Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268 e-ISSN: 2395-3454	Vol. 16	No. 4	pp. 995-1002	2017

Original Research Paper | Open Access

Effect of Immobilized Bacteria on TAN Suppression, Survival Rate, and Biomass Harvest of Pacific White Shrimp, *Litopenaeus vannamei*, Grown in Biofloc Culture System

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 25-10-2016
Accepted: 06-01-2017

Key Words:
Ammonia
Shrimp production
Tambak system
Immobilized bacteria

ABSTRACT

In this study, immobilized bacteria (*Nitrosomonas*, *Nitrobacter* and *Nitrospira*) were added into three shrimp ponds in a concentration of 2×10^6 cfu mL^{-1} that equals to 20 kg beads ha^{-1}. The other three ponds were used as control treatment. All ponds were stocked with PL's (Post Larvae) of Pacific white shrimp at a density of 100 PL's m^{-2}. Shrimps were fed with a commercial diet containing 36% crude protein, 7% fat, 10% ash, and 2% crude fibre. Oxygen was kept near saturation through paddle-wheels. Secchi disc depth, pH and temperature were monitored daily. Total ammonia nitrogen (TAN), nitrite and nitrate were measured every 10 days together with sampling for shrimp body weight and feed adaptation. Average TAN concentration of ponds with nitrifying bacteria was 0.12±0.144 mg L^{-1}, one fourth of the control ponds (0.523±0.564 mg L^{-1}). Average nitrite concentration in control ponds (0.058±0.045 mg L^{-1}) was also significantly higher than the ponds added with immobilized bacteria (0.037±0.038 mg L^{-1}). However, the end product of nitrate was not significantly different (α = 0.607) among the ponds. Although not affecting shrimp growth, TAN suppression significantly increases the survival rate and finally resulted in higher (α = 0.043) biomass harvest, from 12.37 t ha^{-1} (control ponds) to 18.87±2.91 t ha^{-1} at treatment ponds. This study concluded the importance of transforming TAN concentration into nitrate, particularly in intensive culture system. To do this, the presence of immobile nitrifying bacteria is needed.

INTRODUCTION

The first written record on Tambaks, currently known as brackish-water pond for milkfish and/or shrimp farming in Indonesia, was believed to have its origin from Java Island (Thorburn 1982). With intensive technological jump initiated in early 1980s, north coast of Java is nowadays still the largest shrimp farming industry in the country (Hariati et al. 1995). Following a decade of great success, shrimp farming in Java was nearly collapsed in early 1992 (Hariati et al. 1996a, 1996b). Within two years, culture-based shrimp production has decreased by nearly 50%, from around 38,000 t in 1991 to 20,000 t in 1993. Pathogens induced by high accumulation of organic materials due to excessive feed and density dependent factor, were suspected to be the main cause of the collapse (Hariati et al. 1998a). Technological adaptation, from mono-culture *Penaeus monodon* into bi-culture system of *P. monodon* together with *P. merguiensis* did not result in better solutions (Hariati et al. 1998b).

Farmers realized that the expansion of shrimp culture in the region is hampered due to discharge of organic waste into the surrounding water bodies that carry pathogens. Every farm suspected that the incoming water source for the pond would carry diseases until biofloc technology (BFT) was introduced in 2002 (Taw 2005, Avnimelech 2012). Biofloc in this term is defined as a heterogeneous mixture of micro-organisms, including filamentous bacteria, protozoans, particles, colloids, organic polymers, cations and dead cells that together formed a biological flocculant (Schryver et al. 2008, Vilani et al. 2016). Cells in the floc can take nutrients from the wastewater and convert it into microbial biomass. It mainly functions as a natural feed for the shrimps, reduces the onset of pathogens, and to some extent, may stabilize water quality. Application of this new technology has significantly affected shrimp culture production (Devi & Kurup 2015), especially in East Java Province (DKP 2015). The latest production in 2015 exceeded 68,000 t, more than three times of that in 1993 (20,000 t).

Despite the increase of culture-based shrimp production occurred lately, there are still unsuccessful stories of shrimp farming in the region, especially in East Java. Some farms could manage the ponds with TAN concentration of <0.5 mg L^{-1} during the culture period and making profit (Fakhri et al., 2015). However, there are still biofloc systems with TAN concentration of >1.5 mg L^{-1} and resulted in low survival rate and less biomass harvest.

