Application of biofloc to maintain the water quality in culture system of the tiger prawn (*Penaeus monodon*)

[1]Nurhatijah Nurhatijah, [2]Zainal A. Muchlisin, [3]Muhammad A. Sarong, [4]Ahmad Supriatna

[1] Department of Integrated Coastal Resource Management, Graduate School of Syiah Kuala University, Banda Aceh 23111, Indonesia; [2] Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia; [3] Department of Biology, Faculty of Teaching Training and Education, Syiah Kuala University, Banda Aceh, Indonesia; [4] Brackish Water Aquaculture Development Center, Ujong Batee, Aceh Besar District, Indonesia. Corresponding author: Z. A. Muchlisin, muchlisinza@unsyiah.ac.id

Abstract. The objective of the present study was to evaluate the effect of biofloc density on the water quality of the culture media of tiger prawn (*Penaeus monodon*). The completely randomized design method was used in this study. Three levels of biofloc were tested in this study; 5 mL L^{-1}, 10 mL L^{-1} and 15 mL L^{-1}. The biofloc were cultivated in the 2000 L tank using the soil (sediment) from mangrove area. The experiment was conducted in three replicates and prawns were fed an experimental diet three times a day on 07.00 AM 13.00 PM and 18.00 PM for 95 days. The biofloc density was monitored every seven days interval to make sure the density still at tested concentrations. The results showed that the salinity ranged between 20.64 ppt to 20.70 ppt, temperature 26.9-27.4°C, dissolved oxygen (DO) 7.2-7.3 mgL^{-1}, pH 7.8-8.0, total ammonia nitrogen (TAN) 0.10-0.25 mg L^{-1}, nitrite 0.20-0.30 mg L^{-1}, nitrate 18.5-23.2 mg L^{-1} and alkalinity 118.6-125.3 mg L^{-1}. The ANOVA test showed that the floc density did not give the significant effect on the salinity, temperature, pH, DO, TAN and nitrite concentrations ($p > 0.05$), but gave the significant effect on the nitrate and alkalinity concentration ($p < 0.05$). In general, the water quality in all treatments was suitable for tiger prawn, however the water quality in the biofloc treatment was better than control.

Key Words: biofloc, black tiger shrimp, percentage of feed protein, water quality.

Introduction. Water quality is one of limiting factors in prawn culture system. Water does not only serve as a medium to live prawn but also is a place for organic materials accumulation from the remaining feed and feces that toxic for a cultured organism (Avnimelech 2007). Therefore, the water quality management is crucial in the aquatic culture system. The water quality management aimed to provide the comfortable environment and meet the optimum requirement for cultured organisms. Several techniques have been utilized to maintain the water quality in tiger prawn (*Penaeus monodon*) culture system, for example, closed recirculation system (Timmons & Ebeling 2010) and best management practices (Arifin et al 2007). However, these techniques are costly and inefficient in traditional and semi-intensive culture systems.

Biofloc is one of the alternative methods to control and improve the water quality in aquaculture, this method is commonly combined with domestic waste processing (Avnimelech 2009; De Schryver & Verstraete 2009). Biofloc is the floc consisting of micro-organisms such as algae, bacteria, fungus, algae, protozoa, worms, and organic matters from the unconsumed feed and feces, this floc formed due to the mucus which is released by bacteria bound by the filamentous organism or caused by an electrostatic force (De Schryver et al 2008). According to Kuhn et al (2010) the nutrition content of biofloc differs based on carbon source, total suspended solid, salinity, stocking density of cultured organisms, light intensity, phytoplankton and bacteria communities. In general biofloc has 12-50% crude protein, 0.5-12.5% crude lipid, and 13-46% ash (Avnimelech 2009).

Biofloc is believed it could maintain water quality because it contains heterotrophic bacteria which can utilize organic and inorganic materials contained in the water (Ekasari 2009). The biofloc has the ability to suppress toxic compounds such as ammonia and suppresses the growing of harmful bacteria (pathogenic) so that the cultured organisms can grow and develop properly (Schrader et al 2011).

According to Hargreaves (2013), biofloc has several advantages when applied in the culture system, for example stabilize pH, decrease the ammonia content, and recycle sewage (dead algae, feces, and food remains) as organic material for beneficial microbes which can be used as a supplementary food for cultured prawn. Hence, the objective of the present study was to evaluate the effect of biofloc application on the water quality in the culture system of tiger prawn (*P. modonon*).

