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Biofilter efficiency and ultraviolet light on water quality and mortality in *Litopenaeus vannamei* culture

Eficiencia del biofiltro y luz ultravioleta en calidad del agua y mortalidad de *Litopenaeus vannamei* cultivado

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Abstract

Water quality is the primary cause of failure for cultured shrimp projects. This research aimed to evaluate the efficiency of a biofiltration system. Parameters such as ammonium nitrogen, pH, acidity, alkalinity, and mortality were assessed. Six ponds with a water depth of 1.8 m, covered with a plastic ceiling (greenhouses), were used to house four million two hundred thousand *Litopenaeus vannamei* juveniles. Three ponds were equipped with a water pump and FINI-FALCO systems. The FINI-FALCO substrate was oyster shells, and filtration was assessed by adding 1 ppm ammonium chloride to each pond at 24 h for 20 days. Significant differences ($P < 0.05$) in pH, ammonium, nitrite, and nitrate were found among tanks after 28 days. The use of FINI-FALCO reduced ammonium and nitrite levels, and pH fluctuations were observed during nitrification. Mortalities among treatments were reduced but not significantly.

Keywords: Biofilter, Litopenaeus vannamei, nitrifying bacteria, water quality. ultraviolet light

Resumen

La calidad del agua es una de las principales causas del fracaso de los proyectos de cultivo de camarón. El objetivo de esta investigación fue evaluar la eficiencia de un sistema de biofiltración. Para ello, se evaluaron parámetros como el nitrógeno amónico, el pH, la acidez, la alcalinidad y la mortalidad. Se utilizaron seis estanques de 1 ha, con 1,8 m de profundidad de agua, cubiertos con techo plástico (invernaderos), en los que se sembraron cuatro millones doscientos mil juveniles de camarón de la especie *Litopenaeus vannamei*. Tres estanques fueron equipados con una bomba de agua y con sistemas FINI-FALCO. El sustrato utilizado en el FINI-FALCO fue de conchas de ostra y, en cada tanque, se añadió cloruro de amonio a una concentración de 1 ppm cada 24 hr durante 20 días. Los resultados demostraron diferencias significativas ($P < 0,05$) en pH, amonio, nitrito y nitrato entre los tanques tras 28 días. El uso de FINI-FALCO reduce los niveles de amonio y nitrito, y las fluctuaciones en el pH observadas se debieron al proceso de nitrificación. Las mortalidades entre los tratamientos se redujeron, aunque no fueron significativas.

Palabras clave: bacterias nitrificantes, biofiltro, calidad del agua, *Litopenaeus vannamei*, luz ultravioleta

Introduction

In shrimp farming, the determining factors for crop success are well known, without diminishing the importance of other factors. Water quality and biosecurity (maintaining and protecting healthy production) have become increasingly important in recent years and have been the subject of numerous studies, all aimed at improving and maintaining optimal cultivation conditions.

Appropriate water treatment system designs help maintain stable water quality in recirculation systems, eliminating toxic products generated by organism metabolism. If this design is incorrect, the amount of organic sediments and nutrient concentrations could increase, causing stress and poor shrimp growth. Such systems include aeration, degasification, mechanical and biological filters, and, for controlling pathogenic organisms, ozonation and ultraviolet (UV) irradiation (Sansanayuth et al., 1996; FAO, 2014; Fuentes, 2015).

On the other hand, aquatic plants are also used to reduce suspended solids, ammoniacal nitrogen, organic carbon, and phosphorus (Sansanayuth *et al*., 1996; Epp *et al*., 2002; Carbó-Bacaicoa, 2012). Therefore, combining these two systems improves water quality but can increase crop operating costs. This problem increases when pathogenic microorganisms infect shrimp, leading to mortality.

These have led to the development of biosafety systems with closed, recirculating cycles that reduce or eliminate the introduction of pathogens (Otoshi *et al*., 2003, Ochoa, 2004). Given the above and the need to maintain recirculation, high-quality water, and crop biosecurity, it is evident that developing technologies to address these aquaculture problems is necessary.

