

Pond bottom management and probiotic application in extensive Tiger prawn (*Penaeus monodon*) culture on acid sulfate soil

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Abstract. The problems that are often encountered by shrimp culture in acid sulfate soil ponds are disease and degradation of the water quality. The study aimed to determine the effect of pond bottom management and probiotic application on acid sulfate soil for extensive shrimp farming. The research was conducted in Coranwali Village, South Sulawesi, Indonesia. The treatments were: A - probiotics application and pond bottom management, and B - probiotics application without pond bottom management. Each treatment was carried out in three replications. Tiger shrimp seeds were stocked with the density of 5 individuals m^{-2} . The total number of vibrio bacteria (TVB), total plate count (TPC) of sediment and water pond were determined. The soil quality parameters total N and total P were measured. The following water quality parameters were measured: pH, salinity, alkalinity, total organic matter, ammonia, nitrites, nitrates and phosphate. The survival rate, feed conversion ratio and growth of shrimp were observed after three months. The results showed that pond bottom management and probiotic application were able to decrease the total number of vibrio bacteria, to improve water quality, to increase the survival rate and production and to decrease the FCR value. The survival rate and production of shrimp in the probiotics application and pond bottom management treatment ranged between of 56.74-73% ($63.4 \pm 8.52\%$) and 383.3-465 kg ha^{-1} (an average of 436.3 kg ha^{-1}), respectively.

Key Words: pond soil remediation, tiger prawn production, water quality.

Introduction. Tiger shrimp (*Penaeus monodon*) is one of the native commodities of Indonesia, for which the cultivation development is not as fast as for vannamei shrimp (*Litopenaeus vannamei*). Tiger shrimp is promising for both domestic and export markets. The cultivation of tiger shrimp is developing continuously, because it has great potential as an Indonesian resource. However, the tiger shrimp production has decreased due to declining environmental quality, especially regarding water and pond soil in the last 20 years (Poernomo 1992; Pantjara 2014). In addition, shrimp disease, such as *Vibrio harveyi*, White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV), Infectious Hypodermal Haematopoietic Necrosis Virus (IHHNV) also contribute to this decline (Chen et al 2007; George et al 2011; Ting et al 2012). The low level of productivity in the ponds is often the main problem in the cultivation of tiger shrimp (Avnimelech & Ritvo 2003). The soil type is dependent on its physical, chemical and biological factors. Soil quality is an important factor in shrimp pond productivity, as it influences pond bottom stability, pH and salinity. Ponds for shrimp farming are dominated by organic soils and acid sulfate soils or associations of two soil types. The utilization of acid sulfate soil for shrimp production in brackish water aquaculture has occurred extensively in Sumatera, Kalimantan, Java and South Sulawesi, Indonesia. Acid sulfate soil is characterized by the presence of pyrite compounds that vary in depth at each location. This pyrite compounds can cause water acidification (soil has $pH < 3.5$) when oxidized, and can increase the solubility of iron, aluminum, and manganese and decrease phosphorus availability (Pantjara et al 2007). According to Pantjara & Mustafa (2015), one effort to increase the pond productivity is to conduct pond bottom management through soil treatment at a 10-20 cm depth, pond bottom drying, soaking after drying and flushing after soaking to reduce toxic compounds.

Many studies about pond bottom soil improvement for shrimp farming proved that the technique can improve the pond productivity primarily for water quality. If sediment in ponds becomes highly enriched with organic matter, it can become anaerobic. Ammonia, nitrite and hydrogen sulfide originating in anaerobic sediments can also be toxic to shrimp. The use of probiotics is effective in maintaining good water quality during shrimp farming (Castex et al 2010; Holzapfel et al 1998; Hunter-Cevera et al 2005). The probiotic bacteria application to maintain adequate water quality for the growth and survival of tiger shrimp can increase its production (Moriarty & Decamp 2012; Das et al 2006; Han & Zhang 2007).

