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Research Article

The use of different air diffusers on whiteleg shrimp, *Penaeus vannamei* in recirculating aquaculture system: effects on water quality and shrimp growth

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ABSTRACT. This study aimed to evaluate the effects of different air diffusers on the oxygen transfer rate, water quality, and growth performance of the whiteleg shrimp, *Penaeus vannamei*, for 60 days using a recirculating aquaculture system. The first experiment was conducted with six treatments applying two different air diffusers regarding two treatments (TRT): (i) rubber aeration tube and (ii) airstone at three different air pressures (0.025, 0.05, and 0.075 Mpa), respectively. Based on the first experiment's results, the second experiment was designed with two treatments: TRT1 - rubber aeration tube and TRT2 - airstone. Results indicated that the standard oxygen transfer rate increased as the pressure increased. The rubber aeration tube and air stone showed no significant difference at pressure levels. Therefore, the second experiment used a pressure level of 0.025 Mpa for TRT1 and TRT2. The results from the second trial showed that the dissolved oxygen in TRT1 did not differ significantly from that in TRT2. At the same time, the other parameters, such as temperature, pH, and alkalinity, remained steady throughout the culture period. The survival rate and biomass had the highest values in TRT2, but no significant differences were found in growth performance compared to TRT1. The results of this study suggest that either rubber aeration tubes or air stones are feasible and effective alternatives to improve the water quality and growth performance of *P. vannamei* in experimental conditions.

Keywords: *Penaeus vannamei*; air diffusers; water quality; growth performance; aquaculture

INTRODUCTION

Aquatic organisms obtain oxygen from the environment for respiration. The availability of oxygen for aquatic organisms is much less than that for air-breathing organisms, and the dissolved oxygen (DO) content of water may limit the activities of aquatic organisms. In nature, the amount of oxygen supplied to water, which is a combination of diffused oxygen from the air and production from the photosynthesis of aquatic plants, exceeds the amount of oxygen consumption by the aquatic organisms, and oxygen inadequacy for the regular metabolism of the organisms can rarely occur. The biomass of plants, animals, and microbes in aquaculture ponds is much greater than that in natural water bodies. Hence, the oxygen consumption rate on some occasions is faster than replenishing. The low DO concentration and the long period of remaining low can cause the cultured organisms to consume less feed, grow more slowly, have a high food conversion ratio, be more easily infected by diseases, or suffocation and death. Aquaculturists avoid these problems by aerating ponds mechanically to supply normal oxygen concentration (Tucker 2005).

Aeration is the injection of air into the water by different mechanisms. Aeration has two purposes: a) to supply the required oxygen for the organisms, and b) to create a water flow in the culture pond, tank, or others (EPA 1999). There are two types of aeration systems: surface aeration and submerged aeration. The submerged aeration technique has much greater efficiency than surface aeration because of the high oxygen transfer efficiency (OTE) granted by the small bubbles generated from diffusers. In this system, oxygen is compressed and pumped into the water

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through diffusers submerged. Small bubbles are pumped to the bottom of the culture unit and rise to the water column, and the oxygen is passed through the surface of the bubbles into the water.

In a recirculation aquaculture system (RAS), aeration is necessary for the culture units and the biofilter because the nitrification process by the ammonia-oxidizing bacteria and nitrite-oxidizing bacteria is an aerobic reaction. Moreover, the oxygen consumption in the biofilter is very high. The biofiltration capacity of the biofilter depends on the water temperature and pH of the water in the system. A lower pH can reduce the efficiency of the biofilter. The pH of the water further depends on the carbon dioxide (CO_2) production from the fish and the metabolism of nitrifying bacteria in the biofilter (Bregnballe 2015). Nitrification comprises two steps: the ammonia oxidation process from ammonia to nitrite by the ammonia-oxidizing bacteria (AOB) genus *Nitrosomonas* and the nitrite oxidation process from nitrite to nitrate by nitrite-oxidizing bacteria (NOB) genus *Nitrobacter*. Oxygen consumption and the biofilter's consumption rate are very high, so the biochemical oxygen demand (BOD) in the filter system is also high. Therefore, a good and efficient aeration system is required to eliminate the CO_2 in the system (Bregnballe 2015). Many researchers have investigated DO concentration's effect on nitrification rate through pure and mixed cultures. Oxygen plays an important role in nitrification, influencing aerobic bacteria's growth.

According to the above factors, DO becomes crucial in all aspects of a recirculation system. Therefore, more and more efficient aeration or oxygen-supplying techniques are required. Proper oxygen diffusion devices are needed to supply the required DO into the system more efficiently. As mentioned, small bubbles from the air diffusers can improve oxygen diffusion efficiency since smaller bubbles have a slower movement speed than larger ones. This slower speed permits them to remain longer in the water and reduces the loss of bubbles by escaping from the water's surface. The longer the bubbles stay in the water, the greater the oxygen diffusion efficiency. In addition, smaller bubbles result in more bubble surface area per unit volume (EPA 1999). However, due to smaller bubbles, it is hypothesized that the rubber aeration diffuser can be deployed in the biofiltration tank of a recirculation system. If possible, the biofilter's microbial activities can be much facilitating. Therefore, the objective of the present study is to evaluate the effects of different aeration types on water quality and determine the influence of these factors on the growth parameters of *Penaeus vannamei* juveniles reared in a recirculating aquaculture system.

