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Research Article
Effects of Biofloc Application on Survival Rate, Growth Performance and Specific Growth Rate of Pacific Whiteleg Shrimp, *Penaeus vannamei* Culture in Closed Hatchery System

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Abstract
Background and Objective: Biofloc application has been introduced in aquaculture in order to reduce the nutrients level and sustain good water quality. Due to its importance, a study was conducted to identify the effect of biofloc application on shrimp growth performance, specific growth rate and survival rate in a closed hatchery system. **Materials and Methods:** Molasses as carbon sources were applied in ratio 10:1 for biofloc formulation and no addition of molasses in non-biofloc (clear water) treatment. One way ANOVA was applied to analyze the differences between biofloc treatments and clear water. **Results:** The survival rate of the shrimp was ranged between 23.69 and 98.77% for biofloc treatment, whereas 98.15-99.23% for non-biofloc treatment. The lowest survival rate (23.69%) was due to vibriosis infection in one of the biofloc treatment tanks. Growth performance was identified expedite in biofloc especially in (dark green) colour biofloc as compared to non-biofloc. The Specific Growth Rate (SGR) for Body Weight (BW) was identified expedite around (3.25-4.06) g day^{-1} for biofloc treatment compared to non-biofloc around (2.74-3.93) g day^{-1}. The SGR for (TL) also identified expedite around (2.12-2.45) cm day^{-1} for biofloc, compared to non-biofloc (clear water) around (1.71-2.13) cm day^{-1}. **Conclusion:** It can be concluded that the biofloc application successfully improved the shrimp performance and at the same time become the additional natural diet to the shrimp respectively. However, further study needs to be conducted to improve the survival rate and prevent vibriosis infection by using the biofloc system in the future.

Key words: Biofloc technology, molasses, whiteleg shrimp, survival, growth, ecosystem, *P. vannamei*

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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

Biofloc can be defined as an aggregation of algae, bacteria, protozoa and particulate organic matter such as uneaten feed that held together to form a biofloc[1]. Another definition by Ahmad *et al.*[2] defined biofloc as a conglomeratic aggregation of microbial such as phytoplankton, bacteria, living and dead particulate organic matter. Kasan *et al.*[3] defined biofloc as a new green technology which can help to reduce the environmental damage. Previous studies[4-7] found that the application of biofloc in the culture system could enhance and improve shrimp growth performance. The biofloc technology is cost-effective and environmental friendly which helps in developing sustainable aquaculture[8]. The application of biofloc in the grow-out system could improve productivity by 8-43% as compared to the non-biofloc system[9]. Bossier and Ekasari[10] recognized biofloc as a technology that can improve productivity and reduce damage to the ecosystem. Gonzalez *et al.*[11] discovered that the shrimp culture in biofloc treatment have 100% survival rate even though the feeding rate was reduced.

Biofloc can provide an additional food source to the shrimp in the limited or zero water exchange system[12]. There was a variety of concentration of amino acids such as alanine, glutamate, arginine and glycine present in the biofloc that can be consumed as a shrimp diet[13,14]. McIntosh *et al.*[15] identified that the microorganism in the biofloc plays a major roles in nutrient cycling, maintains water quality and nutrition sources to the cultured animals. Ahmad *et al.*[2] discovered that biofloc technology is a sustainable and eco-friendly method in aquaculture to control water quality and as an additional proteinaceous feed to the culture animals. Avnimelech and Kochba[8] found out that biofloc technology is a cheap technology and environmentally friendly to be applied for the sustainable aquaculture. Bossier and Ekasari[10] recognized that biofloc technology can improve the aquaculture production, produce higher productivity and give less impact to the environment. Despite there is many studies on the effectiveness of biofloc in controlling the water quality in aquaculture, there is still lack of studies on the effect of biofloc to the shrimp performance and its effect on the survival rate. Due to the importance of biofloc application in aquaculture sectors, study on the effect of biofloc application to the shrimp growth performance, specific growth rate and survival rate were conducted to determine the effectiveness of biofloc application to the shrimp growth performance and survival rate by culture in the closed hatchery system.

