

Article

Assessment of Water Quality, Growth of *Penaeus vannamei*, and Partial Budget in Super-Intensive BFT and RAS: A Comparison Between Sustainable Aquaculture Systems

Bianca de Oliveira Ramiro ¹, Wilson Wasielesky, Jr. ¹, Otávio Augusto Lacerda Ferreira Pimentel ¹, Taozhu Sun ², Ethan McAlhaney ², Stephen Urick ², Fernando H. Gonçalves ^{2,*}, Jonathan van Senten ², Michael H. Schwarz ² and Dariano Krummenauer ¹

¹ Estação Marinha de Aquicultura, Instituto de Oceanografia, Universidade Federal do Rio Grande, Rio Grande 96210-030, RS, Brazil; biancadeoliveiraramiro@gmail.com (B.d.O.R.); manow@mikrus.com.br (W.W.J.); otavio.pimentel@yahoo.com (O.A.L.F.P.); darianok@gmail.com (D.K.)

² Virginia Seafood Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Hampton, VA 23669, USA; taozhu@vt.edu (T.S.); emcalha07@vt.edu (E.M.); surick@vt.edu (S.U.); jonat86@vt.edu (J.v.S.); mschwarz@vt.edu (M.H.S.)

* Correspondence: ocfernando@vt.edu

Abstract: This study evaluated water quality, growth, and partial budget analysis (PBA) for *Penaeus vannamei*, comparing super-intensive Biofloc Technology (BFT) and Recirculating Aquaculture Systems (RAS). The 69-day trial used 100 L units with two treatments (RAS and BFT), each with three replicates. Shrimp were initially reared in a 30-day nursery to a weight of 0.10 ± 0.04 g and then stocked at 500 shrimp m^{-3} . Biofloc growth in BFT was promoted by maintaining a C:N ratio of 15:1, adding dextrose when total ammonia nitrogen (TAN) reached 1 mg L^{-1} . Probiotics (3 g m^{-3}) were administered daily to both groups. TAN levels in BFT initially spiked but stabilized after 36 days. *Vibrio* abundance was initially higher in RAS, but by the end of the trial, it was higher in BFT. Final weight, weekly growth ratio, and yield were greater in BFT, whereas feed conversion ratio (FCR) and water use were higher in RAS. Survival rates were 83.33% in BFT and 88% in RAS. BFT achieved a superior net benefit/cost compared to RAS. Although RAS more effectively controlled nitrogenous compounds, BFT exhibited better growth performance, with higher final weights, lower FCR, and better *Vibrio* management. The partial budget analysis indicated an economic advantage for BFT, with a net positive benefit of \$2270.09 when shifting from RAS to BFT due to lower operating costs and higher shrimp yield. Among these two sustainable production systems, BFT was more productive while utilizing less natural resources.

Keywords: recirculating aquaculture system; biofloc; nitrogenous compounds; *Vibrio*; sustainable shrimp production



Citation: Ramiro, B.d.O.; Wasielesky, W., Jr.; Pimentel, O.A.L.F.; Sun, T.; McAlhaney, E.; Urick, S.; Gonçalves, F.H.; van Senten, J.; Schwarz, M.H.; Krummenauer, D. Assessment of Water Quality, Growth of *Penaeus vannamei*, and Partial Budget in Super-Intensive BFT and RAS: A Comparison Between Sustainable Aquaculture Systems. *Sustainability* **2024**, *16*, 11005. <https://doi.org/10.3390/su162411005>

Received: 20 November 2024

Revised: 6 December 2024

Accepted: 13 December 2024

Published: 15 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Aquaculture has advanced significantly by incorporating innovative technologies that aim to preserve water resources and reduce environmental impacts. Among these approaches, Recirculating Aquaculture Systems (RAS) and Biofloc Technology System (BFT) operate with minimal water use [1,2]. The practice of *Penaeus vannamei* intensive and super-intensive culture minimizing water exchange meets growing environmental concerns driven by the concept of sustainable development, which seeks to integrate principles of ecological prudence, economic efficiency, and social equity into all human activities [3–5].

RAS provides a high level of control over the aquatic environment, allowing for more efficient production in terms of space and labor use, in addition to substantially reducing water consumption in relation to the biomass produced. According to Timmons et al. [6], these systems facilitate economies of scale, enabling high shrimp production compared to

other aquaculture methods. The configuration of RAS includes devices for water treatment and reuse, such as decanters, mechanical filters, and biological filters [7]. The use of mechanical filters allows the removal of solid waste, including feed remains and feces, whereas biological filters promote the action of nitrifying bacteria to control the levels of ammonia and nitrite in the water [8]. Thus, these systems can achieve high yields with minimal environmental risks, making them one of the most promising technologies for modern aquaculture.

The BFT system represents a complex and dynamic environment characterized by comprehensive microbial diversity, comprising not only bacteria but also algae, protozoa, and organic matter [9,10]. This system adopts an ecologically responsible approach that favors the reuse of water in several cycles, resulting in significant environmental benefits such as reducing pollution in coastal areas [11]. Furthermore, the implementation of BFT has been shown to promote optimized yields in *P. vannamei* cultures at high stocking densities [12,13]. Therefore, this strategy not only increases productivity but also improves environmental control by minimizing or eliminating the need for water changes, thus contributing to the sustainability of the aquaculture sector [12,14,15].

In the context of these systems, the accumulation of nitrogenous compounds results mainly from the ingestion of food by shrimp, their excretion, and the decomposition of organic matter present in the culture environment, including unconsumed feed and feces [10]. Maintaining inadequate levels of total ammonia nitrogen (TAN) and nitrite (NO_2^-) can induce stress and physiological changes in cultured organisms, harming their growth and survival, with a consequent negative impact on production [16]. Therefore, nitrifying bacteria present in bioflocs play a key role in controlling these toxic nitrogenous compounds, facilitating their oxidation to less harmful forms such as nitrate (NO_3^-) [17].

When using RAS and BFT systems in aquaculture, an interconnected approach has emerged, driven by the growing need for sustainability and productivity. Using these systems is essential to mitigate the environmental impacts of intensive aquaculture and promote a more responsible approach to the use of water resources. Although there are studies on BFT and RAS, this research explores, in an unprecedented way, the comparison of specific production costs under super-intensive aquaculture conditions, highlighting the economic and microbiological advantages of bioflocs. This study aimed to evaluate the differences in water quality parameters, *Penaeus vannamei* growth, and partial budget analysis (PBA) between BFT and RAS systems, emphasizing economic factors and *Vibrio* control throughout the cultivation cycle.

2. Materials and Methods

2.1. Design and Experimental Conditions

A *P. vannamei* shrimp grow-out was carried out for 69 days at the Virginia Seafood Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Hampton, VA, USA. *P. vannamei* post-larvae were acquired from Homegrown Shrimp, LLC, Indiantown, FL, USA. Shrimp were initially kept in a 30-day nursery until they reached a weight of 0.10 ± 0.04 g (mean \pm standard deviation) and then were stocked in the 6 experimental units at a density of 500 shrimp m^{-3} .