Probiotics were primarily considered a disease control agent in shrimp aquaculture. In this disease control context, probiotics have been demonstrated to directly inhibit the growth and proliferation of pathogens and to stimulate the dominance of the beneficial microbiota of the host and its rearing environment (Lazado et al 2015). The application of probiotics has a significant advantage not only by being a disease control agent, but as alternatives that promote health and welfare of farmed animals in a larger scope (Lazado & Caipang 2014). Lazado et al (2015) stated that the application of probiotics could improve water quality, pathogen inhibition, growth enhancement, enzymatic contributions, and immunomodulation. The needs of probiotics in ponds are often higher than what can be found in one dosage, so it is sometimes necessary to multiply the probiotic bacteria by multiplication techniques (Gunarto & Mansyur 2009; Irianto & Austin 2002; Soundarapandian et al 2010). This study aims to determine the effect of probiotics and pond soil management on acid sulfate soil ponds for extensive shrimp farming.

Material and Method. This study was conducted from July to October 2015, in community ponds at Sipakainge Farmer Group in Coranwali Village, Tanete Subdistrict, Barru District, South Sulawesi. The experimental ponds used for the research numbered six plots, with a mean area of $10000 \pm 3051.2 \text{ m}^2$ in treatment A and $8753 \pm 1386.5 \text{ m}^2$ in treatment B. The source of the pond water came from the tertiary channels connected to the river estuary, and the water entered the ponds depending on tides. To determine the existing conditions of the ponds, measurements of soil pH were conducted. 15 soil samples (250 g from each pond) and 9 pond water samples (500 mL from each pond) were collected and analyzed. Soil and water samples were immediately placed in leak proof containers (cool boxes), and cooled with dry ice. All the samples were transported to the Research Institute for Coastal Aquaculture (RICA) Laboratory at Maros, South Sulawesi, Indonesia. The ponds used for this study have never succeeded in producing a sufficient harvest of tiger prawns from 2002 to 2014, so the fish farmers shifted to the cultivation of vannamei shrimp (*Litopenaeus vannamei*) or milkfish (*Chanos chanos*) (information obtained from the farmers).

The pond quality improvement was obtained by soil processing, pond bottom soaking and rinsing, and draining to oxidize the organic compounds and toxic materials. Soil drying was carried out for 15 days. The eradication of pests (crustaceans and wild fish) was conducted using saponins ($30\text{-}50 \text{ kg ha}^{-1}$), which were administered at the last draining. The draining of the pond was took place two times. An effort to increase the soil pH in the pond bottom was conducted through providing dolomite at a dose of 500 kg ha^{-1} and to produce natural food by applying urea fertilizer (100 kg ha^{-1}) and Super Phosphate 36 (with a P content of 36%) fertilizer 100 kg ha^{-1} .

The two treatments were: A - probiotics application and pond bottom management, and B - probiotics application without pond bottom management. Each treatment was carried out in three plots. The probiotics were applied one week before stocking tiger shrimp, at the recommended dosage. The probiotics used were *Pseudoalteromonas* spp., *Brevibacillus* spp., and *Pseudomonas* spp. bacteria. The probiotics were cultured, multiplied and then used in ponds (Hunter-Cevera et al 2005; Quigley 2010). The dosage of fermented probiotics in 1 m deep ponds is 5 L ha^{-1} (Atmomarsono et al 2013).

The fries were stocked in ponds with abundant plankton. The stocking density of juvenile tiger shrimp in the ponds was $5 \text{ individuals m}^{-2}$ ($50000 \text{ individuals ha}^{-1}$). To

reduce the stress before stocking, the shrimp seeds were first acclimatized to the temperature and salinity of the pond in plastic bags. The plastic bags were submersed so that the shrimp seeds would enter the pond by themselves. Pellet feed was administered starting after 20 days since stocking the ponds and the estimated availability of natural food in the ponds decreased. The feed was administered daily (3% of the biomass), and the dosage was increased every two weeks.

The total vibrio (TV) and total bacteria (TB) in the pond sediment and water were analyzed (Austin 1988; Buller 2004). Data was tabulated and discussed descriptively. TV was determined by counting the number of bacteria growing on the thiosulfate citrate bile sucrose (TCBS) media, and the total plate counts were determined by counting the number of bacteria growing on the tryptic soy agar (TSA) media.

