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Research Article
Biofloc Application in Closed Hatchery Culture System of Pacific White Shrimp, *Penaeus vannamei* in Sustaining the Good Water Quality Management

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Abstract
Biofloc technology (BFT) as the new application in keeping up good water quality for shrimp culture was conducted in a close hatchery of Universiti Malaysia Terengganu (UMT). Pacific white shrimp, *Penaeus vannamei* was selected to be culture and the study was conducted for 105 days from PL 10 until PL 115. There was no water exchange during 105 days except addition of water to refill the water level after siphoning out wastes. About 6 tanks were used, three tanks for non biofloc/control, (C1, C2 and C3) and three tank for biofloc treatment (T1, T2 and T3), respectively. No mass mortality found out in the biofloc tank except for the first tank, T1 that infected with the *Vibrio* sp. From the observation in the T1, T2 and T3 tanks, water parameter such as DO was recorded in ranged between 5.95 and 9.53 mg L^{-1}, salinity between 31.60 and 36.11 ppt, pH 6.1- 8.2, temperature 26.00-28.66°C, DO% (83.9-107.4%) and the TDS between 31.59 -35.5 mg L^{-1}. Nutrients level include ammonia, nitrite and nitrate were in good condition in biofloc treatment T1, T2 and T3 as compared to the control tank, respectively. All nutrients were high in the middle of culture period but returned to lower reading at the end of culture period as maintain by the biofloc system. The TOC exhibited the same pattern in treatment and control tanks. Biofloc microorganisms successfully work in removing the nitrogenous wasted in the treatment tanks and maintain the nutrients and water quality in the safety level for the shrimp. It can be concluded that biofloc system is really works in maintaining the water quality in the hatchery culture system.

Key words: Biofloc, pacific white shrimp, *Penaeus vannamei*, water quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Now-a-days aquaculture sector is known as a major food producing industry due to growing population density and land use need as compared to other developed industry. Aquaculture practices have to be sustainable to minimize the destruction of the environment[1], maintaining quality and safety standard and enable the use of the land and natural resource efficiently. One of the solutions is by practicing the biofloc technology (BFT) in the culture system. Biofloc can be formed naturally in pond water consist of conglomeration of nitrifying bacteria, organic material, inorganic flocculants and suspended algae[2]. Biofloc particles varied in size but are visible with the naked eye. The floc consists of microbes, uneaten feed particles, shrimp secretion and detritus that flocked together by physiochemical forces and polymer matrices[3]. The BFT is a well established new technology which enhanced water quality in aquaculture by balancing the carbon and nitrogen in the system[4]. It can be done by adding up external carbon sources in the aquaculture system to stimulate the growth of heterotrophic bacteria and the nitrogen uptake through the production of microbial protein in the water system[5]. Production of shrimp with zero water exchange by using the BFT system not only maintain water quality, but also provide essential and higher quality of nutrition to the shrimp to achieve fast growth and low FCR[6]. Algae and plankters in the floc serve as food for the shrimp and nitrifying bacteria convert the nitrogenous waste into simple compounds. Moss[7] and Wasielesky *et al*.[8] reported in their study that water consist of biofloc particles and the associated microbial can significantly improve shrimp growth compared to water lacking these components of floc. Biofloc contributes some nutrition and support fast growth of some penaeid shrimp such as pacific white shrimp, *Penaeus vannamei* by providing additional food sources in intensive and limited exchange of water system[9]. Moss *et al*.[10] and Ju *et al*.[11] discovered that in minimal water exchange in super intensive culture systems formed a dense microbial community including algae, bacteria, zooplankton and fungi that aggregated in the biofloc and responsible for detoxifying and cycling otherwise harmful nutrients. These microorganisms may also supply nutritionally beneficial components to culture animals such as amino acids and vitamins. This improves the water quality and reduces the use of new water thus lead to the sustainability in aquaculture practice and help protecting the environment and ecosystem[2,3]. Through all the advantages BFT gives, therefore the objectives of this study was to determine the effectiveness of biofloc system in closed hatchery system for *P. vannamei* shrimp in maintaining the good water quality in terms of nutrients and organic carbon in shrimp culture treatments.