The experiment was carried out in 100 L experimental units and divided into two treatments, all with three repetitions: RAS (Recirculating Aquaculture System) and BFT (Biofloc Technology System). Seawater (salinity between 28 and 30 g L^{-1}) obtained from mixing tap fresh water with artificial salt (Instant Ocean Sea Salt, Blacksburg, VA, USA) was initially treated with 10 g m^{-3} of sodium hypochlorite and subsequently dechlorinated using ClorAm-x (Reed Mariculture, Campbell, CA, USA).

In the RAS treatment, the water was driven by a 0.75 hp centrifugal pump (Doheny's, model 2601, flow rate of 180 L h^{-1}) to a mechanical Bubble bead filter and then to a biological filter as steps of water treatment before being recirculated among experimental units. The biological filter was composed of K1 Kaldnes Biological Media (Evolution Aqua, Green Brook, NJ, USA) and was constantly aerated with an air injector (Nozzle[®], model

a3, Detroit, MI, USA). Additionally, two air stones were installed inside each RAS tank to maintain optimal oxygen levels. The total volume of the RAS system was approximately 600 L.

In the BFT treatment, biofloc growth was stimulated by maintaining a carbon:nitrogen (C:N) ratio of 15:1 [16,18], by adding dextrose (based on Serra et al. [19]) once the total ammonia nitrogen (TAN) concentration reached 1 mg L^{-1} . In the BFT tanks, a structure containing four porous stones (7.6 cm long \times 2.5 cm wide \times 2.5 cm deep) coupled to a central hose was arranged in each tank to provide oxygenation by an air pump (Intertek, model AP-60, Chickasaw, AL, USA).

In both treatments, the commercial probiotic Sanolife MIC (Inve[®] Aquaculture, Dendermonde, Belgium), composed of *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus* (concentration: 5×10^{10} colony forming units (CFU) g^{-1}) was administered daily at a dosage of 3 g m^{-3} . The probiotics were diluted in water and incorporated into the feed before each feeding.

2.2. Water Quality Variables

Temperature ($^{\circ}\text{C}$), dissolved oxygen (DO, mg L^{-1}) (YSI Pro 2030), pH (Hanna, model HI98107), TAN (mg L^{-1}) [20], and nitrite nitrogen ($\text{NO}_2^{-}\text{-N}$, mg L^{-1}) (Hach method 8507) were measured daily. The concentration of nitrate nitrogen ($\text{NO}_3^{-}\text{-N}$, mg L^{-1}) (Hach method 8039), total suspended solids (TSS, mg L^{-1}) (Hach portable multiparameter colorimeter, model DR900), settleable solids (SS, mL L^{-1}) [21], CO_2 (mg L^{-1}) [22], and alkalinity (mg L^{-1}) [23] were measured weekly. When pH and alkalinity were below 7.5 and 150 mg L^{-1} , respectively, adjustments were made with sodium bicarbonate (NaHCO_3) application, following Furtado et al. [24]. On day 51, a sampling error occurred for $\text{NO}_2^{-}\text{-N}$ in both treatments. Therefore, we decided to present the data only up to day 50.

2.3. Vibrio Community Composition

Water samples were collected from experimental units on days 0, 14, 28, 42, and 63 of the trial to assess the abundance of *Vibrio* spp. in the systems. From each tank, at least two 10 mL water samples were drawn as biological replicates. Samples were collected using sterile techniques to prevent contamination.

The samples were subjected to a standard plate count method to enumerate *Vibrio* spp. Initially, each sample was diluted using sterile phosphate-buffered saline (PBS) to prepare serial dilutions appropriate for counting. The diluted samples were then plated on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, a selective medium for the isolation of *Vibrio* species. Plates were incubated at $35 \text{ }^{\circ}\text{C}$ for 24 h. After the incubation, colonies characteristic of *Vibrio* spp. were counted on the TCBS agar plates. The counts were expressed as colony-forming units (CFU) mL^{-1} , according to the methodology proposed by FDA BAM [25].

2.4. Feed Management

The shrimp were fed Ziegler[®] commercial feed (Gardners, PA, USA), with 35% crude protein, distributed manually three times a day. The amount of feed offered to the animals was calculated according to Jory et al. [26], which is based on the animal's weight and the temperature of the culture water.

2.5. Shrimp Growth, Survival, and Water Use

At the end of the experimental time, shrimp sampling was carried out to determine final weight (g), feed conversion ratio (FCR), survival (%), weekly growth ratio (WGR, g week^{-1}), yield (Kg m^{-3}), and water use ($\text{m}^3 \text{ Kg}^{-1}$).

2.6. Partial Budget Analysis

A partial budget analysis (PBA) was performed considering the costs and revenue associated with shrimp production in RAS compared to BFT systems. Partial budget

analysis measures the net benefit from the difference between the benefits and costs for a small change in the operation [27]. In this case, the RAS and BFT systems have different equipment requirements and procedures that impact on the costs of water use, salt, sodium bicarbonate, dextrose, feed, electricity, labor, operating interest, and depreciation.

The PBA considered two different scenarios to estimate the net benefit cost of a RAS system turning into a BFT system (RAS to BFT) and a BFT system turning into a RAS system (BFT to RAS), based on the performance obtained in the grow-out experiment. The indicators utilized in the PBA are similar to those used by Kruppenauer et al. [28] and are described as follows. Additional revenue was estimated based on the difference in gross receipts between RAS and BFT systems for each scenario. Reduced costs were estimated based on the difference in the input supply items, operating interest, and equipment depreciation between RAS and BFT systems. Total additional benefits = Additional revenue + Reduced costs. In each scenario, additional costs were estimated based on the difference in input supply items, operating interest, and equipment depreciation between RAS and BFT systems. Reduced revenue was estimated based on the difference in gross receipts between RAS and BFT systems for each scenario. Total additional cost = Reduced revenue + Additional costs. Net benefit/cost = Total additional benefits—Total additional costs.

2.7. Data Analysis

Water quality data were tested for normality and homoscedasticity with the Shapiro-Wilk and the Levene test, respectively. Differences between treatments were tested with a repeated measures analysis of variance (ANOVA). When necessary, data were transformed to fulfill parametric assumptions. For non-parametric data (NO_2^- -N), the Friedman test was used.

Vibrio abundance (analyzed separately for each day sampled), shrimp growth, and water use data were tested for normality and homoscedasticity with the Shapiro-Wilk and the Levene test, respectively. Differences between treatments were tested with the T-test. When necessary, data were transformed to fulfill parametric assumptions. Non-parametric data (*Vibrio*—day 28 and survival) were analyzed with the Wilcoxon test.

The graphs, T-test, Wilcoxon, and Friedman tests were performed in the software R 4.3.1 [29] using the packages car [30], stats [29], rstatix [31], and ggplot2 [32]. Repeated measures ANOVA was performed using Past 4.03 2020 software [33].

3. Results

3.1. Water Quality

Temperature was 28.21 °C in RAS and 29.82 °C in the BFT System, dissolved oxygen was above 5 mg L⁻¹, and pH was close to 8 in both treatments (Table 1).

