# CHAPTER IV
# RESULTS AND DISCUSSION

## 4.1 Preparation and Characterization of The Oil Sludge Samples

The oil sludge samples used in all the experiments were prepared in the same manner in order to assure the consistency of the samples by decantation of the original sludge from PTT PLC followed by air-drying. Initially, the water portion of the sludge was separated by decantation and then the excess moisture was removed by drying the oil sludge at open atmosphere in a petri dish for a fixed amount of time. After that, the air-dried sludge was kept in refrigerator. Figures 4.1 and 4.2 show the oil sludge samples obtained from PTT PLC before and after drying, respectively.

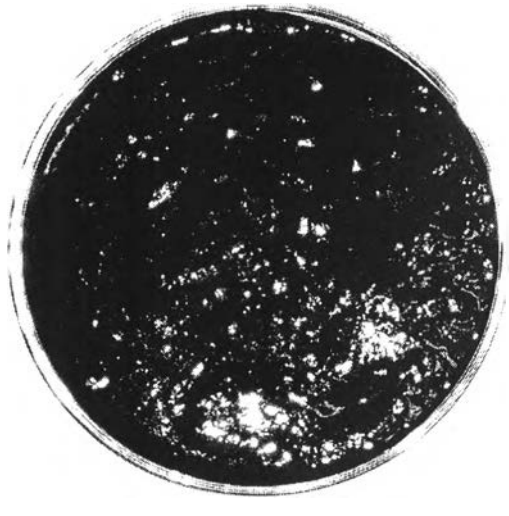

**Figure 4.1** Oil sludge samples which obtained from PTT PLC.

**Figure 4.2** Oil sludge samples after preparation.

The composition of hydrocarbons in oil sludge was analyzed by using GC/MS, which is presented in Table 4.1. It was found that the oil sludge from PTT PLC contains mainly polyaromatic hydrocarbons (PAHs > 90%) with a small

amount of paraffins. PAHs found are three-, four-, and five-ring compounds such as phenanthrene, anthracene, pyrene, and their derivatives.

**Table 4.1** Type of hydrocarbons in oil sludge samples

| Type of Hydrocarbons | Molecular Weight | % |
|---|---|---|
| 1. 2,4-Dimethylheptane | 128.26 | 6.4 |
| 2. Phenanthrene | 178.23 | 1.8 |
| 3. 2-Methylphenanthrene | 192.26 | 9.9 |
| 4. 1-Methylanthracene | 192.26 | 28.1 |
| 5. 9,10-Dimethylanthracene | 206.28 | 28.3 |
| 6. 4-Methylpyrene | 216.28 | 16.6 |
| 7. 1-Methylpyrene | 216.28 | 6.6 |
| 8. 5-Methylchrysene | 242.32 | 2.2 |

## 4.2 Enhanced Solubilization of Hydrocarbons in Oil Sludge by Nonionic Surfactants

### 4.2.1 Determination of Time Required for Solubilization of Oil Sludge by Nonionic Surfactants

In order to study the effect of nonionic surfactants on solubilization of petroleum hydrocarbons in the oil sludge sample, it is imperative to first determine the amount of time required for the solubilization process to reach equilibrium. Three nonionic surfactants, Brij 30, Tween 80 and Triton X-100, at predetermined concentrations were added into three separated sets of flasks containing oil sludge and with constant mixing. Samples were taken and analyzed for solubilization of hydrocarbons at specific time intervals. The solubilization was reported in terms of "Enhanced Solubilization" as it had been substracted by the solubilization amount in the control (no addition of surfactant). Figure 4.3 shows the enhanced solubilization as a function of time for all three surfactants. It can be seen that the solubilization occurred very rapidly during 6 to 24 h. for all cases, and then became quite constant

after 48 h. Thus, it can be concluded that 48 h. or 2 days is adequate for the solubilization to complete and, consequently, in all experiments in the enhanced solubilization studies, samples were taken for analysis after at least 48 h.

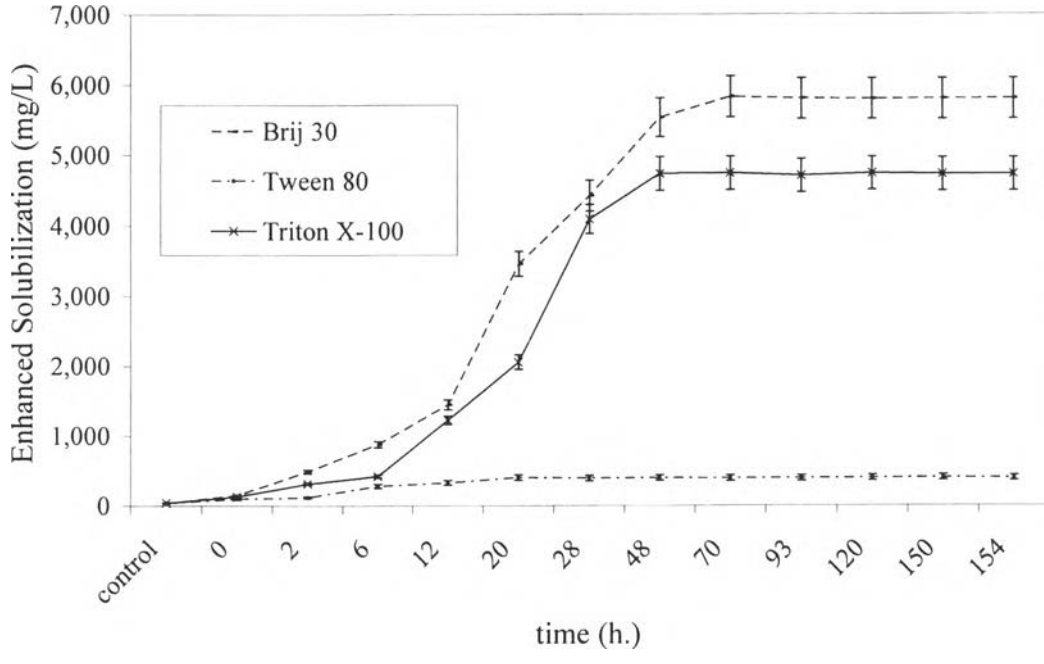


**Figure 4.3** Effect of contact time on solubilization of oil sludge by nonionic surfactants.

The composition of hydrocarbons in oil sludge in the system that added nonionic surfactants is presented in Table 4.2. It was found that the solubilization of hydrocarbons in oil sludge was enhanced by adding nonionic surfactants which mainly polyaromatic hydrocarbons (PAHs > 90%) with a small amount of paraffins.

