Microbubble Aeration: The Effects on the Nursery Stage of *Litopenaeus Vannamei* Biofloc Culture System

Noor Ezzah Rahimah Ahmad Samsuri[1], P. Ganesan[1]*, Iswadi Jauhari[1], G.Y.A Tan[2]

[1]Department of Mechanical Engineering, Faculty of Engineering, Universiti Malaya, 50603, Kuala Lumpur, Malaysia

[2]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

*Corresponding Author

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ABSTRACT

This study presents the effects of nano/micro bubble (MB) aeration on *Litopenaeus vannamei* (whiteleg shrimp) for 30 days of nursery stage. Similar studies were carried out using conventional aquarium air stones that produce larger air bubbles (LB) as a point of reference. The water quality parameters: dissolved oxygen (DO), temperature, ammonia (TAN), nitrite, nitrate, and settleable solids (SS) were recorded throughout the culture days. The shrimp growth parameters in terms of body weight and length, feed conversion ratio (FCR), and survival rate for 30 days of culture were compared. The results showed that microbubble aeration significantly improved the DO of the water, accelerated TAN to nitrate conversion and yielded a higher amount of biofloc than the LB system. Despite the microbubble considerably raising the temperature and lowering the pH, the water quality parameters of MB aeration remained consistent and within the acceptable range. The growth of shrimps in the MB aeration system was significantly larger (increased by 34%) and had achieved a lower FCR value (reduced by 26%) in comparison to that of the LB aeration system. This study found that MB aeration promotes the production of biofloc and the growth of *L. vannamei*.

Keywords: Microbubble, Aeration, Biofloc, Litopenaeus vannamei, Feed conversion ratio (FCR), Aquaculture.

1 Introduction

Microbubbles are defined as bubbles with a diameter of less than 100 μm. Compared to larger bubbles (mm-cm), the properties of microbubbles are very distinct. Microbubbles have high inner pressure, a negatively charged surface, a wide interfacial area, and a slow rising velocity, which makes them rapidly dissolve. [1, 2]. Evidently, microbubble-producing diffused air systems are advantageous to aquaculture development in general. The successful implementation of microbubble aeration systems for aquaculture has been reported for shellfish and fish culture farms in Japan, particularly [3-6]. The effects of microbubble aeration on the cultivation of white shrimp have, however, received relatively little research.[7]

The whiteleg shrimp, *Litopenaeus vannamei*, is the most commonly grown in many parts of the world, particularly in tropical areas [8]. Whiteleg shrimp are a great option for intensive, biosecure, and controlled grow-out operations because of their quick development, strong survival in high-density culture, and resistance to illness[9]. The intensive shrimp farming, on the other hand, prompted various questions about water quality control, environmental and ecological sustainability. Bioflocs technology (BFT) was intended to address the majority of problems associated with closed intense shrimp aquaculture[10-12]. This technology is used to regulate the water quality for shrimp culture and is based on dense and active heterotrophic microorganisms [13-15]. BFT improves agricultural biosecurity, protects local water resources, and reduces wastewater output [16-18]. One requirement for the growth of bioflocs in the culture

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tanks is a high level of oxygenation. Both shrimp culture and aerobic heterotrophic bacteria can be sustained by the use of microbubble aeration, which offers high oxygenation.

Shrimp farming can be divided into various stage. The nursery stage (or nursery pond) is more like a controlled environment for postlarvea of *L. vannamei* for about 30 days before it is transferred to a grow-out pond (60 days). The benefits of this nursery stage are to increase survival rate, to improve feeding efficiencies, and to enhance the growth performance [19-22]. One of the motivations of the present study is to encourage farmers of adopting intensive nursery stage culture coupled with nano/micro bubble aeration and biofloc technologies for better productivity.