MATERIALS AND METHODS

Experimental animals

Whiteleg shrimp *P. vannamei* with a size of ~0.5 g reared at the wet lab of the Faculty of Aquatic Biology and Environmental Science, College of Aquaculture and Fisheries, Can Tho University, was used for the experiment. Five culture tanks of 500 L were filled with 400 L of filtered brackish water each. Sodium bicarbonate ($NaHCO_3$) was used to control alkalinity and pH in a suitable range for shrimp. Shrimp were stocked at the density of 100 individuals per tank. Water quality parameters for culture conditions were maintained as follows: temperature: 28-30°C, salinity: 20, pH: 8-8.5, and the temperature and salinity in the system were maintained at a uniform level. Cultured shrimps were fed with commercial pellet feed with 40% crude protein at a feeding rate of 3-5% by body weight divided into four times per day at 08:00, 11:00, 14:00, and 17:00 h, respectively. Every uneaten portion was collected after 1 h of feeding and then immediately oven-dried at 80°C for 24 h. The amount of all diets fed was calculated by subtracting the uneaten portion, and data were recorded daily (Huynh et al. 2018). No water exchange was performed during the culture; only the water lost due to evaporation was compensated. At the end of 60 days of culture, growth performance and survival of shrimp were assessed.

Experimental design

Experiment 1: effects of the different air diffusers and pressures on the standard oxygen transfer rate

Six treatments were implemented with two different air diffusers at three different flow rates for each diffuser (Table 1). The rubber aeration tube and air stone diffusers purchased from the market in Can Tho city that is commonly used in aquaculture for aeration purpose were connected to the air compressor by a 6 mm pipe, and the pressure levels were maintained with the pressure switch manifold regulator gauges. The different pressures included 0.025, 0.05, and 0.075 MPa. As mentioned above, the tanks were filled with 450 L of brackish water (salinity at 20) and were installed with different air diffusers.

Before the trial was started, the water was deoxygenated to nearly 0 mg L^{-1} with sodium sulfite (Na_2SO_3) (Amstrong & Boyd 1982, Pipoppinyo & Boyd 1993, Al-Ahmady 2006). Then, the tanks were aerated, and the DO concentrations were recorded continuously with a DO electrode up to the stable condition (saturation point). Water temperature, pH, salinity, DO concentration (O_2 mg L^{-1}) at 10, 70, and

Table 1. Experimental design for oxygen transfer rate test.

Treatment names	Air diffusers	Air pressure (Mpa)
TRT1	Rubber aeration tube	0.025
TRT2	Rubber aeration tube	0.050
TRT3	Rubber aeration tube	0.075
TRT4	Air stone	0.025
TRT5	Air stone	0.050
TRT6	Air stone	0.075

100% saturation, and time taken were recorded. The experiment was triplicated. The standard oxygen transfer rate (SOTR, O_2 g h^{-1}) was calculated according to the methods by Pipoppinyo & Boyd (1993), Boyd (1998, 2020), and Redmon (2018). The air pressure levels that gave each diffuser's optimum oxygen transfer rate were selected and used for further experiments.

Oxygen transfer coefficient at the temperature T of the test water h^{-1}:

$$(K_La)_T = [\ln (DO_s - DO_1) - \ln (DO_s - DO_2)] / [t_2 - t_1]$$

Oxygen transfer coefficient at 20°C h^{-1}:

$$(K_La)_{20} = (K_La)_T / 1.024^{T-20}$$

Standard oxygen transfer rate at 20°C h^{-1}:

$$SOTR = (K_La)_{20} \times DO_{S20} \times V$$

where DO_s is the dissolved oxygen concentration at 100% saturation at the temperature T (mg L^{-1}), DO_1 and DO_2 are the dissolved oxygen concentration at 10 and 70% saturation (mg L^{-1}), t_1 and t_2 are the times taken the dissolved oxygen concentration to reach 10 and 70% (h), SOTR is the standard oxygen transfer rate (O_2 g h^{-1}), DO_{S20} is the dissolved oxygen concentration at 100% saturation at 20°C (mg L^{-1}), and V is the volume of the test water (m^3).

Experiment 2: effects of different air diffusers on water quality, bacteria, and growth performance of *P. vannamei*

This experiment was performed with two treatments (three replicates for each): (i) treatment 1 (TRT1) was implemented with a rubber aeration tube, and (ii) treatment 2 (TRT2) was implemented with the air stone. Biofiltration tanks were filled with 400 L brackish water (salinity at 20) and were installed with two different air diffusers in each tank. A steady oxygen transfer efficiency was maintained for each treatment by adjusting the pressures according to the first experiment with pressure switch manifold regulator gauges. A RAS including sedimentation tanks, trickling filters, biofiltration tanks, and culture tanks were used for this experiment. A 150-L sedimentation tank was filled with 100-L brackish water. Bioballs (model: PE50; size: Φ25 × 4; surface area: 600 m^2 m^{-3}) created moving bed biofilters. The water flow was adjusted with valves to maintain an estimated flow rate of 6 L min^{-1} to obtain 500% of water volume circulation per day. Only the oxygenated water supplies the required oxygen for the shrimp; there was no direct aeration in the culture tanks. An air compressor was used to pump the air into the system. A schematic diagram of the full RAS used in this study is shown (Fig. 1).

