Vol 1 No 4 December 2020 Pages 127-132 e-ISSN 2708-5694

Journal of Agriculture, Food and Environment (JAFE)

Journal Homepage: http://journal.safebd.org/index.php/jafe

http://doi.org/10.47440/JAFE.2020.1419

Original Article

Assessing the efficiency of a recirculating fluidized bed biofilter in white leg shrimp (*Litopenaeus vannamei*) broodstock aquaculture

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A B S T R A C T

This paper shows the results of the efficiency of a circulating fluidized bed biofiltration system using polyethelene as a filtering material. Changes in water quality parameters and the growth rates of shrimp (broodstock and commercial production) were used to assess the efficiency of water treatment system. The concentration of $NH4^+$ in waste water from the broodstock tanks decreased 61% after three months of treatment. The final concentration of $NH4^+$ after treatment was 0.07 mg/ml which meet the Vietnamese regulations for discard of aquaculture waste into coastal waters. Biological oxygen demand decreased 48%. The corresponding figures for treating waste water from commercial shrimp tanks were decreases of $NH4^+$ by up to 61% with outlet concentration 0.07 mg/ml accepted to discard according to Vietnamese Standard and biological oxygen demand by 55%. Outlet water providing from broodstock and commercial shrimp tanks were treated with COD concentration decreased from 3.12 down to 2.62 mg/ml and 4.62 down to 2.68 mg/ml, or 16% and 41.9% of COD removal, respectively.

Article History

Received: 16 November 2020

Revised: 09 December 2020

Accepted: 15 December 2020

Published online: 31 December 2020

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Keywords

Shrimps, culture wastewater, Fluidized Bed Biofiltration, Bio-Filter, Treatment

Introduction

The farming of white leg shrimp (*Litopenaeus vannamei*) has been growing quickly as a part of aquaculture development, bringing socio-economic benefits [1]. The shrimp industry is challenged to both expand production volumes and yet meet strict quality requirements of the markets and environmental regulations. Specifically, one of the main challenges is the control of water quality; untreated wastewater has been discharged directly into the environment which has seriously damaged [2], [3] the ecosystem by high organic loading, pollutants and pathogens [4], [5], [6].

Recirculating biofiltration technology does not aim to completely eliminate addition of new water or discharge of used water during a crop production cycle. The primary purpose is to keep concentrations of total ammonia nitrogen (TAN) low with minimal cost for water replacement. During a recent workshop session in Hawaii, Tom Losordo of North Carolina State University defined "closed" recirculating systems as those in which up to 20% of the water volume is exchanged for new water each day, with 5 to 10% being typical. These percentages are minimal and necessary in systems that remove settleable or filterable solids, in addition to processing ammonia. Non-recirculating culture systems use various amounts of new water during production, depending on stocking density and the degree of ammonia processing and other support by natural or artificial processes. Fishes and shrimps can be grown in earthen ponds.

Recently, there were priority of focusing on enhancement of non-specific immune response to protect shrimps from deseases [7]. Henky *et al*., (2011) studied enhancement of non-specific immune response of *Litopenaeus vannamei* by oral control of nucleotide [8]. In 2004, Burgents researched on disease resistance of Pacific white shrimp, *Litopenaeus vannamei*, by combining yeast and food [9]. In another report proved that growth, innate immunity and intestinal morphology of Pacific white shrimp able to be improved by dietary nucleotide-rich yeast supplementation. Vairous biofiltration systems are considered as an effective method for treatment of aquaculture waste water and environmental management [10], [11].