TAN, NO_2^- -N, and NO_3^- -N, and turbidity were higher in the BFT treatment than in the RAS treatment (Table 2). The BFT treatment had spikes in TAN concentration on days 17 (7.25 mg L⁻¹) and 30 (9.78 mg L⁻¹), being controlled from day 36 of the trial (Figure 1a). NO_2^- -N and NO_3^- -N showed an increasing pattern throughout the experimental time in the BFT, while RAS remained stable (Figure 1b,c).

Table 1. Water quality variables during a *Penaeus vannamei* super-intensive grow-out with biofloc technology (BFT) and recirculating aquaculture systems (RAS). Different letters in the same line indicate significant differences between treatments ($p < 0.05$).

Variables	Treatments	
	RAS	BFT
Temperature (°C)	28.21 ± 1.12	29.82 ± 0.79
DO (mg L ⁻¹)	5.75 ± 0.07	5.51 ± 0.16
pH	8.10 ± 0.17	8.11 ± 0.21
TAN (mg L ⁻¹)	1.89 ± 0.60 ^b	3.52 ± 2.00 ^a

Table 1. Cont.

Variables	Treatments	
	RAS	BFT
NO ₂ ⁻ -N (mg L ⁻¹)	0.09 ± 0.11 ^b	2.38 ± 2.23 ^a
NO ₃ ⁻ -N (mg L ⁻¹)	8.85 ± 5.18 ^b	52.73 ± 65.80 ^a
Alkalinity (mg L ⁻¹)	166.20 ± 31.17	199.00 ± 34.62
CO ₂ (mg L ⁻¹)	2.17 ± 0.35	2.69 ± 1.28
TSS (mg L ⁻¹)	31.39 ± 28.75 ^b	217.10 ± 114.95 ^a
SS (mL L ⁻¹)	0.00 ± 0.00 ^b	14.56 ± 15.28 ^a
Turbidity (NTU)	20.38 ± 17.19 ^b	179.90 ± 104.25 ^a

Data are mean ± standard deviation. DO: dissolved oxygen; TAN: total ammonia nitrogen; NO₂⁻-N: nitrite nitrogen; NO₃⁻-N: nitrate nitrogen; CO₂: carbon dioxide; TSS: total suspended solids; SS: settleable solids.

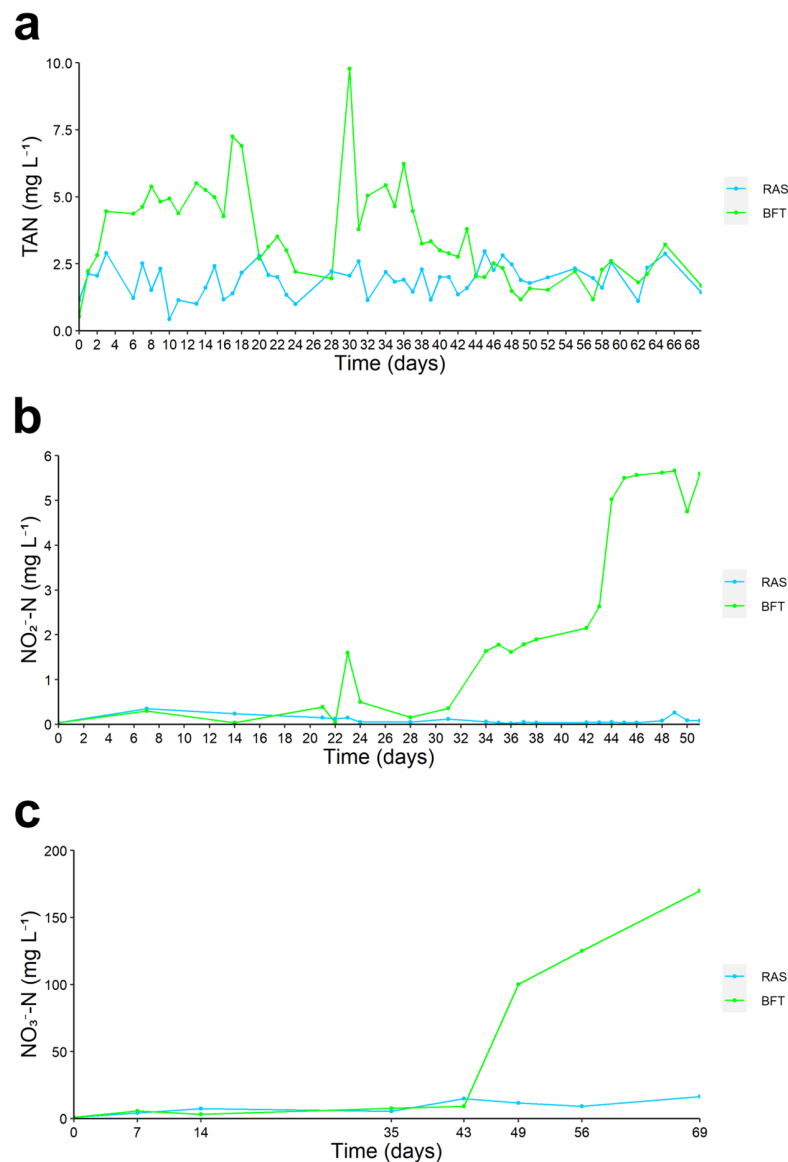


Figure 1. Concentration of total ammonia nitrogen (TAN, (a)), nitrite nitrogen (NO₂⁻-N, (b)), and nitrate nitrogen (NO₃⁻-N, (c)) during a *Penaeus vannamei* super-intensive grow-out with biofloc technology (BFT) and recirculating aquaculture systems (RAS).

The TSS was higher in the BFT treatment, with a tendency to increase throughout the trial (Table 1, Figure 2). The SS was higher in the BFT treatment compared to the RAS (Table 1).

Table 2. *Penaeus vannamei* growth, survival, and water use at the end of a super-intensive grow-out with biofloc technology (BFT) and recirculating aquaculture systems (RAS). Different letters in the same line indicate significant differences between treatments ($p < 0.05$).

Variables	Treatments	
	RAS	BFT
Initial weight (g)	0.10 ± 0.04	0.10 ± 0.04
Final weight (g)	8.14 ± 1.47 ^b	13.56 ± 1.22 ^a
WGR (g week ⁻¹)	0.80 ± 0.15 ^b	1.35 ± 0.12 ^a
FCR	2.81 ± 0.49 ^a	1.91 ± 0.12 ^b
Survival (%)	88.00 ± 0.00	83.33 ± 9.24
Yield (Kg m ⁻³)	3.58 ± 0.65 ^b	5.62 ± 0.33 ^a
Water use (m ³ Kg ⁻¹)	2.13 ± 0.36 ^a	1.82 ± 0.12 ^b

Data are mean ± standard deviation. WGR: weekly growth rate; FCR: feed conversion ratio.