MATERIALS AND METHODS

Water preparation and stocking density: The experiment was conducted in the close marine hatchery of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu for 105 days until reach the juvenile size of post larvae PL 115 days (PL115). Rounded tank with the capacity of 8 t was used in the experiment (depth 1.2 m, diameter 3.3 m), 6.5 t of sea water at 30 ppt was restocked in the tanks and were pre-treated with chlorine 30 ppm t^{-1} water. After 24 h of chlorination, anti chlorine, sodium thiosulfate was added for dechlorination. Pacific white shrimp, *Penaeus vannamei* PL 10 from the private hatchery as the study candidate was restocked in each tank with stocking density 100 PL per cubic meter. The shrimp were fed four times daily (0800, 1400, 2000 and 0200 h) with formulated diet from Gold Coin brand started from Starter Pack No.1 until Grower Pack No. 4 at 10% of biomass which pellet contains nutrition such as crude protein 35.0%, crude fibre 4.0%, crude fat 5.0%, moisture 12.0% and ash 15.0%. The faeces and uneaten food were siphoned once a week.

Experimental design: Treatment tanks consist of biofloc which are T1, T2 and T3 and control tank consists of C1, C2 and C3 without application of biofloc were prepared. Six tanks with capacity 8 t were used and setup in the hatchery. All the aerations supplies were setup in adequate numbers for sufficient oxygen supply for biofloc formation and shrimp consumption. The PL 10 was stocked in all tanks during the early culture period (Day 1, PL 10). All of the treatments and controls were cultured for 105 days of culture period until reached harvest size PL 115.

Biofloc formation and measurement: Molasses as carbon source was applied in the experiment. Molasses as additional carbon sources was added to the water about 840 g to break down 84 g N leave in water. The C:N ratio, 10:1 was applied in the experiment (Fig. 1). About 1 kg of molasses was mixed with 38.08 g grain pellet of 16 g N as protein sources for nitrogen break down process in the water and was well prepared in the 10 L basin. The mixture was fermented for 24 h before being poured into the treatment tank. The same procedure was applied for all treatment T1, T2 and T3. Control tanks were cultured normally for 105 days without

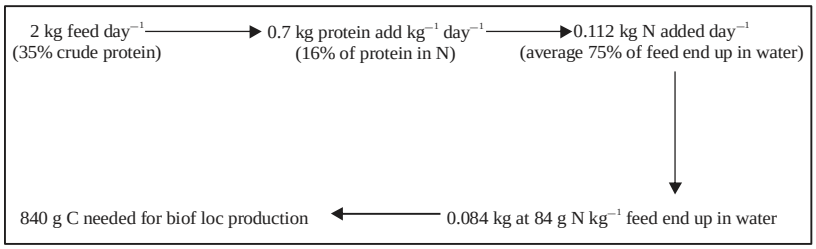

Fig. 1: Schematic diagram of carbon required to remove the nitrogen wastes from uneaten feed and shrimp faeces. About 16% of protein in N[20], 75% of feed end up in water ammonia+shrimp faeces[21] and C:N ratio 10:1 Avnimelech[5] was applied

biofloc application. The procedures were repeated for every 2 days until the biofloc formation in the water column. The biofloc volume was measured every week using Imhoff cone and the size of the biofloc conglomerate was observed and measured via advance microscope Nikon 80i. Plankton assimilate were observed and identified using light microscope and advance microscope Nikon 80i and the incorporate bacterial was identified using API Biomerieux kit. All data collected was recorded until the last week of treatment.

Water parameter, nutrient and TOC analysis: Water parameters were recorded *in situ* using YSI multiprobe model 556 for Dissolved Oxygen (DO), pH, salinity, Total Dissolved Solid (TDS), temperature and DO%. For nutrient such as nitrite, nitrate and ammonia analysis, samples of water were taken and were brought back to laboratory for analysis. The water samples were filtered using filtration set and GFC Whatman filter paper of 0.1 nm was used. Ammonia was analyzed using salicylate method (Standard method 8155) (DR/2400 procedure manual 2002)[12]. Nitrite, NO_2^- was analyzed using diazotization method (Standard method 8507) (DR/2400 procedure manual 2002)[12] and nitrate was analyzed using cadmium reduction method (Standard method 8192) (DR/2400 procedure manual 2002)[12]. All the data for ammonia, nitrite and nitrate were measured using DR/2400 spectrophotometer and all data were recorded. Total Organic Carbon (TOC) in tank treatment and control was analyzed using TOC Analyzer: TOC-L SHIMADZU. Non-purgeable organic carbon or (NPOC) or direct method was used by preacidifying and prepurging the sample to remove the Total Inorganic Carbon (TIC) and Particulate Organic Carbon (POC) in the water samples. Suspended particles were removed through acidification with HCl at pH 2 by adding small portion of acid and the Inorganic Carbon (IC) was removed from the acidified sample by purging with the purified gas. The TOC was converted to carbon dioxide, CO_2 and then were carried using oxygen carrier gas to non-dispersive infrared gas analyzer (NDIR) and the concentration of CO_2 was measured. The TOC analyzer was calibrated with standard solution (Potassium hydrogen phthalate) for accurate TOC measurement.

