

Water quality and bacteriology in an aquaculture facility equipped with a new aeration system

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Abstract

The HOBAS aeration system was tested to compare changes in environmental and bacteriological parameters in ponds growing *Penaeus monodon* during a single production cycle. The stocking density in the aerated pond was doubled to 12 post-larvae (PL) m⁻² in contrast to the non-aerated pond with 6 (PL) m⁻². Microbial abundance in the ponds ranged between 10⁵⁻⁶ cells ml⁻¹. Among the physiological groups of bacteria enumerated, the heterotrophs dominated with an abundance of 10⁴ CFU ml⁻¹. Of the nitrogen and sulfur cycle bacteria, the nitrifiers flourished in the aerated pond and could maintain ammonia-N concentration within permissible levels. Bacterial activity also maintained sulfide concentrations at <0.03 mg l⁻¹. Non-aerated conditions promoted denitrification maintaining nitrate concentration between 0.32-0.98 μM NO₃⁻-N l⁻¹. However, a marked increase in ammonium content was observed in the non-aerated pond at the end of the culture period. Thus in high density ponds, the aerators served to stimulate bacterial growth and activity which consequently maintained the quality of the water to match that of low density ponds. Accordingly, these aerators could be effectively used to sustain higher yields. The effluent from the aerated pond is less likely to alter the redox balance of the receiving waters.

Keywords: Shrimp · Culture · Water · Inorganic nitrogen · Sulfide · Bacteria

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Introduction

Intensification can often lead to deterioration of water and sediment quality in shrimp culture ponds leading to stressed environment that culminate in diseases. In open waters, nutrients determine the potential fertility of water masses. It is well known that nutrient cycling in coastal and brackish waters is related to biotic and abiotic factors. In contrast, enclosed systems such as shrimp culture ponds exhibit comparatively less dynamic conditions. However, modified extensive shrimp ponds have been observed for their low risk, low cost and operational strategies unlike the intensive farming where pond environment is controlled by modern aeration techniques which makes the pond dynamic in terms of biotic and abiotic factors which are again interdependent.

Biological fertility of brackish water aquaculture system is normally decided by the concentration of inorganic nitrogenous species in the water column. Most of the N (90%) enters the farm/pond as formulated shrimp food of which 22% is converted to harvested shrimp, 14% remained in the sediment and the remainder 57% is discharged to the environment (Christopher et al. 2003). Among the inorganic nitrogen species, ammonium is preferred by phytoplankton for growth (Kanda and Hattori 1988). However, Joint et al. (2002) suggest that heterotrophic bacteria out-compete phytoplankton for available inorganic nutrients resulting in nutrient limitation. The microbially mediated nitrification process by which ammonium is oxidized to nitrite and consequently to nitrate plays an important role in maintaining dissolved ammonium concentrations in aquatic systems. The main sources of ammonium in culture systems are shrimp excretion and decomposition of detritus (Montoya et al. 2002). At relatively high concentrations, unionized ammonia could pose threat to cultivable animals. Unionized ammonia (NH_3^+) has been reported to be more toxic than ionized ammonia (NH_4^+) due to its ability to diffuse across cell membranes (Chen and Kou 1993). Studies by Frias-Espericueta et al. (2000) have shown cessation of feeding in post-larvae of *Litopenaeus vannamei* on exposure to ammonia concentrations $\geq 20 \text{ mg l}^{-1}$. Similarly, high levels of nitrite occurring as an intermediate product during nitrification can cause mortality by imparting toxicity to shellfish (Mevel and Charmroux 1981).

Like ammonium and nitrite, hydrogen sulphide in its non-ionic form is extremely toxic and can cause rapid and massive mortalities in aquaculture ponds (Krom et al. 1985). Sulfide toxicity is known to cause mortality due to hypoxia (Bagarinao and Lantin-Olaguer 1999). Aqua farms with high organic load, limited water exchange,

circulation, no aeration, high stocking and feeding rates can become oxygen-depleted and allow sulfide from the sediments to appear in the water column poisoning free-swimming fish (Bagarinao and Lantin-Olaguer 1998).

Most marine aquaculture farms are located along the coast. Water from nearby brackish water bodies is used to fill the culture ponds. Discharge of water contaminated with excessive nutrients or chemicals into the adjoining water bodies at the end of the culture period could have undesirable environmental impacts. Microbial processes affect water quality parameters such as dissolved oxygen, ammonium, nitrite, nitrate etc. (Moriarty 1996). Hence, bacteria in aqua ponds could play an important role in maintaining the water column chemistry. The study compares observations of an aerated higher density pond having 12 PL m⁻² with a lower density non-aerated pond with 6 PL m⁻². It presents the contribution of pertinent groups of bacteria that could serve to maintain the water quality of the pond through a single production cycle.

