

Application of biofloc technology (BFT) in shrimp aquaculture industry

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Abstract. An environmental friendly technology known as Biofloc Technology (BFT) has been implemented to reduce environmental damages and to sustain production of Pacific Whiteleg shrimp, *P. vannamei* aquaculture industry. Since there was no intensive application of BFT being conducted in industrial scale, this study aimed to assess the impact of BFT to water quality and shrimp health. To achieve maximum growth of BFT in *P. vannamei* cultures, an isolated biofloc boost-up bacteria inoculum was added during each new stocking program of shrimp post-larvae (PL). Samples of water, shrimp and biofloc were collected in every ten days interval started from new stocking of shrimp PL up to harvesting periods (± 100 days). Interestingly, biofloc was observed started to be formed as early as 10 days after shrimp cultivation periods, where the biofloc formation was speed up by the addition of the inoculum and thus effectively enhanced good water quality which resulted in the increasing shrimp biomass. By transferring the important knowledge of BFT to shrimp industrial scale, aggregation of microbial communities in biofloc were responsible in maintaining water quality and optimizing shrimp survival and production. Thus, knowledge on microbial composition in biofloc is deemed important for successful design and application of BFT for sustainable shrimp aquaculture industry.

Keywords: Biofloc technology, *P. vannamei*, shrimp post-larvae, water quality and shrimp health.

1. Introduction

Conventionally, biological filter was introduced in aquaculture system which beneficial microbes was used to promote the growth of cultured organisms. It was also helps in maintaining the water quality in recirculating or closed loop systems to remove ammonia, nitrates and dissolved organic solids. In addition to the conventional biofilter systems, the biofloc technology (BFT) is recently proposed as an alternative solution for wastewater treatment and feed re-utilization [1][2], yet it is not completely suitable for small farms due to their intensive aeration, regular waste removal and requirement of additional carbon source to stimulate heterotrophic bacteria growth.

BFT is a technique of enhancing water quality through the addition of carbon sources to the aquaculture system that contain in the feed or external carbon sources. This technology could minimize the water exchange and water usage in aquaculture systems through maintaining adequate water quality within the culture unit, while producing low cost biofloc rich in protein, which in turn can serve as a feed for aquatic organisms [3][4]. Unlike the conventional methods such as biofilter,



BFT support nitrogen removal when the organic matter and biological oxygen demand of the water system is high [5]. The BFT is composed of a variety of microorganisms, uneaten feed, feces, detritus and suspended particles with water propulsion and aeration. The application of BFT system showed successful development in most countries around the globe such as South Korea, Indonesia, Malaysia, Thailand, China, Australia, Hawaii, Brazil, Ecuador, Peru, USA, Mexico, Guatemala and Belize [6]. However, certain BFT systems are not exposed to natural light for algal growth but installed indoor with no exposure to natural light. This system known as “brown-water” biofloc system when there only bacterial processed control the water quality in the system [7]. Thus, ammonia concentration in the tanks that are kept indoor and not exposed to sunlight are not controlled by the algal community but instead mainly controlled by the heterotrophic bacteria found in the system [8].

As compared to conventional method used in aquaculture, BFT provides more economical alternative by decreasing water treatment expenses more than 30%. Furthermore, a potential gain on feed expenses (e.g: the efficiency of protein utilization is twice as high in biofloc technology systems when compared to conventional method) making it economically sustainable for future development [1]. In general, BFT system would increase growth rate of *P. vannamei* as the shrimps that feed on the biofloc would obtained additional supplement nutrition from the assimilated nutritious planktons, bacteria and organic compounds. This study aimed to investigate the effect of BFT towards water quality condition, shrimp health and pond productivity. Thus, by implementation of BFT in Pacific Whiteleg shrimp, *P. vannamei* pond of business entity, shrimp will be healthier and grow optimally in aquaculture systems that contain high level of algae, bacteria and other natural biota.