Statistical analysis: One-way ANOVA from SPSS (version 17.0) with *post hoc* test and Tukey test was applied to analyze the water parameter such as pH, temperature, DO, TDS and DO%, used to determine the differences among treatment. One-way ANOVA was also used to analyse data nutrient for ammonia, nitrite and nitrate and also total organic carbon, TOC for each treatment during 105 days. Data for biofloc volume was analyzed using two-way ANOVA analysis to see the differences between the biofloc levels for every week in 105 days in each treatment tank. Correlation Pearson (1-tailed) was used to analyze the linkage between the length and width of biofloc conglomerate size for every week for 105 days in each biofloc treatment tank.

RESULTS

Different coloration was observed in biofloc treatment tanks (T1, T2 and T3). At the end of culture period, T1 changed from light green to dark green in colour, T2 changed from light green to brownish green and T3 changed from light brown to dark brown (Fig. 2). For the water parameter of DO, there is no significant different between the treatment tank and control where ($p = 0.653$, $p>0.05$) and $F = 0.551$. The highest DO reading was 9.53 mg L^{-1} from T1 and the lowest reading was 6.08 mg L^{-1} from control C3. For salinity there were significantly difference between the treatment and control tank where ($p = 0.000$, $p<0.05$) and $F = 12.628$. The highest reading was 36.11 ppt from treatment tank 1 and the lowest was 31.6 ppt from treatment tank 3. For pH there were significantly different among treatment and control where ($p = 0.000$, $p<0.05$) and $F = 11.485$. The highest pH reading is 8.24 from C3 and the lowest pH reading is 6.11 at the end of culture period of C3. There were significantly difference for TDS where ($p = 0.000$, $p<0.05$) and $F = 15.025$. The TDS was

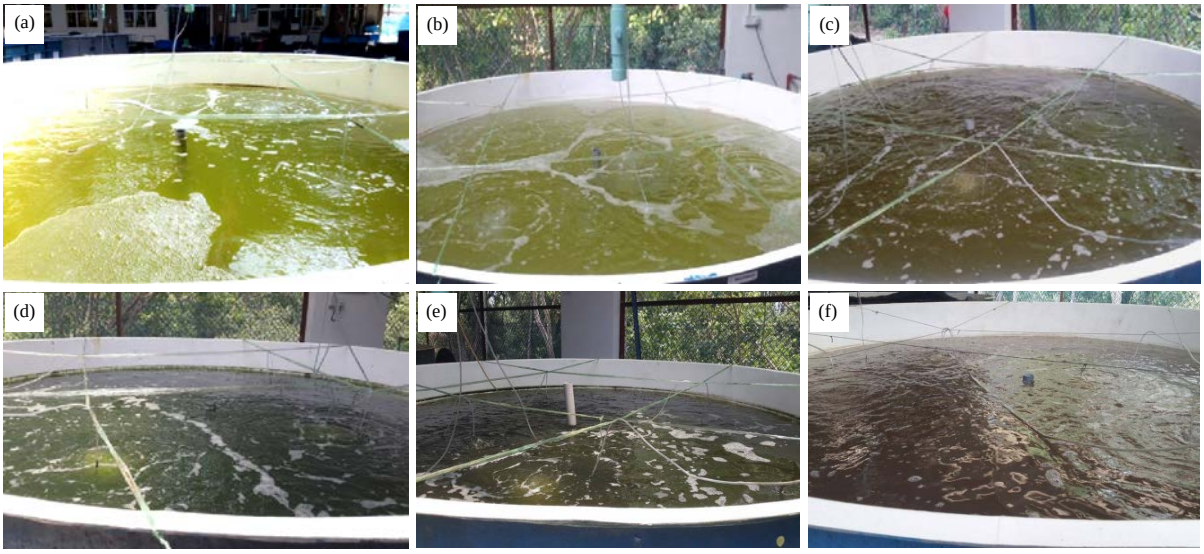

Fig. 2(a-f): Different coloration of biofloc observed during 105 days of cultured period, (a) T1 at early culture period, (b) T1 at end of culture period, (c) T2 at early culture, (d) T2 end of culture period, (e) Represents T3 at early culture period and (f) Represent T3 at end of culture period

