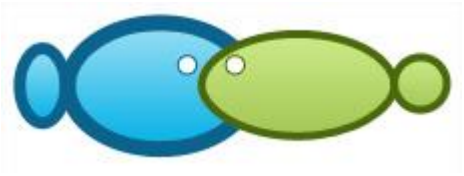

Effect of different biofilter media in recirculating aquaculture system application for whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) farming

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Abstract. The effect of different biofilter media on the environment and growth performance of whiteleg shrimp cultured in a recirculating aquaculture system (RAS) was evaluated. Three different biofilter media applied in this study included type 1 biofilter media with a specific surface area (SSA) of 750 $m^2(m^3)^{-1}$ (control), type 2 biofilter media with an SSA of 1,000 $m^2(m^3)^{-1,}$ and type 3 biofilter media with an SSA of 600 $m^2(m^3)^{-1}$ which were set to get the surface area of 22 m^2 across all treatments. After 56 days of rearing, all used biofilter media showed benefits in water quality maintenance, and the total count of *Vibrio* spp. was limited and varied around 10^3 CFU mL^{-1}. Regarding feed efficiency, the type 1 biofilter helped to reduce the FCR (feed conversion ratio) significantly compared with other biofilter media. Other growth parameters were not significantly different across the treatments. Generally, the study reviewed that the total surface area of biofilter media is the key factor in RAS application in shrimp farming, rather than the amount of those media.

Key Words: biofilter media, growth performance, feed conversion ratio, RAS, *Vibrio* spp.

Introduction. The whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) is the predominant crustacean species in aquaculture, constituting 53% of total farmed crustacean production as of 2018 (FAO 2020). Its key characteristics include fast growth, disease resistance, and high survival rates, making it suitable for intensive closed grow-out systems (Cuzon et al 2004). However, the expansion of shrimp aquaculture has raised several concerns regarding water quality management, environmental sustainability, farm biosecurity, efficient land and water resource usage, cost-effectiveness, and disease prevention in production processes. According to Jackson et al (2003), in intensive shrimp farming models, only 22% of feed nitrogen is converted to shrimp, 57% is discharged into the environment, 14% is deposited at the bottom of the pond, and only 3% of that can evaporate into the air as ammonia. Therefore, research on the use of biological agents is a positive trend that contributes to stabilizing the environment and limiting diseases in ponds. There is a need for technological advancements and innovations to mitigate negative impacts, optimize natural resource utilization, and improve cost efficiency in shrimp-intensive systems. One such innovation is the Recirculating Aquaculture System (RAS), a sustainable approach that improves water quality management while reducing the environmental footprint. Raising aquatic species in RAS systems has many benefits, such as stable environmental factors with little fluctuation, limiting diseases, and not using antibiotics or chemicals, so the farmed products meet food safety and hygiene standards. Although the first RAS was developed in Japan in the 1950s for carp farming (Murray et al 2014), significant advancements and widespread adoption of RAS in aquaculture did not occur until the 1970s (Emmanuelle et al 2009; Martins et al 2010, Ahmed & Turchini 2021, Li et al 2023). Nowadays, RAS is widely adopted globally, with at least 46 fish species, 11 crustacean species, 7 mollusk species, and 7 echinoderm species successfully reared in

these systems (Li et al 2023). From the perspective of a hierarchical analytical process, Zulkarnain et al (2020) concluded that RAS is best suited for intensive whiteleg shrimp farming with advantages such as minimal land requirements, suitability for brackish water locations, environmental sustainability, energy efficiency, and enhanced biosecurity.