2. Materials and methods

2.1. Setting-up biofloc facilities at *P. vannamei* culture ponds.

Pre-treatment of pumped seawater was prepared following standard operation procedure (SOP) developed by shrimp farmer (e.g: holding water, filtration, fertilized water etc.). Prior stocking of shrimp post-larvae (PL), two super-intensive 2-phase biofloc shrimp ponds consists of nursery (total area of 110 m³) and grow-out (total area of 1500 m³) ponds lined with High Density Polyethylene (HDPE) were constructed for setting-up the biofloc facilities. These ponds were set up with optimized designs and positions of paddle wheel in order to maximize the flow rates for BFT formation while enhancing shrimp growth performance.

As the bio-security features are improved, concurrently, it also allows super-intensive farming with stocking density of up to 350 PL per meter square (m²) were practiced. This could compensate the additional cost spent for the development of the new system. In the ratio of one liter inoculum (10^{x9}) per hectare pond, isolated biofloc boost-up bacteria was used as inoculum and injected ‘Figure 1’ to the prepared ponds during each new stocking program of shrimp PL and transferring of adapted shrimp PL into grow-out program (±30 days). As a control, similar design of 2-phase super intensive shrimp ponds were also stocked with shrimp PL following prepared SOP by the shrimp company.

2.2. Formulated conditions for biofloc formation

The most important parameter that needs to be controlled during the implementation of BFT is the ratio between carbon (C) and nitrogen (N) [5]. The C/N ratio of 15 was controlled through addition of commercialized molasses as carbon sources ‘Figure 2’. To calculate C/N ratio, protein percentage in the formulated feeds which contain nitrogen and carbon was firstly identified [9][5]. In our trials, we used 35% of feeds containing protein and daily addition of 630 gram of molasses to maintain C/N ratio of 15 throughout culture periods.

2.3. Development of biofloc technology

In every 10 days interval, the measurement of biofloc formation was performed in-situ using Imhoff cone [1]. The biofloc biomass was obtained after allowing the water to be completely settled for half an hour. The volume of formed biofloc was control between 4–8mL/L by daily addition of molasses to obtain maximal growth of BFT while maintaining water quality and shrimp productivity.

Similarly to biofloc sampling, measurements of water quality parameters including temperature ($^{\circ}\text{C}$), pH, salinity (ppt), dissolved oxygen (DO), nitrite, orthophosphate and ammonium were measured during each sampling date. The in-situ monitoring of temperature, pH, salinity, DO were measured using a multi-parameter probe (YSI data logger). On the other hand, nitrite, orthophosphate and ammonium were analysed following standard method for Examination of Water and Wastewater[10] using diazotization, ascorbic acid and phenate method, respectively.

The occurrence of potential disease in both pond water and shrimp culture were determined by randomly sampled of water and shrimps from each pond. Those samples were analyzed using nPCR (e) for detection of Early Mortality Syndromes (EMS) in water, while nPCR for White Spot Syndromes Virus (WSSV), EMS and Enterocytozoonhepatopenaei (EHP) in shrimp PL.



Figure 1. Injection of prepared biofloc boost-up bacteria as inoculum (starter culture).



Figure 2. Addition of molasses as carbon sources to achieve C/N ratio of 15.

3. Results and discussion

Water quality parameters including temperature ($^{\circ}\text{C}$), salinity (ppt), pH, dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2^-) and ortho-phosphate (PO_4^{3-}) were measured during each sampling day.

3.1. Assessment of the physicochemical properties of water from *P. vannamei* ponds

3.1.1. Temperature. In this study, temperature of *P. vannamei* pond was measured *in-situ* using YSI multi-parameter. The mean temperature throughout each day of culture (DOC) was ranged between 27.6°C and 30.5°C (table 1). The optimum water temperature for shrimps culture was reported between 25°C to 31°C [11]. In this study, range of temperature in *P. vannamei* pond was between 27.6°C and 30.5°C and was still within the optimum range. This is important as temperature can influence shrimp metabolism, feeding rate, oxygen consumption as well as survival and tolerance towards toxic metabolites [12]. It was reported that low temperature can influence outbreak of White Spot Syndrome Virus (WSSV) in shrimps as sudden change in temperature affects shrimps immune system [12].