The objective of this study was to evaluate the effects of microbubble aeration on the water quality and biofloc as well as to determine the influence of these factors on the growth parameters of *L. vannamei.*

2 Material and Methods

2.1 Experimental setup

This bench scale experiment was conducted in an indoor aquaculture laboratory located at Universiti Malaya, Kuala Lumpur. Specific pathogen-free (SPF) *L. vannamei* post-larvae (PL19) (average weight of 0.014 ± 0.01 g) were randomly stocked in six round shape containers, with an initial stocking density of 600 shrimp/m^2 and tested for 30 days. Each container had an operational volume of 150 L and and dimensions of 45.72 cm (bottom diameter), 60.96 cm (top diameter), and 91.44 cm (height). The polyethylene (PE) bioballs with extruded cyclinder surface was contained into pvc mesh netting and two-layers of that was used to construct an additional horizontal surface near the tank bottom. The shrimps were fed four times a day (0800 h, 1200 h, 1600 h, & 2000 h) with a commercial diet, namely Blanca 7701 (Crude Protein: 36%), with a feeding rate of 3% to 6% of body weight, as suggested in Jory et al. (2001)[23].

There are mainly two types of culture setups (Fig.1). For type 1, the tank is based on the microbubble (MB) aeration system. Type 2 is based on the large bubble (LB) system. For both types, there were two duplicates. Therefore, a total of six experiments were conducted parallelly. For micro/nano air bubble aeration (MB), a special type of microbubble generator pump (KTM20F; Nikuni Co. Ltd., Japan) was used. The pump produced an approximately 1.02 m^3/h (17 LPM) water flow rate (Q) with a mixture of about 8% (0.03–0.06 m^3/h) of air. The pump set-up consisted of a separator tank, pressure gauges at the pump suction and discharge, and a rotameter for the air inlet. The setup of this pump is in accordance with the operating standard recommended by the manufacturer, and the pump is designed to produce microbubbles at a size of 5 to 20 μm. The pressure of the discharge liquid (a mixture of water and air) is raised from 300 to 400 kPa (3-4 bar). A separator tank was used to remove any large bubbles from the discharged liquid before it flowed to the culture tanks. For the production of larger bubbles, a spherical shaped air stone (diameter: 3 cm), which was bought from a local aquarium shop, and together with an air pump (Atman; HP-4000) were used to aerate the tanks. The air stone produced air bubbles with sizes of 1.5 to 3 mm. This size is estimated near the air stones' surface. Hereinafter, this aeration type will be referred to as ‘large bubbles’ (LB).

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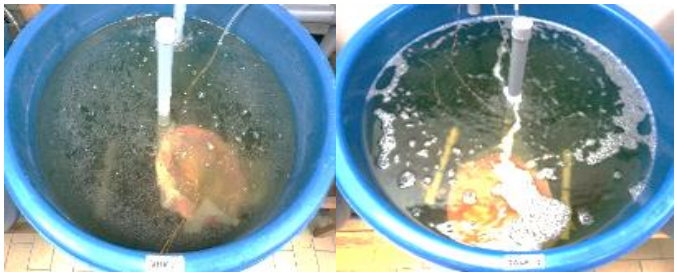

(a) (b)

Figure 1: *Water and biofloc condition of a) Micro-bubble (MB) and b) Large bubble (LB) tanks.*

The water was prepared for 4-5 weeks for an abundance of biofloc microorganisms prior to post-larvae (PL) stocking. Briefly, the tanks were filled with filtered freshwater and treated with two to seven parts of sodium thiosulfate to neutralise one part of chlorine. The water was mixed with artificial sea salt to increase the water salinity up to 18-20 ppt. Molasses as carbohydrate or carbon sources at a C:N ratio of 10:1 was added to the tanks to stimulate the appearance of predominantly heterotrophic bacterial biota. In addition, to initiate the population of nitrifying bacteria and biofloc formation, the tanks were inoculated with 5ppm of a commercial inoculum of nitrifying bacteria (MICROBE-LIFT NITE-OUT II Nitrifying Bacteria) and Effective Microorganism (EM bacteria)[24, 25].