The following soil quality parameters were determined: field pH of soil in water (pH_F), field pH of soil in 30% hydrogen peroxide solution (pH_{Fox}), total-N and total phosphate. The samples were dried in a quick-drying oven at 80–85°C to reach constant weight. Samples were spread out in trays with a depth of 2–3 cm to allow rapid drying. After drying, samples were mixed and sieved through a 2 mm sieve (Ahern et al 2004). Water quality was determined every 20 days, and the water quality parameters measured were pH, salinity, ammonia, total organic matter, alkalinity, nitrite, nitrate, and phosphate (APHA 2005). The survival rate, feed conversion ratio (FCR) and growth of the shrimp were determined after 3 months of rearing. Final size, survival rate, FCR and production values were analyzed using the T-Test.

Results and Discussion

Total vibrio and total bacteria. TV and TB contents in the sediment and in the water had lower values in treatment A than in treatment B (Table 1). This indicates that after pond bottom management, the soil quality was better and the application of probiotics to prevent the development of pathogenic bacteria was effective.

The TB in pond sediment fluctuated at every observation. In treatment A it had a mean value of $3.69 \times 10^6 \pm 4.82 \times 10^5$ cfu g⁻¹. It was slightly higher than that of treatment B, which had a mean of $1.78 \times 10^6 \pm 2.15 \times 10^5$ cfu g⁻¹. The mean TB in water in treatment A was lower than that of treatment B, $6.04 \times 10^4 \pm 3.63 \times 10^4$ cfu mL⁻¹ and $7.11 \times 10^4 \pm 4.26 \times 10^3$ cfu mL⁻¹, respectively.

Table 1
Total vibrio and total bacteria in sediment and pond water

Water parameter	Treatments	Measurement unit	Mean±SD
Total vibrio in sediment	A	cfu g ⁻¹	$2.05 \times 10^4 \pm 0.21 \times 10^1$
	B	cfu g ⁻¹	$3.64 \times 10^4 \pm 0.14 \times 10^1$
Total vibrio in water	A	cfu mL ⁻¹	$3.66 \times 10^2 \pm 0.39 \times 10^1$
	B	cfu mL ⁻¹	$7.07 \times 10^2 \pm 0.72 \times 10^1$
Total bacteria in sediment	A	cfu g ⁻¹	$3.69 \times 10^6 \pm 4.82 \times 10^5$
	B	cfu g ⁻¹	$1.78 \times 10^6 \pm 2.15 \times 10^5$
Total bacteria in water	A	cfu mL ⁻¹	$6.04 \times 10^4 \pm 3.63 \times 10^4$
	B	cfu mL ⁻¹	$7.11 \times 10^4 \pm 4.26 \times 10^3$

Note: A - treatment with probiotics application and pond bottom management; B - treatment with probiotics application without pond bottom management.

Soil quality. The results of the analyses confirmed that the soil at the study site was acid sulfate soil. The mean value of pH_{Fox} reached 2.67 ± 1.731 and the pH_F and pH_{Fox} difference values had a mean of 2.67 ± 1.733 . Total-N content in pond soil had an average value of 0.07 ± 0.013 mg kg⁻¹, and P₂O₅ had a mean of 38.95 ± 36.887 mg kg⁻¹. The pH

value measured directly *in situ* (pH_F) in the ponds before the experiment had a value of 7.38 in the pond for treatment A, lower than that of the ponds in treatment B (7.53). The pH_{Fox} value of the soil in treatment A, after adding peroxide (H_2O_2) reached 3.39, higher than that of the soil in treatment B (1.95). The potential acidity of pond soil calculated from the mean value of pH_F and pH_{Fox} difference in treatment B (5.59 ± 0.211) was higher than that of treatment A (3.99 ± 2.495). Pantjara et al (2010) reported that 37.6% of the productivity of ponds built on acid sulfate soils was determined by pH_F , pH_{Fox} , organic matter, sulfate, Fe^{2+} , and Al^{3+} ; 19.41% was determined by the values of total potential acidity, total sulfidic acidity and FeS_2 ; and 42.99% was influenced by other environmental factors, such as water source, water fertility and pollution, all affecting water quality.

Water quality. Water quality is the most critical factor affecting shrimp health and performance in a shrimp production system. Low water quality can be a trigger for the development of pathogens that can attack shrimp cultures.

