CHAPTER IV

RESULTS

4.1 Shrimp pond characteristics

4.1.1 Shrimp ponds characteristics at Nong Suea (R1 and R2 ponds), Pathum Thani

Two shrimp ponds in Pathum Thani, assigned as R1 and R2, were operated under closed system without water exchange throughout the crop. The culture condition and pond management such as feed, water, aeration and shrimp density were similar.

Sediment characteristics were analyzed every 2 weeks after releasing shrimps until harvesting. The total cultivation crop was 114 days; from December 29, 2003 to April 20, 2004). Temperature and salinity were measured in the water at 50 cm below water surface, while others parameters were measured from dried sediment samples.

4.1.1.1 Temperature

As shown in Figure 4-1, water temperature in all ponds fluctuated between 29.8 and 35 °C. Increase of temperature in both ponds was found during the last four weeks due to seasonal change. However, there was no significant different between water temperature in ponds R1 and R2.

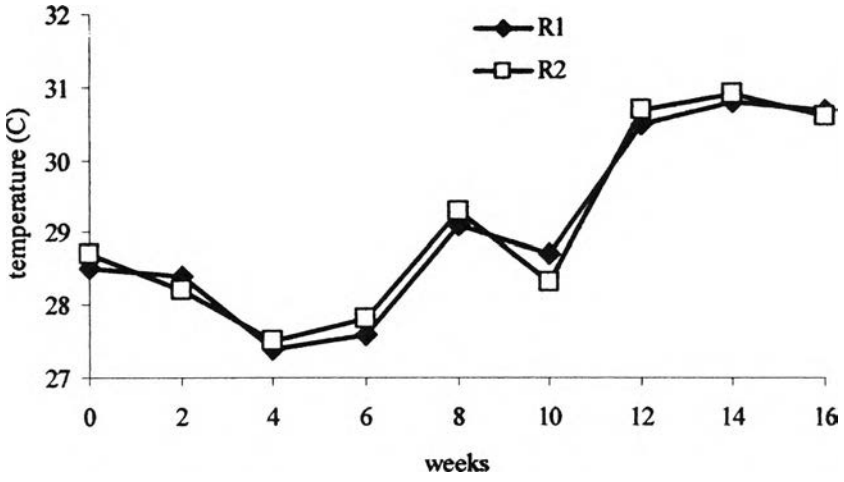

Figure 4-1: Water temperature in R1 and R2 ponds at Pathum Thani Province (measured at 10:00 am).

4.1.1.2 pH

The results of sediment pH in both ponds are shown in Figure 4-2. It was found that pH in both ponds, measured at approximately 10:00 was gradually decrease. In pond R1, pH decreased from 8.90 at the initial to 7.70 at the end of the culture crop while pH of pond R2 also decreased from 8.70 to 7.44. There was no significant different between pH in sediment from ponds R1 and R2.

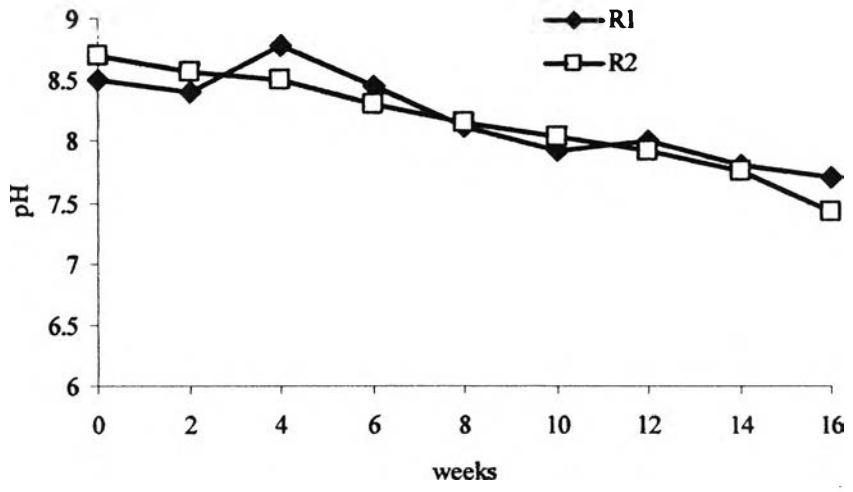

Figure 4-2: pH of the sediment in R1 and R2 ponds at Pathum Thani Province.

4.1.1.3 Salinity

At the beginning of culture, both R1 and R2 had the same salinity at 6 psu. Decrease in salinity of all ponds during week 6-10 was due to raining. However, salinity in all ponds was steadily rose after day 40 due to evaporation. (Figure: 4-3).

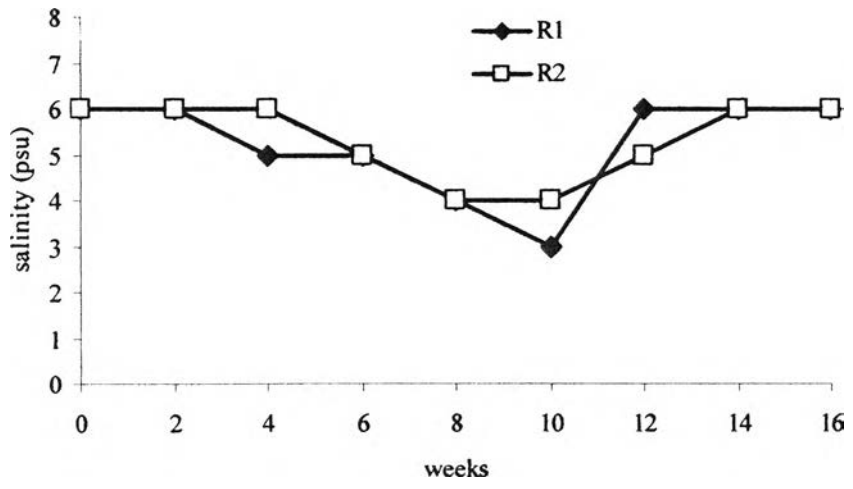

Figure 4-3: Water salinity in ponds R1 and R2 at Pathum Thani Province (measured at 10:00 am).

4.1.1.4 Water content and organic matter content

The results in Figure 4-4 shows that organic matter and water contents in both R1 and R2 ponds had the same trend, which were gradually increased over the culture period. The lowest organic matter content was found at the beginning with 11.70% and 14.55% for ponds R1 and R2 respectively. At the last week, the highest organic matter content of pond R1 was 41.29% while substantially increase of organic matter in pond R2 was found during weeks 14 and 16. This made the highest organic content matter content in Pond R2 was extremely high, up to 56.94%.

With water content, the lowest water contents were found in the first measurement, 21.69% and 18.47%, and the highest water content was found in the last measurement at 34.67% and 35.97% for ponds R1 and R2, respectively.

4.1.1.5 Chlorophyll *a*

Chlorophyll_*a* concentration in R1 and R2 was highly fluctuated (Figure 4-5). The low chlorophyll_*a* concentration was found at the first two weeks (11.51 mg-Chlorophyll *a*/kg (DW) in R1 and 27.21 mg- Chlorophyll *a* /kg (DW) in R2). After that, chlorophyll_*a* in both ponds were suddenly increased to 130-180 mg- Chlorophyll *a* /kg (DW) in week 4. This indicated the bloom of phytoplankton in both ponds. Peak of chlorophyll_*a* concentration was found again in R1 at week 12 while chlorophyll_*a* in R2 was slightly decreased.

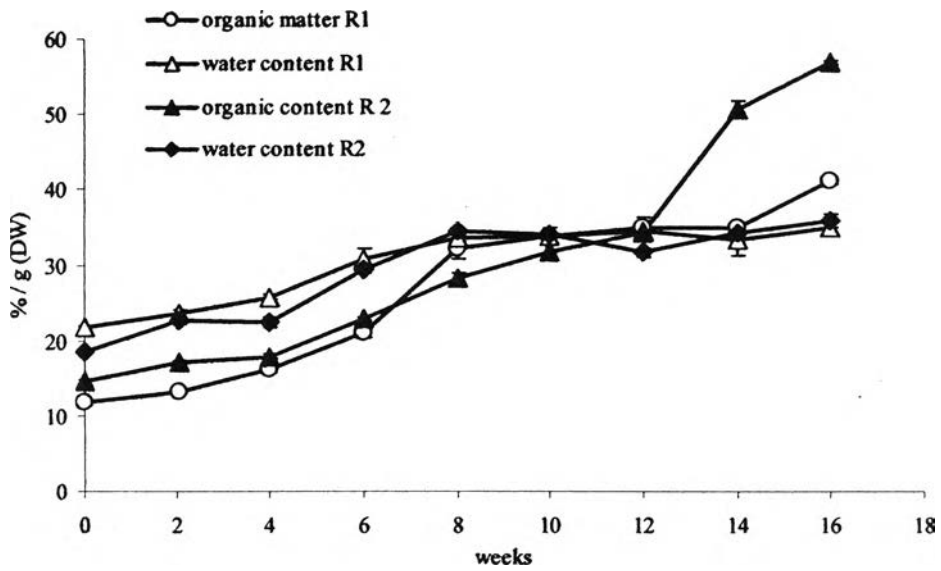

Figure 4-4: Water content and organic matter content in sediment obtained form ponds R1 and R2 at Pathum Thani Province.

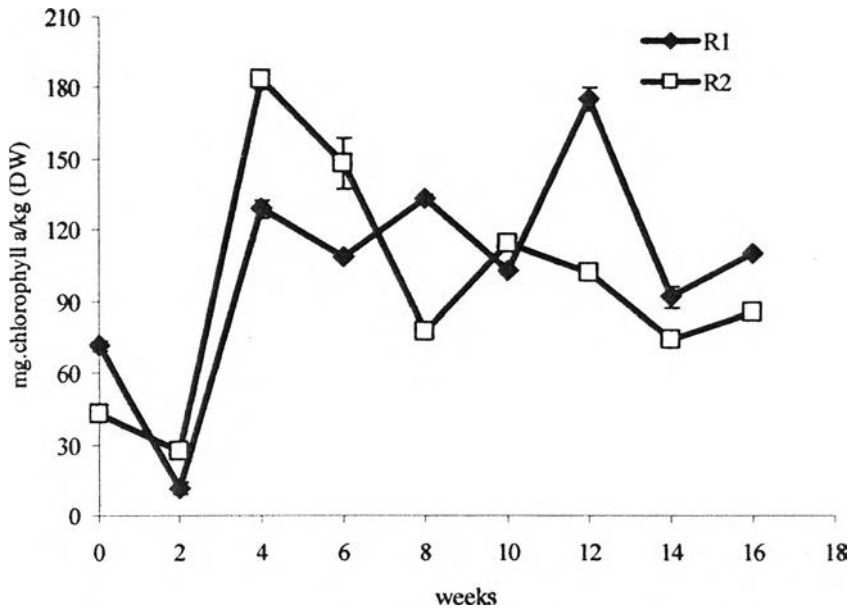

Figure 4-5: Chlorophyll *a* concentration in sediment obtained from ponds R1 and R2 at Pathum Thani Province.

4.1.1.6 Total ammonia

Ammonia concentration in pond R1 fluctuated within 2.9 and 25.4 NH_4^+-N/kg (DW) (Figure 4-6). The highest ammonia concentration was found in week 6 at 25.4 NH_4^+-N/kg (DW) and the lowest ammonia concentration was found in week 8 at 2.9 NH_4^+-N/kg (DW). In pond R2, ammonia concentration was fluctuated within 1.2 and 18.9 NH_4^+-N/kg (DW). The highest ammonia concentration was found in week 0 at 18.9 NH_4^+-N/kg (DW) and the lowest ammonia concentration was found in week 14 at 1.2 NH_4^+-N/kg (DW).

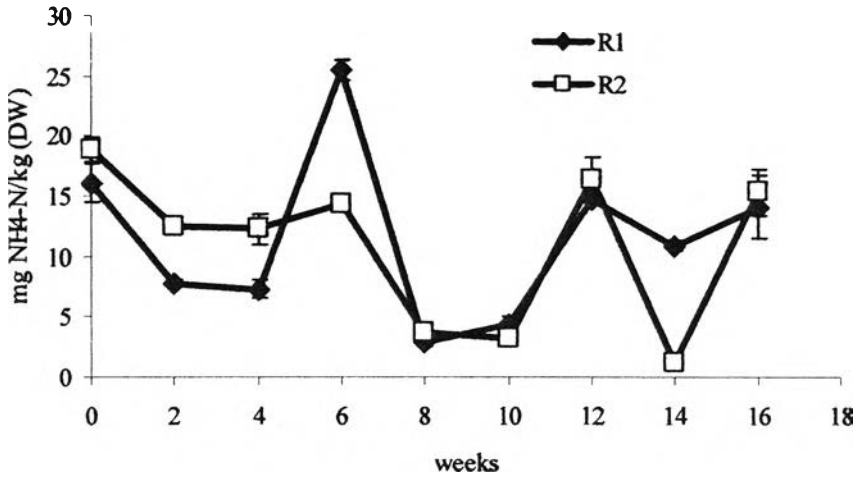

Figure 4-6: Ammonia concentration in sediment obtained from pond R1 and R2 at Pathumthani Province.

