CHAPTER V
DISCUSSION

5.1 Changes in sediment characteristics during shrimp cultivation crops

5.1.1 Organic content

During shrimp cultivation, the change of sediment characteristics in shrimp ponds was detected. Accumulation of organic materials was found especially in the pond with water reuse such as at Pathum Thani (R1 and R2 ponds) and Ban Pho (P1 and P2 ponds). Sediment in ponds R1, R2 had high organic content with the black decomposition layer underneath the sediment surface since the first day of shrimp culture. This was because these two experimental ponds were continuously used for two crops of shrimp culture without pond cleaning between each crop. On the other hand, pond bottom of P1 and P2 ponds at Ban Pho was low in organic content at the beginning of shrimp culture due to the good standard of pond preparation *e.g.* cleaning and drying. Since the shrimp culture was performed in a completely closed system without water exchange, substantially accumulation of organic content was later found even the shrimp was released at the normal stocking density of approximately 45 shrimps/m^2.

Decomposition of organic matters in sediment, incorporated with shrimp excretion, induced an accumulation of toxic waste especially ammonia and nitrite. In normal practice of shrimp cultivation, water must be exchanged so waste was therefore diluted. The use of water recirculating systems with high shrimp stocking density obviously increases the chance of waste accumulation in shrimp pond so it must be seriously concerned.

Sediment from commercial shrimp pond at Bang Khla (K1), especially at the end of the crop, contained high organic content without black hydrogen sulfide layer. This indicated that the regular water exchange between shrimp pond and the reservoir could reduce the risk of sediment decomposition. With good pond bottom condition, high shrimp production was therefore obtained.

Moderately high organic content was also found in sediment samples from extensive shrimp ponds (T1-T3) at Bang Khun Thian. It is known that extensive shrimp culture was performed without feeding and shrimp density was substantially low. Low organic content was therefore expected. However, the bloom of phytoplankton in surrounding waters that regularly be exchanged with the water in shrimp ponds induced the accumulation of chlorophyll-*a* (phytoplankton debris) in the sediment. Decomposition of organic matter in the sediment therefore produced high ammonia especially at the bottom sediment due to low vertical mixing in the pond.

The most serious problem due to an accumulation of organic matter was found in pond R2 at Pathum Thani. As the final organic content was extremely high, up to 56%, and oxygen supply through five air diffusers in the pond seemed to be insufficient, mass reduction of sulfate to hydrogen sulfide (H_2S) was suddenly occurred. This made the whole pond system collapsed and all shrimps in pond R2 were died in just two days. In general, this could be pointed that the organic load into the pond was higher than the carrying capacity of the pond.

5.1.2 Temperature and salinity

Generally, temperature is an uncontrollable factor in outdoor shrimp pond. However, it is fortunately that temperature in tropical region such as in Thailand is ranged within the optimum temperature for penaeid shrimp. Temperature in all ponds of this study was between 28.0-31.4°C, depended on seasonal change. Change of salinity during shrimp culture, on the other hand, is not only due to seasonal change but it also depend on other factors especially pond management. Addition of freshwater into shrimp pond during shrimp culture is among a normal practice of shrimp cultivation in the area far from the sea. This type of low-salinity shrimp culture is mostly found in the central part of Thailand.

In this study, most of the shrimp ponds were operated with low-salinity water. Water in ponds R1 and R2 in Pathum Thani was prepared with a mixing of high salinity water obtained from salt farm and freshwater to the final salinity of 5-6 PSU. Shrimp ponds at Bang Kla and Ban Pho were also operated at low salinity. One of the main reasons of using low salinity was to prevent the infection of marine pathogenic bacteria especially with the genus *Vibrio*. In contrast, extensive shrimp ponds at Bang Khun Thian (T1-T3) were located near the river mount. Salinity of extensive pond was therefore related with surrounding estuarine environment which was varied from approximately 10 to 28 PSU, depended on season.

5.1.3 Chlorophyll *a*

Chlorophyll-*a* is a parameter used to indicate the occurrence of phytoplankton in shrimp pond. Increase in chlorophyll-a concentration in both water and sediment usually related with the bloom of phytoplankton. Factors that induce phytoplankton bloom include nutrients (nitrogen and phosphorus) concentration, light, temperature, salinity etc. With high nutrient concentration, environmental condition of shrimp pond could sometime designate as eutrophic condition or eutrophication (Massana *et al.*, 1997; Eyre, 2002).

In shrimp ponds with high organic content in sediment such as ponds R1 and R2 at Pathum Thani, high phytoplankton density which turned the water color to dark green or dark brown was found since the beginning of the crop. Apart from shrimp ponds in Pathum Thani, chlorophyll-a concentration in shrimp ponds at Ban Pho (P1 and P2) and Bang Khla (K1) tend to increased along with the culture period. Peaks of chlorophyll-*a* during cultivation revealed bloom and drop of phytoplankton population. Accumulation of chlorophyll-*a* in sediment was therefore a result from the collapse of plankton population (Hopkins *et al.*, 1994; Eyre, 2002).

5.1.4 Nutrients concentration

5.1.4.1 Total ammonia

Total ammonia in sediment was high with fluctuation in the ponds with high organic content such as R1, R2, P1 and P2 ponds. However, ammonia in pond K1 at Bang Khla which was a commercial shrimp pond was increased with the culture period. The highest ammonia concentration in K1 pond was detected at the last day of the crop.

