CHAPTER 4

RESULTS AND DISSCUSION

4.1 Characteristic of contaminated soil

As mentioned in chapter 3 the soil samples were taken site 3 and site 7. They were mixed in a ratio of 40:60 in order to reach percentage porosity > 25%. The characteristic of the soil sample is shown in table 4.1.

Table 4.1 Physical and chemical properties of the soil of the bioremediation experiment

Properties	Value	Unit
Sand	84.70	%
Silt	4.50	%
Clay	10.80	%
Soil texture	Loamy sand	-
pH	7.14	-
TPH	1.06×10^5	mg/kg
Organic carbon (OC)	17.46	g/100g
Organic matter (OM)	30.10	g/100g
Nitrogen	0.22	g/100g
Phosphorous	6.76	mg/kg

Note: OM = OC*1.724, Method of soil analysis, part 2, chemical and microbiological properties (1982) 2th edn., page 574, American-society of agronomy, soil science society of America, Madison.

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4.2 Soil pH and density

The adjustment of pH, soil pH was measured, when soil pH between 6 and 8 then the soil was chosen. No chemicals were added to adjust the soil pH. The result of soil pH and bulk density measurement of each site is shown at table 4.2.

Table 4.2 Soil pH and bulk density of the sampling sites

Site	pH	Bulk density (t/m^3)
1	7.37	1.973
2	8.05	0.933
3	5.36	2.026
4	6.66	1.976
5	6.9	2.076
6	6.65	1.838
7	6.22	0.891
8	6.71	2.095

After 17 weeks of biopile experiment, all the samples of soil pH showed a change from the slightly alkaline to acidic. The result of pH changes is attached at table 4.3.

Table 4.3 Variation of soil pH during biopile experiment

Treat-ments	Soil pH during biopile experiment					
	14-Feb-05	12-Mar-05	31-Mar-05	22-Apr-05	9-May-05	25-May-05
Tank 1	7.15± 0.01	6.76 ± 0.12	6.01 ± 0.02	6.09 ±0.04	6.09±0.01	6.01 ±0.04
Tank 2	7.21±0.01	7.09 ± 0.07	7.13 ± 0.01	6.32± 0.05	6.71±0.01	6.86±0.02
Tank 3	7.29±0.07	7.10 ± 0.02	7.11 ± 0.06	6.36± 0.01	6.74±0.06	6.92±0.01
Tank 4	7.25±0.08	7.12 ± 0.06	7.19 ± 0.02	6.25± 0.07	6.84±0.04	6.94±0.01
Tank 5	7.16±0.01	7.17 ± 0.01	7.11 ± 0.02	6.31± 0.04	6.78±0.04	7.04±0.02
Tank 6	7.25±0.05	7.21 ± 0.01	7.01 ± 0.03	6.10± 0.01	6.82±0.01	7.04±0.01

Note: The experiment was done for 17 weeks

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The soil pH in the control group or tank 1, was lowest than the treated-soil group. Its trend indicated sharp drop for the first month of biodegradation, and subsequently dropped again becoming more acidic at the conclusion of the experiment that its pH reached 6.01 $\pm$ 0.04 (table 4.3) which was not favorable for biodegradation (Vidali 2001) (figure 4.1).

At zero time, the treated-soil group (tank 2 through 6) showed level of soil pH differed very little (7.15<pH<7.25). At this time the nutrients were amended. During biodegradation the soil pH in the treated-soil group was slightly acidic. And at the of the experiment the soil pH was observed to have a nearly neutral pH. The lower of soil pH was tank 2 (6.86 $\pm$ 0.02) and the highest was tank 5 and tank 6. Their soil pH levels were the same (7.04 $\pm$ 0.01) (table 4.3).

Leahy *et al*., 1990 reported that Dibble and Bartha observed that a soil pH of 7.8 is optimal for the mineralization of oily sludge of petroleum hydrocarbons.

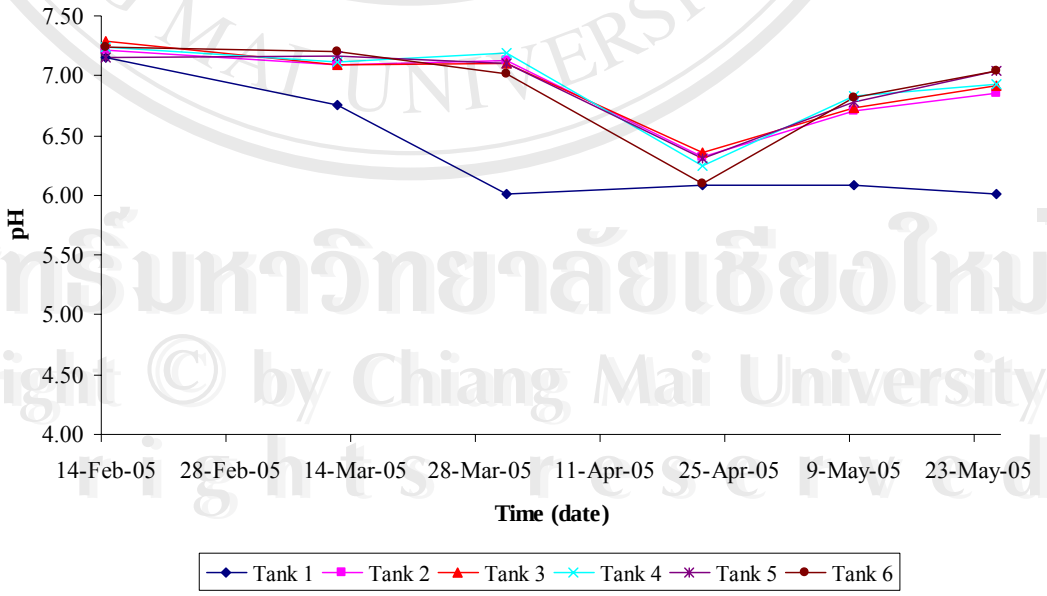

Figure 4.1 Temporal variation of soil pH during biopile experiment

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Furthermore, Vidali (2001) notes the optimum value of soil pH for an oil degradation is 6.5-8.0.

Verstrate *et al*., (1976) found that adjustment of soil pH from acidic conditions (pH 4.5) to near-neutral conditions (pH 7.4) resulted in a doubling of the rate of biodegradation of gasoline in soil (Katherine *et al*., 1994).

Statistically (appendix 8), the soil pH indicates a significant difference at 95% level ($p < 0.05$). There are 4 groups which showed significant differences. The first group was tank 1 showing differences to the other tanks. The second group was tank 2, and the third group were tanks 3 and 4 , and the last group were tanks 5 and 6 (table 4.4).

Table 4.4 The soil pH after biopile experiment

Treatments	Mean $\pm$ SD	Groups
Tank 1 (control group)	6.015 $\pm$ 0.035	(a)
Tank 2	6.855 $\pm$ 0.021	(b)
Tank 3	6.920 $\pm$ 0.014	(c)
Tank 4	6.935 $\pm$ 0.007	(c)
Tank 5	7.035 $\pm$ 0.021	(d)
Tank 6	7.040 $\pm$ 0.014	(d)

Note: -. the letter in the bracket showed homogenous subset
- the experiment was done for 17 weeks

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4.3 Adjustment of soil porosity

As mentioned in chapter 3, the representative soil sample was taken from site 3 and site 7. These samples were blended with three different compositions of 70%:30%, 50%:50% and 30%:70%. The results showed in order to reach 25% of soil void volume, the composition should be higher than 30%:70%. In this experiment, the soil composition of site 3 (sandy matrix soil) and site 7 (clayey matrix soil) was 40:60 (table 4.5). This experiment was designed to be of 25 kg in overall weight, so that the amount of soil from site 3 was 10 kg and soil site 7 was 15 kg.

Table 4.5 Mixing composition between soil site 3 and soil site 7

Mix no.	Soil from site 3	Soil from site 7	H
A	70%	30%	0.69
B	50%	50%	0.64
C	30%	70%	0.43

Note : η = porosity= volume of void / total volume

4.4 Soil temperature

The temporal variations of soil temperature during biopile experiment is shown at figure 4.2 and appendix 5. In all tanks, during the experiment there was observed two variations of temperature viz. the first variation was from the beginning through 15 weeks of the experiment, the soil temperature indicated an increase and toward the end of experiment the soil temperature slightly decreased.