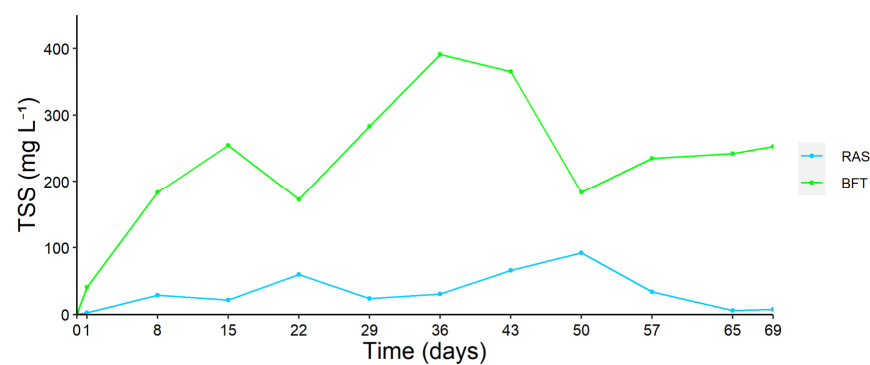


Figure 2. Concentration of total suspended solids (mg L⁻¹) during a *Penaeus vannamei* super-intensive grow-out with biofloc technology (BFT) and recirculating aquaculture systems (RAS).

3.2. *Vibrio* Community Composition

At the beginning of the trial and on days 28 and 42, the RAS treatment had more *Vibrio* than BFT (Figure 3). On day 63, BFT had more *Vibrio* than RAS (Figure 3).

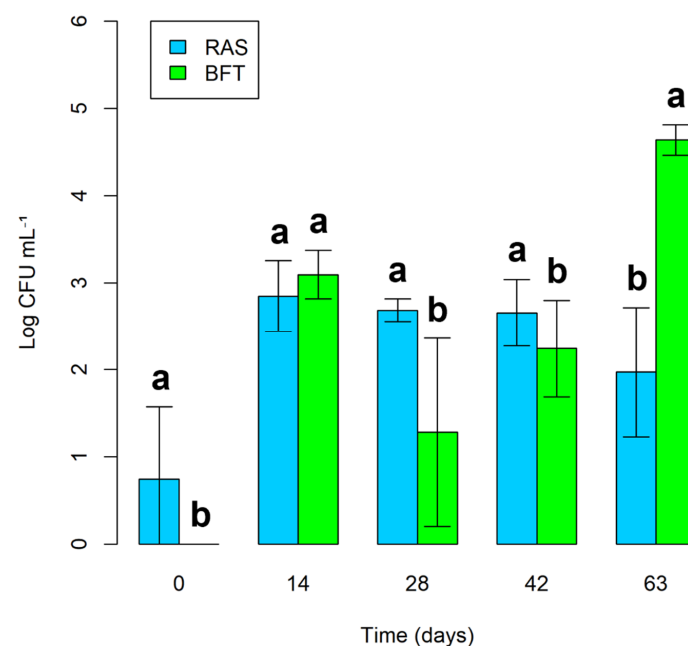


Figure 3. Abundance (Log CFU mL⁻¹) of *Vibrio* spp. during a *Penaeus vannamei* super-intensive grow-out with biofloc technology (BFT) and recirculating aquaculture systems (RAS). Different letters indicate significant differences between treatments ($p < 0.05$).

3.3. Shrimp Growth, Survival, and Water Use

At the end of the trial, final weight, WGR, and yield were higher in the BFT treatment than in the RAS treatment (Table 3). FCR and water use were higher in the RAS treatment than in the BFT treatment (Table 3). Survival was 83.33% in the BFT treatment and 88% in the RAS treatment (Table 2).

Table 3. Price of items considered in the Partial Budget Analysis (PBA) for *Penaeus vannamei* production comparing RAS and BFT systems.

Input	Description	Unit Price (\$)	Total Cost (\$)	
			RAS	BFT
Water	Rate per gallon	3.69	2.92	3.98
Salt	Box 27.2 Kg	50.00	143.77	196.14
Sodium Bicarbonate	Bag 22.68 Kg	34.46	0.07	0.03
Probiotic	500 g	104.00	16.97	16.97
Dextrose	997 g	18.00	-	6.45
Feed	25 Kg	47.79	5.81	6.33
Labor	Wage hour ⁻¹	12.00	346.00	407.00
Electricity	Rate per kWh	2.49	2380.20	137.42
Operating interest	5% interest rate	-	144.79	38.72
Equipment	Description	Unit price (\$)	RAS	BFT
Aeration pump	50 Watts	413.00	413.00	413.00
Water pump	0.75 HP	443.00	443.00	-
Sump	180 gallons	470.00	470.00	-
KMT media	per cu.ft.	45.00	45.00	-
Mechanical sand filter	Bubble bead filter	352.00	352.00	-
Equipment depreciation	\$ year ⁻¹	172.30	32.57	6.16
Production	Description	Unit	RAS	BFT
Final biomass	Experiment yield	G	1074.07	1684.97
Sales price (\$/kg)	Farmer's market	\$ Kg ⁻¹	26.43	26.43
Revenue	From yield	\$	28.39	44.58

3.4. Partial Budget Analysis

The PBA considered economic variables such as costs of water, electricity, labor, and other inputs. These data were collected for both systems and allowed the assessment of the economic cost-benefit comparison. The costs of supplies and equipment were quantified for the scale of the experiment based on the local market prices of goods and services. The items utilized to perform the PBA are listed in Table 3.

The Partial Budget Analysis (PBA) findings (Table 4) indicate that the BFT system is more advantageous compared to shrimp production in RAS. The scenario considering a change from RAS to BFT treatment had a positive net benefit cost of \$2270.09. The scenario considering a change from BFT to RAS requires a series of equipment and extra costs, especially with electricity, which caused the negative net benefit cost of -\$2270.09.

Table 4. Partial budget analysis comparing changing scenarios between RAS to BFT System to produce *Penaeus vannamei*.

Scenarios	Benefits			Costs			Net Benefit/ Cost
	Additional Revenue	Reduced Costs	Total Additional Benefits	Additional Costs	Reduced Costs	Total Additional Costs	
RAS to BFT	16.19	2375.31	2391.50	121.41	0	121.41	2270.09
BFT to RAS	0	121.41	121.41	2375.31	16.19	2391.50	-2270.09

Values are expressed in dollars on a per-cycle basis for the experimental scale.

4. Discussion

In this study, the water quality variables were maintained within the recommended ranges for *Penaeus vannamei* culture, as indicated by Ponce-Palafox et al. [34], Gaona et al. [35], Furtado et al. [24], Maicá et al. [36], and Van Wyk et al. [37]. Temperature, which is a crucial factor for shrimp growth and survival, was monitored carefully. *P. vannamei* can tolerate temperatures ranging from 15 to 35 °C [37]. In both treatments, RAS and BFT, the temperature was maintained above 28.21 °C, which falls within the optimal range for shrimp growth [34].

RAS treatment provided the best conditions for controlling nitrogenous compounds. This was because of the constant mechanical and biological filtration processes used in this treatment. The presence of artificial substrates in one of the water treatment stages provides an increase in the surface area for the growth of chemoautotrophic bacteria, which are responsible for the transformation of toxic nitrogen compounds [38,39]. Furthermore, backwashing was responsible for discarding most of the nitrate produced in the system and maintaining a low concentration throughout the trial [40]. These results are in line with those found by Ray & Lotz [41], who compared the *P. vannamei* culture in RAS and BFT, using a density of 250 shrimp m⁻³, and found the highest control of ammonia and nitrite in RAS, and attributed this to the external filtration process.