Methods and Materials

Sampling location

The shrimp culture ponds were located at Kumta town along the west coast of India (14.42°N & 74.40°E). Two ponds with an area of approximately 0.8 hectares and average depth of 1.2 m were selected for the study. The ponds are tide-fed from an adjoining creek located about 3 km from the sea. The experimental pond was aerated using aerators (Fig. 1) developed by HOBAS Water Engineering, Norway. The core unit consists of a pump, a valve, an air inlet hose and a specially adjusted raft for vertical implementation in the pond. Air is introduced directly into the pump housing and is mechanically mixed with water under vacuum.

Before stocking, the two ponds were dried for a period of one month and lime was applied to the bottom of the pond. They were then filled with water five days prior to stocking during high tide with the help of sluice gates and electric pump. Stocking of ponds was done in late evenings for which seeds were transported from the nearby hatchery. Bags consisting of shrimp larvae were kept in pond water for acclimatization for about two hours until release. The HOBAS aerators in the experimental pond were placed towards each corner of the pond (Fig. 2). The stocking density in the non-aerated pond was 6 post larvae (PL) m⁻² of *Penaeus monodon* while in aerated pond it was doubled to 12 PL m⁻². Shrimps were fed with pelleted feed four times a day (6 am, 11 am,

6 pm & 11pm).

Sampling procedure

Water samples were collected in triplicate from each pond at fortnightly intervals from April to September 2004 covering one production cycle. The samples were always collected during early hours of the day.

Physico-chemical analyses

On the day of sampling, water temperature was measured *in situ* with a hand held mercury thermometer (GH Zeal Ltd, England). The pH of water samples were measured with a PHAN pH meter (Lab-India) with an accuracy of ± 0.01 pH units. The oxidation-reduction potential (ORP) of the pond water was measured with the help of microprocessor-based analyzer (Lab-India, model PHAN) fitted with an *Orion* combination platinum/Ag/AgCl electrode.

Salinity of pond water was determined titrimetrically (Strickland and Parsons 1972). Dissolved oxygen (DO) phosphate, ammonium, nitrite and nitrate in the water samples were measured following standard analytical methods (Parsons et al. 1984). Hydrogen sulfide content was measured as described by Pachmayr (1960).

Bacterial quantification

For microbiological analysis, surficial water was collected in sterile polyethylene bottles. The samples were preserved in an icebox and processed within a few hours of collection. The method of Hobbie et al. (1977) was used for the enumeration of Total Bacterial Counts (TC) by epifluorescence microscopy. Maximum viable counts were achieved using half-strength nutrient agar (HiMedia, India) during preliminary observations and this concentration was used for the enumeration of total heterotrophic bacteria (THB).

Nitrifiers (NTB) and sulfide oxidizing denitrifiers (*Thiobacillus denitrificans* like organisms; TDLO) were enumerated on selective media. About 100 μ l of water sample was spread plated on modified Winogradsky's medium (Ram et al. 2001) for the enumeration of ammonium oxidizers or type I nitrifiers. The plates were incubated for ~15 days at $28 \pm 2^\circ\text{C}$ and counts were recorded as Colony Forming Units (CFU). TDLO were enumerated by the agar shake method on modified Leiske's medium (Loka Bharathi 1989; Loka Bharathi et al. 1988). Sulfate reducing bacteria (SRB) were enumerated on

shake agar using modified Hatchikian's medium (Loka Bharathi et al. 1992). After inoculation, the culture tubes were incubated for 45 days at $28\pm 2^{\circ}\text{C}$. Bacterial counts were recorded as CFU ml^{-1} of water sample.

Statistical analyses

The results of the bacterial counts were processed by \log_{10} transformation. The correlation coefficient values between the bacterial counts and nutrient concentrations were calculated using Analysis tool pack in Microsoft Excel. Only significant relationships have been discussed.

Results

Physico-chemical analyses

Water quality parameters in both the ponds showed similar trends and average values have been presented in Table 1. Statistically, no significant variation in the physico-chemical parameters between the ponds could be observed. Water temperature in the ponds ranged from $27\text{-}35^{\circ}\text{C}$ (± 0.10). As the southwest coast of India receives rainfall from June-September, lower salinity values were encountered towards the end of the culture period. Hence, salinity values ranging from $7.20\text{-}43.18$ (± 0.03) were recorded during the observation period. The pH of pond waters exhibited an increase with increasing days of culture varying between $7.09\text{-}8.50$ (± 0.06).

Dissolved oxygen levels were high throughout the culture period. A decrease in dissolved oxygen to a minimum of 2.86 (± 0.10) mg l^{-1} was observed in the non-aerated pond towards the end of the culture period. Eh values in the aerated pond ranged between $62.80\text{-}112.77$ (± 4.95) whereas in the non-aerated pond the range was relatively lower varying from $54.60\text{-}94.60$ (± 3.43).