4.1.1.7 Nitrate and nitrite

Figure 4-7 shows trend of nitrite and nitrate concentrations in ponds R1 and R2. Nitrate concentration in both two ponds was fluctuated during culture period. Nitrate concentration was low at the first measurement (week 0) before it was suddenly increased

in week 2. Highest nitrate concentration was found in week 2 at 0.41 mg NO_3^--/kg (DW) in pond 1 and 0.25 mg NO_3^--/kg (DW) in R2. After that, nitrate in both two ponds was gradually decreased since week 12. Peak of nitrate concentration was found again in week 14. However, nitrate concentration in R2 seemed to be lower than R1 throughout the culture period.

On the other hand, nitrite concentration in both ponds was less fluctuated. It was stable until week 10 before nitrite in R2 was suddenly increased in week 12.

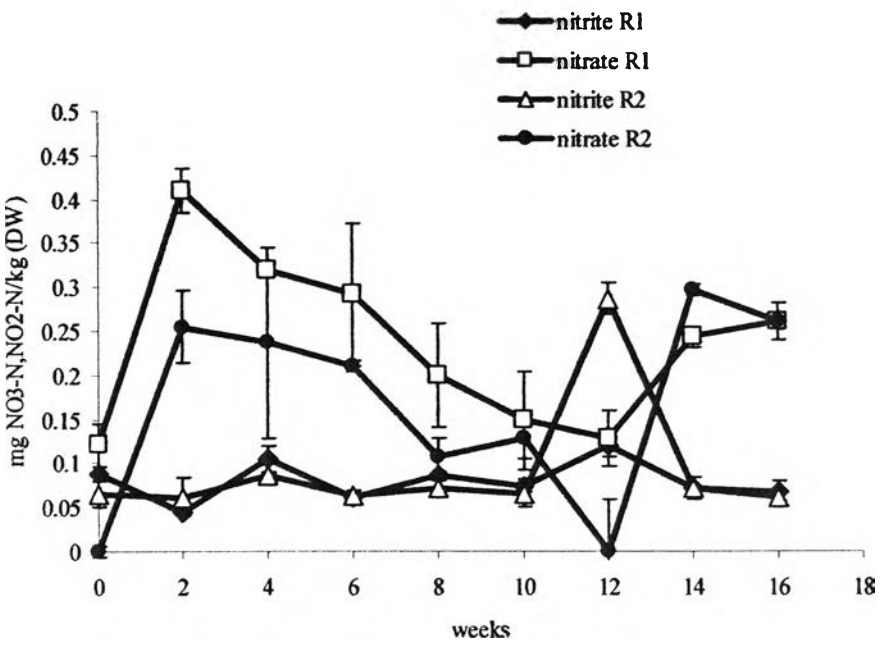

Figure 4-7: Nitrate and nitrite concentrations in sediment obtained from ponds R1 and R2 at Pathum Thani Province.

4.1.1.8 Phosphate phosphorus

Phosphate concentration in both R1 and R2 ponds had the similar trend. As showed in Figure 4-8, phosphate in sediment from both ponds was relatively stable for 12 or 14 weeks, with phosphate concentration range between 0.5-1.5 mgPO_4^--/kg (DW). At the last week, accumulation of phosphate up to 2.1 and 2.28 mgPO_4^--/kg (DW) was found in ponds R1 and R2 respectively.

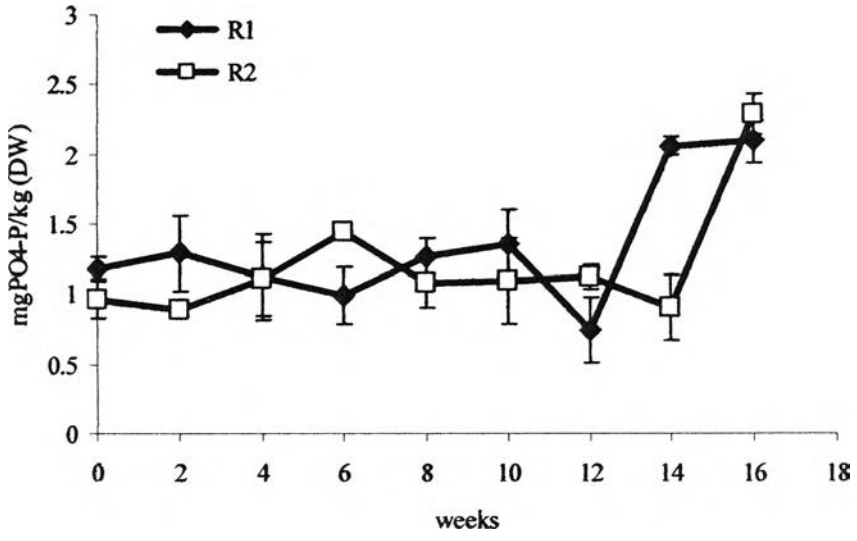

Figure 4-8: Phosphate concentration in sediment obtained from R1 and R2 ponds at Pathum Thani Province.

4.1.1.9 Total Nitrogen and total phosphorus

The result in Figure 4-9 delineated total nitrogen and total phosphorus in both two ponds. Total nitrogen in pond R1 and pond R2 were similarly. It was fluctuated within 10-50 mg/kg (DW) and highest peak of total nitrogen was found in week 6. Different from total nitrogen, total phosphorus was stable within 3-7 mg/kg (DW) during culture period.

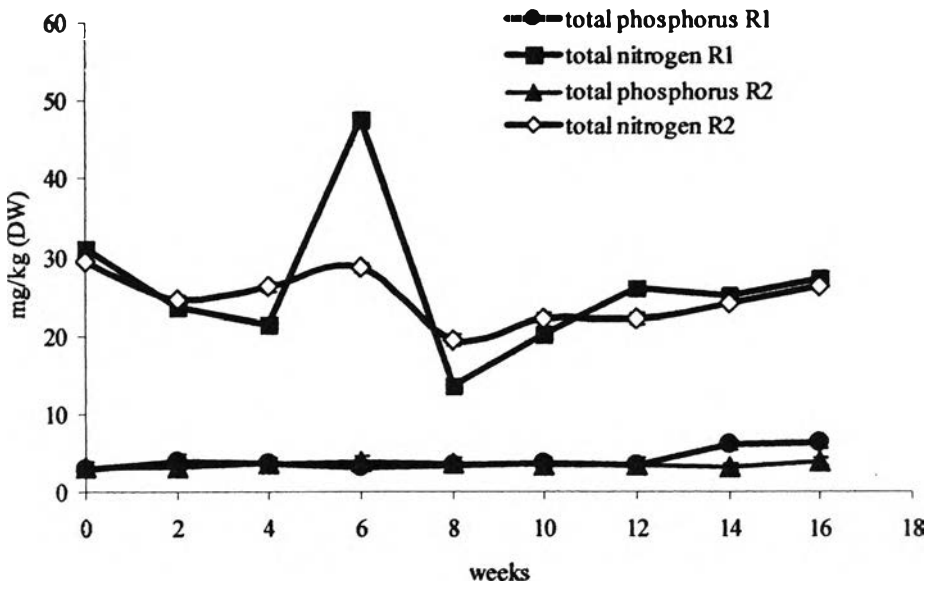

Figure 4-9: Total nitrogen and total phosphorus concentrations in sediment obtained from ponds R1 and R2 at Pathum Thani Province.

4.1.2 Shrimp ponds characteristics at Ban Pho (P1 and P2)

Two shrimp ponds in Ban Pho District, Chacheongsao, were represented the closed system of shrimp ponds that used a combination of ozone for water treatment and chitosan for enhance sedimentation.

Ozonation and addition of chitosan were applied to the first pond (P1) which assigned as experimental pond while control pond (P2) was operated without ozone and chitosan. Other procedures for shrimp cultivation such as shrimp density and aeration in both ponds were similar in practice. Lime was added to the bottom of both ponds at 0.5 kg/ square meter during pond preparation in order to adjust the soil pH before filling the ponds with seawater. Ozone gas generated with ozone generator (E-Base Co. Ltd.) with

pure oxygen was then injected into the P1 pond using submersible jet-aerator for 4 days. Ozonation was temporary stopped before shrimp release and was turned on again after one month. This was to reduce the harmful effect of ozone to young shrimps. The density was 26 shrimps/ square meter. The period of shrimp culture was 3 months from 18 March to 28 May 2004.

4.1.2.1 Temperature

As shown in Figure 4-10, water temperature in both ponds fluctuated between 28.5 and 31.7 °C. Slightly increase of temperature due to seasonal change was found in both ponds. However, there was no significant different between water temperature in P1 and P2 ponds.

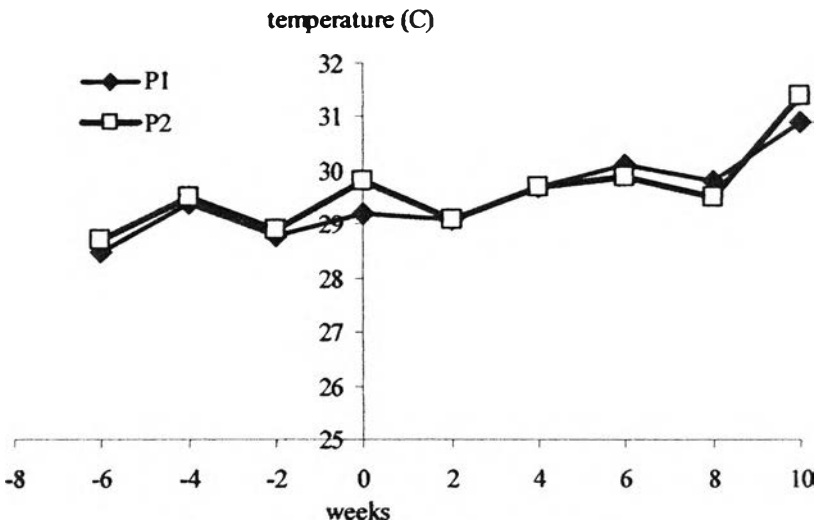

Figure 4-10: Water temperature in ponds P1 and P2 at Ban Pho (monitored at 10:00 am).

4.1.2.2 pH

During pond preparation, $CaCO_3$ lime was applied into both ponds in order to increase the pH. The result of pH monitoring at 10:00 am in both ponds are shown in Figure 4-11. In both ponds, pH was fluctuated between 7.6 and 8.0. Increase of pH in pond P1 from 7.6 in week 2 to 7.9 in week 10 was found while pH in pond P2 was almost constant with slightly increase of pH in the last two weeks.

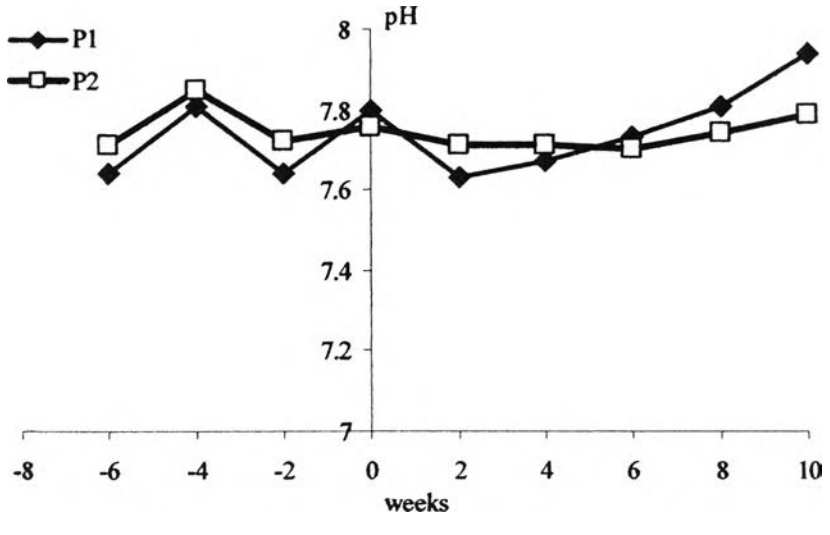

Figure 4-11: pH of the sediment in P1 and P2

4.1.2.3 Salinity

As shown in Figure 4-12, at the beginning of the culture, both ponds had the same salinity at 3 psu. Salinity was decreased throughout the culture period due to rain and some addition of freshwater.

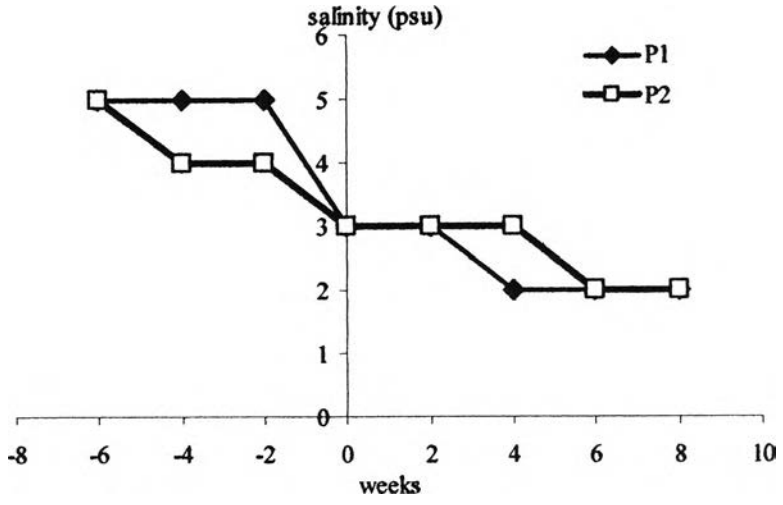

Figure 4-12: Water salinity in P1 and P2 (monitored at 10:00 am).