In the BFT treatment, the nitrification process was observed because TAN concentrations were controlled from day 31 of the trial, and nitrate began to increase from day 43. The reduction in nitrite concentrations may not have occurred because nitrite-oxidizing bacteria were not fully established in the system. The observed TAN and nitrite spikes are typical of a newly started system [42]. This behavior was also reported by Ren et al. [43], who observed TAN spikes within the first two weeks of culture and elevated nitrite levels until the end of the seventh week of the trial. Biofloc development in aquaculture tanks requires a certain period until a stable maturity state is achieved [44]. A reliable BFT system is often established 30 days after the initial application of organic carbon to water [45]. Consequently, dangerous spikes in TAN and nitrite commonly occur during the initial weeks of BFT culture [43,46,47].

Under these conditions, management strategies are adopted to maintain the nitrogenous compound concentration within the appropriate limits for the species being cultivated. In contrast, the nitrogen cycling process does not begin to be carried out by chemoautotrophic bacteria. High concentrations of TAN are controlled by manipulating the Carbon:Nitrogen (C:N) ratio of water with the addition of an organic carbon source, which stimulates TAN immobilization through the growth of heterotrophic bacteria [48]. Nitrite concentration can be managed by water changes, which is the most effective strategy for eliminating part of this compound in the absence of the oxidation process of this compound by nitrite-oxidizing bacteria.

The composition of the *Vibrio* sp. community in the water exhibited several distinct patterns. It is important to highlight that the presence of these bacteria can be disadvantageous since some species have pathogenic potential and can cause diseases, such as vibriosis in shrimp [49]. Our findings revealed that the abundance of *Vibrio* was higher in the RAS treatment during most of the experiment. The difference in *Vibrio* abundance between the RAS and BFT systems throughout the experiment suggests that the characteristics of the bioflocs may play a significant role in bacterial control, especially in the initial days, with a lower abundance of *Vibrio* in the BFT. According to Decamp & Moriarty [50], the inclusion of *Bacillus* sp. as a probiotic in diets increases the survival of cultured shrimp and reduces the presence of *Vibrio* sp. in the water and sediment of the tank. Ferreira et al. [51] indicated that microbial bioflocs can serve as a source of Gram-positive probiotic bacteria of the genus *Bacillus* and are effective in controlling opportunistic *Vibrio* bacteria. It is essential to adequately monitor and control *Vibrio* communities in water, in addition to implementing good management practices, to ensure the health of cultured shrimp [11,52,53].

Probiotic inoculation contributed to microbial stability in both systems; however, the biofloc environment in the BFT may have enhanced the action of probiotics, providing more

effective control of *Vibrio*. In addition to helping control water quality conditions, probiotic inoculation also improves the growth of aquatic organisms [54]. A study by Zokaeifar et al. [55] demonstrated that juvenile *Penaeus vannamei* that received probiotics containing *Bacillus subtilis* strains presented significantly higher values of final weight, weight gain, specific growth rate, and survival compared to systems that did not use probiotics. According to Balcázar et al. [56], probiotic bacteria can reduce or eliminate the incidence of pathogenic microorganisms in the intestine, which is extremely important for the animal's immune system, increasing nutrient absorption and, consequently, improving their performance. The use of probiotics in aquatic organisms has shown positive effects in several experiments and cultivation practices, including the control of bacterial diseases [55,57–59].

In terms of final weight and growth rate (WGR), the RAS system showed inferior performance. This result may be associated with the greater presence of *Vibrio* on days 0, 28, and 42 of the experimental time, despite the daily applications of probiotics. In the BFT system, the presence of microorganisms, combined with the inoculation of probiotics, contributed significantly to the growth of *Penaeus vannamei* [60,61]. In addition, the microbiota in the BFT system, composed of protozoa, rotifers, and other microorganisms, promoted better growth performance for shrimp [62]. These findings contrast with the studies by Ray et al. [63] and Ray & Lotz [41], who compared the intensive cultivation of *P. vannamei* in the RAS and BFT systems, observing superior performance and survival in the RAS system. In our results, we observed that, on certain points, the RAS system presents a higher abundance of *Vibrio*, while on others, the BFT system has higher values. This suggests that the two systems influence the presence of *Vibrio* in different ways over time.

In the BFT system, in addition to the feed provided, the shrimp were able to benefit from the microbial flocs as an additional food source, increasing the culture yield. According to Jory et al. [26] and Tacon et al. [64], bioflocs have high levels of proteins and other essential nutrients that complement the shrimp diet [65]. When analyzing the nutritional influence using the stable isotope technique in the BFT system, it was observed that the microbial community present in the bioflocs was reflected in the tissues of *P. vannamei*, representing a nutritional contribution. These studies corroborate with our results, where the yield was higher in the BFT treatment than in the RAS.

The results obtained in this study revealed a significant difference in water use between RAS and BFT in the super-intensive culture of *Penaeus vannamei*. The BFT system demonstrated superior efficiency, using only 1.82 m³ of water per kilogram of shrimp produced, while RAS required 2.13 m³ of water per kg of shrimp. By using less water per shrimp produced, BFT is more aligned with sustainability principles, as it reduces dependence on this natural resource. This aspect is especially important in a global scenario where the demand for aquatic products continuously increases while water resources become scarce due to climate change, urbanization, and population growth.

The overall growth performance was superior in the BFT system compared to RAS. The BFT advantage over RAS can be significantly improved by the addition of micro and nanobubble technology [66] and artificial substrates [67]. These management strategies for aeration and bacteria colonization improve the nitrification process, the microbial community composition, and the growth of *Penaeus vannamei* [66,67]. However, the economic implications of the adoption of micro and nanobubble technology and artificial substrate in super-intensive biofloc systems have never been quantified.

The Partial Budget Analysis (PBA) findings reinforce the advantages of the BFT system over RAS from an economic standpoint. Even though the BFT system presented higher costs with water, salt, dextrose, feed, and labor, the increased productivity of the BFT system yielded larger shrimp, which can potentially access a different market price compared to RAS-produced shrimp. The RAS system's dependence on equipment such as water pumps, sump, KMT media, and sand filters adds challenges to investment capital allocation and increases energy consumption and electricity costs, causing a negative net benefit cost for this system. However, this analysis considered the scale of the experiment, and most of the equipment utilized is over-dimensioned for the experiment's needs. Even though this

PBA is limited by the experimental scale and is not representative of commercial farms, this method serves as a tool for short-term financial evaluation and decision-making to improve efficiency and profitability [27]. However, aspects of the long-term viability and sustainability of the production systems should be assessed via a feasibility analysis [28], considering real-world farming conditions

5. Conclusions

The results of this study indicate that the BFT system is technically and economically more advantageous than shrimp production in RAS, particularly under the specific conditions of the United States of America. The natural productivity from aggregates in BFT provides better zootechnical parameters, offering valuable insights for improving the management of intensive *Penaeus vannamei* culture. The use of probiotics and bioflocs is essential for controlling *Vibrio* sp. and enhancing productive indices. However, these results are closely tied to the characteristics of local water, climate, and regulations in the U.S., which favor the effectiveness of BFT over RAS. Due to the delayed maturation of nitrifiers in BFT, it would be beneficial to initiate the development of stable biofloc before the introduction of shrimp, considering the specific temperature and water quality conditions in the region.