Measurements

Water quality parameters were recorded daily, including temperature, DO, and pH. Alkalinity, chemical oxygen demand (COD), total ammonia nitrogen (TAN), nitrite (NO_2^--N), nitrate (NO_3^--N), and phosphate (PO_4^{3-}-P) were analyzed once every week by following the Standard methods for water and wastewater examination (APHA 2017) (Table 2). Water samples were collected in the culture tanks at a depth of 30 cm from the surface and then preserved at 4°C until analyzed.

For enumeration of AOB and NOB, 1 g piece of bioball sample from each biofiltration tank was separately added to a test tube containing 9 mL sterilized physiological saline (0.9% NaCl) and then sonicated for 10 min using a sonicator (Branson SFX250) to detach cells (Suantika et al. 2016) to obtained the first dilution tube (10^{-1}) and further serially 10-fold diluted to 10^{-5} dilution in the same solution. Decimal dilutions of multiple sample aliquots were inoculated into liquid mineral media containing ammonium ions for AOB enumeration or nitrite ions for NOB enumeration. Then, the inoculated cultures were incubated at 28°C for 2-3 weeks under dark conditions. The most probable number (MPN) of each group of nitrifying bacteria was determined from the distribution of positive and negative responses using the Griess-Ilosvay reagent among the inoculated tubes. The results were expressed as MPN 100 g^{-1} from a standard MPN table (APHA 2017).

The survival rate (SR) was evaluated at the end of the experiment period. Growth performance factors such as weight gain (WG), daily weight gain (DWG), daily length gain (DLG), specific growth rate (SGR), biomass (B), and feed conversion rate (FCR) were calculated as previously described by Tacon et al. (2002), Niu et al. (2015), and Antunes et al. (2018).

WG (g) = final weight - initial weight

DWG (g d^{-1}) = [final weight - initial weight] / days of culture

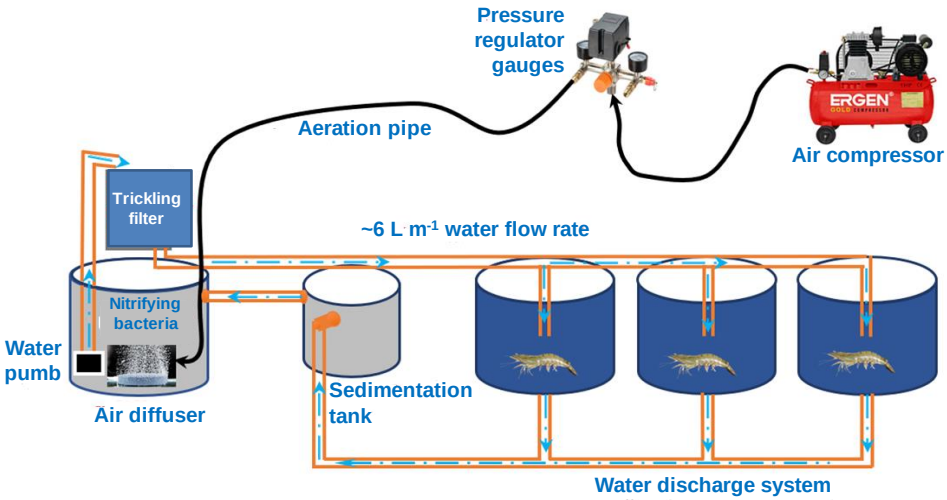

Figure 1. Schematic diagram of recirculation aquaculture system used in this study.

Table 2. Methods used for the analysis of water quality. DO: dissolved oxygen, COD: chemical oxygen demand, TAN: total ammonia nitrogen.

Parameter	Method
Temperature, DO, pH	Multiparameter water quality portable meter (HI9828/10-01)
Alkalinity	2320 B. Titration method
COD	Alkaline potassium permanganate
TAN	4500-NH_3 F. Phenate method
NO_2-N	4500-NO_2^- B. Colorimetric method
NO_3-N	Brucine method (EPA 1971)
PO_4-P	4500-P D. Stannous chloride method

DLG (cm d^{-1}) = [final length - initial length] / days of culture

SGR (% d^{-1}) = 100 × (ln final mean weight - ln initial mean weight) / days of culture

SR (%) = 100 × [number of final shrimp / number of initial shrimp]

B (kg m^{-3}) = [body weight × number of shrimp] / volume of water

FCR = dry feed intake / wet weight gain

Statistical analysis

The data were presented as the mean ± standard error (SE). Both one-way and two-way analysis of variance (ANOVA) were used for experiment 1. One one-way ANOVA was used for experiment 2 to identify the differences among the treatments, followed by Tukey's multiple range test to examine significant differences among treatments using SPSS version 22.0. Before analysis, percent data (SR) were normalized using an arcsine transformation. The significant differences among treatments were considered at the $P < 0.05$.