The range of soil temperatures at tank 1 or the control group, was (26-35^{0}C). This result was higher than the other tanks. For the treated-soil group the range of

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soil temperature differed slightly were tank 2 (24-32^{0}C), tank 3 (24-31^{0}C), tank 4 (24.5-32^{0}C), tank 5 (23-31^{0}C) and tank 6 (24-32^{0}C).

All tanks especially in the control group experienced higher than the optimum value for microbial activity, especially for oil degradation (Vidali 2001).

It was noticed, the experiment was conducted in the summer season that the ambient temperature usually is higher than both rainy and cold season. In order to maintain soil temperatures in the rainy and cold season, the soil may be covered with a clear plastic as Mohn *et al*., 2001 reported that a clear plastic cover will reduce convective heat loss from soil, while permitting solar radiation to warm water and also a plastic cover will prevent drying of the soil, a process that would limit hydrocarbon degradation.

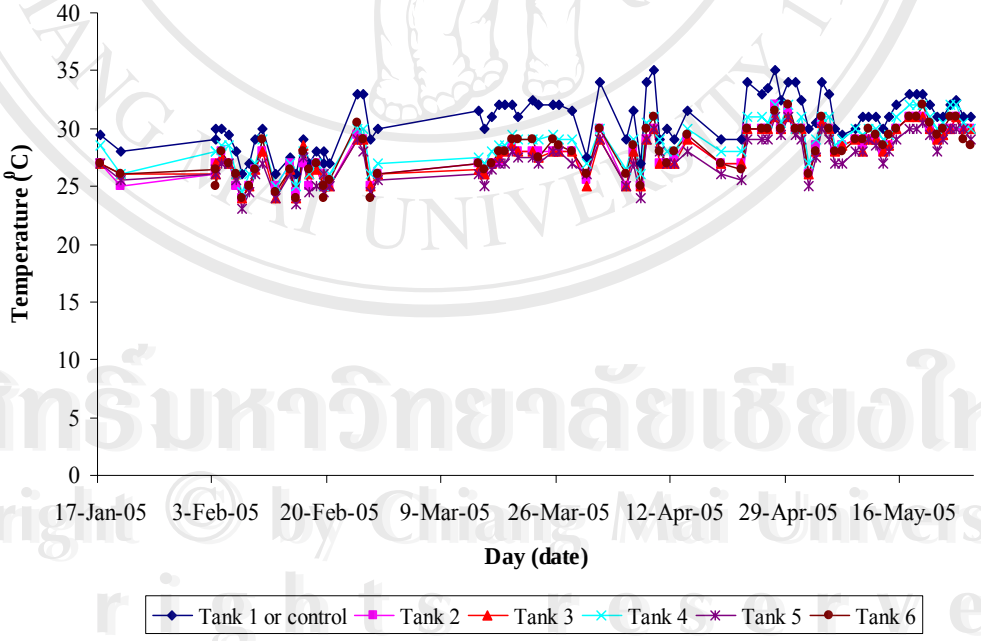

Figure 4.2 Temporal variation of soil temperature for all tanks during biopile experiment

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One way analyses (ANOVA) was done which the result (appendix 8) shows a significant difference at level 95% ($P<0.05$). There are three groups which showed significant differences. The first group was tank 5 and 6 that among them there were not significant differences but differ to the other tanks. The second group was tank 2 till tank 4 and the last group was tank 1 or the control (table 4.6).

Table 4.6 Soil temperature at all tanks after biopile experiment

Treatments	Mean $\pm$ SD (^{0}C)	Groups
Tank 1	30.75 $\pm$ 0.35	(c)
Tank 2	29.75 $\pm$ 0.35	(b)
Tank 3	29.75 $\pm$ 0.35	(b)
Tank 4	29.75 $\pm$ 0.35	(b)
Tank 5	28.75 $\pm$ 0.35	(a)
Tank 6	29.75 $\pm$ 0.35	(a)

Note: -. the letter in the bracket shows group
-. the experiment was done 17 weeks

4.5 Soil nutrients

The levels of nitrogen and phosphorous in the soil may be very critical as these may limit the biodegradation rates because of an active process occurring between the nutrients (Gogoi, *et al*., 2003).

Initially, the soil nutrient, was showed at table 4.1, indicated the concentration of nitrogen and phosphorous was low especially nitrogen. In order to get a ratio C:N:P = 100:10:1 amounts of nitrogen and phosphorous were added. The calculation for

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addition of nitrogen and phosphorous is attached at appendix 1. These amounts were 1907.9 g N and 217.45 g DAP, respectively.

Sources of nitrogen and phosphorous were urea $CO(NH_2)_2$ and diammonium phosphate $(NH_3)_2HPO_4$ or DAP. Urea was chosen as nitrogen source because of its reaction with water yielding ammonia as reaction $CO(NH_2)_2 + H_2O \rightarrow CO_2 + NH_3$, that it is inexpensive, easy to handle and readily available or assimilated in bacterial metabolism but it does create an additional oxygen demand and it will be oxidized to nitrite and nitrate by indigenous bacteria (Cookson 1995).

The temporal variation of the concentration of nitrogen and phosphorous during 17 weeks of biopile experiment is showed at table 4.7 and 4.8, respectively.

Table 4.7 Temporal variation of nitrogen during biopile experiment

Treat-ments	Concentration of nitrogen during biopile experiment (g/100g)					
	14-Feb-05	12-Mar-05	31-Mar-05	22-Apr-05	9-May-05	25-May-05
Tank 1	0.28± 0.00	0.29 ± 0.02	0.28 ± 0.01	0.32 ±0.02	0.34±0.01	0.29 ±0.04
Tank 2	1.58±0.06	1.73 ± 0.06	1.57 ± 0.09	1.52± 0.00	1.35±0.05	1.44±0.13
Tank 3	1.43±0.04	1.49 ± 0.11	1.32 ± 0.01	1.15± 0.07	1.16±0.08	1.19±0.03
Tank 4	1.58±0.09	1.58 ± 0.01	1.43 ± 0.09	1.25± 0.15	1.25±0.04	1.19±0.03
Tank 5	1.52±0.02	1.72 ± 0.08	1.50 ± 0.03	1.33± 0.09	1.41±0.01	1.26±0.05
Tank 6	1.49±0.06	1.52 ± 0.03	1.48 ± 0.05	1.39± 0.04	1.31±0.02	1.33±0.15

Note: The experiment was done for 17 weeks
Total nitrogen was as NH_4-N

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The trend line changes of the concentration of nitrogen during biopile experiment is shown at figure 4.3. In tank 1, or the control group, the concentration of nitrogen remained relatively unchanged. However, in the treated-soil group (tank 2

until tank 6) there was a gradual decrease. The available nitrogen was higher in tank 2 (1.44±0.13 g/100g) and the lower was in tank 3 and 4 which their concentration showed same level (1.19±0.03 g/100g).

Table 4.8 Concentration of phosphorous during biopile experiment

Treat-ments	Concentration of phosphorous during biopile experiment (mg/kg)					
	14-Feb-05	12-Mar-05	31-Mar-05	22-Apr-05	9-May-05	25-May-05
Tank 1	17.61± 0.72	13.45 ± 3.26	28.39 ± 2.45	21.50± 10.47	31.63± 0.53	31.71 ± 3.14
Tank 2	371.56± 29.87	372.76 ± 68.71	397.06 ± 4.48	484.19± 29.13	271.93± 48.82	374.44± 16.27
Tank 3	297.63± 14.94	374.88 ± 119.49	265.04 ± 89.62	226.94± 5.92	263.56± 13.31	204.98± 16.27
Tank 4	388.46± 5.97	315.73 ± 32.86	300.95 ± 95.59	322.14± 31.07	277.16± 17.75	269.26± 71.69
Tank 5	388.46± 65.72	539.11 ± 105.30	275.60 ± 11.95	292.85± 34.02	209.16± 16.27	241.59± 20.71
Tank 6	405.35± 17.92	379.10 ± 20.91	284.05 ± 23.90	342.01± 23.67	348.29± 8.88	192.95± 36.24

Note: The experiment was done for 17 weeks

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An earlier study viz. Banat *et al*., (2003) reported that a reduction in ammonium ion concentration was associated with stripping of nitrogen in ammonia, and the oxidation of ammonium ion into nitrate. The bacterial mixture may preferentially use ammonium as a nitrogen source (Yuan *et al*., 2003).