A system that improves water quality combines the use of nitrifying bacteria in an air substrate with an immersed substrate that helps maintain biotic balance in the water, substantially reducing toxic waste. In addition, the use of UV light lamps in the system helps disinfect water, wholly or partially eliminating harmful microorganisms that could harm the crop. Together, the biofilter and UV lamps in a shrimp-farming pond would reduce ammonia levels and disinfect the water, thereby increasing production.

Aquaculture in closed systems relies on biofilters to maintain pond water at satisfactory quality levels. To establish a biofilter, it is necessary to establish nitrifying bacterial populations using an initiator that mimics natural processes while accelerating them in a shorter time (Moriarty 1999; TECA-FAO 2015). Several studies have shown that the activity of nitrifying bacteria can be accelerated by using ammonium chloride (NH4Cl) as a culture initiator (White et al., 2002; Chun-Hung & Jiann-Chu, 2004; Melgar Valdes et al., 2013). Some of the unknowns regarding nitrifying bacteria stem from the lack of techniques to accurately identify, locate, and quantify microbial populations in situ; determine the physicochemical microenvironment; and measure in situ activity (Moriarty 1999, Solórzano 2017).

This study aimed to evaluate the effects of bacterial nitrifiers on water quality in shrimp culture, as well as the effectiveness of a biofiltration system and ultraviolet lamps in greenhouse ponds for improving water quality.

Materials and methods

Study site

The study was conducted at the Latimar shrimp farm in Tumbes, Peru. The ponds were stocked in an intensive system in a tropical environment with seawater (32 ppt) and discharged into an artificial, planted wetland.

Experimental design

For the study, two treatments were implemented: the FINI-FALCO system (Figure 1) and a control treatment, each with three replicates. These treatments were maintained in six identical ponds (250 m² each), each 1.8 m deep, and were used in a 1 ha area under a greenhouse system covered externally with transparent plastic; the bottoms of each pond were lined with high-density plastic. The six ponds were aerated with blowers (28 HP per pond), underwent a 5% daily water replacement, and used commercial probiotics. At the start of this experiment, 4.2 million juvenile *Litopenaeus vannamei* shrimp, on day 70 of culture and with an average individual weight of 10–20 g, from three different commercial laboratories in Central America, were distributed among the six ponds at an approximate population density of 70 individuals m². The shrimp were fed commercial feed (40% crude protein).

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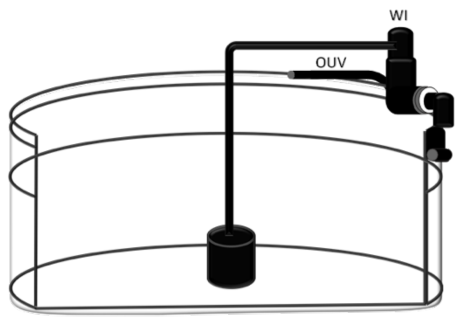

Figure 1. Horizontal view of a fishery designed system with UV light and a biofilter. The diagram shows the filter immersed in crushed oyster shell and FALCO (an external filter with UV light, synthetic fiber, and activated charcoal), the water intake before UV light (WI), and the water exit after UV light (OUV).

Biofilter system

The conditioning of nitrifying bacteria was conducted in a 25,000 L fiberglass tank (White et al., 2002; Chun-Hung & Jiann-Chu, 2004; Coronado et al., 2014; Gallego-Alarcón & García-Pulido, 2017; Del'Duca *et al*., 2019). Marine water was used at 32 UPS of salinity (S) and 30-33°C temperature (T). Styrofoam coolers with a capacity of approximately 15 L were used to store the substrate.

Water quality analysis

Crushed oyster shell was used as a bacterial substrate. Ten coolers with substrate were submerged in water and subjected to a sack with beach sand to provide bacteria. Each cooler was equipped with air diffusers to oxygenate the substrate. To accelerate bacterial populations, ammonium chloride (NH4Cl) (Merck) was used at a rate of 38.2 g of product per liter of distilled water, yielding a stock solution at a final concentration of 10,000 mg L-1. This solution was added twice a week in sufficient quantities to maintain a constant level of at least 1 mg L-1 of ammonium (NH4+) in the water. Ammonium and nitrite were monitored weekly for 16 weeks. For measurements of ammonium and its derivatives (NO_2, NO_3), an SVQ118 spectrophotometer (Merck) and reagents (Hach®) were used.