Increased organic matter content was observed in both treatments due to heavy rains that contributed to the high turbidity of the water source. The organic matter values in the water of each plot during the study were: $87.51 \pm 1.783 \text{ mg L}^{-1}$ in A treatment, with $85.89 \pm 10.839 \text{ mg L}^{-1}$ in A1, $89.78 \pm 8.746 \text{ mg L}^{-1}$ in A2, and $86.85 \pm 7.292 \text{ mg L}^{-1}$ in A3. The organic matter in treatment B was $91.83 \pm 2.0847 \text{ mg L}^{-1}$, with $89.13 \pm 2.334 \text{ mg L}^{-1}$ in B1, $92.54 \pm 4.089 \text{ mg L}^{-1}$ in B2 and $93.83 \pm 6.487 \text{ mg L}^{-1}$ in B3 (Figure 1).

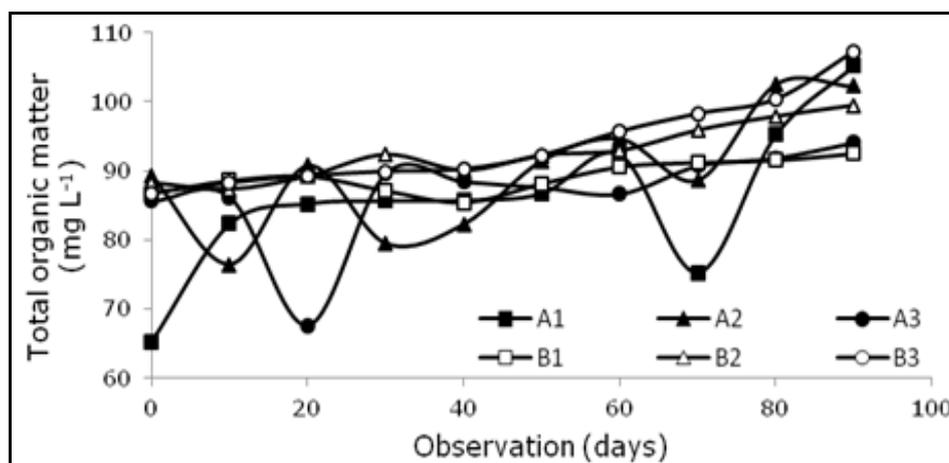


Figure 1. The fluctuation of total organic matter (mg L^{-1}) during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

Ammonia accumulates in water due to the decomposition of organic solids such as excess feed and feces. Ammonia is more toxic than nitrite, which is more toxic than nitrate (Boyd 1990; Nath & Bolte 1998). In natural conditions, ammonia is instantly diluted to safe levels by the surrounding water. Ammonia is produced from organic nitrogen through ammonification by microorganisms (Li et al 2007). Ammonia concentrations in pond water during the study are presented in Figure 2. The ammonia concentrations in the ponds were: in treatment A it was $0.247 \pm 0.0186 \text{ mg L}^{-1}$, with $0.231 \pm 0.1188 \text{ mg L}^{-1}$ in A1, $0.275 \pm 0.1098 \text{ mg L}^{-1}$ in A2 and $0.233 \pm 0.0828 \text{ mg L}^{-1}$ in A3. In treatment B it had a mean of $0.232 \pm 0.0283 \text{ mg L}^{-1}$, with $0.202 \pm 0.1209 \text{ mg L}^{-1}$ in B1, $0.304 \pm 0.1078 \text{ mg L}^{-1}$ in B2 and $0.189 \pm 0.0667 \text{ mg L}^{-1}$ in B3. During the study, the ammonia content was high, above the recommended limits for shrimp farming. Ammonia concentration in pond water should be below 0.5 mg L^{-1} . Boyd (1990) reported that the ammonia content safe for shrimp life is less than 1 mg L^{-1} . According to Pantjara et al (2007), ammonia is responsible for gill hyperplasia and lamella fusion in tiger shrimp in acid sulfate soil ponds and it restricts water flow over the gills, leading to respiratory stress.