5.1.4.2 Nitrate and nitrite

Variation of nitrate concentration was substantially different in the pond in each area. At Pathum Thani (R1 and R2 ponds), nitrate concentration was fluctuate during shrimp culture with the concentration higher than a shrimp pond at Bang Khla (K1). However, accumulation of nitrate was found in shrimp pond at Ban Pho. In general, accumulation of nitrate in aquaculture pond is unusual. One of the reason is that nitrate was converted from ammonia and nitrite *via* nitrification process under sufficient oxygen condition. This was because the ponds P1 and P2 were installed with the high efficiency aeration systems including two sets of paddle wheel and two submersible air injectors in each 40x40 m^2pond.

Although the fluctuation of nitrite in sediment of most ponds was found, however, concentration of nitrite was relatively low especially when compared with ammonia. It is known that nitrite is an intermediate substance of nitrogen conversion *e.g.* nitrification and denitrification processes in the sediment. Low concentration of nitrite and nitrate in the sediment therefore indicated that both complete nitrification-denitrification processes were occurred and most of the nitrogen in sediment was released as nitrogen gas to the atmosphere *via* denitrification process (Deinema, 1985; Boyd *et al.*, 2001; Chou, 2002).

5.1.4.3 Phosphate

In this study, accumulation of phosphate during shrimp culture was found in shrimp ponds in different areas. It was interesting that accumulation of phosphate was found only in pond P1 but not in pond P2 at Ban Pho although these two ponds were located side by side. In comparison with other sites, high phosphate concentration in sediment of ponds R1 and R2 at Pathum Thani was found only in the last week while phosphate in pond K1 at Bang Kla increased with the culture period.

Since the study of bacteria related with phosphate cycle in shrimp or fish ponds was very limited, and the phosphate deposition process in the sediment was concerned as chemical reaction between dissolved reactive phosphate and structure of the sediment. As mentioned by Avnimelech and Lacher (1979), 80% of phosphorus from feed is not recovered in harvested fish but deposited in the pond bottom.

Polyphosphate accumulating organisms (PAOs) processes couple a front-end anaerobic zone with a subsequent aerobic zone to biologically removing phosphate (Mino *et al.*, 1998; Khoshmanesh *et al.*, 1999). In the anaerobic stage, the responsible micro-organisms may utilize energy derived from polyphosphate hydrolysis for carbon uptake end storage, and later under the aerobic stage, the previously, stored carbon can be used for growth and for polyphosphate formation (Boschker *et al.*, 2001). However, most of the study on PAOs was done at low temperature environment, activity of natural PAOs in tropical climate has never been reported.

Phosphate-accumulating bacteria can use nitrate as terminal electron acceptor instead of oxygen. The active phosphate accumulating-bacteria were also the efficient denitrifying bacteria such as *Agrobacterium tumefaciens*, *Aquaspirillum dispar*, and *Agrobacterium radiobacter* (Merzouki *et al.*, 1999).

Moreover, the role of some bacteria related with phosphate cycle especially phosphate accumulation in tropical aquaculture pond was still unknown and required further studies.

5.1.4.4 Total nitrogen and total phosphorus

Concentration of inorganic nitrogen compounds especially ammonia and nitrate in the sediment of all shrimp ponds were fluctuate with the trend reflecting the fluctuation of total nitrogen. This trend was also found with phosphate and total phosphorus. Hence, it might be estimated that the exchange of nitrogen and phosphorus compound between sediment and water was rather high.

5.2 Change of culturable bacteria (i.e. aerobic bacteria and *Vibrio* spp.) in sediment of shrimp ponds

Avnimelech and Ritvo (2003) defined that sediment accumulated in shrimp pond are highly reduced, enriched in organic matter, nitrogen and phosphorus. In this study, number of total aerobic bacteria increased with the shrimp cultured period and the number of bacteria was rapidly increased during the last week. The similar results were commonly reported in shrimp ponds elsewhere such as at Khung Krabaen Bay, Chantaburi Province in Thailand (Ruangpan *et al.*, 1996) and also in the U.S.A. (Burford *et al.*, 1998).

In general, high input of organic matter in the pond, principally as pellet feed, increased the concentration of carbon and nutrient source that promote the growth of bacteria. The pond bottom is therefore a favorable site for microbial growth due to the available of organic matter (Avnimelech and Ritvo, 2003). This suggests that bacteria were rapidly decomposed particulate organic substances into soluble compounds. Since bacterial numbers were highly correlated with organic carbon and total Kjeldahl nitrogen in the sediment, it could be summarized that these were limiting factors to bacterial growth (Burford *et al.*, 1998, Burford, and Lorenzen, 2004).

Sediment at the pond bottom is generally a proper environment for both aerobic and anaerobic bacteria. Organic matters derived from uneaten feed, feces, phytoplankton and zooplankton debris, shrimp shell and even shrimp carcass are decomposed by bacteria (Alonzo-Rodriguez. and Paes-Osuna, 2003; Gross *et al.*, 2003). In fact, practical pond management during shrimp culture especially aeration and lime supplement could support the activity of aerobic bacteria in the water column and also at the water-sediment interface. Water alkalinity between 100-120 mg/L that is usually regulated in commercial shrimp pond is suitable for the activity of the autotrophic nitrifying bacteria (Avnimelech and Ritvo, 2003).

Ammonia oxidizing bacteria (AOB) consist of two major groups that are beta-proteobacteria and gamma-proteobacteria. The common genera that were regularly reported are such as *Nitrosomonas*, *Nitrospira*, *Nitrosococcus* and *Nitrosolobus*. Other group of nitrifying bacteria is nitrite oxidizing bacteria (NOB) including *Nitrococcus*, *Nitrospira* and *Nitrobacter* (Wood, 1986, Boyd 1992; Pillay 1992; Smith 1993; Hopkins et al. 1994). These nitrifying bacteria are strictly aerobic chemolithotrophic bacteria that use inorganic carbon such as carbon dioxide or bicarbonate as a sole carbon source and oxidize nitrogen as an energy source (Prosser, 1986; Rittmann and McCarty, 2001; Rowan et al., 2003).

