CHAPTER IV
RESULTS AND DISCUSSION

4.1 Enhanced Solubilization of Hydrocarbons in Oil Sludge by Nonionic and Anionic Surfactants

4.1.1 Determination of Contact Time Required for Solubilization of Oil Sludge by Single- and Mixed-Surfactants Systems

In order to study the effect of anionic, nonionic and mixed surfactants on the solubilization of petroleum hydrocarbons in the oil sludge, it is imperative to first determine the amount of time required for the solubilization process to reach equilibrium. Tween 80 (nonionic surfactant), SDS (anionic surfactant) or mixed surfactants (Tween 80 and SDS), at a predetermined concentration, was added into three separated sets of flasks containing oil sludge with constant mixing. 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 shown 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. Figure 4.1 presents the enhanced solubilization as a function of time for all three surfactant systems compared with the control where no surfactant was added. It can be seen that the solubilization occurred very rapidly during 6 to 28 hours for all cases, and then became quite constant after 48 hours. Therefore, it can be concluded that equilibration time of 48 hours is adequate for the solubilization to complete. Consequently, in all experiments in the next parts of the study, the samples were taken for analysis after 48 hours or 2 days.

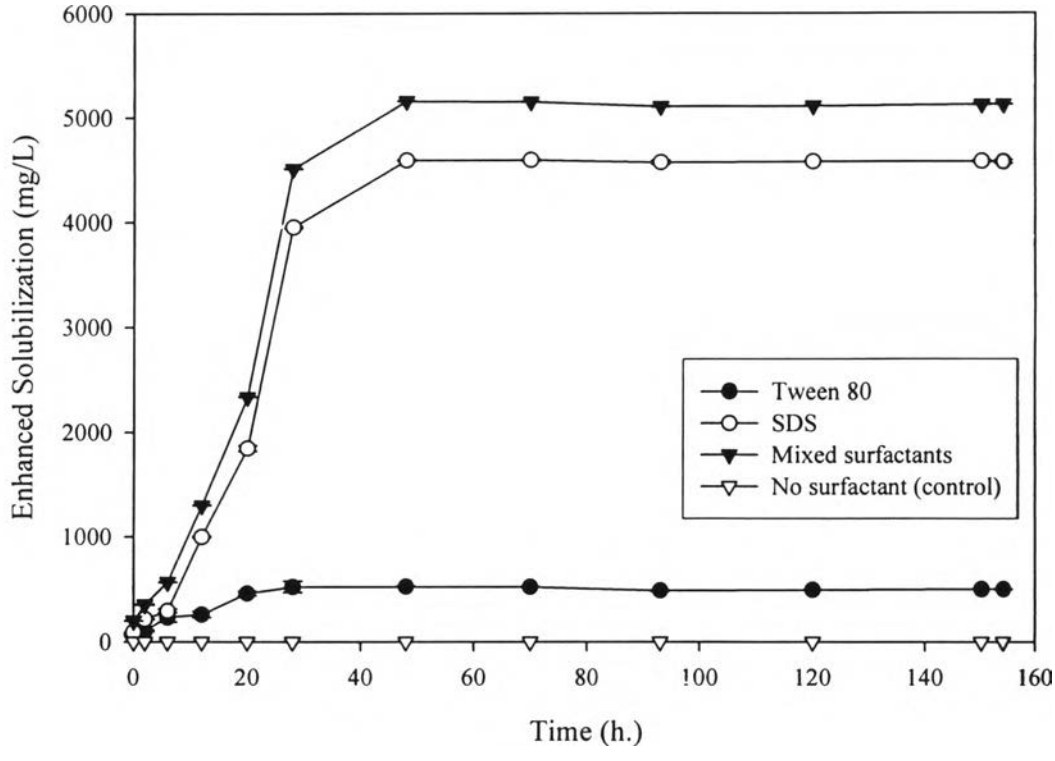

Figure 4.1 Contact time profile of the enhanced solubilization of oil sludge by single and mixed- surfactants systems.

4.1.2 Effect of Single and Mixed 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 hydrocarbons in oil sludge sample without adding surfactant was relatively low. The net solubilization in the control flask as measured by TOC (Total Organic Carbon) and COD (Chemical Oxygen Demand) was found to be 38.39 and 114 ppm, respectively.

In order to improve the solubilization of the hydrocarbons in oil sludge, the subsequent experiments were carried out with addition of the surfactants. Varying amount of the surfactant, SDS (anionic surfactant) or Tween 80 (nonionic surfactant) was added into the flasks containing the same amount of oil sludge sample and their effect on the solubilization of hydrocarbons in oil sludge was examined by measuring TOC and COD of the filtered solution taken from the flasks.

The solubilization of hydrocarbon in oil sludge as enhanced by the addition of SDS and Tween 80 as a function of added surfactant concentration are shown in Figures 4.2 (measured as TOC) and 4.3 (measured as COD), respectively. For both surfactants, it was found that initially the solubilization of the hydrocarbons increased with increasing the added concentration of surfactant in the system. Then the enhanced solubilization reached its maximum value at a specific concentration for each surfactant. For SDS, maximum solubiiization of 537.9 ppm TOC or 5700 ppm COD was achieved at approximately 2 % w/v. For Tween 80, maximum solubilization of 403.3 ppm TOC or 2,814.31 ppm COD was achieved at approximately 0.1 %w/v. Beyond these concentrations, the solubilization gradually decreased as the surfactant concentration increased. It can be seen that SDS had greater effect on enhanced solubilization of hydrocarbon in the sludge, however, the effect exerted by SDS occurred at a much higher concentration when compared with Tween 80. In contrast, although the enhancing effect of Tween 80 on solubilization was not as much as that of SDS but it required much lower concentration. From the results, it is obvious that the addition of these surfactants can enhance solubilization of the hydrocarbons in the sludge. In this aspect, further study was carried out using mixed-surfactant system of SDS and Tween 80. In this set of experiments, Tween 80 concentration in mixed-surfactant system was fixed at 0.1%w/v (where the highest solubilization occurred) while SDS concentration was varied. For the mixed-surfactant system, maximum solubilization of 1,678 ppm TOC or 8,478 ppm COD was obtained at 2%w/v SDS which is quite similar to the single-surfactant system. It can also be seen that the greater enhancing effect on hydrocarbon solubilization was observed in the mixed-surfactant system compared to the single-surfactant systems where Tween 80 or SDS was added alone.

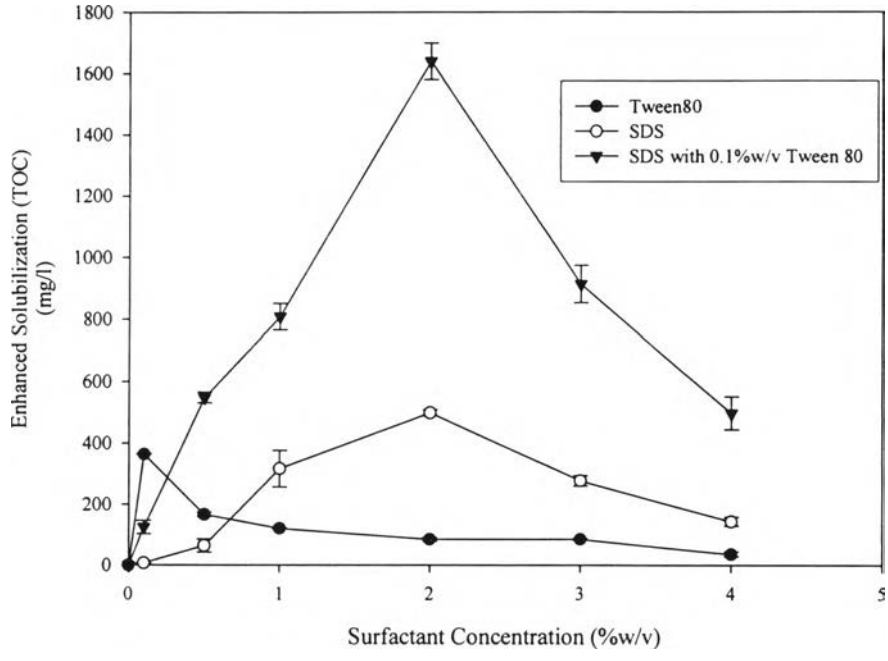

Figure 4.2 Enhanced solubilization (TOC) of hydrocarbons in oil sludge by the addition of SDS, Tween 80 or mixed surfactants at various concentrations.

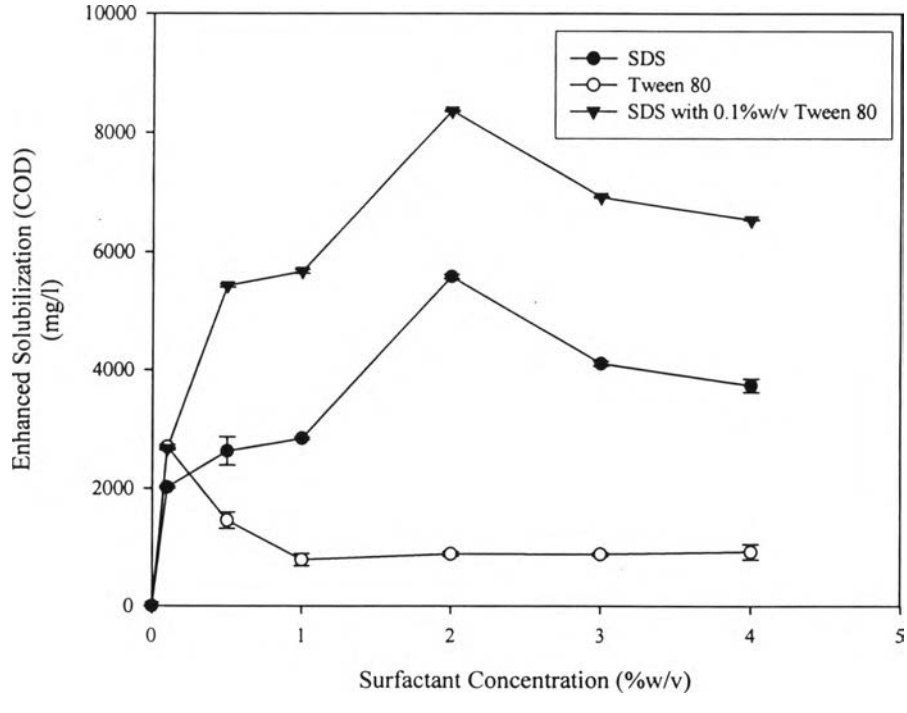

Figure 4.3 Enhanced solubilization (COD) of hydrocarbons in oil sludge by the addition of SDS, Tween 80 or mixed surfactants at various concentrations.

