

Effects of Commercial Microbial Products on Water Quality in Tropical Shrimp Culture Ponds

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Abstract

A study was conducted to compare the efficacy of different microbial products in enhancing the water quality of commercial shrimp grow-out ponds. A total of nine ponds, with three ponds for each treatment designated as T1 (Product 1), T2 (Product 2) and control were used. Physical and chemical parameters of pond water were analyzed fortnightly throughout the culture cycle of 110 days. Pore water of the pond bottom sediment, collected on days 45 and 100 of culture, was analyzed for the same parameters. Total ammonia N, nitrite-N, nitrate-N, total nitrogen, and hydrogen sulphide increased steadily with the culture period in T1 and T2 ponds. Similarly, nutrient concentrations in the pore water increased significantly ($P < 0.05$) from the beginning to the end of the culture cycle except for the dissolved silica, which decreased significantly ($P < 0.05$) during the same period. Water transparency was highest during the initial phase of culture but gradually declined towards the end of the cycle. Total nitrogen and ammonia concentrations in T1 were lower than those in other treatments in the first two phases of the culture period. Values of all other physical and chemical parameters were not significantly different ($P > 0.05$) among treatments. In this study, the addition of commercial microbial products to shrimp ponds did not result in significant improvement of water quality over the control. However, relatively better water quality in the early phase of the culture period and higher shrimp production in T1 ponds indicated that certain microbial products have the potential to enhance pond environment and shrimp yields.

Introduction

In intensive culture systems, there is usually an accumulation of high load of organic material in the pond bottom due to uneaten feed, feces and plankton die-offs. Thus, water quality in intensive aquaculture systems is to

a large extent controlled by the microbial biodegradation of organic residues (Avnimelech et al. 1995). Microbial processes affect water quality mainly due to utilization of oxygen, regeneration of inorganic nutrients and production of toxic metabolites like ammonia, nitrite and sulphide (Moriarty 1996). Therefore, microbes are very important and have a critical role in aquaculture systems, in both the hatchery and the grow out stages, because water quality and disease control are directly related and closely affected by microbial activity (Jory 1998).

During the past decades the use of bacterial amendments has been recommended for use in aquaculture ponds. Some of the benefits of using bacterial products include the reduction of blue green algal populations thus preventing off-flavor, nitrate, nitrite, ammonia and phosphate levels, increased dissolved oxygen concentrations and promotion of organic matter decomposition (Boyd 1995). However, a reliable documentation on the positive benefits of applying bacterial products in aquaculture systems is difficult to find (Queiroz and Boyd 1998). According to Boyd (1995) bacterial amendments or bacterial extracellular enzymes, which solubilize organic matter, are unlikely to improve pond soil and water quality conditions. In addition, without the proper environmental condition, applications of bacterial amendments or enzymes neither enhance bacterial activity nor improve water quality (Boyd 1995, Shariff et al. 2001). Boyd et al. (1984) also did not find any significant effect of bacterial suspension at different dosage on the nutrient concentration in pond water. Likewise, Timmerson and Gerard (1990) reported the same observation after using two commercial bacterial suspensions tried under laboratory conditions.

On the other hand, Moriarty (1996) strongly advocates the use of microbial biotechnology in pond aquaculture. He emphasized that the successful use of probiotic to promote sustainable aquaculture greatly depends on an understanding of the nature of competition between species and strains of bacteria. Suhendra et al. (1997) supported this view as he found that routine use of commercial probiotics in a shrimp farm in West Java, resulted in reduced incidence of *Vibrio* and other viral outbreaks, enhanced environmental conditions, reduced organic matter accumulation, improved water quality and increased shrimp size and total production. In addition, Moriarty (1998) found that the use of probiotics could prevent luminescent *Vibrio* infestation by either lowering or completely eliminating luminous *Vibrio* in pond water and sediment. Furthermore, works of Rengpipat et al. (1998) showed similar results; although no obvious effect was found with regards to water quality in shrimp ponds, the effect of probiotics was reflected in the significantly higher survival of shrimp.