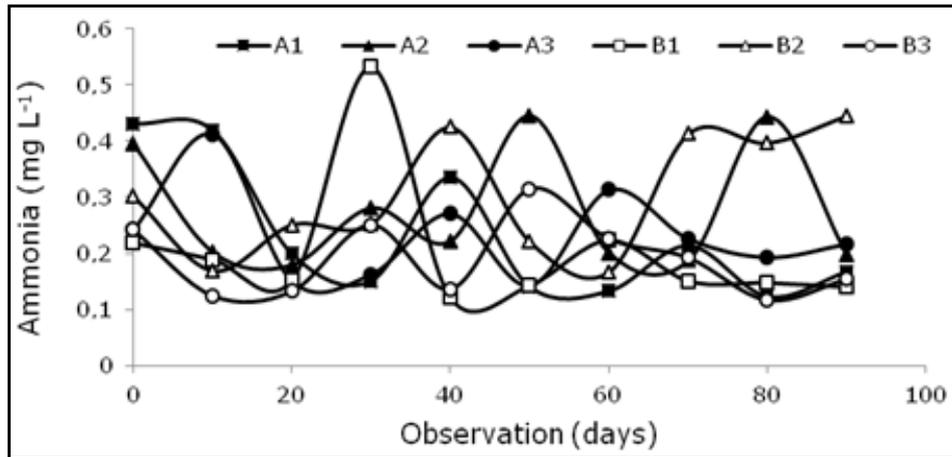


Figure 2. The fluctuation of ammonia (mg L^{-1}) during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

Nitrite toxicity has effects on the circulatory and immune systems of an aquatic organism. Boyd (1990) and Hudson et al (2003) reported that nitrite enters the bloodstream and inhibits the binding of oxygen to the iron molecule of hemoglobin. The process of nitrification carried out by microorganisms takes place in the soil and water (Choo & Tanaka 2000). The results of nitrite analysis during the study appeared to fluctuate, but tended to be similar in all treatments. The nitrite concentrations in each pond were $0.015 \pm 0.0019 \text{ mg L}^{-1}$ in treatment A, with $0.008 \pm 0.0073 \text{ mg L}^{-1}$, $0.026 \pm 0.0436 \text{ mg L}^{-1}$ and $0.008 \pm 0.0073 \text{ mg L}^{-1}$ in A1, A2 and A3, respectively, and $0.032 \pm 0.0230 \text{ mg L}^{-1}$ in treatment B, less than 0.25 mg L^{-1} and still within the tolerable range for tiger shrimp survival (Figure 3). According to Boyd (1990), the safe nitrite content for shrimp life is $<0.1 \text{ mg L}^{-1}$. At the end of the study, the nitrite concentration tended to be lower than at the beginning. The nitrification process took place because of the high content of organic matter and supporting environmental conditions for bacterial activity, especially for *Nitrosomonas* sp., which converted NH_4^+ to NO_2^- . This was due to the ammonification and nitrification processes of NH_4^+ (Moriarty & Decamp 2012). The nitrite content was $0.041 \pm 0.0634 \text{ mg L}^{-1}$ in B1, $0.035 \pm 0.0634 \text{ mg L}^{-1}$ in B2 and $0.0211 \pm 0.0230 \text{ mg L}^{-1}$ in B3.

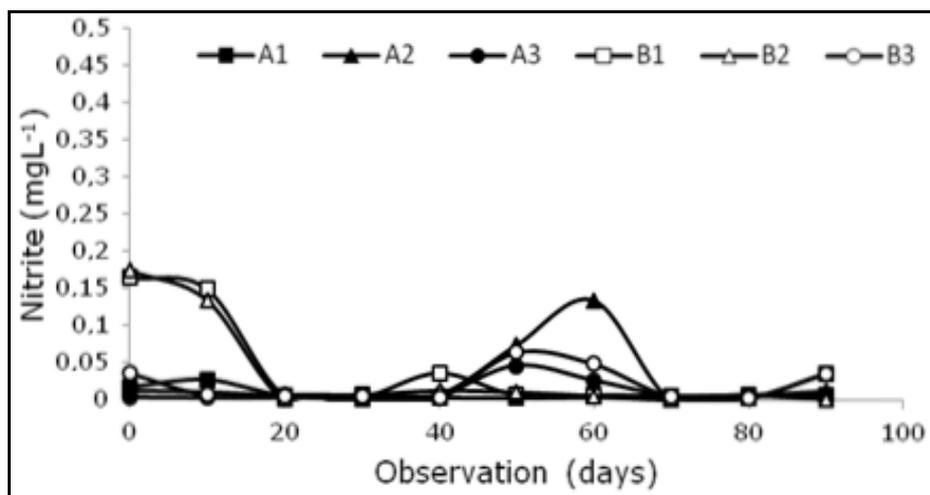


Figure 3. The fluctuation of nitrite (mg L^{-1}) during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