In order to quantify the effect of nonionic and anionic surfactants on the solubilization of individual hydrocarbons present in oil sludge, the oil sludge samples after equilibrated with Tween 80 (0.1% w/v), SDS (2% w/v) or mixed Tween 80 and SDS (0.1% and 2% w/v) were analyzed by GC/MS and the %enhanced solubilization was calculated with respect to the control (no added surfactant) as shown in Figure 4.4. It can be obviously seen that the addition of the surfactants both in single- and mixed-surfactant systems greatly enhanced the aqueous solubility of most of the hydrocarbons in the oil sludge. The solubilization of hydrocarbons present in the sludge increased from approximately 20% to as high as 100% when compared to the control with no addition of surfactant. When comparing between Tween 80 and SDS, Tween 80 was shown to have slightly higher enhancing effect on the solubilization of hydrocarbons with lower number of rings (i.e., phenanthrene) than SDS whereas SDS had more effect on the solubilzation of hydrocarbons with higher number of rings (i.e., pyrene). However, in general the mixed-surfactant system was shown to have the highest effect on solubilization of most of the petroleum hydrocarbons in oil sludge. Thus, the enhancing effect on the solubilization of hydrocarbons is in the following order: mixed SDS and Tween 80 > pure SDS > pure Tween 80.

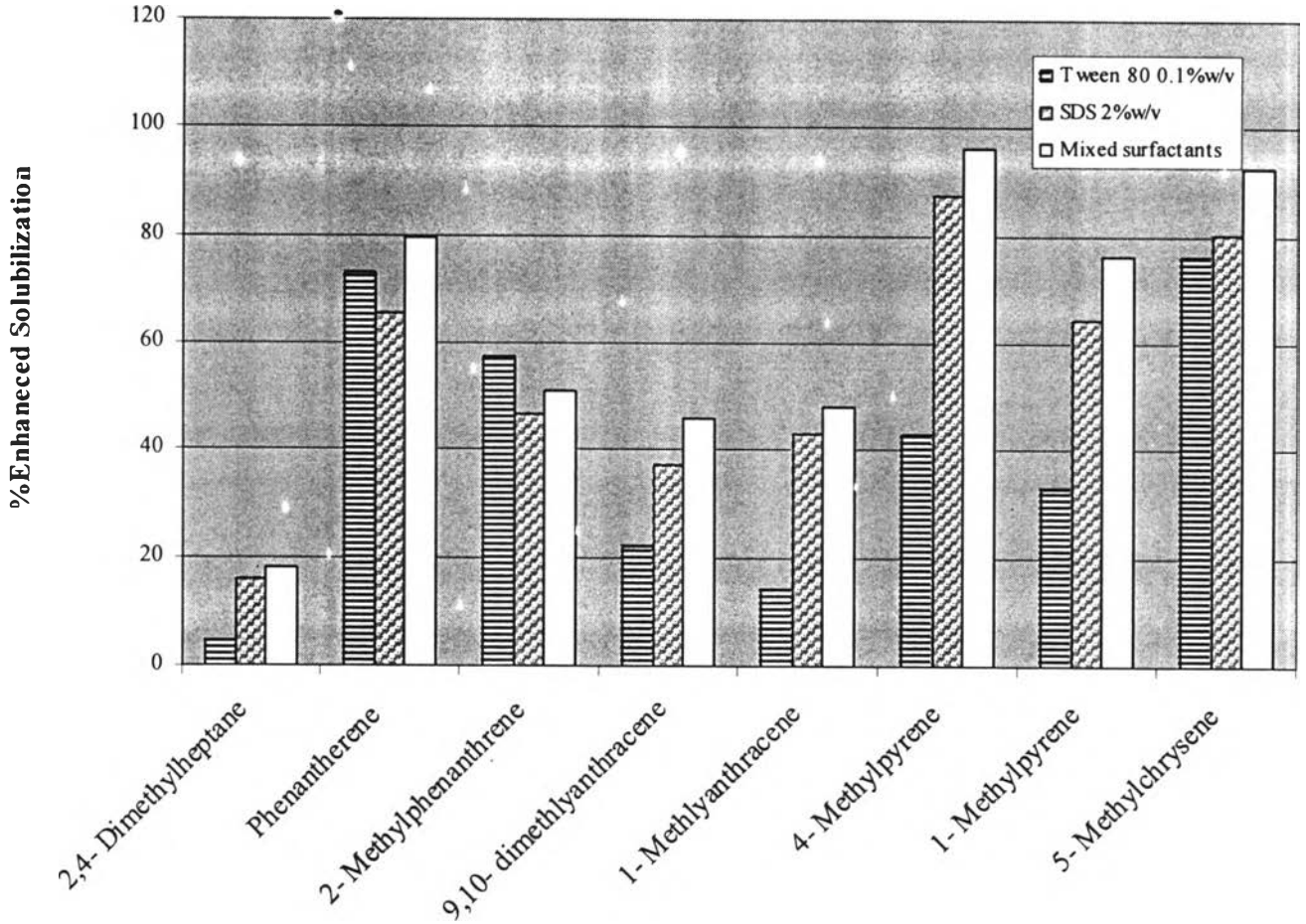

Figure 4.4 Effect of surfactant on enhanced solubilization of various types of hydrocarbons in oil sludge.

4.2 Enhanced Biodegradation of Hydrocarbons in Oil Sludge by Single and Mixed Surfactants

In this part of the study, the effect of single and mixed surfactants on biodegradation of hydrocarbons in oil sludge was investigated by using two types of microorganisms; the indigenous bacteria consortia originally present in the oil sludge and *Pseudomonas aeruginosa*, isolated from the petroleum contaminated site in Thailand. The effect was examined in two aspects: the growth and hydrocarbon biodegradation of the bacteria in the presence of surfactants and the results were compared to the systems without addition of surfactants. The growth of the bacteria was measured as dry weight cell method whereas the biodegradation of hydrocarbons in the oil sludge was calculated based on total petroleum hydrocarbon (TPH) being degraded by the microorganism as measured by TPH solvent extraction technique.

4.2.1 Growth and TPH Degradation of Indigenous Bacteria in the Absence of Surfactant

As the oil sludge was found to contain indigenous bacteria capable of growing on and degrading hydrocarbons present in the oil sludge, it is important to first examine the growth and hydrocarbons biodegradation of the indigenous bacteria. In this part of the study, the control was the flasks which were autoclaved in order to terminate all living microorganisms. The extent of biodegradation of hydrocarbons in oil sludge sample was presented as TPH degradation obtained by determining the loss of TPH during biodegradation (based on a fixed volume of 30 ml) with respect to the autoclaved control flasks. The growth of indigenous bacteria was reported as dry weight cell mass. Figure 4.4 shows the growth of indigenous bacteria in the oil sludge in comparison with the control experiments (autoclaved). The typical growth curve shown in the figure revealed that the indigenous bacteria present in the sludge was capable of growing on the sludge. The course of the bacteria growth took 7 days to reach the stationary phase at the cell concentration of approximately 1 mg/L (dry weight). Figure 4.5 shows that the biodegradation of hydrocarbons in the sludge was slow during the first few days and increased rapidly

during the 4^{th}-5^{th} day and became constant after that. This can be explained that it would require some time for the bacterial culture to build up cell mass high enough to degrade the hydrocarbons. After 7 days, the amount of TPH degradation by indigenous bacteria was approximately 90 mg based on a fixed volume of 30 ml.

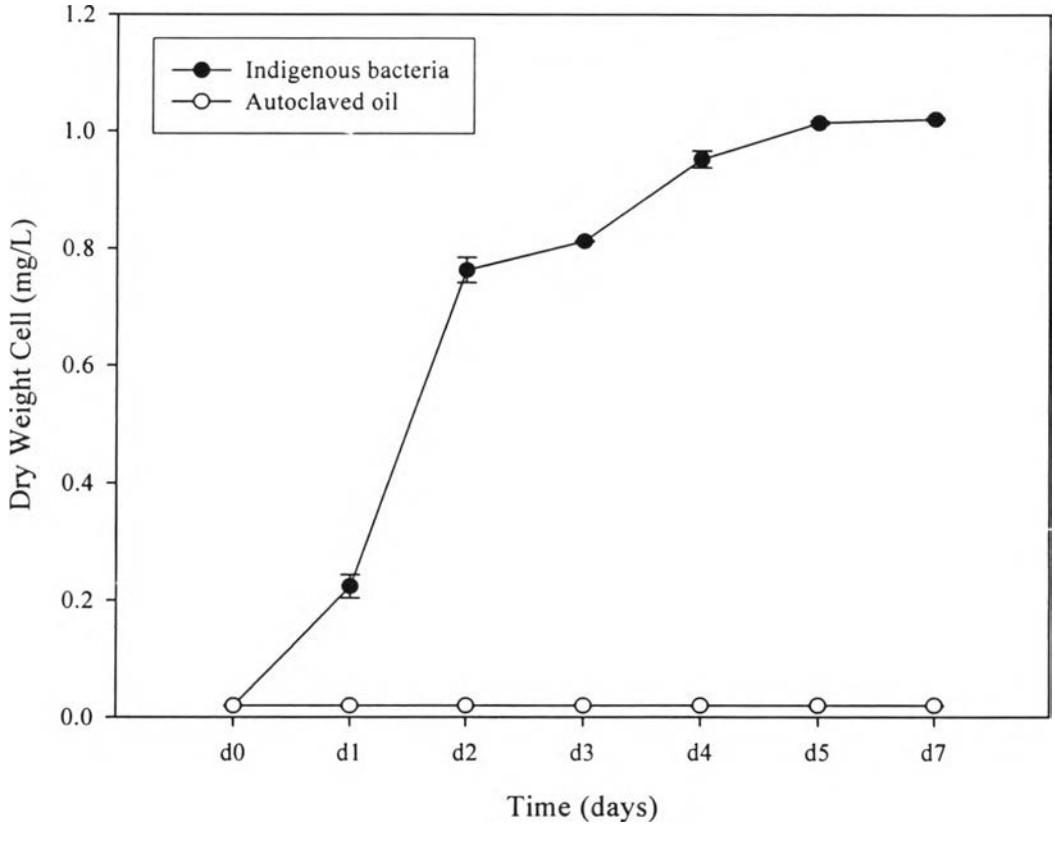

Figure 4.4 The growth of indigenous bacteria in oil sludge without addition of surfactant.