**Table 4.2** % Type of hydrocarbons in oil sludge samples when added nonionic surfactants

| Type of Hydrocarbons | % | | |
|---|---|---|---|
| | Brij 30 | Tween 80 | Triton X-100 |
| 1. 2,4-Dimethylheptane | 1.1 | 4.1 | 3.4 |
| 2. Phenanthrene | 19.7 | 4.7 | 5.1 |
| 3. 2-Methylphenanthrene | 12.4 | 15.2 | 13.5 |
| 4. 1-Methylanthracene | 21.7 | 22.1 | 23.3 |
| 5. 9,10-Dimethylanthracene | 28.2 | 25.9 | 28.4 |
| 6. 4-Methylpyrene | 7.6 | 18.1 | 17 |
| 7. 1-Methylpyrene | 6.5 | 6.4 | 6.7 |
| 8. 5-Methylpyrene | 2.8 | 3.5 | 2.6 |

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

In this part of the study, the control experiment was first carried out to quantify the solubility of the hydrocarbons in the oil sludge in the absence of the surfactant. The solubility of hydrocarbons in aqueous phase was determined using two techniques by measuring Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC), in ppm or mg/L. This control experiment reveals that the solubilization of the hydrocarbons in the oil sludge samples without the addition of the surfactant was quite low as expected. The total solubilization in the control flasks as measured by COD and TOC was found to be only 115 ppm and 37.7 ppm, respectively. In order to improve the solubilization of the hydrocarbons in the oil sludge, the subsequent experiments were carried out with the addition of various nonionic surfactants. Varying amounts of the nonionic surfactants, Brij 30, Triton X-100, and Tween 80, were added into the flasks containing the same amount of the oil sludge samples and their effect on the solubilization of hydrocarbons in the oil sludge were examined in a similar manner by measuring TOC and COD of the filtered

solution from the flasks. At this point, another set of control experiments was performed in order to determine the contribution to COD and TOC from the added surfacftant itself. The solubility obtained from the first control and the contribution from surfactant measured in the second control were substracted from the solubility in the presence of surfactant to yield "Enhanced Solubilization". Figure 4.4 shows the solubilization of hydrocarbons in the oil sludge as enhanced by the addition of the three nonionic surfactants or "Enhanced Solubilization" as a function of added concentration in terms of the CMC and %w/v of the surfactant.

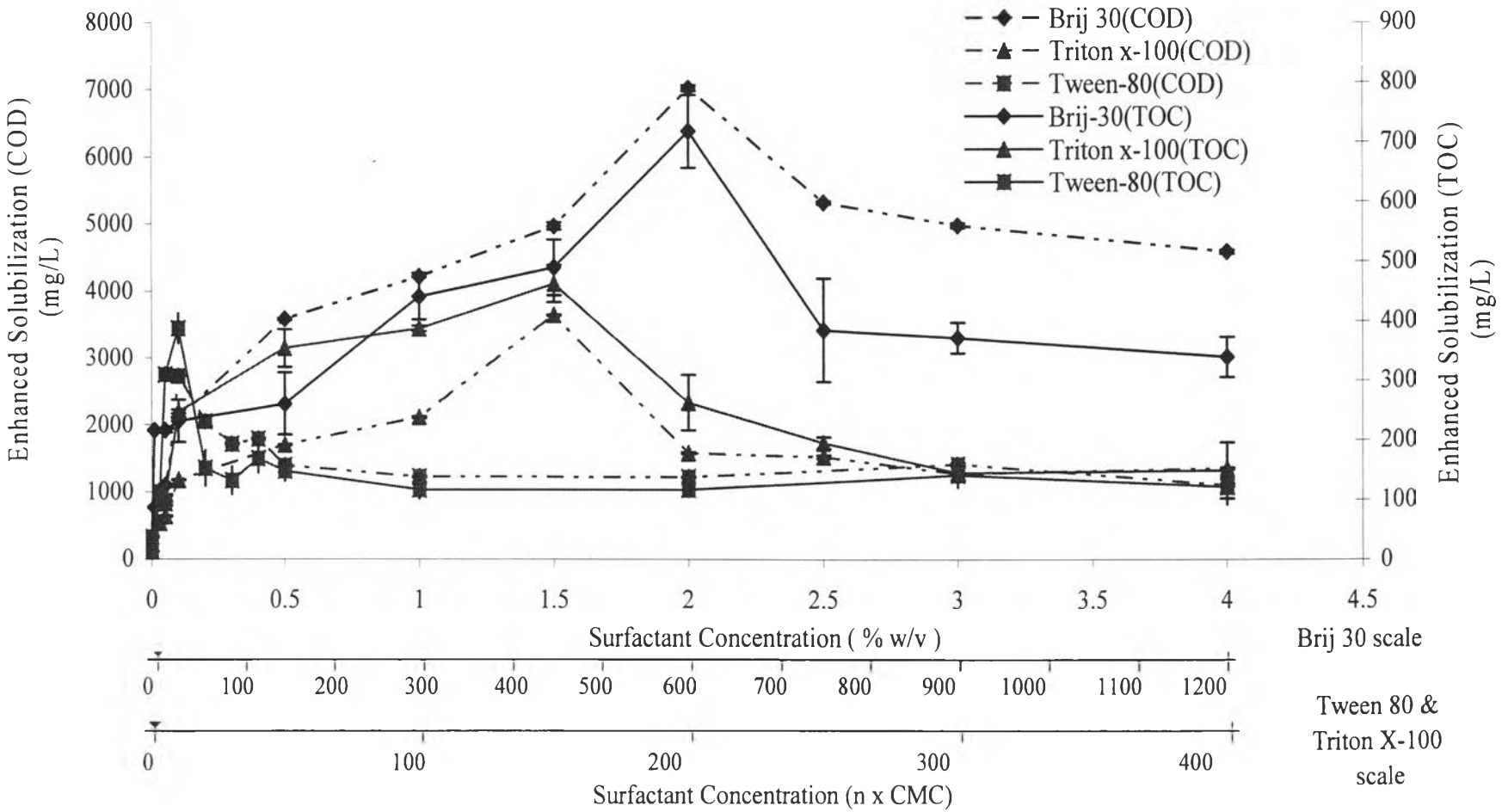


**Figure 4.4** Solubilization of hydrocarbons in the oil sludge as enhanced by the addition of the three nonionic surfactants.

For all three surfactants, a general trend can be observed that initially the solubilization of the hydrocarbons significantly increased with increasing the amount of the surfactant added to the system. Then the enhanced solubilization reached its maximum value at a specific amount/concentration of individual nonionic surfactant. For Brij 30, maximum solubilization of 7,000 ppm COD or 700 ppm of TOC was achieved at the concentration approximately 600 times of its CMC or 2 %w/v. For Triton X-100, maximum solubilization of 4,500 ppm COD or 450 ppm of TOC was achieved at approximately 150 times of its CMC or 1.5 %w/v. For Tween 80, maximum solubilization of 2,800 ppm COD or 400 ppm of TOC was achieved at approximately 60 times of its CMC or 0.1 %w/v. Beyond these concentrations, the solubilization gradually decreased as the surfactant concentration increased. From the results, it is obvious that the addition of the nonionic surfactant in an appropriate range of concentration significantly enhanced the solubilization of hydrocarbons in oil sludge. There is an optimal concentration for each surfactant at which the solubilization reaches its maximum value. However, if the added concentration exceeds the optimal concentration, it may create an excess of micelles which coalesce, leading to a decrease of the emulsion stability and partial flocculation of hydrocarbons. It can also be seen that the addition of Brij 30 had much greater effect on the solubilization than the addition of Triton X-100 and Tween 80. However, the effect exerted by Brij 30 occurred at a much higher concentration when compared to the other two surfactants. On a contrary, although the enhancing effect on solubilization is not as much as that of Brij 30 but Triton X-100 and Tween 80 require much lower concentration.