Nitrate concentration was similar to that of nitrite, with a tendency of lower nitrate concentrations at the end of the study (Figure 4). The nitrate concentrations in each pond were as follows: in treatment A there was a mean of 0.087 ± 0.0527 mg L⁻¹, with 0.112 ± 0.1361 mg L⁻¹, 0.190 ± 0.1321 mg L⁻¹, and 0.041 ± 0.0429 mg L⁻¹, in A1, A2 and A3, respectively. In treatment B, the mean value was 0.073 ± 0.0692 mg L⁻¹, with 0.104 ± 0.1387 mg L⁻¹ in B1, 0.095 ± 0.1379 mg L⁻¹ in B2 and 0.022 ± 0.0183 mg L⁻¹ in B3.

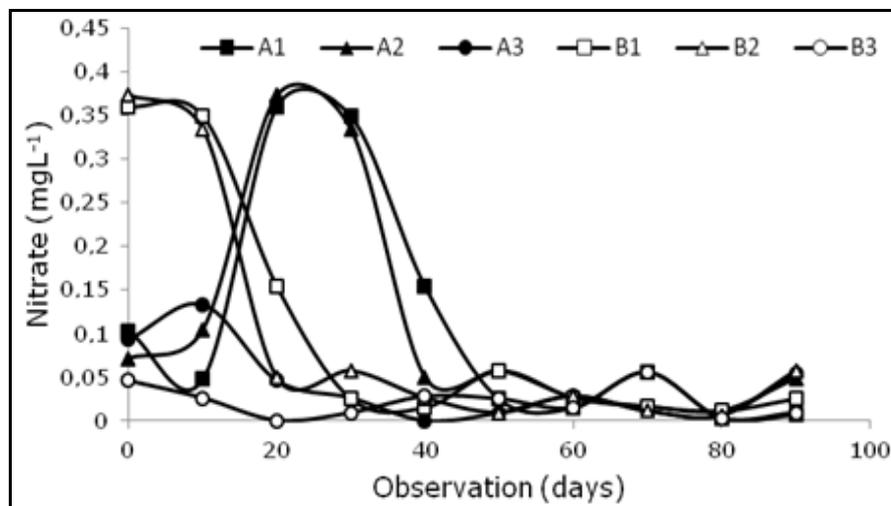


Figure 4. The fluctuation of nitrate (mg L⁻¹) during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

Other parameters such as pH, Fe²⁺, and alkalinity fluctuated, but they were still within the tolerable range for the survival of tiger prawns (Figures 5 to 7). SO₄²⁻ had high concentrations, >400 ppm (Figure 8).

The pH value in the pond water was 8.2 ± 0.06 in treatment A, with 8.2 ± 0.24 in A1, 9.3 ± 0.32 in A2, and 8.1 ± 0.34 in A3. In treatment B, the pH value was 8.3 ± 0.07 , with 8.3 ± 0.24 in B1, 8.4 ± 0.36 in B2, and 8.2 ± 0.34 in B3 (Figure 5).

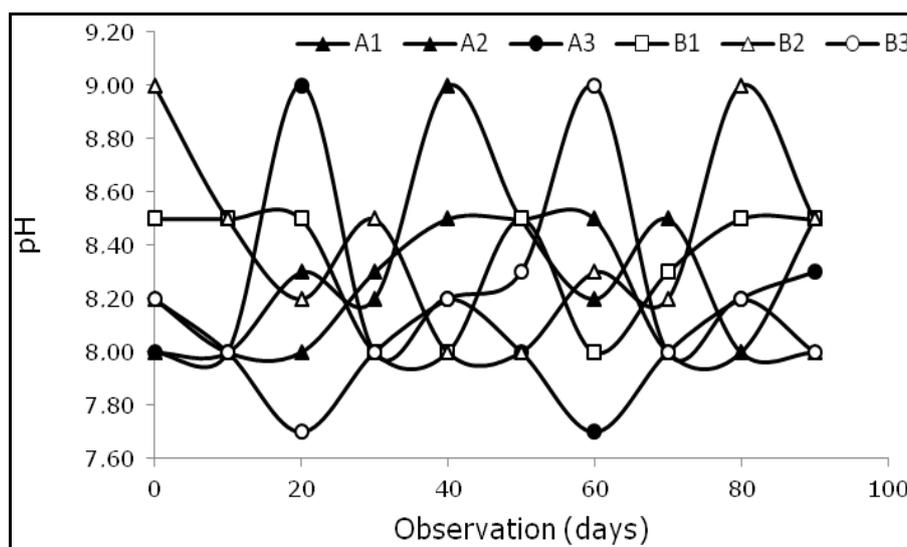


Figure 5. The fluctuation of pH during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