Material and Method

Time, location and experimental design. The experiment was conducted on June to August 2015 in Laboratory of Ujung Batee Brackish Water Aquaculture Development Center, Aceh Besar District, Indonesia.

The completely randomized design was utilized in this study. Three levels of biofloc density were tested in this study: 5 mL L^{-1}, 10 mL L^{-1} and 15 mL L^{-1} and every treatment was done at four replicates. The post larvae (PL-30) tiger prawns were reared in the 12 cylindrical high density polyethylene (HDPE) tanks (total volume 100 L) and every tank has 25 prawns. The prawns were fed the commercial diet with 35% crude protein for 95 days.

Biofloc cultivation. The biofloc was cultivated in a fiber plastics tank (vol. 2000 L). The tank was fulfilled with sea waters (20 ppt) and a total of 1000 tilapia fish (*Oreochromis niloticus*) with the average size of 3-5 cm were stocked into the tank and the tank was equipped with aeration. Approximately 20 g of soil sediment from mangrove area were put in the tank as the biofloc inoculant. The fish were fed the commercial diet with 38% crude protein at feeding level of 5% body weight and the feed were mixed with molasses at the ratio of 2:1 (feed: molasses) before being given to the tilapia fish. The biofloc were harvested after 45 days and applied into the experimental tanks at respective density.

Biofloc harvests and stocked. The aeration was switched off to allow the floc to settle on the bottom of the cultivation tank. The biofloc were siphoned into a glass beaker at the respective volume of 400 mL, 800 mL and 1200 mL to result in the required density of 5 mL L^{-1}, 10 mL L^{-1} and 15 mL L^{-1} in the experimental tanks. The density of biofloc in the experimental tanks was monitored for 7 days interval to make sure the biofloc density is still at expected value.

Water quality parameters. The dissolved oxygen (DO), pH and water temperature were measured directly in the experimental tanks every day using the digital water checker (YSI-550 A, ASTM, Alla, France). While, the total ammonia nitrogen (TAN) and nitrate (NO_3) were measured using the spectrophotometric method (Eaton et al 2005), nitrite (NO_2) was examined using calorimetric method and alkalinity was measured *ex situ* using the titrimetric method (Eaton et al 2005).

Data analysis. The data were subjected to analysis of variance (One-way ANOVA) and followed by the Duncan multi-range test at the confidence level of 95% using SPSS ver. 16.0 software.

Results and Discussion. The results showed that the salinity ranged from 20.41‰ to 20.52‰, water temperature 26.95-27.21°C, DO 7.2-7.3 mg L^{-1}, pH 7.90-7.96 (Table 1), TAN ranged from 0.10 mg L^{-1} to -0.25 mg L^{-1}, nitrite 0.20-0.30 mg L^{-1}, nitrate 18.8-23.2 mg L^{-1} and alkalinity 117.7-122.9 mg L^{-1}(Table 2). The ANOVA test showed that the floc density did not give the significant effect on the salinity, temperature, pH, DO, TAN and nitrite concentrations ($p > 0.05$), but gave the significant effect on the nitrate and alkalinity values ($p < 0.05$). In general, the range of water quality parameters is still within the standard ranges for tiger prawn cultivation (Arifin et al 2007). The direct observation showed that the water in the floc treatment looks murky, which is caused by the formation of biofloc. According to Ekasari (2009) heterotrophic bacteria in the biofloc have the ability to utilize organic and inorganic nitrogen contained in water of cultures media and therefore the biofloc can maintain the water quality.

Table 1

The average values (mean±standard deviation) of salinity, water temperature, pH, and dissolved oxygen concentrations in respective treatments

Biofloc concentration	*Salinity (ppt)*	*Temp. (°C)*	*pH*	*DO (mg L^{-1})*
Control	20.47 ± 0.0^{b}	27.14 ± 0.0^{a}	7.93 ± 0.0^{a}	7.2 ± 0.0^{b}
5 mL L^{-1}	20.52 ± 0.0^{c}	27.16 ± 0.1^{a}	7.96 ± 0.0^{a}	7.3 ± 0.0^{a}
10 mL L^{-1}	20.46 ± 0.0^{b}	27.21 ± 0.2^{a}	7.96 ± 0.0^{a}	7.3 ± 0.0^{a}
15 mL L^{-1}	20.41 ± 0.0^{a}	26.95 ± 0.2^{a}	7.90 ± 0.0^{a}	7.3 ± 0.0^{a}

The mean values followed by the different superscripts are significantly different ($p < 0.05$).