4.1.2.4 Water content and organic matter content

The results in Figure 4-13 show organic matter and water contents in both pond P1 and P2. Both parameters were gradually increased during culture period. The lowest organic matter was found in the first week just after filling ponds with water. During pond preparation between week -4 to week 0 and during shrimp cultivation from week 0 to week 8, organic content of the sediment was increased at almost constant rate. However, sharply increase of organic content was detected in the last week (week 10). This was related with mass mortality of shrimps in both ponds.

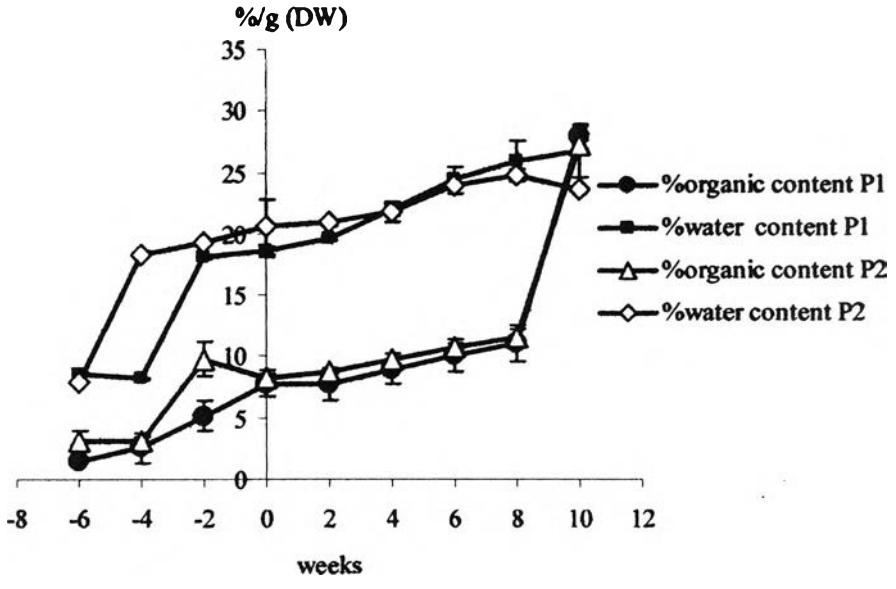

Figure 4-13: Water content and organic matter content in sediment obtained from ponds P1 and P2 at Ban Pho.

4.1.2.5 Chlorophyll *a*

Trend of chlorophyll_*a* concentration in sediment of ponds P1 and P2 was quite different (Figure 4-14). Initially, chlorophyll_*a* in P1 was low, less than 30 mg-Chlorophyll *a* /kg (DW) (6 week before shrimp released). Chlorophyll_*a* was gradually increased after week 0. The highest chlorophyll_*a* concentration in pond 1 was found in week 8 at 73.5 mg-pigment/kg (DW). In pond P2, the highest concentration was found in week-4 before in was gradually decreased during culture period.

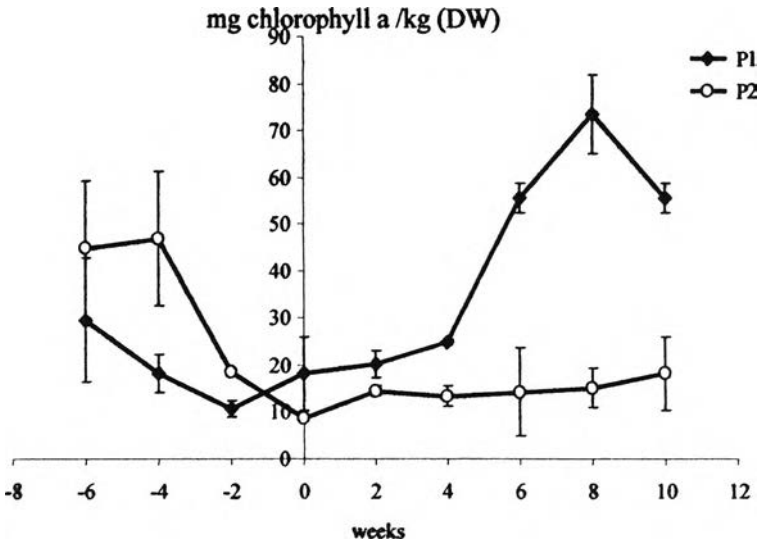

Figure 4-14: Chlorophyll *a* concentration in sediment obtained from pond P1 and P2 at Ban Pho.

4.1.2.6 Total ammonia

Ammonia concentration in sediment fluctuated between 1.5 and 9.8 mg NH_4^+-N/kg (DW) in Pond P1 and between 9.87 and 13.8 mg NH_4^+-N/kg (DW) in Pond P2 (Figure 4-15). High ammonia concentration was found during pond preparation (week -6). Thereafter, ammonia was apparently decreased after water filling in week -4. After shrimp released (week 0) ammonia concentration in pond P1 and P2 was different in which pond P1 had much higher ammonia with a peak of ammonia up to 9.87 mg NH_4^+-N/ kg (DW) in week 4. In pond P2, on the other hand, ammonia concentration was less than 3.69 mg NH_4^+-N/ kg (DW) through out the culture period.

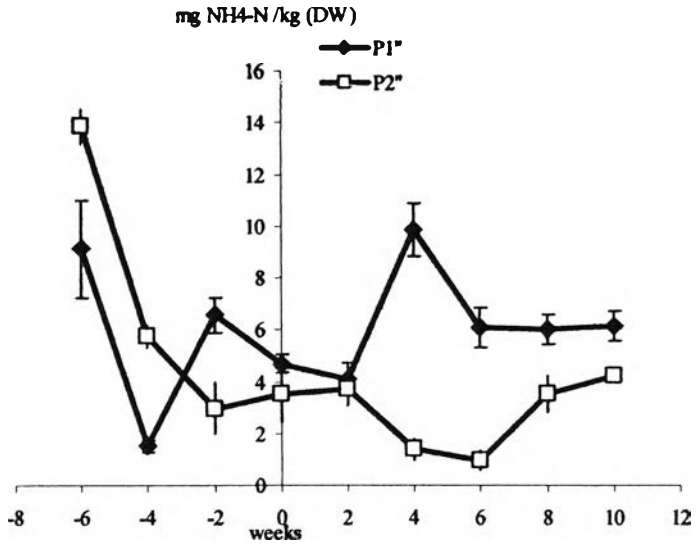

Figure 4-15: Ammonia concentration in sediment obtained from ponds P1 and P2 at Ban Pho.

4.1.2.7 Nitrite and nitrate

Nitrite concentration in both P1 and P2 ponds was less than 0.1 mgNO_2^--N/kg (DW) through out the culture period (Figure 4-16). On the other hand, nitrate concentration in both ponds was low and stable within the first four week of cultivation. Thereafter, sharply increase of nitrate was found after week 6 to the highest concentration of 1.09 mg NO_3^--N/kg (DW) in pond P1 and 1.26 mg NO_3^--N/kg (DW) in pond P2 at week 10.

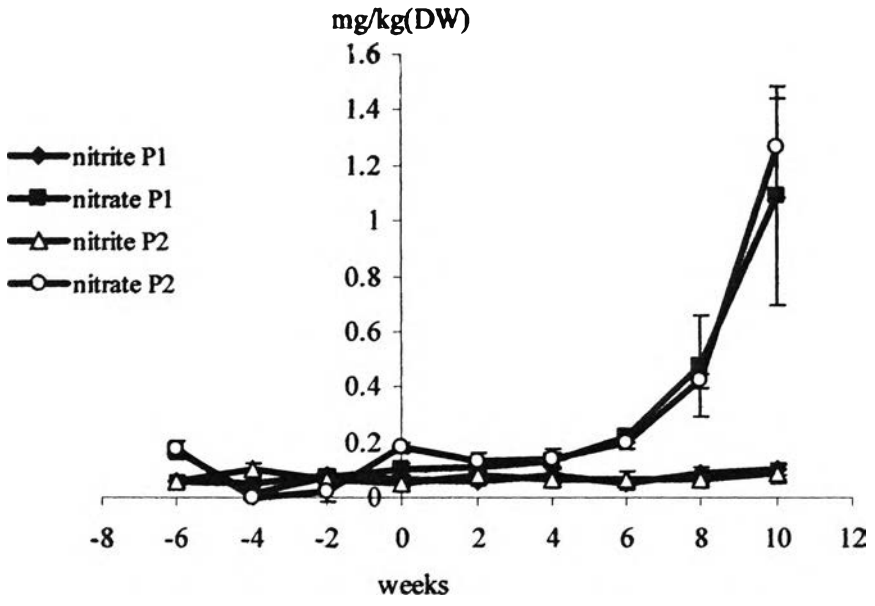

Figure 4-16: Nitrate and nitrite concentrations in sediment obtained from P1 and P2.

4.1.2.8 Phosphate

Phosphate concentration in sediment from ponds P1 and P2 during pond preparation (week -6 to week 0) was almost constant. However, after starting shrimp culture with regular feeding from week 0 until the end of the experiment, accumulation of phosphate in the sediment was found. As shown in Figure 4-17, phosphate in sediment of pond P1 was accumulated to the concentration of 26.9 mg PO_4-P/kg (DW) in the last week while phosphate in pond P2 was only 3.5 mg PO_4-P/kg (DW).

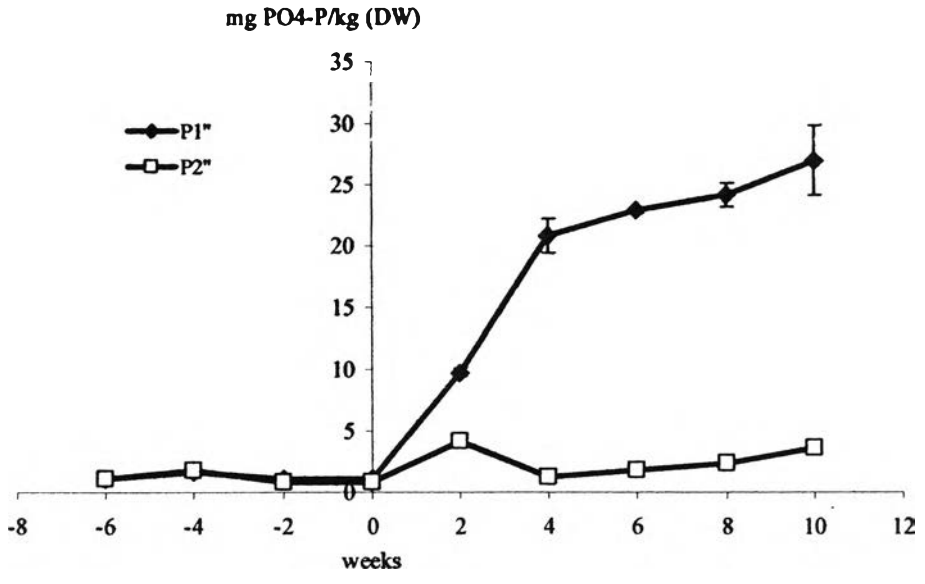

Figure 4-17: Phosphate concentration in sediment obtained from ponds P1 and P2 at Ban Pho.

4.1.2.9 Total Nitrogen and total phosphorus

The result in Figure 4-18 shows total phosphorus and total nitrogen in ponds P1 and P2. Total phosphorus in both ponds was gradually increased since week 0 and total phosphorus in pond 1 was significantly higher than pond 2. The highest total phosphorus in pond 1 was found in week 10 at 41.0 mg/kg (DW) and the highest phosphorus in pond 2 was found in week 10 at 16.5 mg/kg (DW). Accumulation of total nitrogen in sediment during shrimp culture was found in both ponds and total nitrogen in pond P1 was higher than pond P2 throughout the culture period.

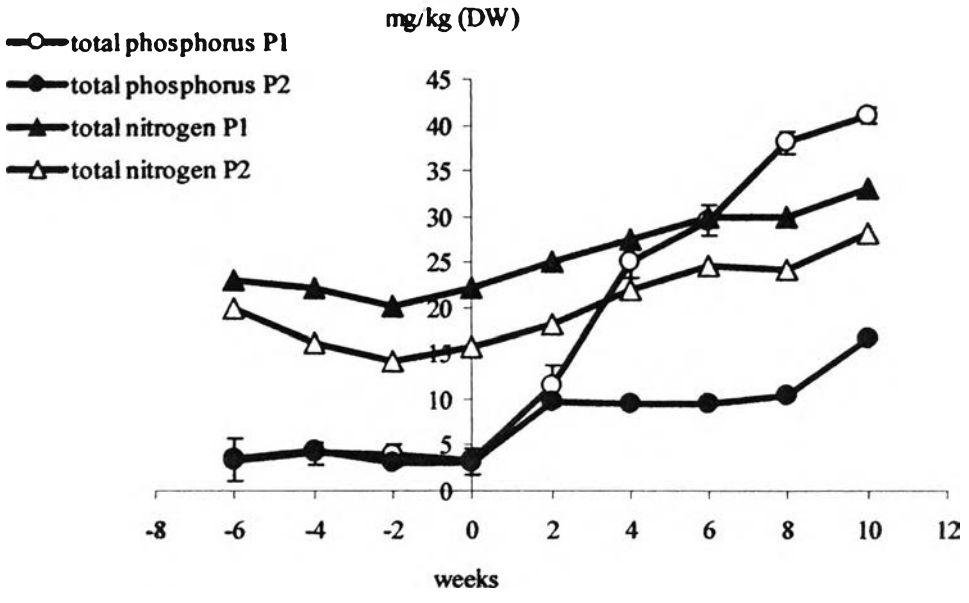

Figure 4-18: Total nitrogen and total phosphorus concentrations in sediment obtained from ponds P1 and P2 at Ban Pho.