One way analyses (ANOVA) of the concentration of total nitrogen shows a significance difference ($P < 0.05$) where there were 3 groups viz. the first group was

tank 1 showed a significant difference to the other tanks. The second group was tank 3, 4, 5 and 6 and the last group was tank 2, 5 and 6 (appendix 8 and table 4.9.).

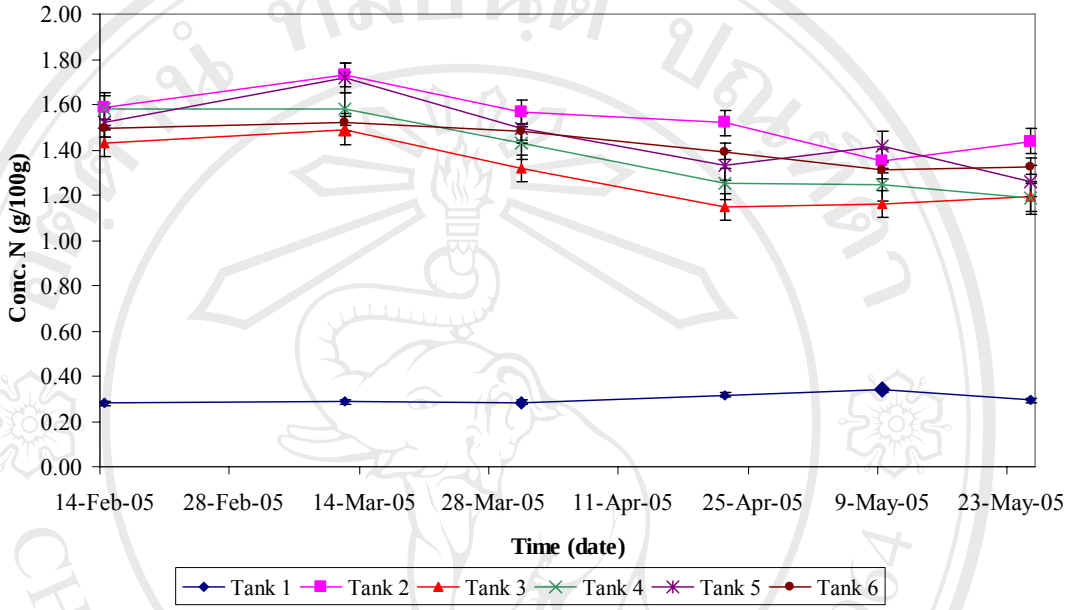

Figure 4.3 The variation of nitrogen during biopile experiment

The concentration of phosphorous in the tank 1 (control group) was lowest than the treated-soil group (tank2 until 6), but its concentration during biopile experiment increased from (17.61± 0.72 mg/kg) to be (31.71 ± 3.14 mg/kg). Generally, in treated-soil group, the concentration of phosphorous decreased from a range (297.63 to 405.35 mg/kg) to (192.95 to 374.44 mg/kg) (table 4.3).

The addition of phosphorous can stimulate the biodegradation of oil hydrocarbons, but various P-source can have different effect on biodegradation, depending on the solubility of the P-source and their toxicity (Margesin *et al*., 2000).

Statistically (appendix 8), the concentration of phosphorous shows significant difference ($P<0.05$) where there are 3 groups (table 4.8). The first group was tank 1

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different to the other tanks. The second group was tank 2 and the last group was tank 3 through 6.

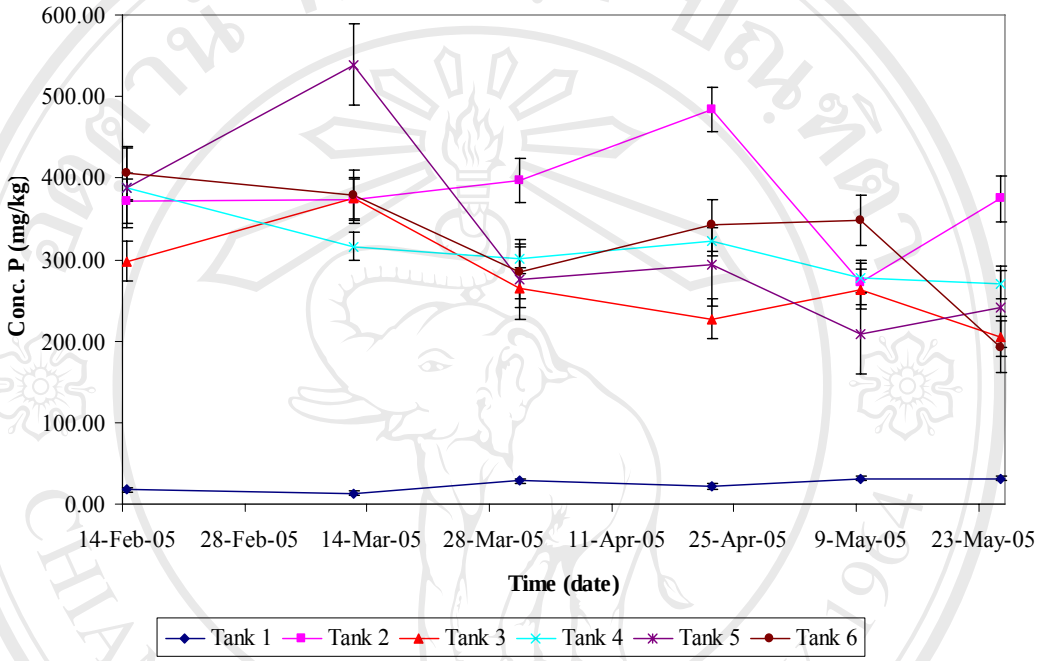

Figure 4.4 The variation of phosphorous during biopile experiment

The supply of nutrient (nitrogen and phosphorous) in this experiment was conducted only one time and it was amended at beginning of experiment. The feeding of nutrients (urea and DAP) was done in dry powder form and no water addition. Because of the concentration of TPH or equal to the present hydrocarbon in the soil was 100,000 mg/kg it was considered high enough so that the ratio of C:N:P was applied at 100:10:1.

Von Fahnestock *et al.*, 1996 suggested that the amended of nutrient is one time, after the initial inorganic nutrients amendment is made, no further nutrient addition will be required, and the addition of nitrogen and phosphorous are

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particularly to be needed if the available carbon levels are high which the ratio of C:N:P was 100:10:1.

Table 4.9 Concentration of nitrogen and phosphorous after biopile experiment

Treatments	Mean and Standard Deviation			
	Nitrogen (g/100g)		Phosphorous (g/100g)	
Tank 1	0.295 ± 0.035	(a)	0.317 ± 0.031	(a)
Tank 2	1.440 ± 0.127	(c)	3.744 ± 0.163	(b)
Tank 3	1.190 ± 0.028	(b)	2.050 ± 0.163	(c)
Tank 4	1.185 ± 0.035	(b)	2.693 ± 0.717	(c)
Tank 5	1.260 ± 0.056	(bc)	2.416 ± 0.207	(c)
Tank 6	1.325 ± 0.148	(bc)	1.930 ± 0.362	(c)

Note: -. the letter in the bracket shows group
- the experiment was done for 17 weeks

This ratio is also suggested by previous studies, such as by Maila *et al*., 2004; Aichberger *et al*., 2005 and Lyes *et al*., 2005.

However, it is rarely necessary to add the total amount of nitrogen and phosphorous called for by this theoretical C:N:P-ratio, because nutrients are recycled during the course of treatment as microorganisms die. Adding the large quantities of nitrogen that a 100:10 C:N-ratio suggests are needed, especially all at once, can be toxic to microorganisms and other soil microorganisms (McMillen, *et al*).

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Furthermore, addition of inorganic nutrients has been reported to affect both positive and negative different aspects of pollutant degradation kinetics, such as lag time, degradation rate and degradation extent (Leys *et al*., 2005). However, inorganic nitrogen and phosphorous addition have been shown to increase transformation rates of hydrocarbon, without apparent increase in microbial biomass (Breedveld *et al*., 2000).

