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Biofloc Technology for Sustainable Growth of Shrimp Species: *Litopenaeus Vannamei*

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Abstract

*Aquaculture stands as the fastest-growing food-producing sector globally, undergoing continuous development, expansion, and intensification across all regions. Despite the anticipation of higher production levels, the global growth of aquaculture is constrained by the severe scarcity of water and the limited availability of suitable land. Biofloc systems have emerged as a viable solution tailored for water-scarce areas. This innovative system offers a potential food source for shrimp reared in an environmentally friendly, limited or zero water exchange environment, with minimal effluents released into the surrounding ecosystem. The present study was conducted at a corporate aquaculture farm facility located in Cuddalore district, Tamilnadu. The research focused on evaluating the water quality, survival rates, and growth performance of Litopenaeus vannamei, ranging from post-larvae (PL-9) to 26.6 grams, over a 120-day period within a Biofloc system. Three cement tanks, designated as C1 (control) and C2, C3 (test), were utilized, each with a stocking density of 120 PL/sq.m. After reaching a weight of **2** grams, the shrimp were transferred to grow-out ponds. The results indicated that shrimp reared in Biofloc tanks exhibited significantly higher final weights, survival rates, and favorable water quality parameters compared to the control group, which relied solely on commercial feed. Significantly, as the biofloc volume rose, there was a noteworthy decrease in the requirement for feed application, thereby establishing the Biofloc system as a financially efficient option for shrimp cultivation.*

Keywords: *Biofloc technology; Litopenaeus vannamei; Aquaculture sustainability; Water quality management; Shrimp growth performance*

1. Introduction

Aquaculture, as a pivotal contributor to global food production, faces challenges associated with the generation of substantial waste, primarily stemming from the use of manufactured feeds essential for augmenting production beyond natural capacities (Iwama 1991). The consequential discharge of aquacultural effluents into natural water bodies raises concerns about the proliferation of excess organic pollutants, posing threats of toxicity and environmental imbalance (Piedrahita 2003). This not only leads to the outbreak of diseases but also disrupts biodiversity and ecosystem equilibrium. In contrast to conventional aquaculture methods, Biofloc technology (BFT) emerges as an economically viable and sustainable alternative. This approach distinguishes itself by minimizing water exchange and reducing feed input, thus promoting sustainable development (Avnimelech and Kochba 2009; De Schryver et al. 2008).

At the core of BFT lies the principle of converting toxic nitrogenous wastes into beneficial microbial protein, accomplished by maintaining an optimal Carbon-to-Nitrogen (C/N) ratio, thereby enhancing water quality (Ahmad et al., 2017). The biofloc system tends to exhibit elevated nitrite levels due to the conversion of ammonia to nitrite by heterotrophic bacteria, subsequently utilized by microalgae for growth (Avnimelech 1999; Ebeling et al., 2006). While nitrate, the final product of ammonia nitrification, is considered less harmful to shrimp (Van Wyk and Scarpa 1999), the biofloc environment fosters the utilization of nitrogen sources by heterotrophic bacteria, mitigating toxic levels. The development of Biofloc systems stems from a necessity to enhance environmental control within aquaculture production. In regions facing water scarcity or where land costs are prohibitive, the adoption of more intensive aquaculture practices becomes imperative for cost-effective production. Additionally, the inception of BFT serves as a preventive measure against the spread of diseases from one farm to another, aligning with the overarching goal of improving biosecurity. A deliberate reduction in water exchange emerges as a strategic choice to fortify farm biosecurity, underscoring the multifaceted advantages of Biofloc technology in addressing contemporary challenges in aquaculture. Therefore, the overarching objective is to highlight the significance of Biofloc technology as a transformative approach, not only for economic considerations in water-scarce areas but also as a strategy for enhancing biosecurity and mitigating environmental impacts. Through this exploration, we aim to contribute to the ongoing discourse on sustainable aquaculture practices, providing practical insights that can inform future endeavors in the pursuit of a more resilient and eco-friendly aquaculture industry.