RESULTS

Experiment 1: effects of different air diffusers and pressures on the standard oxygen transfer rate

The standard oxygen transfer efficiency of the rubber aeration tube and air stone diffusers ranged from 2.19-2.84 and 2.19-2.77 O_2 g h^{-1}, respectively. The two treatments had no significant difference ($P > 0.05$) (Table 3). The pressure tended to have a positive correlation with the SOTR. The SOTR at an air pressure of 0.05 Mpa ranged from 2.23-2.74 O_2 g h^{-1} and was not significantly different from the other two ($P > 0.05$). The lowest and highest air pressures (0.025 and 0.075 Mpa) caused the SOTR to range from 2.19-2.44 and 2.19-2.74 (O_2 g h^{-1}) but were not significantly different from each other ($P > 0.05$) (Table 4).

The optimum pressure levels for each air diffuser for the second experiment were determined from the combined results of the air diffuser and pressures. The SOTRs of the rubber aeration tube and air stone diffuser ranged between 2.19-2.74 and 2.19-2.72 O_2 g h^{-1}, respectively. No significant difference was observed

Table 3. Standard oxygen transfer rates (SOTR, O_2 g h^{-1}) of different air diffusers. The data in the table are mean SOTR values from the two diffusers ± standard error by one-way ANOVA. The insignificant difference ($P > 0.05$) is shown with the same superscript letters in the column.

Diffuse	SOTR
Rubber aeration tube	2.45 ± 0.06^a
Air stone	2.51 ± 0.08^a

Table 4. Standard oxygen transfer rates (SOTR, O_2 g h^{-1}) at different air pressures under experimental conditions. The data in the table are mean SOTR values from the three pressures ± standard error by one-way ANOVA. The insignificant difference ($P > 0.05$) is shown with the same superscript letters in the column.

Pressures	SOTR
0.025 Mpa	2.29 ± 0.05^a
0.050 Mpa	2.51 ± 0.08^a
0.075 Mpa	2.55 ± 0.09^a

between the pressure levels ($P > 0.05$) (Table 5). The lowest SOTR was observed at the lowest pressure (0.025 Mpa) in this treatment and was not significantly different from the other two treatments ($P > 0.05$). Regarding efficiency at different air pressure levels, the rubber aeration tube and air stone diffusers at 0.025 Mpa were suitable for assessing *P. vannamei* (Table 5).

Experiment 2: effects of different air diffusers on water quality, bacteria, and growth performance of *P. vannamei*

Water quality

Temperature, pH, DO, and alkalinity were not significantly different between the treatments ($P > 0.05$) and ranged from 28.3-28.4ºC, 8.07-8.1, 4.48-4.49, and 135-138 mg of $CaCO_3$ L^{-1}, respectively (Table 6).

Total ammonia nitrogen (TAN) was under 0.1 mg L^{-1} on day one and raised to a range of 0.425 to 0.505 mg L^{-1} on day 7. The results were significantly different between the treatments. After that, it dropped to a range of 0.064 to 0.222 mg L^{-1} and remained steady throughout the culture period (Table 7). Nitrite (NO_2^--N) concentrations in all treatments increased rapidly by day 7-21 with the ranges of TRT1; 1.12 ± 0.008 mg L^{-1}, and TRT2; 0.965 ± 0.003 mg L^{-1}. The concentrations of NO_2^--N in the TRT1 and TRT2 sharply dropped on day 28. At the end of the experiment, NO_2^--N in the treatments ranged from 0.231-0.233 mg L^{-1}, and the

Table 5. Air diffusers standard oxygen transfer rates (SOTR, O_2 g h^{-1}) at different pressures. The data in the table are mean values of the three replications ± standard error. The insignificant difference ($P > 0.05$) is shown with the same superscript letters in the column.

Treatment	SOTR
(TRT1) Rubber aeration tube × 0.025 Mpa	2.29 ± 0.07^a
(TRT2) Rubber aeration tube × 0.050 Mpa	2.48 ± 0.07^a
(TRT3) Rubber aeration tube × 0.075 Mpa	2.58 ± 0.1^a
(TRT4) Air stone × 0.025 Mpa	2.29 ± 0.08^a
(TRT5) Air stone × 0.050 Mpa	2.54 ± 0.16^a
(TRT6) Air stone × 0.075 Mpa	2.52 ± 0.17^a

Table 6. Temperature, pH, dissolved oxygen, and alkalinity during the culture period. The data in the table are mean values of the three replications ± standard error. The insignificant difference ($P > 0.05$) is shown with the same superscript letters in the same row.

Parameters	Treatment	
	TRT1	TRT2
Temperature (°C)	28.3 ± 0.3^a	28.4 ± 0.4^a
pH	8.07 ± 0.07^a	8.10 ± 0.06^a
Dissolved oxygen (mg L^{-1})	4.49 ± 0.03^a	4.48 ± 0.03^a
Alkalinity ($CaCO_3$ mg L^{-1})	137 ± 3.9^a	135 ± 3.8^a

concentrations were insignificantly different ($P > 0.05$) between the treatments (Table 8).