Author Contributions: Conceptualization, B.d.O.R. and D.K.; methodology, B.d.O.R., D.K., F.H.G., M.H.S., J.v.S., S.U., E.M. and T.S.; formal analysis, B.d.O.R., O.A.L.F.P., F.H.G. and T.S.; investigation, B.d.O.R., O.A.L.F.P. and T.S.; resources, W.W.J., D.K., M.H.S. and J.v.S.; data curation, B.d.O.R., O.A.L.F.P., F.H.G. and T.S.; writing—original draft preparation, B.d.O.R.; writing—review and editing, W.W.J., O.A.L.F.P., F.H.G., T.S., S.U., E.M. and D.K.; visualization, B.d.O.R., O.A.L.F.P., F.H.G. and T.S.; supervision, W.W.J., D.K., M.H.S., J.v.S., E.M. and S.U.; project administration, W.W.J., D.K., M.H.S. and J.v.S.; funding acquisition, W.W.J., D.K., M.H.S. and J.v.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Council for Scientific and Technological Development (CNPq, process number: 307741/2022-2 and 313514/2023-2), Foundation for Research Support of the State of Rio Grande do Sul—FAPERGS (process number: 21/2551-505 0002225-6 and 17/2551-0000955-0), and the Coordination for the Improvement of Higher Education Personnel (CAPES; process number: 88887.595816/2020-00, 88887.61299 3/2021-00, and 88887.826108/2023-00).

Institutional Review Board Statement: This study was carried out following the bioethics standards of the Virginia Seafood Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, USA.

Data Availability Statement: The data presented in this study are available on request.

Acknowledgments: The authors would like to thank CAPES and CNPq for their support and the entire VSAREC team. Special thanks to Homegrown Shrimp USA for the post larvae, Ziegler for the feed, and INVE for the probiotic.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Verdegem, M.C.J.; Bosma, R.H.; Verreth, J.A.J. Reducing Water Use for Animal Production through Aquaculture. *Int. J. Water Resour. Dev.* **2006**, *22*, 101–113. [[CrossRef](#)]
2. De Schryver, P.; Crab, R.; Defoirdt, T.; Boon, N.; Verstraete, W. The Basics of Bio-Flocs Technology: The Added Value for Aquaculture. *Aquaculture* **2008**, *277*, 125–137. [[CrossRef](#)]
3. Macintosh, D.; Phillips, M. Environmental Issues in Shrimp Farming. In *Shrimp 92, Proceedings of the 3rd. Global Conference on the Shrimp Industry Hong Kong, China, 1992*; Saram, H., Singh, T., Eds.; Infofish: Kuala Lumpur, Malaysia, 1992.
4. Primavera, J.H. Environmental and Socioeconomic Effects of Shrimp Farming: The Philippine Experience. *Infofish Int.* **1994**, *1*, 44–49.
5. Rosenthal, H. Fish Farm Effluents and Their Control in EC Countries: Summary of a Workshop. *J. Appl. Ichthyol.* **1994**, *10*, 215–224. [[CrossRef](#)]
6. Timmons, M.; Ebeling, J.; Piedrahita, R. *Acuicultura En Sistemas de Recirculación*; NRACE Publications n. 101-2009; Cayuga Aqua Ventures: New York, NY, USA; Fundación Chile: Santiago, Chile, 2009. (In Spanish)