4.1.3 Shrimp pond characteristics at Bang Khla (K1)

A shrimp pond at Bang Khla (K1) District in Chachoengsao Province was selected to represent commercial shrimp farm. The pond as previously used as rice field was newly converted to shrimp pond. Shrimps were cultured using water exchanged from the reservoir within the farm area and pond management was according to normal practice of commercial shrimp farm. The density was 26 shrimps/ square meter. The period of shrimp culture was 4 months from 8 April to 18 August 2004.

4.1.3.1 **Temperature**

As shown in Figure 4-19, water temperature in K1 was almost stable through out the culture period. The water temperature measured at approximately 10:00 am was between 28 and 29.5 °C.

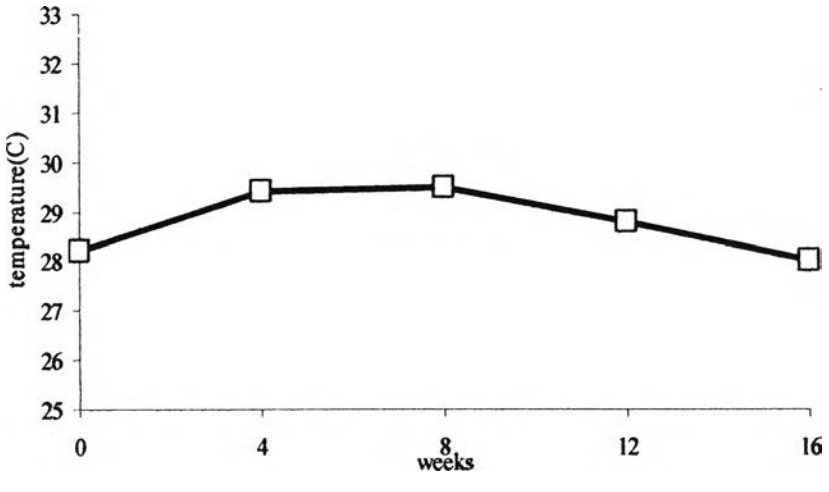

Figure 4-19: Water temperature at 10:00 am in pond K1at Bang Khla.

4.1.3.2 **pH**

Figure 4-20 shows that pH measured at 10:00 am in pond K1 was between 7.1-7.8. It has to be noted that, as the normal practice of commercial shrimp cultivation, calcium lime was regularly added in the pond to maintain the water alkalinity. This was therefore regulated the pH with low fluctuation of between 7.12 to 7.81.

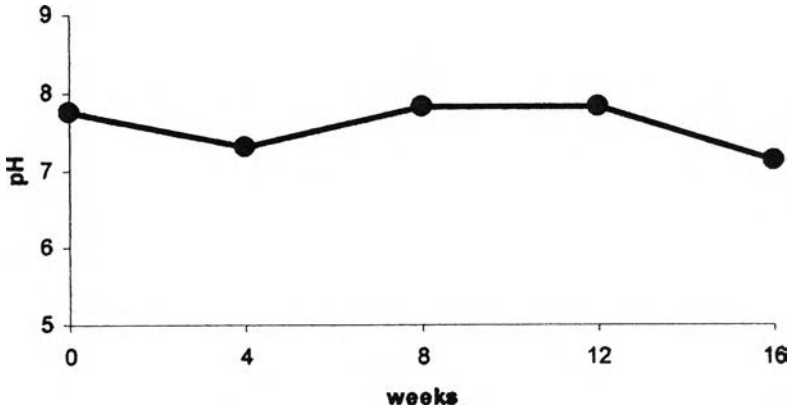

Figure 4-20: pH of sediment obtained from pond K1 at Bang Khla.

4.1.3.3 Salinity

Figure 4-21 shows water salinity in K1 pond at Bang Khla site. Salinity was decrease from 6.0 PSU at the beginning to 3.0 PSU at the end of the crop. This was mostly related with water exchanged between shrimp pond and lower salinity water in the reservoir.

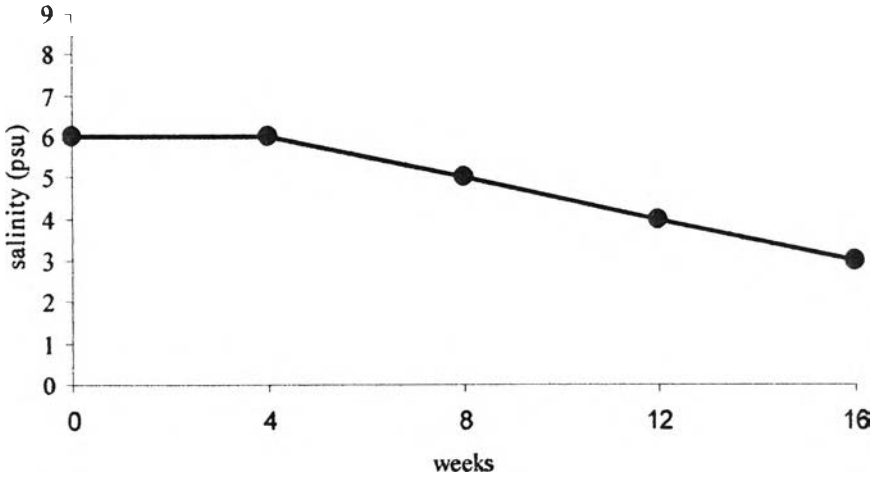

Figure 4-21: Water salinity in pond K1 at Bang Khla monitored at 10:00 am.

4.1.3.4 Organic matter and water content

Figure 4-22 shows percentage of organic matter and water contents in sediment from pond K1. It was found that both organic matter and water content was gradually increased during the culture period. The highest organic matter and water content in week 16 was 22.34 % and 32.40%, respectively.

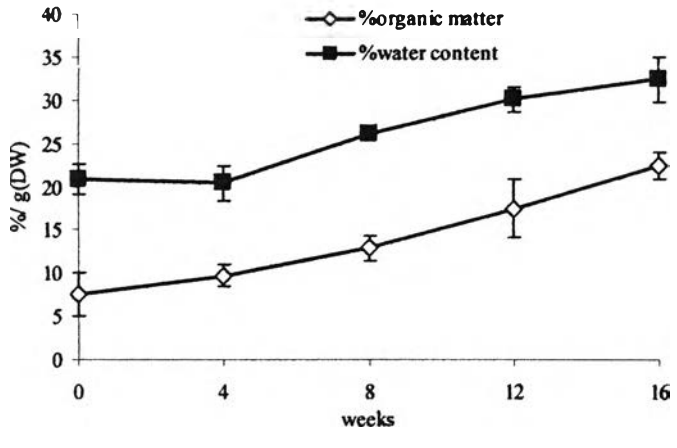

Figure 4-22: Water content and organic matter content in sediment collected from pond K1 at Bang Khla.

4.1.3.5 Chlorophyll *a*

Chlorophyll_*a* concentration in pond K1 was shown in Figure 4-23. The highest chlorophyll_*a* concentration was found in week 0 at 58.12 mg- Chlorophyll *a* /kg (DW) before it was rapidly decreased to 11.16 mg- Chlorophyll *a* /kg (DW) in week 4. After that, it was fluctuate within 36-42 mg- Chlorophyll *a* /kg (DW) until the end of the culture period.

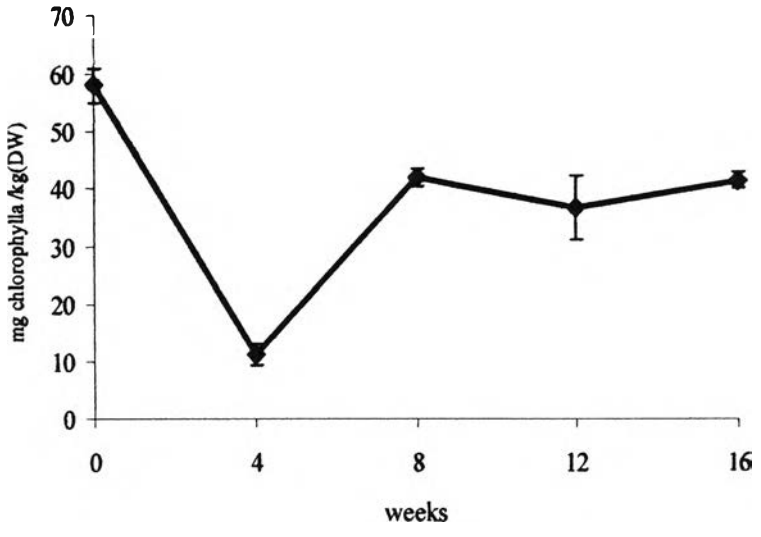

Figure 4-23: Chlorophyll *a* concentration in sediment collected from pond K1 at Bang Khla.

4.1.3.6 Total ammonia

Ammonia concentration in sediment from pond K1 was shown in Figure 4-24. Ammonia concentration was gradually increased from 10.44 mg NH_4^+-N/kg (DW) in week 0 to 17.53 mg NH_4^+-N/kg (DW) in week 16.

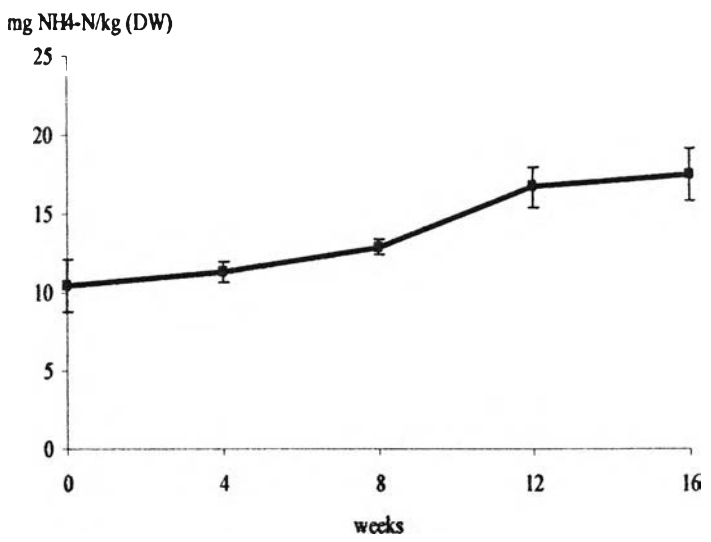

Figure 4-24: Ammonia concentration in sediment collected from pond K1 at Bang Khla.

4.1.3.7 Nitrate and nitrite

Nitrate and nitrite concentration of sediment collected from pond K1 were illustrated in Figure 4-25. Nitrite was slightly increased from 0.008 mg NO^{-}_{2}-N/kg (DW) in week 0 to 0.06 mg NO^{-}_{2}-N/kg (DW) in week 4 and then stable at the concentration below 0.1 mg NO^{-}_{2}-N /kg (DW) until the end of the experiment. Unlike nitrite, nitrate was gradually increased from 0.14 mg NO^{-}_{3}-N/kg (DW) in week 0 to0.28 mg NO^{-}_{3}-N/kg (DW) in week 16.

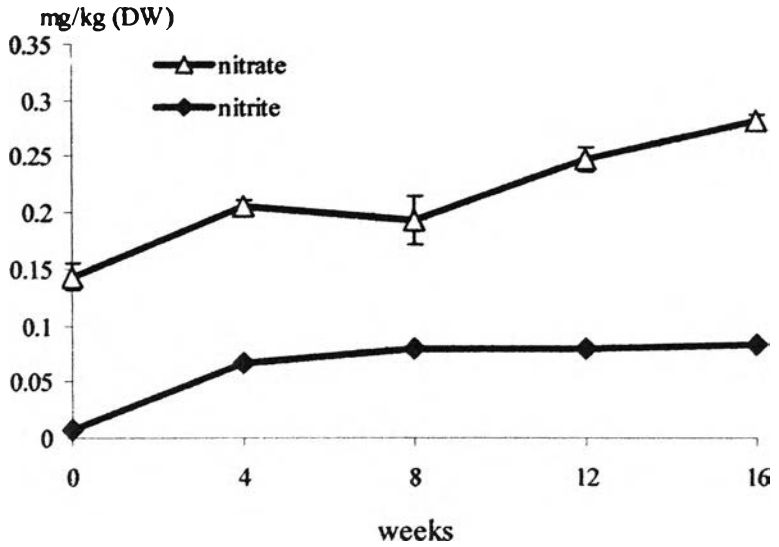

Figure 4-25: Nitrate and nitrite concentrations in sediment collected from pond K1 at Bang Khla.