Denitrification, on the other hand, is a very important process occurred in anoxic sediment when nitrate is an electron acceptor instead of oxygen. When nitrate depleted, iron, manganese, sulfate and CO_2 then consequently serve as electron acceptors (Christensen *et al.*, 2000; Braker *et al.*, 2001). Since the direct measurement of denitrification in the sediment is rather complicate and the attempt to measure *in-situ* denitrification in shrimp ponds (R1 and R2) at Pathum Thani was not succeed, denitrification monitoring was therefore excluded from this study. One of the reasons is that the sediment in these ponds was rich with sulfate reducing layer from the beginning of the crop. With this condition, most of the carbon degradation up to 50-80% was possibly an anaerobic process that coupled with sulfate reduction (Suplee and Cotner, 1996; Burford and Longmore, 2001; Avnimelech and Ritvo, 2003). This might affected the availability of carbon for other process including denitrification. An attempt to measure denitrification in the sediment taken from extensive shrimp ponds in Vietnam by Alongi *et al.* (1999) showed that neither denitrification nor methanogenesis could be detected. This was reported as an unexpected phenomenon since there was the abundance of nitrate and lack of free sulfides. In detail, the carbon oxidation estimated in their study revealed that 40-60 % of carbon was oxidized by O_2 respiration following by Mn reduction (7-22%) and Fe reduction (5-25%) while sulfate reduction took place at between 13-26%. Regarding to their measurement that performed only once in two shrimp ponds, the data should suspected as an incomplete result and therefore need to be confirmed.

Apart from total aerobic bacterial count, bacteria in the genus *Vibrio* (gamma-proteobacteria) were particularly monitored by TCBS selective medium. This genus is well known as a common marine bacterium, and some species such as *V. harveii* and *V.*

cholerae were reported as the important pathogenic bacteria in marine shrimps (Aono, 1997; Ravenschlag *et al.*, 2001). The highest concentration of *Vibrio* spp. was found in the sediment from extensive shrimp ponds (K1-K3) at Bang Khun Thian while *Vibrio* concentration was substantially low in other shrimp ponds that located inland. It has to be noted that the number of *Vibrio* in inland shrimp ponds was low and almost stable throughout the culture period. This was due to limitation of sodium which is essential for growth of *Vibrio* spp. (Bowman and McCuaig, 2003; Gomoz-Gil, 2003).

5.3 Change of bacterial diversity during shrimp culture period

The sequence differences in 16s rRNA gene allow DNA from various organisms to be physically separated in a denaturing gradient gel, thereby allowing one to generate profiles of bacterial community. The profiles are visible as bands (or lines) in a gel. The banding patterns and relative intensities of the bands provide a measure of difference populations among the communities. Gel bands representing dominant species, which constitute at least 1% of the total bacterial community can be excised and sequenced. Sequence analysis of individual bands is used to infer the identity of the source organism based on database searches and phylogenetic methods (Muyzer *et al.*, 1993; Kowalchuk *et al.*, 1997; Ogino *et al.*, 2001).

DGGE profile of R1 and R2 ponds at Pathum Thani showed that the r1 band was predominant in both shrimp ponds throughout the culture period. After cloning, sequencing, and comparing with 16S rDNA database (BLAST), the sequence of band r1 was resembled to *Pseudomonas alcaligenes* with 98% similarity. In contrast, bands r2 to r9 had visibly shifted during shrimp culture. The BLAST results indicated that bands r2, r6 and r8 were possibly *Serratia* sp. *Halomonas* sp. *Vibrio* sp. respectively with percent similarity of more than 97%). However, five other bands (r3, r4, r5, r7 and r9) were similar to various uncultured bacteria with percent of similarity between 93 and 100%. These uncultured bacteria were reported as soil bacteria (r3, r4 and r5) or bacteria found in the river sediment (r9).

Since the DGGE bands generally illustrated the dominant bacterial groups in sediment samples, the results indicated that five of the ninth dominant bacterial groups are uncultured. Their characteristics and roles of these uncultured bacteria in environmental process in shrimp pond are therefore unexplored. In fact, these uncultured bacteria perhaps include the strictly anaerobic bacteria which were not present in aerobic agar plate of the standard counting method or they might need special nutrients or minerals to support their growth (Ward *et al.*, 1995; Liesack *et al.*, 1997; Gafan *et al.*, 2005).

The dominant bacteria in shrimp ponds (P1 and P2) at Ban Pho analyzed by DGGE composed of three major bands (p1, p2 and p8) that were appeared throughout the culture period. These bands were identified as *Pseudomonas* (100% similarity), uncultured bacteria (99% similarity) and *Serratia* sp. (97% similarity), respectively. Density of other bands apart from p1, p2 and p8 were increase with the culture period (see lanes 3,4,5 of P1 and lanes 8,9,10 of P2 in Figure 4-36). Results from sequencing and BLAST showed that band p3, p4, p5, p6 and p7 were possibly *Desulfovibrio* sp. (100% similarity), uncultured bacterium (97% similarity), *Bacillus* sp. (99% similarity), *Pseudomonas borealis* (100% similarity) and *Halomonas* sp. (98% similarity), respectively. However, the most abundant bacterium in this site was uncultured bacteria (p2 band).