The diseases originated from organic polution water is a high risk of shrimp farm. Beside shrimp non-specific immune improvement studies, another effective way to administrate the shrimp culture by using biological filter system is a potential tendency and necessary. Biofilteration is one of the most important method used to improve water quality before water is discharged from a production process**.** In particular, biofilters used to help maintain water quality in recirculating systems for varous targets. It is mentioned as a very important and essential key especially for recirculating aquaculture or aquarium systems [11], [12]. In general, there are two types of aerobic microorganisms that colonize in biofilters for aquaculture. Heterotrophic bacteria utilize the dissolved carbonaceous material as their food source. Chemotrophic bacteria such as *Nitrosomonos sp*. bacteria utilize ammonia as a food source and produce nitrite as a waste product [12], [13]. Chemotrophic bacteria such as *Nitrospira sp.* utilize nitrite as a food source and produce nitrates as a wasteproduct. *Nitrosomonos* and *Nitrospira* will both grow and colonize the biofilter as long as there is a food source available. Depending on design and application, biofilters have the ability to accomplish the three main functions as known remove ammonia, remove nitrites and remove dissolved organic solids.

In addition, recirculating biofiltration technology does not aim to completely eliminate addition of new water or discharge of used water during a crop production cycle. The primary purpose is to control concentrations of total ammonia nitrogen (TAN), BOD and COD low with minimal cost for water replacement. Non-recirculating culture systems use various amounts of new water during production, depending on stocking density and the degree of ammonia processing and other support by natural or artificial processes. Fishes and shrimps can be grown without discharge of used water during the entire crop production cycle. A small amount replacement of evaporation water losses may require addition of water at a rate of 1 to 3% or more of the pond volume per day. In nonrecirculating (flow-through) tank systems, considerable water exchange is required to dilute the ammonia produced by the fish or shimp at most commercially useful stocking densities.

The heart of recirculating biofilter system is bioactor with many types of biological filters play an important role in treating aquaculture waste water around the world, including trickling filters [14], submerged filters [15], [16], rotary biological contactors [17], [18], bio-drum filter [19] and fluidized bed filtration [20]. As mentioned above, the recirculation biofiltration systems helps control the deaseas, and also reduce consumptive water use and waste discharge in aquaculture systems, often dramatically, but business operators may lack the capital or otherwise hesitate to make the initial investment. The fluidized bed biofilters have price comparable to the other systems [21].

The filter material determines the efficiency of the fluidized bed filtration systems [22], [23], [24]. Filter materials must be able to handle different aspects of water treatment, including organic substances, nutrients. The non-moving surface area is to provide a substrate for various bacteria to attach and grow. In order for the bacteria to do their mission effectively, the biofilter and packing design must provide an even distribution of nutrients and oxygen while removing dissolved and suspended waste products. Synthetic plastic materials such as polyurethane, polyethylene, polystyrene has suitable to design a large-surface material to permit adherence of micro organisms [25], [26]. Moreover, these plastic materials is easy to be changed to a parking type to promote easy and constant movement of the fitered materials by calculating suitable parking density which in turn increases the interactive areas between micro-organism and pollutants. Therefore, the study has assessed the efficiency of a filter material made from polyethylene synthetic plastic in a circulating fluidized bed biofilter system. The advantages and disadvantages of the models was investigated for selecting which controls water quality in commercial shrimp and broodstock culture.

2. Materials and methodology

2.1. Filter materials for circulating culture system

The circulating shrimp culture system includes: 3 tanks of broodstock (85 m^3/tank), 3 bio-filter tanks (1.5m^3 / tank) and 1 tank (0.5m^3) which is a primary filter tank, 2 pumps (7 m^3/h),and a gas supply system for shrimp. UV lights (2 Philips TUV 55W HO LAMP), 1 Triton II pressured sand filter (1 of Pentair Pool Products, Sanford, NC - Moopark, CA, USA).

According to preliminary review, polyethylene could be a suitable filter material for the fluidized bed biofilter [24], [27]. Another consideration is that our method of designing biological filtration systems should ideally comply with the technical standards of the wastewater treatment for aquaculture [21], [28]. Also ideally, the design must meet simple requirements, low cost and equipment that could be used in water.