continuously observed higher in T1, T2 and T3 of biofloc tank. For C1, C2 and C3, the TDS reading dropped started from days 90 until end of culture period. For temperature there is no significantly different among treatment and control where ($p = 0.396$, $p>0.05$) and $F = 1.041$. The temperature lowest reading is from C3 which is 22.97°C and the highest reading is 28.62°C from C2. For DO%, there were no significantly different among the treatment and control tank where ($p = 0.589$, $p>0.05$) and $F = 0.655$. The lowest DO% is 82.9% and highest reading is 116.8% from T1 (Fig. 3). For data analysis of nutrients such as ammonia, there was no significantly difference among the treatment and control tank where ($p = 0.066$, $p>0.05$) and $F = 2.802$. Ammonia was seen continuously increased in C1 C2 and C3 until end of culture in contrast to the biofloc treatment T1, T2 and T3 the ammonia reading slightly decreased from month 3 until end of culture period. For nitrite, there was a significant difference among treatment and control where ($p = 0.004$, $p<0.05$) and $F = 6.218$. The same situation observed in control C1, C2 and C3 where the nitrite increased until the end of culture period, in contrast to the biofloc treatment T1, T2 and T3 that began to decrease in month 3 and end culture was at 0.5 mg L^{-1}. For data analysis of nitrate, there was significantly difference among treatment and control tank ($p = 0.000$, $p<0.005$) and $F = 9.551$. Nitrate reading slightly decreased in biofloc treatment T1, T2 and T3 at the end of culture to 5.0 mg L^{-1} as compared to the control tank where the nitrate level increased to 20 mg L^{-1} at the end of culture period. For TOC data analysis, there were no significant differences among the treatment where ($p = 1.000$, $p>0.05$) and $F = 0.00$. The TOC was observed higher in both biofloc and control tank, however, in the biofloc treatment tank the TOC was low, ranging from 4.55-4.67 ppm when reached 60 days as compared to control tanks that have TOC reading 6.09-6.63 ppm. However, there was only a small difference in the TOC reading between control and biofloc treatment tank (Fig. 4).

For the biofloc conglomeration size, there were bigger sizes of floc observed from T1 and T2 as compared to the floc in tank 3 (Fig. 5). From observation, green algae and cyanobacteria dominated in the water medium in T1 with the presence of protozoan *Vorticella* sp. Meanwhile, Bacillariophyceae or diatom and cyanophyte were observed dominated in the water in T2 with the presence of rotifer zooplankton. In T3 Bacillarophyceae or diatom and dinoflagellates dominated the water column with the presence of parasite nematode, ciliate protozoa, copepod and rotifer zooplankton. Heterotrophic bacteria were identified dominated in the water column in T1, T2 and T3 of *Aeromonas* sp. and *Pseudomonas* sp. For biofloc volume level, there was no significantly differences in each treatment where ($p = 0.083$, $p>0.05$) and $F = 1.786$ for every week in 105 days. The highest biofloc reading was observed in T1 which is 7.0-8.0 mL L^{-1} at the end of culture period as compared to tank 3 which have 4.0-5.0 mL L^{-1} of biofloc