The dominant bacteria in shrimp pond at Bang Khla (K1) during the first week of the crop were also uncultured bacteria (k6 band). However, the diversity was clearly changed after one month of shrimp culture and then stabled until the end of the crop. At this period, the dominant bacteria illustrated as bands k1, k3, k4 and k5 were *Pseudomonas* sp. (100% similarity), *Marinobacter sedimentalis* (100% similarity), *Halomonas* sp. (100% similarity) และ uncultured gamma proteobactria (99% similarity), respectively.

DGGE bands found in the sediment of extensive shrimp ponds (T1 - T3) was similar in the number of bands but different in the intensity. Some band such as t2 (100% similarity to *Bacillus* sp.) was found dominant in T1 pond but not in T2 and T3. With this site, dominant bacteria that found in all ponds were uncultured marine bacterium (band t1 with 100% similarity), *Vibrio harveyi* (band t3 with 100% similarity) and *Halomonas* sp. (band t4 with 94% similarity).

Pseudomonas spp. are ubiquitous in the environment and often constitute a substantial fraction of the microflora in any given area (Braun-Howland and Nierzwicki-Bauer, 1993; Andersen *et al.*, 2000). They are present in soils, freshwater, seawater and sewage. They are substantially active species in aerobic decomposition and biodegradation, and play a key role in the carbon cycle. *Pseudomonas* growth is relied on respiration for the catabolism of various compounds, including aliphatic and aromatic hydrocarbons, fatty acids, insecticides and other environmental pollutants (Austin and Allen, 1982). This made *Pseudomonas* a common species found and has been reported in aquaculture pond (Pankhurst *et al.*, 1996; Rao *et al.*, 2000; Al-Harbi and Uddin, 2004) and other aquatic environment (Fry, 1987; Lightner, 1993b; Thorseth *et al.*, 2001). In addition it could be easily detected by the classical culture and counting technique. *Pseudomonas* spp. are also the denitrifying bacteria (Anderson *et al*, 1989; Rao *et al.*, 2000). They can use nitrate (NO_3) as an electron acceptor. With this process, NO_3 is reduced to NO_2 (nitrite) and then to a gaseous form of nitrogen such as N_2 or N_2O (nitrous oxide) or ammonia (NH_3) (Andersen *et al.*, 2000; Rao *et al.*, 2000).

Halomonas spp. are another dominant bacteria in shrimp ponds of this study. They were found in 4 out of 27 clones of DGGE bands derived from all 4 sites (Pathum Thani, Ban Pho, Bang Khla and Bang Khun Thian). In general, *Halomonas* and phylogenetically related bacteria are common in various habitat, such as saline pond, sea water and sea sediment (Fuhrman, 1993; Bowman *et al.*, 2000; Bowman and McCuaig, 2003; Kaye *et al*, 2004). The *Halomonas* are members of gamma proteobacteria with gram negative and rods shape. They are extremophile with an ability to tolerate (and in some case require) medium containing high salt. *Halomonas* are capable of anaerobic growth with glucose (in absence of nitrate). Some are capable of carrying out denitrification, reducing nitrate to nitrogen and gaining energy in the process (Ventosa *et al*, 1998; Braker *et al*, 2001; Nogales *et al*, 2002; Okamoto *et al*, 2004). Yoshie *et al* (2004) suggested that members of the genus *Halomonas* are dominant denitrifying bacteria in an acetate-fed saline wastewater treatment system.

The gram negative, rod shape, gamma proteobacteria, *Serratia* spp. were found in shrimp ponds at Pathum Thani (R1 and R2) and Ban Pho (P1, P2). The genus *Serratia* was found in soil and water as well as in the guts of insects and vertebrates. They are facultative anaerobic bacteria (Bowman and McCuaig, 2003). Röling *et al* (2004) found that the oil-degrading bacterial community on DGGE were related to *Serratia plymythica*. Some species such as *Serratia marcescens* is a chitinaseproducing microorganism (Torsvik *et al.*, 1996; LeCleir *et al*, 2004). Colonies of *Serratia* have long been known for their red pigment. However, pigmentation is only present in just a small percentage of isolated culture (Head, 1998; Ogino, 2001).

Vibrio spp. are gamma proteobacteria with gram negative and straight or curved rod shape. They occur in both marine and freshwater habitats and in associations with aquatic animals (Aono *et al.*, 1997; Arias *et al.*, 1999). Some species are bioluminescent and live associations with fish and other marine life. Other species are pathogenic for fish, eels, and frogs, as well as other vertebrates and invertebrates. They are facultative anaerobic bacteria but not the denifrifying bacteria (Ravenschlag *et al.*, 2001; Thompson, 2004). Several *Vibrio* are capable of both respiratory and fermentative metabolism (Sobecky *et al.*, 1998; Arias *et al.*, 1999).

Marinobacter sp are heterotrophic and thermotolerant marine bacterium. They are gram negative, rod shaped gamma proteobacteria. Some are capable of carrying out denitrification, reducing nitrate to nitrogen and gaining energy in this process (Sobecky *et al.*, 1998; Gorshkova *et al.*, 2003; Shivaji *et al*, 2005).

Bacillus spp. are facultative anaerobic, spore forming bacteria. They can resist to hostile physical and chemical conditions. Spores of many *Bacillus* species are even resisted to heat, radiation, disinfectants and desiccation. They are nitrifier and commonly found in soil water and seawater. *Bacillus* spp. are bacteria that have a high capability of organic matter degradation (Pichinoty, 1979; Wright *et al.*, 1997; Sobecky *et al.*, 1998; Thorseth, 2001).