In addition, most packings for aquaculture are injection molded from polypropylene (PP) or high density polyethylene (HDPE). PP and HDPE are excellent polymers with high heat resistance and excellent chemical resistance. Injection molding is an expensive way to create surface area. Howerver, the recerculing systems are offen small where high cost is not a factor. As the result, the polypropylene plastic filter material was chosen for the research.

2.2. Preparation of Broodstocks and commercial production

White leg parents: 700 adult broodstock, equal proportions of males and females. After spawning, post larvae were nourished in 4000 PL45 (0, 4g/PL).

2.3. The method of broodstocks management of commercial shrimps

2.3.1. Raising broodstocks

This stage was conducted to raise broodstocks of shrimp with good quality for the the commercial shrimp cultivation. To ensure juvenile shrimps grow up without diseases, the water for nourshing tanks was filtered by biological filtration systems. At this phase, the broodstocks were fed with squid, insects.

After 7-10 days of breeding, the eyes of females were cut to stimulate the development of ovaries to be ready for reproduction. After 3-5 days of eye ablation the females have eggs and are ready to reproduce. Then males and females were put in the same tank for breeding. After the female reproduction, nauplii were quantified then move to the nursing tank to PL45 size.

2.3.2. Commercial white leg shrimps

After breeding broodstock stage, the tank was cleaned for next commercial shrimp culture in June. Similar to the broodstock stage, waste water was treated by a fluidized bed bio-filter system and returned to the tank as a cycle of water.

2.4. Operation of fluidized bed biofiltration circulation system

Three biofilteration tanks (Fig. 1 –part 3) designed by polyethelene materials as shown in Fig. 2 to able to treat waste water originated 70 m^3 from broodstock culture and 100 m^3 waste water of disease-free commercial shrimp (SPF). There were three broodstock tanks with 85 m^3/tank (Fig. 1 part 1) and water level was 0.5 - 0.6 m height, ensured the total volume of 3 tanks was 60 up to 70 m^3. In the commercial s……………………..hrimp culture, two commercial white leg shrimp tanks were conducted at 1.5 m of water level or 100 m^3 , and waste water also was pumped continuously through biofilteration tank to control the environmental parameters.

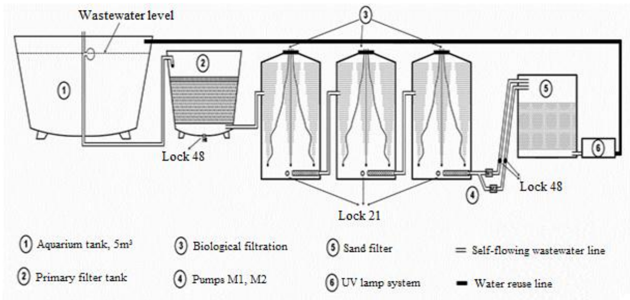
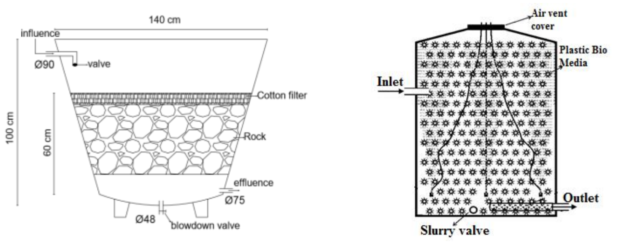


Fig. 1. Water balance diagram of fluidized bed filter model in Cat Ba



A B

Fig. 2. Structure of the filter tanks (longitudinal section), A: Primary filter tank, B: Biological fiteration tank

The water from shrimp culture tank was pumped into the primary filter tank (Fig. 2-A) to remove solid organic matter by the cotton layer over the filter (this layer could be cleaned easily). Then the water runs into the biological filtration system consisting of 3 interconnected tanks (1.5 m^3 /tank, Fig. 2-B). The amount of polyethelene filter material in the filter tank accounted for 35% of biological filter tank. The air supply was put at the bottom to provide Oxygen for aerobic reaction by *Nirosomonas* and *Nitrobacter* and helps the filter material move around the water layers. Next, the water was filtered by the sand filter (Fig. 1- part 5) then moved to the UV lamp tank (Fig. 1-part 6) before moving back to the shirmp culture tanks.