The potential of RAS technology in whiteleg shrimp farming has been reported in several previous studies (Suantika et al 2018; Bauer et al 2021; Chen et al 2021a; Hai & Viet 2022), primarily under experimental conditions. Therefore, one proposed solution involves piloting the existing RAS systems for use in medium-scale farms (around 10,000 m^2). This initiative aims to evaluate innovations and potential upscaling opportunities in the future. RAS are designed to address key aspects such as nitrogenous waste treatment, oxygenation optimization, removal of suspended solids, and control of organic compound buildup (Brazil 2006). In RAS, the filters play a pivotal role in managing ammonia accumulation, a common concern in aquaculture systems. Ammonia buildup in RAS is regulated primarily through water exchange and the use of biofilters (Brazil 2006). The effectiveness of this process relies on efficient biofilters, particularly nitrifying bacteria, which are capable of converting the toxic ammonia produced by aquatic organisms into nitrates, a comparatively less harmful compound. A biological filter typically consists of non-corroding materials such as plastic, fiberglass, ceramic, or rock, providing ample surface area for nitrifying bacteria colonization (Ebeling & Timmons 2012). To enhance the efficiency and compactness of biofilters, materials with a high specific surface area (SSA) are usually chosen. In simpler terms, biofilter media with higher surface area provides bacteria with more surface area to grow, thereby increasing the nitrification capacity. This allows for higher feed rates to be sustained (DeLong & Losordo 2012).

Taking all factors into account, this study aims to evaluate the performance of different types of biofilter media installed in RAS in nitrogen removal from a shrimp farming system. It provides a useful case and operation parameter with wide potential applications.

Material and Method

Experimental animals. Healthy postlarvae (PL) of whiteleg shrimp (PL 15) were obtained from the shrimp hatchery of the College of Aquaculture and Fisheries, Can Tho University (CTU), Vietnam. The postlarvae were reared in a 40 m^3 composite tank for three weeks under standard laboratory conditions and fed a commercial diet (40% protein), four times a day (at 7:00, 11:00, 16:00, and 21:00) applying bio-floc technique (Avnimelech 2009). After three weeks of nursery, the pathogen-free and uniform juveniles of 0.38±0.09 g (4.15±0.50 cm) were selected and used for the experiment.

Biofilter media. In this study, three types of biofilter media were evaluated. The control biofilter media (Type 1) is the conventional media used at CTU, with SSA of 750 $m^2(m^3)^{-1}$ and dimensions of 15 × 15 mm, supplied by RK BioElements, Denmark. The other two types, provided by Greenpacks Vietnam Co., Ltd., are Type 2, with an SSA of 1,000 $m^2(m^3)^{-1}$ and dimensions of 10 × 10 mm, and Type 3, with an SSA of 600 $m^2(m^3)^{-1}$ and dimensions of 20 × 20 mm (Figure 1).

Experimental design. The three aforementioned types of biofilter media were evaluated. The experiment was a one-factor design with three treatments, each representing one of the three biofilter media types, with three replicates per treatment. The amount of biofilter media used is adjusted to get a consistent surface area of 22 m^2 across all treatments. Juveniles were stocked at a density of 150 individuals $(m^3)^{-1}$ and cultured in water with a salinity of 15 ppt for 56 days. The system included a 1 m^3 culture composite tank, a 0.2 m^3 settling composite tank, and a 0.2 m^3 biofilter composite tank. To establish the nitrification process, ammonium chloride (NH_4Cl) was added as an ammonia source for bacterial colonization in the biofilter before shrimp stocking. No commercial bacterial inoculants were introduced, allowing autochthonous nitrifying bacteria to develop naturally over two weeks. The progression of nitrification was monitored by measuring TAN, nitrite, and nitrate levels to confirm biofilter maturation before stocking shrimp.

Type 1

Type 2

Type 3

Figure 1. Experimental biofilter media.

Experimental management. Shrimps were fed 3-10% of total body weight four times daily at 7:00, 10:30, 14:00 and 17:30. During the experiment, the amount of diet given was progressively changed and adjusted according to the appetite of the shrimp by checking the bottom of the tanks for excess feed remaining 2 hours after feeding. In this way, overfeeding was minimized, and shrimps were fed close to satiation.

Water temperature, dissolved oxygen (DO), and pH were recorded every 7 days at 7 a.m. and 2 p.m., using a Handy Polaris (OxyGuard, Denmark) meter for temperature and DO and a Hanna HI98107 (Hanna Instruments, USA) meter for pH. Alkalinity, total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were measured every 7 days in the morning using the HI83303 multiparameter photometer (Hanna Instruments, USA) following the manufacturer's protocol.