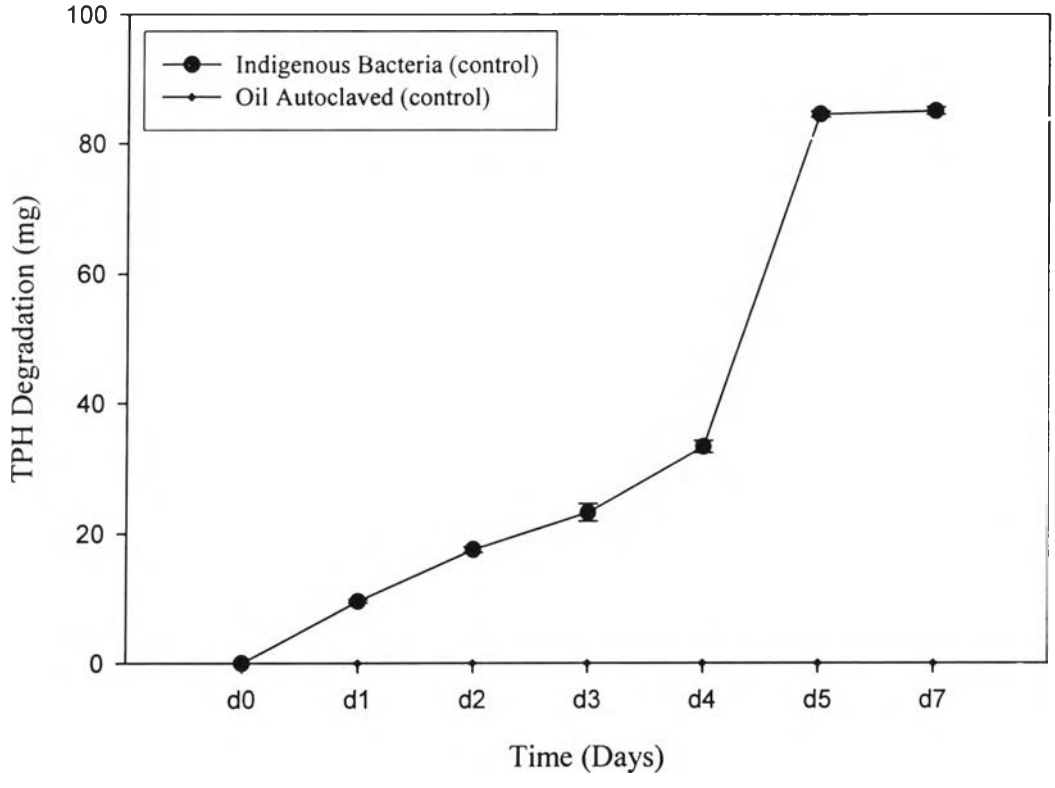

Figure 4.5 TPH Degradation of oil sludge (based on a fixed volume of 30 ml) by indigenous bacteria without addition of surfactant.

4.2.2 Effect of Single and Mixed Surfactants on Growth and Biodegradation of Indigenous Bacteria

From the solubilization study in the previous section (4.1.2), the added surfactant concentration that resulted in the highest solubilization of hydrocarbons was chosen for each surfactant to further study the effect on biodegradation of hydrocarbons in the oil sludge. These concentrations were 2%w/v and 0.1%w/v for SDS and Tween 80, respectively, and 0.1%w/v + 2%w/v for mixed surfactants (Tween 80 + SDS). The surfactant was added into the culture containing oil sludge (2%w/v) at the predetermined concentration described above. The culture was then incubated at room temperature on an orbital shaker at 140 rpm for 7 days. The time course of the growth of indigenous bacteria (dry weight cell mass) and TPH degradation in the cultures with and without the addition of surfactants are presented

in Figures 4.6 and 4.7, respectively. The control curves (in the absence of surfactant) were the same as shown in Figures 4.4 and 4.5. In general, the growth curves of the bacteria in the presence of single and mixed surfactants were similar to that of the control (no surfactant). It can be obviously seen that the bacterial growth in the presence of surfactant was significantly higher than the growth of the control (in the absence of surfactant). While the growth of the control was only 1 mg/l, the growth in the presence of surfactant (single and mixed) was in the range of 1 – 1.26 mg/l with the mixed-surfactant system having the highest growth of 1.26 mg/l.

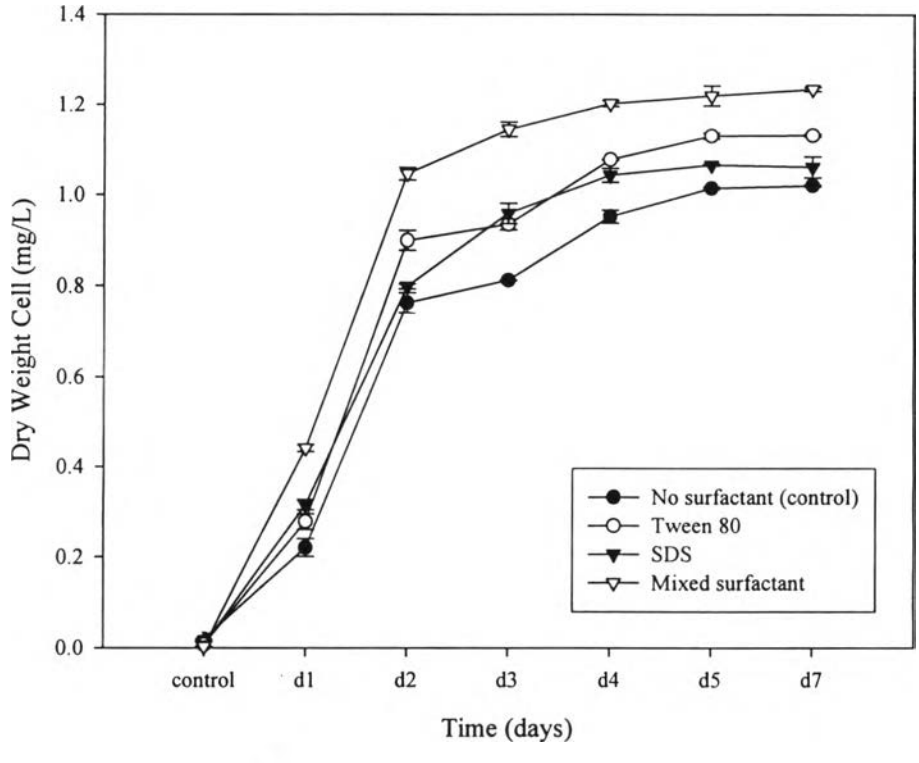

Figure 4.6 The effect of single and mixed surfactants on growth of indigenous bacteria.

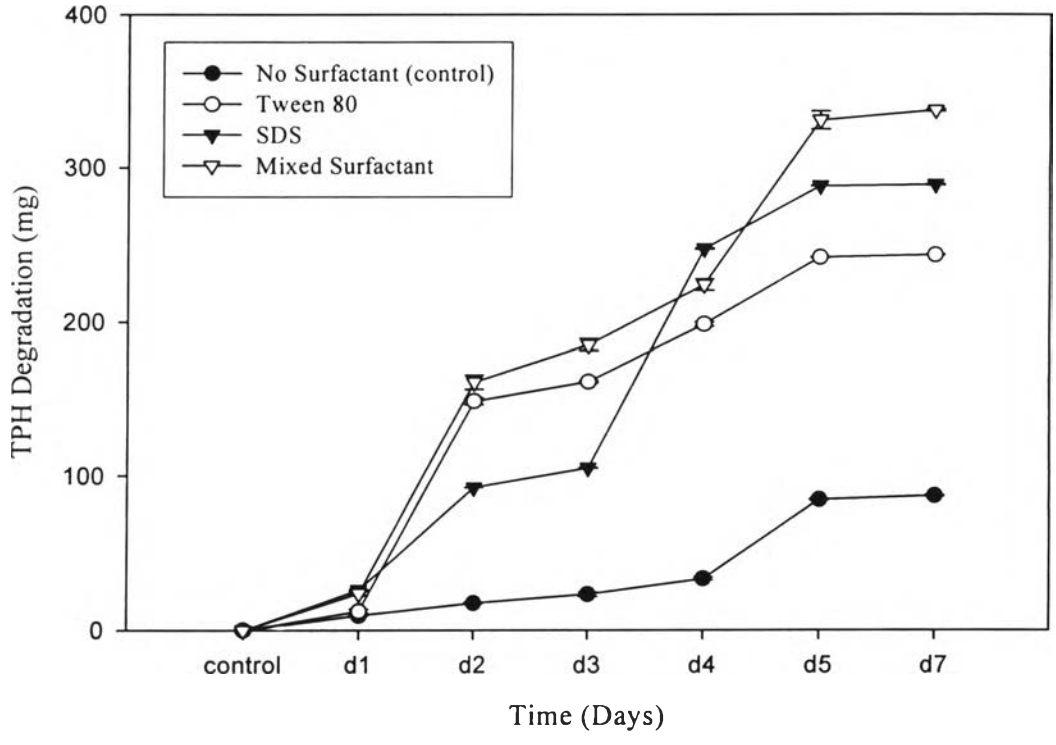

Figure 4.7 The effect of single and mixed surfactants on biodegradation (based on a fixed volume 30 ml) of indigenous bacteria.