2.6. Water quality monitoring

Water in inlet and outlet of the filtration system was regularly sampled to evaluate treatment efficiency. Water analysis was carried out at the laboratory of National Institute for Marine Fisheries (NCFI). Parameters measured included: pH, DO, salinity, BOD5, COD, N-NO2-, N-NO3-, N-NH4$^+$ and temperature. Sampling was conducted once per month. The analysis method were followed by Boyd and Tucker [29].

2.7. Monitoring survival rate of shrimp

Growth rate of shrimp were monitored periodically every two months to evaluate the efficiency of water treatment by the filter system.

Total length was measured by a ruler with precision of 0.1 cm, each sample time measured 30 individuals.

Whole weight was measured by semi-quantitative weight with accuracy of 0.01 gram, each time measured 30 individuals.

2.8. Data analysis

The experimental data was conducted in triplicate and presented as mean ± standard diviation (SD). Mean differences among treatments were assessed using SPSS 24.0 version, p-values less than 0.05 were considered as a statistically significant.

3. Results and discussion

3.1. The water quality monitoring in the biofilter system

3.1.1 The variation of phycical environmental parameters

The temperature, pH, salinity in the shrimp culture and commercial white leg shrimp culture tanks were tested each time point. The results showed that except pH, other parametters were comparatively stable and suitable for shrimp aquaculture (Table 1 and Table 2). The temparature in broodstock tank was stable in summer and suitable for broodstock rearing (Table 1).

The concentration of oxygen in the outlet of the broodstock tank was statistically significantly lower than that in the inlet (ANOVA F..degrees of freedom factors and errors, P<0.05) (Table 1). This suggests that the microorganisms attached in the filter material used oxygen resulting a part of oxygen in the main tank was consumed.

Table 1. Water quality parameters measured in brookstocks culture tanks

		March	April	May
Temperature (^{o}C)		26.6±2.8	28.6±1.0	28.0±1.2
Salinity S ‰)		31.0±0	31.0±0	30.2± 0.8
pH		8.2±0.2	8.2±1.9	8.2±0.2
DO (mg/l)	Inlet	5.4±0.1	5.5±0.1	5.3±0.2
	Outlet	5.2±0.2	4.6±0.1	4.8±0.2

From June to October, during the first 4 months of growout, temperatures in the commercial shrimp tanks were relatively stable, varying between 26.7 to 30.3^{o}C and these temperatures were suitable for normal shrimp growth (Table 2). However, the temperature fell sharply to 23^{o}C in November because of northeast monsoon and during this time the shrimp grew slowly. The pH fluctuated between 7.73 to 8.06 and was within the range for normal shrimp growth.

The concentration of DO continuously showed statistically significant decreases after the filter process in the commercial tanks (ANOVA F..degrees of freedom factors and errors, P<0.05) (Table 2). This also suggests that the microorganisms attached in the filter material used oxygen resulting a part of oxygen in the commercial shrimp tank was consumed.

Table 2. Water quality in commercial shrimp culture tanks

		June	July	August	September	October	November
Temperature (^oC)		30.3±0.3	30.2±0.4	29.8±0.6	29.5±0.6	26.7±1.0	23.1±2.4
Salinity S (‰)		25.0±0.0	25.7±0.9	22.2±1.8	21.9±0.2	22.0±0.0	20.9±1.0
pH		8.0±0.1	7,9±0.1	7.9±0.1	8.0±0.1	7.7±0.2	7.7±0.2
DO (mg/l)	Inlet	5.3±0.3	5.4±0.2	5.2±0.3	5.4±0.1	5.4±0.2	5.6±0.1
	Outlet	5.2±0.1	5.0±0.1	4.5±0.1	4.6±0.2	4.6±0.0	4.8±0.0

3.1.2. The variation on nutrients

BOD_5 and COD concentrations in broodstock culture tanks were decreased and the differences considering the time points as replicates are statistically significant (ANOVA F..degrees of freedom factors and errors, P<0.05, ANOVA F..degrees of freedom factors and errors, P<0.05, respectively, Table 3).