In order to quantify the effect of the nonionic surfactants on enhancing solubilization of individual hydrocarbon present in oil sludge, the oil sludge samples with the addition of surfactant were analyzed by using GC/MS and presented in terms of % enhanced solubilization with respect to the control (no surfactant addition). Figure 4.5 shows the % enhanced solubilization of various hydrocarbons in oil sludge by three nonionic surfactants used in this study. It is obvious that the addition of nonionic surfactants enhanced the apparent solubility of most of the hydrocarbons in oil sludge. Among the three surfactants, Brij 30 was shown to give the highest effect on the solubilization of petroleum hydrocarbons in

oil sludge. The enhanced solubilization of hydrocarbons was in the order of Brij 30, Triton X-100 and Tween 80. For Brij 30, the highest enhanced solubilization was observed (6769 %) for phenanthrene present in the sludge whereas the lowest was observed for 2,4-dimethylheptane. The enhanced solubilization of other hydrocarbons was in the range of 200-700%. The relative degree of enhanced solubilization decreased in the order 5-methylchrysene, 2-methylphenanthrene, 9,10-dimethlyanthracene, 1-methylpyrene, 1-methylanthracene, and 4-methylpyrene. Similar enhanced solubilization behavior was observed when using Tween 80 and Triton X-100 but to a much lower extent. The enhanced solubilization was in the range of 100-400 % for systems having Triton X-100 and Tween 80. This may be attributed to the different numbers of polyoxyethylene (POE) of the surfactants. Surfactants with a short POE chain have a high capacity for enhancing the solubilization of hydrocarbons. On the other hand, the HLB was designed for matching surfactant structure to an organic chemical to be emulsified, and high HLB number reflects high water solubility of the surfactant. The percentage of enhanced solubilization of the hydrocarbons can be correlated to their water solubility which nonpolar solubilizates are in general less soluble in micelles than their polar. Therefore, the solubilization should be in the order of; aromatic > cyclic aliphatic > linear aliphatic as observed in this study.

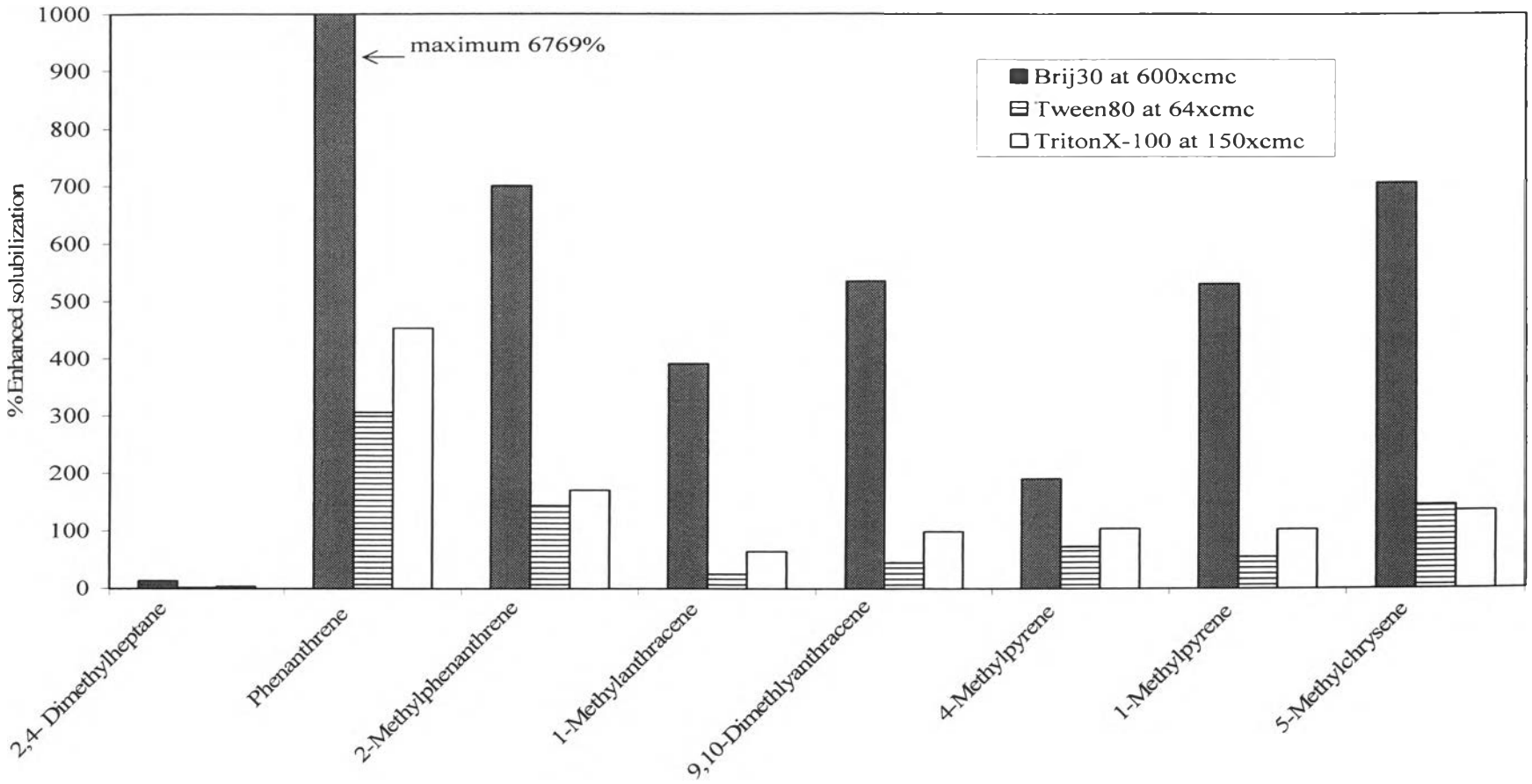


**Figure 4.5** % Enhanced solubilization reported as type of hydrocarbons.

## 4.3 Enhanced Biodegradation of Hydrocarbons in Oil Sludge by Nonionic Surfactants

In this part of the study, the effect of nonionic 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 hydrocarbons biodegradation of the bacteria in the presence of nonionic surfactants and the results were compared to the systems without addition of nonionic surfactants. The growth of the bacteria was measured as dry weight cell (mg) per fixed volume of culture whereas the biodegradation of hydrocarbons in the sludge was calculated based on total petroleum hydrocarbon (TPH) being degraded by the microorganism as measured by TPH solvent extraction technique.

### 4.3.1 The Growth and TPH Degradation of Indigenous Bacteria

As the oil sludge may contain indigenous bacteria capable of growing on and degrading hydrocarbons present in the sludge, it is important to first examine the growth and hydrocarbons biodegradation of the indigenous bacteria. In this part of the study, the control flasks were autoclaved to terminate all living microorganisms. The extent of biodegradation of the hydrocarbons in oil sludge sample was presented as TPH degradation obtained by determining the loss of TPH during biodegradation with respect to the autoclaved control flasks. The growth of indigenous bacteria was reported as dry weight cell as described above. Figure 4.6 presents that the growth and TPH degradation of indigenous bacteria in comparison with the control experiments (autoclaved). Typical growth curve having lag phase, exponential phase and stationary phase was observed. The biodegradation was slow during the first few days and increased rapidly during 3-4 days, and became constant after 5 days which was also the stationary phase of indigenous bacteria. According to this figure, without addition of surfactant, the amount of TPH degradation by indigenous bacteria was approximately 50 mg.