2.2 Measurement of water physicochemical parameters

Dissolved oxygen (DO) and temperature were measured using a DO meter (Hanna Instrument; HI98193; ±1.5%) twice daily (0700 h & 1900 h). Salinity is measured using a refractometer [26], while pH is measured using a pH meter (Hanna Instrument; HI8314; ±0.01 pH). The analysis of total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3) and alkalinity was performed once every 5 days using a commercial chemical reagent test kit (API Multiparameter test, USA) according to APHA (2012)[26]. Alkalinity was measured once a week using an acid titration method of phenolphthalein and bromophenol blue and maintained around 130-140 mg/L. For quantitative evaluation of the biofloc, settleable solids (SS) was determined weekly using APHA methods of # 2540 F [26].

2.3 Growth performance

The shrimps' physical state was continually monitored for signs of skin discoloration, fungal growth, and other abnormalities. At the end of the experiment, the weight of the shrimp was measured using a digital scale (KERN KB 2000-2N; 0.03 g). The survival rate (1), feed conversion ratio (FCR) (2), and production (3) were calculated according to the following formulas [27, 28]:

$$\text{Survival (\%)} = \left[\frac{\text{Remained shrimps}}{\text{Stocked shrimps}}\right] \times 100 \quad (1)$$

$$\text{The feed conversion ratio (FCR)} = \frac{\text{total dry weight of diet feed (g)}}{\text{wet weight gain}} \quad (2)$$

$$\text{Production} = \frac{\text{Total biomass (g)}}{\text{Tank volume (m2)}} \quad (3)$$

2.4 Statistical analysis

A one-way analysis of variance (ANOVA) was applied to compare parameters at a significance level of $P < 0.05$. Tukey's multiple range test was applied when significant differences were detected. Statistical analyses were carried out using the statistical package of SPSS (SPSS; version 27; IBM; New York, USA).

3 Results and Discussion

3.1 Water quality

The statistical data are shown in Table 1. There were significant differences in the DO values between the MB and LB tanks ($P<0.05$). The DO of MB was generally higher than the DO of LB (Fig. 2). The MB pump system raises the pressure of the mixture (water and air) to 350 to 400 kPa (3.5–4 bar or around 4 atm). Consequently, it is plausible to anticipate a higher DO value than that at 1 atm (i.e., 8.24 ppm at 25 °C according to Hendry's rule). The average difference between these two types of aeration was 1.93 ppm. This is predicted considering micro/nano bubbles are known to significantly increase the dissolved oxygen concentration in water. [3, 4].

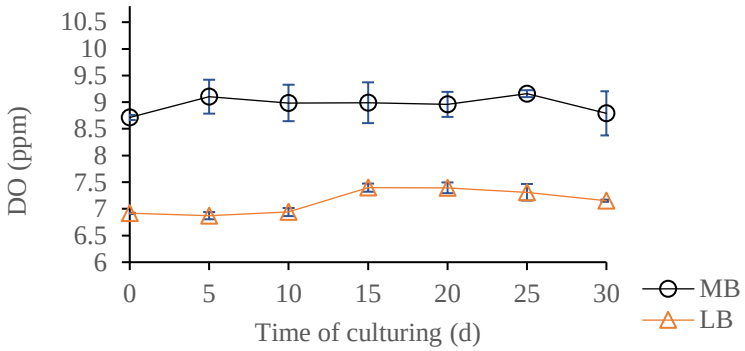

Figure 2: *DO for MB and LB tanks*

The temperature of the MB and LB tanks differed significantly ($P<0.05$) (Table 1). MB tanks generally had a greater temperature than LB tanks. As was previously mentioned, the MB pump system setup for microbubble production causes the pressure of the mixture (water and air) to be raised to 350–400 kPa (3.5–4 bar or around 4 atm). With increasing pressure, a rise in temperature is expected. The temperature rise is noticeable for the current bench scale due to the small volume of water used in the tanks. However, both MB and LB tanks temperature were within the acceptable range [29, 30].

In general, the pH of MB tanks was significantly lower ($P< 0.05$) than LB tanks, which the difference was about 0.1. This implies that the water becomes a little bit more acidic as a result of the MB system. There were differences in temperature between the MB and LB tanks, as seen in Table 1. Based on the idea of chemical equilibrium, Le Châtelier's Principle states that a small pH level decrease may occur as water temperature rises [31]. According to the stated theory, MB tanks' temperature was greater, which resulted in a lower pH level.