During the feeding trial period, dead shrimp were removed and recorded. Total *Vibrio* spp. count was weekly checked. The bacterial count in culture water was determined using the spread plate method on thiosulfate citrate bile salts sucrose (TCBS, Himedia, India) agar. Serial 10-fold dilutions were performed in a sterile saline solution (0.85% NaCl). The TCBS plates were incubated at 28°C for 24 hours, after which all bacterial colonies were counted and recorded as CFU mL^{-1}.

Shrimp sampling. Shrimp body weight and length were recorded every two weeks by measuring 10 individuals per tank. At the end of the experiment, all the shrimp in the experimental tanks were weighed in bulk, and the wet weight of 30 shrimp per tank was measured individually.

Growth performance parameters were calculated as follows:

Weight gain (Wg, g) = Wf – Wi

Length gain (Lg, cm) = Lf – Li

Daily weight gain (DWG, g day^{-1}) = (Wf – Wi) / 56

Daily Length gain (DLG, g day^{-1}) = (Lf – Li) / 56

Feed conversion ratio (FCR) = (consumed feed) / (weight gain)

Survival rate (SR, %) = (Final no. of shrimp) / (Initial no. of shrimp)*100,

where the initial weight (Wi, g) and length (Li, cm) were determined at the beginning of the experiment, and the final weight (Wf, g) and length (Lf, cm) were determined after the 56 days of culture.

Total *Vibrio* spp. count in rearing water was determined using the spread plate method on Thiosulfate citrate bile salts sucrose (TCBS, Himedia, India) agar. Serial 10-fold dilutions were performed in a sterile saline solution (0.85% NaCl). The TCBS plates were incubated at 28°C for 24 hours, after which all bacterial colonies were counted and recorded as CFU mL^{-1}.

Statistical analysis. All data were presented as mean value ± standard deviation. Mean differences of parameters among treatments were tested using SPSS Statistics Version 21. The differences were considered significant at $p<0.05$.

Results and Discussions

Water quality parameters. Water temperature varied between 27.47°C and 29.11°C. Dissolved oxygen (DO) levels were consistently maintained above 7.5 mg L^{-1} across all treatments, while pH values ranged from 8.13 to 8.24. Alkalinity in all treatments was maintained at a concentration of over 160 mg $CaCO_3$ L^{-1} (Table 1).

Table 1

Water quality parameters during the experimental period

Parameters	*Time*	*Type 1*	*Type 2*	*Type 3*
Temperature (℃)	am	27.47±0.08	27.48±0.02	27.5±0.06
	pm	29.09±0.06	29.08±0.03	29.11±0.04
pH	am	8.14±0.03	8.13±0.01	8.13±0.01
	pm	8.24±0.05	8.23±0.02	8.21±0.0
DO (mg L^{-1})		7.70±0.07	7.67±0.11	7.68±0.06
Alkalinity (mg $CaCO_3$ L^{-1})		160.50±3.11	161.46±1.56	163.88±0.90

Note: Values are presented as mean±SD.

The highest TAN concentration (1.92±0.03 mg L^{-1}) was recorded in the Type 3 treatment on day 14. However, this level was reported to have no impact on white leg shrimp farming. Subsequent measurements maintained TAN levels below 1 mg L^{-1} (Figure 2).