Nitrate (NO_3^--N) concentrations of all the treatments ranged from 0.022-0.159 mg L^{-1} during the first week of the experiment, and no significant difference was shown ($P > 0.05$). However, starting from day 14, the concentrations significantly differed between the treatments ($P < 0.05$) and increased as long as the culture continued. At the end of the experiment, the NO_3^--N concentrations of TRT1 and TRT2 were 27.63 ± 0.49 and 24.03 ± 0.33 mg L^{-1}, respectively, and all the treatments were significantly different from each other ($P < 0.05$) (Table 9).

Likewise, the orthophosphate (PO_4^{3-}-P) concentrations positively correlated with the experimental time; the longer the experiment, the higher the concentration. No significant difference was observed up to day 7 ($P > 0.05$), and from day 14, the concentrations became significantly different ($P < 0.05$). The concentration of PO_4^{3-}-P in TRT1 and TRT2 was 4.51 ± 0.012 and 4.51 ± 0.008 mg L^{-1}, respectively, at the end of the experiment. The concentration of PO_4^{3-}-P did not find a significant difference between TRT1 and TRT2 ($P > 0.05$) (Table 10).

Table 7. Weekly fluctuation of total ammonia nitrogen (mg L^{-1}). The data in the table are mean values of the three replications ± standard error. The significant difference ($P < 0.05$) is shown with different superscript letters a and b in the same row.

Days	Treatment	
	TRT1	TRT2
1	0.019 ± 0.001^{a}	0.019 ± 0.001^{a}
7	0.505 ± 0.001^{b}	0.425 ± 0.002^{a}
14	0.066 ± 0.004^{a}	0.113 ± 0.002^{b}
21	0.064 ± 0.001^{a}	0.104 ± 0.002^{b}
28	0.082 ± 0.006^{a}	0.090 ± 0.002^{a}
35	0.084 ± 0.001^{a}	0.133 ± 0.001^{b}
42	0.217 ± 0.002^{b}	0.141 ± 0.002^{a}
49	0.222 ± 0.010^{a}	0.219 ± 0.005^{a}
56	0.123 ± 0.001^{a}	0.129 ± 0.002^{a}

Table 8. Weekly fluctuation of NO_2^--N (mg L^{-1}). The data in the table are mean values of the three replications ± standard error. The significant difference ($P < 0.05$) is shown with different superscript letters a and b in the same row.

Days	Treatment	
	TRT1	TRT2
1	0.005 ± 0.000^{a}	0.005 ± 0.000^{a}
7	0.179 ± 0.003^{b}	0.158 ± 0.000^{a}
14	1.04 ± 0.011^{b}	0.820 ± 0.001^{a}
21	1.12 ± 0.008^{b}	0.965 ± 0.003^{a}
28	0.368 ± 0.003^{b}	0.302 ± 0.002^{a}
35	0.317 ± 0.009^{b}	0.248 ± 0.004^{a}
42	0.575 ± 0.009^{b}	0.533 ± 0.006^{a}
49	0.414 ± 0.008^{b}	0.355 ± 0.003^{a}
56	0.233 ± 0.001^{a}	0.231 ± 0.002^{a}

Between the treatments, chemical oxygen demand (COD) steadily ranged from 4.22 to 7.61 mg L^{-1} until day 21. After that, it increased to a range of 8.68-10.83 mg L^{-1} at day 28 and finally reached an average concentration of 26.6 ± 0.411 mg L^{-1} in TRT1 and TRT2 at day 56 and not different between the two treatments ($P > 0.05$) (Table 11).

Growth performance of *P. vannamei*

The growth performance of whiteleg shrimp was assessed at the end of the experiment period. The growth parameters were not statistically different between the treatments regarding WG, DLG, DWG, and SGR ($P > 0.05$). There was a significant difference in SR between treatments ($P < 0.05$). The best SR was recorded in TRT2 (85.33 ± 1.86%), and TRT1 was the lowest with a value of 76.67 ± 1.86%. The final biomass

Table 9. Weekly fluctuation of NO_3^--N (mg L^{-1}). The data in the table are mean values of the three replications ± standard error. The significant difference ($P < 0.05$) is shown with different superscript letters a and b in the same row.

Days	Treatment	
	TRT1	TRT2
1	0.022 ± 0.001^{a}	0.023 ± 0.001^{a}
7	0.156 ± 0.017^{a}	0.159 ± 0.003^{a}
14	2.19 ± 0.018^{b}	1.95 ± 0.061^{a}
21	4.30 ± 0.030^{a}	4.51 ± 0.025^{b}
28	6.74 ± 0.301^{a}	7.13 ± 0.170^{b}
35	8.41 ± 0.298^{a}	9.18 ± 0.309^{a}
42	18.6 ± 0.130^{b}	17.4 ± 0.207^{a}
49	23.5 ± 0.390^{a}	22.1 ± 0.278^{a}
56	27.6 ± 0.487^{b}	24.1 ± 0.334^{a}

Table 10. Weekly fluctuation of PO_4^{3-}-P (mg L^{-1}). The data in the table are mean values of the three replications ± standard error. The significant difference ($P < 0.05$) is shown with different superscript letters a and b in the same row.