MATERIALS AND METHODS

Water parameter, biofloc formulation and shrimp culture in hatchery: This study was conducted at the marine hatchery of the Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu (UMT) from July until September of 2016. Six treatments were introduced consists of biofloc (dark green, brown green, dark brown) and clear water system (non-biofloc 1, non-biofloc 2, non-biofloc 3) with a capacity of 10 ton per tank. Postlarvae, PL8 were stocked in the treatment tank with a stocking density of 100/m^3. Water in the clear water system was an exchange about 20% of the total capacity once in two weeks. Water parameters were monitored by using YSI multiprobe 556 for in-situ measurement of temperature, salinity, pH, Total Dissolved Solids (TDS) and Dissolved Oxygen (DO).

For biofloc formulation in the biofloc treatment tank, molasses as carbon sources were used in C: N ratio 10: 1. Molasses were mix up with the artificial pellet before being poured into the biofloc treatment tank. This process was done three to four times in a week until the formation of biofloc in the biofloc culture tank. The feed from Gold Coin brand was fed to the shrimp with 10% biomass started from feed no 1 until feed no 4 which was the growth up pellet size. Shrimp were fed four times daily at 08:00, 14:00, 20:00 and 02:00 hrs. All the uneaten feed and the dead organic matter in the pond bottom were siphoned twice a week to maintain the good water quality in the culture system.

The coloration of the biofloc was identified from the treatment tank in the hatchery and data were recorded. For the samples collection and identification, the biofloc samples were collected using 20 micron sieve net and were fix in 10% formalin. Biofloc samples containing microorganisms were identified referred to ALGAEBASE database and identification was done using compound Advance microscope Nikon 80i in the laboratory.

Shrimp growth performance based on specific growth rate (SGR) and survival rate (SR): For the analysis of shrimp growth performance, 20 shrimp samples were collected randomly from each treatment tank. The Body Weight (BW) of shrimps was measured using a microbalance with an accuracy of 0.0001 g and the Total Length (TL) was measured using digital vernier calliper with an accuracy of 0.1 mm, respectively. The shrimp were culture for 100 days until reach market size for the final measurement of TL and BW. The Specific Growth Rate (SGR) was calculated using the formula by Bautista-Teruel *et al.*[16]. The SGR and survival rate were measured by using formula as:

$$\text{SGR by BW} = \frac{100\ [\ln \text{ mean final BW (g)} - \ln \text{ mean initial BW (g)}]}{\text{Culture period (day)}}$$

$$\text{SGR by TL} = \frac{100\ [\ln \text{ mean final TL (mm)} - \ln \text{ mean initial TL (mm)}]}{\text{Culture period (day)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of shrimp}}{\text{Initial number of shrimp}} \times 100$$

Statistical analysis: All data were analyzed by using SPSS Statistics 17.0 software. For data Specific growth rate SGR, survival rate and growth performance, T-Test and One-Way ANOVA was used to analyze the differences between the biofloc treatments and control (clear water). The relationship between total BW and TL were analyzed using correlation (Two-Tailed Test).

RESULTS

Water parameter and biofloc composition: From the result of the water parameter (Table 1), it was determined that DO was lower in the biofloc treatment (5.9-7.3 mg L^{-1}) as compared to non-biofloc (clear water) treatment (6.9-7.5 mg L^{-1}) observed in the middle of the culture period. For salinity, temperature, TDS and pH there was some differences between the biofloc and non-biofloc treatment tank.