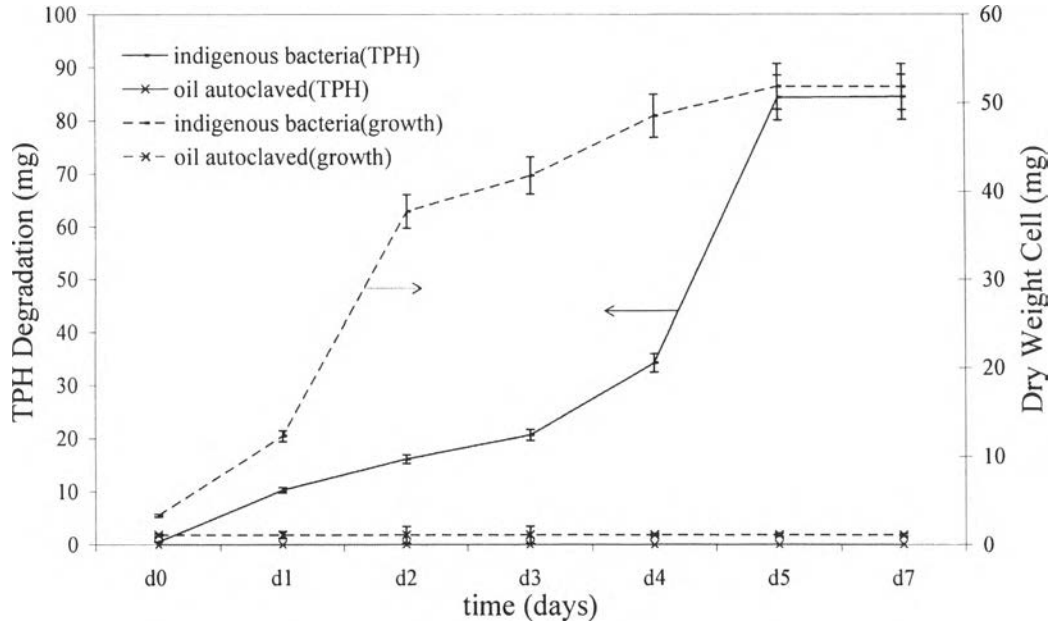


**Figure 4.6** The growth and TPH degradation compare indigenous bacteria.

4.3.2 Effect of Nonionic Surfactants on Growth and Biodegradation of Indigenous Bacteria

From the solubilization studies, the concentration that provided highest solubilization was chosen for each surfactant to further study the effect on biodegradation of hydrocarbons in the sludge. Therefore, Brij 30, Tween 80 and Triton X-100 were added into the culture containing oil sludge (2% w/v) at the concentration of 2 %w/v, 1.5 %w/v and 0.1 %w/v, respectively. The culture was incubated at room temperature on an orbital shaker set at 170 rpm for 7 days. Time-course for dry weight cell of indigenous bacteria and TPH degradation in cultures with and without the addition of surfactant were presented in Figure 4.7. In the absence of nonionic surfactants, the results were the same as seen in Figure 4.6. After incubation for 7 days, the growth and TPH degradation in the control flask were approximately 50 mg of dry weight cell control and 84 mg TPH, respectively. The growth curves of the bacteria in the presence of nonionic surfactant were similar to that observed in the control (no surfactant). The lag phase, exponential phase and stationary phase on growth of indigenous bacteria were observed in all of cultures with the addition of these surfactants. For all systems with the addition of nonionic

surfactants, the growth of the bacteria was shown to be in the range of 45-65 mg with Brij 30 having the highest growth of 62 mg and Triton having the lowest growth of 46 mg. For biodegradation, the TPH degradation was highest in the presence of Brij 30. Approximately 350 mg TPH was degraded by the microorganism after 7 days. In the presence of Tween 80, the extent of degradation was the second with the total degraded TPH of 240 mg after 7 days. The biodegradation was lowest in the presence of Triton X-100 where the TPH degradation of only approximately 50 mg was observed after 7 days.

The oil sludge samples after the biodegradation were analyzed again by GC/MS in order to quantify the degradation of individual hydrocarbons present in the sludge. Figure 4.8 shows the degradation of each hydrocarbon in the sludge as a percentage of the amount originally present in the sludge before biodegradation as determined from the autoclaved control. By comparing with the culture without addition of surfactant, it can be seen that the biodegradation of most hydrocarbons in the sludge was greatly enhanced by the addition of the nonionic surfactants, especially Brij 30 and Tween 80 where the TPH degradation was much higher than that of the control (no surfactant). For Brij 30, the highest enhancement on the biodegradation was observed in almost of hydrocarbon compounds. In the presence of Brij 30, TPH degradation was approximately 8-12 times of the degradation in the culture without surfactant. For Tween 80, the enhancement on the biodegradation was slightly lower than the enhancement by Brij 30, in general, except for 2-methyl phenanthrene. For Triton X-100, the enhancement exerted by the surfactant on the biodegradation was essentially insignificant when compared to the culture without addition of surfactant. When comparing between various types of hydrocarbons present in the sludge, the extent of TPH degradation in the presence of Brij 30 was highest for phenanthrene whereas the degradation of 1-methylpyrene, 1-methyl anthracene, 2-methylphenanthrene were comparable and 4-methylpyrene was the lowest. Similar biodegradation behavior was observed in the culture with the addition of Tween 80, except that the degradation of 2-methylphenanthrene was the highest. For the culture having Triton X-100, the biodegradation of the hydrocarbons was quite similar to that observed in the culture containing Brij 30, but the extent of the degradation was much lower. For most of the hydrocarbons present

in the oil sludge, the degraded amount was comparable to the culture with no surfactant.