2. Materials And Methods

Tank Preparation

The study took place at a commercial aquaculture farm in Cuddalore district, Tamil Nadu, India. Post-larvae (PL) were obtained from a hatchery in Puducherry and confirmed negative for the white spot syndrome virus (WSSV) through a PCR assay. PL were transported in oxygenated double-layered polythene bags with crushed ice packs to maintain an optimal temperature. All tanks were thoroughly cleaned and disinfected using bleaching powder and dried for 2 days. Additionally, there was a reservoir tank of 220 sq.m. It was filled with 6ppt water, treated with 5ppm bleaching powder, and left for 3 days for dechlorination. Filter bags were checked properly in the inlet and outlet pipes. PL were brought to the farm and treated with potassium permanganate. Then, tank water was slowly added to adjust salinity and pH. Subsequently, the seeds were released into tanks C1, C2, C3 (each 54.6 sq.m) at a stocking density of 120/m2. Before transferring shrimp into grow-out ponds, the ponds were leveled, fixed with HDPE sheets, filled with chlorinated water, and equipped with aerators. Shrimp, along with floc water, were pumped into grow-out ponds P1, P2, P3 (each 200 sq.m) upon reaching 2.0g. C1, P1 served as controls, while C2, C3, P2, P3 were considered test groups.

Floc Preparation in the Tanks

Ammonium chloride was added on the first day to initiate nitrogen in the system. Carbon sources were added on the third and fifth days, with double the quantity on the seventh day. PL 10 were introduced on the ninth day. The water's color changed to light brown, indicating floc formation. Imhoff cones were used to measure the quantity of biofloc, settling within 10 to 20 minutes. The optimum volume was maintained between 10 to 15ml/lit for good functionality in biofloc systems for *Litopenaeus vannamei*. The floc volume increased gradually from 0.1ml to 15ml/lit.

Water Quality Parameters:

Throughout the study, water sampling was conducted daily until harvest, usually between 7.00 a.m. to 09.30 a.m. pH was measured using a pH meter, temperature with a mercury thermometer, transparency with a Secchi disk, and salinity using a hand refractometer. Dissolved oxygen was estimated with a DO meter. Alkalinity, calcium, magnesium, ammonia, nitrite, and nitrate were estimated using titration methods (APHA 2005).

Microbiological Test

Regular microbiological tests were conducted. 0.1 ml of culture water was spread on TCBS (Thiosulfate citrate bile salts sucrose) agar from Hi-Media, India, and incubated overnight at 37°C to measure vibrio prevalence.

Feed Management

Up to PL 14, they were fed with PL feed, and then Blanca feed pellets (CP Aquaculture, India private limited) were provided four times daily at 6 am, 10 am, 2 pm, and 6 pm. No water exchange occurred during the culture period, and daily sludge removal was performed to control nitrite and TSS in the system**.**

Statistical analysis

All the values presented in the manuscript were calculated as the mean of three experiments, each conducted in triplicate, and accompanied by their respective standard deviations (SD).

3. Results and Discussion

The comprehensive evaluation of water quality parameters over a 120-day culture period is detailed in Table 1. Observations of pH, temperature, and dissolved oxygen (DO) were recorded during both the early mornings and late evenings. Throughout the culture period, the pH exhibited a consistent fluctuation pattern. In the early mornings, the pH ranged between 7.9 and 8.4, while during the evenings, it fluctuated between 8.1 and 8.5. This indicates a normal variation in acidity levels, within acceptable limits for the thriving aquaculture environment. Dissolved oxygen levels were diligently maintained above 5.5 through continuous aeration. According to Muthu (1980), maintaining DO levels above 3.5 ml/lit is essential for shrimp culture ponds. However, in the biofloc system, Avnimelech (2012) suggests a higher threshold of more than 5.0 mg/lit, a standard consistently met in our study.

Table 1. Water quality parameters analysis for a 120-day culture period

Parameters	Biofloc	Control
Temperature (◦C)	28.56 ± 0.21	28.48 ± 0.22
Oxygen (mg L^{-1})	5.87 ± 0.18	5.49 ± 0.20
pH	7.92 ± 0.07	7.54 ± 0.05
Salinity (g L^{-1})	6.54 ± 0.15	6.98 ± 0.18
NH_3-N (mg L^{-1})	0.025 ± 0.03	0.03± 0.02
NO_2 −-N (mg L^{-1})	0.60 ± 0.50	1.65 ± 0.69
NO_3 −-N (mg L^{-1})	90 ± 40.62	42.22 ± 19.86
Alkalinity (mg L^{-1})	144.44 ± 29.83	98.55 ± 19.96