Growth performance, specific growth rate (SGR) and survival rate: For the growth performance of non-biofloc (clear water), the result for final BW was in ranged between 11.55 and 12.93 g, meanwhile, for biofloc treatment, the final BW was around 15.74-23 g. The biofloc 1 (dark green) recorded the highest shrimp growth performance for BW followed by biofloc 2 (brown green) and biofloc 3 (dark brown) (Fig. 1). For growth performance final TL for non-biofloc treatment the results was 10.14-10.56 cm and for biofloc treatment tank was around 13.32-14.72 cm. The biofloc 1 (dark green) also has the highest post-larvae performance for TL followed by biofloc 2 (brown green) and biofloc 3 (dark brown). The non-biofloc (clear water) have slow growth performance as compared to culture in biofloc system (Fig. 2). The mean±SD for BW was 15.38±4.16 g and the mean total length; TL was 12.15±2.01 cm. For the statistical analysis using

Table 1: Water parameter identified in the middle of culture period of *P. vannamei* shrimp for biofloc and non-biofloc (clear water) treatments

Water parameter	Non-biofloc 1	Non-biofloc 2	Non-biofloc 3	Biofloc 1 (dark green)	Biofloc 2 (brown green)	Biofloc 3 (dark brown)
DO (mg L^{-1})	7.54	6.90	6.92	7.30	5.90	6.37
Salinity	32.55	33.46	32.41	31.60	33.45	31.28
pH	7.42	7.83	7.99	7.19	7.38	7.43
Temperature	27.53	27.04	26.57	27.90	27.89	27.91
DO (%)	103.30	99.40	98.90	94.00	87.20	92.10
TDS	33.49	33.13	32.43	32.60	32.53	32.40

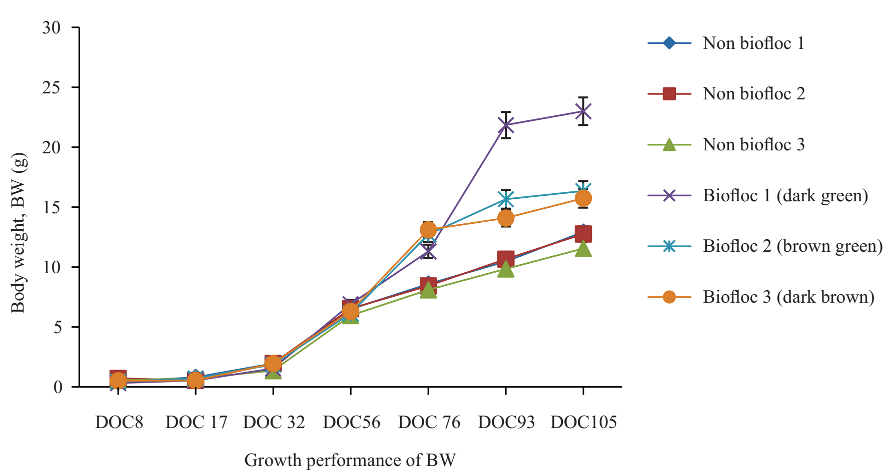

Fig. 1: Growth performance of body weight from non-biofloc (clear water) and biofloc treatments

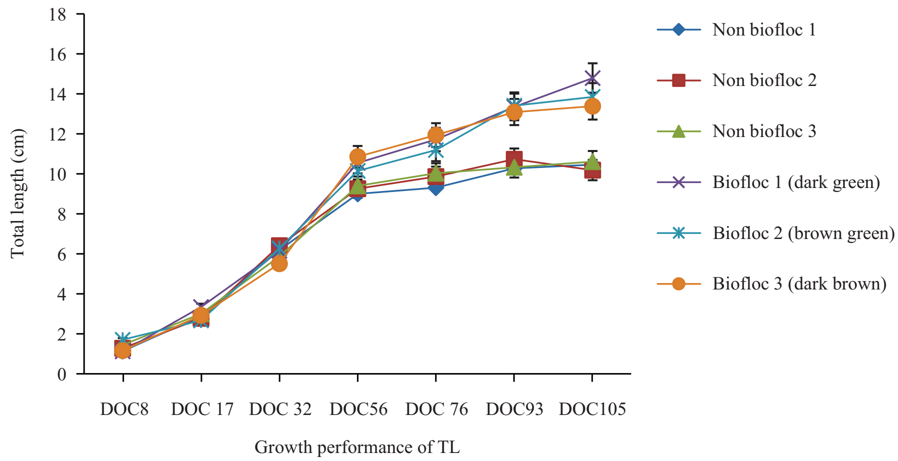

Fig. 2: Growth performance of total length from non-biofloc (clear water) and biofloc treatments