Intensive shrimp culture is characterized by excessive feed with rich protein content. This is the main source of total ammonium nitrogen (TAN) that is toxic to the shrimps. Biofloc, to some extent, can control this nitrogen source by feeding bacteria with carbohydrates, subsequently uptake the nitrogen and convert into microbial proteins (Avnimeleh 1999, Xu et al. 2016). Toxic TAN can also be converted into nitrate, which is not toxic through the work of nitrifying bacteria. A consortia of nitrifying bacteria (*Nitrobacter*, *Nitrosomonas*, *Nitrospira*) has been isolated from local tambaks in East Java (Hariati et al. 2011a) and its nitrite oxidoreductase activities were tested. However, these bacteria are unstable in the water, unless immobilized. This study was aimed to analyse the effect of three bacteria in converting TAN into final end product of nitrate within biofloc culture system.

MATERIALS AND METHODS

Immobile bacteria: Nitrifying bacteria used in the experiment were isolated in 2010 from local shrimp ponds in East Java. All bacteria were confirmed as *Nitrosomonas* (AOB), *Nitrobacter* (NOB), and *Nitrospira* (NOB) based on morphological, biochemical, and molecular (16S rRNA) examination (Hariati et al. 2011a). In-vitro test showed the effectiveness of these bacteria in reducing the total ammonia nitrogen (Hariati et al. 2011b). The bacteria were then immobilized in beads made of saw-dusks at diameter varying from 700 to 1,000 µ. The ratio among *Nitrosomonas* (AOB), *Nitrobacter* (NOB) and *Nitrospira* (NOB) in the bead was 3.0:0.5:0.5, to reach 2×10^6 cfu mL^{-1} that equal to 20 kg beads ha^{-1} (Table 1).

Pond experimental setup: The field experiment was conducted in Tuban Regency, East Java, Indonesia. The farm was chosen based on the farm experience in applying biofloc technology (BFT) and willingness of the farm owner to cooperate. In total, six ponds were assigned for the experiment, with pond areas varying between 2,500 and 3,500 m^{-2}. Three ponds were randomly selected as control, having regular BFT management system. The other three were assigned to having regular BFT system and supplemented with immobile bacteria, composed of *Nitrosomonas*, *Nitrobacter*, and *Nitrospira*. All ponds were dried out at almost the same time for 20 days, top soil was reversed for aeration and then limed at the same dose of 300 kg ha^{-1}. Water replenishment for all the ponds were taken from the same water input and completed almost at the same time (48 hr) (Table 1). Pond aeration was prepared using paddle wheels and propellers (± 70 HP ha^{-1}) that were placed at four to six different sites allowing for maximum water movement.

PL's stocking and feed: All the ponds were stocked with PostLarvae (PL10) of Pacific white shrimp, *Litopenaeus vannamei*, from one hatchery source with specific pathogen free (SPF) certificate, land transported ±290 km from the farm (Situbondo Regency of East Java). PL's density was made equal for each pond (100 individual m^{-2} that estimated based on sampling). PL's were fed three times daily with a commercial diet containing 36% crude protein, 7% fat, 10% ash, and 2% crude fibre. In the first 30 days of the culture period, no sampling on shrimp individual body weight was done. Based on farm long experience, feeding was estimated to reach nearly satiation. Starting at day 30 of the culture period, sampling was done for every 10 days to estimate shrimp individual body weight. Feeding level was then assigned at 5% of shrimp biomass per day. Total amount of feed given per day would be different based on the estimate of individual body weight at specific day of interest.

Supplementation of molasses to maintain C/N ratio: Molasses was used as the only source for carbohydrate to meet C/N ratio equal to 12:1. This ratio was calculated based on the total amount of protein in the feed given to the pond, where nitrogen source in the pond being equal to 0.16 of protein content in the feed. The liquid molasses was given every 5 days, equally spread into the pond surface.

Water quality parameters: Water temperature and pH of all ponds were measured twice a day, 6:00 a.m. and at 16:00 p.m. Secchi disc visibility and dissolved oxygen (DO) were measured once a day, Secchi being at noon (16:00 p.m.) and DO early morning. When DO reached < 4.5 mg L^{-1}, all the propellers were operated to reach an optimal oxygen concentration in the pond water. DO, temperature and pH were measured using portable DO-HI98196 (Table 2). Turbidity was measured based on Secchi disc visibility. Total ammonia nitrogen, together with nitrite (NO_2-N), and nitrate (NO_3-N) were measured every 10 days starting from day-0. The measurement followed all the Nessler and colorimetric (spectrophotometer).

Sampling for estimation of shrimp individual body weight and growth: Sampling for estimation of shrimp body weight was initiated at day 30 of culture period. Shrimps were caught using a scatter net of diameter 3 m. Total shrimp in the net was counted and weighed. Shrimp individual body weight was calculated by dividing the total shrimp weight

Table 1: Pond (characteristics and pretreatment) used in the experimental setup.