Table 4.10 The percentage removal of organic carbon during biopile experiment

Treatments	Initial OC	Final OC	Percentage lost OC (%)	Ratio OC to N (OC:N)	Notes
Tank 1	13.50	13.94	-3	47 or 470:10	Minus indicated the concentration was increasing. In tank 1 without addition nutrient
Tank 2	19.60	16.17	17	11 or 110:10	
Tank 3	18.41	17.25	6	14 or 140:10	
Tank 4	17.04	14.90	13	13 or 130:10	
Tank 5	18.22	15.39	16	12 or 120:10	
Tank 6	16.49	12.50	24	9 or 90:10	

Note: OC = organic carbon; N = nitrogen
The experiment was done for 17 weeks

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The percentage of removal of the organic carbon and the ratio between organic carbon and nitrogen is shown table 4.10. In the tank 1, or control group, the concentration of organic carbon slightly increased (3%) compared to the initial

experiment. However, in the treated-soil group (tank 2 through tank 6) was a gradual decrease during the biopile experiment for 17 weeks. The higher removal of organic carbon was in the tank 6 (24%) and the lower was in the tank 3 (6%).

Table 4.10 also indicates at the end of biopile experiment, that in tank 1 or control group, the ratio of organic carbon and total nitrogen was 47 or 470:10. This condition is far from a favorable for bioremediation. For the treated-soil group, the ratio of organic carbon and nitrogen generally increased in tank 2 through 5, but in the tank 6 it decreased. The higher ratio C:N was in tank 3 (140:10) and the lower was in tank 6 (90:10).

Mishra *et al*., 2001 stated a change in the C:N ratio at the end of the study also suggests increased bacterial activity.

4.6 Soil moisture content

The moisture content of all the samples were observed as decreasing continuously during the biodegradation experiment (appendix 6 and figure 4.5). In tank 1 the moisture went down below 5% after 77 days. This was fastest than other tanks. This was followed by tanks 3 and tank 4 where moisture went down below 5% after 80 days. The last were tank 2, tank 5 and tank 6 was 95 days since at zero time (Von Fahnestock *et. al*., 1996). At the end of experiment the highest water content was in tank 6 (3.8 $\pm$ 0.198 %) followed by tank 5 (3.7 $\pm$ 0.184%) and the lowest was in tank 1 (2.4 $\pm$ 0.156 %).

Von Fahnestock *et. al*., (1996) reported in a previous study (Leeson and Hinchee 1995) found out of 123 sites surveyed in a bioventing field experiment, the soils at 114 sites contained 5% to 25% water by weight. A slight increase in

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biodegradation with increasing moisture was detected, but the results did not show a strong correlation between the biodegradation rate and moisture content.

Furthermore Von Fahnestock *et. al*., 1996 stated a biopile would be expected to demonstrate similar behavior with an optimum moisture of 10% to 20% by weight and 5% to 30% being acceptable.

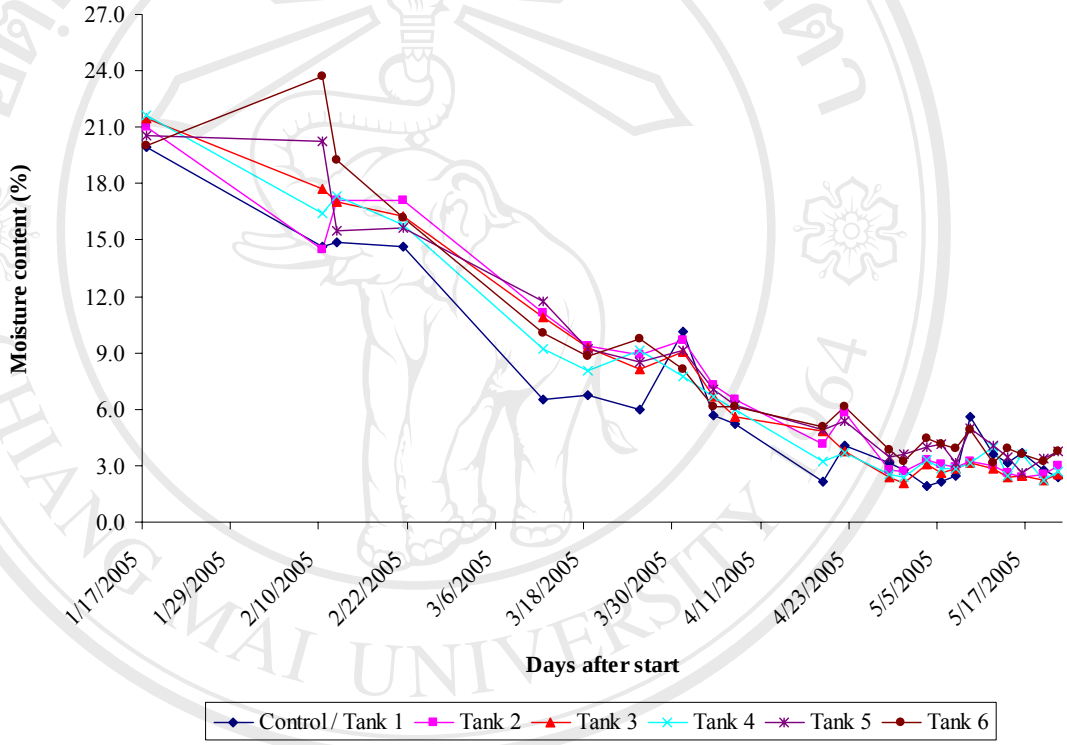

Figure 4.5 Percentage of moisture content of the tank 1 until 6 during biopile experiment

Statistically (appendix 8) was done which the results shows a significant difference ($P<0.05$) which there are 3 group. The first group was tank 1, tank 3 and tank 4 which amongst themselves were the same but different to the other tanks. The second group was tank 2 and tank 4 and the last group was tank 5 and tank 6 (table 4.11).

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Table 4.11 The final moisture content of all tanks

Treatments	Mean ± SD (%)	Groups
Tank 1	2.38 ± 0.156	(a)
Tank 2	2.99 ± 0.028	(b)
Tank 3	2.54 ± 0.057	(a)
Tank 4	2.69 ± 0.156	(ab)
Tank 5	3.73 ± 0.184	(c)
Tank 6	3.76 ± 0.198	(c)

Note: -. the letter in the bracket shows group
-. the experiment was done for 17 weeks

Because the moisture content of each of the tanks differed at the initial experiment so that the percentage of total lost moisture was calculated by dividing between the final and initial moisture and the result is shown in table 4.12. The highest loss of moisture content was experienced in the tank 1, 3 and 4, which showed the same level (88%) and the lowest was in tank 6 (81%) (figure 4.6 and table 4.12).

Air normally will enter biopile at less than 100% relative humidity. The air will tend to remove moisture as it moves through the biopile and become saturated with water, thus reducing the moisture content. However, at the same time, the biodegradation process is converting hydrocarbons to CO_2 and H_2O, thus renewing the moisture content to some degree. Approximately 0.68 kg of H_2O is produced per 0.45 kg of TPH degraded (Von Fahnestock, *et al*. 1996).

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Table 4.12 Percentage of moisture content lost during biopile experiment

Treatments	Initial moisture (%)	Final moisture (%)	Total lost of moisture content (%)
Tank 1	19.93	2.38	88
Tank 2	21.05	2.99	86
Tank 3	21.51	2.54	88
Tank 4	21.62	2.69	88
Tank 5	20.58	3.73	82
Tank 6	20.04	3.76	81

Note: -. the experiment was done for 17 weeks

The main mechanism of water removal was the evaporation of water as a consequence of microbial heat generation. Water evaporation caused a continuous heat removal both in tank 1 and treated-soil group (tank 2 till tank 6), progressively. The continuous decrease in the moisture content during composting is an indication of organic matter decomposition (Kulcu *et al*., 2004).

In general. biodegradation of contaminants is optimal at a soil moisture content between 30 and 80% of the soil's WHC or Water Holding Capacity (Miller *et al*., 2004).