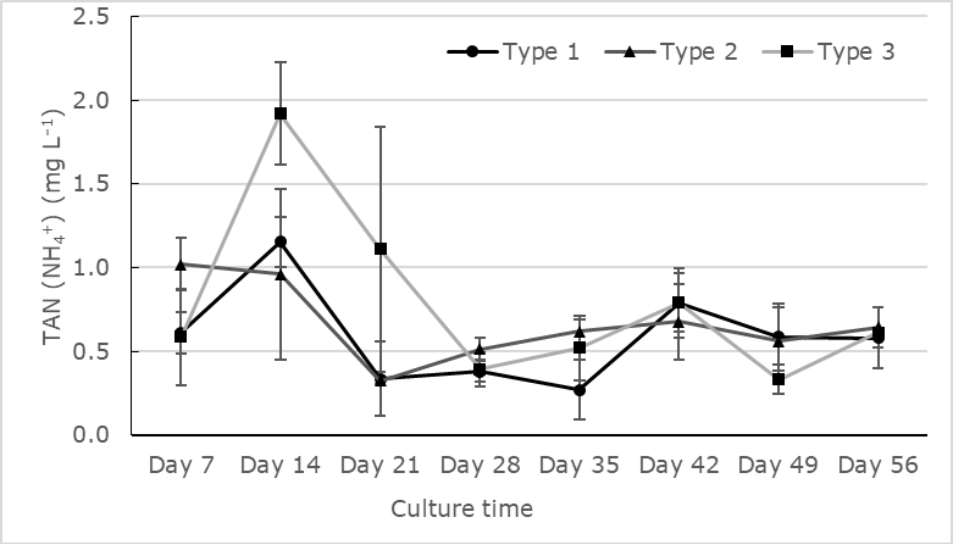

Figure 2. TAN (NH_4^+) concentration at different sampling times throughout the experiment.

On the other hand, nitrite concentrations in the present study showed an increasing trend throughout different sampling times. The differences, but not significant, were found at the later samplings (Day 49 and Day 56), where the lowest nitrite levels were noted at Type 2 compared to the other media types (Figure 3).

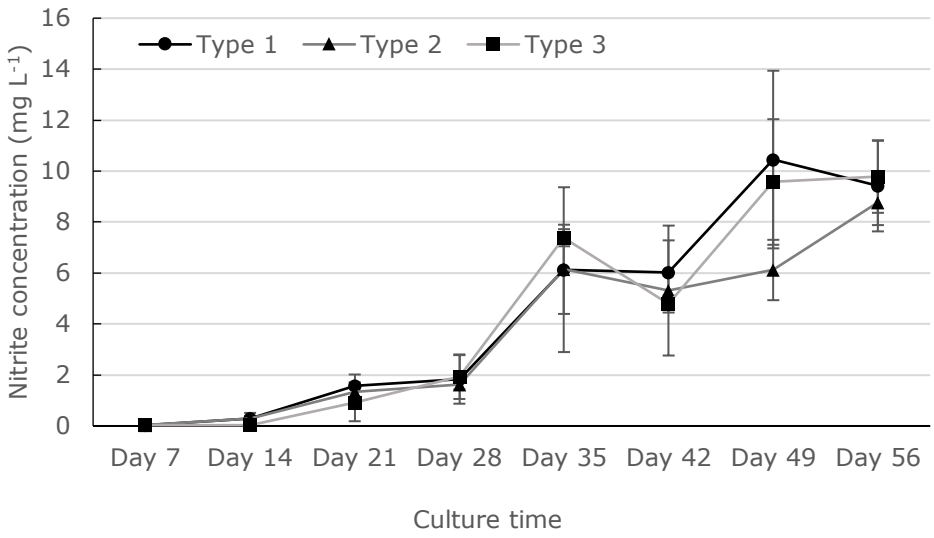

Figure 3. Nitrite concentration at different sampling times throughout the experiment.

In the current study, nitrite concentrations were below or equal to 6 mg L^{-1} until Day 42, except in Type 3 on Day 35. On Day 49, the nitrite concentration in the type 2 treatment remained at 6.12±1.19 mg L^{-1}, whereas those in types 1 and 3 increased to 10.45±3.49 mg L^{-1} and 9.57±2.46 mg L^{-1}. In the last measurement, nitrite concentration in the three treatments ranged between 8.87 and 9.79 mg L^{-1} (Figure 3).