UV disinfection system

The biofiltration system, consisting of an immersed filter with crushed oyster shell and an external filter with ultraviolet light, synthetic fiber, and activated carbon (Tracy et al., 2006), was designed to be easy to assemble, disassemble, disinfect, and transport. Each of the systems used in three ponds of this study consisted of a FINI-FALCO consisting of a plastic tank with a cover with a capacity of 100 L with a crushed and previously conditioned oyster shell substrate, with a 1½" PVC pipe fixed in the center like water intake. A water pump, (Jacuzzi® 1 HP), with an inlet and outlet diameter of 1½" and a capacity of six gallons per minute was used in the system. The non-immersed filter was also used (Tracy *et al*., 2006), with a conical-bottom fiberglass tank with a capacity of 250 L. The non-immersed filter used plastic fiber as a substrate to fix nitrifying bacteria, and it was equipped with a UV light system with four 40 W lamps. The study was conducted over 16 weeks.

Statistical analysis

For mortality, a two-way statistical analysis was used; for the evaluation of water physicochemical parameters, a two-way analysis of variance (Yancheva et al., 2015) with subsampling was used. Data were statistically evaluated using SPSS® for Windows V. 11. In both analyses, differences were considered significant at $P < 0.05$.

Results

Bacterial conditioning performance

The development and performance of nitrifying bacteria in assimilating NH4Cl and converting it to nitrite were observed. The results show that during the second week, nitrite formation increases exponentially, reaching a maximum in the sixth week, after which the nitrite concentration begins to decrease (Figure 2). Only ammonium, nitrite, and nitrate measurements were made in this study.

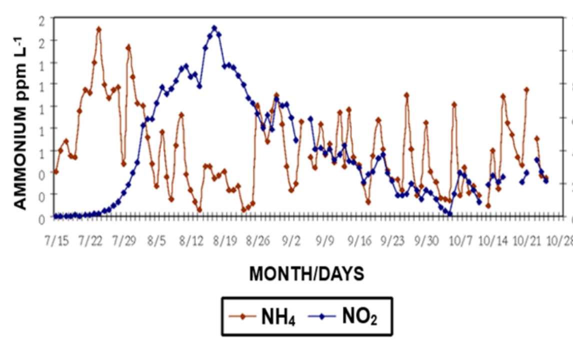

Figure 2. Effects of conditioning nitrifying bacteria on the ammonium (NH4) and nitrite (NO2) levels in ppm over time, using NH4Cl as an ammonium source; x-axis refers to month/day.

Water quality parameters

After the seventh week, the concentration of ammonium and nitrites established the balance. The ammonium rebounds observed in the graph are the cause of the addition of NH_4Cl. After each rebound of ammonium, one of nitrite is observed with a difference of approximately three to five days (Figure 2).

System efficiency (FINI-FALCO). The statistical analysis of the FINI-FALCO system study shows that, overall, the system improves water quality by removing ammonium and nitrate (Table 1).

The system's use in removing NH4 and NO3 showed significant differences ($P < 0.05$), whereas for NO2, no difference was found between ponds with and without the system ($P < 0.05$) (Table 1).

Table 1. Ammonium, nitrite, and nitrate levels (mean ± standard deviation) in ponds with the FINI-FALCO system (Treated) and the control group (Non-treated) without the system.

Tanks	Ammonium (NH_4)	Nitrite (NO_2)	Nitrate (NO_3)
Treated	0.0025 ± 0.0020	0.27 ± 0.18	7.67 ± 2.25
Non-treated	0.0036 ± 0.0016	0.39 ± 0.19	8.94 ± 2.18
	P = 0.046 (*P* < 0.05)	*P* = 0.067 (*P* > 0.05)	*P* = 0.043 (*P* < 0.05)

Shrimp survival and biomass

Shrimp collected from the drain during each pond replacement were counted and compared. The final count indicates that there was no difference ($P < 0.05$) in survival between ponds with a system and ponds without a system (Table 2).

Table 2. Number of shrimps stocked, recovered, and dead in the three ponds with the system and the three ponds without the system.