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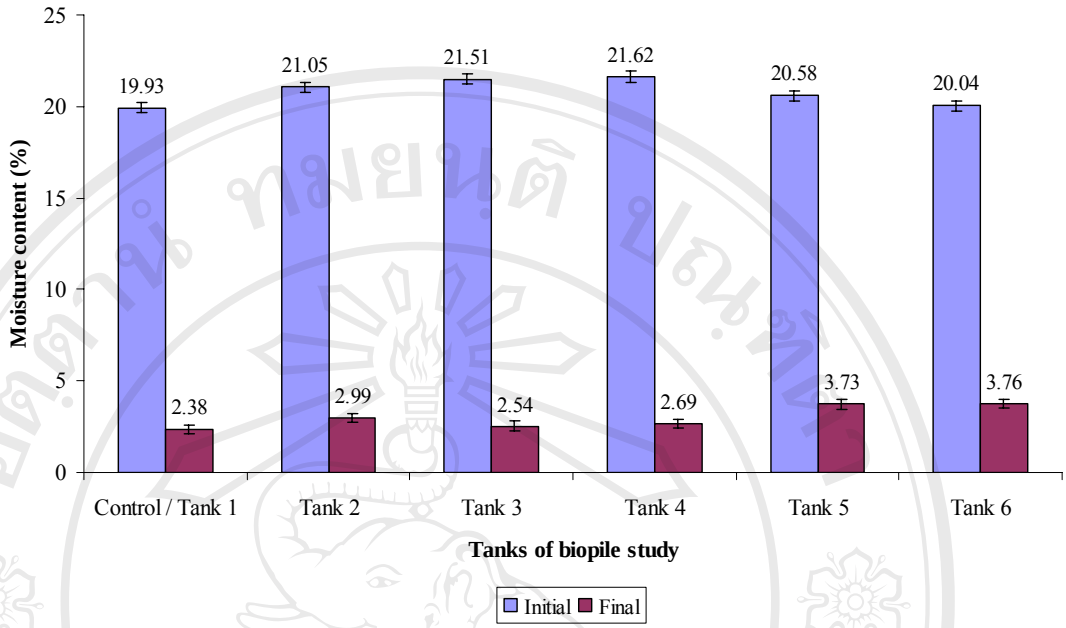

Figure 4.6 The change of moisture content in the biopile experiment during 17 weeks

4.7 Enumeration of total heterotrophic bacterial

The culturable heterotrophic microbial population for all tanks is shown in figure 4.7 and appendix 7. In the tank 1 or control group, the duration of the lag period (acclimation time) of the heterotrophic microbial was 3 weeks, since zero time. Then the exponential phase was apparent for 6 days during which it was noted that the number of heterotrophic microbial increased by two-fold ($5.5x10^8$ CFU/g-of-dry-soil) from zero time ($5.6x10^6$ CFU/g-of-dry-soil). Then during the stationery phase for 16 days, the growing rate was slow and finally, the death phase where the number of heterotrophic microbial declined ($1.3 x10^7$ CFU/g-of-dry-soil) (figure 4.8).

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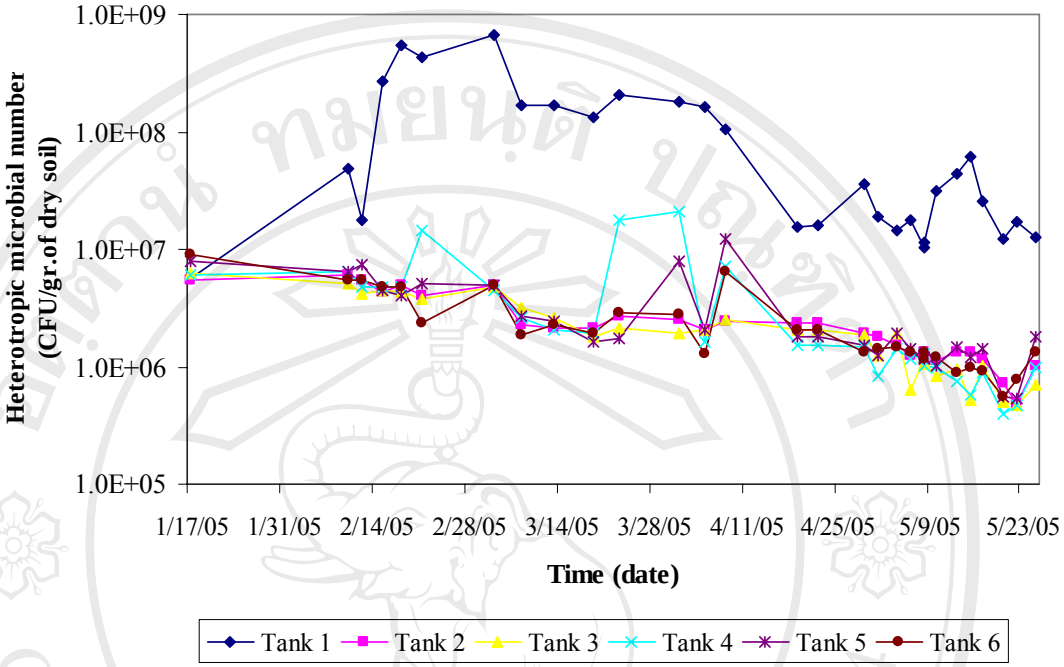

Figure 4.7 Temporal variation of heterotrophic bacterial population in the all tanks during biopile experiment

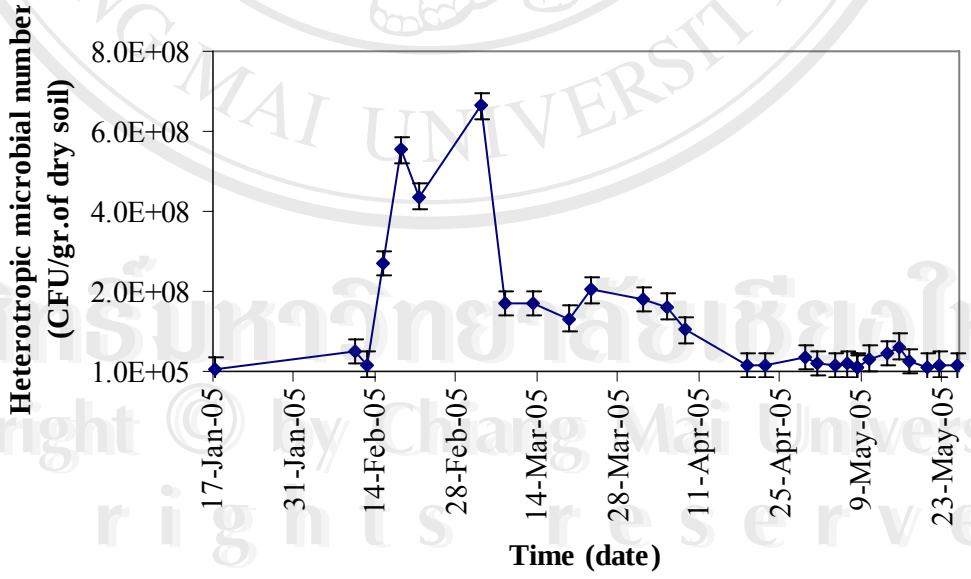

Figure 4.8 Temporal variation of heterotrophic bacterial population in the tank 1 or control group during biopile experiment

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It was observed that the population of the total heterotrophic microbial for treated-soil groups (tank 2 through to tank 6) remained relatively unchanged during biopile experiment (figure 4.9).