4.1.3.8 Phosphate

The result of phosphorus concentration in sediment collected from K1 pond is shown in Figure 4-26. At the beginning, Phosphate concentration was 0.55 mg PO_4-P/kg (DW). Then, phosphorus concentration was sharply increased to the highest

concentration of 1.90 mg PO_4-P/kg (DW) at week 8 and remained at this concentration, between 1.66-1.9 mg PO_4-P/kg (DW), until the end of the crop.

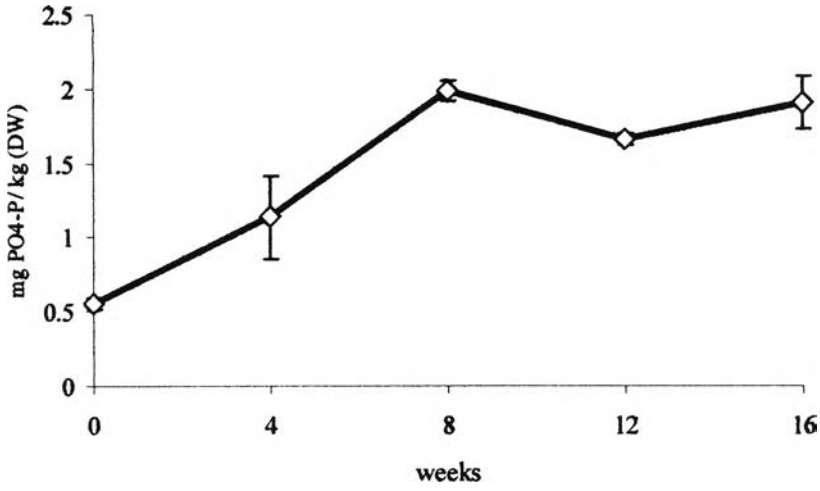

Figure 4-26: Phosphate concentration in sediment collected from pond K1 at Bang Khla.

4.1.3.9 Total nitrogen and total phosphorus

The result in Figure 4-27 delineated total nitrogen and total phosphorus in sediment from pond K1. Total phosphorus was almost stable during culture period, with the concentration of less than 5 mg/kg (DW). On the other hand, total nitrogen was tended to increase and fluctuate within 18-32 mg/kg (DW) in which the lowest total nitrogen was found in week 0 at 18.23 mg/kg (DW) and the highest total nitrogen was found in week 16 at 31.14 mg/kg (DW).

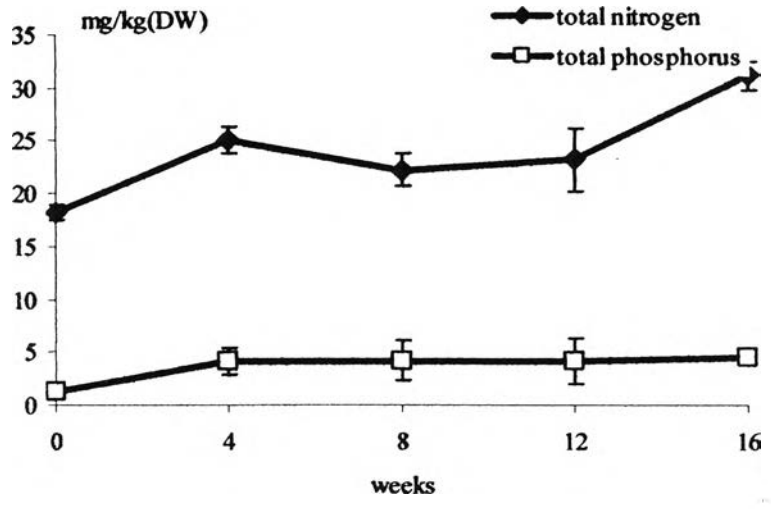

Figure 4-27: Total nitrogen and total phosphorus concentrations in sediment collected from pond K1 at Bang Khla.

4.1.4 Shrimp pond at Bang Khun Thian (T1, T2 and T3)

Three shrimp ponds (T1, T2 and T3) represented the extensive shrimp ponds in Bang Khun Thian District, south of Bangkok. The sediment samples were collected only once in August 2004. The environmental condition during sediment sampling and sediment characteristics are shown in Table 4-1 and Table4-2 respectively

Table 4-1: Environmental condition during sediment sampling at three extensive shrimp ponds in Bang Khun Thian District.

	Pond T1	Pond T2	Pond T3	Average±SD
Temperature	29.0	29.8	30.0	29.6±0.04
pH	7.42	7.46	7.19	7.36±0.07
Salinity (PSU)	20.9	20.5	20.5	20.63±0.00

Table 4-2: Sediment characteristics at three extensive shrimp ponds in Bang Khun Thian District

	Pond T1	Pond T2	Pond T3	Average±SD
% Organic matter content	13.34	14.96	16.32	14.87± 1.24
% Water content	17.55	20.17	21.54	19.75±2.58
Chlorophyll-a (mg Chl/kg (DW))	50.74	31.38	19.58	33.9±8.52
Total ammonia (mg NH_4-N/kg DW)	10.21	9.32	7.61	9.04±1.20
Nitrite (mg NO_2-N/kg DW)	0.34	0.31	0.33	0.32±0.00
Nitrate (mg NO_3-N/kg DW)	0.40	0.31	0.36	0.35±0.00
Phosphate (mg PO_4-P/kg DW)	5.06	13.76	9.56	9.46±2.14
Total nitrogen (mg N/kg DW)	15.55	18.67	20.90	18.37±1.48
Total phosphorus (mg P/kg DW)	20.31	13.44	19.17	17.64±1.75

4.2 Bacterial analysis

4.2.1 Bacterial analysis using conventional methods

Bacteria in sediment were investigated by serial dilutions and spread plated technique. After incubation period, colony forming unit (CFU) were counted and recorded. Total aerobic bacteria were counted using Plate Count agar. *Vibrio* count was observed using TCBS agar after 3 to 5 days incubation.

4.2.1.1 Aerobic Bacteria counted

4.2.1.1.1 Aerobic bacteria in sediment from Nong Suea sampling site

Number of aerobic bacteria in sediment from ponds R1 and R2 were shown in Figure 4-28. In both ponds, the lowest bacterial densities were detected during the first week at $2.67x10^5$ for pond R1 and $3.17x10^5$ CFU / g (DW) for pond R2. Thereafter, number of bacteria was found increase throughout the culture period with the highest colony number of $2.98x10^6$ and $2.97\ x10^6$ CFU / g (DW) in ponds R1 and R2, respectively

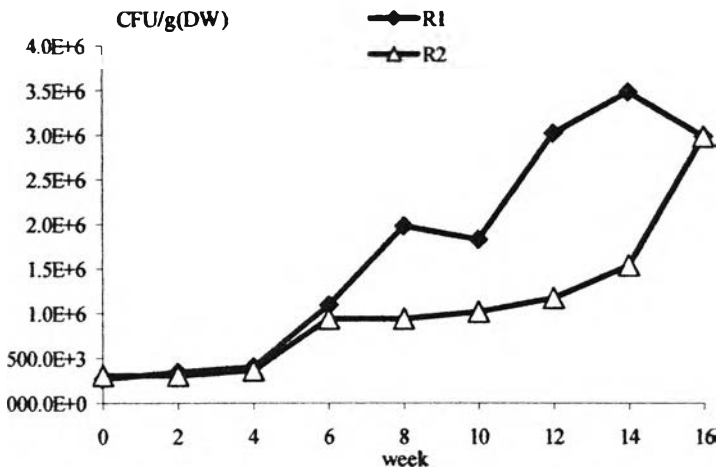

Figure 4-28: Number of aerobic bacteria colonies in sediment derived from ponds R1 and R2 at Pathum Thani Province.

4.2.1.1.2 Aerobic bacteria in sediment derived from Ban Pho site

Since water in shrimp pond P1 at Ban Pho was treated with ozone while pond P2 was only aerated, number of aerobic bacteria in sediment from both ponds was therefore significantly different (Figure 4-29). In ozone treatment pond (P1), the aerobic bacterial in sediment decreased sharply in the first two weeks of ozonation (Week -2). This made the lower bacterial number in the sediment of pond P1 than that found in pond

P2 for 8 weeks of shrimp culture. However, bacteria in sediment of pond P1 was gradually increase and the number was close to that found in pond P2 at week 10.

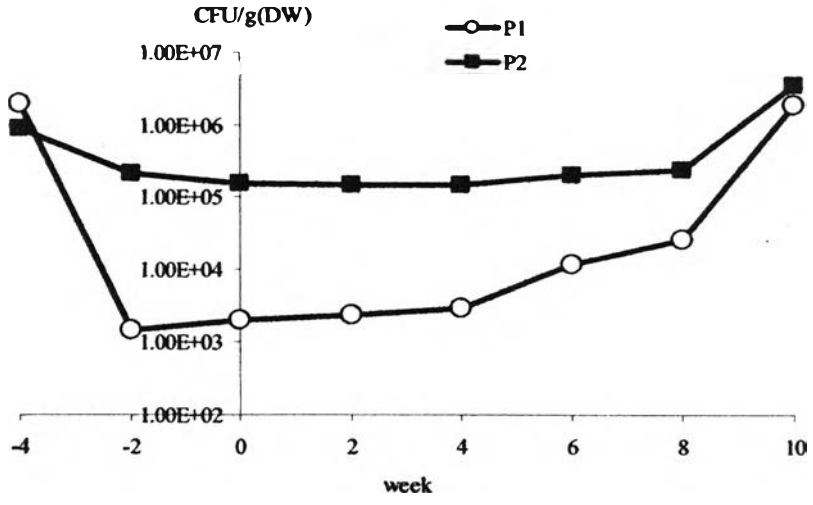

Figure 4-29: Number of aerobic bacterial colonies in sediment derived from ponds P1 and P2 at Ban Pho site.

4.2.1.1.3 Aerobic bacteria in sediment from Bang Khla sampling site

The number of aerobic bacteria in sediment taken from Bang Khla (pond K1) was shown in Figure 4-30. The results revealed that the colony number increased with culture period from 2.51×10^5 CFU/g (DW) at initial to 8.52×10^5 CFU/g (DW) at week 16.

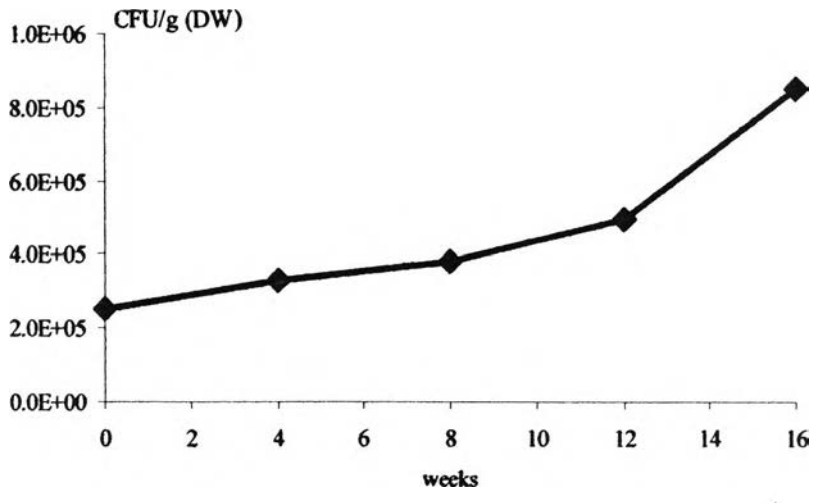

Figure 4-30: Number of aerobic bacteria colonies in sediment derived from pond K1 at Bang Khla site.

4.2.1.1.4 Aerobic bacteria count in sediment taken from Bang Khun Thian sampling sites

The number of aerobic bacteria in sediment derived from three extensive shrimp ponds at Bang Khun Thian was $1.75x10^7$, $1.06x10^7$ and $8.40x10^6$ CFU / g (DW) for ponds T1, T2 and T3 respectively. These figures are the highest bacterial concentration of all sites in this study.

4.2.1.2 *Vibrio* sp. count on TCBS agar plate

4.2.1.2.1 *Vibrio* sp. counted in sediment taken from Nong Suea sampling site

Observation of *Vibrio* colonies on TCBS agar plates (Figure 4-31) showed that *Vibrio* colonies in both ponds were similar. The *Vibrio* colonies in both P1 and P2 ponds were fluctuate within 200-2,700 CFU/g (DW). The highest number of *Vibrio* in

sediment was found in the last week (Week 16), which contained 2,733 and 1,784 CFU/ g (DW) for ponds R1 and R2 respectively.

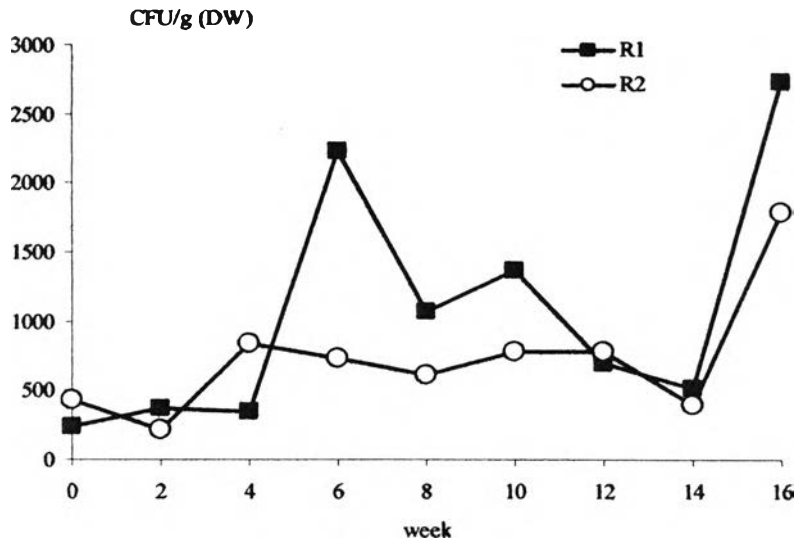

Figure 4-31: Number of *Vibrio* colonies in sediment taken from ponds R1 and R2 at Pathum Thani Province.