Pond Area (m^2)	Treatment	Soil aeration and lime (days; kg)	Water depth (cm)	PL's stocking (ind)	Bead (kg)
A = 2,500	BFT (control)	2 days; 75 kg	100	250,000	5
B = 3,000	BFT (control)	5 days; 90 kg	90	300,000	6
C = 3,000	BFT+bacteria	5 days; 90 kg	100	300,000	6
D = 2,500	BFT+bacteria	4 days; 75 kg	100	250,000	5
E = 3,500	BFT (control)	4 days; 105 kg	95	350,000	7
F = 3,000	BFT+bacteria	5 days; 90 kg	100	300,000	6

Table 2: Water quality parameters and methods (tools) for measurement.

Water quality parameter	Methods/Tools for measurement	Unit of measurement	Resolutions of measurements
Temperature	DO-HI98196	(p C)	0.1
pH	DO-HI98196	(pH unit)	0.01
Dissolved oxygen	DO-HI98196	(mg L^{-1})	0.01
Salinity	Refracto CD-18736	(ppt)	0.5
TAN	Spectrophotometer	(mg L^{-1})	0-2
NO_2-N	Spectrophotometer	(mg L^{-1})	0-2
NO_3-N	Spectrophotometer	(mg L^{-1})	0-2
Shrimp body weight	TANITA KD-200	(g)	0.01
Biomass harvest	OCS-L digital-100	(kg)	0.01

to its total weight (g). Shrimp growth was calculated based on specific growth rate (SGR) with the following formula:

$$SGR\ (\%\ BW\ day^{-1}) = (\ln(W_t) - \ln(W_0))/(t - t_0)$$

Where:

BW = body weight
W_t = weight (g) at day culture of t
W_0 = weight (g) at initial culture day
ln = natural logarithm 2.71282
t = culture day at time t (day)
t_0 = initial day of culture (day)

During harvest, all shrimp were weighed using OCS-L digital 100 kg (Table 2). Sampling to estimate the average individual body weight at harvest was done based on a procedure explained above. This estimate of individual body weight was, in reverse, used to calculate total shrimp at harvest through:

$$N_h = B_h / IBW_h$$

Where:

N_h = an estimate of total number of shrimp at harvest
B_h = total biomass harvest (g)
IBW_h = average individual body weight at harvest (g)

Survival Rate (SR) was then estimated based the calculation:

$$SR\ (\%) = (N_h / N_0) \times 100$$

Where:

N_0 = total number of PostLarvae (PL=10) stocked

Data analysis: Water quality such as pH and Secchi visibility were presented in a graph. TAN, nitrite and nitrate, per 10 days measurements, were also shown in a graph. Statistical analysis to test the effect of treatment (supplementation of nitrifying bacteria) was performed using SPSS v.16.0 software package. The *t*-test ($\alpha = 0.05$) was also done to examine the difference of water quality, SR, SGR and biomass harvest.

RESULTS

Farm owner decided to harvest all the ponds at day 100 of the culture period, assuming shrimp individual body weight of 25 g. This harvest strategy was influenced by market price, shrimp size, and risk assessment on the onset of shrimp diseases. All ponds used in this experiment produced in total 30.5 t of shrimp biomass within total pond's area of ±1.9 ha.

Water quality: There was no temperature and salinity differences observed among ponds and between treatments. Pond's water depth was maintained at 90 cm the lowest level and up to 100 cm. Input water was added intermittently in accordance with pH and secchi visibility. Dissolved oxygen was managed to be > 4.5 mg L^{-1} for all ponds through paddle wheel and propeller. Fluctuations of pH in the control (biofloc) ponds were relatively higher than treatment (biofloc + bacteria) ponds. In control ponds, pH varied between 6.4 to 8.0 (Fig. 1a). In the treatment ponds, pH fluctu-