All *Desulfovibrio* are sulfate reducing bacteria with gram negative, non spore forming, curved rod shaped cell and motile. Members of the genus *Desulfovibrio* commonly exist in soil and aquatic habitats (Devereux *et al.*, 1992; Teske *et al.*, 1996; Carlos Frazão *et al.*, 2000). Since these organisms are strictly anaerobe, they are found in both aquatic and terrestrial environments with anoxic condition resulting from microbial decomposition (Aono *et al.*, 1997; Sobecky *et al.*, 1998). The most favorable environment is usually rich in organic material and sulfate. Since it is the common bacteria in both aquatic and terrestrial habitats, this genus is among the most studied of the sulfate-reducing proteobacteria (Devereux *et al.*, 1990; Devereux and Stahl, 1993; McDougall, 1997).

The DGGE analysis illustrated that autotrophic nitrifying bacteria such as *Nitrosomonas*, *Nitrosococcus*, *Nitrobacter* and *Nitrospira* were not the dominant group of bacteria in shrimp pond sediment. However, the experiment regarding nitrification process carried out in R1 pond by Sutti (2004) showed that the conversion of ammonia to nitrite and nitrate occurred within the shrimp pond. The present of nitrifying bacteria which are the autotrophic bacteria should possibly a minor population and might be appeared as a smear in DGGE gel.

5.4 Relation of bacterial diversity and culture period based on DGGE analysis

Considering bacterial diversity illustrated by DGGE bands, number of species or diversity was increased with culture period. When applying diversity index calculation derived from number and intensity of DGGE bands, it was clearly that diversity index was increased from approximately 0.22 on the starting day to more than 0.6 at the last day of shrimp culture. One of the reasons is an accumulation of both organic and inorganic nutrients in the sediment. This accelerated growth of the bacteria and became an optimum condition for various kinds of bacteria (see 5.2) (Vetriani *et al.*, 1999; Bowman *et al.*, 2000; Luna *et al.*, 2004).

5.5 Effect of ozone on bacterial communities in shrimp pond

The results from both bacterial count and DGGE analysis showed that ozonation in the pond P1 at Ban Pho significantly reduce the number of bacteria in the sediment. This made the DGGE band from sediment sample at 2 weeks after ozone treatment had very low in DNA content (see band number 2 in Figure 4-36).

Ozone has strong cleaning and disinfection properties which will rapidly kill viruses, bacteria, fungi and protozoa. Basically, ozone has the same properties as chlorine, but with seven times the oxidation capacity. Ozone is used in many aquaculture systems for water treatment and disinfection. However, too high ozone concentration can even oxidize the body surface of aquatic animal. Limiation of ozone concentration and exposure time (e.g. lethal concentration) must be evaluated for each animal species (Summerfelt and Hocooeimer, 1997; Bullock, 1997; Joret, 1997).

Although ozonation in shrimp pond could reduce the number of bacteria, this might not be an advantage of ozone for shrimp culture. It is also known that ozone could also affect all microorganisms in the water including phytoplankton and zooplankton. Dead plankton and bacterial debris after ozone treatment was then sank to the pond bottom. At the last day of culture, concentration of organic matter, chlorophyll-*a* and the thickness of black hydrogen sulfide layer in sediment from P1 was significantly higher than in P2 ponds. This condition was not good for regularly pond management.

5.6 Nitrogen conversion in sediment from shrimp pond under laboratory condition

This additional experiment was carried out in order to evaluate the activity of natural bacterial populations in organic nitrogen conversion. Since the monitoring of nitrogen conversion in shrimp pond is rather complicate and the preliminary experiment was not success, laboratory study using a custom made chamber was carried out instead. Shrimp feed pellets after sterilized by autoclave, were used as an organic nitrogen source.

The results indicated that the sedimentary bacteria decomposed protein in shrimp feed and converted to ammonia. Thereafter, ammonia concentration was steady for approximately 15 hours (hour 18-33). Since water in the reactor was in aerobic condition, ammonia was possibly converted to nitrite and nitrate via nitrification process. This was confirmed by nitrite peak at hour 9. Constant concentration of ammonia in the reactor during hours 18-33 suggested that the rate of ammonia production by ammonification process was approximately equal to the rate of ammonia conversion to nitrite and nitrate by nitrification process. When the organic nitrogen source depleted, the decrease of ammonia was detected. On the other hand, nitrate concentration in the water and in the sediment from hours 0-48 was continuously decreased (Figure 4-44). This was probably due to denitrification process in the sediment as indicated by ORP values between –326 and –331 mV that is the suitable ORP range for nitrate reduction process.

Addition of high organic nitrogen (1 g shrimp feed or 67 g/m^2) into the reactor at hour 87 resulted the release of ammonia in the reactor up to 8 mg NH_4-N/L. At this nitrogen loading of 4.7 g-N/m^2, ammonia production rate was calculated as 0.13 mg NH4-N/reactor/h or 8.7 mg-NH_4-N/m^2/h. Comparison the data between hours 87-136 in both Figures suggested that the conversion of ammonia to nitrate occurred in the sediment layer. Thereafter, denitrification process, as indicated by ORP between -372 to –412 mV, removed nitrate from the reactor by convert it to nitrogen gas. Concentration of ammonia and nitrate in the reactor at hours 154-251 was almost constant. This indicated the balance between ammonia production (ammonification), ammonia conversion to nitrate (nitrification) and nitrate conversion to nitrogen gas (denitrification).

The results from this experiment suggested that bacterial populations from R2 ponds at Pathum Thani had an ability of nitrogen conversion from organic nitrogen to inorganic nitrogen possibly through ammonification : nitrification : denitrification processes. Ammonifying bacteria seemed very active since they were rapidly decompose organic nitrogen (shrimp feed) to ammonia within a few hours. With bacterial diversity as already described in the result chapter, it is clearly found that nitrifying bacteria were minor species within the sediment. However, these bacteria were also active and quickly convert ammonia to nitrite and nitrate. Conversion of nitrate to nitrogen gas was occurred in the sediment layer. Many species of denitrifying bacteria such as *Pseudomonas* spp. and *Bacillus* spp. (see Table 4-3) were dominated in the sediment sample. The denitrification was, therefore, probably driven by these bacteria.