7. Xiao, R.; Wei, Y.; An, D.; Li, D.; Ta, X.; Wu, Y.; Ren, Q. A Review on the Research Status and Development Trend of Equipment in Water Treatment Processes of Recirculating Aquaculture Systems. *Rev. Aquac.* **2019**, *11*, 863–895. [CrossRef]
8. Martins, C.I.; Eding, E.H.; Schneider, O.; Rasmussen, R.; Olesen, B.; Plesner, L.; Verreth, J.A.J. *Recirculation Aquaculture Systems in Europe. Position Paper*; CONSENSUS Working Group 3. Recirculation Systems; Wageningen University & Research: Oostende, Belgium, 2005.
9. Hargreaves, J.A. *Biofloc Production Systems for Aquaculture*; Factsheet 4503; Southern Regional Aquaculture Center: Stoneville, NC, USA, 2013.
10. Robles-Porchas, G.R.; Gollas-Galván, T.; Martínez-Porchas, M.; Martínez-Cordova, L.R.; Miranda-Baeza, A.; Vargas-Albores, F. The Nitrification Process for Nitrogen Removal in Biofloc System Aquaculture. *Rev. Aquac.* **2020**, *12*, 2228–2249. [CrossRef]
11. Krummenauer, D.; Samocha, T.; Poersch, L.; Lara, G.; Wasielesky, W. The Reuse of Water on the Culture of Pacific White Shrimp, *Litopenaeus vannamei*, in BFT System. *J. World Aquac. Soc.* **2014**, *45*, 3–14. [CrossRef]
12. Krummenauer, D.; Peixoto, S.; Cavalli, R.O.; Poersch, L.H.; Wasielesky, W. Superintensive Culture of White Shrimp, *Litopenaeus vannamei*, in a Biofloc Technology System in Southern Brazil at Different Stocking Densities. *J. World Aquac. Soc.* **2011**, *42*, 726–733. [CrossRef]
13. da Silveira, L.G.P.; Krummenauer, D.; Poersch, L.H.; Rosas, V.T.; Wasielesky, W. Hyperintensive Stocking Densities for *Litopenaeus vannamei* Grow-out in Biofloc Technology Culture System. *J. World Aquac. Soc.* **2020**, *51*, 1290–1300. [CrossRef]
14. Samocha, T.M. *Sustainable Biofloc Systems for Marine Shrimp*; Academic Press: San Diego, CA, USA, 2019.
15. Schweitzer, R.; Baccarat, R.F.C.; Gaona, C.A.P.; Wasielesky, W.; Arantes, R. Concentration of Suspended Solids in Superintensive Culture of the Pacific White Shrimp *Litopenaeus vannamei* with Biofloc Technology (BFT): A Review. *Rev. Aquac.* **2024**, *16*, 785–795. [CrossRef]
16. Ebeling, J.M.; Timmons, M.B.; Bisogni, J.J. Engineering Analysis of the Stoichiometry of Photoautotrophic, Autotrophic, and Heterotrophic Removal of Ammonia–Nitrogen in Aquaculture Systems. *Aquaculture* **2006**, *257*, 346–358. [CrossRef]
17. Del’Duca, A.; Cesar, D.E.; Freato, T.A.; Azevedo, R.d.S.; Rodrigues, E.M.; Abreu, P.C. Variability of the Nitrifying Bacteria in the Biofilm and Water Column of a Recirculating Aquaculture System for Tilapia (*Oreochromis niloticus*) Production. *Aquac. Res.* **2019**, *50*, 2537–2544. [CrossRef]
18. Avnimelech, Y. *Biofloc Technology—A Practical Guide Book*, 2nd ed.; The World Aquaculture Society: Baton Rouge, LA, USA, 2012.
19. Serra, F.P.; Gaona, C.A.P.; Furtado, P.S.; Poersch, L.H.; Wasielesky, W. Use of Different Carbon Sources for the Biofloc System Adopted during the Nursery and Grow-out Culture of *Litopenaeus vannamei*. *Aquac. Int.* **2015**, *23*, 1325–1339. [CrossRef]
20. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 1981.
21. Eaton, D.E.; Clesceri, L.S.; Greenberg, A.E. *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; American Public Health Association: Washington, DC, USA; American Water Works Association: Denver, CO, USA; Water Environment Federation: Alexandria, VA, USA, 1995.
22. Timmons, M.B.; Ebeling, J.M. *Recirculating Aquaculture*, 3rd ed.; Ithaca Publishing Company LLC: Ithaca, NY, USA, 2013.
23. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2017.
24. Furtado, P.S.; Poersch, L.H.; Wasielesky, W. Effect of Calcium Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical Performance of Shrimp *Litopenaeus vannamei* Reared in Bio-Flocs Technology (BFT) Systems. *Aquaculture* **2011**, *321*, 130–135. [CrossRef]
25. Kaysner, C.A.; DePaola, A., Jr.; Jones, J. *Bacteriological Analytical Manual (BAM)*; U.S. Food & Drug Administration: Washington, DC, USA, 2004; Chapter 9: Vibrio.
26. Jory, D.E.; Cabrera, T.R.; Dugger, D.M.; Fegan, D.; Lee, P.G.; Lawrence, A.L.; Jackson, C.J.; McIntosh, R.P.; Castañeda, J. A Global Review of Shrimp Feed Management: Status and Perspectives. The New Wave—Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture. *World Aquac. Soc.* **2001**, 104–152.
27. Engle, C. The Enterprise Budget and Partial Budgeting in Aquaculture. In *Aquaculture Economics and Financing*; Wiley: New York City, NY, USA, 2010; pp. 117–130.
28. Krummenauer, D.; Pimentel, O.A.L.F.; Bezerra, A.; Gonçalves, F.H.; Poersch, L.H.; Wasielesky, W. The Use of Automatic Belt Feeders in a *Penaeus vannamei* Pilot Scale Super-Intensive Nursery and Grow-out with Biofloc System. *Aquac. Eng.* **2024**, *107*, 102453. [CrossRef]
29. The R Core Team. *R: A Language and Environment for Statistical Computing, version 3.1.1*; R Foundation for Statistical Computing: Vienna, Austria, 2023.
30. Fox, J.; Weisberg, S. *An R Companion to Applied Regression*, 3rd ed.; SAGE: Thousand Oaks, CA, USA, 2019.
31. Kassambara, A. *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package version 0.7.2, 2023. Available online: <https://CRAN.R-project.org/package=rstatix> (accessed on 1 November 2024).
32. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Springer: New York City, NY, USA, 2011.
33. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Paleontol. Electronica* **2001**, *4*, 9.
34. Ponce-Palafox, J.; Martinez-Palacios, C.A.; Ross, L.G. The Effects of Salinity and Temperature on the Growth and Survival Rates of Juvenile White Shrimp, *Penaeus vannamei*, Boone, 1931. *Aquaculture* **1997**, *157*, 107–115. [CrossRef]
35. Gaona, C.A.P.; Poersch, L.H.; Krummenauer, D.; Foes, G.K.; Wasielesky, W.J. The Effect of Solids Removal on Water Quality, Growth and Survival of *Litopenaeus vannamei* in a Biofloc Technology Culture System. *Int. J. Recirc. Aquac.* **2011**, *12*, 54–73. [CrossRef]