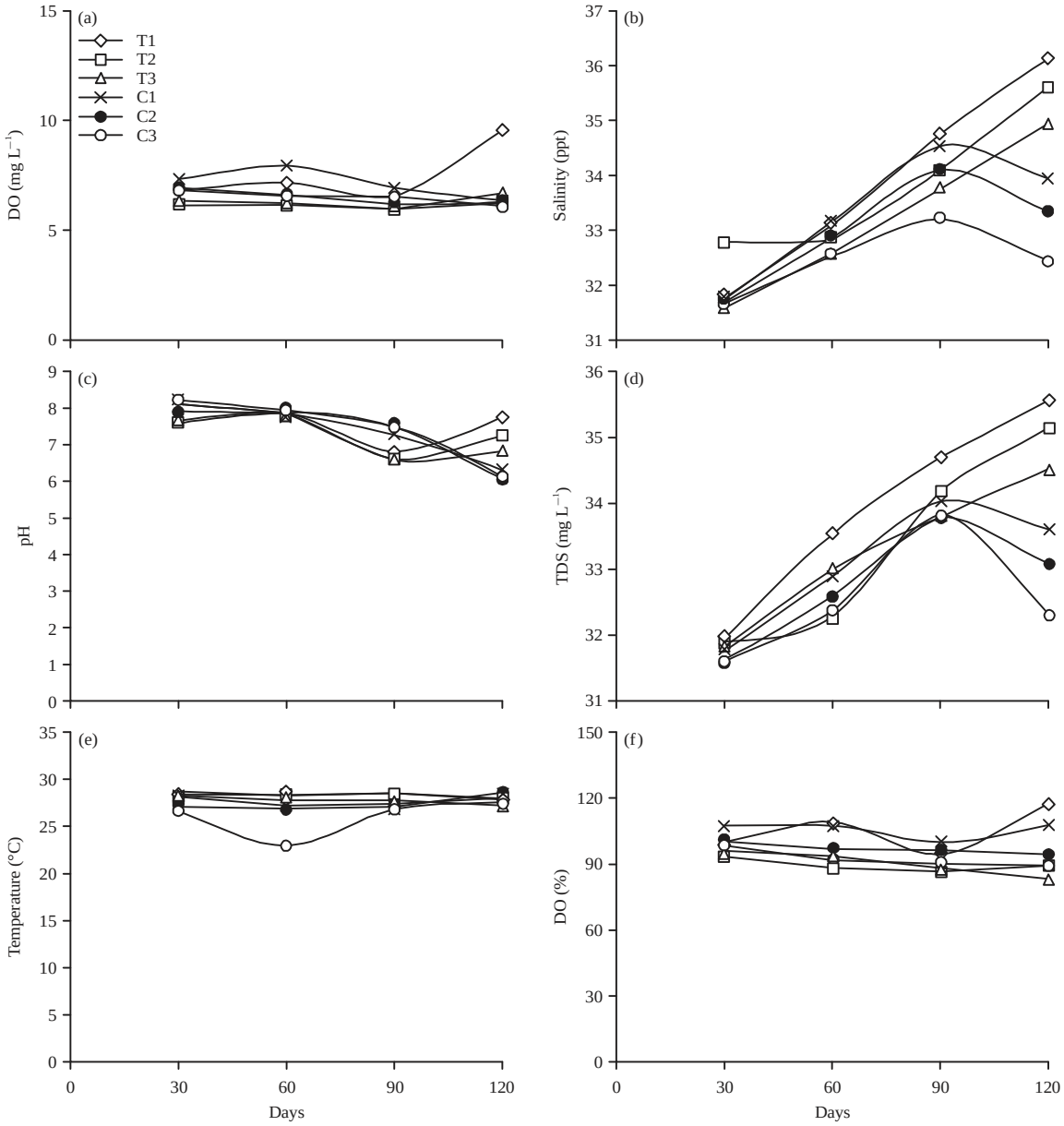

Fig. 3(a-f): Water parameters for 105 days of shrimp *Penaeus vannamei* during the culture period applied with biofloc system. Parameters include (a) DO, (b) Salinity, (c) pH, (d) TDS, (e) Temperature and (f) DO% for all treatment (T1, T2 and T3) and control tanks (C1, C2 and C3)

volume as observed in the end of culture period (Table 1). Apart from that, correlation analysis showed that there was a significant positive relation between the length and width of the biofloc conglomerate size, where ($r = 0.387$, $p<0.05$) where the correlation is significant at 0.01 level (1-tailed).