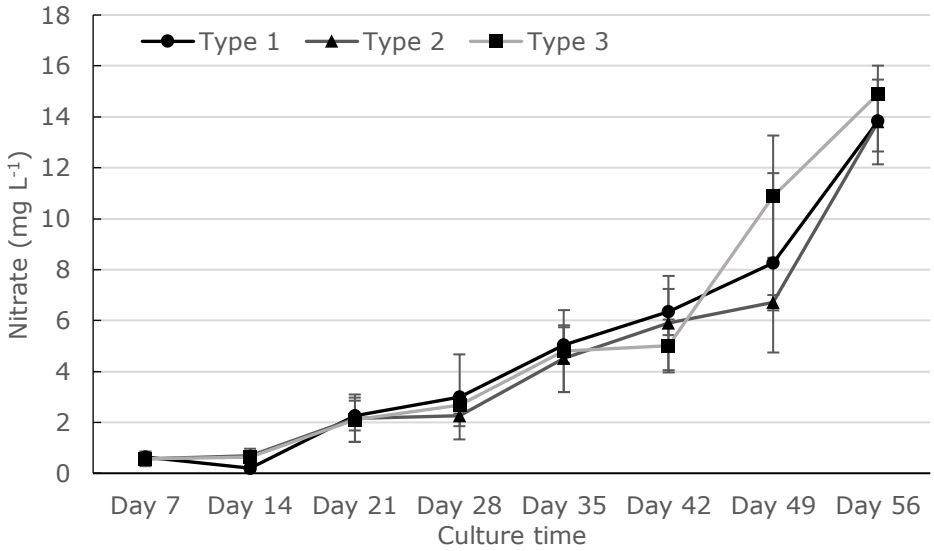

Figure 4. Nitrate concentration at different sampling times throughout the experiment.

There were no significant differences in nitrate concentrations among the three biofilter media types at any sampling points ($p>0.05$). Noticeable variations appeared on Day 49, with the lowest nitrate concentration observed in the Type 2 treatment (6.70±0.30 mg L^{-1}) and the highest in Type 3 (10.87±2.40 mg L^{-1}). Type 3 continued to have the highest nitrate level on Day 56 (14.90±1.11 mg L^{-1}), while the other two treatments showed similar concentrations (1.80 and 1.83 mg L^{-1}) (Figure 4).

Growth performance Indicators: Generally, all growth indices showed no significant differences among the experimental media types ($p > 0.05$). The data on shrimp weight indicates steady growth across all treatments over the 56 days. Initially, all shrimp started with a weight of approximately 0.38 g. By day 14, shrimp in Type 3 biofilters demonstrated slightly higher growth (1.71±0.25 g), although this difference was not statistically significant compared to Types 1 and 2. By day 28, shrimp in Type 2 biofilters exhibited the

highest weight (4.35±0.32 g), maintaining a slight lead through day 42 (8.59±1.10 g). By day 56, final shrimp weights across the treatments were comparable, with Type 1 at 14.18±0.62 g, Type 2 at 14.27±0.35 g, and Type 3 at 13.89±1.21 g (Figure 5). Throughout the experiment, no statistically significant differences were observed in shrimp weight among the treatments, indicating that all biofilter media types provided similar conditions for weight gain.