Temperature conditions during the culture period remained relatively stable, ranging between 27°C to 28°C, providing an optimal environment for shrimp growth. Salinity was predominantly maintained at 6.5ppt, with occasional peaks observed up to 7ppt, well within acceptable limits for successful culture. Water transparency, measured at 15cms, indicated a clear aquatic environment. Noteworthy was the color transformation of the water, starting with a light brown hue in the initial days, gradually progressing to brown, and ultimately settling into a green tint. This evolution in color reflects the dynamic nature of the aquaculture system. As shown in Fig. 1, the total alkalinity of the water generally stayed within the range of 140 to 160 mg/lit, with occasional decreases during the culture period prompting the addition of sodium bicarbonate to enhance alkalinity levels.

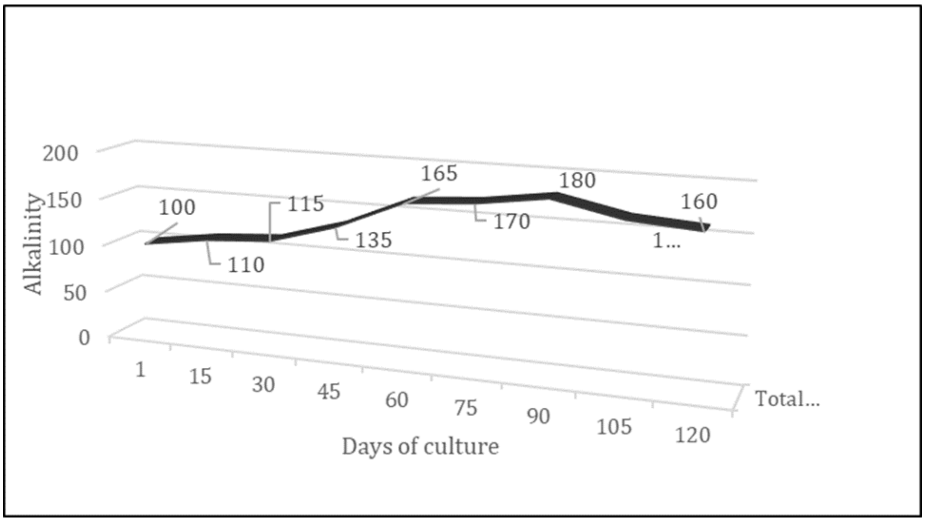

Fig. 1. Dynamics of total alkalinity in the aquaculture system over the culture period

Calcium and magnesium were maintained at a stable ratio of 1:2.5, contributing to the overall stability of water chemistry. Importantly, throughout the entire culture period, hydrogen sulfide (H_2S) levels remained undetectable, indicating a well-managed and healthy aquatic environment. The absence of H2S is crucial, as its presence can negatively impact the well-being of aquatic organisms. These findings provide valuable insights into sustainable aquaculture practices. On the initial day, ammonia in the system was 0.04 when the PL was introduced, attributed to the earlier addition of ammonium chloride to initiate biofloc formation. As illustrated in Fig. 2, ammonia levels increased to 0.1 and then gradually decreased, ultimately reaching nil.