DISCUSSION

The result showed that the Total Dissolved Solid (TDS) was higher in biofloc treatment T1, T2 and T3 as they consist of biofloc aggregate compared to control tank without biofloc treatment. The water parameter as DO was observed fluctuated in 105 days of culture period. All control and treatment tank contained the same number of shrimp until the end of study except for tank 1. Higher DO was observed in T1 as the shrimp number decreased because of the early mortality cause by *Vibrio* sp., infection in the early month. Lower number of shrimp in tank 1 due to the mortality contributed to the higher dissolved oxygen observed in tank 1. The DO was also seen to fluctuate in all treatment

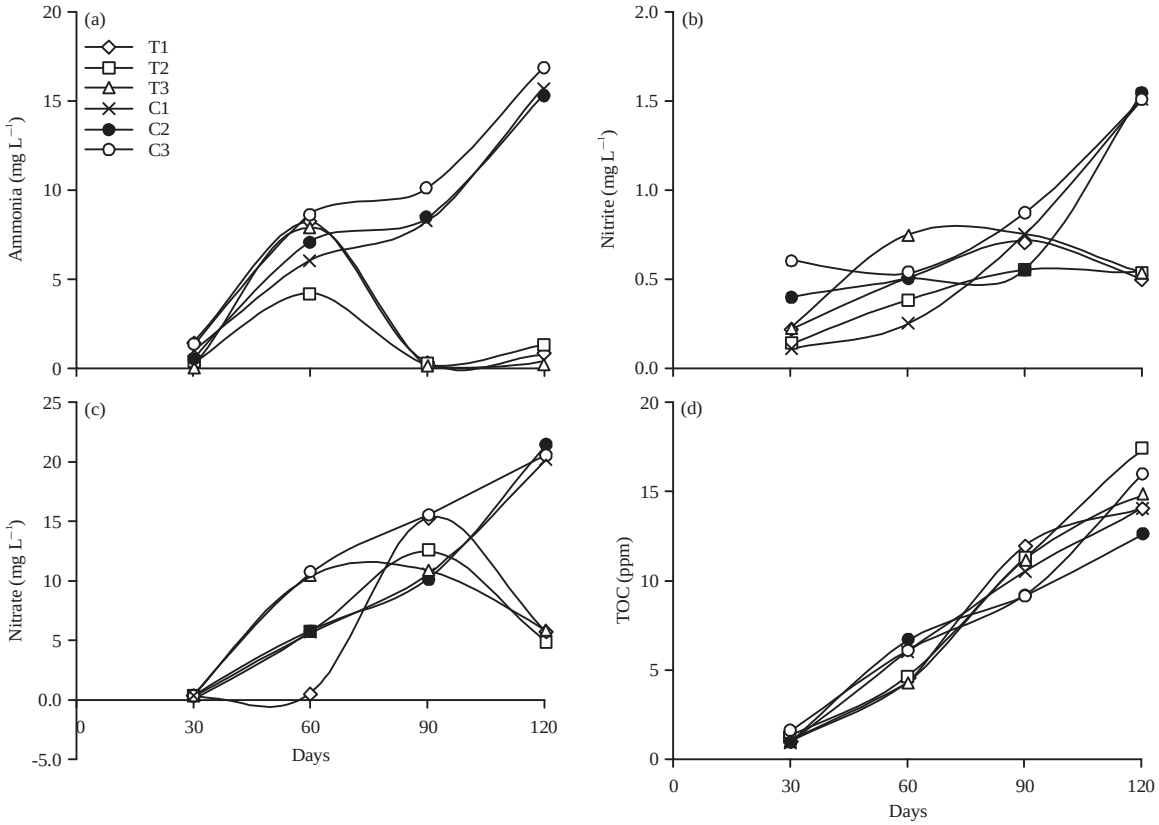

Fig. 4(a-d): Nutrients analysis for (a) Ammonia, (b) Nitrite, (c) Nitrate and (d) Total Organic Carbon (TOC) of water during 105 days of culture period for treatment tanks applied biofloc (T1, T2 and T3) and control tanks without biofloc (C1, C2 and C3)

Table 1: Biofloc volume (mL L^{-1}) measured using Imhoff cone and the biofloc conglomerate size (μm^2) for treatment tank (T1, T2 and T3) observed and size measured under 100x magnification using advance microscope 80i