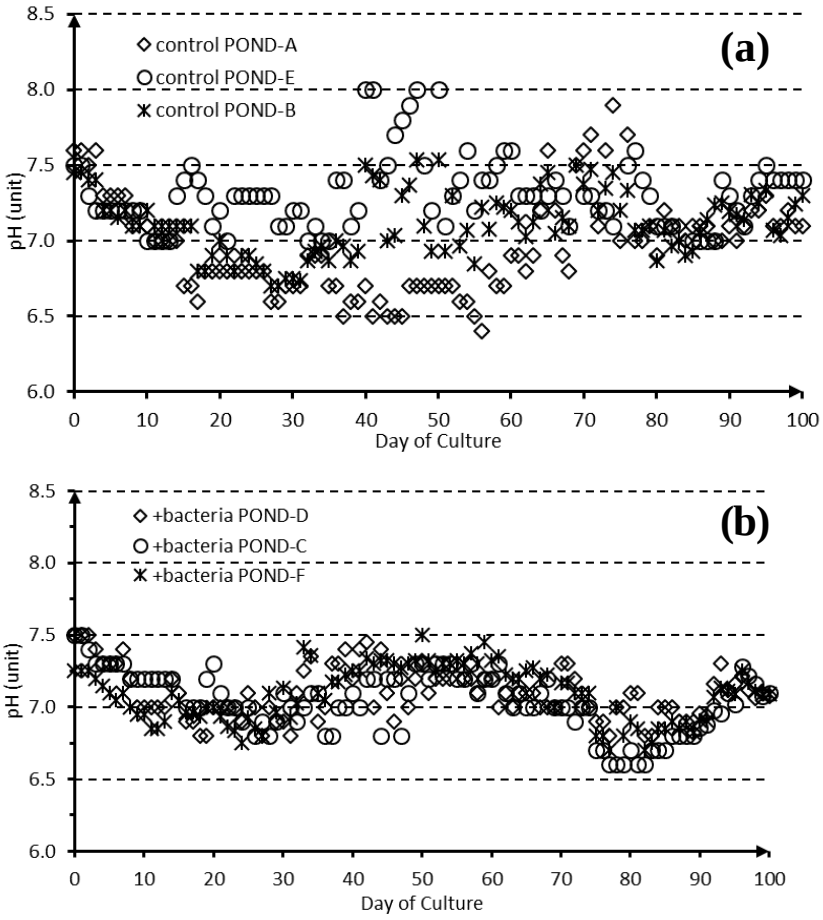

Fig. 1: Daily pH fluctuations (measured at 4:00 PM) during culture period (day 0 to 100). (a) control ponds (n=3), biofloc system without additional bacteria; (b) treatment ponds (n=3), biofloc system with immobilized nitrifying bacteria.

ated between 6.6-7.5 (Fig. 1b). Secchi disc visibility in the treatment ponds was more stabilized toward the end of the culture period. On the contrary, Secchi disc visibility in control ponds tended to increase near the end of the culture period (Fig. 2).

Total ammonia nitrogen (TAN) at ponds treated with nitrifying bacteria was kept nearly constant until the end of the culture period. For control ponds, TAN concentration tremendously increased on day 60 of the culture period. However, toward the end of the culture period, the TAN was gradually decreasing (Fig. 3a). Average TAN concentration in ponds supplemented with bacteria was significantly lower than control ponds and reached about one fourth of the control ponds.

Nitrite concentration in all the ponds tended to increase after day 60 of culture period (Fig. 3b). In the control ponds, nitrite reached its maximum concentration at day 70 of culture period (0.16 mg L^{-1}). At the end of the culture period, nitrite in all ponds decreased to nearly undetectable. Comparison of means for nitrite showed that average nitrite concentration in control ponds was significantly higher than in ponds supplemented with nitrifying bacteria ($\alpha = 0.05$). Nitrate concentration in all ponds showed almost similar patterns (Fig. 3c). The nitrate seemed to increase after the increase of nitrite. Statistically, average nitrate concentration in all ponds was not significantly different ($\alpha > 0.05$).

Shrimp growth, survival rate and biomass harvest: Starting from day 70 of the culture period, shrimp individual body weight in the ponds added with bacteria seemed to be higher than that in the control ponds (Fig. 4). However, the specific growth rate (SGR) was not statistically different ($\alpha = 0.064$) (Table 3). This means that supplemented nitrifying bacteria did not directly affect the shrimp growth.

Treating the shrimp biofloc culture with nitrifying bacteria significantly affected the shrimp survival rate (Table 3). The survival rate increased nearly 15% compared to biofloc system (control). Mean body weight at harvest of shrimp in treatment ponds, although not significant (α =