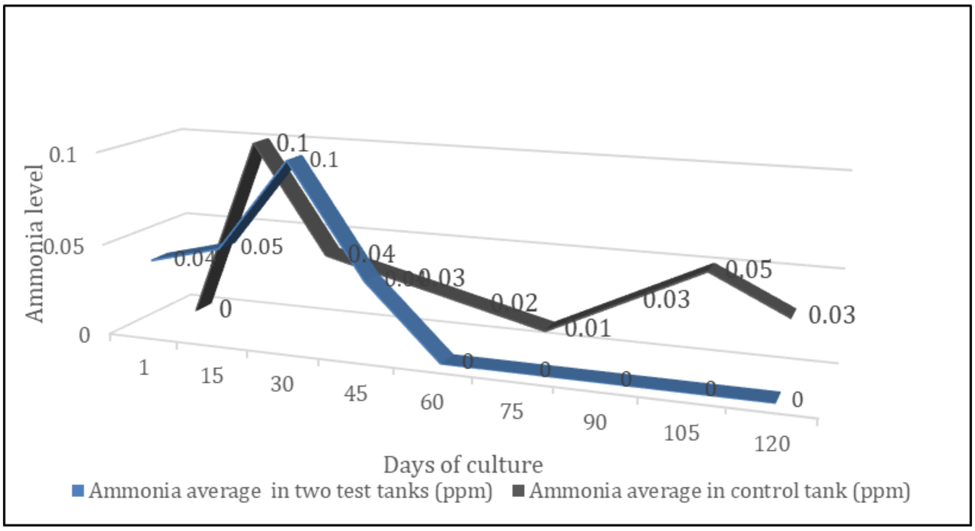

Fig. 2. Dynamic changes in ammonia levels within the aquaculture system over the culture period.

On the initial day, ammonia registered at 0.04 when post-larvae (PL) were introduced, attributed to the prior addition of ammonium chloride to initiate biofloc formation. Subsequently, ammonia levels increased to 0.1 and then gradually decreased, ultimately reaching nil. This temporal pattern suggests an efficient biofloc system capable of managing ammonia levels.

Fig. 3 provides insights into the nitrite dynamics during the culture period. The nitrite level commenced at 0.01, but after 15 days, a notable increase to 1.5 occurred, coinciding with exuvial entrapment of shrimp and subsequent mortality. In response, proactive measures were taken, involving the removal of sludge twice a day and adjustments to the carbon source and commercial feed. This strategic intervention, particularly observed in indoor tanks where unutilized feed was present in the sludge, resulted in a gradual decrease in nitrite levels to 0.2. This highlights the critical role of regular maintenance practices in mitigating nitrite-related stressors in the aquaculture system.

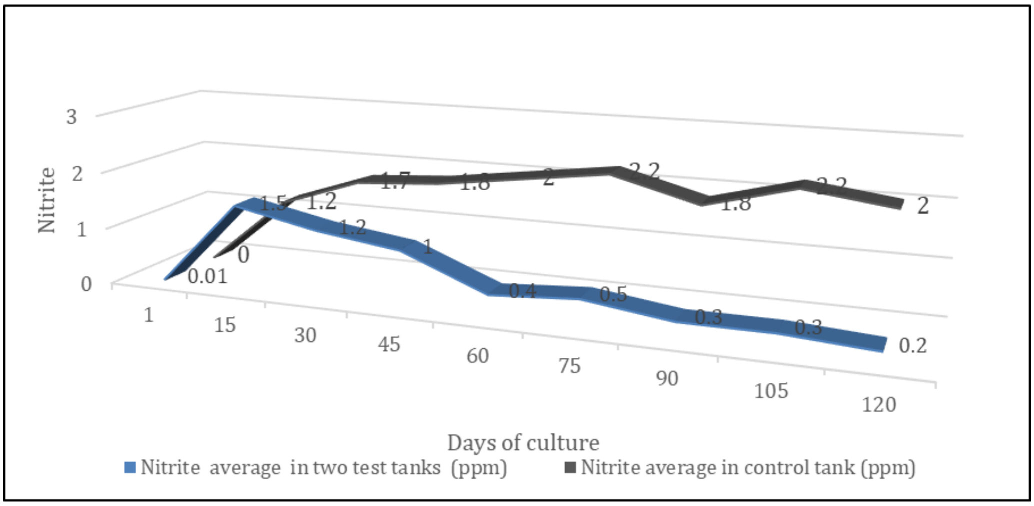

Fig. 3. Dynamics of nitrite levels and mitigation strategies in the aquaculture system over the culture period

Furthermore, the nitrate levels, as depicted in the Fig. 4 data, remained within the range of 60 to 140 mg/lit throughout the entire culture period. The consistent maintenance of nitrate levels within this range suggests effective nitrogen management. Nitrate levels are crucial indicators of nitrogen cycling and, when controlled, contribute to the overall health and sustainability of the aquaculture environment. These results underscore the importance of closely monitoring and promptly addressing water quality parameters in aquaculture systems. The successful management of ammonia, nitrite, and nitrate levels contributes significantly to the overall well-being and growth performance of shrimp (Fig. 5). The presented findings provide valuable insights for refining and optimizing aquaculture practices, ultimately enhancing the sustainability and productivity of shrimp culture systems.