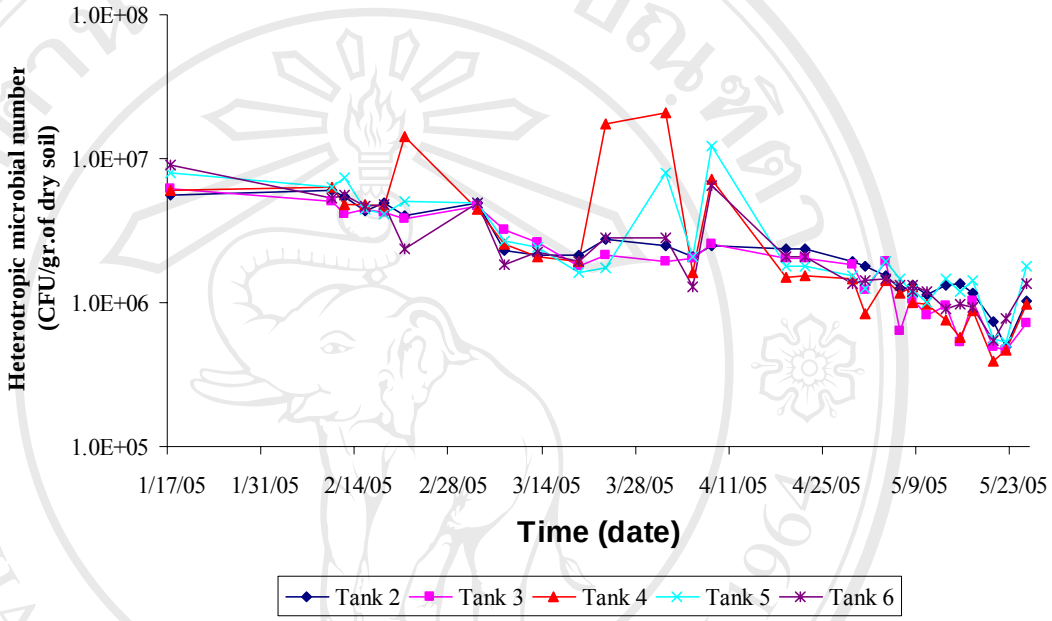

Figure 4.9 Temporal variation of heterotrophic bacterial population in the treated-soil group during biopile experiment

The length of the acclimation periods may be 1 hr or many months because microorganism do not respond immediately. A lag period occurs before any significant degradation, and utilization of electron acceptor occurs (Martin 1999) .

Furthermore, lags of several weeks to several months have been experienced. Lags periods are typical and result from several factor. The most common lag period is the time required for a small population of bacteria to grow before enough specific degraders accumulate to cause measurable increase in chemical transformation. Many factors can effect the growth of small populations. Indigenous protozoa have been shown to extend lag periods as a result of their predator activities. Lags in

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achieving mineralization can be influenced by the buildup of toxic intermediates and the corresponding lag period of the microbial species required to degrade the intermediates (Cookson, 1995).

In this experiment the enumeration of present microbial in the soil was performed by enumeration of total heterotrophic microbial; however, the soil-contaminated-oil-degraders (contaminant-specific degraders) were not.

Determination of the total heterotrophic microbial used peptone yeast extract agar media with the carbon source being peptone. The contaminant-specific degraders are performed in a medium that provides only the necessary inorganic nutrients required for microbial growth, but with no intrinsic carbon sources, the contaminant supplement serves as the sole source of carbon (Gogoi *et al*., 2003).

Furthermore, Balba *et al*., 1998 stated that the use of specific hydrocarbon degrading bacterial counts provide additional information on the hydrocarbon biodegradation potential in a particular soil. The percentage of hydrocarbon-utilizing bacteria to the total heterotrophic bacterial counts usually reflects the extent of microbial acclimation and hydrocarbon degradation activities in an oil-contaminated sites.

At the beginning of the experiment, the initial indigenous population of total heterotrophic microbial was found 10^6 CFU/g-of-dry-soil. In a previous study, it was reported that when the population of indigenous microorganisms capable of degrading the target contaminants is less than 10^5 CFU/g-of-dry-soil, bioremediation will not occur at a significant rate (Mishra *et al*., 2001).

Margesin *et al*., 2000 assumed that changes in bacterial numbers are indicative of a stimulated biodegradation process, but do not represent an accurate measurement

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of the actual biodegradation. The quantification of viable soil microorganisms alone gives no information about the efficiency of the populations, and it is well known that only a small part of soil microorganisms can be isolated and cultivated on laboratory media. Therefore, the quantification of soil biological activities is often to interpret the intensity of microbial metabolism in soil

The temporal number of heterotrophic microbial is shown at appendix 7. Statistically, the number of heterotrophic microbial is significantly different ($P<0.05$) as shown in appendix 8. The significance differences among the tanks are divided into 3 groups. The first group was tank 2, 3, 4, and 6 which within themselves were the same but were different to the other tanks. The second group consisted of tank 5 and 6. The last group was only tank 1 (table 4.13).

At the end of the experiment, it was observed that the number of total heterotrophic microbial in the tank 1 or control groups was (10^6 -10^8) CFU/g-of-dry-soil which was higher than others. In the treated-soil group, the range of the number of total heterotrophic microbial remained relatively unchanged during the biopile experiment however the highest number was in tank 5 (1.8×10^6 CFU/g-of-dry-soil) and the lowest was tank 3 (7.1×10^6 CFU/g-of-dry-soil) (table 4.13).

The same result was experienced by Margesin *et al*., 2000 where it was observed that the number of hydrocarbon degraders increased after stimulation of the biodegradation process. However, this increase was also in soil without added nutrients amendments.

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Table 4.13 The heterotrophic microbial number after biopile experiment

Treatments	Mean of the heterotropic microbial number (CFU/g of dry soil)	Groups
Tank 1	1.3.E+07	(c)
Tank 2	1.0E+06	(a)
Tank 3	7.1E+05	(a)
Tank 4	9.7E+05	(a)
Tank 5	1.8E+06	(b)
Tank 6	1.3E+06	(ab)

Note: -. the letter in the bracket shows the group
-. the biopile experiment was done for 17 weeks

Even in the absence of added N and P, biodegradation continues in soil albeit at a slow rate. This is probably a consequence of nutrient regeneration that is a recycling of the elements as they are first assimilated into microbial cells and then are converted back to the inorganic forms as the cell lyse or are consumed by predators or parasites, both of which release some of the N and P contained in their prey or host. Under such circumstances, the rate of biodegradation will be governed by the rate at which the limiting nutrients are recycled (Alexander *et al.*,1999).

Additionally, Margesin *et al.*, 2000 found that the number of heterotrophic microorganisms remain almost constant during the whole incubation, whereas the number of hydrocarbon-degrading microorganisms increased with time. Furthermore, Leahy *et al.*, 1990 reported that this phenomena was also experienced by some previous studies such as Cobet *et al.*, 1973, Hollaway *et al.*, 1980, Olsen *et al.*, 1979

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and Pinholt *et al*., 1979 which stated that the diversity of heterotrophic populations was shown to be unchanged.

Even though there was little change in the total heterotrophic microbial population this does not mean that TPH could not be degraded by surviving populations. The indigenous microbes must have been somewhat tolerant to TPH since the initial concentrations were relatively high (Miller *et al*., 2004).

4.8 Total petroleum hydrocarbon (TPH)

The remains of total petroleum hydrocarbon (TPH) in all tanks during biopile experiment for 17 weeks are showed in table 4.14. In tank 1 or the control group the remains of petroleum hydrocarbon for 17 weeks of experiment was (44103 $\pm$ 321) mg/kg. Its reduction was 58.5%, which was lowest than the other tanks. In the treated-soil group the highest reduction of petroleum hydrocarbon was in the tank 4 (68.13%) followed by tank 5 (67.32%).

Aichberger *et al*., 2005 conducted the suspension flask experiments for 3 sites with drilling soil from Tuttendorf, Vienna. He found the initial TPH concentrations of 2360, 2710 and 7010 mg kg^{-1} in course of full experiment of 61 days there was reduction by 79,81, 66%, respectively.

Li *et al*., (2004) found there was reduction of 24% (from 20,318 to 15,497 mg/kg of TPH) and 26% (from 17,514 to 12,946 mg/kg of TPH) within 30 days for aeration system with horizontal perforated pipes placed in random fashion and a vertical perforated that penetrates the center of the pile, respectively.

