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The effects of ammonium loading rates and salinity on ammonium treatment of wastewater from super-intensive shrimp farming

Tran Manh Hai[1,*], Nguyen Thanh Tung[1], Nguyen Trieu Duong[1], Nguyen Cam Tu[2], Nguyen Truong Quan[3], Nguyen Hoai Chau[1]

[1]*Institute of Environmental Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay district, Ha Noi, Viet Nam*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet street, Cau Giay district, Ha Noi, Viet Nam*

[3]*Research Centre for Environmental Technology and Sustainable Development, VNU University of Science, Vietnam National University, Ha Noi, 334 Nguyen Trai street, Thanh Xuan district, Ha Noi, Viet Nam*

[*]Email: haitm.iet@gmail.com

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Abstract. Treatment of wastewater from super-intensive shrimp farming (SISF) for discharge or recirculation purposes is currently attracting the attention of managers and researchers. The fixed bed biofilm reactor (FBBR) has been successfully used for biological treatment of drinking water as well as for wastewater treatment in aquaculture farm. Ammonium and salinity are important factors affecting the efficiency of pollutants treatment. This paper presents the results of research on ammonium treatment in super-intensive shrimp wastewater by aerobic microbiological process using FBBRs. The results showed that: at ammonium loading rates of 0.014; 0.028; 0.049 and 0.070 $kg/m^3/d$, at salinity of 10 ‰, the ammonium removal efficiencies were 98 - 99; 97.7 - 98.8; 96.8 - 98.7 and 95.7 - 98.0 percent, respectively (ammonium concentrations in effluent were 0.05 - 0.1; 0.12 - 0.23; 0.23 - 0.56 and 0.51 - 1.07 mgN/l, respectively); at salinity of 15 ‰, the ammonium removal efficiencies were 95.8 - 96.0, 94.5 - 92.0, 93.1 - 92.3 and 66.8 - 68.8 percent, respectively (ammonium in effluent were 0.20 - 0.21; 0.55 - 0.8; 1.20 - 1.35 and 7.8 - 8.3 mgN/l, respectively); and at salinity of 20 ‰, the ammonium removal efficiencies were 92.0 - 96.0, 87.0 - 89.0, 69.1 - 70.9 and 59.6 - 66.0 percent, respectively (ammonium in effluent were 0.2 - 0.4; 1.1 - 1.3; 5.1 - 5.4 and 8.5 - 10.1 mgN/l, respectively). This result showed that the influence of ammonium loading and salinity on ammonium treatment efficiency was very significant.

Keywords: super-intensive shrimp wastewater, fixed bed biofilm, ammonium loading, salinity.

Classification numbers: 3.4.2; 3.7.1; 3.7.2.

1. INTRODUCTION

Brackish-water shrimp farming in Viet Nam, especially super-intensive shrimp farming (SISF), driven by its greater yields and a high success ratio, has been growing in terms of total farmed area and output. In a single province of Ca Mau, the land area devoted for SISF has increased rapidly throughout the years - from 175 ha in 2016 to about 1750 ha in 2018 [1] and 2500 ha in 2020 [2, 3]. Intensification in shrimp farming has become the development trend of the Mekong Delta as well as of the whole country.

SISF operations typically consist of feeding, water quality management and wastewater treatment, and disease prevention. Results from a survey conducted by the Institute of Environmental Technology at a number of SISF in 4 provinces (Ca Mau, Bac Lieu, Tien Giang and Thai Binh) show that the farm owners are implementing wastewater treatment systems as presented in Figure 1.

(1)	**(2)**	**(3)**	**(4)**	**(5)**
→ Settling pond	→ Treatment pond	→ Ready pond	→ Shrimp pond	→ Wastewater treatment
↑	↑		↓	↑
PAC: 20 - 30 g/m^3	$Ca(OCl)_2$: 20 - 30 g/m^3	Air stripping to reduce residual chlorine ~ 1 ppm	(i) Daily water renewal (10 - 50 % of all water volume) (ii) Siphoning of sludge	(i) probiotic supplementation (ii) air stripping (iii) disinfection

Figure 1. Scheme of wastewater treatment systems in shrimp farming

Table 1. Evolution of quality of water and wastewater from siphoning in super-intensive white-leg shrimp ponds [4].