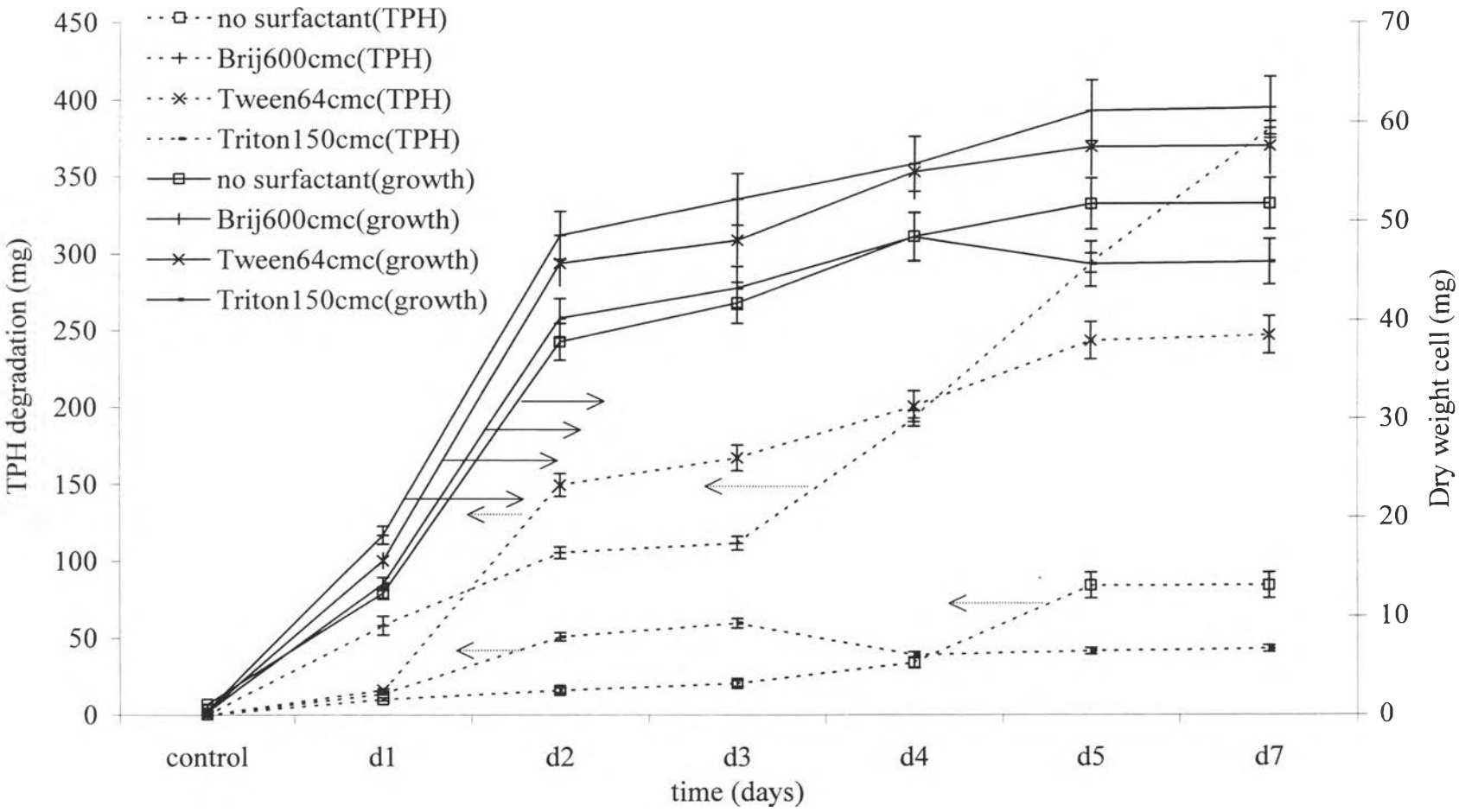


**Figure 4.7** The effect of nonionic surfactants on growth and biodegradation of indigenous bacteria.

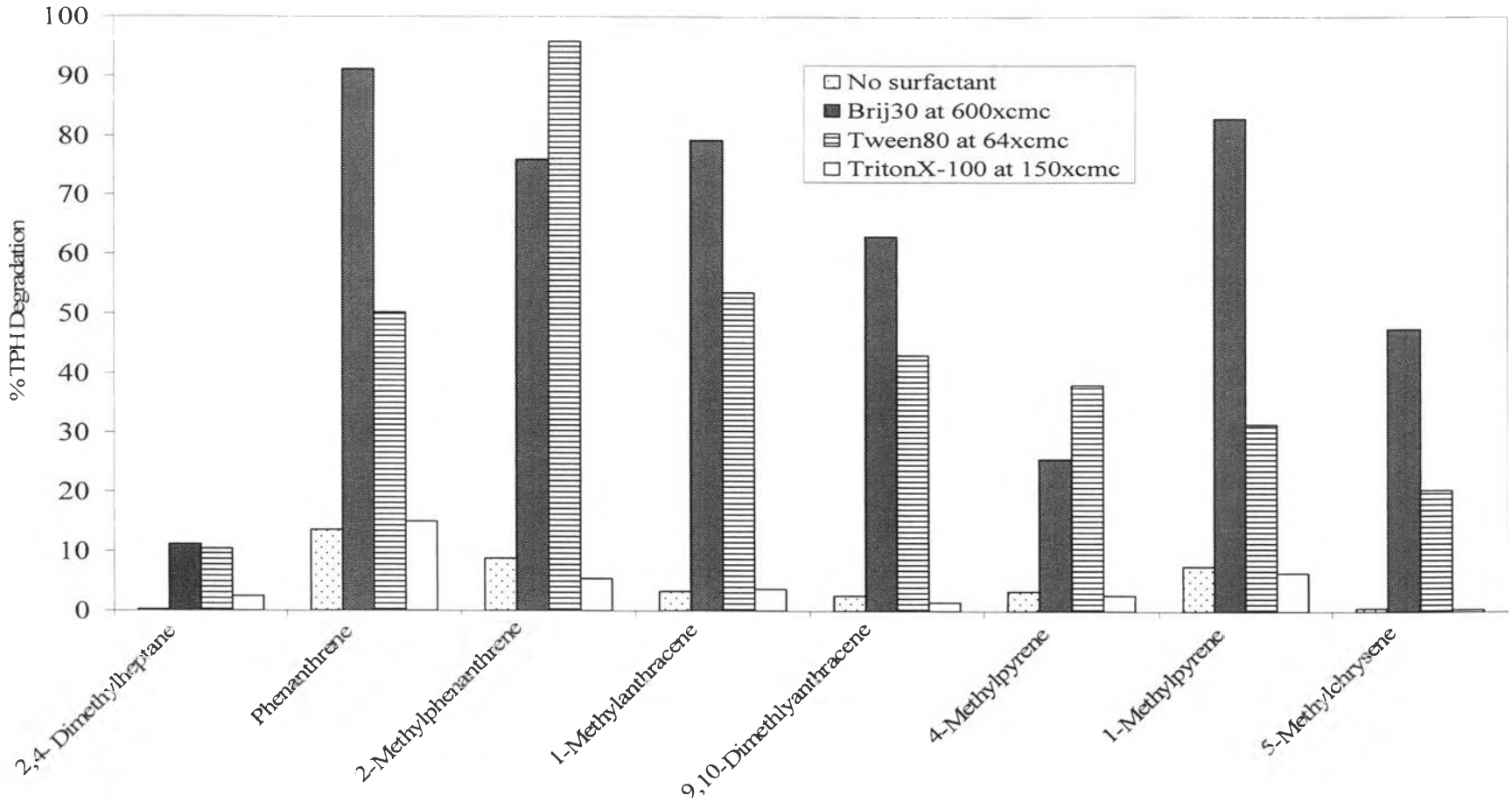


**Figure 4.8** The effect of nonionic surfactants on biodegradation by indigenous bacteria (reported as type of hydrocarbons).

### 4.3.3 Effect of nonionic surfactants on growth and biodegradation of *Pseudomonas aeruginosa*

Similar procedures to the biodegradation experiments using indigenous bacteria were used in this part of the study but *Pseudomonas aeruginosa* was used as a degrading microorganism instead. The cultures were incubated, sampled, and then analyzed in the same manner as previously described. Time-course for dry weight cell of *Pseudomonas aeruginosa* and TPH degradation in the cultures with and without the addition of surfactant were presented in Figure 4.9. In the absence of nonionic surfactants, after incubation for 7 days, the growth and TPH degradation in the control flask were approximately 58 mg of dry weight cell control and 91 mg TPH, respectively. The growth curves of the bacteria in the presence of nonionic surfactant were similar to that observed in the control (no surfactant). The lag phase, exponential phase and stationary phase on growth of indigenous bacteria were observed in all of cultures with the addition of these surfactants. For all systems with the addition of nonionic surfactants, the growth of the bacteria was shown to be in the range of 55-65 mg with Brij 30 having the highest growth of 62 mg and Triton having the lowest growth of 55 mg. For biodegradation, the TPH degradation was highest in the presence of Brij 30. Approximately 384 mg TPH was degraded by the microorganism after 7 days. In the presence of Tween 80, the extent of degradation was the second with the total degraded TPH of 248 mg after 7 days. The biodegradation was lowest in the presence of Triton X-100 where the TPH degradation of only approximately 92 mg was observed after 7 days.

From the GC/MS results, the biodegradation of each hydrocarbon in the sludge could be calculated as a percentage of the amount originally present in the sludge before biodegradation as determined from the autoclaved control as shown in Figure 4.10. By comparing with the culture without addition of surfactant, it can be seen that the biodegradation of most hydrocarbons in the sludge was greatly enhanced by the addition of the nonionic surfactants, especially Brij 30 and Tween 80 where the TPH degradation was much higher than that of the control (no surfactant). For Brij 30, the highest enhancement on the biodegradation was observed in almost of hydrocarbon compounds. In the presence of Brij 30, TPH degradation was approximately 2-27 times of the degradation in the culture without

surfactant. For Tween 80, the enhancement on the biodegradation was slightly lower than the enhancement by Brij 30, in general, except for 2-methyl phenanthrene, 9,10-dimethylanthracene, 4-methylpyrene. For Triton X-100, the enhancement exerted by the surfactant on the biodegradation was essentially insignificant when compared to the culture without addition of surfactant. When comparing between various types of hydrocarbons present in the sludge, the extent of TPH degradation in the presence of Brij 30 was highest for phenanthrene whereas the degradation of 1-methylanthracene, 9,10-dimethylanthracene, 1-methylpyrene, 2-methylphenanthrene, 4-methylpyrene were comparable and 2,4dimethylheptane was the lowest. Similar biodegradation behavior was observed in the culture with the addition of Tween 80, except that the degradation of 2-methylphenanthrene was the highest. For the culture having Triton X-100, the biodegradation of the hydrocarbons was quite similar to that observed in the culture containing Brij 30, but the extent of the degradation was much lower. For most of the hydrocarbons present in the oil sludge, the degraded amount was comparable to the culture with no surfactant.