As in many other countries, shrimp farming in Malaysia is also threatened by various water quality and disease problems. Shrimp farmers desperate for a solution to overcome the problems use commercial probiotics as a practice in shrimp farming although there are no clear evidence on the effectiveness of the products. This study was carried out to evaluate the effectiveness of different commercial microbial products on the water quality parameters and shrimp production of *Penaeus monodon* grow-out ponds.

Materials and Methods

Location

Nine commercial *P. monodon* intensive grow-out ponds with an area of 0.5 ha each, located in Kuala Selangor, Peninsular Malaysia, 3° 17' N and 101° 17' E, were used in the experiment. Three ponds were used for microbial product 1 (T1), another three for product 2 (T2) and the rest were used as the control.

Pond management

Bottom sludge accumulated after previous cropping in all the experimental ponds was removed and washed off using a high-pressure water hose. Ponds were drained and left to dry completely under the sun for one week before ploughing was done. Ponds were limed using agricultural lime and hydrated lime at the rate of 2 t·ha⁻¹ and 1.5 t·ha⁻¹, respectively. The ponds were filled with water, treated with chlorine (40% active ingredients) and allowed to stand for at least three days with aeration before probiotic products were applied and shrimp post larvae (PL 15) were stocked at 37 post larvae·m⁻², a rate normally used in intensive shrimp farming in Malaysia. Dolomite (20 kg·ha⁻¹) and tea seed cake (100 kg·ha⁻¹) were applied in the ponds 5 days after stocking. Addition of sucrose and zeolite at the rate of 20 kg·ha⁻¹ and 6-10 kg·ha⁻¹, respectively were maintained on a regular basis (10 days intervals). A pair of paddlewheel aerators was used for the first two months working at least 4 to 12 h daily. Subsequently, it was increased to four aerators working at least 12 to 20 h daily. Shrimp were fed with commercial pellets twice a day during the first month, four times a day for the next two months and six times a day until harvest. The daily feeding rate is 6 to 10% of shrimp body weight during the first month and 4 to 5% for the rest of the culture period.

Microbial product description and application

Two commercial microbial products were selected for the study and were applied to the culture ponds according to the manufacturers' recommendations. Product 1 (T1) contained a bacterial cell density of 10⁸ cfu·ml⁻¹ with *Bacillus* sp. as the major species and 5.6 x 10⁵ cells·ml⁻¹ of *Saccharomyces* sp. It was in the form of tiny dry granules aseptically packed in air-tight bags (Devaraja et al. 2002). It was prepared by diluting 1 kg of the product into 5 l of seawater. Initial application was done a day before stocking at a rate of 8 kg·ha·week⁻¹. For 8 weeks after the first application, the product was applied at a rate of 5 kg·ha·week⁻¹. Then the rate was increased to 7 kg·ha·week⁻¹ until the end of the culture period of 110 days.

Product 2 (T2) was in liquid form, packed in plastic containers. It contained *Bacillus* sp., *Nitrosomonas* sp. and *Nitrobacter* sp. with a total cell density of 10⁸ cfu·ml⁻¹ (Devaraja et al. 2002). Stock solution was prepared by

diluting 1 l of the product in 20 l of freshwater. The stock was activated by mixing with previously boiled mixture of 2 kg rice bran, 2 kg soybean meal and 8 kg fish meal. The active solution was then aerated for 6 h before manually spreading on the pond surface at a rate of 150 l·ha⁻¹ four times a week until the end of the culture period.

Collection and analysis of samples

Water samples were collected four days after stocking and subsequently every fortnightly. All samples were taken from the middle of the ponds where the water was well mixed with the paddle wheels installed in all the corners of the ponds. Water quality parameters such as temperature, dissolved oxygen (DO), pH and salinity were measured *in situ* using the Hydro lab Surveyor 3 multimeter. Hydrogen sulphide was analyzed using the Hach Kit model DREL 2010, and water transparency using the Secchi disk. Samples for nutrient, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) analyses were taken using the Van Dorn water sampler and transported to the laboratory in an icebox.