For the biodegradation of hydrocarbons in the sludge by indigenous bacteria, Fig. 4.7 clearly shows that the TPH degradation in the cultures with addition of surfactant was much higher than that of the control culture (without addition of surfactant). While the TPH degradation in the control was only 100 mg (based on 30 ml volume), the TPH degradation with the addition of surfactant was in the range of approximately 240-350 mg after 7 days, reflecting 2.4-3.5 times higher than the control. Among these cultures, the culture having mixed surfactants yielded the highest hydrocarbons degradation followed by the cultures having SDS (280 mg) and then Tween 80 (240 mg), respectively.

The oil sludge samples after the biodegradation were further analyzed by GC/MS in order to quantify the degradation of individual hydrocarbons present in the oil sludge. Figure 4.8 presents the biodegradation of individual hydrocarbons in the oil sludge as a percentage of the amount originally present in the

oil sludge before biodegradation (determined from the autoclaved control). From this figure, it can be seen that the biodegradation of all hydrocarbons in the oil sludge was greatly enhanced by the addition of single and mixed surfactants, especially SDS and the mixed surfactants (Tween 80 and SDS) when compared to the control (no added surfactant). In the presence of these surfactants, TPH degradation was found to increase in the range of approximately 2-10 times of the control. In general, the mixed-surfactant system yielded the highest enhancing effect for most of hydrocarbons present in the sludge followed by SDS and Tween 80, respectively. When comparing between various types of hydrocarbons present in the oil sludge, the biodegradation of an alkane (heptane) was the lowest whereas the biodegradation of polyaromatic hydrocarbons with low number of rings (phenanthrene) was shown to be the highest and the extent of biodegradation gradually decreased with the compounds with higher number of rings or with branching. For example, methylpyrene and methylchrysene were biodegraded to the lowest extent. This can be explained that the degrading microorganism is capable of breaking the ring hydrocarbon compounds rather than the straight chain compounds like heptane. This behavior is frequently found in many species of soil bacteria capable of degrading hydrocarbon compounds. For polyaromatic hydrocarbons, it has been generally known that the higher number of rings in the structure of the hydrocarbon compounds, the more difficult the compounds being degraded by the microorganism. The results observed here are in good agreement with this general rule.

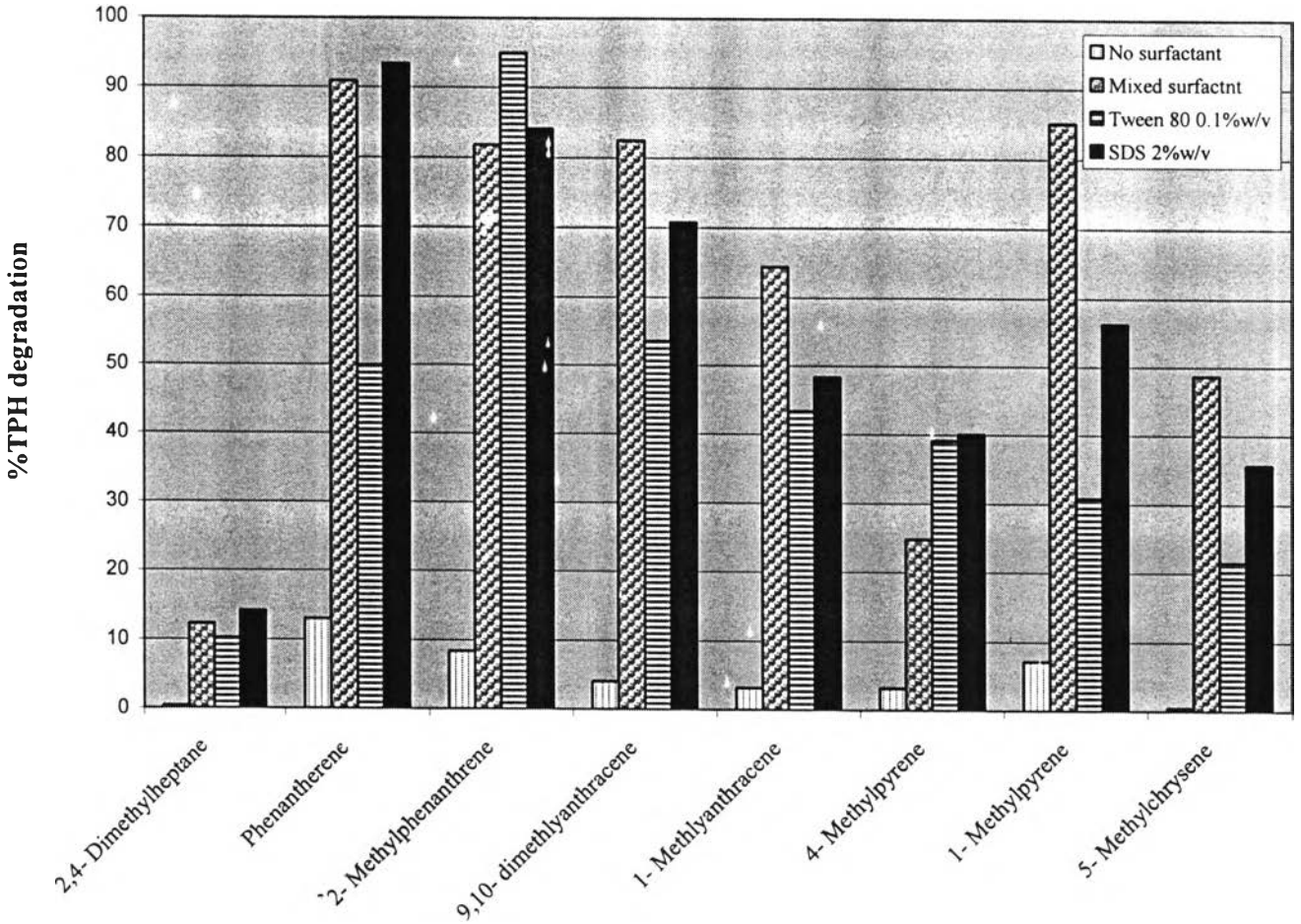

Figure 4.8 The effect of single and mixed surfactants on biodegradation of various hydrocarbons in the oil sludge by indigenous bacteria (reported as type of hydrocarbons).

4.2.3 Effect of Single and Mixed Surfactants on Growth and Biodegradation of *Pseudomonas aeruginosa*

Similar procedures to the biodegradation of hydrocarbons in the oil sludge by indigenous bacteria shown in the previous section were used in this part of the study but *Pseudomonas aeruginosa* isolated from contaminated site in Thailand was used as a degrading microorganism instead. The cultures were incubated, sampled, and analyzed in the same manner as previously described. The growth of *Pseudomonas aeruginosa* and the TPH degradation in the cultures with and without added surfactants are presented in Figures 4.9 and 4.10, respectively. The results were found to be quite similar to those obtained by using indigenous bacteria which indicates a resemblance between the two cultures used in this study. The growth of the bacteria in the presence of single and mixed surfactants was higher than that of the control (no surfactant). For the systems with the addition of surfactants, the growth of the bacteria was shown to be in the range of 1-1.3 mg/l (dry weight cell mass) with the mixed-surfactant system having the highest growth followed by SDS and Tween 80, respectively. For the biodegradation of hydrocarbons in the sludge, it can be obviously seen that the biodegradation in the cultures with the addition of surfactant in both single- and mixed-surfactant systems was significantly higher than that of the control culture (no addition of surfactant). After incubation for 7 days, the TPH degradation in the control flask was only approximately 95 mg based on the same fixed volume of 30 ml whereas the TPH degradation of the cultures with the addition of surfactant was in the range of 270-330 mg on the same basis. This is approximately 3-4 times higher than the control and the culture containing mixed surfactants was shown to have the highest degradation followed by the cultures with the addition of SDS and Tween 80 in single-surfactant systems, respectively.

The samples from the biodegradation cultures were further analyzed by GC/MS in order to determine the biodegradation of individual hydrocarbon present in the oil sludge and the results were presented as a percentage of the amount originally present in the sludge before biodegradation (or control) as shown in Figure 4.11.