Moreover, the data from this experiment is substantially useful for the evaluation of the carrying capacity of shrimp pond sediment to nitrogen waste loading. High ammonia in shrimp pond, *e.g.* more than 2 mg-N/L, must be avoided since it is toxic to shrimp. Since ammonia was produced by both shrimp excretion and decomposition of uneaten feed, loading of organic nitrogen, in which most are from protein in shrimp feed, must be seriously concerned. At normal practice, shrimp feeding between 20-40 g/m^2/day was regularly performed by farmers. This loading is subjected to safe level or nitrogen input within the pond carrying capacity. Further study is essentially needed in order to estimate the carrying capacity of shrimp pond to nitrogen loading. This is also needed as an important data for the development of proper technology to improve carrying capacity toward the super-intensive shrimp production system.

5.7 Correlation between bacterial communities and sediment characteristics

Many studied have demonstrated that bacterial communities in aquatic sediment show considerable variation in time and may exhibit the relationship with the environment (Wagner-Dobler *et al.*, 1992; Martin *et al.*, 2003; Byron *et al.*, 2004).

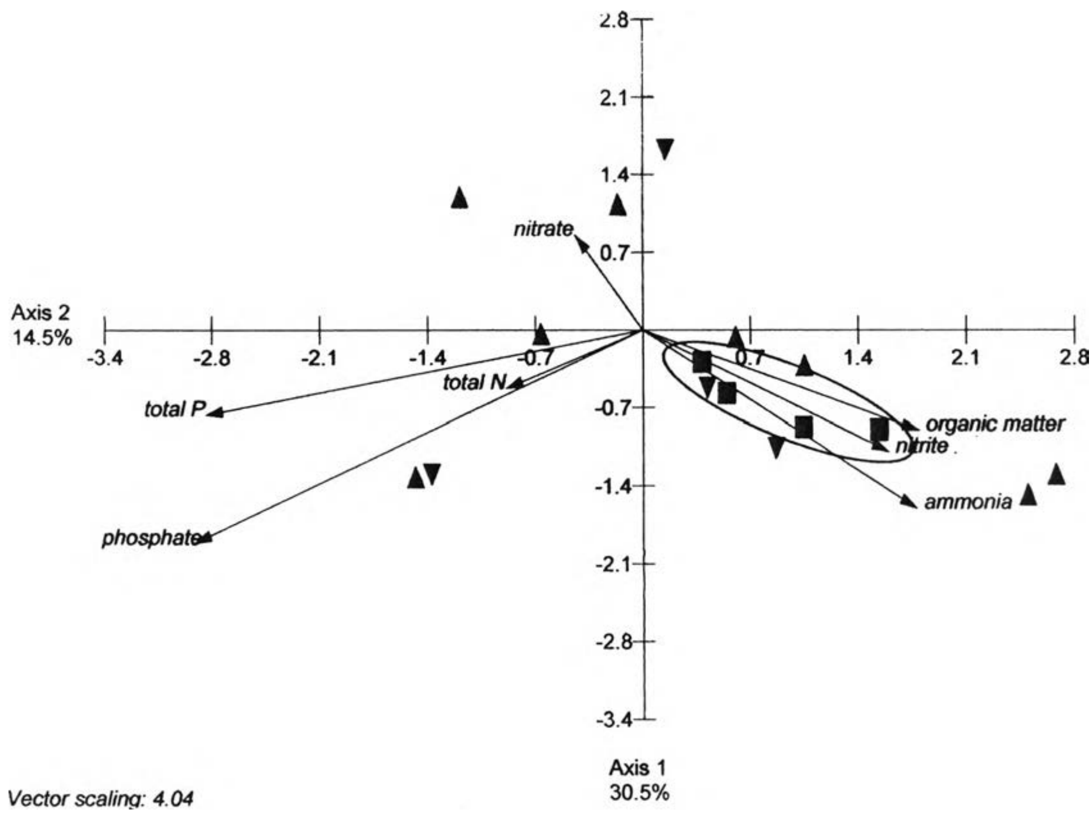

Figure 5.1: Canonical Correspondence Analysis of data generated from the DGGE profiles of amplified bacterial 16S rDNA and sediment chemical characteristics. Symbols: ▲, bacterial communities from week 0-4; ▼, bacterial communities from week 6-10; and ■, bacterial communities from week 12-16. Raw data was derived from shrimp ponds at Pathum Thani and Ban Pho.

In this study, correlation between bacterial communities and sediment characteristics was analyzed by canonical correspondence analysis (CCA), which is a multivariate direct gradient analysis that combined the environmental data sets with community profiles in a joint analysis. This statistically tool is generally used to explain how the variation observed between different denaturing gel electrophoresis banding patterns could be associated with the variations of measured environmental variables (Fromin *et al.*, 2002). CCA of bacterial communities from DGGE analysis and various sediment chemical characteristics in shrimp ponds at Pathum Thani and Ban Pho is shown in Figure 5-1. The first and second CCA axes describe 30.5% and 14.5% of the data, respectively. Consequently, about 45% of the variation between bacterial communities could be explained by the sediment chemical characteristics of the shrimp ponds.

