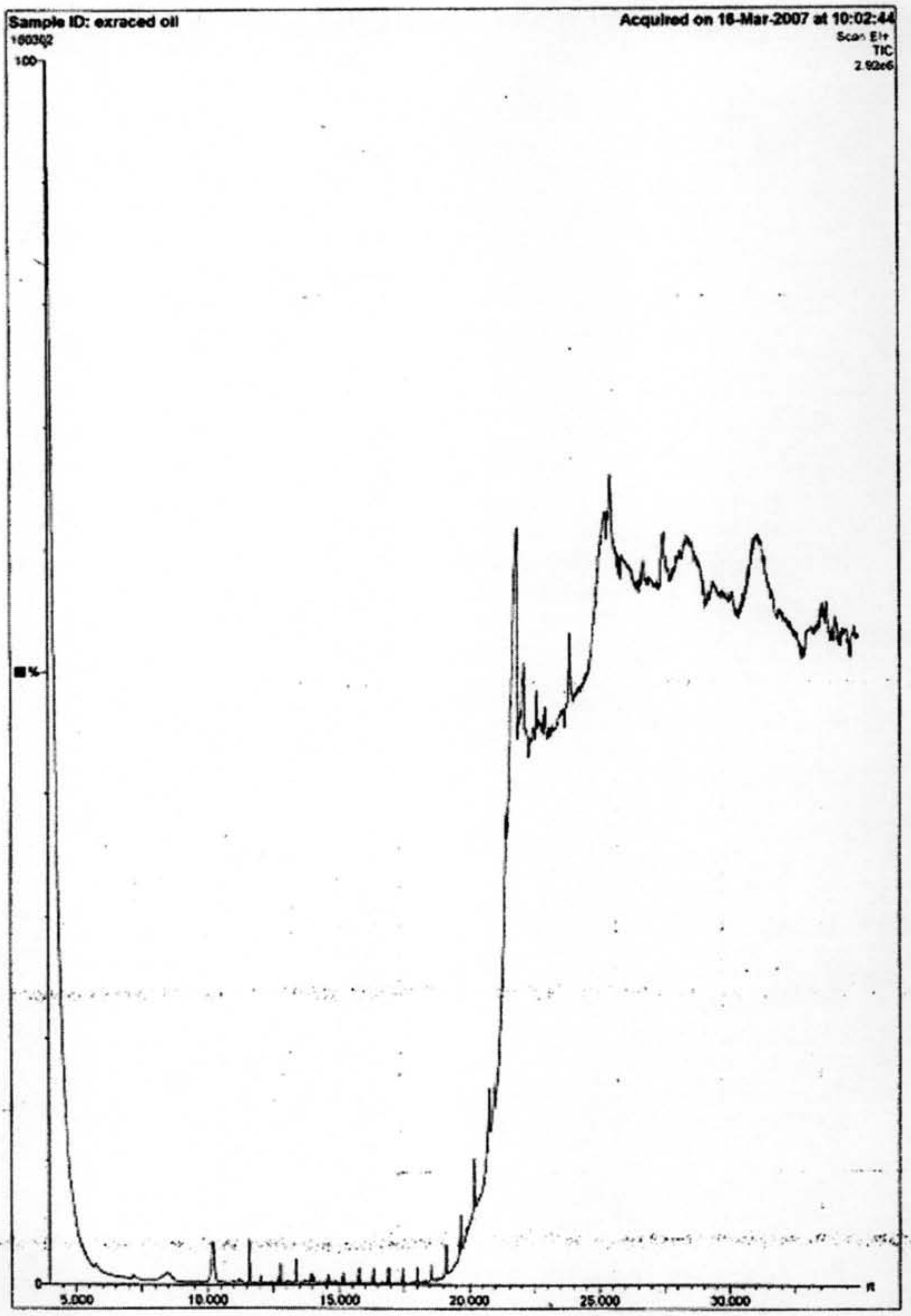
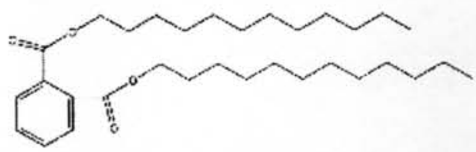
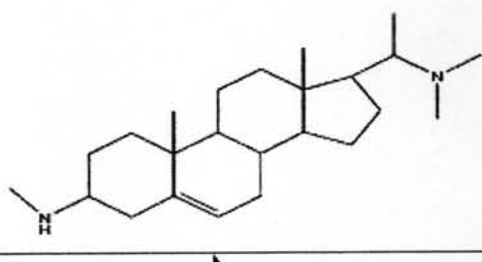
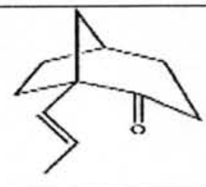


Figure 4.38 GC/MS analysis of the reactor solution after biodegradation process.