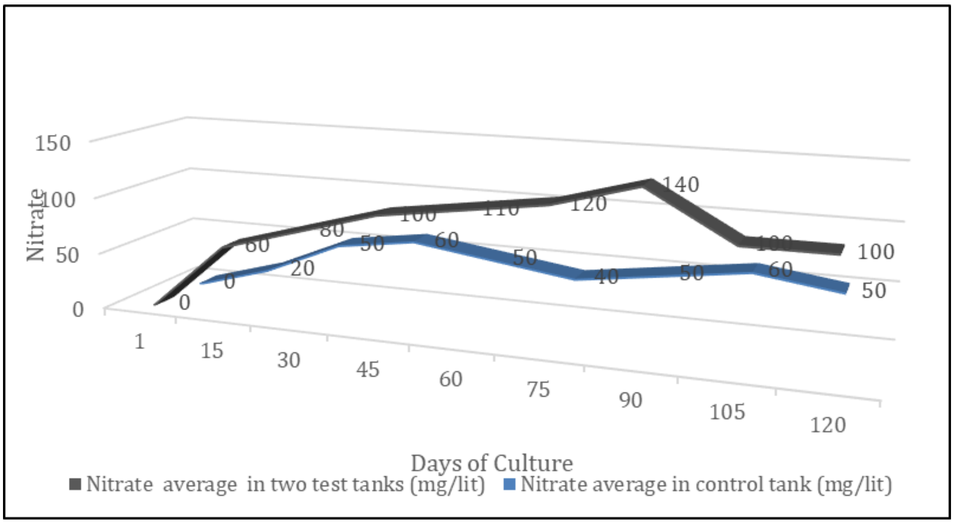

Fig. 4. Nitrate dynamics and nitrogen management in the aquaculture system over the culture period

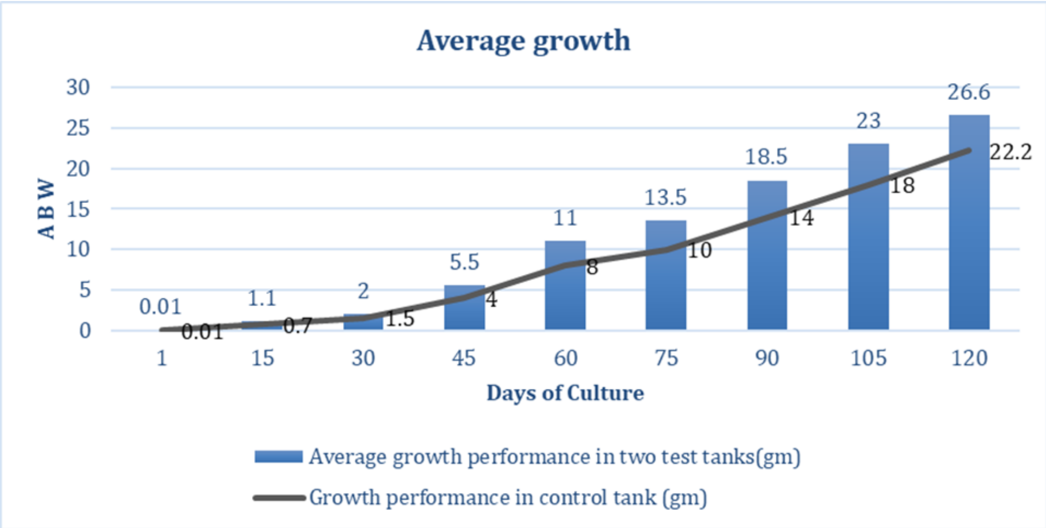

Fig. 5: Impact of water quality management on shrimp well-being and growth performance in aquaculture systems

Sampling, a crucial aspect for assessing shrimp health, growth, and survival, was conducted every 15 days throughout the study. The initial sampling at the 15th day of culture (DOC) yielded an average weight of 1 gm. Subsequent sampling at the 30th DOC, with an average weight of 2 gm, marked the transfer of shrimp to grow-out ponds (P1, P2, P3), where they were cultured until harvest. At the time of harvest, shrimp in P2 and P3 ponds achieved sizes of 27.0 gm and 26.3 gm, respectively, contributing to an average weight of 26.6 gm. The survival rates in P2 and P3 ponds were 77% and 78%, respectively, resulting in an overall average survival rate of 77.5%. In contrast, the control group exhibited a lower survival rate of 62%, with an average weight of 22.2 gm (Fig. 6).