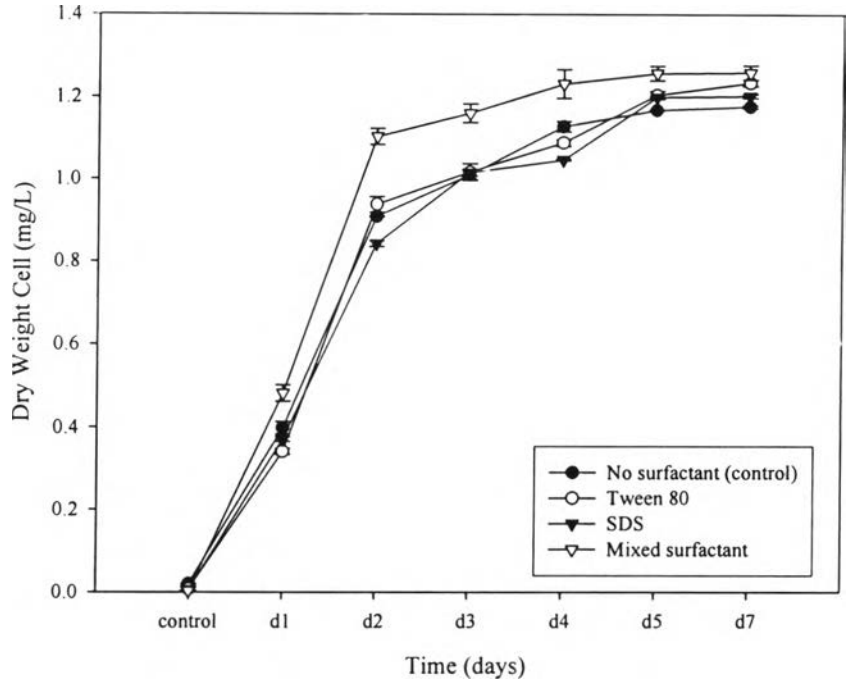

Figure 4.9 The effect of single and mixed surfactants on growth of *Pseudomonas aeruginosa*.

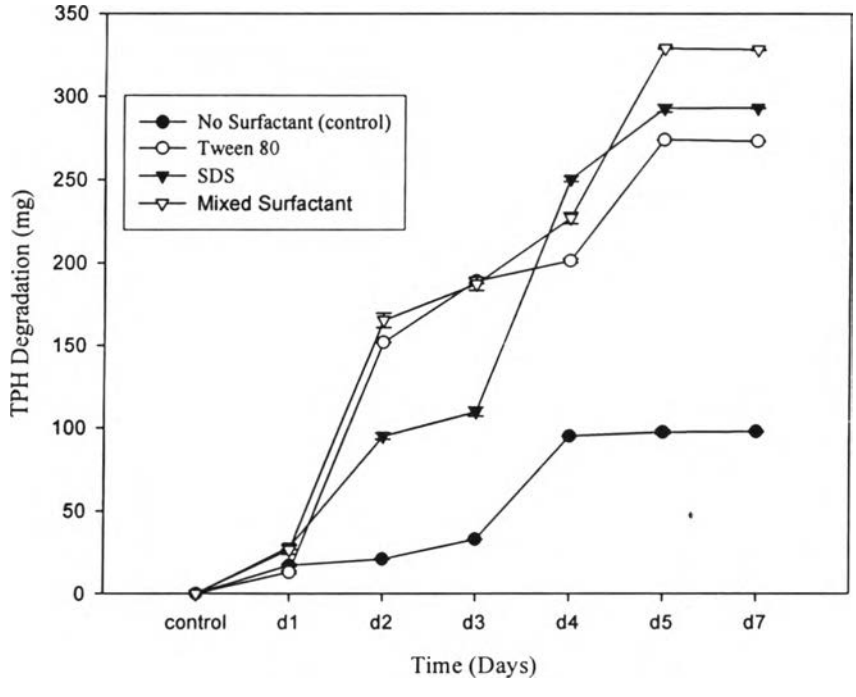

Figure 4.10 The effect of single and mixed surfactants on biodegradation (based on a fixed volume of 30 ml) of *Pseudomonas aeruginosa*.

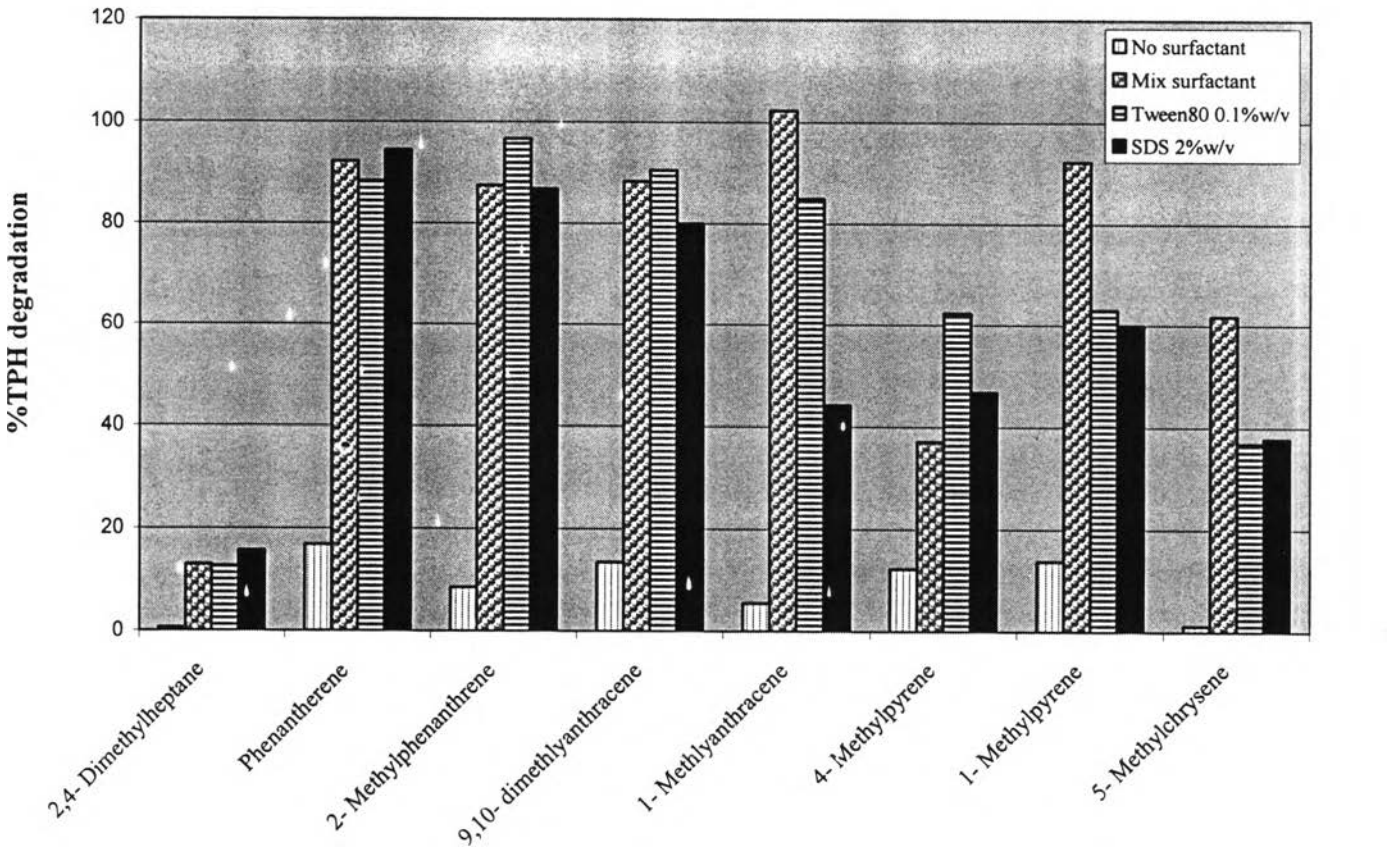

Figure 4.11 The effect of single and mixed surfactants on biodegradation of various hydrocarbons in the oil sludge by *P. aeruginosa* (reported as type of hydrocarbons)

By comparing with the control culture without the addition of surfactant, it can be seen that the biodegradation of most hydrocarbons in the oil sludge by *Pseudomonas aeruginosa* was greatly enhanced by the addition of surfactants in both single- and mixed-surfactant systems, especially in the cultures having mixed surfactants and SDS. In the presence of these surfactants, TPH degradation was found to increase in the range of approximately 4-12 times of the control. For a straight chain hydrocarbon (heptane) and relatively small polyaromatic hydrocarbons such as phenanthrene, all three surfactant systems provided nearly the same enhancing effect on the biodegradation. However, for bigger polyaromatic compounds such as anhtracene, pyrene and chrysene, the mixed-surfactant system of SDS and Tween 80 was shown to have a greater effect on the biodegradation than the single-surfactant systems.

When comparing between various types of hydrocarbons present in the oil sludge, similar results as the biodegradation by indigenous bacteria previously discussed were observed. That is the biodegradation of an alkane (heptane) was the lowest whereas the biodegradation of polyaromatic hydrocarbons with low number of rings (phenanthrene) was shown to be the highest and the extent of biodegradation gradually decreased with the compounds with higher number of rings or with branching. For example, pyrene and chrysene were biodegraded to a much lowest extent when compared to phenanthrene and anthracene. This can be explained that the degrading microorganism is capable of breaking the ring hydrocarbon compounds rather than the straight chain compounds like heptane. The same explanation to that given in the previous section of the hydrocarbons biodegradation by indigenous bacteria could be offered here.

There is important to note that a set of experiments was conducted to determine whether the single and mixed surfactants used in this study can be utilized by microorganisms for their growth. For all three surfactant systems, the surfactant at the concentration used in the biodegradation studies was added into the bacterial culture containing MSM but without the oil sludge. The cultures were monitored by measuring the growth of the bacteria as optical density at 600 nm throughout the cultivation course (7 days) as shown in Figure 4.12. It can be seen that essentially no growth was observed in all cultures having Tween 80, SDS, or mixed surfactants.

Thus, it can be concluded that the bacteria could not utilized surfactants as a carbon source and the observed growth of the bacteria and biodegradation in the sludge seen in the previously sections were the result of the biodegradation of the hydrocarbons, not the surfactant added into the sludge.