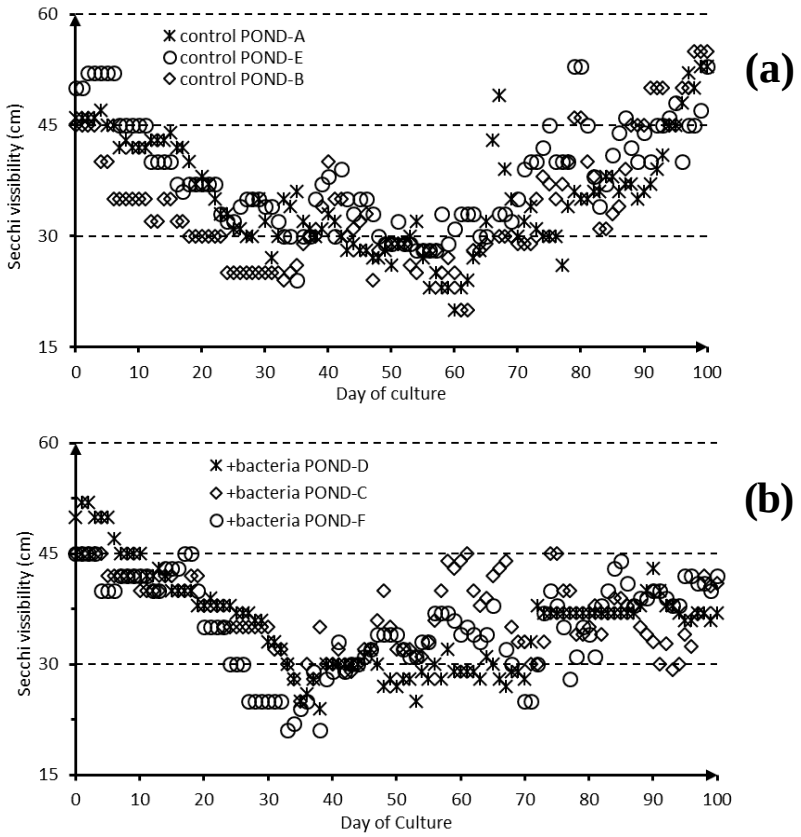

Fig. 2: Daily Secchi disc visibility fluctuations (measured at 4:00 PM) during culture period (day 0 to 100). (a) control ponds (n=3), biofloc system without additional bacteria; (b) treatment ponds (n=3), bifloc system with immobilized nitrifying bacteria.

Table 3: Shrimp final body weight (IBW), growth (SGR), survival rate (SR) and biomass harvest in each pond, and with the result of mean comparison test.

POND	IBW(g ind^{-1})	SGR (%BW day^{-1})	SR (%)	Harvest (t ha^{-1})
A (control biofloc)	19.95	2.99	55.6	11.1
E (control biofloc)	20.60	3.03	52.2	10.7
B (control biofloc)	24.80	3.21	61.5	15.3
D (biofloc + bacteria)	25.00	3.22	64.4	16.1
C (biofloc + bacteria)	33.40	3.51	65.6	21.9
F (biofloc + bacteria)	28.60	3.35	65.0	18.6
	Result of mean comparison test (α = 0.05)			
Control (biofloc)	21.8±2.63^{a}	3.08±0.12^{a}	56.4±4.7^{b}	12.4±2.51^{b}
Biofloc+bacteria	29.0±4.21^{a}	3.36±0.14^{a}	65.0±0.6^{a}	18.9±2.92^{a}

0.056), was relatively higher than that in control ponds. Survival rate and mean body weight at harvest result in significantly higher biomass harvest in ponds supplemented with immobile nitrifying bacteria (Table 3).

DISCUSSION

Water quality, such as salinity and temperature may affect shrimp growth (Fakhri et al. 2015). In this experiment, both the parameters were not significantly different in all ponds during culture period. Hence, the probable effect of the above parameters can be ignored. Although not significantly different, pH in the control ponds (biofloc) showed a higher fluctuation compared to treatment ponds (Fig. 1a). This pH fluctuation resulted in unstable biofloc population as indicated by the higher Secchi disc visibility of control ponds (Fig. 2b). Supplementing biofloc system with more nitrify-