Table 2

The average values (mean±standard deviation) of total ammonia nitrogen, nitrite, nitrate, and alkalinity concentrations in respective treatments

Biofloc concentration	*TAN (mg L^{-1})*	*Nitrite (mg L^{-1})*	*Nitrate (mg L^{-1})*	*Alkalinity (mg L^{-1})*
Control	0.25 ± 0.0^{b}	0.30 ± 0.0^{a}	18.8 ± 1.3^{a}	117.7 ± 1.6^{a}
5 ml L^{-1}	0.10 ± 0.0^{b}	0.20 ± 0.0^{a}	20.3 ± 0.7^{a}	119.1 ± 2.2^{a}
10 ml L^{-1}	0.15 ± 0.0^{b}	0.20 ± 0.0^{a}	23.2 ± 1.0^{b}	122.9 ± 2.0^{b}
15 ml L^{-1}	0.10 ± 0.0^{a}	0.20 ± 0.0^{a}	19.5 ± 0.2^{a}	118.7 ± 1.7^{a}

The mean values followed by the different superscripts are significantly different ($p < 0.05$).

The result showed that the salinity ranged between 20.41ppt to 20.52 ppt, and there were no significant differences in the salinity among the treatments. These values are still in the optimum level for tiger prawn (Tarsim 2004). According to Rosenberry (2001) tiger prawn is an euryhaline aquatic organism, which could tolerate the salinity between 3 ppt to 45 ppt, and however the best growth performance occurred at 15-30 ppt.

The water temperature ranged from 26.95°C to 27.21°C, indicating that the water temperature variations between treatments were low. However, the lower water temperature was recorded at treatment B (10 mL L^{-1}). In general, the ranges of water temperature in all treatments are still suitable for tiger prawn growing. This is in agreement to Pillay & Kutty (2005) who stated that the optimum range of water temperature for tiger prawn is between 12.0^{o}C to 37.5^{o}C. Beside affecting the prawn, the water temperature is also affecting the floc formations (De Schryver & Verstraete 2009). Furthermore, De Schryver & Verstraete (2009) stated that the best temperature for biofloc was between 18^{o}C to 20^{o}C. Therefore, the study indicates that the water temperature values in all treatments were higher than the optimum level for biofloc, however, it still can be tolerated by the cultured prawn. In addition, the activity of microbes in the floc was affected by temperature, for example the extracellular polysaccharide as the primary form of floc produced by microbes are higher at 25^{o}C (Krishna & Van Loosdrecht 1999).

The DO ranged from 7.2 mg L^{-1} to 7.3 mg L^{-1}, and pH ranged from 7.90 to 7.96, indicates no significant differences among the treatments. In general the DO values are suitable for tiger prawn. According to Boyd & Tucker (1998) the minimum DO requirement for prawn culturing is about 4 ppm. The higher of DO concentration in the experiment tanks are probably due to all the tanks were equipped with continuously aeration. Beside to trigger oxygen diffusion from the air (atmosphere) into the water, aeration is also needed for mixing water and avoid deposition of biofloc, food remains and feces which could increase the levels of ammonia (Hargreaves 2013).

The study revealed that the pH values were still in optimum range for penaeid shrimps. According to Wickins & Lee (2002) the penaeid shrimps need the pH value between of 7.6 to 8.3. There are two types of ammonia in culture system i.e. NH_3 (unionized ammonia) and NH_4^+ (ionized ammonia) and the total amount of the two forms of ammonia is called total ammonia nitrogen (Ebeling et al 2006) while pH is one of the parameters that affect the level of ionized and non-ionized ammonia in the water.