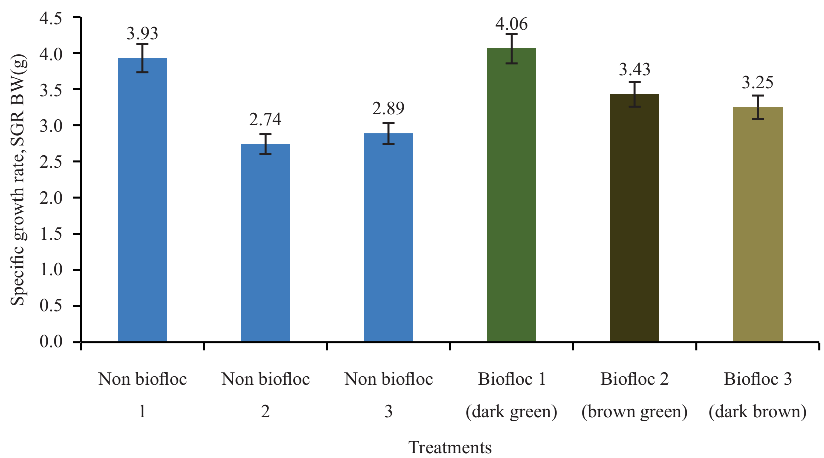

Fig. 3: Specific growth rate, SGR for body weight for non-biofloc (clear water) and biofloc system

One way ANOVA, for body weight, BW there is no-significant differences among treatment where $p = 0.880$, $p>0.05$, $F = 0.349$ and for total length, TL there were also no-significant different among biofloc and non-biofloc (clear water system) where $p = 0.960$, $p>0.05$ and $F = 0.200$. For the relationship between BW and TL, there was a significantly different where $p = 0.018$, $p<0.05$ using correlation two-tailed test between the BW and TL of biofloc and non-biofloc (clear system) treatment tank.

For specific growth rate of body weight, SGR BW the result for non-biofloc was around (2.74-3.93) and for biofloc was around 3.25-4.06 (Fig. 3). The SGR TL for non-biofloc was around 1.01-2.13 and for biofloc treatment, the SGR TL was around 2.12-2.45 (Fig. 4). The mean±SD for SGR BW was (3.38±5.35) and the mean±SD for SGR TL was (2.13±0.24). The statistical analysis by using One way ANOVA found out that there was non-significantly different $p>0.05$ where $p = 0.06$ for SGR TL and $p = 0.287$ for SGR BW between the treatments.

For survival rate, there was a higher survival rate identified from non-biofloc around (98.15-99.23%) as compared to biofloc treatment tank around (23-98.7%) (Fig. 5). The mean±SD survival rate identified was around (83.71±12.24%). Statistical analysis using the T-test identified that there were significantly different $p<0.05$ where $p = 0.001$ between the survival rate from non-biofloc and biofloc treatment tank.

Microorganisms identified in the biofloc: Figure 6a showed the colour of the biofloc for biofloc 1 (dark green) floc with appearance of zooplankton copepod, (b) biofloc 2 (brown green) floc with appearance of rotifer, (c) biofloc 3 (dark