Sediment samples collected on days 45 and 100 of the culture period, using an Ekman grab, were placed in plastic bags and kept in a portable cooler to minimize deterioration during travel. Immediately upon arrival, all the samples were extracted using a 50 ml fitted centrifuge holder at the speed of 5,000 rpm. Extracted pore water, with the exception of the samples for total nitrogen (TN) and total phosphorus (TP), were filtered through a glass fiber filter (GF/C) and 0.45 mm membrane filters before analysis. Samples were then analyzed for total ammonia nitrogen, nitrite-nitrogen, soluble reactive phosphorus (SRP) and TP following Parsons et al. (1984), nitrate and TN following Kitamura et al. (1982), and dissolved silica using modified silicomolybdate method from Hach. Unionized ammonia nitrogen values were computed according to Trussel (1972).

Water quality parameter data were analyzed based on the three different phases of the culture period: initial (0 to 34 days), mid (35 to 76 days) and final (77 to 110 days). Data were analyzed using ANOVA and Duncan's Multiple Range Test at $p < 0.05$ using the statistical analysis system package.

Results and Discussion

There were no significant differences ($P > 0.05$) in physical parameters such as temperature, DO, pH, salinity and transparency among treatments and they were all at optimum level for *P. monodon* culture (Table 1). Water temperature and pH fluctuations showed similar pattern among the treatments, being constant throughout the culture cycle. Likewise, DO fluctuations were similar among treatments, increasing from the initial phase, reaching a peak during mid phase, and decreasing towards the end of the culture cycle. Generally, DO and pH values in all the ponds never reached

critical levels (Table 1), probably due to aeration. According to Neori et al. (1989) the DO levels and to a certain extent the pH are generally kept within acceptable limits using aeration devices. Salinity values in all the treatment ponds increased as the culture progressed indicating very little water exchange during the culture period. Water transparency was highest at the beginning of culture and gradually declined towards the end of the cycle, probably due to increased phytoplankton production as indicated by the increased chlorophyll *a* concentrations (Yusoff et al. 2002).

Table 1. Water quality parameters in marine shrimp culture ponds Values are means $\pm$ standard errors. Ranges of values are given in the parentheses. Means in a row with different superscripts are significantly different at $p < 0.05$.