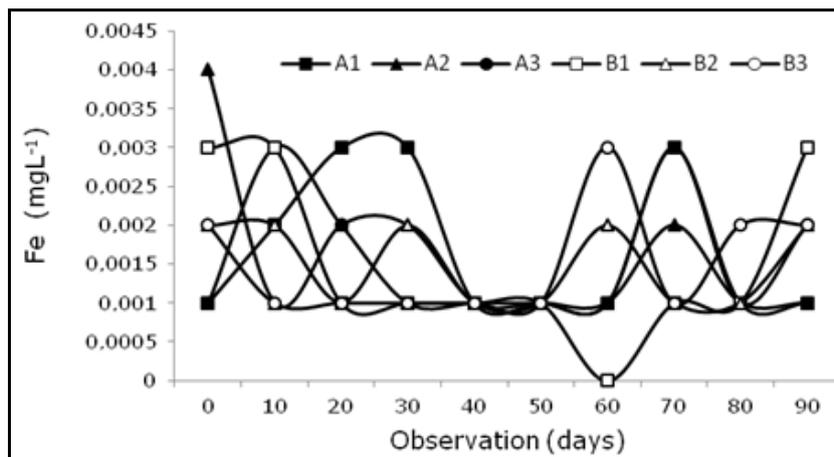


Figure 6. The fluctuation of Fe (mg L^{-1}) during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

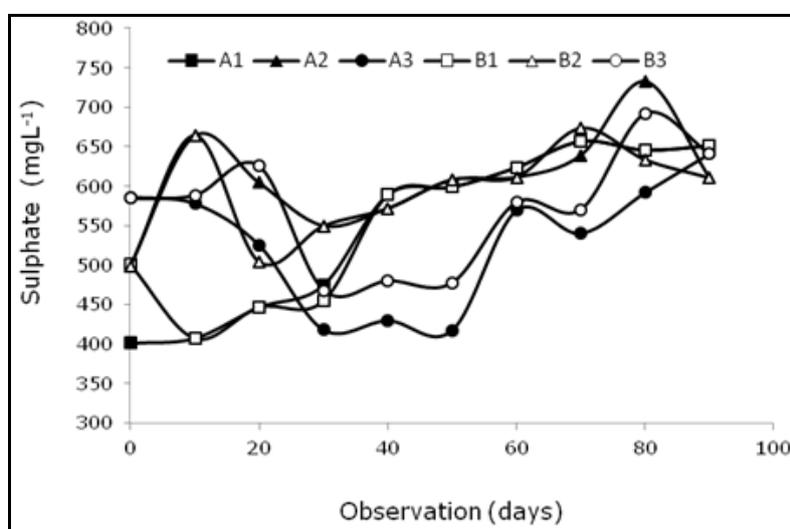


Figure 7. The fluctuation of sulphate (mg L^{-1}) during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

The alkalinity concentration of the pond water indicates its capacity to neutralize the acid or to buffer the changes in pH. Seawater has a primary source of sodium cations and magnesium, while the source of the anions is chloride. At the beginning, the pond water alkalinity was less than 100 mg L^{-1} (Figure 8). The rain probably caused the alkalinity dilution, lowering the value of salinity and alkalinity of the water and the pH. According to Boyd (1990), the process of algae photosynthesis requires CO_2 . Low water pH can also be caused by the formation of hydroxide ions due to the hydrolysis of bicarbonate and carbonate.

The water alkalinity in all experimental lots was adequate for tiger shrimp life (between $80\text{--}200 \text{ mg L}^{-1}$) (Boyd 1990). The alkalinities of pond water in all treatments were high, ranging from 86 to 160 mg L^{-1} in treatment A and from 90 to 194 mg L^{-1} in treatment B.