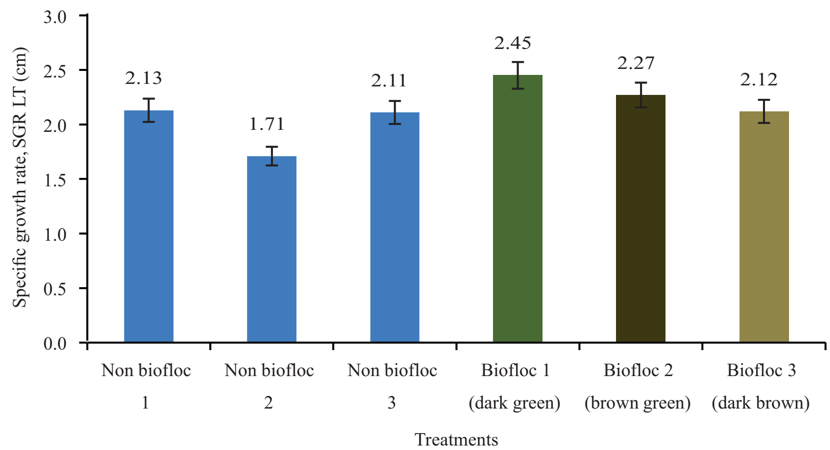

Fig. 4: Specific growth rate, SGR for total length for non-biofloc (clear water) and biofloc system

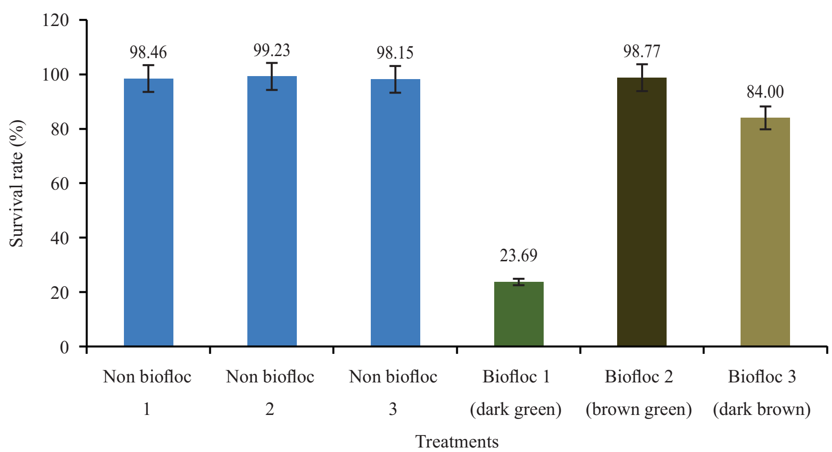

Fig. 5: Percentage of survival rate in non-biofloc (clear water) and biofloc treatments

brown) floc with appearance of *Navicula* sp. diatom and (d) for non-biofloc (clear water system) with appearance of diatom *Nitzschia* sp. All the differences coloration of biofloc contains difference type of phytoplankton and microorganisms that aggregates together and makes the biofloc colour differences (Table 2). In the dark green biofloc treatment, the type algae come from green algae, diatom and from blue green algae or cyanobacteria also zooplankton such as copepod and rotifer group. In the brown green typically from diatom, green algae and dinoflagellate and also from rotifer group and in dark brown contain diatom, green algae, copepod, rotifer and protozoa. In the clear water system mostly contains of diatom, dinoflagellate and protozoa group.

DISCUSSION

From the result of the water parameter in the middle of culture period, there was lower dissolved oxygen identified from the brown green color of water in biofloc tank. This might be due to the organisms and algae in the biofloc that used the oxygen for respiration and also for the degradation process of the uneaten feed and dead algae by the heterotrophic bacteria in the biofloc. Hargreaves[1] also discovered the fluctuation of dissolved oxygen in the biofloc system where algal activity in predominant due to the high loaded of nutrient from the feeding. Other study done by Manan *et al.*[17] also identified that abundance of phytoplankton, zooplankton, protozoa, nematodes, algae also heterotrophic bacteria from *Pseudomonas* sp. and *Aeromonas* sp. accumulated together in the biofloc. Oxygen consumption by this type of organism might be the reason for the lower DO in biofloc (brown green) colour during culture treatment. Hargreaves[1] also found out the in indoor brown water biofloc system have normally about 6 mg L^{-1} that containing bacteria and algae due to respiration was quite similar with the result of DO achieved in biofloc (brown green) colour treatment.