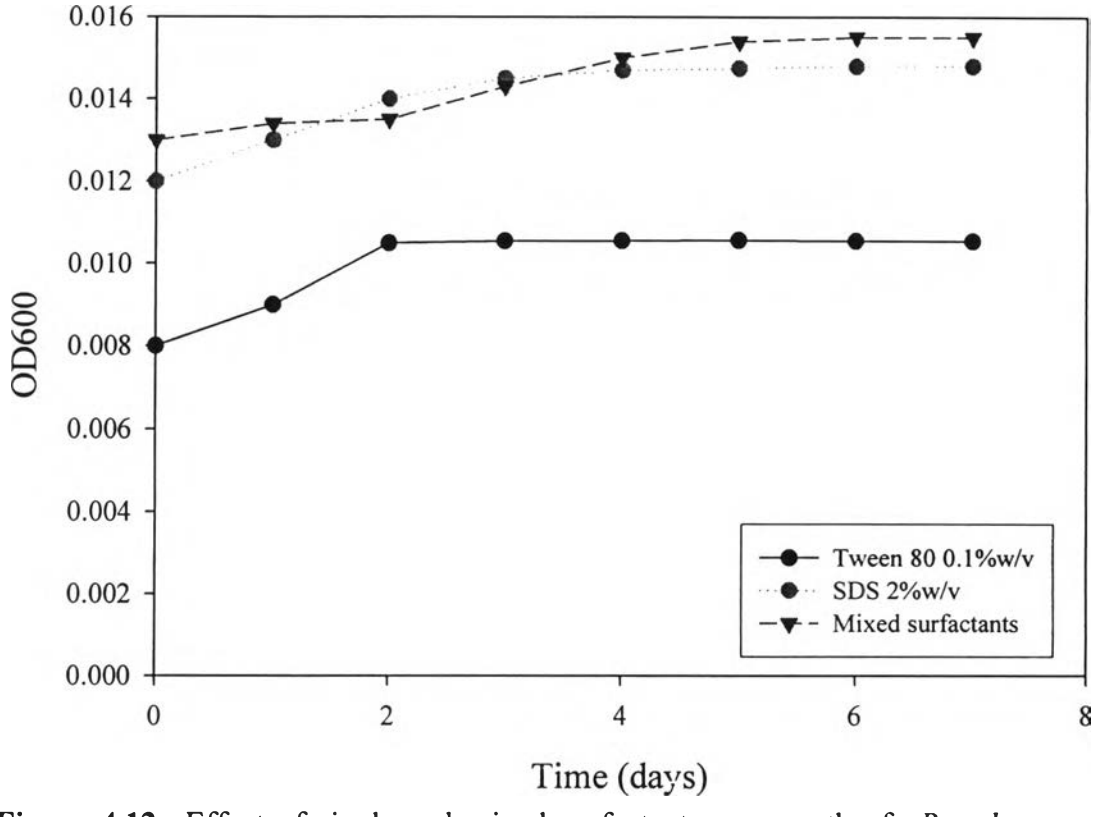

Figure 4.12 Effect of single and mixed surfactants on growth of *Pseudomonas aeruginosa.*

4.3 Rate and Yield of TPH Degradation by Indigenous Bacteria and *Pseudomonas aeruginosa*

In the previous sections, the degradation of hydrocarbons in the oil sludge by the microorganisms was reported as the total TPH degradation (mg) during the course of the biodegradation. It is also important to observe the rate in which the hydrocarbons were degraded biologically and yield in which the microorganism

utilize hydrocarbons for their growth. In this part, the rate and the yield were presented and discussed.

Figure 4.13 shows the rate of biodegradation of hydrocarbons (mg TPH/day) by indigenous bacteria and *Pseudomonas aeruginosa* for all three surfactant systems (Tween 80, SDS and mixed surfactants) in comparison with the control (no added surfactant). It can be seen from this figure that the cultures with the addition of the surfactant had a much higher rate than the control culture. The rates of hydrocarbons biodegradation in three surfactant systems were approximately 3-4 times of the rate obtained in the control with the mixed-surfactant system had the highest biodegradation rate of approximately 48 mg/day. The results can be explained that in the cultures having surfactant, most of hydrocarbons were solubilized in the micellar phase formed by the surfactant molecules in the aqueous phase. In other words, one can say that the solubilized hydrocarbons were being concentrated in the micellar phase where the biodegradation occurred. Consequently, the degrading microorganisms can utilize the hydrocarbons at a much higher rate than the control culture.

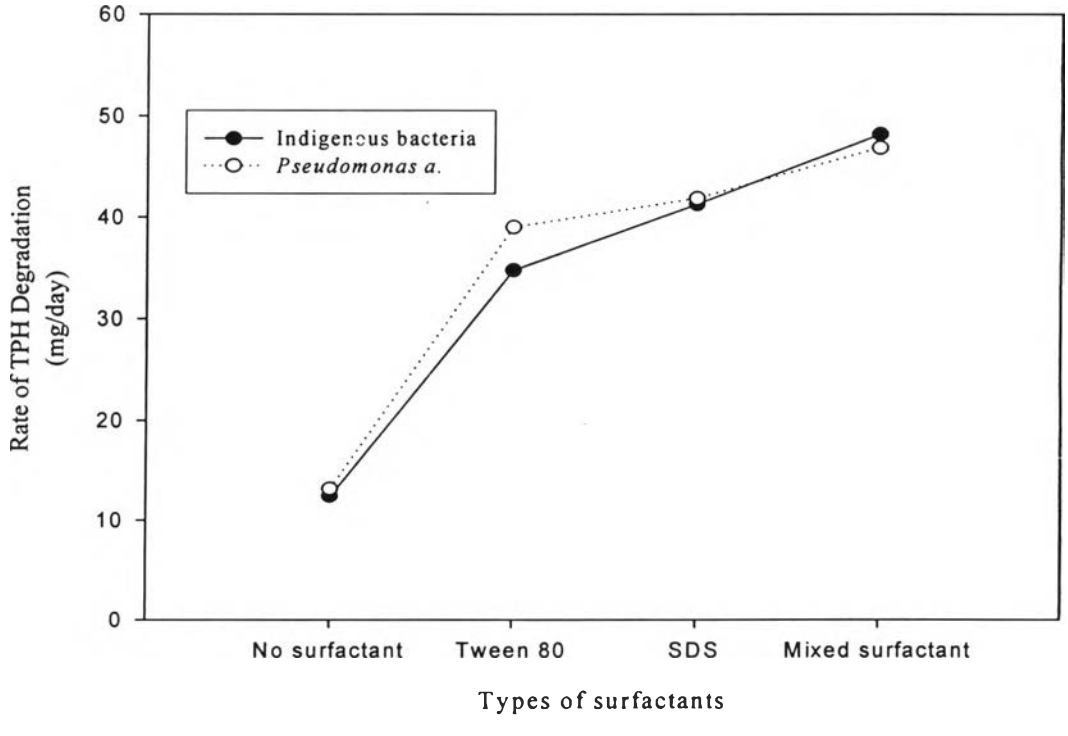

Figure 4.13 The rate of TPH degradation by indigenous bacteria and *P. aeruginosa.*

Figure 4.14 shows the yields (mg degraded TPH/mg dry weight cell) of the hydrocarbons biodegradation of indigenous bacteria and *Pseudomonas aeruginosa* in all three surfactant systems in comparison with the control (no added surfactant). Similar trend to the results observed in Figure 4.13, it can be seen that the yields obtained in the cultures with the addition of surfactant for all three surfactant systems were much higher than the yield obtained in the control culture for both indigenous bacteria and *P. aeruginosa*. The highest yield was observed in the culture having mixed surfactants (5.21) followed by the cultures having SDS (4.88) and Tween 80 (4.43), respectively, whereas the lowest yield (1.67) was observed in the control culture. This can be explained that, although the growth in the cultures with the addition of surfactant was slightly higher than the growth in the control, but the TPH degradation in these cultures was much higher than that of the control. Thus, the yields of the cultures with the addition of surfactant for all three surfactant systems were higher than that of the control.

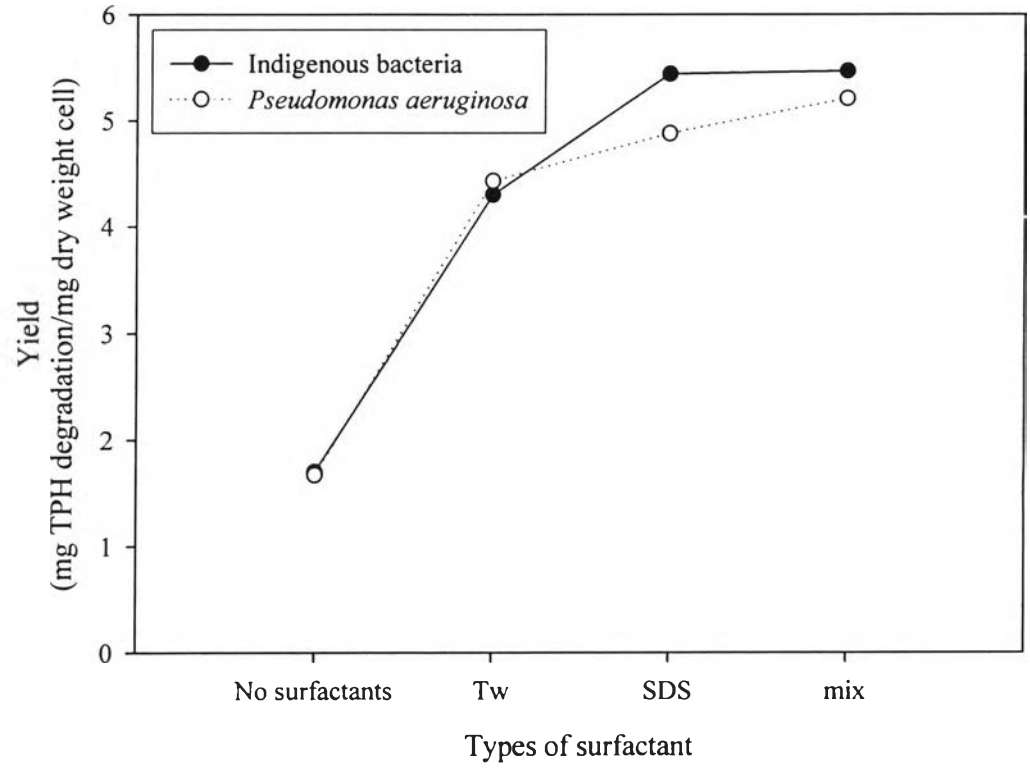

Figure 4.14 The yield of indigenous bacteria and *Pseudomonas aeruginosa.*

Since different amount of surfactant was used in each system, it is important to evaluate the effect of the surfactant on hydrocarbons degradation on the same weight basis. The total TPH degradation and the rate of biodegradation per weight of added surfactant are shown in Tables 4.1 and 4.2, respectively. It can be seen that both total TPH degradation and TPH degradation rate per weight of added surfactant for both microorganisms were highest in the cultures with addition of Tween 80 followed by the cultures having mixed surfactants and SDS, respectively. This is due to the fact that Tween 80 was used at the lowest concentration (0.1 %w/v) among the three surfactant systems, yet provided a reasonable biodegradation of hydrocarbons in the oil sludge.