A steady decrease in phosphate content was observed in the aerated pond. A significant positive relationship of $r=0.83$, $p<0.001$ between phosphate and ammonium concentrations in the aerated pond water was also observed. The concentration of the nutrient in the non-aerated pond varied inconsistently (Fig. 3a) during the culture period.

Low ammonium content was recorded in the ponds during the initial period of culture (Fig. 3b). It increased gradually as the cycle progressed. This gradual increase to a maximum of 4.76 (± 0.31) $\mu\text{M NH}_4^+\text{-N l}^{-1}$ was observed in non-aerated conditions and

was responsible for 72% variation in nitrite concentration ($r=0.84$; $p<0.001$). Under oxygenated conditions, ammonium level of up to $0.15 (\pm 0.06) \mu\text{M NH}_4^+\text{-N l}^{-1}$ could be recorded at the end of the culture cycle.

Nitrite concentrations in the pond were generally low throughout the cycle in the aerated pond (Fig. 3c). In the non-aerated pond, a maximum of $3.42 (\pm 0.04) \mu\text{M NO}_2^-\text{-N l}^{-1}$ was recorded towards the end of the culture period coinciding with the ammonium maxima. Though an initial value of $4.72 (\pm 0.23) \mu\text{M NO}_3^-\text{-N l}^{-1}$ was measured at the start of the cycle in the aerated pond, only $0.30 (\pm 0.03) \mu\text{M NO}_3^-\text{-N l}^{-1}$ could be detected towards the end. The variation in nitrite brought about a variation in nitrate concentration up to 43% ($r=0.65$, $p<0.05$). In the non-aerated pond, nitrate concentrations varied between $0.33\text{-}0.98 (\pm 0.23) \mu\text{M NO}_3^-\text{-N l}^{-1}$ (Fig. 3d).

Hydrogen sulfide concentration in both the ponds peaked on the 45th day of the culture period (Fig. 3e) followed by a steady decrease. Towards the end of the culture period (120 days), a significant increase in its concentration to $1.26 (\pm 0.08) \mu\text{g l}^{-1}$ was observed in the aerated pond than in the adjacent non-aerated pond.

Bacterial abundance

Total bacterial counts in both the ponds varied by an order ranging between 10^{5-6} cells ml^{-1} (Fig. 4a). The culturable bacterial abundance in the pond followed the following order: heterotrophs > nitrifiers and denitrifiers > sulfate reducers (Table 2). The heterotrophic population (Fig. 4b) in both the ponds were generally stable increasing gradually from 10^3 CFU ml^{-1} at the start of the culture period to reach 10^4 CFU ml^{-1} . The heterotrophs were found to negatively influence the variation in phosphate concentration ($r= -0.66$, $p<0.05$) in the culture ponds by up to 44%.

Among the bacteria capable of inorganic nitrogen cycling, the nitrifiers (Fig. 4c) were generally higher than the denitrifiers (Fig. 4d) and were most abundant in the aerated pond water towards the end of the observation period where they reached a maximum of 0.76×10^3 CFU ml^{-1} . In the non-aerated pond, the nitrifiers were maximum during the initial days of culture at 0.89×10^3 CFU ml^{-1} and a steady decrease in their abundance followed thereafter. A negative relationship of heterotrophs with nitrifiers ($r= -0.66$, $p<0.01$) in non-aerated conditions was also observed. In contrast to nitrifiers, abundance of denitrifiers in the ponds decreased over time ranging between $0.005\text{-}0.12 \times 10^3$ CFU ml^{-1} and $0.001\text{-}0.067 \times 10^3$ CFU ml^{-1} in the aerated and non-aerated pond

respectively. About 42% ($r=0.65$, $p<0.05$) of the total bacteria were found to relate positively to TDLO in the non-aerated pond.

A decrease in the population of sulfate reducers over time was observed in the non-aerated pond. Similar decrease in their numbers with time was recorded in the aerated pond with the maximum SRB abundance (Fig. 4e) of 0.025×10^3 CFU ml⁻¹ on the 30th day of the culture period. The SRB abundance remained low and hydrogen sulfide content in the aerated pond increased towards the end of the culture period.

Discussion and conclusions

Water quality is an important factor that influences the growth of cultured shrimps. Aeration of shrimp culture ponds has been effectively used to maintain good water quality conditions (Martinez-Cordova 1998). Nitrogen (N) plays a key role in the dynamics of aquaculture systems due to its dual role in various forms as a nutrient and toxicant (Burford and Lorenzen 2004). Heterotrophs mineralize a major portion of organic matter in the ponds to produce ammonium. In addition to high feed loading, shrimp excretion can also contribute to high ammonia concentrations (Shan and Obbard 2001). Kuenen and Robertson (1994) suggest that some bacteria are able to convert NH₄⁺ and other reduced nitrogen compounds in combined nitrification/denitrification processes. As microbes primarily mediate nitrogen cycling, they could be significant in mitigating elevated inorganic nitrogenous compound from accumulating to toxic levels. Ammonia-N concentrations as high as 0.283-1.714 mg l⁻¹ have been reported by Rao and Karunasagar (2000) in aerated shrimp culture ponds situated on the southwest coast of India. Comparative analyses between a lower shrimp density non-aerated pond and an aerated culture pond with a higher shrimp loading in the present study has indicated that toxic ions like ammonium were within the safe levels of <1 mg l⁻¹ recommended for shrimp culture (MPEDA 1992) in the latter.