Statistically, there was no significant difference in TAN between MB and LB tanks. However, there was a significant difference ($P<0.05$) in the concentration of nitrite and nitrate between the MB and LB tanks, with MB having significantly lower nitrite and greater nitrate levels. These results implied several findings. First, it is possible that the MB system aided in the nitrifying bacteria's quick growth, which made the conversion of TAN to nitrite and nitrate relatively quick and efficient.

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The SS of LB tanks were significantly lower than MB tanks ($P<0.05$). This finding indicate that MB aeration encourages the development of biofloc compared to the LB aeration. The presence of biowalls of equivalent size to the volume of water in the tanks resulted in a lower SS value than the suggested 5 to 15 mL/L range by Emerenciano et al. (2017)[[32].

Table 1: *Water quality parameters in all tanks.*

Parameters	MB	LB
DO (ppm)	9.01 ± 0.33^{a}	7.08 ± 0.24^{b}
pH	7.79 ± 0.10^{b}	7.89 ± 0.08^{a}
Temp (°C)	28.58 ± 0.68^{a}	26.67 ± 0.28^{b}
TAN (mg/L)	0.08 ± 0.12	0.12 ± 0.15
Nitrite (mg/L)	0.33 ± 0.12^{b}	0.44 ± 0.11^{a}
Nitrate (mg/L)	56.67 ± 24.97^{a}	22.5 ± 24.45^{b}
SS (ml/L)	0.63 ± 0.30^{a}	0.33 ± 0.11^{b}

The values are the means of replicates ± SD. Different superscript letters indicate that the averages differ significantly ($P<0.05$).

3.2 Growth performance

Table 2 shows the shrimp growth at Day 30 (the end of the culture). Weight gain, FCR, and productivity were all significantly different between MB and LB tanks ($P<0.05$; Table 2). Shrimps in MB tanks gained more weight and length than shrimp in LB tanks, with differences of approximately 34% and 13%, respectively. The microbubble aeration water culture appeared to be advantageous for shrimp growth based on the better results found in MB tanks as compared to LB tanks. It is obvious that microbubbles greatly accelerate shrimp growth. When compared to LB tanks, the FCR values of MB tanks were significantly lower by 26% ($P<0.05$). Microbubbles clearly produce substantially lower FCR readings. The heterotrophic bacteria in the water may be a contributing factor since they supply the shrimp with additional food sources.

Table 2: *Average growth performance in all tanks.*

Parameters	MB	LB
Weight gain (g)	0.41 ± 0.09^{a}	0.29 ± 0.03^{b}
Length gain (cm)	2.36 ± 0.45	2.06 ± 0.65
Survival rate (%)	80.36 ± 18.12^{a}	69.25 ± 7.77^{b}
FCR	0.69 ± 0.10^{b}	0.90 ± 0.08^{a}
Production (g/m^2)	196.66 ± 25.90^{a}	124.68 ± 7.60^{b}

The values are the means of replicates ± SD. Different superscript letters indicate that the averages differ significantly ($P<0.05$).

4 Conclusions

The effects of MB aeration to the *L.vannamei* biofloc culture were evaluated and compared with LB aeration. In conclusion, MB aeration provided the culture an improved and consistent DO. MB aeration also accelerates the conversion of TAN to nitrate, and aids the production of biofloc in measures of SS. Other water quality parameters such as pH and temperature remained stable and within the acceptable range for *L.vannamei* despite the fact that the heat dissipation from the bench scale MB system causes the MB generator to raise the water temperature by 2 °C and reduce the pH level significantly. Additionally, MB

aeration improved shrimp growth performance, with greater mean weight gains of 34%, higher mean length gains of 13% and lower FCRs of 26% when compared to LB aeration.

5 Declarations

5.1 Acknowledgements

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5.2 Competing Interests

There is no conflict of interest.

5.3 Publisher's Note

AIJR remains neutral with regard to jurisdiction claims in published maps and institutional affiliations.

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