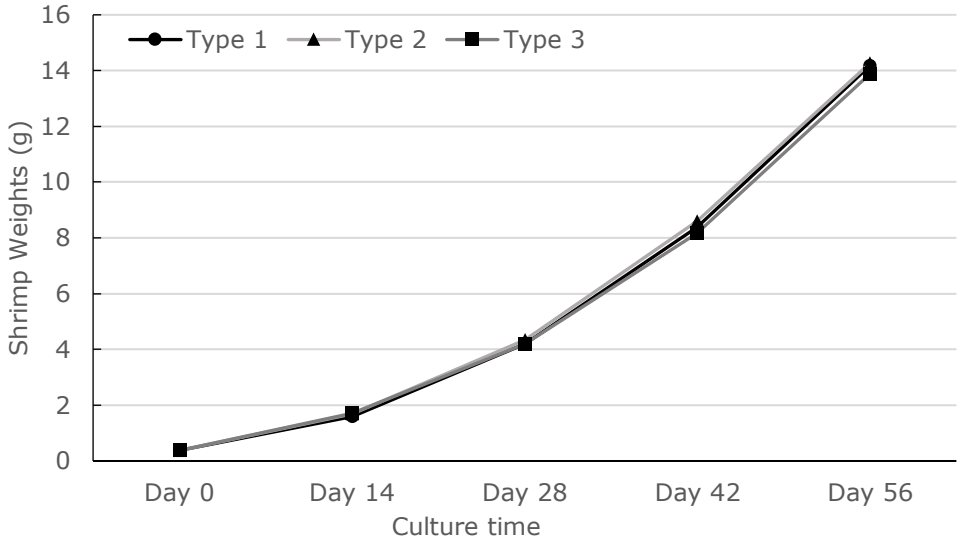

Figure 5. Shrimp weight (g) at different sampling times

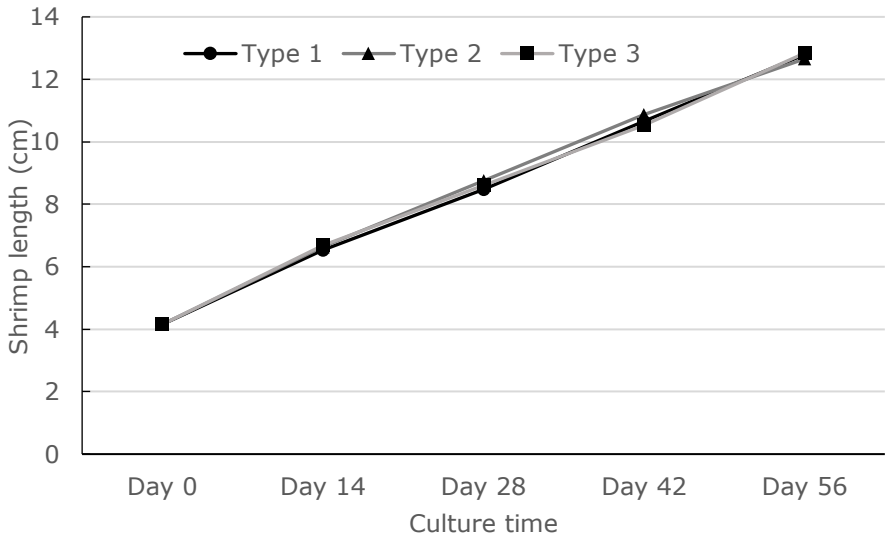

Figure 6. Shrimp length (cm) at different sampling times.

Shrimp length also showed consistent growth, with no statistically significant differences between treatments at any sampling point. All groups started at approximately 4.15 cm on Day 0. By Day 14, shrimp in Type 3 showed a slightly higher length (6.69±0.31 cm) than the other treatments. By Day 28, shrimp in Type 2 had a marginally higher length (8.76±0.08 cm) compared to the other treatments. By Day 56, the final lengths were closely aligned, with Type 1 at 12.79±0.29 cm, Type 2 at 12.65±0.14 cm, and Type 3 at 12.84±0.5 cm (Figure 6). Overall, no significant differences in shrimp length were observed among the different biofilter treatments throughout the culture period.

Table 2

Growth performance of whiteleg shrimp in RAS using different biofilter media

Growth indices	*Type 1*	*Type 2*	*Type 3*
LG (cm)	8.64±0.29	8.50±0.14	8.68±0.50
WG (g)	13.8±0.62	13.9±0.35	13.5±1.21
DLG (cm day^{-1})	0.15±0.01	0.15±0.00	0.16±0.01
DWG (g day^{-1})	0.25±0.01	0.25±0.01	0.24±0.02
FCR	$0.95±0.02^{a}$	$0.97±0.03^{ab}$	$1.01±0.03^{b}$
SR (%)	92.7±3.46	92.2±1.39	90.2±4.29
Biomass (kg $(m^3)^{-1}$)	1.97±0.06	1.97±0.02	1.87±0.07

Note: Values are presented as means±SD; the data in a row with similar subscript letters (a,b) show no significant difference.