Among bacteria involved in nitrogen cycling in the present study, the ammonium oxidizers were found to be generally high in aerated conditions. Ammonium oxidizers are usually sensitive to sudden change in temperature, reduction in available nutrients and H₂S (Joye and Hollibaugh 1995). They also require an optimum temperature between 20 and 30°C (Foch and Verstate 1977). Most importantly, nitrification rates are dependent on ambient dissolved oxygen concentrations. Gunderson and Mountain (1973) found that nitrification rate decreased if dissolved oxygen concentration was less than 0.3 mg L⁻¹. In

addition, the optimal pH for nitrification is 7-7.5 (Wong Chong and Loehr 1975). Cheng and Liu (2001) have shown that aeration created favorable conditions for nitrifying bacteria at dissolved oxygen concentrations of 4–6 mg L⁻¹ and an oxidation-reduction potential ranging between 80–100 mV. In the present study, the ponds were well oxygenated (2.93-4.06 mg l⁻¹) with Eh ranging between 63-113 mV. In addition, no significant variation in pH was observed in the culture ponds. The study therefore indicates that optimal conditions prevalent in the aerated pond were favourable for nitrifier growth and consequently the nitrification process. Continuous monitoring at definite intervals (hours) showed that NH₄⁺, NO₂⁻ and NO₃⁻ levels were always within permissible limits due to dynamic cycling. Fortnightly intervals generally showed more marked variation. Both bacterial counts and chemical parameters like NH₄⁺, NO₂⁻ and NO₃⁻ clearly suggest that the bacterial processes (oxidation and reduction) could be responsible for maintaining the level of these parameters within desirable limits. The low levels of nitrogen compounds in the aerated pond especially towards the end of the observation period are only indicative of optimal conditions favourable for nitrifier growth and consequently the nitrification process. Besides, HOBAS technology has been shown to display a better utilization of nutrients (Drengstig et al. 2004). Experimental studies done elsewhere for example in constructed wetlands treating domestic sewage has revealed high nitrification rates in aerated systems (Cottingham et al. 1999). Studies by Mota et al. (2005) to assess the effects of aeration cycles on nitrifiers and nitrogen removal in intermittently aerated reactors have shown that under non-aerated conditions (1-4 hours duration) nitrification occurs only partially followed by denitrification.

In the present study, elevated ammonium concentration in the non-aerated pond were accompanied by a nitrite maxima and low nitrate concentration. Further, the positive relationship between ammonium and nitrite in the non-aerated pond could be suggestive of higher ammonium generation rates through organic matter decomposition and consequent nitrite production through nitrification/denitrification. The marginally lower nitrate values in the non-aerated pond observed between 45 – 105 days of culture could be indicative of higher denitrification activity. A positive correlation ($r=0.65$, $p<0.05$) between the total bacteria and denitrifiers is suggestive that the microbes in the system could be participating in the denitrification process. An inverse relationship observed between heterotrophs and nitrifiers could be indicative of higher heterotrophic abundance constraining the oxygen requirement of nitrifiers in non-aerated conditions.

Cottingham et al. (1999) suggest that increased competition for available oxygen between heterotrophic bacteria and nitrifiers results in lower rates of nitrification but more efficient denitrification. Aeration using devices like blowers and paddle wheels have been shown to be favorable for shrimp growth and health (Martinez-Cordova 1998; Calle 2003). Though phytoplankton uptake dominates total ammonium nitrogen transformations under normal pond conditions (Lorenzen 1999), Antony and Philip (2006) state that bacteriological nitrification is the most practical method for the removal of ammonium from closed aquaculture systems. Shan and Obbard (2001) have demonstrated that cultures of indigenous nitrifying bacteria enriched from intensive prawn aquaculture pond water were capable of removing total ammoniacal nitrogen up to 200 mg l⁻¹. In the present study, although the aerated pond bears a higher stocking density of 12 PL m⁻², the HOBAS aeration technology promoted higher abundance of nitrifiers compared to the non-aerated pond. This consequently helped to maintain low concentration of the reduced nitrogenous species in the system.