Days	Treatment	
	TRT1	TRT2
1	0.038 ± 0.001^{a}	0.041 ± 0.003^{a}
7	0.069 ± 0.001^{b}	0.064 ± 0.001^{a}
14	0.197 ± 0.002^{a}	0.190 ± 0.003^{a}
21	0.270 ± 0.003^{a}	0.281 ± 0.002^{a}
28	0.667 ± 0.019^{a}	0.685 ± 0.019^{a}
35	1.55 ± 0.020^{b}	1.33 ± 0.027^{a}
42	2.04 ± 0.014^{b}	1.74 ± 0.021^{a}
49	3.02 ± 0.023^{b}	2.91 ± 0.023^{a}
56	4.51 ± 0.012^{a}	4.51 ± 0.008^{a}

and FCR were not significantly different between TRT1 and TRT2 ($P < 0.05$) (Table 12).

Ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB)

AOB populations in the biofiltration systems had steadily increased from day 1 to day 7 without any significant difference between the treatments ($P > 0.05$). On day 14, AOB in TRT1 tended to be significantly higher than TRT2 ($P < 0.05$), reaching an average of $4.87 \pm 0.07 \times 10^5$ MPN 100 g^{-1}. On day 28, AOB in TRT1 increased to $9.77 \pm 0.05 \times 10^5$ MPN 100 g^{-1}, which was not significantly higher than that in TRT2 ($P < 0.05$). After that, the AOB population in all treatments remained in a steady range of $3.1 \pm 0.24 \times 10^5$ to $7.23 \pm 0.06 \times 10^5$ MPN 100 g^{-1} until the end of the culture period with no significant difference between the treatments ($P > 0.05$) (Table 13).

Table 11. Weekly fluctuation of chemical oxygen demand (mg L^{-1}). The data in the table are mean values of the three replications ± standard error. The significant difference ($P < 0.05$) is shown with different superscript letters a and b in the same row.

Days	Treatment	
	TRT1	TRT2
1	4.41 ± 0.076^{a}	4.22 ± 0.129^{a}
7	7.61 ± 0.218^{a}	7.49 ± 0.192^{a}
14	6.92 ± 0.044^{b}	6.64 ± 0.056^{a}
21	5.85 ± 0.563^{a}	7.44 ± 0.127^{b}
28	8.68 ± 0.268^{a}	10.8 ± 0.164^{b}
35	10.2 ± 0.232^{a}	10.3 ± 0.706^{a}
42	14.6 ± 0.299^{a}	16.1 ± 0.595^{a}
49	19.1 ± 0.765^{a}	19.5 ± 0.299^{a}
56	26.7 ± 0.253^{a}	26.5 ± 0.568^{a}

Table 12. Growth performance of *P. vannamei* after 60 days of culture. IMW: initial mean weight, FMW: final mean weight, WG: weight gain, DWG: daily weight gain, DLG: daily length gain, SGR: specific growth rate, SR: survival rate, IB: initial biomass, FB: final biomass, FCR: feed conversion rate. The data in the table are the mean values of the three replications ± standard error. The significant difference ($P < 0.05$) is shown with different superscript letters a and b in the same row.

Growth parameter	Treatment	
	TRT1	TRT2
IMW (g)	0.43 ± 0.01^{a}	0.44 ± 0.01^{a}
FMW (g)	10.9 ± 0.35^{a}	10.2 ± 0.13^{a}
WG (g)	10.5 ± 0.355^{a}	9.76 ± 0.120^{a}
DWG (g d^{-1})	0.174 ± 0.006^{a}	0.163 ± 0.002^{a}
DLG (cm d^{-1})	0.120 ± 0.002^{a}	0.115 ± 0.001^{a}
SGR (% d^{-1})	5.38 ± 0.074^{a}	5.25 ± 0.014^{a}
SR (%)	76.7 ± 1.86^{a}	85.3 ± 1.86^{b}
IB (g $m^{-3)}$	0.436 ± 0.004^{a}	0.439 ± 0.006^{a}
FB (kg m^{-3})	2.08 ± 0.114^{a}	2.19 ± 0.044^{a}
FCR	1.38 ± 0.082^{a}	1.34 ± 0.028^{a}

Similarly, the NOB population in all treatments steadily increased until day 14, reaching a range of $2.57 \pm 0.06\times10^{4}$ to $3.43 \pm 0.22\times10^{4}$ MPN 100 g^{-1} with no significant difference between the treatments ($P > 0.05$). Then, the NOB population in TRT1 and TRT2 raised to $2.24 \pm 0.21\times10^{5}$ and $2.07 \pm 0.20\times10^{5}$ MPN 100 g^{-1}, respectively on day 21. On day 28, the NOB TRT2 decreased to $7.03 \pm 0.25\times10^{4}$ MPN 100 g^{-1} but did not find significant differences compared to the NOB TRT1 ($P < 0.05$). During the subsequent weeks of the experiment, the NOB populations remained steady

Table 13. Weekly fluctuation of ammonia oxidizing bacteria (MPN 100 g^{-1} of bioballs). The data in the table are the mean values of the three replications ± standard error. The significant difference ($P < 0.05$) is shown with different superscript letters a and b in the same row.