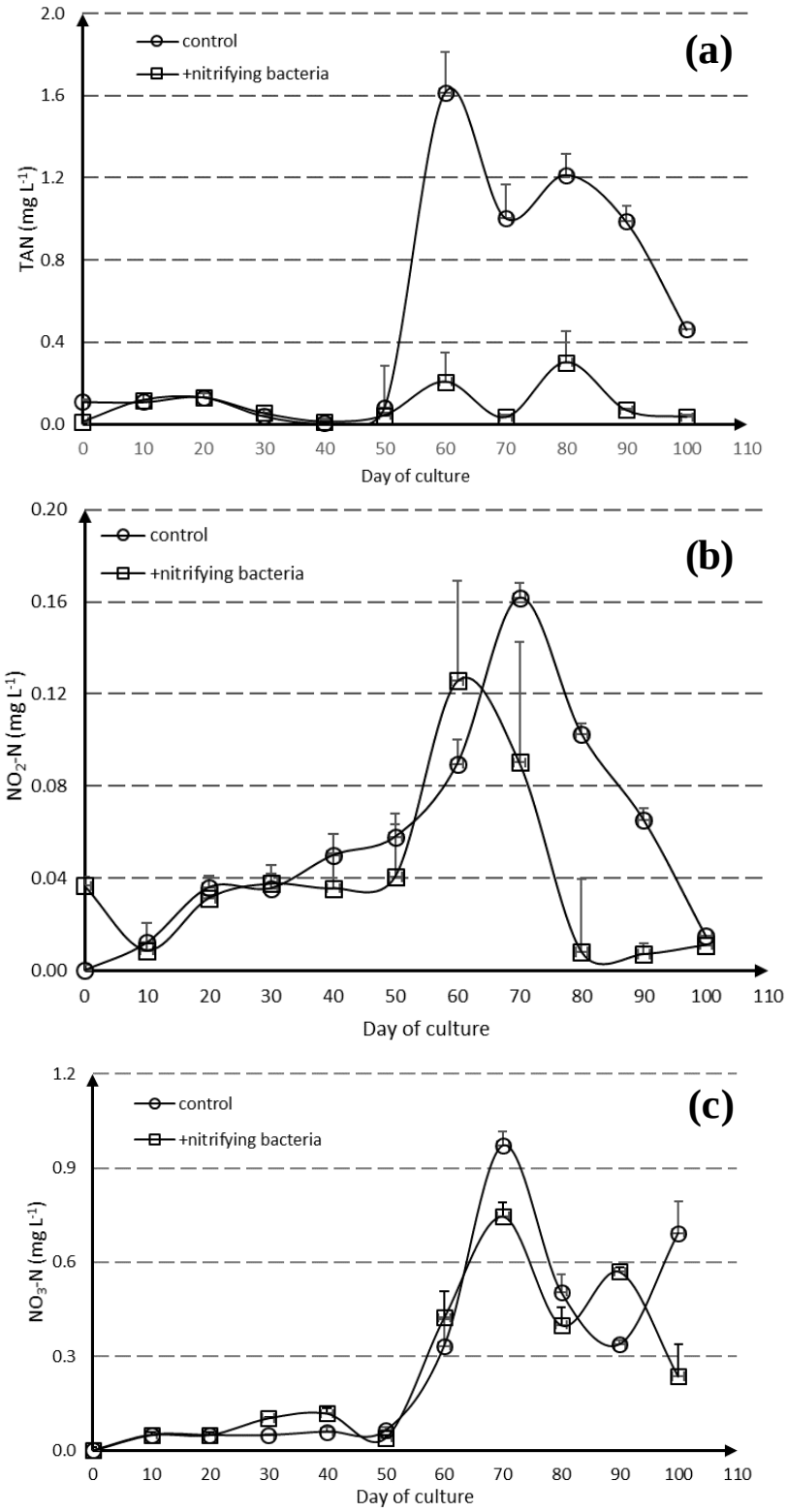

Fig. 3: Water quality fluctuation sampled every 10 days for each pond. (a) Total ammonia nitrogen (mg L^{-1}), (b) Nitrate (mg L^{-1}), (c) Nitrate (mg L^{-1}).

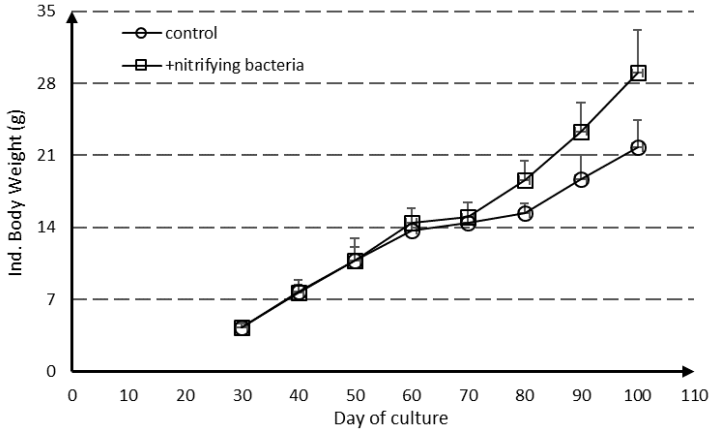

Fig. 4: Estimates of shrimp individual body weight at control (n=3) and treatment ponds based on sampling done every 10 days, initiated from 30 days of culture period.

ing bacteria will result in dominant production of HCO_3^-, instead of CO_2 (Xu et al. 2016). This will increase the alkalinity and lead to more stabilized system.