When comparing the growth and the biodegradation of hydrocarbons of both indigenous bacteria and *Pseudomonas aeruginosa*, the results obtained from the culture having Triton X-100 reveal a contradiction between the growth and the biodegradation since normal growth was observed but the degraded amounts of all hydrocarbons were unreasonably low. At this stage, it is important to conduct a set of experiments to determine whether the nonionic surfactants used in this study can be utilized by the microorganisms for their growth. Surfactant at the concentration used in the biodegradation studies was added into the bacterial culture containing MSM but without sludge. The utilization or degradation of the surfactant was monitored by measuring the growth of the bacteria as optical density for 7 days as shown in Figure 4.11. It can be seen that essentially no growth was observed in the cultures having Brij 30 and Tween 80. In contrast, significant growth was observed in the culture containing Triton X-100, indicating that *Pseudomonas aeruginosa* could utilize this surfactant as a source of carbon for its growth. This well explains the contradiction observed in the biodegradation in the presence of Triton since the bacteria growth was a result of the degradation of the surfactant, not the hydrocarbons originally present in the sludge.

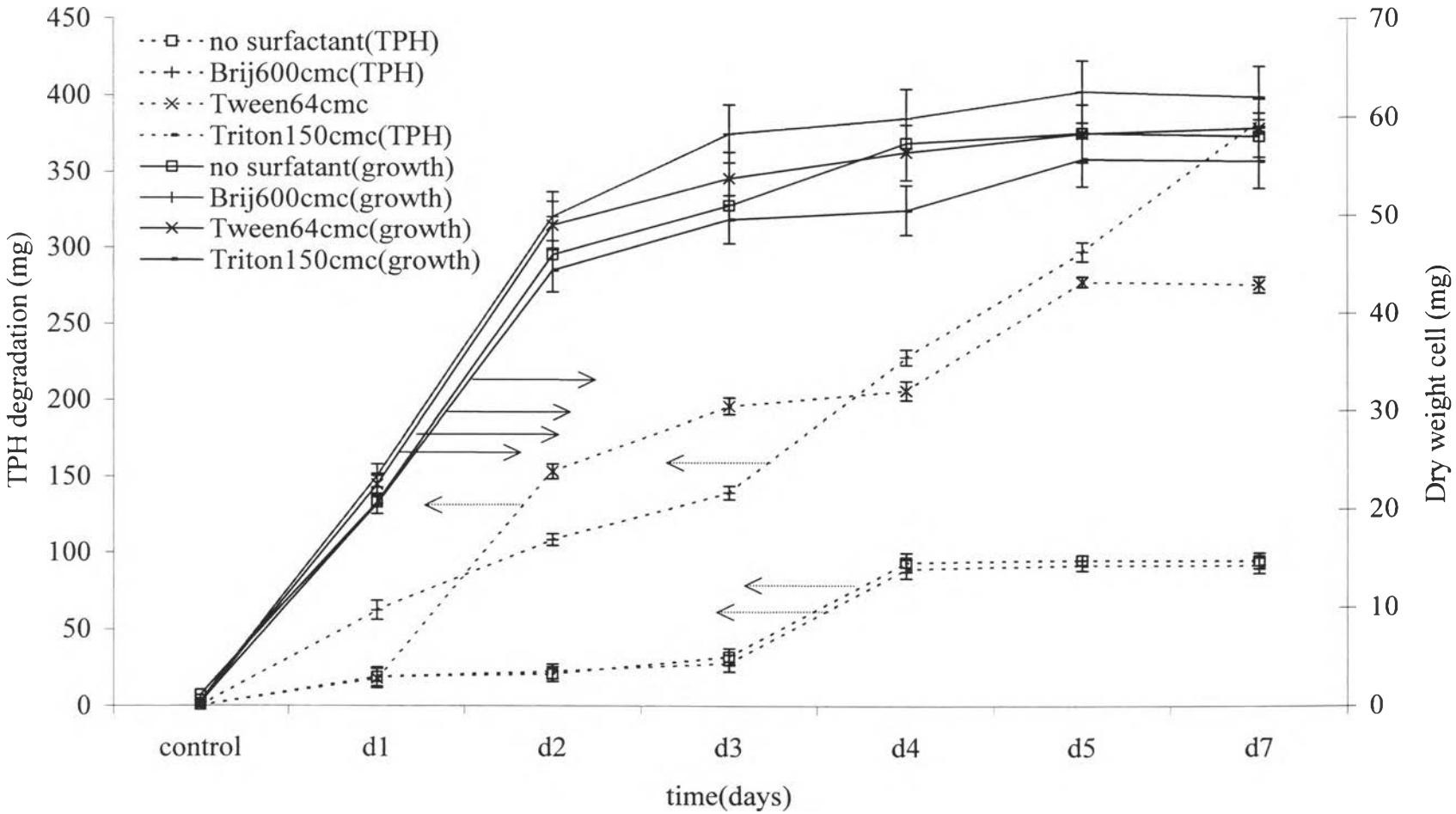


**Figure 4.9** The effect of nonionic surfactants on growth and biodegradation of *Pseudomonas aeruginosa*.

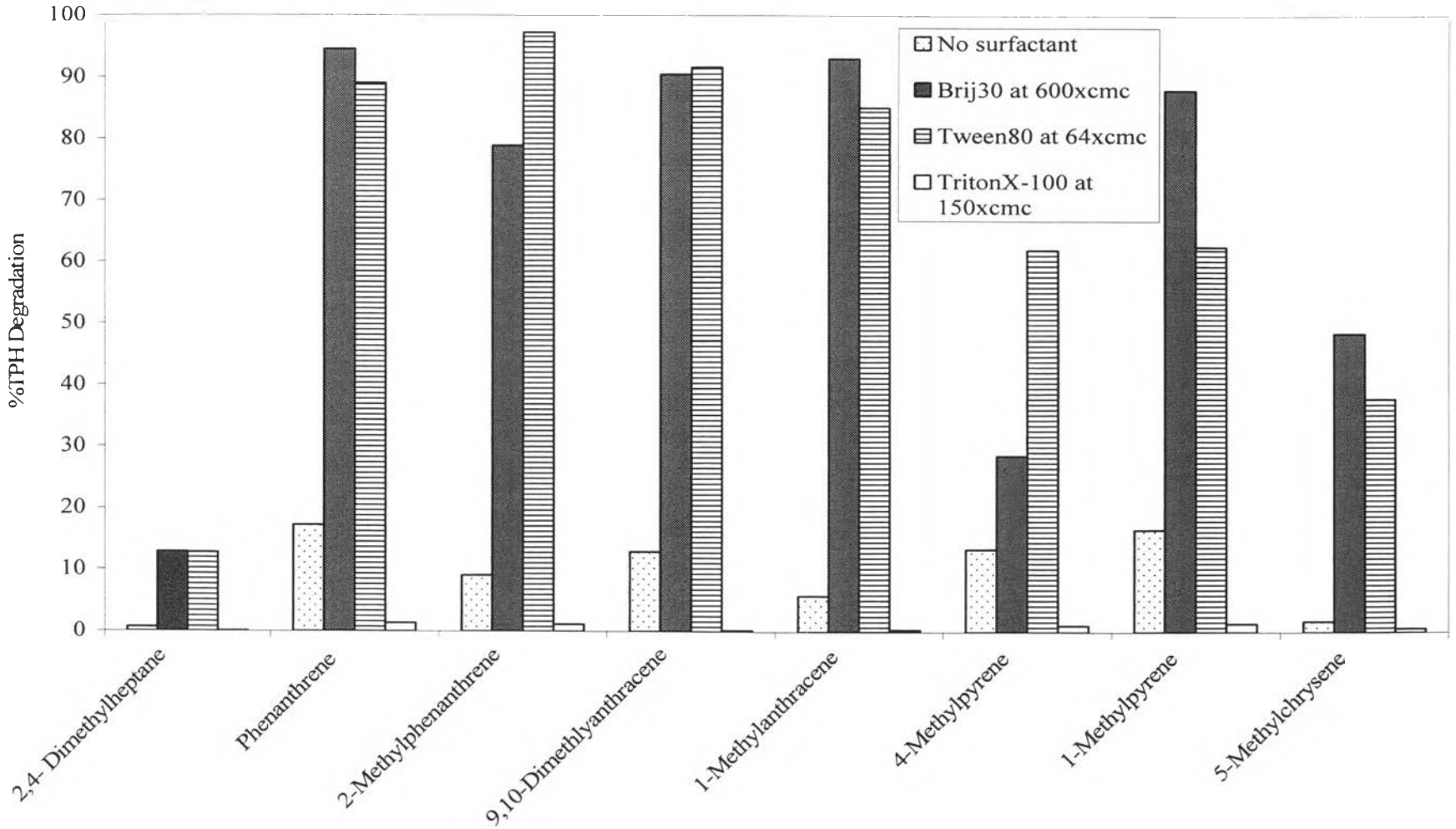


**Figure 4.10** The effect of nonionic surfactants on biodegradation by *P. aeruginosa* (reported as type of hydrocarbons).

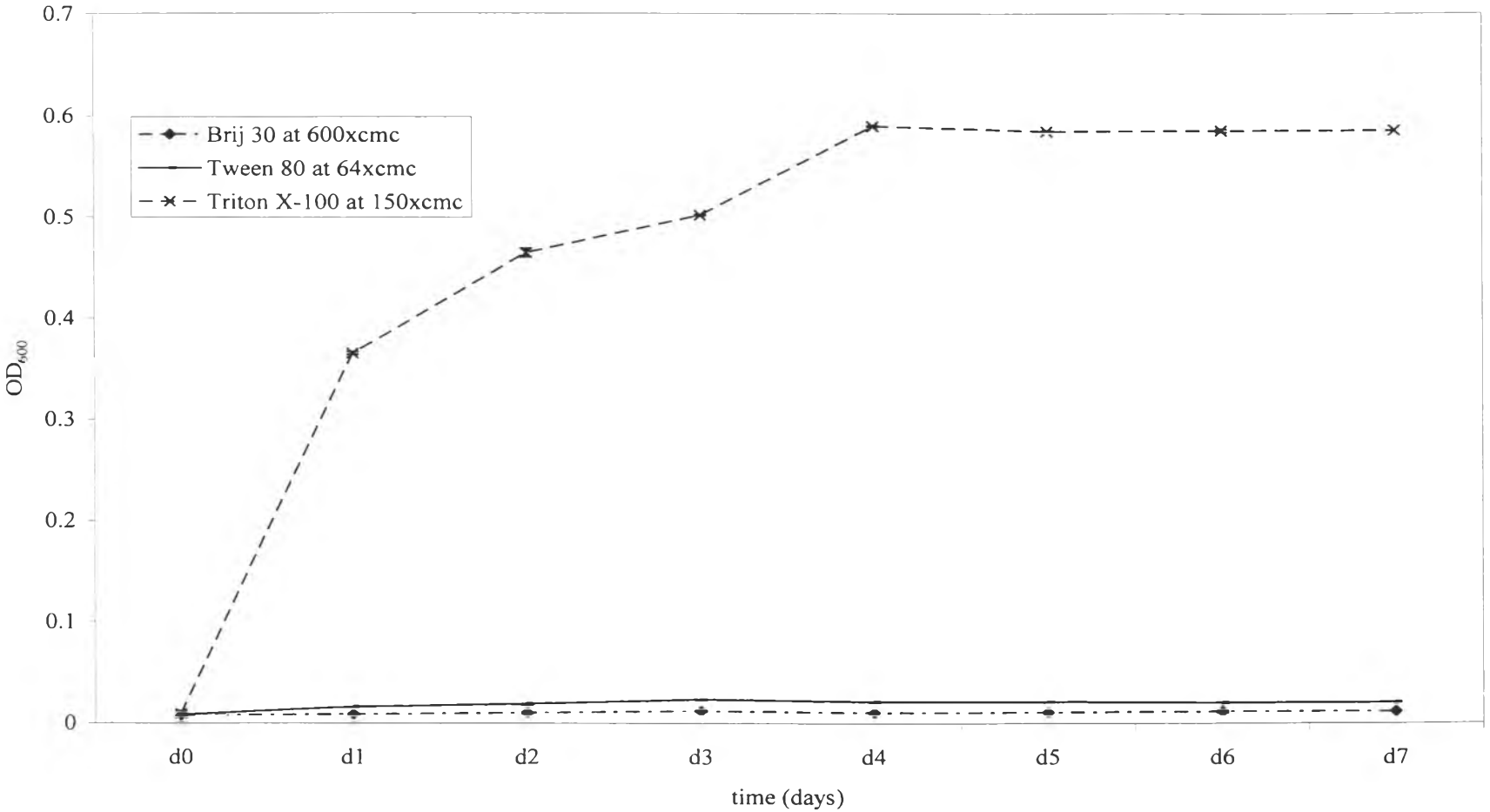