Days	Treatment	
	TRT1	TRT2
1	30.0 ± 0.102^{a}	19.0 ± 0.015^{a}
7	$4.50 \pm 0.084\times10^{3a}$	$4.57 \pm 0.120\times10^{3a}$
14	$4.87 \pm 0.074\times10^{5b}$	$1.70 \pm 0.060\times10^{5a}$
21	$1.56 \pm 0.098\times10^{6a}$	$1.58 \pm 0.112\times10^{6a}$
28	$9.77 \pm 0.053\times10^{5a}$	$1.21 \pm 0.089\times10^{6a}$
35	$3.67 \pm 0.162\times10^{5a}$	$3.10 \pm 0.024\times10^{5a}$
42	$6.83 \pm 0.083\times10^{5a}$	$4.97 \pm 0.193\times10^{5a}$
49	$7.23 \pm 0.060\times10^{5a}$	$5.43 \pm 0.101\times10^{5a}$
56	$4.80 \pm 0.140\times10^{5a}$	$4.77 \pm 0.103\times10^{5a}$

Table 14. Weekly fluctuation of nitrite-oxidizing bacteria (MPN 100 g^{-1} of biofilm). The data in the table are the mean values of the three replications ± standard error. The insignificant difference ($P > 0.05$) is shown with the same superscript letters in the same row.

Days	Treatment	
	TRT1	TRT2
1	$0.03 \pm 0.09\times10^{2a}$	$0.02 \pm 0.11\times10^{2a}$
7	$0.79 \pm 0.07\times10^{2a}$	$0.84 \pm 0.08\times10^{2a}$
14	$2.57 \pm 0.06\times10^{4a}$	$3.43 \pm 0.22\times10^{4a}$
21	$2.24 \pm 0.21\times10^{5a}$	$2.07 \pm 0.20\times10^{5a}$
28	$1.25 \pm 0.07\times10^{5a}$	$7.03 \pm 0.25\times10^{4a}$
35	$7.43 \pm 0.16\times10^{5a}$	$6.63 \pm 0.06\times10^{5a}$
42	$6.57 \pm 0.11\times10^{4a}$	$3.57 \pm 0.07\times10^{4a}$
49	$2.53 \pm 0.15\times10^{5a}$	$3.47 \pm 0.17\times10^{5a}$
56	$3.2 \pm 0.15\times10^{5a}$	$2.57 \pm 0.14\times10^{5a}$

without showing any significant difference between the treatments ($P > 0.05$) (Table 14).

DISCUSSION

Water quality is of great importance in aquaculture systems. DO is one of the important factors affecting the growth and the health of the whiteleg shrimp, *P. vannamei*, and it is also an important limiting factor for cultivation activities of all aquatic organisms to survive and grow (Supriatna et al. 2017). Since an intensive aquaculture system, ponds are heavily stocked with fish and a high feed supply. In these artificially fed fish ponds, problems like organic pollution, deficiency of oxygen, increased levels of free CO_2, and a total increase in ammonia-nitrogen nitrite-nitrogen ratio are frequently occurring (Sultana et al. 2017). Therefore,

better aeration systems and aerators are required. Aerators are widely used in aquaculture ponds to enhance the concentration of DO and provide water circulation (Boyd & Watten 1989) as cited in Pipoppinyo & Boyd (1993). However, the question is how to choose a proper aerator for a specific purpose. There are two ways of describing aerator performance: the SOTR (O_2 kg h^{-1}) and SAE (O_2 kg kW^{-1} h^{-1}) (Tucker 2005). In this study, the SOTR was used to describe the performance of the aerators. Regarding aerator performance, there was no significant difference between the two diffusers.

In this study, the water temperature was maintained between 27 to 30ºC in all treatments, which was within the optimal ranges for the growth of shrimp (Christopher 2008). In addition, the optimal pH value for *P. vannamei* shrimp culture is 7.5-8.5, with a fluctuation range of 0.5 (Reddy & Mounika 2018). Most aquaculture species' desired total alkalinity level lies between 50-150 mg of $CaCO_3$ L^{-1} but no less than 20 mg L^{-1} (Wurts 2002). Usually, the recommended DO concentrations in shrimp farms should be higher than 5 mg L^{-1} (Islam et al. 2004), meaning that the DO concentration in TRT1 and TRT2 was under the required level. According to Galang et al. (2019), the level of oxygen consumption is related to the ability of individuals to absorb oxygen to support their life processes. In the present study, the *P. vannamei* shrimp was cultivated with nanobubbles and an aerator. That study showed that the level of the *P. vannamei* shrimp's oxygen consumption in the treatment with nanobubbles was in a lower range than that in the other treatment. Low DO consumption level values are because oxygen in nanobubbles can last longer in the water and are more easily absorbed by the shrimp (Galang et al. 2019).