3.1.2. Salinity. Salinity level for each DOC of shrimp pond was measured using YSI multi-parameter. In this study, salinity was ranged between 20.0 ppt and 37.0 ppt (table 1). According to [13], the optimum salinity for shrimp farming was between 15 ppt to 25 ppt. Although the obtained salinity range in this study were exceeded the optimum range, it has been reported that *P. vannamei* can tolerate with a wide range of salinity between 1 ppt and 40 ppt [15]. Salinity is dependent on amount of rainfall and rate of evaporation [16]. This explained high salinity for almost all DOC due to hot and sunny day weather conditions during El-nino phenomenon in mid-2016 which increase water temperature drastically. Less amount of rainfall and high evaporation rate contributed to high salinity in shrimp pond.

3.1.3. pH. pH refers to the degree of alkalinity or acidity in the water sample. In this study, the pH value of each DOC of shrimp pond was measured (table 1). pH is very crucial for survival and growth of shrimp culture as it affects metabolism and other physiological process of shrimps [12]. [11]Has reported that the ideal pH for shrimps culture was between 6.8 to 8.7. In this study, range of pH recorded was in the optimum range for shrimp ponds. Low pH in shrimp ponds has been reported as the cause for infection of White Spot Syndrome Virus (WSSV) in shrimps[12]. pH range from 7.0 to 8.5 has reported to be the optimum pH range for biofloc production which favors functioning of biological cycles in biofloc systems [14]. While most bacteria grow best in pH range near neutrality; 6.5 to 7.5, it was reported that the optimum pH for nitrifying bacteria was 8.

3.1.4. Dissolved oxygen. DO of each DOC in shrimp pond was showed in (table 1). DO was an important variable that influences the growth of aquatic organisms [13]. According to [14], DO level in aquatic systems should not less than 5 mg/L and optimum DO level range for maintaining biofloc system was between 7 and 8 mg/L in order to avoid competition of oxygen demand. As bacteria and algae that form biofloc also have oxygen demand, competition can easily occur in a biofloc pond system [14].

Aeration will also improve growth of microalgae [15] and production of oxygen from microalgae through photosynthesis will increase DO level in the shrimp ponds. According to [17], DO level in shrimp pond depends on physical, chemical and biochemical activities prevailing in the water body. Factors such as sediment oxygen uptake, photosynthesis, plankton respiration, animal respiration and air-water gas transfer influenced the DO concentration in aquaculture [17].

Table 1. Summary of physicochemical parameters of water during sampling periods in both treatment and control ponds of *P. vannamei* culture. Noted that all parameters were under acceptable ranged for *P. vannamei* culture.

Parameters	Unit
Temperature	27–30.5°C
pH	6.7–8.5
Salinity	20.0–37.0ppt
Dissolved Oxygen (DO)	4.80–7.0 mg/L

3.2. The impact of biofloc application in reducing ammonia, nitrite and ortho-phosphate concentration

In this study, ammonia, nitrite and ortho-phosphate were analysed following standard method by using phenate, diazotization method and ascorbic acid [18], respectively. 'figure 3' showed ammonia, nitrite and ortho-phosphate concentration for each DOC of shrimp pond. It was found that the concentration of ammonia was highest during DOC 50 with 2.3 mg/L whereas, the lowest concentration of ammonia was shown during DOC 0 with 0.03 mg/L. Ammonia concentration showed the lowest concentration during DOC 0 and increased gradually towards DOC 40. However, ammonia concentration in the shrimp pond once again showed declining at DOC 50 and DOC 60.