During 0-12 weeks of culture period, there was no significant correlation between bacterial communities and sediment characteristics. Meanwhile, the data at week 16 (in circle) illustrated the correlation between organic matter, ammonia and nitrite with bacterial communities. It was found that organic matter, ammonia and nitrite had a strong relationship, while nitrate had an opposite correlation with those three parameters. Total nitrogen, total phosphorus and phosphate were found independent from organic matter in the sediment. Regardless of pond locations, this CCA model suggests that sedimentary bacterial communities were depended on the accumulation of organic matter, ammonia, and nitrite in these ponds.

Moreover, correspondence analysis (CA) of DGGE data, as shown in Figure 5.2, also illustrated the close relationship of bacterial community compositions in shrimp ponds during week 12-16. The data indicated that similar bacterial populations were found after 12-week shrimp cultivation. These probably resulted from the changes of sediment properties especially the amounts of organic matter, ammonia, and nitrite as seen in CCA.

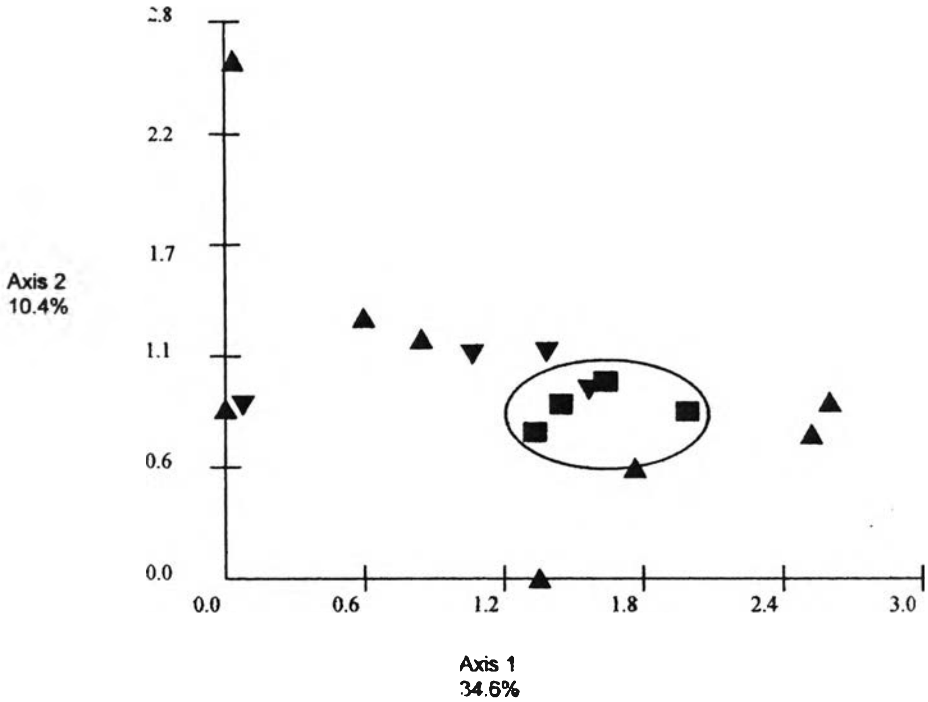

Figure 5.2: Correspondence Analysis of data generated from the DGGE profiles of amplified bacterial 16S rDNA. Symbols: ▲, bacterial community from week 0-4; ▼, bacterial community from week 6-10; and ■, bacterial community from week 12-16. Raw data was derived from shrimp ponds at Pathum Thani and Ban Pho.

The effect of organic matter, ammonia, and nitrite concentrations on microorganisms was later confirmed by regression analysis using data from shrimp ponds in Bang Khla (k1 pond). As shown in Figure 5.3, the regression representing the increase of microorganisms as a function of increase organic matter concentration (with $R^2 = 0.949$). With 10% increase of organic matter concentration, the numbers of microorganisms increased about 0.281 logCFU/g.

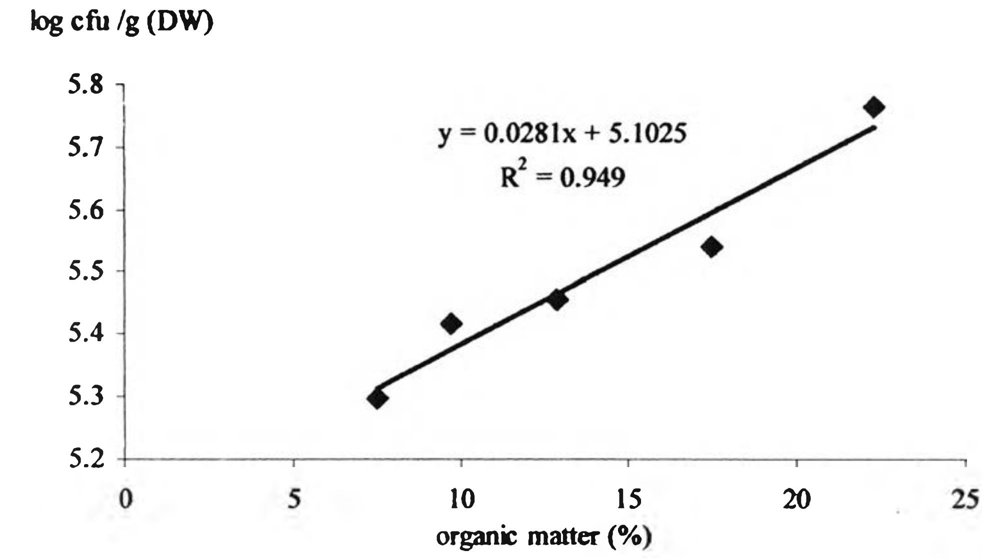

Figure 5.3: The relation of organic matter content and bacteria number in K1 pond.