The unavailability of sufficient oxygen in the water causes high ammonia. Low oxygen is only sufficient for ammonia formation, so the ammonia oxidation process into nitrite and nitrate (nitrification) does not run perfectly (Galang et al. 2019). In aquaculture systems, TAN concentrations are usually maintained below 2 mg L^{-1}. A linear relationship between the TAN conversion rate and TAN concentration in the filter is normally found at these levels. When the values exceed 3-4 mg L^{-1}, the nitrification rate usually does not increase further. The turnover rate and physical movement of ammonia nitrogen from the culture tank to and through the biofilter can limit the activity of a biofilter (Losordo et al. 2015).

According to Effendi & Elizal (2020), the optimal NO_2^--N (nitrite) is <0.05 mg L^{-1} with a tolerance of 0.1-1 mg L^{-1}. Nitrite is the parameter of water quality that has the highest influence on the growth rate of *P. vannamei*, by 75.2%, compared with the other factors, such as DO and total *Vibrio* (Ariadi et al. 2019). Gross et al. (2004) suggested that long-term exposure to nitrite concentrations of less than 1 mg L^{-1} NO_2^--N might negatively affect *P. vannamei* growth and survival when grown in slightly saline water. According to Li et al. (2006), TAN and NO_2^--N concentrations were not significantly affected by either stocking density or DO concentration in the study of DO concentration and stocking density on growth and non-specific immunity factors in Chinese shrimp, *Fenneropenaeus chinensis.*

The nitrogenous compounds important in the rearing of aquatic organisms include TAN, nitrite (NO_2^--N), and nitrate (NO_3^--N). Nitrogenous compounds can injure gill tissue, affecting oxygen consumption in organisms during rearing or causing mortality (Lin & Chen 2001, 2003, Kuhn et al. 2010). The results obtained in the study by Furtado et al. (2015) showed that nitrate concentrations up to 177 mg L^{-1} are acceptable for rearing *P. vannamei* in systems with bioflocs without water renewal at a salinity of 23. In this study, histopathological damage was observed in the gills and hepatopancreas of shrimp exposed to concentrations ≥300 mg L^{-1} for 42 days, with poorer zootechnical performance and lower survival observed in the shrimps reared at concentrations ≥300 mg L^{-1} at a salinity of 23.

According to Tan et al. (2013), an investigation in a municipal wastewater treatment process of the effects of dissolved oxygen on simultaneous nitrification and denitrification in polyurethane foam contact oxidation reactors showed that nitrate could be removed at low DO levels. However, the higher the DO concentration level of 6.0 mg L^{-1}, the lower the removal rate. It has been verified that different bacteria have different suitable ranges of DO. When DO levels reach 0.5-1.0 mg L^{-1}, ammonia-oxidizing bacteria are detected, but no nitrite-oxidizing bacteria (Tan et al. 2013).

High protein feeds benefit shrimp production because nitrogen and phosphorus influence shrimp metabolism and production. Nevertheless, shrimp do not feed all the feed applied, and many leftovers are settled in the sediment and mixed with bottom soil in ponds (Xia et al. 2004). Phosphorus toxicity is virtually nonexistent. However, high concentrations of phosphate should be avoided because this may lead to the occurrence of harmful cyanobacteria blooms in culture systems (Smith 1983, Anderson et al. 2002), as cited in Silva et al. (2013). Very low phosphorus concentrations negatively affect nitrification (Nishio et al. 2008).

The positive effect of DO on shrimp growth was recorded in the study of Nonwachai et al. (2011). The *P. vannamei* shrimps reared above 4 mg L^{-1} had significantly higher body weight gain than those reared at 2-4 mg L^{-1} and below 2 mg L^{-1}. Moreover, the survival rate of the shrimps was also higher at higher DO levels. During the work of Li et al. (2006) on Chinese shrimp, the DO concentration inserted an interactive effect with the stocking density on shrimp weight gain.

A study by Princic et al. (1998) on the effects of pH, oxygen, and ammonium concentrations on the community structure of nitrifying bacteria from wastewater showed that the original or more conventional nitrifiers were quite active when ammonium concentration was high. The nitrification rate was directly interconnected with environment pH, delayed when the environmental pH was low but not in a high-pH environment. The population of nitrifying bacteria has a positive correlation with their activities and denitrification activities (Bodelier et al. 1996). The concentration of DO saturation in water decreases with the increasing temperature. Studies have shown that the biofilter performance at temperatures between 14 and 27°C is more significantly affected by oxygen concentration than temperature variation (Losordo et al. 2015). Salinity also affects the activity of the biofilter. Marine species generally tend to be more sensitive to high levels of ammonia-nitrogen concentrations. The effects of seawater on nitrification have been a topic of argument. According to previous studies, the nitrification rate in saltwater biofilters was around 40% lower than in similar freshwater systems (Losordo et al. 2015).

In conclusion, the SOTR of the rubber aeration tube showed no significant difference compared to that of stone diffusers *in vitro* conditions (Experiment 1). Moreover, the SOTR of the two air diffusers did not remarkably respond to pressure rises. Therefore, these air diffusers did not significantly impact the oxygen transfer rate. However, in the experimental condition of whiteleg shrimp culture in the recirculation system (Experiment 2), the effect of different air diffusers on water quality, the shrimp's growth performance, and the visibility of AOB and NOB were not demonstrated.

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