4.2.1.2.2 *Vibrio* sp. counted in sediment taken from Ban Pho sampling site

The results of *Vibrio* count in Ban Pho shrimp ponds sediment, as illustrated in Figure 4-32, show that number of *Vibrio* in control pond (P2) was increase with culture period while number of *Vibrio* in ozone treatment pond (P1) was relatively low throughout the experiment. This suggested that ozonation in pond P1 clearly inhibit growth of *Vibrio* in the sediment. The highest number of *Vibrio* colonies in sediment from pond P2 was 7,359 CFU/g (DW) at the last week of culturing while the highest number *Vibrio* colonies in pond P1 was 1,423 CFU/g (DW) during pond preparation before ozonation at week -4.

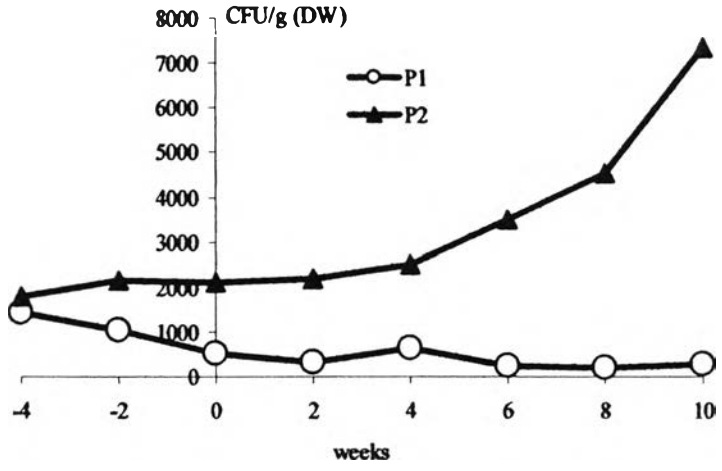

Figure 4-32: Number of *Vibrio* colonies in sediment taken from ponds P1 and P2 at Ban Pho site.

4.2.1.2.3 *Vibrio* sp. counted in sediment taken from Bang Khla sampling site

Trend of *Vibrio* colony from sediment in shrimp pond at Bang Khla was shown in Figure 4-33. The highest *Vibiro* colonies number was found in the last week of culturing period at the concentration of 852 (CFU /g (DW).

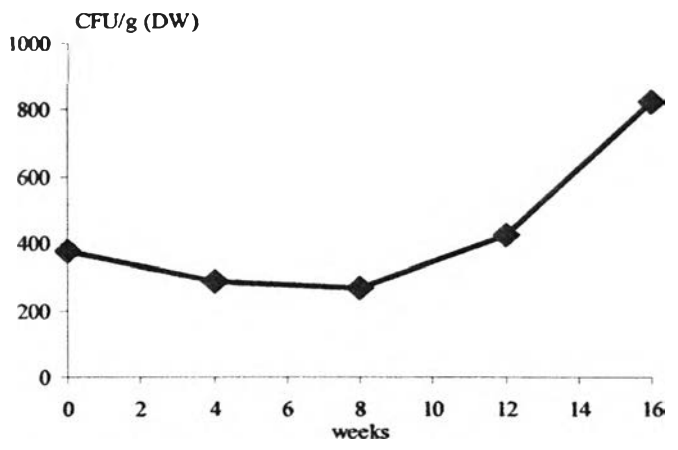

Figure 4-33: Number of *Vibrio* colonies in sediment obtained from pond K1 at Ban Khla.

4.2.1.2.4 *Vibrio* sp. counted in sediment taken from Bang Khun Thian sampling site

The number of *Vibrio* in sediment derived from three extensive shrimp ponds at Bang Khun Thian was $1.04x10^5$, $9.3x10^4$ and $6.06x10^4$ CFU / g (DW) for ponds T1, T2 and T3 respectively. Figures 4-34 showed the highest *Vibrio* concentration of all sites in this study.

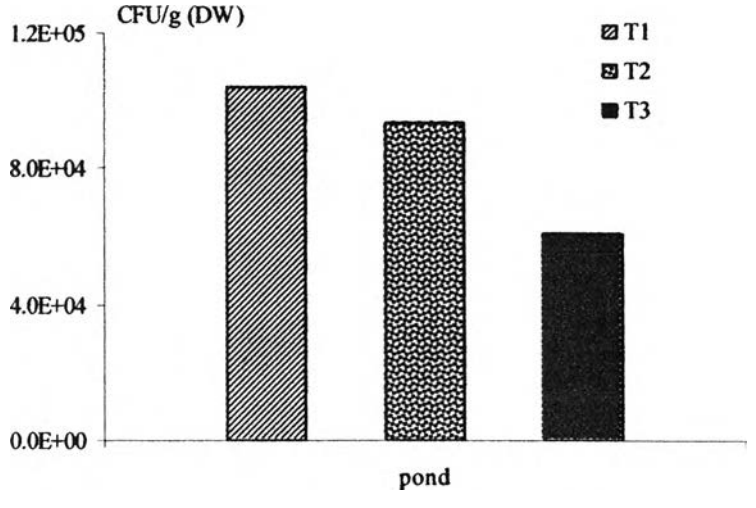

Figure 4-34: Number of *Vibrio* colonies in sediment obtained from pond T1-T3 at Bang Khun Thian.

4.2.2 Bacterial analysis using PCR-DGGE method

Sediment samples from eight shrimp ponds in four locations, *i.e.* Pathum Thani, Ban Pho, Bang Khla and Bang Khun Thian, were used for the study on diversity and dynamics of the dominating microbial populations during shrimp culture period. With DGGE technique, the appearance and disappearance of amplicons in the DGGE pattern indicate important shifts in the microbial community structure. The intensity of an individual band is a semi quantitative measurement for the relative abundance of a specific bacteria population in the communities (Muyzer *et al.* 1993).

Bacterial DNA from sediment samples was extracted. Cells were lysed directly in the sediment by using a combined approach which included bead beating and incubation with SDS-PVPP. Purification of total DNA by extraction once with cold phenol and phenol-chloroform-isoamyl alcohol resulted in a brown DNA solution which could not be used for PCR-mediated amplification of bacterial 16SrDNA, probably due to the presence of inhibitory substances like humic acid.

Amplification of sediment DNA with PRBA338f and PRUN518r primers which correspond to position 338 to 518 relative to the *Escherichia coli* rRNA sequences resulted in PCR products from each sample. Migration of PCR amplicons in a denaturing gel is a function of both fragment length and nucleic acid sequences. This enables the separation of equal-length PCR fragments that have different sequences. When used in combination with 16S rDNA-targeted PCR, the number of DGGE bands resolved reflects the number of distinct 16S rDNA genes amplified from the DNA extract and consequently the number of bacteria species in each sediment sample.

4.2.2.1 Bacterial analysis by DGGE-PCR method for sediment obtained form shrimp ponds at Nong Suea, Pathum Thani Province

Analysis of Nong Suea sediment taken during shrimp culturing, ranging from shrimp released to shrimp harvested, was showed in Figure 4-35. Each lane derived from one sediment sample. It was found that, based on the fragment intensities; about 2-5 predominant fragments were observed in each lane. The gel pattern in both R1 and R2 ponds were similar and the number of intense bands increased following the period of cultivation. In both R1 and R2 ponds, the band r1 was the dominant band found in every lane. In addition, r1 and r3 were predominant bands in the first week of both ponds.

Some bands such as r4 and r8 were not major bands in the first week but appeared during shrimp cultivation crop. DGGE bands r1- r9 were excised and used as template for re-amplified. The amplification products were then cloned and sequenced.

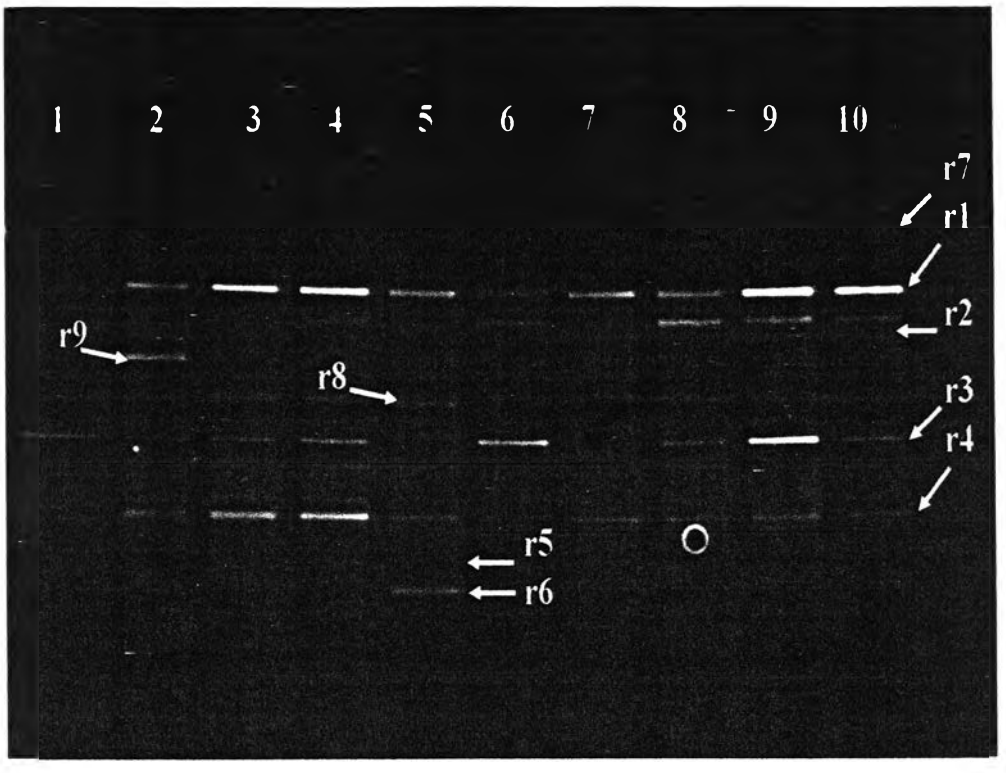

Figure 4-35: DGGE analysis of bacterial community derived from sediment from R1 (lanes 1-5) and R2 (lanes 6-10) ponds at Pathum Thani Province: lanes 1 and 6 represent week 0 which was the day of shrimps released; lanes 2 and 7 were week 4 (1 month); lanes 3 and 8 were week 8 (two months); lanes 4 and 9 were week 12 (three months); and lanes 5 and 10 were week 16 (four months) of shrimp cultivation. Arrows indicated position of bands that were excised from the gel, amplified, cloned and sequenced. These clones were designated as r1-r9.

4.2.2.2 Bacterial analysis by PCR-DGGE method for sediment obtained form shrimp pond at Ban Pho

DGGE profile of total bacterial DNA extracted from two shrimp ponds in Ban Pho District is shown in Figure 4-36. The pond P1 (DGGE lanes 1 to 5) was an ozone treatment pond, while P2 (lanes 6 to 10) was a control pond. Sediment samples in week -4 (lanes 1 and 6), which was four weeks before shrimp released, represents the day that water was filled in the pond during pond preparation, while week -2 (lanes 2 and 7) represents the starting day of ozonation. DGGE profile every two weeks during shrimp cultivation was illustrated in lanes 3-5 for pond P1 and 8-10 for pond P2 respectively.

DGGE profiles illustrated that bacterial communities in both ponds were similar in diversity. The number of intense bands increased following the period of cultivation. Since numbers of DNA bands from P1 and P2 ponds were not different, thus ozone that strongly reduced the number of bacteria in P1 seemed not affect bacterial diversity in the sediment. The dominant bands appeared in every lane was p2. DGGE bands p1- p9 were excised and used as template for re-amplified. The amplification products were then cloned and sequenced.