Parameters	T1	T2	Control	Optimum range
Temperature ($^{\circ}\text{C}$)	30.2 ^a $\pm$ 0.2 (28.5-32.0)	30.7 ^a $\pm$ 0.2 (29.4-33.3)	30.5 ^a $\pm$ 0.2 (28.9-32.9)	27.0-33.0*
Dissolved Oxygen ($\text{mg}\cdot\text{l}^{-1}$)	7.4 ^a $\pm$ 0.3 (5.2-10.2)	8.1 ^a $\pm$ 0.3 (6.0-15.0)	7.6 ^a $\pm$ 0.3 (5.6-10.9)	4.0-8.5 $\text{mg}\cdot\text{l}^{-1}$ ***
pH	7.5-8.4	7.7-8.1	7.6-8.4	7.5-9.0***
Alkalinity ($\text{mg CaCO}_3 \cdot \text{l}^{-1}$)	102.47 ^b $\pm$ 3.88 (71.30-137.20)	123.06 ^a $\pm$ 3.31 (97.20-153.20)	87.65 ^c $\pm$ 5.07 (51.80-140.70)	40-160 $\text{mg}\cdot\text{l}^{-1}$ *
Salinity (ppt)	30.1 ^a $\pm$ 0.8 (24.6-35.8)	29.5 ^a $\pm$ 0.9 (25.1-35.0)	30.2 ^a $\pm$ 0.8 (23.8-35.1)	10.0-30.0*
Transparency (cm)	30.5 ^a $\pm$ 3.0 (12.0-52.0)	29.7 ^a $\pm$ 1.3 (20.5-39.0)	28.8 ^a $\pm$ 2.0 (15.0-55.0)	30.0-40.0 cm**
Total Ammonia- N ($\text{mg}\cdot\text{l}^{-1}$)	0.286 ^a $\pm$ 0.070 (0.00-1.967)	0.317 ^a $\pm$ 0.112 (0.01-1.147)	0.185 ^a $\pm$ 0.045 (0.004-0.892)	< 0.5 $\text{mg}\cdot\text{l}^{-1}$ *
Unionised Ammonia-N ($\text{mg}\cdot\text{l}^{-1}$)	0.012 ^a $\pm$ 0.002 (0.00-0.043)	0.037 ^a $\pm$ 0.018 (0.002-0.330)	0.012 ^a $\pm$ 0.003 (0.000-0.057)	< 0.1 $\text{mg}\cdot\text{l}^{-1}$ ***
Nitrate-N ($\text{mg}\cdot\text{l}^{-1}$)	0.013 ^a $\pm$ 0.004 (0.002-0.085)	0.009 ^{ab} $\pm$ 0.002 (0.002-0.033)	0.005 ^b $\pm$ 0.001 (0.001-0.031)	< 1 $\text{mg}\cdot\text{l}^{-1}$ *
Nitrite-N ($\text{mg}\cdot\text{l}^{-1}$)	0.019 ^a $\pm$ 0.010 (0.001-0.086)	0.012 ^a $\pm$ 0.004 (0.002-0.057)	0.011 ^a $\pm$ 0.003 (0.001-0.051)	< 1 $\text{mg}\cdot\text{l}^{-1}$ *
Total-Nitrogen ($\text{mg}\cdot\text{l}^{-1}$)	0.373 ^a $\pm$ 0.086 (0.013-1.321)	0.374 ^a $\pm$ 0.073 (0.075-1.104)	0.434 ^a $\pm$ 0.080 (0.078-0.973)	N/A
Soluble Reactive Phosphorus ($\text{mg}\cdot\text{l}^{-1}$)	0.117 ^{ab} $\pm$ 0.010 (0.053-0.275)	0.138 ^a $\pm$ 0.014 (0.049-0.315)	0.104 ^b $\pm$ 0.011 (0.055-0.240)	< 3 $\text{mg}\cdot\text{l}^{-1}$ *
Total-Phosphorus ($\text{mg}\cdot\text{l}^{-1}$)	0.263 ^a $\pm$ 0.025 (0.103-0.542)	0.291 ^a $\pm$ 0.030 (0.120-0.586)	0.277 ^a $\pm$ 0.029 (0.073-0.585)	N/A
Hydrogen Sulphide ($\text{mg}\cdot\text{l}^{-1}$)	0.054 ^a $\pm$ 0.007 (0.012-0.137)	0.032 ^b $\pm$ 0.004 (0.004-0.064)	0.044 ^{ab} $\pm$ 0.007 (0.004-0.141)	< 0.1 $\text{mg}\cdot\text{l}^{-1}$ ***
Dissolved Silica ($\text{mg}\cdot\text{l}^{-1}$)	8.13 ^a $\pm$ 0.44 (5.05-12.38)	7.59 ^a $\pm$ 0.44 (4.38-12.81)	7.06 ^a $\pm$ 0.29 (5.11-9.29)	N/A
Chemical Oxygen Demand ($\text{mg}\cdot\text{l}^{-1}$)	870.31 ^a $\pm$ 83.59 (102.0-1228.5)	852.20 ^a $\pm$ 88.95 (107.0-1274.0)	866.25 ^a $\pm$ 84.71 (108.0-1238.0)	N/A
BOD ₅ (25 $^{\circ}\text{C}$) ($\text{mg}\cdot\text{l}^{-1}$)	16.54 ^a $\pm$ 2.53 (1.68 - 40.41)	20.22 ^a $\pm$ 3.30 (2.65 - 46.40)	22.34 ^a $\pm$ 4.37 (2.40 - 76.19)	N/A
Chlorophyll <i>a</i> ($\text{mg}\cdot\text{m}^{-3}$)	105.19 ^a $\pm$ 19.25 (5.66 - 388.59)	89.511 ^a $\pm$ 15.61 (7.32 - 299.84)	121.80 ^a $\pm$ 20.63 (10.15 - 358.98)	N/A