**Figure 4.11** Effect of nonionic surfactants on growth of *Pseudomonas aeruginosa*.

## 4.5 Rate and yield of TPH degradation by indigenous bacteria and *Pseudomonas aeruginosa*

In the previous sections, the degradation of hydrocarbons in the sludge by the microorganisms was reported as the total TPH degraded (mg) during the course of the biodegradation. It is also important to observe the rate in which the hydrocarbons were degraded biologically and the yield in which the microorganisms utilize hydrocarbon for their growth. In this part of the study, the rate and the yield were presented and discussed.

Figure 4.12 shows the rates of biodegradation of hydrocarbons (mg TPH/day) and the rates per cell mass (mg TPH/ day*dry weight cell) by indigenous bacteria and *Pseudomonas aeruginosa* for all three surfactants in comparison with the control (no surfactant). It can be seen from this figure that the cultures with the addition of Brij 30 had the highest rate and rate per cell mass regardless of the type of microorganisms used. The rate and rate per cell mass were approximately 55 mg/day and 0.9 mg/ day*dry weight cell, respectively. The rates obtained in the cultures having Tween 80 (35 mg/day and 0.7 mg/ day*dry weight cell) were slightly lower than those obtained in the culture having Brij 30. The lowest rates were observed in the cultures having Triton X-100 which were approximately 8 mg/day and 0.2 mg/ day*dry weight cell, even lower than the values obtained in the culture with no surfactant addition. The results can be explained that as most of the TPH degradation is present in the micellar phase, the microorganisms must first penetrate the surfactants in order to degrade the hydrocarbons that are dissolved in the micelle. Therefore, the microorganism can biodegrade the hydrocarbons in micelles that are composed of Brij 30 molecules within the highest extent of degradation. In the case of Tween 80, the rates were lower which is probably due to the fact that Tween 80 has more complex structure than Brij 30. For Triton X-100 systems, the rates of biodegradation of hydrocarbons were lowest because of the fact that Triton X-100 could be degraded by the microorganisms and used as a carbon source for their growth.