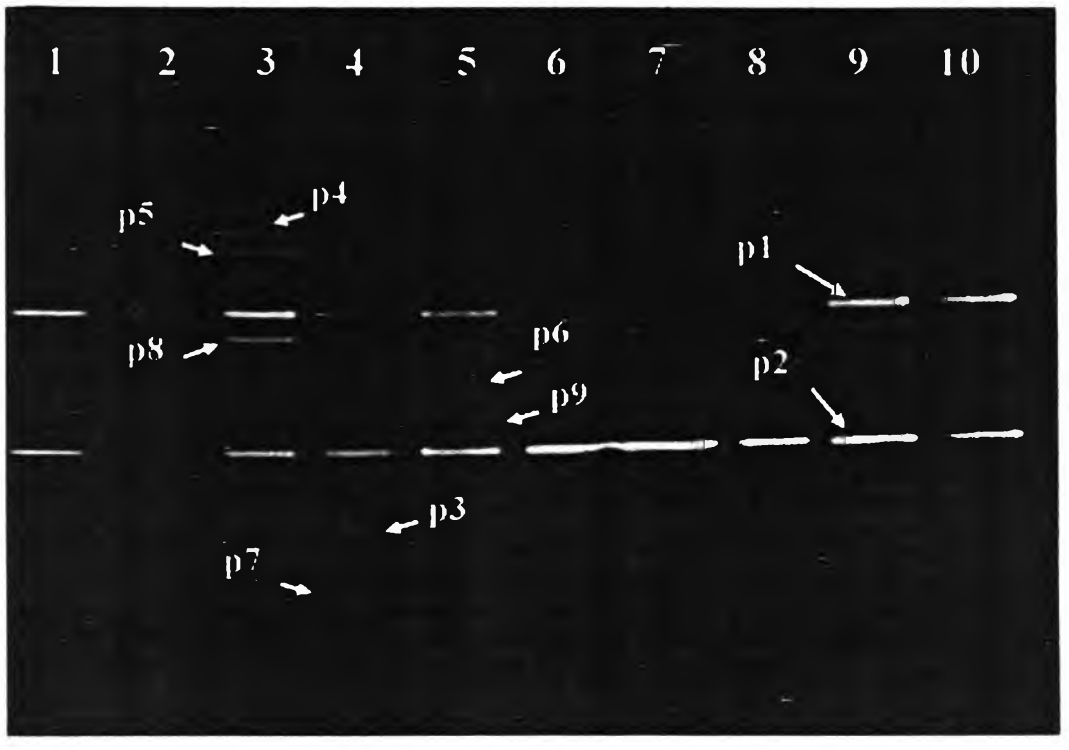

Figure 4-36: DGGE analysis of bacterial community derived from sediment from ponds P1 (lanes 1-5) and P2 (lanes 6-10) at Ban Pho. Lanes 1 and 6 represented bacterial community in pond P1 and P2 on the day of water filling which was four weeks before shrimp released (week -4). Lanes 2 and 7 were the starting day of ozonation* in week -2. Lanes 3 and 8 were from week 0 which was the day of shrimps releasing. Lanes 4 and 9 were from week 4 and lane 5 and 10 were from week 10 of shrimp cultivation. Arrows indicate position of bands that were excised from the gel, amplified, cloned and sequenced. These clones were designated as p1-p9.

*Remark: Ozonation was applied only in pond P1.

4.2.2.3 Bacterial analysis by DGGE-PCR method for sediment obtained from Bang Khla and Bang Khun Thian.

Figure 4-37 (A) shows the bacterial communities in a commercial shrimp pond at Bang Khla. Lane 1 which represented the starting day of shrimp cultivation had the lowest number of DNA bands. During 4, 10 and 16 weeks of shrimp cultivation which represented by lanes 2, 3 and 4 respectively, DGGE profile had very similar band pattern. Bacterial communities from lanes 2, 3 and 4 were apparently more complex than lane 1.

The DGGE profile in Figure 4-37(B) illustrates the bacterial communities in sediment from extensive shrimp ponds T1, T2 and T3 at Bang Khun Thian were similar in number of bands. However, some dominant bacteria in pond T1 (band t2) was appeared at higher density than that found in T2 and T3 ponds. Bands of DGGE gel from Bang Khla (bands k1-k6) and Bang Khun Thian (bands t1-t4) were excised from gels was used as template for re-amplified. The amplification products were then cloned and sequenced.

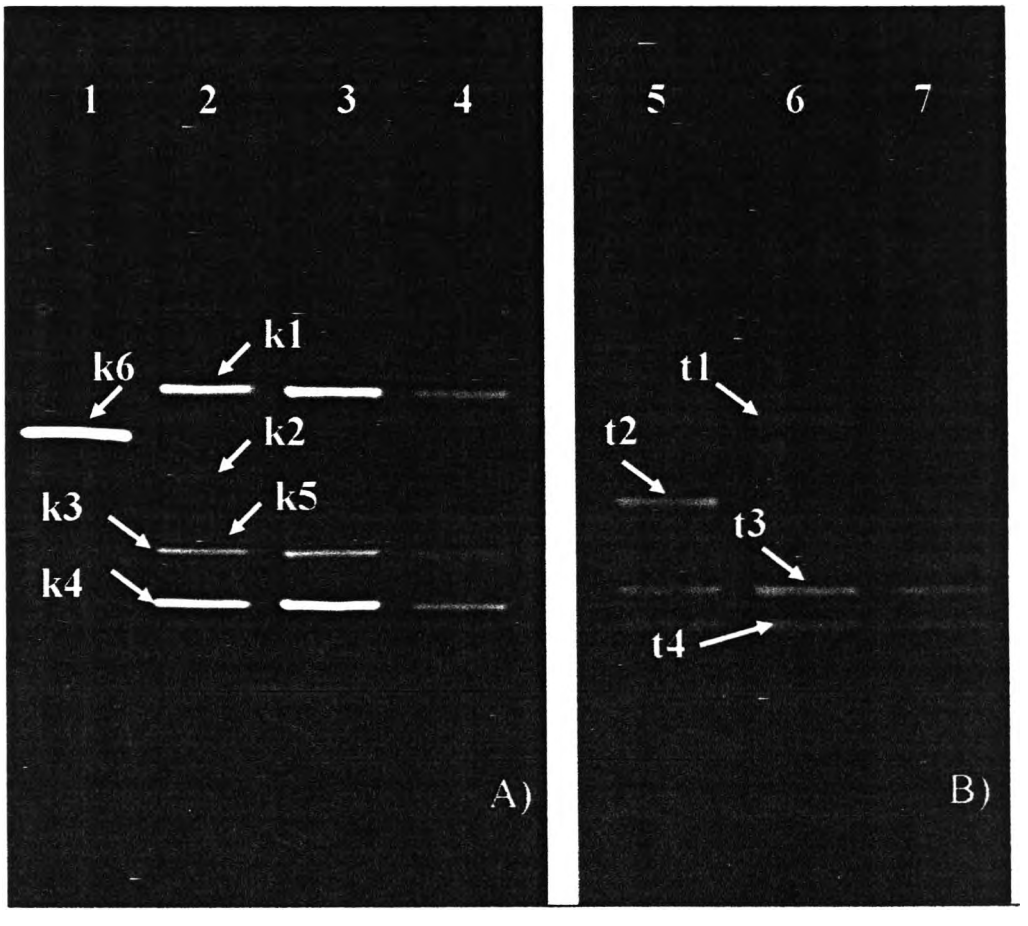

Figure 4-37: DGGE analysis of bacterial communities derived from sediment in a shrimp pond K1 at Bang Khla (A) and three shrimp ponds (T1-T3) at Bang Khun Thian sites (B). In Figure 4-51 A, lanes 1-4 represent bacterial diversity at weeks 0, 4, 12 and 16, respectively, during shrimp cultivation in pond K1. In Figure 4-51 B, lanes 1-3 represent bacterial diversity of ponds T1 to T3 respectively. Arrows indicated position of bands that were excised from the gel, amplified, cloned and sequenced. These clones were designated as k1-k6 and t1-t4.

4.2.3 Cloning and sequencing

Twenty eight excised bands of 16S rDNA fragments from 4 experimental shrimp pond sites were cloned into the pGEM-T Easy Vector (Promega) and then the plasmids were purified using the QIA Miniprep Kit (Qiagen,CA).

Positive clones with an insert of the correct size (200 bases) were selected randomly from each library for sequencing. Sequencing of the band p9 from Ban Pho pond failed probably due to the fact that the band of interest was relative weak. Finally, 27 cloning sequences were compared with the sequences stored in GenBank using BLAST program available at the National Center for Biotechnology Information, U.S.A. (http://www.ncbi.nlm.nih.gov/BLAST/).

Comparisons of partial 16S rDNA cloning sequences with high similarity sequences are shown in table 4-3.

Analysis revealed that eight DGGE bands were closely related to uncultured bacterium. Sequences obtained from DGGE bands r1, k1, p1 and p6 were closely related to those of species from the genus *Pseudomonas*. Sequence analysis shown that DGGE bands r6, p7, k4 and t4 sequences were similar to *Halomonas* species. Only two bands, r8 and t3 related to *Vibrio* species,

Table 4-3: Similarity of sequences obtained from excised DGGE bands (see Figure 4-35 to 4-37) to sequences in the NCBI database.

Strain	Closet known relative	Source	GenBank accession no.	%Similarity	Group
r1	*Pseudomonas alcaligenes* M4-7	Marine	AY835998	98	Gamma Proteobacteria
r2	*Serratia* sp. C1	Soil	AY633490	98	Gamma Proteobacteria
r3	Uncultured soil bacterium clone SO55	Soil	AY037588	95	unidentified
r4	Uncultured bacterium	Soil	AF234076	95	unidentified
r5	Uncultured Bacilli bacterium Clone M10Ba24	Soil	AY360614	100	unidentified
r6	*Halomonas* sp. HA-T	Marine	AB104435	98	Gamma Proteobacteria
r7	Uncultured soil bacterium	Soil	AY234707	93	unidentified
r8	*Vibrio sp.* CH-291	Sea surface water	AJ580582	93	Gamma Proteobacteria
r9	Uncultured gamma proteobacterium clone BS1-0-108	Tidal sediment	AY254934	97	Gamma Proteobacteria
p1	*Pseudomonas* sp. H786	Marine sponge	AY368554	100	Gamma Proteobacteria
p2	Uncultured Bacilli bacterium clone X3ba61	Soil	AY607419	99	unidentified
p3	*Desulfovibrio* sp.A2	Wastewater	AY770382	100	Delta Proteobacteria
p4	Uncultured bacterium	Freshwater sediment	AB127757	97	unidentified
p5	*Bacillus* sp.PL-16	Marine sediment	AJ229238	99	Bacillales
p6	*Pseudomonas borealis*	Estuary	AF321013	100	Gamma Proteobacteria
p7	*Halomonas* sp. HA-T	Marine	AB104435	98	Gamma Proteobacteria
p8	*Serratia* sp. D3	Marine ecosystem	AY745744	97	Gamma Proteobacteria
k1	*Pseudomonas* sp. H786	Marine sponge	AY368554	100	Gamma Proteobacteria
k2	Uncultured gamma proteobacterium	Bay plankton	AY628668	96	Gamma Proteobacteria
k3	*Marinobacter sedimentalis*	Marine sediment	AJ609270	100	Gamma Proteobacteria
k4	*Halomonas* sp.ws31	Deep sea sediment	AJ551141	100	Gamma Proteobacteria
k5	Uncultured gamma proteobacterium clone ccslm2118	Soil	AY133084	99	Gamma Proteobacteria
k6	Uncultured Bacterium A49	Soil	AF158720	97	unidentified
t1	Uncultured marine bacterium clone OMU-3289	Marine	AY937036	100	unidentified
t2	*Bacillus* sp. PL-12	Marine sediment	AF326366	100	Bacillales
t3	*Vibrio harveyi* strain ACMM 645	Marine	AY264934	100	Gamma Proteobacteria
t4	*Halomonas* sp. Capricorn	Marine	AY734435	94	Gamma Proteobacteria

4.2.4 Bacterial diversity analysis

Diversity indices were calculated using band intensity of the DGGE profile. The genetic diversities of the bacterial communities were different when compared between the first and last day of shrimp culturing.

4.2.4.1 Diversity indexes in sediment taken from Nuang Suae sampling site

Shannon-Wiener diversity index values were shown in Figure 4-38. For both R1 and R2 ponds, diversity of bacterial population was increased following the culture period. The bacterial diversity indexes in week 0 to week 16 were ranged from 0.275 to 0.835 and 0.429 to 0.867, respectively. The highest values were found in the last week.

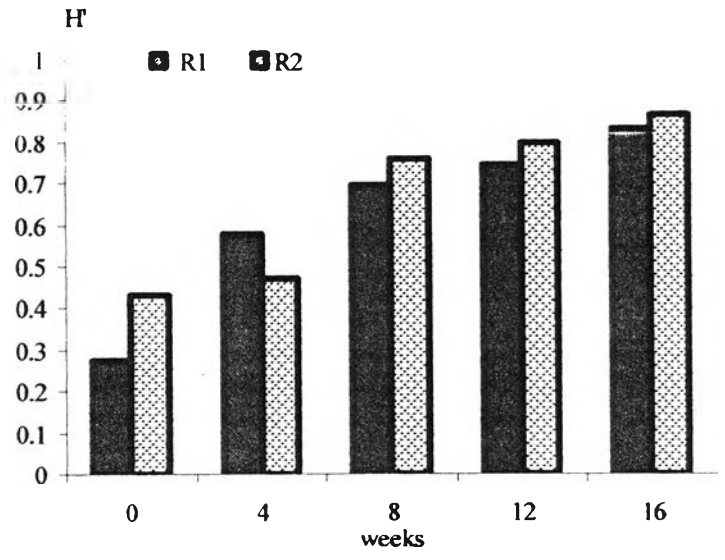

Figure 4-38: Diversity indexes of bacterial communities derived from DNA bands in DGGE gel of R1 and R2 ponds at Pathum Thani.