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Table 4.14 Temporal variations of the TPH content in all tanks during biopile experiment

Treatments	Concentration of total petroleum hydrocarbon in all tanks (mg/kg)				
	20-Jan-05	12-Mar-05	31-Mar-05	9-May-05	25-May-05
Tank 1	106254 ± 471	96139 ± 1544	87881 ± 972	57722 ± 929	44103 ± 321
Tank 2	106254 ± 471	83963 ± 697	75682 ± 822	56243 ± 2404	37078 ± 757
Tank 3	106254 ± 471	114400 ± 3431	83898 ± 2358	73505 ± 747	36590 ± 625
Tank 4	106254 ± 471	113651 ± 2110	86631 ± 891	78186 ± 974	33858 ± 2519
Tank 5	106254 ± 471	76394 ± 1046	64175 ± 3722	50374 ± 782	34723 ± 932
Tank 6	106254 ± 471	86956 ± 578	51540 ± 3516	39200 ± 433	35605 ± 1800

Note: -. The biopile experiment was done for 17 weeks

Margesin *et al*., 2000 stated the soil hydrocarbon content can not be reduced to zero by using biological decontamination, even after a prolonged treatment. The remaining 10-30% consists of hydrocarbons that are structurally less available for biodegradation due to recalcitrance and very limited bioavailability, causing a downward trend in microbial abundance well in advance of exhaustion of all hydrocarbons.

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Statistically (appendix 8), there was significant difference at 95% ($P<0.05$) in that tank 1 was different from the other tanks. However, for the treated-soil group, among themselves it was homogenous or not significant difference (table 4.15).

Table 4.15 The concentration of hydrocarbon after biopile experiment

Treatment	Mean ± SD (mg/kg)	Groups
Tank 1	44103 ± 321	(a)
Tank 2	37078 ± 757	(b)
Tank 3	36590 ± 625	(b)
Tank 4	33858 ± 2519	(b)
Tank 5	34723 ± 932	(b)
Tank 6	35605 ± 1800	(b)

Note: -. the letter in the bracket shows the group
-. The experiment was done for 17 weeks

First-order kinetic for removal of petroleum hydrocarbons from soil were previously observed by some authors such as Cookson, 1995, Kirchmann *et al*., 1995, Li *et al*., 2003, Laleh *et al*., 2003 and Aichberger *et al*., 2005.

The first order kinetic was linearly regressed with relationship between operational period and natural log values of TPH concentration (Namkoong *et al*., 2002).

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The equation for first-order kinetics is:

$$\frac{dH}{dt} = -bH \quad (1)$$

where H is the hydrogen concentration and b is the degradation rate.

The solution of H is :

$$H = H_0 \exp(-bt) \quad (2)$$

Where H_0 is the initial oil content. The logarithm of the equation is:

$$\ln(H) = \ln(H_0) - bt \quad (3)$$

In order to get b or degradation rate thus a weighted regression method may be conducted where b indicate slope of line liner regression. The linear regression for all tanks were given at figure 4.10 until 4.15.

The calculation of the slope and the goodness of fit of the linear line regression, standard deviation were calculated by SPSS program as shown at appendix 9

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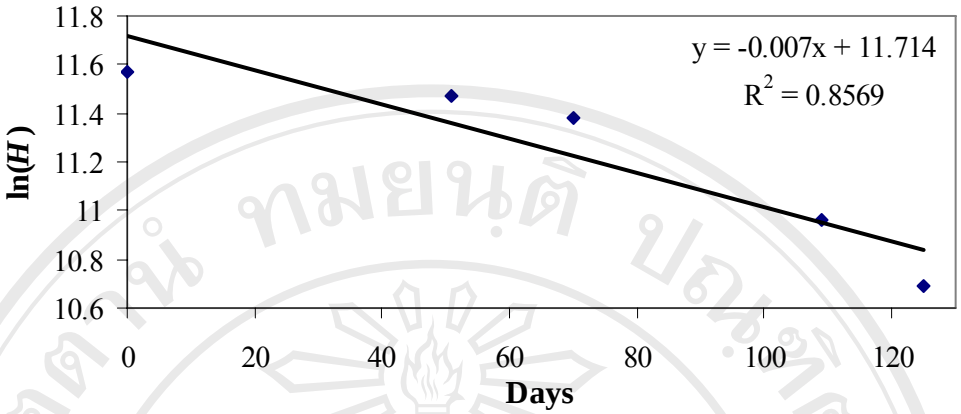

Figure 4.10 Natural logarithm of the TPH and the regression equation for control group or tank 1

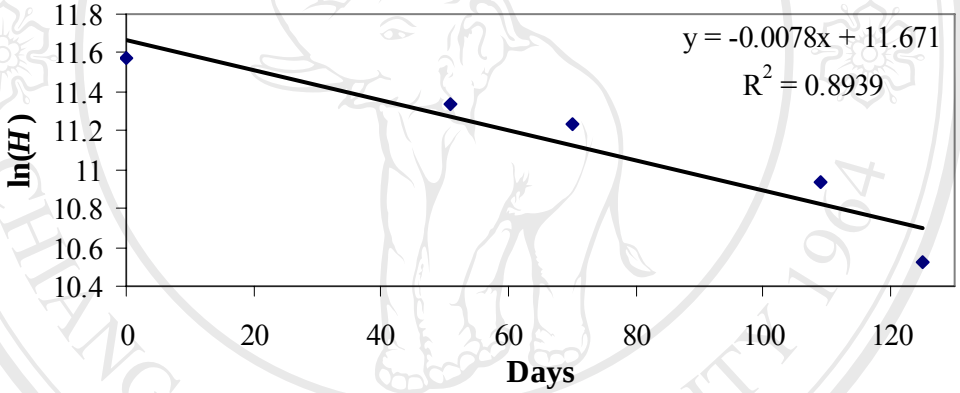

Figure 4.11 Natural logarithm of the TPH and the regression equation for tank 2

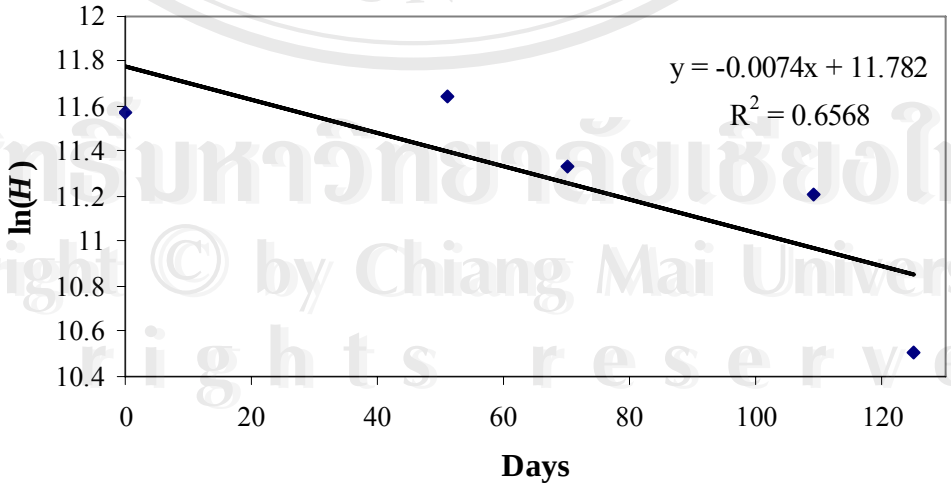

Figure 4.12 Natural logarithm of the TPH and the regression equation for tank 3

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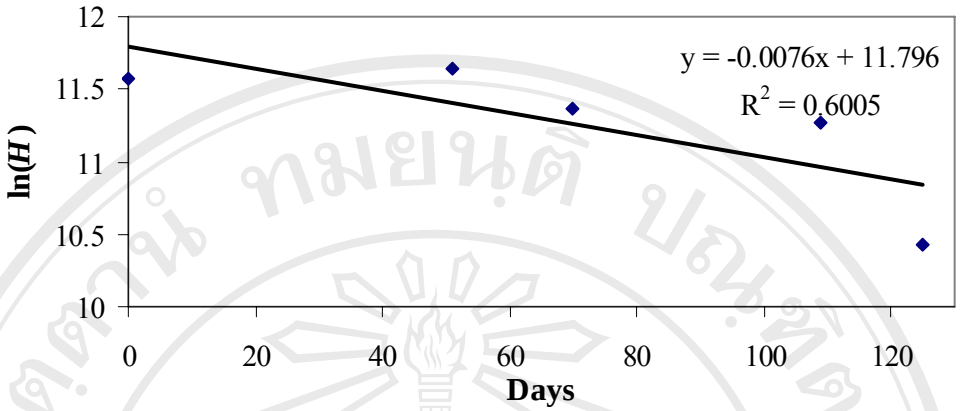