After 56 days of rearing, shrimp exhibited a length gain of 8.64±0.29 to 8.68±0.50 cm and a weight gain of 13.8±0.62 to 13.9±0.35 g. Daily length gain (DLG) and daily weight gain (DWG) were consistent across treatments, with values of approximately 0.15-0.16 cm day^{-1} and 0.24-0.25 g day^{-1}, respectively. The FCR was significantly lower in Type 1 (0.95±0.02) compared to Type 3 (1.01±0.03) ($p>0.05$), suggesting better feed conversion efficiency in Type 1.

Survival rates were relatively high in all treatments, ranging from 90.2±4.29% in Type 3 to 92.7±3.46% in Type 1. Biomass production followed a similar trend, with Type 1 and Type 2 achieving 1.97 kg $(m^3)^{-1}$, while Type 3 had a slightly lower biomass of 1.87±0.07 kg $(m^3)^{-1}$ (Table 2).

Total Vibrio spp. count in rearing water within 56 days of culture. The densities of total *Vibrio* spp. in this study are summarized in Table 3. There were no significant differences in *Vibrio* spp. counts among experimental biofilter media types ($p>0.05$). During the first 35 days, *Vibrio* spp. counts in all treatments remained relatively stable, ranging from 2.27±0.47 to 10.43±8.12×10² CFU mL^{-1}, except Day 14, where a notable increase in bacterial counts was observed (Table 3). After Day 42, the total *Vibrio* spp. counts rose, peaking on Day 49 at 36.33±12.22 to 43.67±4.16×10² CFU mL^{-1}, before declining to levels between 15.13±9.16 and 19.57±5.78×10² CFU mL^{-1}.

Table 3

Total *Vibrio* spp. count at different sampling times (10^2 CFU mL^{-1})

Sampling Times	*Type 1 (×10² CFU mL^{-1})*	*Type 2 (×10² CFU mL^{-1})*	*Type 3 (×10² CFU mL^{-1})*
Day 7	2.57±0.68	3.00±1.31	3.97±1.99
Day 14	25.47±10.09	27.43±9.36	26.10±7.39
Day 21	3.40±0.96	2.53±0.57	2.50±0.53
Day 28	8.57±3.01	6.80±1.85	10.43±8.12
Day 35	3.23±1.33	3.20±1.35	2.27±0.47
Day 42	19.57±14.5	12.10±17.29	1.60±1.73
Day 49	39.33±5.51	43.67±4.16	36.33±12.22
Day 56	19.57±5.78	15.13±9.16	17.63±9.51

CFU: Colony forming unit, values are presented as mean±SD.

Discussion. The water quality parameters during the experimental period were within the optimal range for shrimp farming and showed no significant differences among treatments ($p>0.05$). Generally, the temperature, pH, DO salinity, and alkalinity were all in the encouraged ranges for shrimp farming (Table 1) (Van Wyk & Scarpa 1999, Krummenauer et al 2011; Zahraie et al 2019; Martins et al 2020). Regarding TAN, Lin and Chen (2001) established safe levels of 2.44, 3.55, and 3.95 mg L^{-1} TAN for 2.2 cm juveniles at salinities of 15, 25, and 35 g L^{-1}, respectively. Additionally, in a prior investigation conducted by

Frías-Espericueta et al (1999), a safe threshold of 7.09 mg L^{-1} TAN was identified for 3.8 g of juvenile shrimp at a salinity of 33 g L^{-1}.