The results revealed that the TAN, nitrite and nitrate concentrations ranged between 0.10-0.25 mg L^{-1}, 0.20-0.30 mg L^{-1}, and 18.8-23.2 mg L^{-1}, respectively (Table 2). The study revealed that lower TAN was recorded at 5 mL L^{-1} and 15 mL L^{-1} of biofloc, however these values were not significantly different with 10 mL L^{-1}. While the higher nitrate and alkalinity were recorded at 10 mL L^{-1}, but these was not significantly different with other treatments.

In general, the TAN concentration is still in optimum ranges for penaeids (Taslihan et al 2003). The TAN concentration is the combination between the ionized nitrogen (NH_4^+) and unionized nitrogen (NH_3). The concentration of NH_3 is affected by pH, temperature and salinity where the concentration of NH_3 is higher when the temperature and pH increased (Boyd & Tucker 1998). Biofloc has the ability to balance the concentration of ammonia in the culture system, because the heterotrophic bacteria in biofloc are able to absorb ammonia 40 times faster than nitrification bacteria (Ebeling et al 2006). In addition, De Schryver & Verstraete (2009) stated that biofloc grown in the bioreactor can convert 98% ammonia to nitrate at a concentration of 110 mg L^{-1} day^{-1}. The ability of heterotrophic bacteria to absorb the ammonia is influenced by the ratio of organic carbon and nitrogen (C/N ratio) in water where the ammonia will be absorbed quickly when C/N was higher (Schneider et al 2006).

The nitrite concentrations in all floc treatments are suitable for tiger prawn culture, the highest nitrite concentration was recorded at control treatment. According to Taslihan et al (2003), the optimum maximum level of nitrite was 0.2 mg L^{-1}. The low value of nitrite on the biofloc treatments probably due to the ammonia has converted to nitrite by heterotrophic bacteria in biofloc then utilized by micro algae for growing (Avnimelech 1999; Ebeling et al 2006). This is an indication that the nitrification process occurred in the culture medium. The nitrification is a two-step process in which ammonia is oxidized to nitrite and then nitrite is oxidized to nitrate by nitrification bacteria (Sticney 2005).

Nitrate is the end product of the nitrification process, this compound is toxic for penaeid shrimp at concentrations above 200 mg L^{-1} (Chamberlain 1988). The observation of the nitrate content of culture media during the study shows the range of 18.8-23.2 mg L^{-1}, these values are below the limit for shrimp. This is probably due to the presence of phytoplankton (microalgae) in biofloc. Algae needs nitrate as a source of nutrients that are absorbed from the water and therefore the concentration of nitrate in the water media was lower (Ebeling et al 2006). The concentration of nitrate and nitrite shows the opposite relationship as recorded in this study. The higher nitrite and lower nitrate concentrations were recorded in the control treatment, this is probably due to lack of microalgae in the water media because no biofloc occurred.

The higher alkalinity was recorded at biofloc density of 10 mL L^{-1} and the lower concentration occurred at 15 mL L^{-1}. In general the concentration of alkalinity in all treatments are suitable for tiger prawn. According to Taslihan et al (2003) the optimum range of alkalinity was 90-130 mg L^{-1}. Alkalinity has an important role as a pH buffer. Ebeling et al (2006) suggest alkalinity should be maintained in the range of 100-150 mg L^{-1} $CaCO_3$ with the addition of sodium bicarbonate or agricultural lime if needed. This report is the initial study and the further study is performing to evaluate the effect of biofloc application on the growth performance, survival rate and feed utilization of the tiger prawn.

Conclusions. The biofloc density did not give the significant effect on the salinity, temperature, pH, DO, TAN and nitrite concentrations, but gave the significant effect on the nitrate and alkalinity values. In general, the range of water quality parameters are still within the standard ranges for tiger prawn cultivation in all treatments, but the several water quality parameters (i.e. TAN, nitrite ad nitrate, and alkalinity) of biofloc treatment were better than control.

References

Arifin Z., Adiwidjaya D., Komarudin U., Abidin U. N., Susanto A., Taslihan A., Ariawan K., Mardjono M., Sutikno E., Supito, Syahrul M. L., 2007 [Application of best management practices in the tiger prawn, *Penaeus monodon* intensive culture]. Department of Marine and Fisheries, Directorate General of Aquaculture, Center for Brackish Water Aquaculture Development, Jepara, 67 pp. [in Indonesian]

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

Avnimelech Y., 2007 Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264:140-147.