Sources:

*Fast and Lester, 1992

**Abesamis, 1989

***Tsai, 1989

N/A = not available

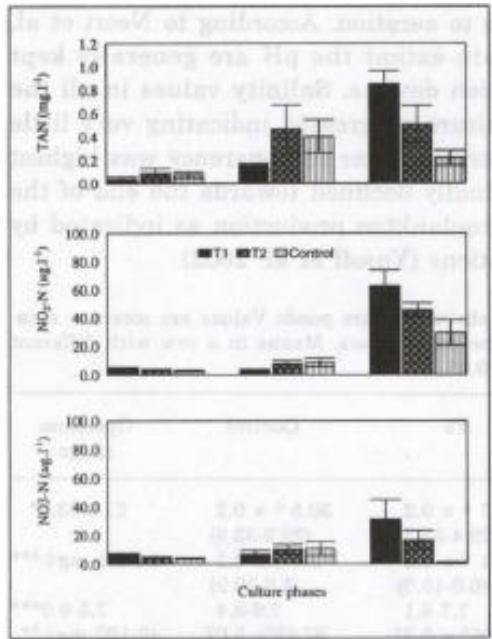


Fig. 1. Concentrations of different forms of nitrogen in the initial phase (0 to 34 days), mid phase (35 to 76 days) and final phase (77 to 110 days) of culture in marine shrimp ponds. Vertical bar indicates standard error of the mean. TAN = Total Ammonia Nitrogen

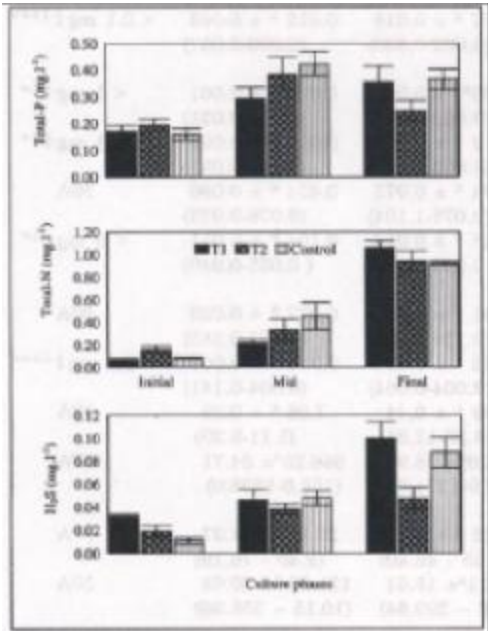


Fig. 2. Concentrations of some important chemical parameters in the initial phase (0 to 34 days), mid phase (35 to 76 days) and final phase (77 to 110 days) of culture in marine shrimp ponds. Vertical bar indicates standard error of the mean.

There were no significant differences in the concentrations of ammonia, nitrate, nitrite and TN during the initial phase among treatments (Figs. 1 and 2). During the mid phase, total and unionized ammonia in T1 were significantly lower ($P < 0.05$) than T2 and the control. However, in the final phase, total ammonia concentration in T1 was the highest compared to other treatments (Fig. 1) probably due to higher shrimp production and thus higher feeding rate and excretory product in the former (Devaraja et al. 2002). Significantly lower ($P < 0.05$) concentrations of total and unionized ammonia, nitrate and nitrite concentrations during the final phase were detected in control ponds compared to T1. Lower concentrations of inorganic nitrogen in the control ponds were probably due to relatively higher phytoplankton populations in this treatment (Yusoff et al. 2002). According to Krom et al. (1989), the regulation of nutrients in aquaculture ponds is mediated primarily by phytoplankton. Hargreaves (1998) reported that ammonia is the preferred nitrogen substrate for phytoplankton. In this study, nitrate-N concentrations were relatively low, especially in T2 and the control, probably indicating that it was a preferred form of nitrogen for some phytoplankton species. In addition, most of the nitrogen was accumulated in the sediment

as evident in the high concentrations detected in the pore water on day 100 (Table 2). According to Lorenzen et al. (1997), 48 to 66% of nitrogen would settle to the pond bottom. In fact in this study, 71 to 77% of total nitrogen of all the ponds settled into the sediment (Table 2).