The water temperature ranged from $28\text{--}35^\circ\text{C}$. In this study, it seemed that the fluctuation of water temperature was influenced by the water depth, which was different in the ponds, from 30 to 50 cm. The low water depth was caused by the relatively high porosity of the pond, and, at the same time, there was no high tide, so the alimentation system did not work at maximum parameters.

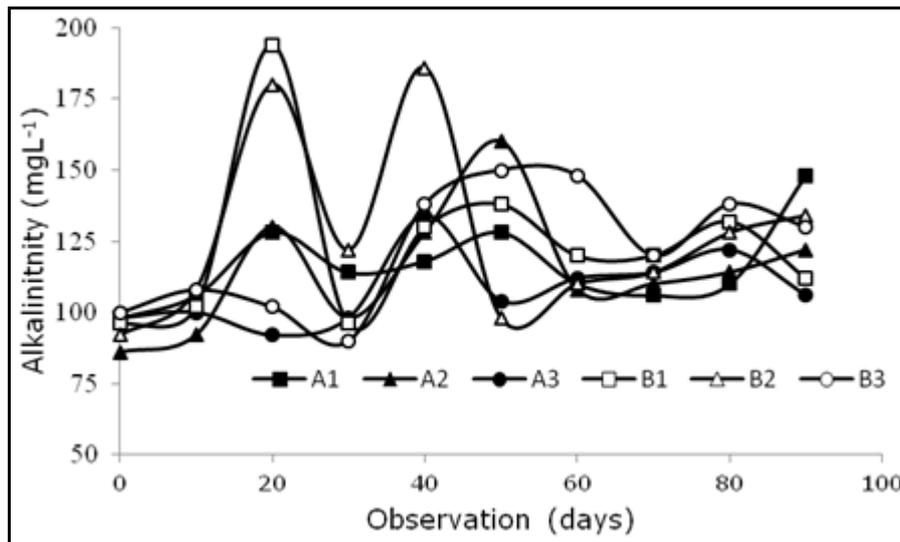


Figure 8. The fluctuation of alkalinity(mg L^{-1}) during the 90 days of observation. Treatment A - probiotics application and pond bottom management; treatment B - probiotics application without pond bottom management.

Tiger shrimp farming performance. In extensive shrimp farming, the management of pond bottom for good water quality is important. Degradation of water quality affects the shrimp growth and survival rate. Application of probiotics and pond bottom management increased the shrimp production, survival rate and decreased the FCR value (Table 2).

Table 2
The effect of probiotic application and bottom pond management on the final size, survival rate, FCR and production of tiger shrimp (*Penaeus monodon*)

Parameter	Treatment A Mean \pm SD	Treatment B Mean \pm SD
Time of rearing (days)	100 \pm 6.80	103 \pm 11.1
Initial size (g ind ⁻¹)	0.001	0.001
Final size (g ind ⁻¹)	13.54 \pm 0.85 ^a	13.57 \pm 1.02 ^a
Survival rate (%)	64.4 \pm 8.5 ^a	41.92 \pm 1.67 ^b
FCR	1 \pm 0.19 ^a	1.08 \pm 0.08 ^a
Production (kg pond ⁻¹)	436.3 \pm 45.92 ^a	249.5 \pm 8.01 ^b
(kg ha ⁻¹)	436.3 \pm 45.92 ^a	285.0 \pm 4.19 ^b

Note: different superscripts in the same row show significant differences ($P>0.05$). Treatment A - probiotics application and pond bottom management; treatment - probiotics application without pond bottom management.

The survival rate and production of tiger shrimp in treatment A ($P<0.05$) was higher than that in treatment B. The survival rate in treatment A was 64.4 \pm 8.5% and in treatment B it was 41.92 \pm 1.67%. Tiger shrimp production in treatment A was 436.3 \pm 45.92 kg ha⁻¹ and in treatment B it was 285 \pm 4.19 kg ha⁻¹. Probiotic application in the ponds was needed to increase the productivity because the bacteria was able to break down the organic carbon and to stabilize the water quality and safety for the growth and life of shrimp.

Conclusions. Application of probiotics and pond bottom management was able to decrease the total number of vibrio bacteria, improve water quality, increase the survival rate and production and decrease the FCR value of tiger shrimp. The production of tiger prawns on the treated ponds was better, with an average 436.3 kg ha⁻¹.

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