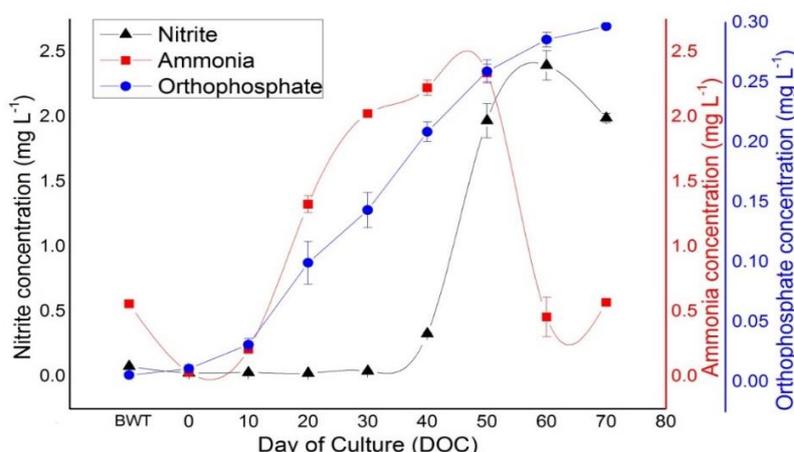


Figure 3. The ammonia, nitrite and ortho-phosphate concentration in each DOC of shrimp pond. The error bar indicates standard deviation of nutrients (nitrite, ammonia and orthophosphate) for each DOC.

Nitrite concentration was the highest during DOC 60 with 2.40 mg/L while DOC 20 was found to have the lowest nitrite concentration with only 0.02 mg/L. Throughout all DOC, nitrite concentration showed increasing trend from DOC 30 to DOC 60. However, at DOC 70, nitrite concentration showed declining.

Apart from nitrite and ammonia, ortho-phosphate was also analysed. The highest ortho-phosphate concentration was in DOC 70 with 0.30 mg/L while DOC 0 showed the lowest ortho-phosphate concentration of 0.01 mg/L. Ortho-phosphate concentration has found to be increased from BWT to DOC 50. However, the concentration has been decreased during DOC 60 and DOC 70.

3.3. The impact of biofloc application in improving shrimp health and productivity

The findings of this program was successfully provide substantial evidence that implementation of biofloc in *P. vannamei* culture had enhanced the protection of shrimp culture towards any diseases.

Evaluation on the effectiveness of biofloc towards the growth of *Vibrio* spp. showed negative results throughout all DOC of shrimp cultures, resulting no indication of disease occurrence which causing EMS, WSSV and EHP outbreak in shrimp pond. Similarly to treatment pond, the control pond showed no disease outbreak during trials as the SOP of the company were also implemented commercial probiotic product to prevent any incoming sources of pathogenic bacteria.

A variation in BFT pond production with an average of 3.1 tons per pond per cycle was achieved (table 2). This is equivalent to average of 2.06 kg/m² of shrimp production. As for comparison, the control pond generates between 0.8 to 1.0 kg/m² of shrimp production. From the population density between 240,000 to 340,000 pieces, an average survival rate (SR) of 89% was achieved. This finding showed that with high stocking density, the 89% of SR achieved is relatively high and sustainable.

As mentioned earlier, the system is designed to accommodate high stocking density of up to 350 juvenile per m² and capable to produce up to 4 tons of shrimp per pond per cycle. The stocking density will slowly be increased and adjusted to achieve the targeted figure in the most optimum condition. It was expected that the new system is confidently promising to be able to generate a consistent and stable production in the future.

Table 2. Comparison of survival rate and shrimp production in control pond and super-intensive 2-phase biofloc system.

Production (1500m ² pond)	Grow-out 1 (Treatment-BFT)	Grow-out 2 (Control)
	Survival Rate (%)/ Production (kg)	Survival Rate (%)/ Production (kg)
Cycle 1	82/2471	-
Cycle 2	98/2646	88/2568
Cycle 3	94/3602	82/2541
Cycle 4	83/3608	82/3170

4. Conclusion

The main output of this program had provided clear understanding of BFT as new inventive technologies which resulted in competitive market pricing in terms of production expansion and sustainability. This study proved that optimum ranged of water quality status for shrimp growth was achieved with low quantity or almost none of sludge was accumulated. Biofloc build-up was purposely speed up by the addition of the inoculum and thus effectively enhance the biofloc formation and contributed towards good water quality which result in the optimum shrimp growth productivity.

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