The hydrocarbon compounds in the oil sludge was utilized by microorganisms for their carbon and energy source. As observed form Table 4.1 and Table 4.2, the microorganisms preferred to utilize carbon in cyclic aromatic compounds than long chain alkane compounds. The high molecular weight compounds were degraded by microorganisms and the hydrocarbons that were difficult to degrade were the hydrocarbon bond with oxide, nitrogen and silica. These components were still presented in the reactor effluent.

Table 4.2 The hydrocarbon contents in the extracted oil from oil sludge before degradation

Type of hydrocarbons in the extracted oil from oil sludge	Molecular Weight	Structure
Didodecyl Phthalate	502	
1-Fluoro-Dodecane	188	
Pregn-5-Eng-3,20-Diamine,N3,N20,N20-Trimethyl-, (3-Beta,205)-	358	
1-(1-Propenyl)-Bicyclo3.2.1.Octan-2-One	164	
1-Dotriacontanol	466	

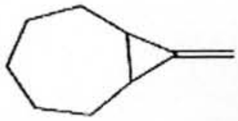
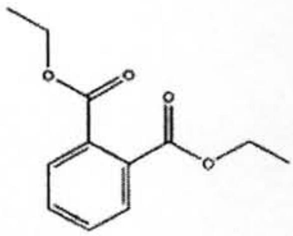
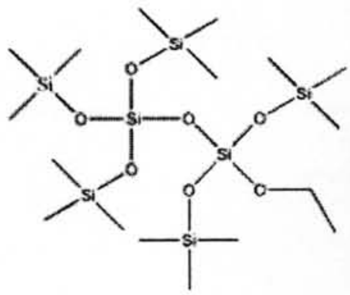
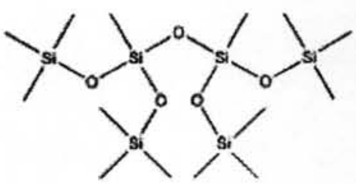
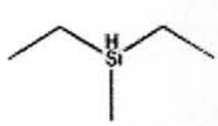
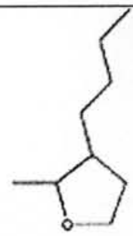
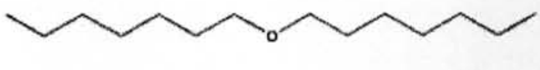
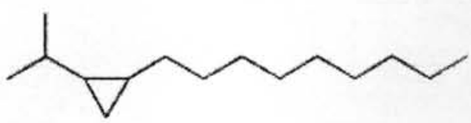
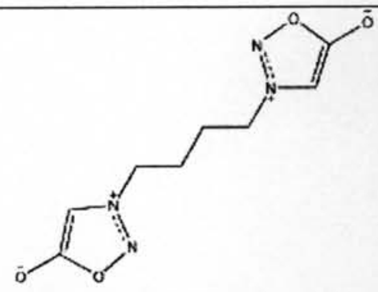
Type of hydrocarbons in the extracted oil from oil sludge	Molecular Weight	Structure
Bicyclo5.1.0 Octane,8-Methylene	122	
Diethyl Phathalate	222	
3-Ethoxy-1,11,7,7,7-Hexamethyl-3,5,5,Tris(Trimethylsiloxy) Tetrasiloxane	562	
1,1,1,3,5,7,7,7-Octamethyl -3,5-Bis(Trimethylsiloxy) Tetrasiloxane	458	
Dodecane	145	

Table 4.3 The hydrocarbon contents in the extracted oil from oil sludge after degradation

Type of hydrocarbons in the extracted oil from oil sludge	Molecular Weight	Structure
Dimethylethylsilane	102	
Hexadecanal Cyclic Ethylene Acetal	284	
Trans-2-Methyl-3-Butyltetrahydrofuran	144	
Dodecane	145	
Bis(1-heptyl) Ether	214	
1-Dodecyl Fluoride	188	
n-Dotriacontanol	466	
Cyclopropane,1-(1-Methylethyl)-2-Nonyl-	210	

Type of hydrocarbons in the extracted oil from oil sludge	Molecular Weight	Structure
Tetramethylenedi- 3,3'-Sydnone	226	
1-(1-Propenyl)-Bicyclo3.2.1.Octan-2-One	164