In the current study, nitrite concentrations were below the safe level until Day 42, except in Type 3 on day 35 (Figure 3). Lin and Chen (2003) determined safe nitrite values of 6.1 mg L^{-1} nitrite for whiteleg juveniles at a salinity of 15 ppt. In the last measurement, nitrite concentration in three treatments ranged between 8.87 to 9.79 mg L^{-1}, but the levels was till at safe levels, Huang et al (2020) indicated that no significant differences in the survival rate of whiteleg shrimp exposed to nitrite levels ranging from 0 to 20 mg L^{-1}, while the growth rate was significantly reduced at the nitrite concentration of 20 mg L^{-1}. In the current experiment, nitrite concentrations were maintained at safe levels for whiteleg shrimp farming until Day 42. At the later stage, increased shrimp biomass directly resulted in a corresponding increase in feed input, which led to elevated nutrient levels in the culture system. In conventional farming, farmers typically reduce density in cases of high survival by either partial harvesting or transferring a portion of shrimp to other ponds. Nitrate concentrations increased throughout the experiment but remained within the acceptable range for whiteleg shrimp aquaculture (Figure 4) (Kim et al 2019; Valencia-Castañeda et al 2020). The nitrate concentrations in the present study are lower than the critical safe levels for whiteleg shrimp (45 mg L^{-1}) (Valencia-Castañeda et al 2020). If comparing RAS and bio-floc system, the ammonia was higher in the RAS, while nitrite, nitrate, and turbidity were all significantly lower (Andrew et al 2017).

The results of growth performance suggest that while there were minor variations in performance, all three biofilter media types supported shrimp growth within similar ranges (Table 2). The growth parameters of whiteleg shrimp in this study were consistent across the different biofilter media types. This suggests that while the experimental biofilter media influences water quality to some extent, it did not have a profound impact on shrimp growth, as all media types appeared to maintain acceptable water conditions for shrimp growth. However, it is evident that the shrimp SR and WG exhibited higher values, but not significantly, in the Type 1 and Type 2 treatments. Consequently, these treatments also yielded a higher, though not statistically significant, biomass of harvested shrimp. These improved results, in turn, contributed to a notable reduction in FCR values (Table 3). Biofilter media differ in their surface, structure, and environments for microbial colonization. These variations create distinct microbial communities that may influence nutrient cycling and availability. Consequently, this could enhance feed digestion and assimilation, thereby improving FCR (Chen et al 2021b).

The growth performance of shrimp in this experiment is better than the experiment set by Andrew et al (2017), even though other input parameters were similar, such as the size of the juvenile, the culture duration (about 56 days), or the water temperature. Moreover, the FCR of this study was much lower (1 compared with 1.5), but the biomass of this experiment was similar (about 2 kg $(m^3)^{-1}$) even if the stocking density was lower (150 compared with 250 individuals $(m^3)^{-1}$. Normally, the growth performance of shrimp culture in RAS is faster than other operation systems like bio-floc due to the excessively concentrated particles, which may increase the oxygen demand of the microbial community, clog shrimp gills, promote nuisance microorganisms, and slow shrimp growth (Ray et al 2009).

In terms of microorganisms, *Vibrio* spp. are prevailing microorganisms in seawater and brackish water, with a share of the bacterial community of up to 40%, and several *Vibrio* spp. form a part of the natural microflora of fish and shellfish (Bauer et al 2021). In this study, *Vibrio* spp. count results align with our previous trials conducted at Can Tho University, where Vibrio counts ranged from 10^2 to 10^3 CFU $mL^{-1,}$ which are in the critical safe limit. Additionally, the *Vibrio* spp. densities observed in the current study were notably lower than those reported in previous research on whiteleg shrimp cultured in recirculating aquaculture systems and bio-floc systems (Chen et al 2018; Khoa et al 2020) recorded Vibrio counts around 10^4 CFU mL^{-1}.

Conclusions. This study demonstrated that all treatments maintained optimal conditions for shrimp farming and no significant differences in water quality, growth performance, and *Vibrio* spp. counts were observed. However, the FCRs were lightly affected by the types of biofilter media. In terms of water quality, the study reviewed that total surface area of biofilter media is the key factor in RAS application in shrimp farming regardless of the amount of that media. However, for commercial scalability, biofilter media selection should consider both biological efficiency and economic feasibility. Future studies should incorporate cost-benefit analyses to assess the viability of different biofilter types, taking initial investment into account. Additionally, investigating microbial community composition across different biofilter media would provide deeper insights into microbial colonization, nutrient cycling, and disease control.

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