4.2.4.2 Diversity indexes in sediment taken from Ban Pho sampling site

The index values in P1 showed the sharp increased between in W-4 to W-2. After that they were little variations with only a small increase in week to W10 (Figure 4-39). Ranges of H' in P1 were between 0.43 and 0.87. In P2 pond, H' values presented little variation in the week-4 to the week 0. After that, they presented a large increased, from 0.26 to 0.96 in week 0 to week-10. H' values in P2 were between 0.26 and 0.96.

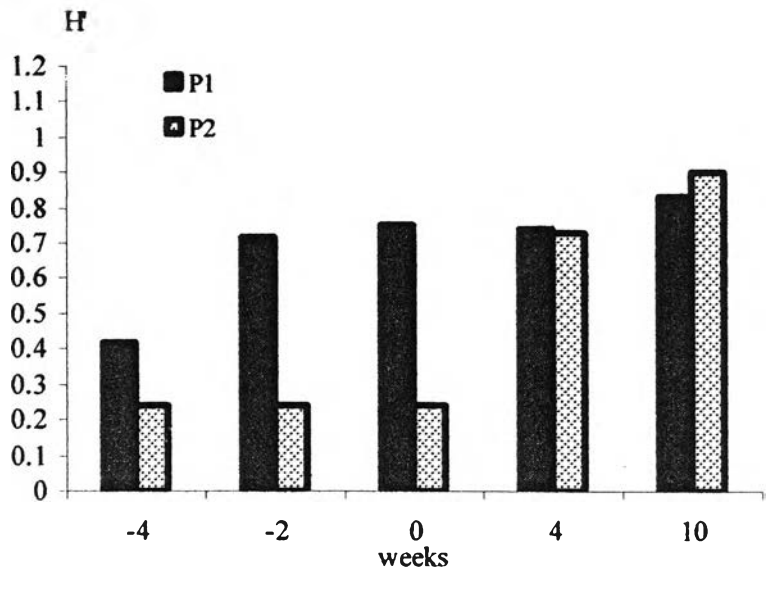

Figure 4-39: Diversity indexes of bacterial communities derived from DNA bands in DGGE gel of P1 and P2 ponds at Ban Pho.

4.2.4.3 Diversity indexes in sediment taken from Bang Khla sampling site

The H' value showed a sharp increased from week 0 to week 4. Then, they presented a small decreased from week 4 until week 16. Diversity index of bacterial community in sediment taken from Bang Khla were ranged from 0.23 to 0.68 (Figure 4-40).

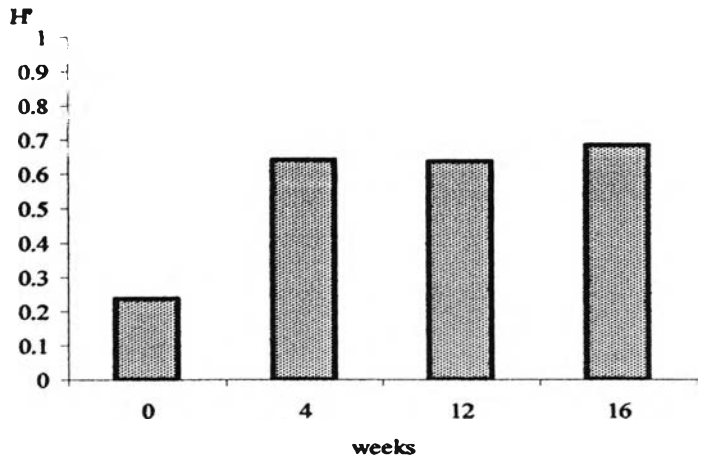

Figure 4-40: Diversity indexes of bacterial communities derived from DNA bands in DGGE gel of K1 pond at Bang Khla.

4.2.4.4 Diversity indexes in sediment taken from Bang Khun Thian sampling site

Diversity indexes in sediment samples collected from Bang Khun Thian are shown in Figure 4-41. H' value from T1 was higher than T2 and T3. Bacterial diversity indexes in three ponds were 0.763, 0.588 and 0.589, respectively.

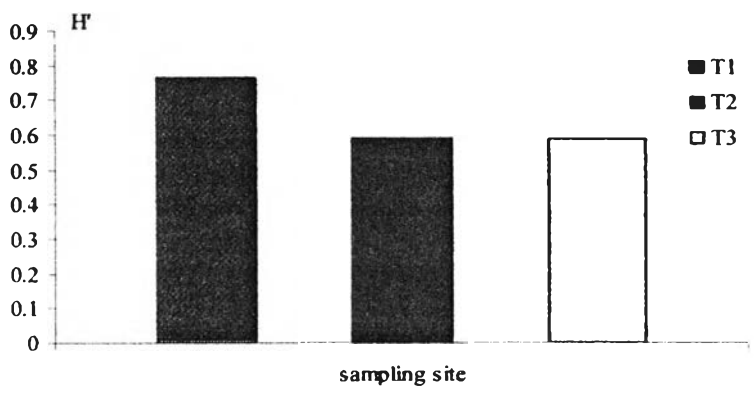

Figure 4-41: Diversity indexes of bacterial communities derived from DNA bands in DGGE gel of T1, T2 and T3 pond at Bang Khun Thian.

4.3 Nitrogen conversion by sediment from shrimp pond under laboratory condition

At the initial, 0.1 g of shrimp feed pellets was added into the chamber in order to supply nutrients for natural bacteria population in the sediment from shrimp pond at Pathum Thani. The experimental period was 48 hours with regularly monitoring of ammonia, nitrite and nitrate. Thereafter, another experiment was carried out with an addition of 1 g of shrimp feed after hour 87. During the experiment, dissolved oxygen in the water was between 5.4 - 8.14 mg/L. The results of nitrogen analysis are shown in Figure 4-42 to Figure 4-44.

After adding 0.1 g (6.7 g/m^2) shrimp feed pellets into the chamber, ammonia concentration in the sediment increased from 1.95 to 4.04 mgNH_4-N/L within 15 hours. (Figure 4-42). A peak of nitrite was found between hours 3-9 (Figure 4-43). Thereafter, ammonia concentration was steady for approximately 15 hours (hour 18-33) before depleted. On the other hand, nitrate concentration in the water and in the sediment from hours 0-48 was continuously decreased (Figure 4-44). ORP values during hours 0-48 was between –326 and –331 mV.

Addition of 1 g shrimp feed into the chamber at hour 87 resulted the release of ammonia in the chamber up to 8 mg NH_4-N/L. Thereafter, concentration of ammonia and nitrate in the reactor at hours 154-251 was almost constant. With this experiment, during hours 87-136, ORP was between -372 to -412 mV.

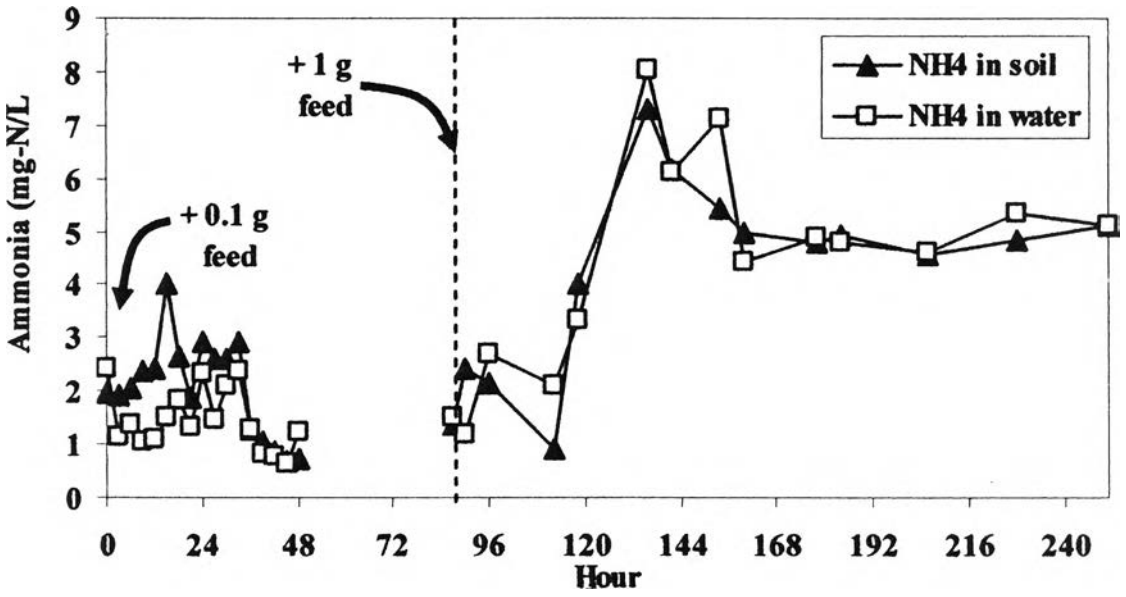

Figure 4-42: Concentration of ammonia in sediment and in water of the reactor shrimp feed was added at hour 0 and hour 87.

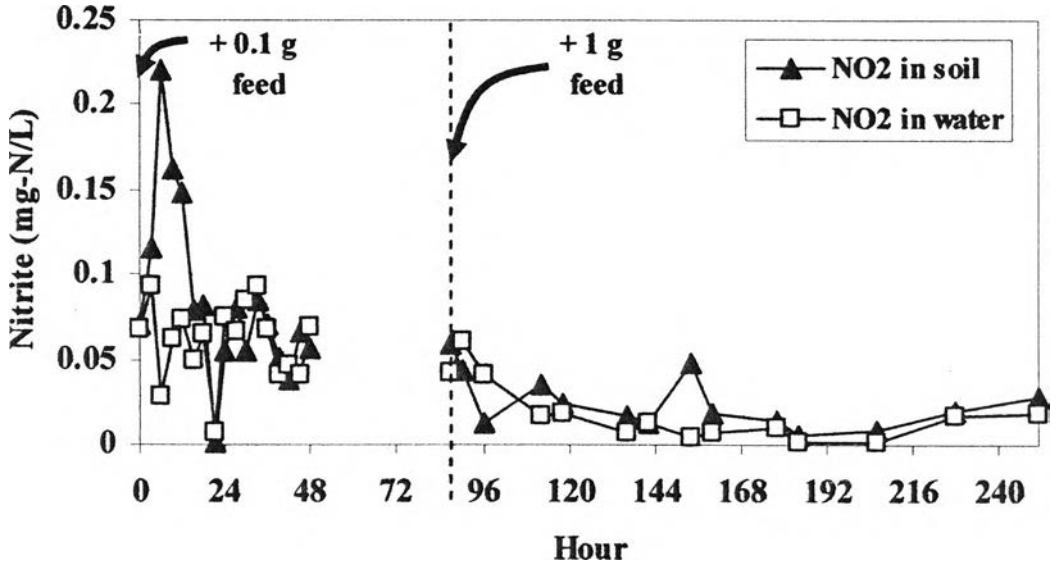

Figure 4-43: Concentration of nitrite in soil and in water of the reactor shrimp feed was added at hour 0 and hour 87.

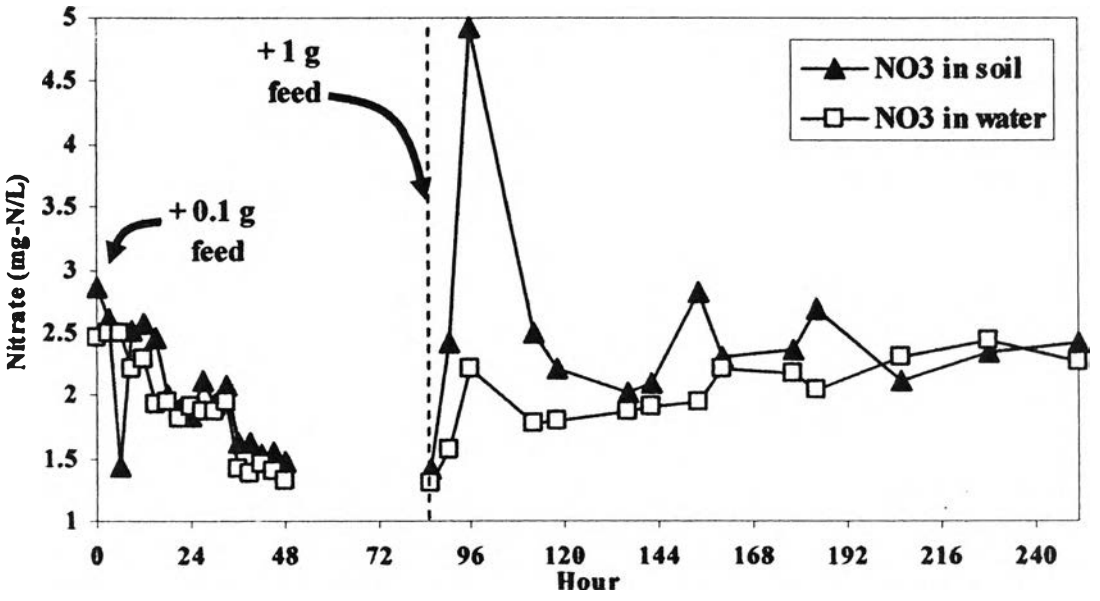

Figure 4-44: Concentration of nitrate in soil and in water of the reactor shrimp feed was added at hour 0 and hour 87.