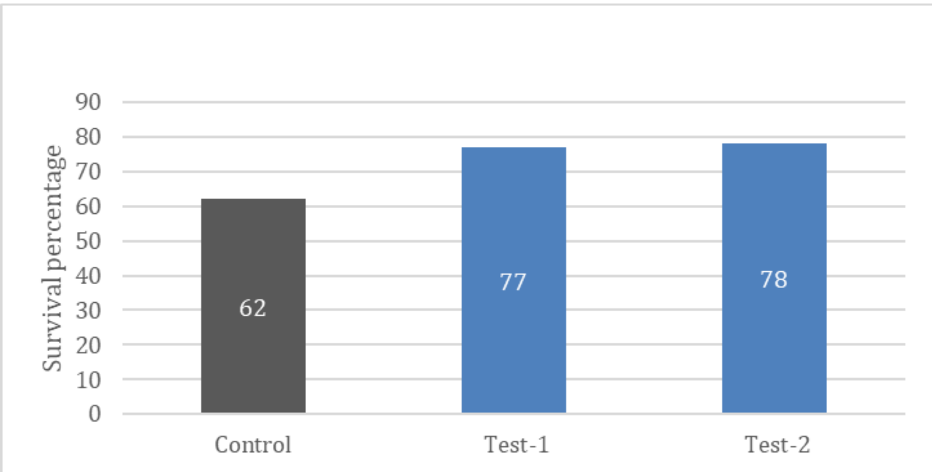

Fig. 6. Comparative survival rates in grow out ponds and control

The initial stocking density in each tank was 6560 (equivalent to 120 PL/sq.m). The total production in the two test tanks reached 5084 individuals. Fig. 7 depicts the dynamics of floc volume, starting at 0.1 mg/lit on day 1 and increasing to 14 ml/lit by the 45th day. The quantity and quality of floc were consistently monitored throughout the culture period. Microscopic examination revealed the presence of rotifers, copepods, algae, etc. No disease outbreaks occurred during the study, and Vibrio sp. were not observed, indicating a healthy and disease-free culture environment.

These results highlight the effectiveness of the sampling strategy, the success of grow-out pond management, and the positive impact of the biofloc system on shrimp survival rates and growth. The absence of disease outbreaks and Vibrio sp. further underscores the overall health and robustness of the aquaculture system, contributing to valuable insights for the refinement and optimization of shrimp culture practices.