Figure 4.13 Natural logarithm of the TPH and the regression equation for tank 4

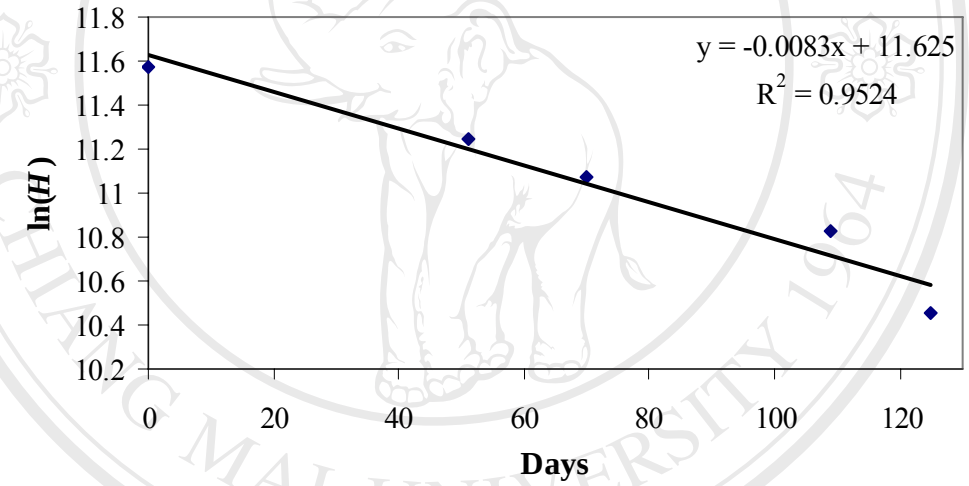

Figure 4.14 Natural logarithm of the TPH and the regression equation for tank 5

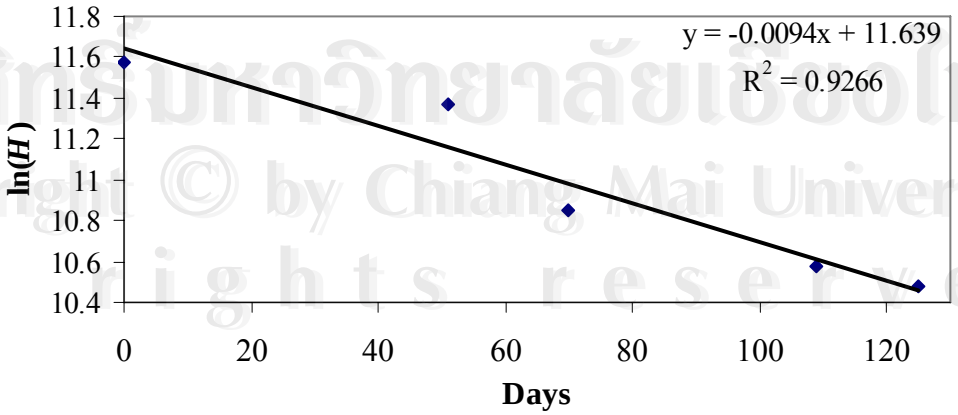

Figure 4.15 Natural logarithm of the TPH and the regression equation for tank 6

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The slope and the goodness for the linear line regression of all tanks during biopile was showed in table 4.16.

Table 4.16. The slope and goodness of fit of the linear line regression for all tanks during biopile experiment

Treatments	Slope (b)	Standard Deviation	The goodness fit (R^2)	Flow rate of air (l/min) ,(SCFH)
Tank 1	-0.006986	0.001648	0.85688	-
Tank 2	-0.007751	0.001541	0.89394	11.8, 24.9
Tank 3	-0.007428	0.003100	0.65676	8.6, 18.1
Tank 4	-0.007606	0.003582	0.60048	6.2, 13.2
Tank 5	-0.008330	0.001075	0.95244	3.8, 8.1
Tank 6	-0.009406	0.001529	0.92658	2.4, 5.2

Note : -. minus indicated degradation
-. the linear regression with level confidence was 95%.
-.the biopile experiment was done 17 weeks .

Tank 1 or control group which was without addition nutrients nor air shows the degradation rate was (0.007 ± 0.001648) day^{-1} with 95% confidence level and its R^2 (0.85688) showed degradation rate constant significantly correlated to those of TPH was shown by ANOVA ($P<0.05$) with 95% confidence interval (appendix 9). With the degradation rate constant at $(0.007 \pm 0.001648)day^{-1}$, the tank 1 or control group was slowest than all tanks.

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For the treated-soil group that received forced air and nutrients addition shows the range of degradation rate hydrocarbon was (0.0074 to 0.0094)day^{-1} with the R^2 range was (0.60048 to 0.95244). The goodness of fit of linear line regression or R^2 of the degradation rate constant for tank 3 and 4 found not significantly correlate to the those TPH was showed by ANOVA ($P>0.05$) with 95% confidence interval. And for tank 2, 5 and 6, their R^2 showed the degradation rate constant significantly correlated to the those TPH was showed by ANOVA ($P<0.05$) with 95% level of confidence (appendix 9).

Tank 6 with an air flow rate of 2.4 l/min showed the highest degradation rate constant (0.0094 $\pm$ 0.001529) day^{-1} with the R^2 was 0.9266, however tank 5 with its air flow rate 3.8 l/min and degradation rate constant (0.0083 $\pm$ 0.001075) day^{-1} had the highest R^2 was 0.9524. So according to their R^2, the degradation rate constant of tank 5 was accepted and tank 6 was rejected (table 4.16).

First order kinetic for removal of petroleum hydrocarbons from soil were previously observed by Yerushalmi for the removal of petroleum hydrocarbon from soil was 0.009 days^{-1} and 0.007 days^{-1} for biostimulated soil and original, respectively (Yerushalmi *et al*., 2003). Also Yerushalmi *et al*., 2003 reported that Taylor and Viraraghavan (1999) found the rate constants for removal petroleum hydrocarbons from soil was 0.01 days^{-1} and 0.03 days^{-1} during the biodegradation of hydrocarbons in non-amended and amended soil, respectively.

Furthermore, Li *et al*., 2004 found the rate constant was (0.0111 $\pm$ 0.0090) and (0.0117 $\pm$ 0.0090) days^{-1} for aeration system with horizontal perforated pipes placed in random fashion and a vertical perforated that penetrates the center of the pile, respectively .

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As a comparison of the degradation rate between the present experiment and previous study is shown at table 4.17.

Table 4.17 The comparison of degradation rate between the present experiment and some previous studies

Authors	The degradation rate constant	
The present experiment	(0.0083 $\pm$ 0.001075) $days^{-1}$ for addition nutrients and air	(0.007 $\pm$ 0.001648) $days^{-1}$ for without addition nutrients nor air
Yerushalmi *et al*., 2003	0.009 $days^{-1}$ for biostimulated soil	0.007 $days^{-1}$ for original soil
Taylor and Viraraghavan 1999	0.03 $days^{-1}$ for amended soil	0.01 $days^{-1}$ for non-amended
Li *et al*., 2004	(0.0111 $\pm$ 0.0090) $days^{-1}$ for aeration system with horizontal perforated pipes placed in random fashion	(0.0117 $\pm$ 0.0090) $days^{-1}$ for aeration system with a vertical perforated that penetrates the center of the pile.

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In this experiment the hydrocarbon content was determination by only TPH. In the recalcitrant biodegradation term, the higher-molecular weight PAHs, resin and asphaltnes are more recalcitrant to biodegradation (Owen *et al*., 2003). So it is essential to measure a comprehension of the same respect of total oil content and its

constituents in terms of the hydrocarbon present. Usually the hydrocarbons present comprise saturates (alkanes and cycloalkanes) and aromatics (mono- and polynuclear). The polar fraction of the petroleum containing nitrogen, sulfur and oxygen is comprised of ashphaltenes and resins (Gogoi *et al*., 2003). The relative potentials for bioremediation of the major types of petroleum compounds decrease in the order, monoaromatic>straight chain alkanes>branched alkanes>napththenes>polynuclear aromatics>polars that microorganisms are selective and attack specific hydrocarbons rather than all the components of the oily sludge (Gogoi *et al*., 2003).

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