	Shrimp		
	Stocked	Recovered	Dead
Tanks equipped with FINI-FALCO	2,100,000	1,914,753	185,247
Non equipped	2,100,000	1,913,396	186,604
Total	4,200,000	3,828,150	371,850

Discussion

Several studies of filtration systems using nitrifying bacteria and their products have described the problem under different types of aquatic systems and microclimates (Xie *et al.*, 1999; Mosquera-Corral *et al.*, 2003; Ong *et al.*, 2003; Schramm 2003; Burford & Lorenzen 2004; Chun-Hung & Jiann-Chu 2004; Calvachi 2015; Fuentes 2015). However, in aquaculture, most research focuses on smaller-scale systems using closed recirculation (Menasveta 2002; White et al., 2002; Cheng *et al.*, 2004; Chun-Hung y Jiann-Chu 2004).

In larger systems, biofiltration is uncommon, even though extensive and semi-extensive systems are standard and also face problems with increasing ammonium and its derivatives. To a lesser extent, ultraviolet light irradiation systems are also not widely used or reported in extensive aquaculture (Summerfelt 2003).

The results of the evaluation of nitrifying bacteria conditioning indicate that the oxidation of nitrogen products is a chain reaction in which ammonium is first transformed into nitrite and subsequently into nitrate (Burford & Lorenzen 2004, Casciotti *et al.*, 2011). Since this experiment design conditioned the nitifying bacteria, it could be suggested that this chain reaction cannot support the natural functions described (Sansanayuth *et al.*, 1996; Epp *et al.*, 2002; Han & Boyd 2018), which involve a balance between nitrogen compounds (Gross *et al.*, 2003; Collazos-Lasso y Arias-Castellanos 2015).

The results of the incorporation of a biofilter in the FINI-FALCO system with the oyster shell substrate previously conditioned with nitrifying bacteria using NH4Cl (Gross *et al.*, 2003; Cheng *et al.*, 2004) accelerate the biofilter initiation process, making it possible to incorporate it into systems already in operation (Gross *et al.*, 2003).

The incorporation of crushed oysters (calcium carbonate) from FINI-FALCO provides alkalinity to the system. It helps regulate the acidity produced by the nitrification process, thereby maintaining a pH favorable to nitrifying bacteria (Antileo *et al.*, 2002). Although the pH was routinely controlled by adding lime (CaCO3) in these ponds, it is possible that crushed oyster shell in FINI-FALCO positively participates in the process, as mentioned earlier, as observed in studies on conditioning nitrifying bacteria.

Previous studies show that the FINI-FALCO system significantly reduces ammoniacal components in recirculating systems, substantially improving water quality and maintaining it at optimal levels for shrimp and juvenile larvae (Luo *et al*., 2013, Wang et al., 2019). The results of the present study showed that both ammonium and nitrate concentrations decreased compared to ponds without a system.

The minimum difference can be attributed to the amount of substrate available in FINI-FALCO. As Burford (2004) and Wang (2019) point out, the area of the fixing substrate is proportional to the amount of active nitrifying bacteria, and the number of bacteria is proportional to the concentrations of ammonium, nitrites, and oxidized nitrates. It can be inferred that the number of colonies of nitrifying bacteria available in the FINI-FALCO system is not sufficient to oxidize the ammoniacal products of a pond of one hectare of shrimp at a density of 70 shrimp m-2.

FINI-FALCO is a promising system that must be studied in detail to be used effectively in aquaculture production systems. It is highly feasible that its use benefits aquaculture systems, including improved management and higher densities, reflected in higher production and, therefore, greater economic performance.

Conclusion

The significant differences in the parameters of ammonium and nitrate indicate that the FINI-FALCO system promotes nitrification. Besides, the present study provides evidence that the scallop substrate can be used in a biofilter system and that the system can be initiated with reagent-grade ammonium chloride, which accelerates nitrification and supports the development of nitrifying bacterial populations. It is recommended to standardize the balance of nitrifying bacteria based on nitrite and nitrate production to control both parameters when starting the FINI-FALCO in the pond.

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Data availability statement

The authors state that the Autonomous University of Tamaulipas, Mexico, does not have a repository cloud. However, raw data from the results, figures, and tables will be available for review upon previous notification by mail to the corresponding author.

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