Among the bacterial groups tested, the heterotrophs outnumbered the nitrifiers, denitrifiers and sulfate reducers. The heterotrophic abundance in the ponds ranged from 10³⁻⁴ CFU ml⁻¹ and were marginally higher in aerated than non-aerated waters. High heterotrophic abundance could be due to the elevated organic carbon availability in the ponds. Shrimps are poor converters of feed of which more than 50% goes as waste into the pond (Rao and Karunasagar 2000). Besides breaking down organic matter, heterotrophs are also superior competitors for phosphate (Drakare 2002) and perhaps maintain optimal levels of this nutrient. Experiments have demonstrated bacterial growth and biomass production to be limited by inorganic phosphorus (P) concentrations (Thingstad et al. 1998; Cotner et al. 2000). The inverse relationship observed between phosphate concentration and heterotrophs in the present study could be indicative of enhanced heterotrophic phosphate uptake. Roden and Edmonds (1997) have reported enhanced liberation of solid-phase PO₄⁻³ to sediment pore waters during anaerobic incubation in the presence of abundant sulfate. Such a mobilization could be minimal when aeration is sufficient.

The sulfur cycle bacteria are equally important in pond ecosystems in which high sulfide concentrations are generally observed towards the end of the culture period. The presence of sulfate reducers and sulfide oxidizers is suggestive of efficient sulfur cycling in an environment (Madrid et al. 2001). However, among the physiological groups of

bacteria enumerated in the present study, the sulfide oxidizers and sulfate reducers were relatively less in their abundance than the heterotrophs and nitrifiers ranging between 1-67 CFU ml⁻¹ and 2-25 CFU ml⁻¹ respectively. Devaraja et al. (2002) have also reported similar abundance of sulfate reducing bacteria at an average density of 2.74 ± 0.27 CFU ml⁻¹ in water of the shrimp culture ponds. However, Rao and Karunasagar (2000) have reported high numbers of sulfur oxidizers and sulfate reducers at a maximum of 10^{2-4} CFU l⁻¹ attributing their abundance to the higher availability of organic matter. Low H₂S levels within the recommended levels of <0.03 mg l⁻¹ (Paulraj et al. 1998) suggested for shrimp culture have been observed in the present study indicating that sulfide generated through SRB activity could be efficiently oxidized by TDLO community.

Though the SRB are known to be anaerobic, their presence is less deterred by oxygen levels in the water but more by non-availability of carbon (Loka Bharathi 2004). There have been several instances where anoxic processes could take place in micro niches like particles in the oxic water column or sediments (Jørgensen 1977; Schramm et al. 1999). Rao and Karunasagar (2000) have also ascribed their occurrence in both sediment and water of shrimp farms to reduced micro-niche in sediment particles suspended in water. In organically rich environments, aerobic heterotrophs consume oxygen, creating anaerobic conditions towards the center of the particles allowing SRB to flourish (Jorgensen 1977). On the other hand, *Thiobacillus denitrificans* has the ability to reduce nitrite to NO, N₂O and N₂ when sulfide is the electron donor (Aminuddin and Nicholas 1973). Kelly and Wood (2000) also affirm the ability of *T. denitrificans* to grow as a facultatively anaerobic chemolithotroph coupling the oxidation of inorganic sulfur compounds to the reduction of nitrate, nitrite and other oxidized nitrogen compounds to dinitrogen. Experiments by Schedel and Trüper (1980) have shown the activation of sulfite reductase in *T. denitrificans* during the oxidation of reduced sulfur compounds. Many heterotrophic bacteria are also known to behave like TDLO in environments where both sulfide and nitrate are available (Loka Bharathi 1989). It is possible that sulfide produced in the ponds gets immediately oxidized either chemically or biologically by sulfide oxidizers consequently utilizing available nitrate.

Nutrients added to intensive shrimp ponds can affect environmental conditions within ponds and surrounding marine environments through effluent discharge (Tookwinas and Songsangjinda 1999). Discharge of nutrient rich effluent culture systems can contribute to the eutrophication of receiving waters potentially impacting both natural

biota and local culture operations (Browdy et al. 2001). The present study suggests that aeration by the HOBAS technology in higher density shrimp pond enhanced microbial activity which effectively prevented the accumulation of ions like ammonium, nitrite and sulfide to toxic levels. The study also suggests that aeration with higher animal loading was as good as pond with lower number of animals with no aeration. The prevailing conditions helped the pond bacteria to maintain the quality of the aquatic environment and consequently contribute to the potential shrimp yield. Hence, the undesirable environmental impact due to discharge of this water into the adjoining creek at the end of the culture cycle would be minimal.

Acknowledgement

This work forms a part of the project "Environmental management of Coastal Aquaculture" funded by the Indo-Norwegian Program for Institutional Co-operation. The authors are thankful to Dr. S.R. Shetye, Director, NIO for the facilities. Sheryl is thankful to CSIR (Govt. of India) for the award of Senior Research Fellowship. This is NIO contribution No. 4520.