The SRP concentrations in all treatments did not show any significant difference throughout the culture period (Table 1), indicating that it was steadily recycled between the water and the phytoplankton. Thus, TP is a better indicator of enrichment than SRP (Wetzel 1983). TP increased significantly ($P < 0.05$) during the midphase in all treatments, and TP in T1 was significantly lower ($P < 0.05$) than the control during this period (Fig. 2). In the final phase, TP in T2 and control decreased probably due to higher consumption by the blue-green algae density and lower phosphorus input than T1, which had a higher shrimp survival and production. According to Krom et al. (1985) feed is a relatively more important source of phosphorus to the pond than it is for nitrogen. In addition, a significant amount of phosphorus in all ponds was adsorbed into the bottom sediment. The accumulation of TP in the sediment pore water increased to 45%, 41% and 16% for T1, T2 and control, respectively on day 100 (Table 2). Krom and Berner (1980) and Boyd and Musig (1981) reported that phosphorus that is lost from the water must have been absorbed by phytoplankton, bacteria, macrophytes, or lost through seepage and evaporation or deposited in the sediments.

Dissolved silica concentration in the pond water was relatively constant throughout the culture cycle, indicating that there was no shortage of silica in the water column for diatom growth. Relatively high concentrations of dissolved silica ranging from 102.22 $\text{mg}\cdot\text{l}^{-1}$ to 108.44 $\text{mg}\cdot\text{l}^{-1}$ were detected in the sediment pore water on day 45 (Table 2). Significant decrease ($P < 0.05$) of silica concentrations in the pore water after 100 days of culture (Table 2) probably indicated that replenishment of silica in the water might have

Table 2. Concentrations of nutrients in pore water from sediment of marine shrimp ponds sampled at 45 and 100 days. Values are means and standard errors. Means in a row with different superscripts are significantly different at $p < 0.05$.

Parameters	Day 45			Day 100		
	T1	T2	Control	T1	T2	Control
TAN (mg/l)	8.62 ^c ± 2.40	17.11 ^b ± 3.15	8.67 ^c ± 1.03	31.22 ^a ± 0.64	29.98 ^a ± 0.18	28.46 ^a ± 0.80
NO ₂ -N (mg/l)	0.018 ^a ± 0.00	0.14 ^a ± 0.01	0.016 ^a ± 0.00	0.006 ^a ± 0.00	0.011 ^a ± 0.05	0.013 ^a ± 0.00
NO ₃ -N(mg/l)	0.10 ^c ± 0.00	0.07 ^d ± 0.01	0.08 ^{cd} ± 0.01	12.17 ^a ± 0.00	12.14 ^b ± 0.01	12.15 ^{ab} ± 0.01
T-N(mg/l)	49.67 ^c ± 0.71	50.89 ^c ± 1.40	44.00 ^d ± 0.16	64.44 ^{ab} ± 3.42	69.06 ^a ± 1.41	62.17 ^b ± 0.16
SRP(mg/l)	8.38 ^a ± 0.52	8.16 ^a ± 0.46	3.01 ^b ± 0.51	6.53 ^a ± 0.42	7.82 ^a ± 1.41	8.14 ^a ± 0.16
T-P(mg/l)	10.20 ^c ± 0.71	11.42 ^c ± 1.41	4.53 ^d ± 0.16	22.47 ^b ± 0.51	27.63 ^a ± 0.46	27.85 ^a ± 0.52
Dissolved Silica (mg/l)	104.06 ^a ± 4.40	108.44 ^a ± 5.70	102.22 ^a ± 1.00	67.62 ^b ± 1.75	75.33 ^b ± 5.70	69.12 ^b ± 1.00

come from the sediment. Conley and Schelske (1989) reported that benthos could disturb the sediment surface and increase the dissolution of dissolved silica into the overlying water.