Addition of certain bacteria into the shrimp culture system, such as *Bacillus subtilis* E20 in the form of probiotic, can increase the survival rate of shrimp. This bacteria was able to compete and inhibit growth of pathogenic bacteria, especially *Vibrio* (Liu et al. 2010, Liang et al. 2014). In this study, immobile nitrifying bacteria (*Nitrosomonas*, *Nitrobacter* and *Nitrospira*) were added into a biofloc culture system to partly convert toxic TAN (through *Nitrosomonas*) into nitrite and further into nitrate (through *Nitrobacter* and *Nitrospira*). This was proven through low and stable TAN concentration in the ponds supplemented with these bacteria (Fig. 3a).

Biofloc is mainly designed to reduce the accumulation of inorganic nitrogen in the culture system (Scryver et al. 2008, Khatoon et al. 2016). It was also done in this study by feeding heterotrophic organisms with carbohydrate, and subsequently uptake of nitrogen into microbial protein. However, biofloc ability in nitrogen uptake depends on different factors (Valsamma et al. 2014, Wei at al. 2016) such as microbial conversion coefficient, microbial composition, carbon source, and oxygen supply (Ekasari et al. 2014b). Supplementing biofloc system with nitrifying bacteria, as in the treatment ponds, could accelerate the process to eliminate toxic TAN in the water.

The effective C/N ratio can be different under different biofloc conditions (Avnimelech 1999, Ekasari et al. 2014a, Cardona et al. 2015). This study applied C/N ratio of 12:1. As TAN concentration increased after day 60 of the culture period (Fig. 3a), farm owner added more molasses into control ponds since day 75 of the culture period to reach C/N ratio of 15:1. This management action seemed to be effective as can be seen from the reduced concentration of TAN concentration at the end of the culture system.

Nitrifying bacteria were suggested to produce more HCO_3^- than CO_2 in the system. This lead to higher alkalinity that prevent high water quality fluctuation, such as pH. The bacteria also function to reduce toxic TAN concentration. More stable water quality and less toxic substances in the pond are avoidance factors to stress for shrimp. These are the main reasons for treatment ponds having a higher shrimp survival rate than control ponds (Table 3). Although similar shrimp body weight at harvest was suggested, higher survival rate finally resulted in higher shrimp biomass production in treatment ponds.

CONCLUSION

Shrimp biofloc culture that supplemented with immobile nitrifying bacteria (Nitrosomonas, Nitrobacter and Nitrospira) resulted in more stable water quality such as less pH fluctuation and Secchi disc visibility. These bacteria were able to reduce the concentration of toxic nitrogenous species (TAN and nitrite) and transform it into nitrate. Under these conditions, shrimps were less exposed to stress and resulted in 10% higher survival rate at harvest compared to control-biofloc ponds. Finally, it produced higher shrimp biomass at harvest.

ACKNOWLEDGEMENT

We appreciate the Ministry of Research, Technology and Higher Education in Jakarta who financially supported this study. Authors also wish to thank Syamsudi, intensive Tambak's owner in Tuban East Java Indonesia, for his kind contribution during the field experiment.

REFERENCES

Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture system. Aquaculture, 176: 227-235.

Avnimelech, Y. 2012. Biofloc Technology-A Practical Guide Book, 2nd ed. The World Aquaculture Society, Baton Rouge, Louisiana, EUA., pp. 272.

Cardona, E., Lorgeoux, B., Geffroy, C., Richard, P., Saulnier, D., Gueguen, Y., Guillou, G. and Chim, L. 2015. Relative contribution of natural productivity and compound feed to tissue growth in blue shrimp (*Litopenaeus stylirostris*) reared in biofloc: Assessment by C and N stable isotope ratios and effect on key digestive enzymes. Aquaculture, 448: 288-297.

Devi, A.C.A. and Kurup, B.M. 2015. Biofloc technology: An overview and its application in animal food industry. International Journal of Fisheries and Aquaculture Sciences, 5(1): 1-20.

DKP 2015. Laporan Tahunan Statistik Perikanan Budidaya di Jawa Timur 2015 [Annual Report on Aquaculture Statistics of East Java Province 2015]. Provincial Fisheries Office, Surabaya, pp. 15-16 [available in Bahasa Indonesia]

Ekasari, J., Angela, D., Waluyo, S.H., Bachtiar, T., Surawidjaja, E.H., Bossier, P. and Schryver, P.D. 2014b. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture, 426-427: 105-111.