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Legend to tables

Table 1: Water characteristics of culture ponds

Table 2: Average bacterial numbers in aerated and non-aerated pond

Legend to figures

Fig. 1 HOBAS aerator installed in the experimental shrimp pond

Fig. 2 Positioning of HOBAS aerators in experimental pond

Figs. 3a-e Variation in chemical species in the aerated and non-aerated shrimp culture ponds

Figs. 4a-e Bacterial abundance in the aerated and non-aerated culture ponds

Table 1

Parameters	Aerated pond (n=9)			Non-aerated pond (n=9)		
	Min	Max	Average	Min	Max	Average
Temp (°C)	27.0	35.0	31.6 (±0.10)	27.00	35.00	31.50 (±0.10)
Salinity (ppt)	7.20	43.18	24.58 (±0.03)	8.10	41.89	24.60 (±0.02)
pH	7.09	8.32	7.67 (±0.06)	7.16	8.50	7.71(±0.06)
Eh (mV)	62.80	112.77	86.29 (±4.95)	54.60	94.60	72.95 (±3.43)
Dissolved oxygen (mg l ⁻¹)	2.93	4.06	3.52 (±0.15)	2.86	4.81	3.83 (±0.19)
PO ₄ -P (µM l ⁻¹)	0.13	3.97	1.78 (±0.48)	0.72	3.91	2.11 (±0.47)
NH ₄ -N (µM l ⁻¹)	0.10	1.93	0.77 (±0.19)	0.31	4.76	1.38 (±0.10)
NO ₃ -N (µM l ⁻¹)	0.30	4.72	1.08 (±0.51)	0.33	0.98	0.59 (±0.23)
NO ₂ -N (µM l ⁻¹)	0.06	0.53	0.28 (±0.06)	0.08	3.42	0.73 (±0.13)
S-S (µg l ⁻¹)	0.43	1.29	0.84 (±0.29)	0.38	1.37	0.77 (±0.24)

Table 2

	Total bacteria (Cells ml ⁻¹)	Heterotrophs (Av. CFU ml ⁻¹)	Nitrifiers (Av. CFU ml ⁻¹)	Denitrifiers (Av. CFU ml ⁻¹)	Sulfate reducing bacteria (Av. CFU ml ⁻¹)
Aerated Pond	2.80x10 ⁵ - 9.65x10 ⁶	2.087x10 ³ - 3.203x10 ⁴	0.003- 0.760x10 ³	0.005- 0.120x10 ³	0.002- 0.033x10 ³
Non-aerated Pond	3.40x10 ⁵ - 9.65x10 ⁶	1.320x10 ³ - 2.036x10 ⁴	0.007- 0.89x10 ³	0.001- 0.067x10 ³	0.003- 0.025x10 ³