Table 3. Nutrients in water of broodstock culture tank

Indicator *(mg/l)*	March		May		June		Regulation *(mg/l)*	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Int*	Vn**
$N\text{-}NH_4^+$	0.07	0.05	0.14	0.06	0.18	0.07	0.5	0.1
$N\text{-}NO_2^-$	0.03	0.02	0.05	0.03	0.08	0.04	0.5	-
$N\text{-}NO_3^-$	0.55	0.40	2.56	2.98	3.12	3.47	100	-
BOD_5	1.87	1.85	1.76	1.08	1.86	0.85	5	-
COD	3.08	2.99	2.98	2.56	**3.12**	**2.62**	10	3

(*) International standard [16].
(**)Vietnamese Sea water quality standard (QCVN 10-MT:2015/BTNMT)

As mentioned above, DO parameters during broodstock and commercial culture were declined indicated that microoranism activated. Differently, remarkable oxygen consumption proved that microorganisms well adapted to surface of filter then tranferring the concentrations of BOD_5 and COD into biomass. In broodstock culture, BOD_5 and COD concentration were decreased highest in June at 1.01 mg/l and 0.5 mg/ml, respectively (Table 3). Consequence, the outlet BOD_5 of tested time points of broodstock tanks meet the internatiional discharge standard, and the outlet COD of tested time points of broodstock tanks meet the Vietnamese standard. Similarly, the highest reduction of BOD_5 and COD in commercial tanks were in October at 1.61 mg/l and 1.94 mg/ml, respectively (Table 4) which help the outlet COD of checked time points of commercials tanks meet the Vietnamese and international discharge standard.

Table 4. Minerals and nutrients in water of commercial shrimps culture tanks

Parameters *(mg/l)*	Time (Month) *June*		*July*		*August*		*September*		*October*		Regulation *(mg/l)*	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Int*	Vn*
$N\text{-}NH_4^+$	0.11	0.10	0.14	0.06	0.18	0.07	0.26	0.23	0.23	0.19	0.5	0.1
$N\text{-}NO_2^-$	0.004	0.002	0.005	0.003	0.008	0.004	0.003	0.002	0.084	0.024	0.5	
$N\text{-}NO_3^-$	0.65	0.72	0.46	0.58	1.10	1.14	1.23	1.24	2.03	2.26	100	
BOD_5	1.67	1.02	1.76	1.08	1.86	0.85	2.56	1.26	2.93	1.32	5	
COD	3.08	2.66	2.98	2.56	3.12	2.62	3.85	2.76	4.62	2.68	10	3

(*) International standard [16].
(**)Vietnamese Sea Water quality standard (QCVN 10-MT:2015/BTNMT)

Concerning changes in nutrients of inlet and outlet of the biofilter system of the broodtock tanks and production tanks, there were reductions in the concentrations of ammonium parameter, about a month after the commencement of the respective experiments (Tables 3, 4). There were correlated increases in nitrate levels expected because the microogarnism (*Nitrosomonas*) living on the surface of the filter layer converted total amonia nitrogen (TAN) to nitrite which is then converted into nitrate. As a result, the biofiltration system could control ammonium level in outlet water making this parameters meet the Vietnamese discharge standard. All in one, the $N\text{-}NH_{4+}$, BOD_5 and COD levels decreased significantly after filtration (Table 3, 4) which suggests that microorganisms used oxygen for conversion of ammonium. These results show that fluidized bed biofiltration is effective in treating the organic nutrients in the tanks.