Avnimelech Y., 2009 Biofloc technology - a practical guide book. The World Aquaculture Society, Baton Rouge, Louisiana, US, 182 pp.

Boyd C. E., Tucker C. S., 1998 Pond aquaculture water quality management. Kluwer Academic Publisher, Massachusetts, 700 pp.

Chamberlain G. W., 1988 Rethinking shrimp pond management. Coastal Aquaculture 5(2):1-20.

De Schryver P., Verstraete W., 2009 Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. Bioresource Technology 100:1162-1167.

De Schryver P., Crab R., Defoirdt T., Boon N., Verstraete W., 2008 The basics of biobiofloc technology: the added value for aquaculture. Aquaculture 277:125-137.

Eaton A. D., Clesceri L. S., Rice E. W., Greenberg A. E. (eds), 2005 Standard methods for the examination of water and wastewater. 21st edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, pp. 2-39.

Ebeling J. M., Timmons M. B., Bisogni J. J., 2006 Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346-358.

Ekasari J., 2009 [Biofloc technology: theory and practical in intensive aquaculture system]. Jurnal Akuakultur Indonesia 8(2):117-126. [in Indonesian]

Hargreaves J. A., 2013 Biofloc production system for aquaculture. Southern Regional Aquaculture Center, Publication factual sheet No: 4503, 12 pp.

Kuhn D. D., Lawrence A. L., Boardman G. D., Patnaik S., Marsh L., Flick G. J. Jr., 2010 Evaluation of two types of biobiofloc derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303:28-33.

Krishna C., Van Loosdrecht M. C. M., 1999 Effect of temperature on storage polymers and settleability of activated sludge. Water Research 33:2374-2382.

Pillay T. V. R., Kutty M. N., 2005 Aquaculture: principles and practices. Second edition, Blackwell Publishing, Iowa - USA, 640 pp.

Rosenberry B., 2001 World shrimp farming 2000. Shrimp News International, San Diego, 324 pp.

Schneider O., Sereti V., Eding E. H., Verreth J. A. J., 2006 Molasses as C source for heterotrophic bacteria production on solid fish waste. Aquaculture 261:1239-1248.

Schrader K. K., Green B. W., Perschbacher P. W., 2011 Development of phytoplankton communities and common off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*). Aquacultural Engineering 45:118-126.

Stickney R. R., 2005 Aquaculture: an introductory text. CABI Publication, Massachusetts, 265 pp.

Tarsim, 2004 [Effect of adding the white legged shrimp (*Penaeus vannamei*) on growth and survival of tiger shrimp (*Penaeus monodon*) in intensive farming]. Jurnal Akuakultur Indonesia 3(3):41-45. [in Indonesian]
Taslihan A., Supito, Sutikno E., Callinan R. B., 2003 [Tiger prawn culture technique]. Direktorat Jenderal Perikanan Budidaya, Balai Besar Pengembangan Budidaya Air Payau, Jepara, 59 pp. [in Indonesian]
Timmons M. B., Ebeling J. M., 2010 Recirculating aquaculture. Second edition, Cayuga Aqua Ventures, New York, 489 pp.
Wickins J. F., Lee D. O. C., 2002 Crustacean farming: ranching and culture. Second edition, Blackwell Publishing, Iowa, 465 pp.

Received: 26 July 2016. Accepted: 18 August 2016. Published online: 30 August 2016.
Authors:
Nurhatijah Nurhatijah, Department of Integrated Coastal Resource Management, Graduate School of Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: nurhatijah.poliven@yahoo.com
Zainal A. Muchlisin, Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: muchlisinza@unsyiah.ac.id
Muhammad A. Sarong, Department of Biology, Faculty of Teaching Training and Education, Syiah Kuala University, Banda Aceh, Indonesia, e-mail: ali_sarong@yahoo.com
Ahmad Supriatna, Brackish Water Aquaculture Development Center, Ujong Batee, Aceh Besar District, Indonesia, e-mail:supriatnaahmad@yahoo.com

How to cite this article:
Nurhatijah N., Muchlisin Z. A., Sarong M. A., Supriatna A., 2016 Application of biofloc to maintain the water quality in culture system of the tiger prawn (*Penaeus monodon*). AACL Bioflux 9(4):923-928.