Hydrogen sulphide concentration in T1 treatment was significantly higher ($P < 0.05$) than other ponds in the initial phase (Fig. 2). Although the hydrogen sulphide steadily increased in T1 and control ponds, there were no significant differences in the later periods, indicating that accumulation of organic loads during the culture period in the control ponds was higher than in T1. Generally, hydrogen sulphide is toxic to most cultured organisms, however, the hydrogen sulphide concentrations observed in this study were still within the safe level for shrimp (Table 1) (Tsai 1989).

Total alkalinity values ($\text{mg CaCO}_3 \cdot \text{l}^{-1}$) in all ponds were within the optimum range for shrimp culture (Table 1). Significantly higher ($P < 0.05$) alkalinity in T1 and T2 ponds than in the control during the initial and the final phases indicated that the former had higher carbon source and buffering capacity than the later. According to Jarrett et al. (1993), high primary production would lead to decline in alkalinity. Thus, maintenance of optimum alkalinity through liming is very important since calcium is also crucial for shrimp moulting and growth (Tseng 1987). In addition, CaCO_3 is used to maintain the buffering capacity of the water (Tsai 1989). In this study, all the alkalinity values seemed to favor the moulting as manifested in the considerably high daily growth rate in shrimp in all treatments. The mean shrimp growth rates for T1, T2 and the control were 0.20, 0.18 and 0.18 $\text{g} \cdot \text{day}^{-1}$ respectively. Allan (1989, in Allan and Maguire 1992) reported that the growth rate of *P. monodon* in a commercial farm in Australia was 0.17 $\text{g} \cdot \text{day}^{-1}$.

In general, nutrients steadily increased throughout the culture period, both in the water and in the sediment, resulting in decrease in water transparency and increase in chemical and biochemical oxygen demand and chlorophyll *a*. However, DO only showed a slight decrease at the end of the culture cycle, probably due to the aeration of the pond water. Although there were no significant differences in the average values of most water quality parameters among treatments, total nitrogen and ammonia were lower than the other treatments in the first two phases of the culture period. In a parallel study, Yusoff et al (2002) reported that blue-green algae were significantly higher in the control than in T1, and Devaraja et al (2002) showed that sediment of T1 ponds had a significantly higher ($P < 0.05$) number of total plate count and presumptive sulphur oxidizing bacteria compared to other treatments. In addition, the feed conversion ratio (FCR) in T1 (1.55) was significantly lower than those in T2 (1.72) and the control (1.73), resulting in higher shrimp production in T1 (5837 $\text{kg} \cdot \text{ha}^{-1}$) compared to 4877 $\text{kg} \cdot \text{ha}^{-1}$ and 5102 $\text{kg} \cdot \text{ha}^{-1}$ in T2 and the control, respectively (Devaraja et al. 2002).

Good water and sediment quality in the beginning of the culture period is essential for high shrimp growth and survival. This study showed that certain commercial microbial products could maintain good water and sediment quality, at least in the beginning of the culture period,

which in turn enhanced shrimp growth and production. Further research is needed to produce effective bioremediation products for improving water quality and shrimp yields in tropical aquaculture ponds.

Acknowledgments

The authors would like to thank the Government of Malaysia (Intensification of Research in Priority Areas, Project No. 01-02-04-165) and the International Foundation for Science (Grant No. A/2496-IF) for supporting this study. The authors are also grateful to J.W. Properties Sdn. Bhd. for the use of the experimental ponds and for providing assistance during fieldwork; Mr. E. Peralta, F. Shishehchian, H. Rezaei and Ms. M.S. Zubaidah for their technical assistance.

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