The time points of $N\text{-}NH_{4+}$ concentrations showed a qualitatively similar pattern of performance for the broodstock and shrimp producion period. After conditioning, the polyethelene biofilter kept TAN at low levels until shrimp was havested. It meant that the polyethelene biofilter adapted, presumably by an increase in bacterial population as well decrease in BOD_5, COD and reduced total ammonia to low levels.

3.2. Results of broodstocks and commercial white legs shrimp culture

3.2.1. The results of imported white leg shrimp domestication, nourshing and breeding.

Disease free (TSV, WWSV, IHHNV, MBV, YHV) broodstock was imported from the High health Company, Hawaii, United States. Their food was culture fish and insects which was sterilized by ozone or 2ppm iodine. The amount of food depends on shrimps' needs and the amount of water change which was sterilized by Philips TUV 55WW Ho Lamp UV lamp, 10 balls / set is 20%. The density was 10 shrimp/m^2, water level was 50-60 cm, temperature was 28-

29°C, salinity was 29 – 32 ppm, pH was from 7.5 to 8.5 and an air supply system ran 24 hours per day.

After domestication, the broodstocks recovered rapidly (it took 50 hours to bring the broodstocks from Hawaii). The mortality rate was around 5% after a week. Survival rate after 4 weeks of domestication was 87% (610 broodstocks: 295 females and 315 males). After 4 weeks, the broodstocks recovered completely and was ready to eye ablation to give birth. Ovaries developed well and the rate of shrimp having eggs was high after eye ablation, over 75% of females participated in spawning with 100,000 - 180,000 eggs/female and eggs hatching rate was about 80%. The results of broodstock's (PL5-10) medical check after 3 months of domestication, and breeding were negative for 05 diseases (TSV, WSV, YHV, IHHNV, MBV). 20% of the total amount of water was changed daily, equal to 1/10 of the amount of water required by the guideline handbook in Hawaii (at least 200% per day without biological filtration).

3.2.2. Results of commercial white leg shrimp culture

In the commercial white leg shrimp culture stage, PL45 (0.4 g/ PL) were nourished in a 85 m^3 tank (40 shrimps/m^3) and the water level was 1.5m. There was a biofilter system for using treated water from the culture tank again. The food was CP combined with vitamins and minerals (feed 4 times per day at 6h, 11h, 17h, 23h). In the first three months, the shrimp grew quickly in length and weight but the growth decreased in the fourth month. By November, the air temperature decreased therefore the water temperature also decreased dramatically to 19.5-25.5^{0}C. As a result, the shrimp growth is insignificant. After 3 months of culture (from 20/6 to 20/9), the average weight was 25g, the highest figure was 29. The survival rate was 86% and the growth rate was 2.1 g/week. After 5 months of culture (from 20/6 to 20/9), the weight increased to 33.5 g, the heaviest shrimp was 40 g. The average growth rate was 1.6 g/week and the survival rate was 85%.

4. Conclusion

The study assessed the efficiency of bio-fluidized bed filtration system in treating water from broodstock culture stage to commercial shrimp culture stage (25/h after 3 months).

The Fluidized bed biological filter model can treat a large volume of water in small biofilter tank (4.5 m^3 filter tank can maintain the quality of 70 m^3 water).

The water quality of the system was maintained well in the whole shrimp culture process. Water quality indicators: BOD_5, COD, N-NH_4^+ and NO_2 were under the permitted limitations and the shrimp were healthy. The concentrations of BOD_5, COD, N-NH_4^+ and NO_2^- met the Vietnamese Sea water quality standard (QCVN 10-MT:2015/BTNMT) and international standards for aquacuture water quality standard [16].

There should be further research to finalize the water treatment model using the fluidized bed filtering method and to standardize the model with specific requirements with the basic design of other filter systems.

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