Ekasari, J., Azhar, M.H., Surawidjaja, E.H., Nuryati, S., Schryver, P.D. and Bossier, P. 2014a. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish & Shellfish Immunology, 41: 332-339.

Fakhri, M., Budianto, B., Yuniarti, A. and Hariati, A.M. 2015. Variation in water quality at different intensive whiteleg shrimp, *Litopenaeus vannamei*, farms in East Java, Indonesia. Nature Environment and Pollution Technology, 14(1):65-70.

Hariati, A.M., Aulani'am, Soemarno, Marsoedi and Verdegem, M. 2011a. Phylogenetic and nitrite oxidoreductase activities of nitrobacteria and nitrospira isolated from shrimp pond sediment in East Java, Indonesia. Journal of Agriculture and Food Technology, 1(12):231-2237.

Hariati, A.M., Sudirdjo, Aulani'am, Soemarno and Marsoedi 2011b. The effect of consortia bacteria on accumulation rate of organic matter in tiger shrimp, *Penaeus monodon* culture. Journal of Applied Environmental and Biological Sciences, 1(12): 592-596.

Hariati, A.M., Wiadnya, D.G.R., Fadjar, M., Muhammad, S., Faber, R., Verdegem, M.C.J. and Boon, J.H. 1998a. The impact of tiger shrimp *Penaeus monodon* Fabricius, postlarvae stocking density on production in traditional tambak system in East Java, Indonesia. Aquaculture Research, 29: 229-236

Hariati, A.M., Wiadnya, D.G.R., Prajitno, A., Sukkel, M., Boon, J.H. and Verdegem, M.C.J. 1995. Recent developments of shrimp, *Penaeus monodon* (Fabricius) and *Penaeus merguiensis* (de Man), culture in East Java. Aquaculture Research, 26:819-829.

Hariati, A.M., Wiadnya, D.G.R., Rini, R.K., Boon, J.H. and Verdegem, M.C.J. 1998b. *Penaeus monodon* (Fabricius) and *Penaeus merguiensis* (de Man) biculture in East Java, Indonesia. Aquaculture Research, 29: 743-750.

Hariati, A.M., Wiadnya, D.G.R., Sukkel, M., Boon, J.H. and Verdegem, M.C.J. 1996b. Pond production of *Penaeus monodon* in relation to stocking density, survival rate and mean weight at harvest in East Java, Indonesia. Aquaculture Research, 27: 277-282.

Hariati, A.M., Wiadnya, D.G.R., Tanck, M.W.T., Boon, J.H. and Verdegem, M.C.J. 1996a. *Penaeus monodon* (Fabricius) production related to water quality in East Java, Indonesia. Aquaculture Research, 27: 255-260.

Khatoon, H., Banerjee, S., Yuan, G.T.G., Harris, N., Ikhwanuddin, M., Ambak, M.A. and Endut, A. 2016. Biofloc as a potential natural feed for shrimp postlarvae. International Biodeterioration & Biodegradation, 113: 304-309.

Liang, W., Luo, G., Tan, H., Ma, N., Zhang, N. and Li, L. 2014. Efficiency of biofloc technology in suspended growth reactors treating aquacultural solid under intermittent aeration. Aquacultural Engineering, 59: 41-47.

Liu, K.F., Chiu, C.H., Shiu, Y.L., Cheng, W. and Liu, C.H. 2010. Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. Fish & Shellfish Immunology, 28: 837-844.

Scryver, P.D., Crab, R., Defoirdt, T., Boon, N. and Verstraete, W. 2008. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture, 277:125-137.

Taw, N. 2005. Shrimp farming in Indonesia evolving industry responds to varied issues. Global Aquaculture Advocate Magazine, pp. 65-67.

Thorburn, C. 1982. Teknologi Kampungan: A Collection of Indigenous Indonesian Technologies. Stanford, Volunteers in Asia, pp. 30-31

Valsamma, J., Haseeb, M., Ranjit, S., Anas, A. and Bright Sing, I.S. 2014. Shrimp production under zero water exchange mode coupled with bioremediation and application of probiotics. Journal of Fisheries International, 9(1): 5-14.

Vilani, F.G., Schveitzer, R., Arantes, F.R., Vieira, F.N., Santo, C.M.E. and Seiffert, W.Q. 2016. Strategies for water preparation in a bioûoc system: effects of carbon source and fertilization dose on water quality and shrimp performance. Aquaculture Engineering, 74: 70-75.

Wei, Y.F., Liao, S.H. and Wang, A.L. 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. Aquaculture, 465:88-93.

Xu, W.J., Morris, T.J. and Samocha, T.M. 2016. Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. Aquaculture, 453: 169-175.