Table 4.1 Total TPH degradation and the rate of TPH degradation per weight of surfactant for indigenous bacteria.

Types of surfactant	TPH degradation (g / g surfactant)	TPH degradation rate (g / day / g surfactant)
Tween 80	4.87	0.696
SDS	0.29	0.04
Mixed surfactant	0.32	0.046

Table 4.2 Total TPH degradation and the rate of TPH degradation per weight of surfactant for *Pseudomonas aeruginosa*.

Types of surfactant	TPH degradation (g / g surfactant)	TPH degradation (g / day / g surfactant)
Tween 80	5.46	0.78
SDS	0.293	0.05
Mixed surfactant	0.313	0.045

4.4 Enhanced Biodegradation of Oil Sludge by Mixed Surfactant in Bioreactor

In the previous sections, the effects of single and mixed surfactants on biodegradation of hydrocarbons in the oil sludge were examined in the batch system. In this part of the study, the surfactant-enhanced biodegradation was further examined in a semi-batch bioreactor (0.5 l) using a fill-and-draw technique. As providing the highest biodegradation of hydrocarbons in the batch system, the mixed surfactants (0.1 % Tween 80 and 2% SDS) was consequently used as a model surfactant system in the semi-batch bioreactor system containing 2%w/v of oil sludge in MSM. The basic operation of the semi-batch bioreactor can be described as follows: for each day a predetermined volume of the medium (50 ml) was drawn from the reactor and analyzed for the bacterial growth and hydrocarbon degradation, and then the same volume of the fresh medium containing different amount of oil sludge and surfactant was filled back into the reactor. The two microorganisms, indigenous bacteria and *Pseudomonas aeruginosa*, used in the previous sections

were employed in this bioreactor study. For each culture species, four reactors with different amounts of oil sludge and surfactant ranging from 5-30 ml were used as shown in Table 4.3. The control reactor was the reactor with no addition of surfactant. In addition, one reactor (reactor 5) was operated with the addition of glucose in order to examine the effect of glucose on the bacterial growth and hydrocarbon biodegradation in the bioreactor. Similar to the previous sections, the effects were examined in two aspects: the growth and the hydrocarbons biodegradation of the bacteria in the mixed-surfactant system. The results were compared to the control culture without the addition of surfactant. The growth of the bacteria was measured as dry weight cell (mg/l) whereas the biodegradation of hydrocarbons in the oil sludge was calculated based on total petroleum hydrocarbon (TPH) being degraded by the microorganism as measured by using TPH solvent extraction technique.

Table 4.3 The conditions of bioreactors used in this study.

Reactor	Total volume of 50 mL filled each day		Total volume drawn in each day (mL)	Bacteria
	oil sludge + mix surfactant MSM at same ratio(mL)	MSM (mL)		
Reactor1 (In)	5	45	50	Indigenous
Reactor2 (In)	10	40	50	Indigenous
Reactor3 (In)	20	30	50	Indigenous
Reactor4 (In)	30	20	50	Indigenous
Reactor5* (In)	30	20	50	Indigenous
Reactor1 (P)	5	45	50	Pseudomonas a.
Reactor2 (P)	10	40	50	Pseudomonas a.
Reactor3 (P)	20	30	50	Pseudomonas a.
Reactor4 (P)	30	20	50	Pseudomonas a.
Control	0	50	50	Autoclaved

Reactor5* = Add Glucose

4.4.1 The Effect of Mixed Surfactant on Growth and Hydrocarbon Biodegradation of Indigenous Bacteria in Bioreactor

From Table 4.3, it can be seen that, for this fill-and-draw operation, although the same amount of sample (50 ml) was drawn from all reactors, during the fill period each reactor was filled with different amount of oil sludge and surfactant

in MSM in each day during the operation. For example, in reactor 1, each day 50 ml of sample was drawn from the reactor first and then 45 ml of MSM plus 5 ml of MSM containing 2%w/v oil sludge mixed with the mixed surfactants was filled back to the reactor. For reactor 2, the same volume (50 ml) was drawn but only 40 ml of MSM plus 10 ml of MSM containing 2%w/v oil sludge mixed with the mixed surfactants was filled back to the reactor. This would result in the same fill-and-draw operation for all reactors but at different concentration or loading of oil sludge in each reactor and the reactors were run for the total of 10 days. In reactor 5, the same operation and condition as reactor 4 but glucose was added at the concentration of 0.04 g/l to examine the effect of glucose on the growth and biodegradation of indigenous bacteria in the bioreactor. The results are shown in Figures 4.15 and 4.16 for the growth and TPH degradation, respectively. From Figure 4.15, it can be seen that the bacterial growth in all reactors was relatively constant after 5 days of the operation. Nearly the same growth was observed in reactors 1-4 which was in the range of 0.56 g/l and was clearly shown to be higher than the growth observed in the control reactor (0.48 g/l). When compared to the reactors without glucose (reactors 1-4), the higher growth was obtained in reactor 5 with the addition of glucose.

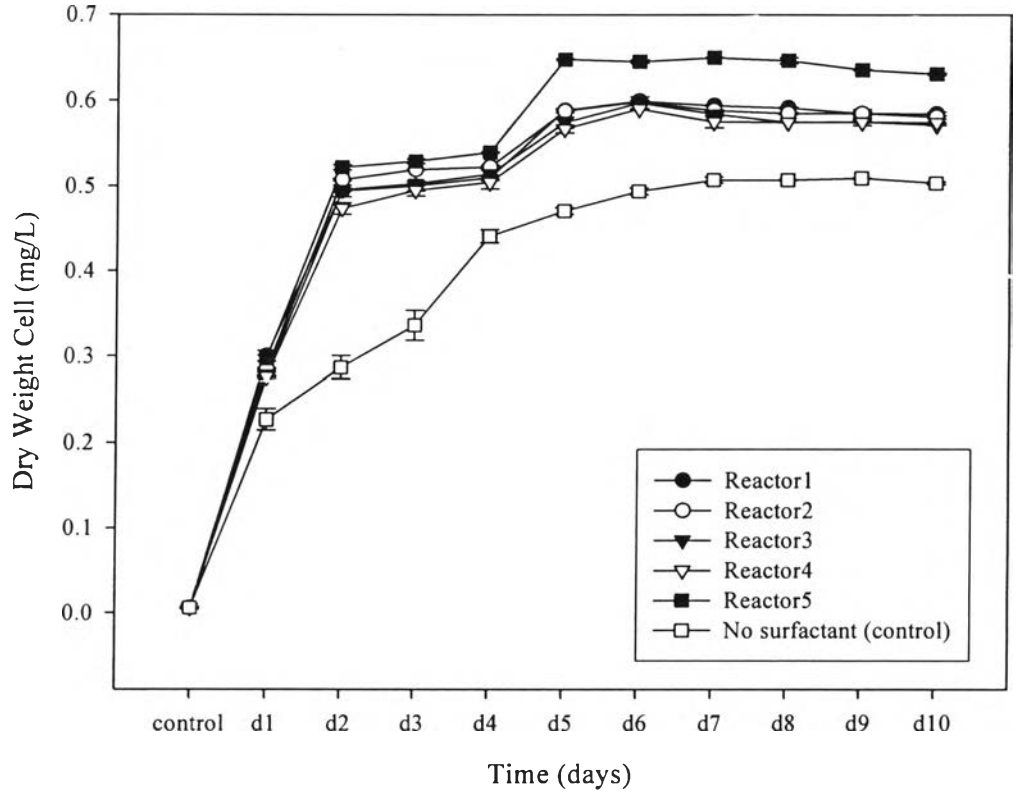

Figure 4.15 The growth of indigenous bacteria in bioreactor under various conditions.