Fig. 1

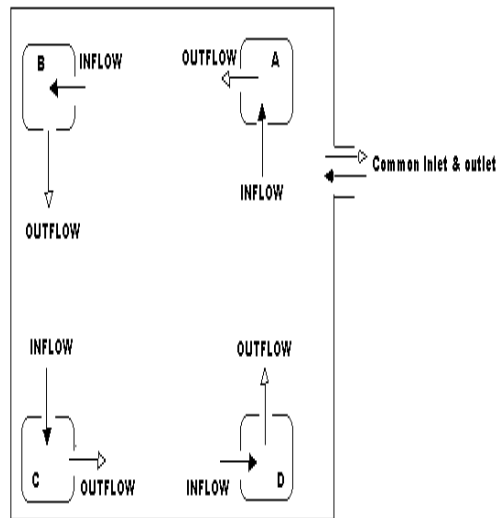


Fig. 2

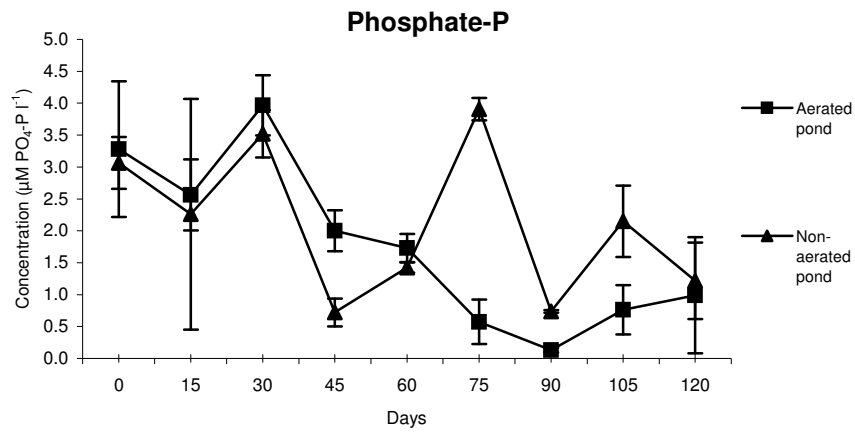


Fig. 3a

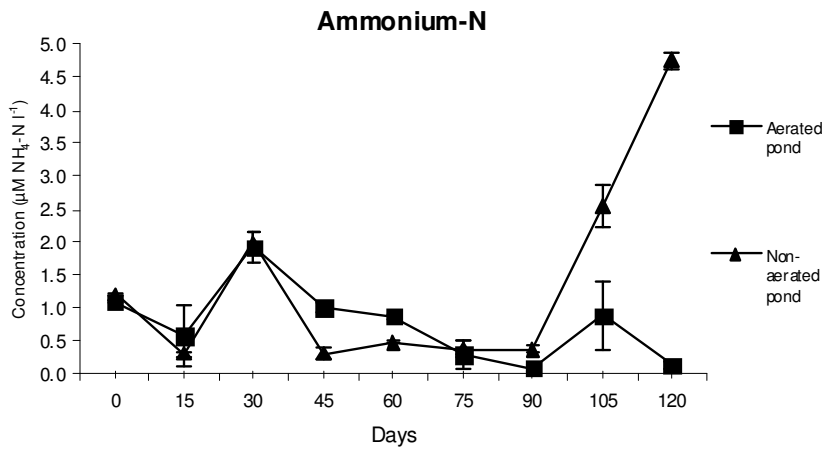


Fig. 3b

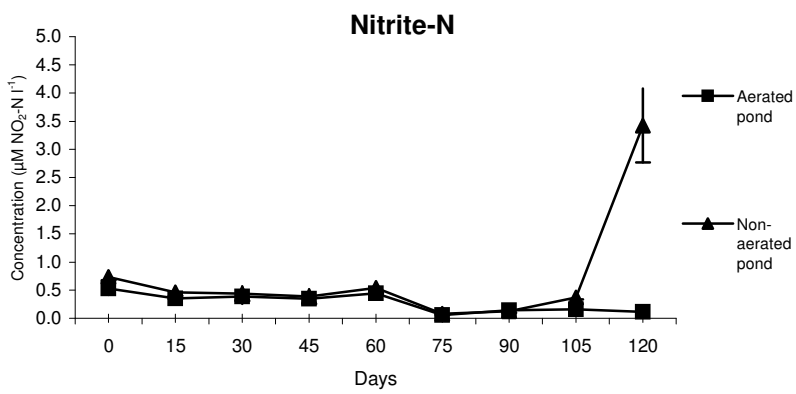


Fig. 3c

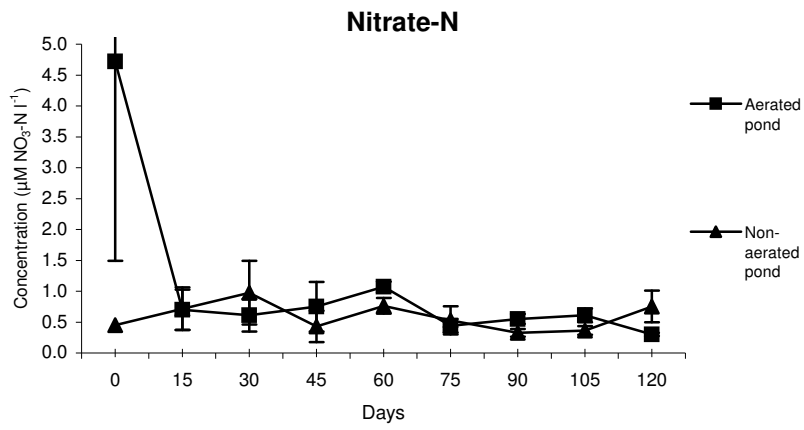


Fig. 3d