Table 2: Biofloc composition in each treatment for biofloc and clear water treatments

Treatment	Color type	Phytoplankton	Zooplankton
Biofloc 1	Dark green	*Gomphoperia* sp.	*Brachionus* sp.
		Oscillatoria sp.	Protozoa
		Chlorella sp.	Nematode
		Chlamydomonas sp.	Copepod
		Tetraselmis sp.	
		Nitzschia sp.	
Navicula sp.			
Licmophora sp.			
Amphora sp.			
Cymbella sp.			
Biofloc 2	Brown green	*Nitzschia* sp.	Copepod
		Leptocylindrus sp.	*Brachionus* sp.
		Navicula sp.	Protozoa
		Cyclotella sp.	Nematode
		Chlorella sp.	
		Borodinellopsis sp.	
		Scenedesmus sp.	
		Tetraselmis sp.	
		Protoperidinium sp.	
		Alexandrium	
Biofloc 3	Dark brown	*Nitzschia* sp.	Copepod
		Leptocylindrus sp.	*Brachionus* sp.
		Cyclotella sp.	Gastrotrich
		Licmophora sp.	protozoa
		Navicula sp.	nematode
		Chlorella sp.	
		Gloeocapsa sp.	
		Borodinellopsis sp.	
Non-biofloc 1	Clear water	*Cosinodiscus* sp.	*Brachionus* sp.
		Navicula sp.	Protozoa
		Chlamydomonas sp.	
		Amphora sp.	
		Cymbella sp.	
Non-biofloc 2	Clear water	*Nitzschia* sp.	Copepod
		Chlorella sp.	Protozoa
		Cosinodiscus sp.	
		Melosira sp.	
		Navicula sp.	
		Protoperidinium sp.	
Non-biofloc 3	Turbid	*Nitzschia* sp.	Protozoa
		Cosinodiscus sp.	*Paramecium* sp.
		Navicula sp.	

The shrimp cultures in biofloc system also were identified to have the fastest growth performance as compared to culture in clear water system. Hargreaves[1] identified from the research been conducted that the shrimp culture in biofloc water containing growth enhancing factors such as microbial, animal proteins that boost up the shrimp performance. Previous study by Xu and Pan[7] identified that biofloc can improve the growth performance and feed utilization of the shrimp through the supplemental food source from the biofloc. Other recent studies[13,18,19] identified that the dietary in the biofloc successfully enhance the growth performance of the shrimp culture. Lee *et al*.[20] also found out that biofloc is a good source of dietary which give beneficial effects on growth performance and health of the shrimp culture. Kim *et al*.[21] also found that biofloc contribute to the growth of *P. vannamei* shrimp larvae however; do not affect on the survival and growth of other penaeids shrimp. All of the recent studies totally supported that the biofloc is effective in enhancing the growth performance of the shrimp in the culture system.

The survival rate was identified low in the dark green biofloc as compared to the other two type of biofloc that have higher survival rates such as in the clear water treatment. This is due to the mortality during early stages because of the vibriosis infection to the shrimp post-larvae in the dark green biofloc treatment. From a previous study done by Luis-Villasenor *et al*.[22] identified vibrionaceae in both biofloc