		Biofloc volume (mL L^{-1}), biofloc conglomerate size (width×length, μm^2)							
Treatments	Weeks	Month 1 (30 days)		Month 2 (60 days)		Month 3 (90 days)		Month 4 (105 days)	
T1	1	0.5	338.81×225.81	5.0	385.73×329.25	6.0	335.06×250.04	7.0	351.33×213.45
	2	2.0	253.76×278.19	5.0	326.24×178.91	7.0	298.56×166.54	8.0	396.70×375.96
	3	2.0	293.26×438.19	5.0	290.79×279.49	7.0	327.63×163.59	8.0	301.78×104.11
	4	3.0	149.19×311.24	5.0	159.49×275.80	7.0	174.43×245.35	8.0	289.43×210.29
T2	1	0.5	141.83×165.48	2.0	171.26×172.08	3.0	284.35×193.98	5.0	224.31×112.45
	2	1.0	92.07x 247.36	2.0	162.67×111.20	3.0	196.93×239.43	5.0	208.69×168.02
	3	2.0	110.45×107.63	3.0	106.83×176.69	4.0	134.50×133.40	7.0	184.02×231.98
	4	2.0	120.58×401.89	3.0	132.49×171.67	4.0	114.93×127.45	7.0	299.04×163.48
T3	1	0.5	32.36×21.53	2.0	171.35×115.82	3.0	116.63×84.49	4.0	104.38×135.39
	2	0.5	177.76×114.47	2.0	160.43×200.47	3.0	109.69×177.80	4.0	192.98×160.83
	3	1.0	82.13×77.44	2.0	275.03×131.10	4.0	102.41×215.18	5.0	217.65×137.32
	4	1.0	125.25×98.51	3.0	148.93×124.27	4.0	226.60×119.59	5.0	243.19×97.09

*Biofloc volume control (<5-15 mL L^{-1})[19]

tanks, especially in the biofloc treatment tank. According to Browdy *et al.*[13], dissolved oxygen gets fluctuated as in super intensive culture system where the microbial community consume as much DO. Ammonia nitrite and nitrate in the biofloc in T1, T2 and T3 was observed decreased started from day 60 to day 90 until end of culture. The TOC decreased only in biofloc treatment T1 while, higher TOC was recorded in other treatment tank. According to Avnimelech[14] biofloc technology can support nitrogen removal even when the organic matter and biological oxygen demand is higher in the system. Perez-Rostro *et al.*[2] found out that the microorganisms present in the biofloc maintain the water quality because they decrease nitrogen compound. From this study, the bacteria in the biofloc uptake the ammonium and

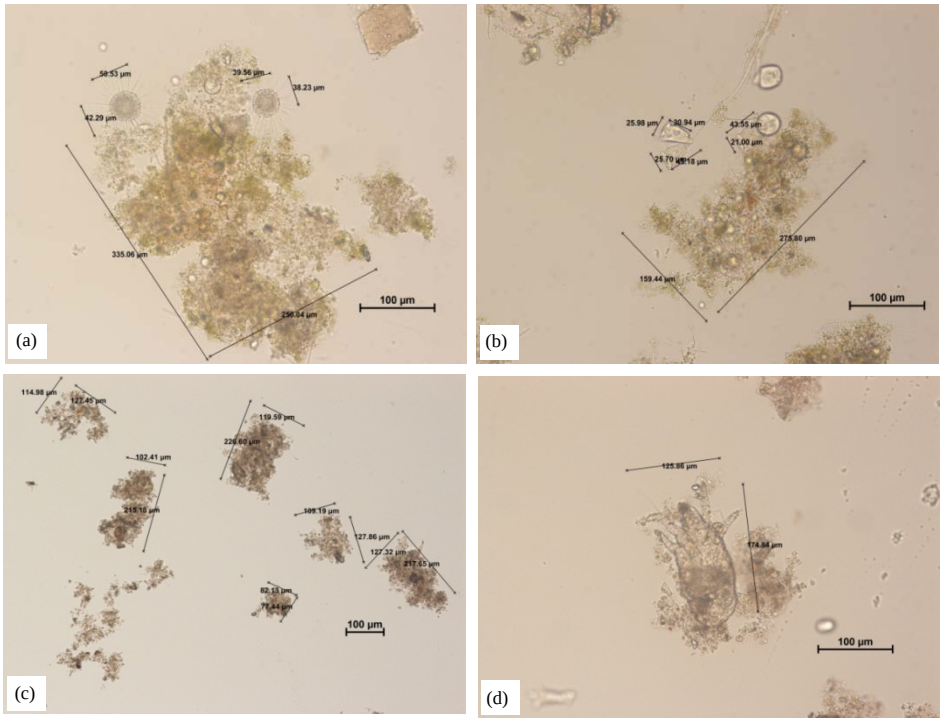

Fig. 5(a-d): Microscopic observation of water samples from treatment tanks under 100x magnifications via advance microscope 80i, (a) Large size of biofloc conglomeration with appearance of radiolarian from T1, (b) Medium biofloc conglomeration size was observed with the present of protozoa *Vorticella* sp., from T2, (c) Small conglomerate of biofloc from T3 and (d) biofloc with nauplii copepod from T3