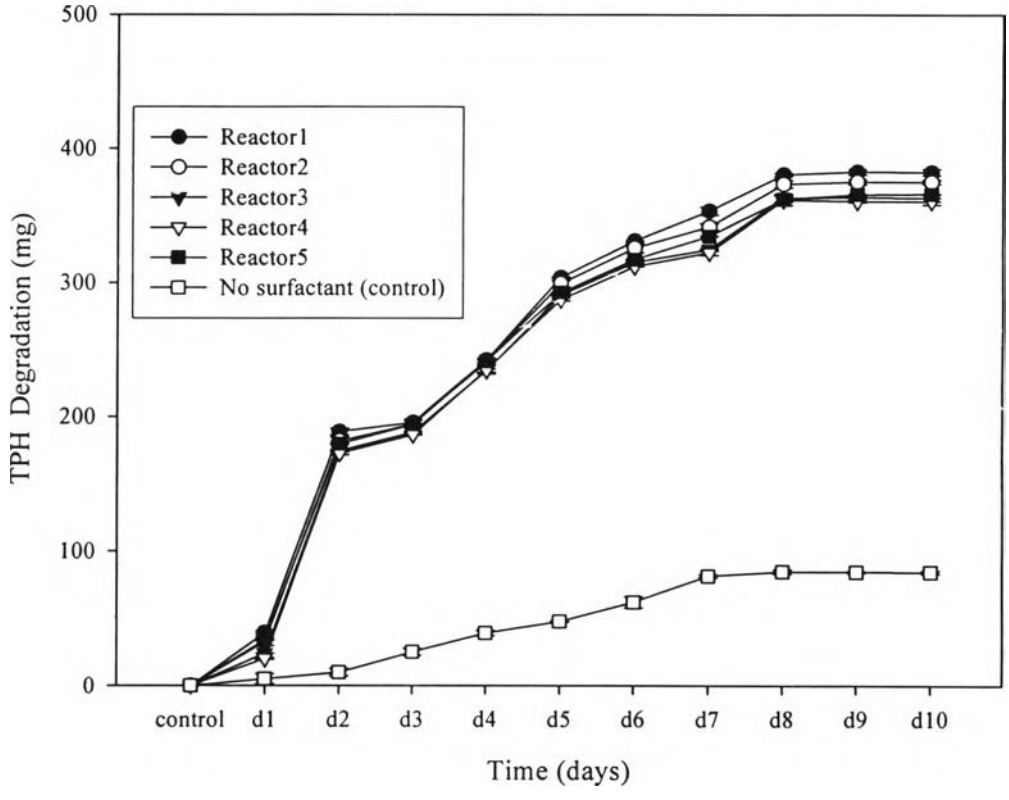

Figure 4.16 The biodegradation of hydrocarbons in the oil sludge by indigenous bacteria in the bioreactor under various conditions.

Fig. 4.16 shows that the TPH degradation became relatively constant after 8 days of operation and approximately 330-350 mg of TPH degradation (based on a fixed volume of 30 ml) could be obtained for all test reactors (1-5). This is approximately 4 times higher than the biodegradation obtained in the control reactor. The results clearly indicate that the surfactant-enhanced biodegradation of hydrocarbons in the oil sludge could be well achieved in the semi-batch bioreactor. It is interesting to note that, although the bacterial growth in reactor 5 (with glucose addition) was the highest as seen in Fig. 4.15, but the extent of hydrocarbon biodegradation was not much different from other test reactors. This suggests that the bacteria prefer to utilize glucose as a source of carbon for their growth over the hydrocarbons and the higher growth observed was a result of glucose utilization rather than hydrocarbons degradation.

4.4.2 The Effect of Mixed Surfactant on Growth and Hydrocarbon Biodegradation of *Pseudomonas aeruginosa* in Bioreactor

Similar biodegradation experiments as discussed in the previous section using indigenous bacteria in the bioreactor were carried out in this part of the study but using *Pseudomonas aeruginosa* as the degrading microorganism instead. The culture was incubated and the reactors were operated in the same manner as previously described. The time course of the growth of *Pseudomonas aeruginosa* and TPH degradation in the bioreactors are presented in Figures 4.17 and 4.18, respectively. It can be seen that the results were similar to those of the indigenous bacteria obtained in the bioreactors. For the bacterial growth, the growth in the test reactors (reactors 1-4) was considerably higher than the control reactor (no added surfactant). However, when compared among the test reactors, the growth of the bacteria in reactor 1 (lowest sludge loading) was the highest whereas that of reactor 4 (highest sludge loading) was the lowest. This suggests that the *Pseudomonas aeruginosa* culture in the semi-batch reactor may not be able to degrade the oil sludge being fed into the reactor at high sludge loading. The difference in the growth between the indigenous bacteria and *Pseudomonas aeruginosa* cultivated in the semi-batch bioreactor may be due to the fact that the indigenous bacteria present in the oil sludge as a consortia (mixed culture), not a single species like *Pseudomonas aeruginosa* culture used in this study. Therefore, the synergism between the microorganisms co-exist in the consortia may be an important factor in biodegradation of hydrocarbons in the oil sludge. For the biodegradation of hydrocarbons shown in Fig. 4.18, similar trend to the growth was also observed for *Pseudomonas aeruginosa*. That is the biodegradation of hydrocarbons in the oil sludge in the test reactors (reactors 1-4) was higher than that of the control reactor. The TPH degradation was in the range of approximately 350-400 mg based on a fixed volume of 30 ml which was approximately 4 times higher than that of the control. The biodegradation was highest in reactor 1 and lowest in reactor 4 as also observed in the bacterial growth for which the same explanation previously discussed can be offered.

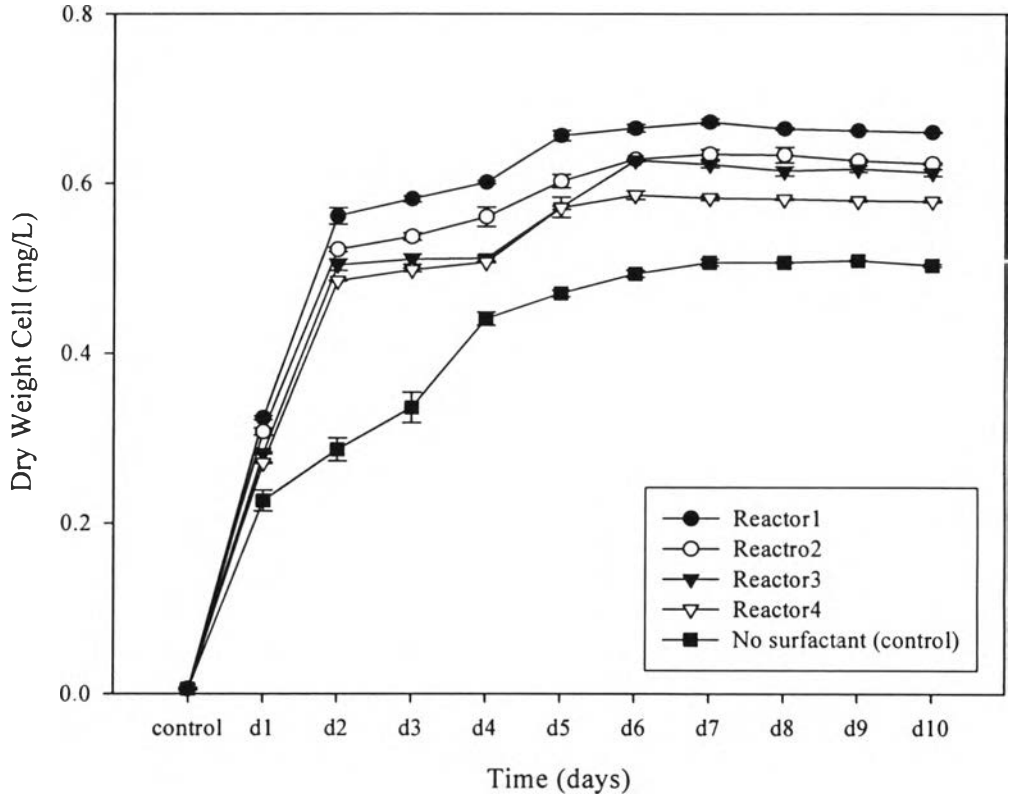

Figure 4.17 The effect of mixed surfactant on growth of indigenous bacteria in the bioreactor under various conditions.

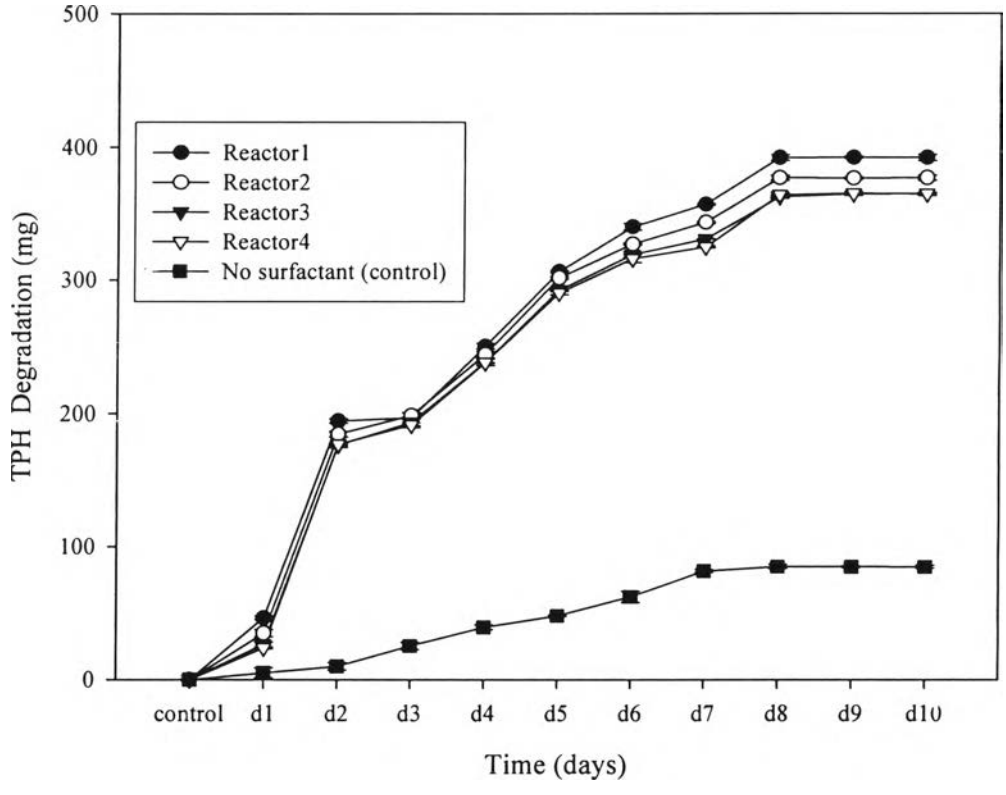

Figure 4.18 The biodegradation of hydrocarbons in the oil sludge by *Pseudomonas aeruginosa* in the bioreactor under various conditions.