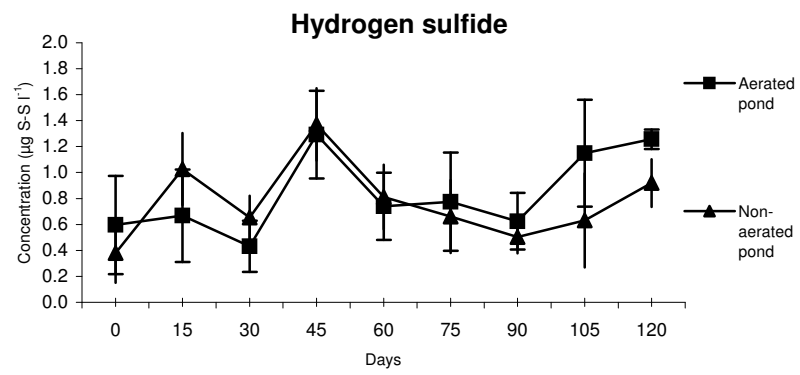


Fig. 3e

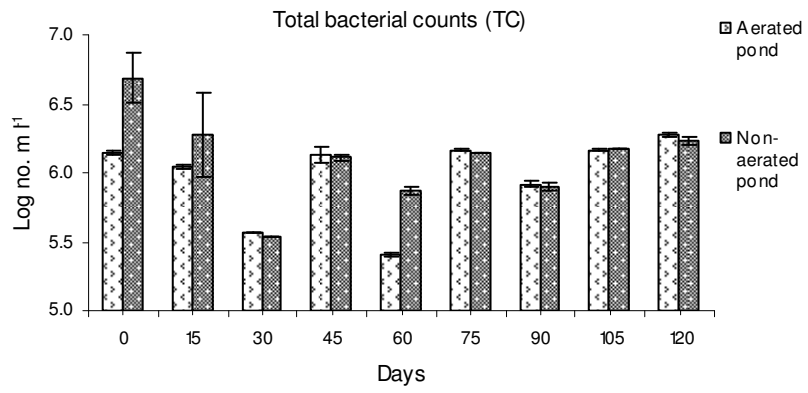


Fig. 4a

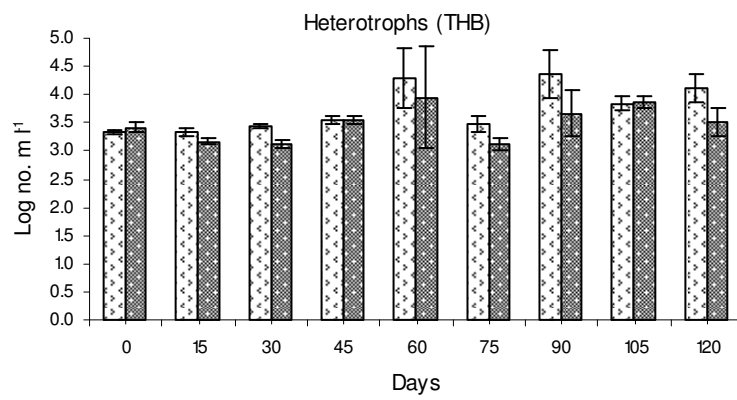


Fig. 4b

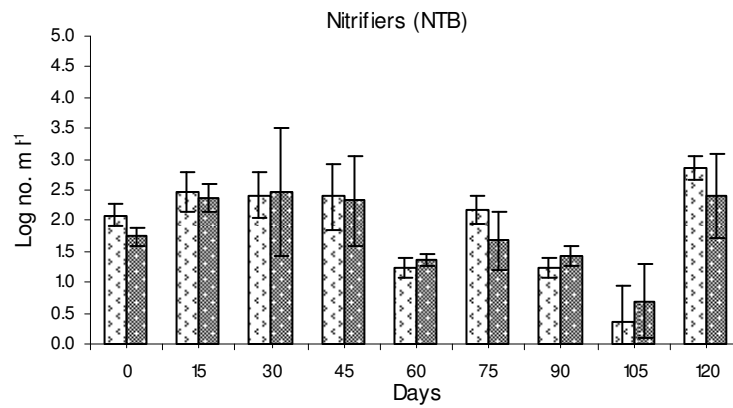


Fig. 4c

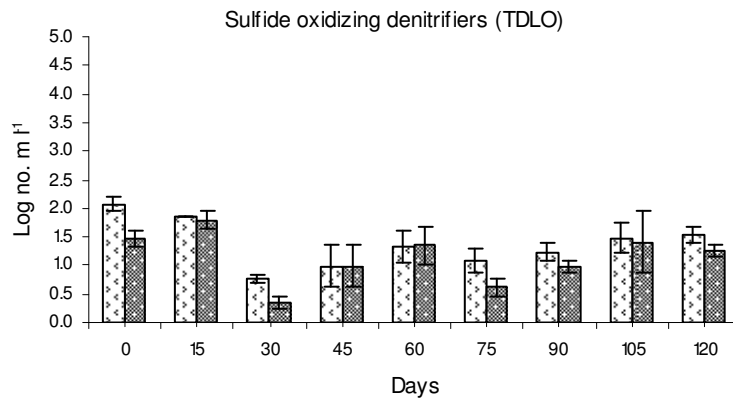


Fig. 4d

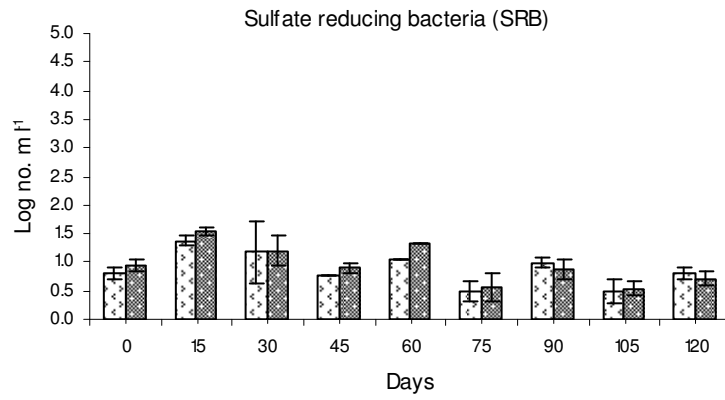


Fig. 4e