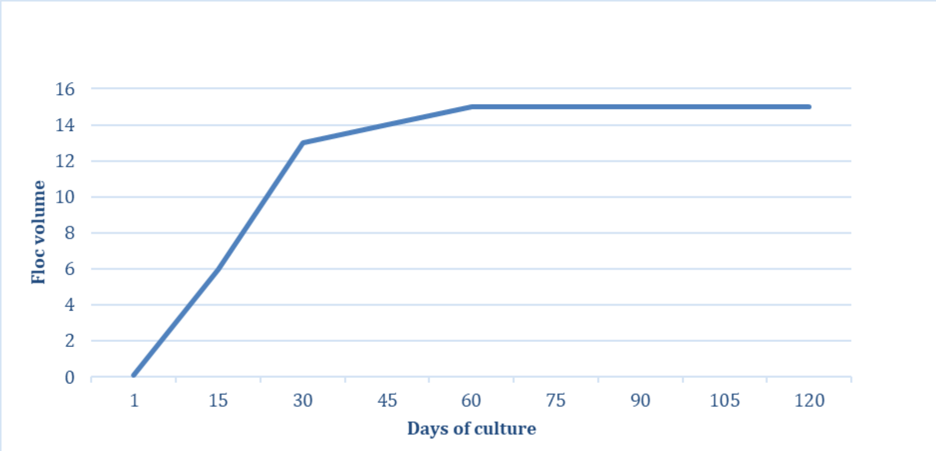

Fig. 7. Dynamics of floc volume (mg/ml) in biofloc aquaculture systems

The growth performance data presented in Table 2 underscores significant disparities in the average growth of shrimp within the two test tanks in contrast to the growth observed in the control tank over the 120-day culture period. The table delineates the comparative growth performance of shrimp in Biofloc test tanks and a control tank during this culture period. The results consistently reveal a notable advantage in favor of the Biofloc test tanks. Initially (Day 1), there is no substantial difference in shrimp weight between the two groups. However, as the culture period advances, the Biofloc test tanks consistently manifest higher growth rates compared to the control tank. By Day 120, shrimp in the Biofloc test tanks attain a weight of 26.6 gm, while those in the control tank weigh 22.2 gm. These findings suggest that the adoption of Biofloc technology positively impacts shrimp growth, likely attributable to improved water quality, nutrient recycling, and enhanced feed utilization within the Biofloc system.

Table 2. Comparative growth performance of shrimp in Biofloc test tanks and control tank over a 120-day culture period

DOC	Average growth performance in two test tanks(gm)	Growth performance in control tank (gm)
1	0.01	0.01
15	1.1	0.7
30	2.0	1.5
45	5.5	4.0
60	11.0	8
75	13.5	10
90	18.5	14.0
105	23.0	18.0
120	26.6	22.2

This growth trend continues, with the test tanks consistently outperforming the control tank at subsequent time intervals. By DOC 120, the average growth in the test tanks reaches 26.6 gm, while the control tank lags behind at 22.2 gm. The observed differences in growth performance can be attributed to various factors, including the implementation of the biofloc system in the test tanks. The biofloc environment likely provided additional nutrients and a more favorable habitat for shrimp growth, resulting in the observed higher growth rates. The consistent maintenance of water quality parameters, as discussed in the previous section, played a crucial role in creating an optimal environment for shrimp growth. Adequate dissolved oxygen, stable pH, and controlled salinity are known to positively influence shrimp development. Moreover, the presence of biofloc in the test tanks might have contributed to enhanced nutrient availability, promoting better feed utilization and growth. The biofloc system, with its microbial community, can serve as a supplementary food source for shrimp, fostering improved growth conditions. This finding aligns with the current understanding of the benefits associated with biofloc technology in aquaculture, emphasizing its potential for enhancing productivity and sustainability.

4. Conclusion

The implementation of Biofloc Technology in shrimp aquaculture presents a promising eco-friendly approach with multifaceted benefits. The key advantages include a significant reduction in pollution, achieved through zero or minimal water exchange. Notably, BFT effectively addresses water quality concerns by reducing Ammonia, Nitrite, and Hydrogen Sulfide (H2S) levels in the system through the efficient recycling of nitrogenous waste. One of the standout features of BFT is its positive impact on operational costs. The technology leads to a reduction in feed costs, as the microbial floc, a byproduct of the system, serves as a nutritious food source for shrimp. This not only contributes to cost savings but also enhances the overall nutritional value of the shrimp diet, resulting in commendable rates of survival and growth. A noteworthy outcome of the study is the absence of disease outbreaks throughout the entire culture period. The biofloc itself acts as a probiotic, fostering a healthy microbial environment that supports the well-being of shrimp. This observation underscores the potential of BFT not only as a production-enhancing method but also as a preventative measure against diseases.

The success observed in this study using BFT opens avenues for future research and applications in shrimp aquaculture. Future studies could delve deeper into optimizing biofloc systems, exploring variations in system parameters to enhance efficiency further. Additionally, investigations into the long-term sustainability and environmental impact of BFT on larger scales would be valuable for broader industry adoption. The integration of advanced monitoring technologies and data analytics could provide real-time insights into the performance of biofloc systems, allowing for proactive management and continuous improvement. Furthermore, research into the potential applications of BFT in diverse aquaculture settings and with different species could broaden the scope of its benefits. In conclusion, the findings from this study highlight the potential of Biofloc Technology as a sustainable and economically viable approach in shrimp aquaculture. Its capacity to address water quality, reduce operational costs, and promote disease resistance positions BFT as a key player in the evolution of modern aquaculture practices. Further research and innovations in this field hold the key to unlocking even greater efficiencies and benefits for the aquaculture industry.

Conflict of Interest: No

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