36. Maicá, P.F.; Furtado, P.S.; MARTINS, Á.C.d.S.; Filho, K.C.M.; Wasielesky, W., Jr. Efeito da Alcalinidade no Consumo Alimentar de Juvenis de Camarão Branco do Pacífico Cultivados Em Sistema de Água Clara E Bioflocos. *Bol. Do Inst. De Pesca* **2018**, *44*, e222. [[CrossRef](#)]
37. Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K.L.; Mounta, J.; Scarpa, J. *Farming Marine Shrimp in Recirculating Freshwater Systems*; Harbor Branch Oceanographic Institution: Fort Pierce, FL, USA, 1999.
38. Crab, R.; Avnimelech, Y.; Defoirdt, T.; Bossier, P.; Verstraete, W. Nitrogen Removal Techniques in Aquaculture for a Sustainable Production. *Aquaculture* **2007**, *270*, 1–14. [[CrossRef](#)]
39. Calone, R.; Pennisi, G.; Morgenstern, R.; Sanyé-Mengual, E.; Lorleberg, W.; Dapprich, P.; Winkler, P.; Orsini, F.; Gianquinto, G. Improving Water Management in European Catfish Recirculating Aquaculture Systems through Catfish-Lettuce Aquaponics. *Sci. Total Environ.* **2019**, *687*, 759–767. [[CrossRef](#)] [[PubMed](#)]
40. Timmons, M.B.; Ebeling, J.M.; Wheaton, F.W.; Summerfelt, S.T.; Vinci, B.J. *Recirculating Aquaculture System*, 2nd ed.; Cayuga Aqua Ventures: New York, NY, USA, 2002.
41. Ray, A.J.; Lotz, J.M. Shrimp (*Litopenaeus vannamei*) Production and Stable Isotope Dynamics in Clear-water Recirculating Aquaculture Systems versus Biofloc Systems. *Aquac. Res.* **2017**, *48*, 4390–4398. [[CrossRef](#)]
42. Prangnell, D.I.; Samocha, T.M.; Staresinic, N. Water. In *Sustainable Biofloc Systems for Marine Shrimp*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 37–58.
43. Ren, W.; Li, L.; Dong, S.; Tian, X.; Xue, Y. Effects of C/N Ratio and Light on Ammonia Nitrogen Uptake in *Litopenaeus vannamei* Culture Tanks. *Aquaculture* **2019**, *498*, 123–131. [[CrossRef](#)]
44. Xu, W.-J.; Morris, T.C.; Samocha, T.M. Effects of C/N Ratio on Biofloc Development, Water Quality, and Performance of *Litopenaeus vannamei* Juveniles in a Biofloc-Based, High-Density, Zero-Exchange, Outdoor Tank System. *Aquaculture* **2016**, *453*, 169–175. [[CrossRef](#)]
45. Abu Bakar, N.S.; Mohd Nasir, N.; Lananan, F.; Abdul Hamid, S.H.; Lam, S.S.; Jusoh, A. Optimization of C/N Ratios for Nutrient Removal in Aquaculture System Culturing African Catfish, (*Clarias gariepinus*) Utilizing Bioflocs Technology. *Int. Biodeterior. Biodegrad.* **2015**, *102*, 100–106. [[CrossRef](#)]
46. Bakhshi, F.; Najdegerami, E.H.; Manaffar, R.; Tukmechi, A.; Farah, K.R. Use of Different Carbon Sources for the Biofloc System during the Grow-out Culture of Common Carp (*Cyprinus carpio* L.) Fingerlings. *Aquaculture* **2018**, *484*, 259–267. [[CrossRef](#)]
47. Luo, G.; Gao, Q.; Wang, C.; Liu, W.; Sun, D.; Li, L.; Tan, H. Growth, Digestive Activity, Welfare, and Partial Cost-Effectiveness of Genetically Improved Farmed Tilapia (*Oreochromis niloticus*) Cultured in a Recirculating Aquaculture System and an Indoor Biofloc System. *Aquaculture* **2014**, *422–423*, 1–7. [[CrossRef](#)]
48. Avnimelech, Y. Carbon/Nitrogen Ratio as a Control Element in Aquaculture Systems. *Aquaculture* **1999**, *176*, 227–235. [[CrossRef](#)]
49. Tan, D.; Gram, L.; Middelboe, M. Vibriophages and Their Interactions with the Fish Pathogen *Vibrio anguillarum*. *Appl. Environ. Microbiol.* **2014**, *80*, 3128–3140. [[CrossRef](#)] [[PubMed](#)]
50. Decamp, O.; Moriarty, D.J.W. Probiotics as Alternative to Antimicrobials: Limitations and Potential. *World Aquac.* **2006**, *73*, 60–62.
51. Ferreira, G.S.; Bolívar, N.C.; Pereira, S.A.; Guertler, C.; Vieira, F.d.N.; Mouriño, J.L.P.; Seiffert, W.Q. Microbial Biofloc as Source of Probiotic Bacteria for the Culture of *Litopenaeus vannamei*. *Aquaculture* **2015**, *448*, 273–279. [[CrossRef](#)]
52. Hostins, B.; Lara, G.; Decamp, O.; Cesar, D.E.; Wasielesky, W. Efficacy and Variations in Bacterial Density in the Gut of *Litopenaeus vannamei* Reared in a BFT System and in Clear Water Supplemented with a Commercial Probiotic Mixture. *Aquaculture* **2017**, *480*, 58–64. [[CrossRef](#)]
53. Chen, S.; Ling, J.; Blancheton, J.-P. Nitrification Kinetics of Biofilm as Affected by Water Quality Factors. *Aquac. Eng.* **2006**, *34*, 179–197. [[CrossRef](#)]
54. Wang, Y.-B. Effect of Probiotics on Growth Performance and Digestive Enzyme Activity of the Shrimp *Penaeus vannamei*. *Aquaculture* **2007**, *269*, 259–264. [[CrossRef](#)]
55. Zokaeifar, H.; Balcázar, J.L.; Saad, C.R.; Kamarudin, M.S.; Sijam, K.; Arshad, A.; Nejat, N. Effects of *Bacillus Subtilis* on the Growth Performance, Digestive Enzymes, Immune Gene Expression and Disease Resistance of White Shrimp, *Litopenaeus vannamei*. *Fish Shellfish. Immunol.* **2012**, *33*, 683–689. [[CrossRef](#)] [[PubMed](#)]
56. Balcazar, J.; Blas, I.; Ruizzarzuola, I.; Cunningham, D.; Vendrell, D.; Muzquiz, J. The Role of Probiotics in Aquaculture. *Vet. Microbiol.* **2006**, *114*, 173–186. [[CrossRef](#)] [[PubMed](#)]
57. Gatesoupe, F.J. The Use of Probiotics in Aquaculture. *Aquaculture* **1999**, *180*, 147–165. [[CrossRef](#)]
58. Wang, Y.-B.; Li, J.-R.; Lin, J. Probiotics in Aquaculture: Challenges and Outlook. *Aquaculture* **2008**, *281*, 1–4. [[CrossRef](#)]
59. Newaj-Fyzul, A.; Al-Harbi, A.H.; Austin, B. Review: Developments in the Use of Probiotics for Disease Control in Aquaculture. *Aquaculture* **2014**, *431*, 1–11. [[CrossRef](#)]
60. Bauer, W.; Prentice-Hernandez, C.; Tesser, M.B.; Wasielesky, W.; Poersch, L.H.S. Substitution of Fishmeal with Microbial Floc Meal and Soy Protein Concentrate in Diets for the Pacific White Shrimp *Litopenaeus vannamei*. *Aquaculture* **2012**, *342–343*, 112–116. [[CrossRef](#)]
61. Emerenciano, M.; Ballester, E.L.C.; Cavalli, R.O.; Wasielesky, W. Biofloc Technology Application as a Food Source in a Limited Water Exchange Nursery System for Pink Shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquac. Res.* **2012**, *43*, 447–457. [[CrossRef](#)]
62. Wasielesky, W.; Atwood, H.; Stokes, A.; Browdy, C.L. Effect of Natural Production in a Zero Exchange Suspended Microbial Floc Based Super-Intensive Culture System for White Shrimp *Litopenaeus vannamei*. *Aquaculture* **2006**, *258*, 396–403. [[CrossRef](#)]

63. Ray, A.J.; Drury, T.H.; Cecil, A. Comparing Clear-Water RAS and Biofloc Systems: Shrimp (*Litopenaeus vannamei*) Production, Water Quality, and Biofloc Nutritional Contributions Estimated Using Stable Isotopes. *Aquac. Eng.* **2017**, *77*, 9–14. [[CrossRef](#)]
64. Tacon, A.G.J.; Cody, J.J.; Conquest, L.D.; Divakaran, S.; Forster, I.P.; Decamp, O.E. Effect of Culture System on the Nutrition and Growth Performance of Pacific White Shrimp *Litopenaeus vannamei* (Boone) Fed Different Diets. *Aquac. Nutr.* **2002**, *8*, 121–137. [[CrossRef](#)]
65. Krummenauer, D.; Abreu, P.C.; Poersch, L.; Reis, P.A.C.P.; Suita, S.M.; dos Reis, W.G.; Wasielesky, W. The Relationship between Shrimp (*Litopenaeus vannamei*) Size and Biofloc Consumption Determined by the Stable Isotope Technique. *Aquaculture* **2020**, *529*, 735635. [[CrossRef](#)]
66. Ramiro, B.d.O.; Wasielesky, W.; Pimentel, O.A.L.F.; Poersch, L.H.d.S.; Advent, B.; Gonçalves Júnior, G.F.; Krummenauer, D. The Effect of Using Nano and Microbubbles as Aeration Strategies on the Nitrification Process, Microbial Community Composition, and Growth of *Penaeus vannamei* in a Super-Intensive Biofloc System. *Aquaculture* **2024**, *587*, 740842. [[CrossRef](#)]
67. Ramiro, B.d.O.; Wasielesky, W.; Pimentel, O.A.L.F.; San Martin, N.P.; Borges, L.d.V.; Krummenauer, D. Different Management Strategies for Artificial Substrates on Nitrification, Microbial Composition, and Growth of *Penaeus vannamei* in a Super-Intensive Biofloc System. *Aquaculture* **2025**, *596*, 741853. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.