Similarly, the relation of nitrite and aerobic bacteria in pond K1 is shown in Figure 5.4. The regression represents the increase of microorganisms as a function of increase nitrite concentration (with $R^2 = 0.914$).

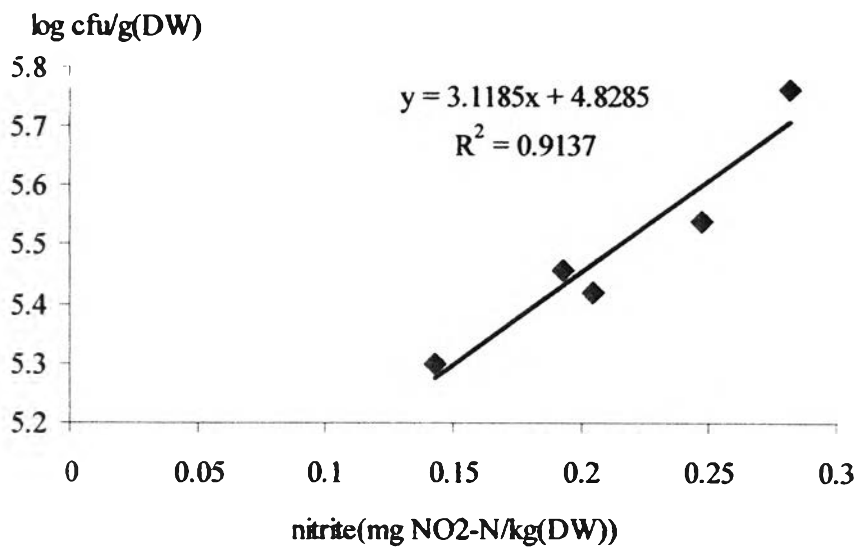

Figure 5.4: The relation of nitrite concentration and bacteria number in K1 pond.

In addition, the relation of ammonia concentration and aerobic bacteria in K1 pond is showed in Figure 5.5. The regression representing the increase of microorganisms as a function of organic matter concentration (with $R^2 = 0.832$). With 10% increase of ammonia concentration, the numbers of microorganisms increase about 0.435 logCFU/g.

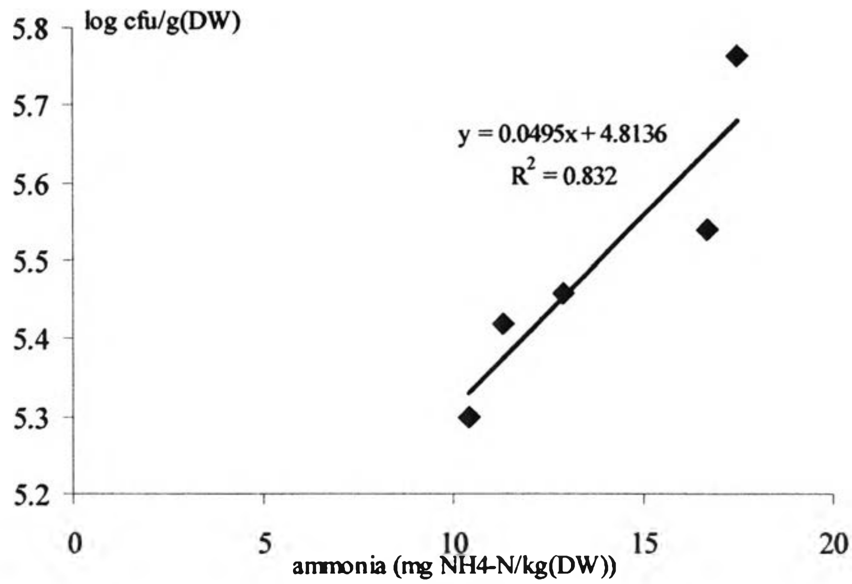

Figure 5.5: The relation of ammonia concentration and bacteria number in K1 pond.

Organic matter in shrimp ponds generally came from shrimp feed, shrimp excretion, organism debris, shrimp shell and even shrimp carcass. In general, decomposition of organic nitrogen induces an increase of ammonia in the water. This condition influenced phytoplankton bloom in the pond that was commonly found in this study. This was following by plankton die off and sink to the bottom of the pond. From Figure 5.1-5.5, it could be concluded that the accumulation of organic matter, ammonia, and nitrite led to the growth of certain sedimentary bacteria that probably related with carbon and nitrogen cycle in the pond. Activity of bacteria in the sediment reflected that role of biogeochemical processes and finally conducted the carrying capacity of the pond. During shrimp culture, increase of bacteria population due to the accumulation of organic matter can deplete oxygen in the pond that can directly affect shrimp. Oxygen requirement at the last period of the crop was therefore much higher than that of the starting period.

Data from section 5.6 showed that, after adding organic nitrogen (0.1 g shrimp feed or 6.7 g/m^2) in the reactor, sedimentary bacteria decomposed protein in shrimp feed and converted to ammonia. Then, ammonia was possibly converted to nitrite and nitrate via nitrification process. Constant concentration of ammonia in the reactor suggested that the rate of ammonia production by ammonification process was approximately equal to the rate of ammonia conversion to nitrite and nitrate by nitrification process. When the organic nitrogen source depleted, decrease of ammonia was finally detected. On the other hand, nitrate concentration in the water and in the sediment continuously decreased. This was probably due to denitrification process in the sediment.

In contrast, addition of high organic nitrogen (1 g shrimp feed or 67 g/m^2) in the reactor resulted the release of high concentration of ammonia. This reflecting the important of basic knowledge concerning the safe quantity of nitrogen input within the carrying capacity of the pond. Further study is substantially needed with more accurately measurement of the carrying capacity both in term of nitrogen waste and other waste substances.