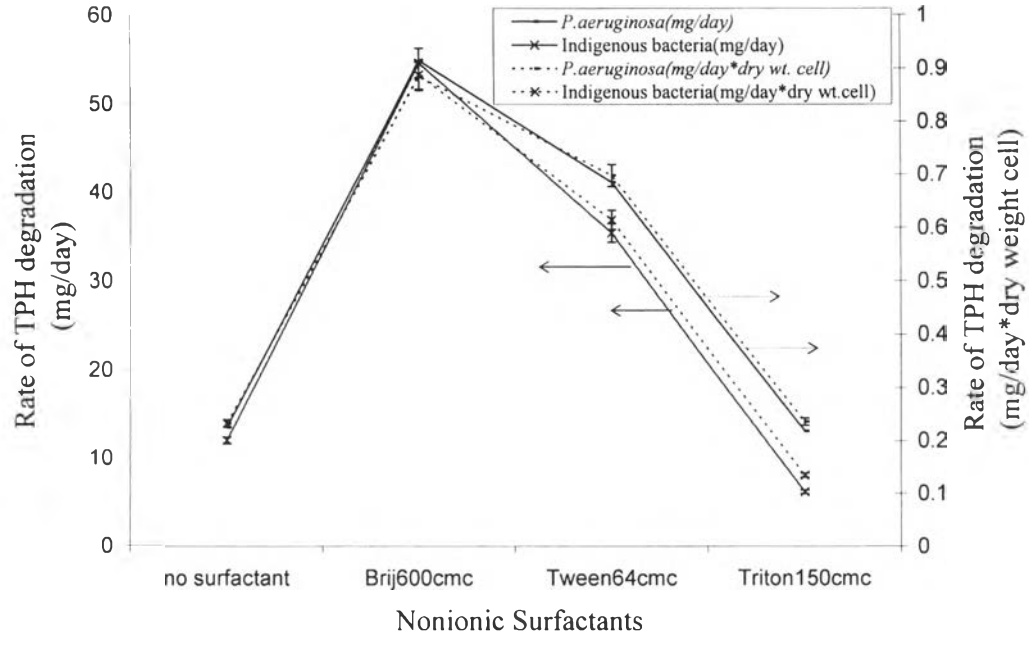


**Figure 4.12** Rate of TPH degradation by indigenous bacteria and *P. aeruginosa*.

Figure 4.13 shows the yields (mg dry weight/mg degraded TPH) of indigenous bacteria and *Pseudomonas aeruginosa* in all three surfactant systems in comparison with the control (no surfactant). In contrast to the results observed in Figure 4.12, it can be seen that the yields of both microorganisms were lowest in the Brij 30 cultures (0.2). The yields obtained in Tween 80 cultures were only slightly higher (0.25). The highest yields were observed in the cultures having Triton X-100 (0.6-1) which were slightly higher than those obtained in the control (0.6). This can be explained that since the growth obtained in all cultures were quite similar (in the range of 50-60 mg dry weight cell), and thus, the yield was basically determined by the amount of degraded TPH (denumerator). For the cultures having Brij 30 and Tween 80, the biodegradation was high so that large amount of TPH degraded was obtained and used to calculate yields, resulting in low values. In contrast, considerably low TPH degradation was observed in the control culture and the cultures having Triton X-100 and, consequently, low amounts of degraded TPH were used to calculate the yields resulting in high values.

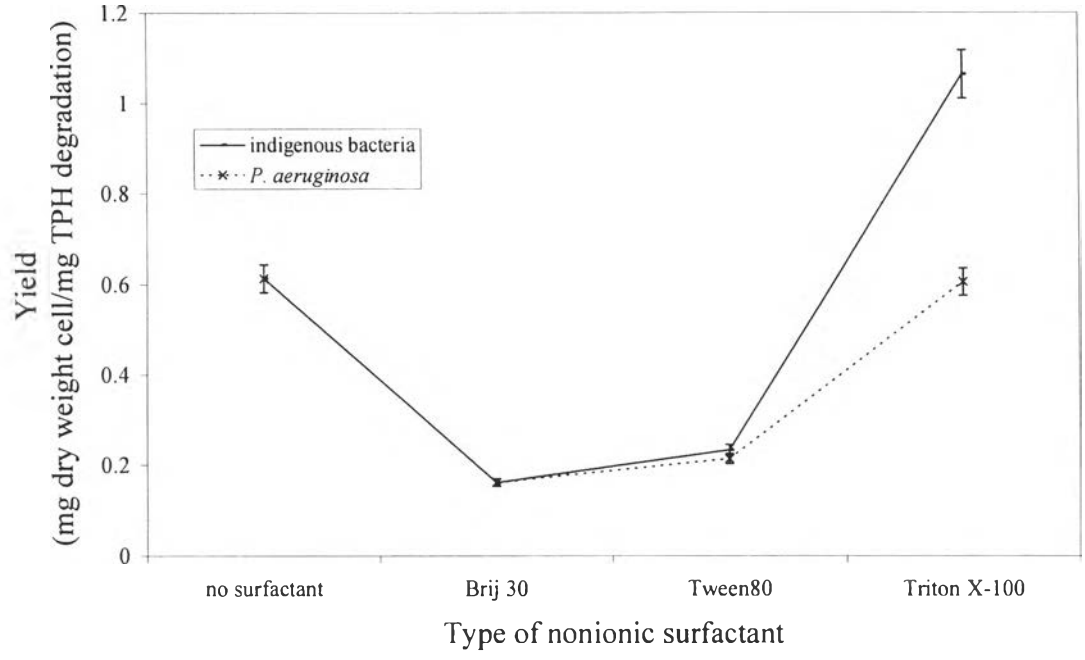


**Figure 4.13** Yield of indigenous bacteria and *Pseudomonas aeruginosa*.

Since different amount of each surfactant was used in the biodegradation studies, it is important to evaluate the effect of the surfactant on hydrocarbon degradation on the same weight basis. Table 4.3 (a) and (b) shows the total amount of TPH degradation and the rate of TPH degradation per weight of added nonionic surfactant. It can be seen that both total TPH degradation and the TPH degradation rate per weight of added nonionic surfactant for both microorganisms were highest in the cultures with Tween 80. For indigenous bacteria, the amount of degraded TPH per g of Tween 80 was found to be 4.93 g TPH/g Tween 80 whereas the degradation rate was found to be 0.70 g TPH/day*g Tween 80. For *Pseudomonas aeruginosa*, the amount of degraded TPH per g of Tween 80 was found to be 5.49 g TPH/g Tween 80 whereas the degradation rate was found to be 0.78 g TPH/day*g Tween 80. Both amount of degraded TPH and the degradation rate obtained in the cultures with addition of Tween 80 were approximate 10.15 times those obtained in cultures containing Brij 30 and Triton X-100. The results clearly showed that the addition of Tween 80 had the most effect on enhanced biodegradation, and may be the most suitable surfactant.

**Table 4.3 (a)** Indigenous bacteria, the total TPH degradation and the rate of TPH degradation per weight of nonionic surfactant

| Type of nonionic surfactant | TPH degradation (g)/ wt of surfactant (g) | TPH degradation (g)/ day*wt of surfactant (g) |
|---|---|---|
| Brij30 | 0.38 | 0.05 |
| Tween 80 | 4.93 | 0.70 |
| Triton X-100 | 0.58 | 0.08 |

**Table 4.3 (b)** *Pseudomonas aeruginosa*, the total TPH degradation and the rate of TPH degradation per weight of added nonionic surfactant

| Type of nonionic surfactant | TPH degradation (g)/ wt of surfactant (g) | TPH degradation (g)/ day*wt of surfactant (g) |
|---|---|---|
| Brij30 | 0.38 | 0.05 |
| Tween 80 | 5.49 | 0.78 |
| Triton X-100 | 0.12 | 0.02 |