control the product of waste in the tank. According to Ju *et al.*[15] microalgae aggregate in the biofloc assimilate ammonia and nitrate in the water and utilize the compound to build cellular protein which some algae mostly diatom provide nutritional feed to the shrimp. Kuhn *et al.*[16] found out that bacteria from chemoautotrophic nitrifiers can oxide ammonia and reduce toxicities of nitrate produced in the water. In this study, the biofloc in T1 was observed have the higher volume of biofloc and larger size of aggregate floc compared to T2 and T3. This is because the water in T1 was observed with green colour ofwater which contained dominant phototropic organisms which efficiently use the energy from sunlight for food synthesizing. That is why higher volume of biofloc and larger size of floc aggregation was observed in T1. According to Perez-Rostro *et al.*[2] in the microbial biofloc system productivity, microbe depends directly on the sunlight where sunlight is the main sources of energy for phototropic microorganism like algae and vascular plant. In this study it can be observed that different coloration of tank water consists of different group of microbes. The condition was caused by the phytoplankton, which was dominant in the tank that blooms earlier than other type of plankton and dominate in the water medium. In T1, the transition in the colour of water was observed from light green to dark green colour indicating the abundance of phytoplankton consist of green algae like *Chlorella* sp., euglena, cyanobacteria or phototrophic bacterial like *Ossilatoria* sp., *Gomphosperia* sp. and also protozoan like *Vorticella* sp. The water in T2 tank was brownish green indicating the presence of phytoplankton from class bacillariophyte such as *Navicula* sp., *Cyclotella* sp., *Cosinodiscus* sp. and cyanobacteria such as *Ossilatoria* sp., *Gomphosperia* sp., also presence of zooplankton rotifer in the water medium. In T3 the water was dark brown in colour which indicates it consist of diatom such as *Navicula* sp., *Nitzchia* sp. and *Cosinodiscus* sp. Dinoflagellates such as *Protoperidinium* sp. and *Alexandrium* sp., was observed in the water column with the appearance of nematode parasite, ciliate and *Vorticella* sp., protozoan and zooplankton grazers such as copepod and rotifer observed in the water column. Heterotrophic bacteria such as *Pseudomonas aeroginosa* also was indentified from the water column that consist of floc aggregation. Decomposer bacteria such as *Aeromonas salmonicida* and *Aeromonas hydrophilla* also was identified from the floc that decomposed all the organic matter in the culture tank. The mean size of PL 115 shrimp in the biofloc

tanks was around 15.7-23 gram per individual shrimp as compared to shrimp in control tank with average weight around 11.5-12.9 gram per individual shrimp. This proved that biofloc not only stabilized water quality, but also supplied nutritional protein diet to the cultured shrimp. Schneider *et al.*[17] found out that with BFT, the nitrogenous waste was converted into bacterial biomass that contains protein through additional of carbon sources. De Muylder *et al.*[6] and Emerenciano *et al.*[18] stated that biofloc provides essential high quality protein for the shrimp as additional feed that enable fast growth in the shrimp with low Feed Conversion Ratio (FCR). Perez-Rostro *et al.*[2] also reported that Malaysian prawn, *Macrobrachium rosenbergii* cultured in the biofloc system reached a higher weight around 15 g than those cultured in the normal culture system. One of the advantages of biofloc systems is reducing the amount of water required for water quality maintenance which no water replacement is needed during the whole culture period. In this study, no water exchange was done in biofloc culture tanks. However, a small amount of water was added after the removal of wastes in the tank to maintain the water level. Biofloc help maintaining the water quality and prevent it from fluctuation. Avoiding the water exchange can also increase Biosecurity as the water is often the source of pathogen[2,6].

CONCLUSION

The BFT was meant to maintain the good water quality for better aquaculture management. Other than supplying additional nutritional diet to the shrimp, BFT can help reduce environmental damage cause by aquaculture, reduce the use of water, decrease the FCR and feed costing as well as enabling efficient energy use. With BFT application system, expectedly will help spur the sustainable aquaculture practise in the future.

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