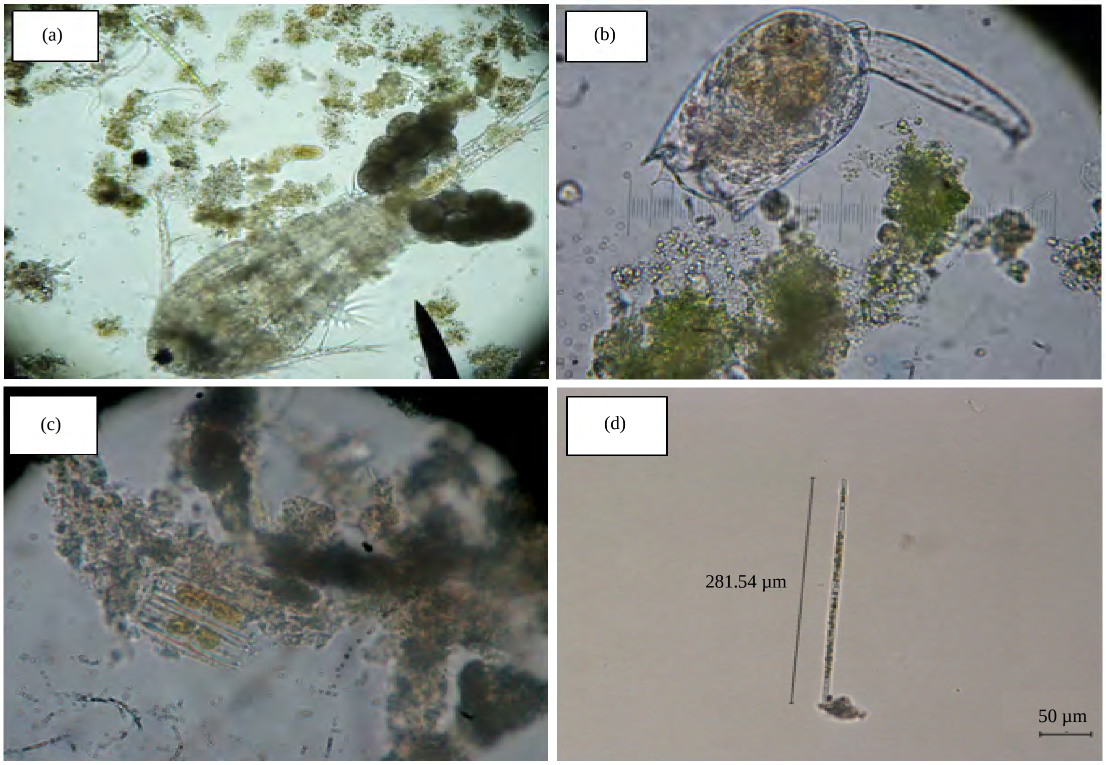

Fig. 6(a-d): Difference coloration of biofloc
(a) Biofloc 1 (dark green), (b) Biofloc 2 (brown green), (c) Biofloc 3 (dark brown) and (d) From non-biofloc (clear water) treatments

and non-biofloc systems of culture however the diversity was higher in control compared to biofloc treatment. However for other biofloc treatment, the survival rate was reasonably high up to 98%, as similar to the survival rate of clear water system. Manan *et al.*[17] also identified higher survival rate with more than 90% in the culture system using biofloc treatment. Other study conducted by Emerenciano *et al.*[12] also found out the presence of biofloc increased the survival and growth rate of *F. paulensis* shrimp due to the nutritional source from the biofloc to the postlarvae shrimp. Kim *et al.*[23] also discovered that biofloc can control pathogenic bacteria and enhance shrimp immunity and other study by Hotowitz and Horowitz[24] found out that culture using biofloc system can reduce the potential of disease outbreaks. All of the recent studies support that biofloc can increase the chances of the survival rate for the culture animals. However the lower survival rate achieved in dark green biofloc could be treated if early detection of the vibriosis infection was identified in the biofloc treatment.

CONCLUSION

From the results achieved, it can be concluded that shrimp culture in the biofloc system was really helpful in improving the shrimp growth performance. The shrimp has the fastest growth rate as compared to culture in non-biofloc (clear water system) respectively. A variety of microorganisms became sources of protein in the biofloc and also as an additional diet to the shrimp. The biofloc also identified successfully as a shrimp growing promoter. However, further study should be conducted to improve the survival rate of the shrimp and also to prevent the vibriosis infection during early stage of culture with the application of biofloc technology. With some modification, good adjustment and practices, hopefully, biofloc technology could help optimize the shrimp survival rate and performance and also could help increase the shrimp production in the future.

SIGNIFICANCE STATEMENT

This study discovered that shrimp that cultured in the Biofloc system have a faster growth rate as compared to the shrimp cultured in clear water system. Biofloc as the proteinaceous diet proved in improving the growth performance and survival rate of the whiteleg shrimp, *P. vannamei* during the culture period.

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