No	Parameters	Unit	30 days	40 days	55 days	Wastewater from siphoning	Regulations	
							(*)	(**)
							Supply water	Discharge water
1.	NH_4^+-N	mg/l	2.9	5.1	8.2	41	-	-
2.	NH_3-N	mg/l	0.37	0.65	0.87	-	< 0.3	-
3.	T-N	mg/l	-	-	-	145	-	-
4.	H_2S	mg/l	-	-	-	0,35	< 0.05	-
5.	NO_2^-N	mg/l	5.2	10.1	12.2	-	< 0.35	-
6.	BOD_5	mg/l	26	41	65	-	<30	≤ 50
7.	COD	mg/l	65	100	145	1.200	-	≤ 150
8.	TSS	mg/l	40	65	110	475	-	≤ 100

(*) 45/2010/TT-BNNPTNT*: Providing* for *conditions on food and safety and hygiene-guaranteed in intensive tiger shrimp and white-leg shrimp-rearing establishment and zones.*

(**) QCVN 02 – 19:2014/BNNPTNT*: National* technical *regulation on brackish water shrimp culture farm – Conditions for veterinary hygiene, environmental protection and food safety.*

The amount of daily water renewal is gradually increased, from 10 to 50 % (about 30 % on average) of all water volume, by the age of maturity of the shrimps. In cases where shrimps display signs of poor health, the amount of water renewal can be increased up to 100 %. The amount of wastewater from sludge siphoning is about 3 % of the renewed water volume. It should be noted that the amount of shrimp excrement is about 70 % of feed, and the bigger the shrimps the more the food is given, which means the greater the amount of waste and thus pollutants in the water, feed conversion ratio (FCR) was 1.1 to 1.2. Table 1 shows the water quality regarding the age of maturity of the shrimps as well as the amount of wastewater (containing suspended and settleable solids) collected from siphoning.

Disease outbreaks are the primary cause of shrimp production loss [5]. One outbreak can devastate an entire area of shrimp farming, including that of brackish water shrimp farming. In recent years, Early Mortality Syndrome (EMS) spread widely, causing heavy losses especially among giant tiger shrimps. EMS is caused by *Vibrio parahaemolyticus*, which produces a toxin that causes acute hepatopancreatic necrosis syndrome (AHPNS) in cultured shrimp [6 - 8]. EMS can be transmitted through cohabitation with infected shrimp larvae - both wild-caught and cultured larvae [9, 10], exchange process of contaminated water [11], and contaminated residual feed.

Strict control of the quality of supply and discharge water (including wastewater treatment and circulation) is the fundamental measure to protect the environment and limit the spread of diseases. Many provinces in Viet Nam with a developed shrimp industry have imposed stricter regulations on treatment of supply water and wastewater from SISF. For examples, the maximum pond area is required to not exceed 20 % of the total farming area; the area of waste treatment has to be at least 15 % of the total area [12]; waste treatment ponds must be lined with tarpaulin to limit soil and groundwater pollution; the levels of pH, BOD, COD, NH_4^+, total N, total P, residual chlorine, total grease, suspended solids (SS) and Coliform after treatment have to follow the Vietnam’s national standards QCVN 40:2011/BTNMT [13].

In terms of wastewater treatment of organic matter and nitrogen compounds (in dissolved and/or suspended form), the most commonly used process is biological methods. This process, which involves the use of biological organisms in the removal of pollutants, has been used for many years [14 - 17]. It has many advantages such as very high efficiency (can be up to 90 - 99 %), lesser use of chemicals, and lower energy cost per volume unit of treated water compared to other methods, all of which bring about economic benefits.

Due to their simple structure and ability to reproduce quickly, bacteria can be very good at processing wastes of natural origin produced by humans and animals. More importantly, they can change themselves and adapt well to the environment, thus natural bacteria, and microorganisms in general can be "trained" to process certain man-made toxins. Treatment technologies of water containing SS, BOD/COD, NH_4^+, and microorganisms usually employ the following steps: SS removal, organic/nitrogen removal, and disinfection [14, 18, 19].

There are several major factors that influence nitrification, which is affected by the levels of dissolved oxygen (DO), pH, toxicants, metals, free ammonia and nitrous acid, ammonia-oxidizing bacteria, and salinity [20 - 22]. Studies show that, as the salinity increases, the microbial activity decreases and so does the treatment efficiency. With salinity from 3 to 20 g/l and sludge retention time from 3 to 20 days, organic loading rates of 0.5 to 2 kg COD/kg VSS-day could be reached [23]. At salinities of 10 and 20 g/l, TOC removal efficiency decreased by 35 - 37 %, and both BOD removal and nitrification efficiency also decreased [24]. Microbial activity decreased sharply with the increase of salinity, biomass recovered over a period of several weeks in reaction to tanks with the salinity of 10 and 20 g/l; at a salinity of 30 g/l, BOD removal efficiency was reduced by about 30 %. At a salinity of between 35 and 45 g/l, sludge tended not to settle [25], and at a salinity of 40 g/l microbial activity was completely unrecoverable [26].

How a technology as well as a treatment process is selected depends on the characteristics of the wastewater to be treated (flow, pollutant components, concentration of substances, etc.). Previous studies show that, so far, improvements have been focused mainly on materials for microbial attachment and different ways of using them in tanks. Two types of technology usually used to are: fixed bed biofilm reactors (FBBR) and moving bed biofilm reactors (MBBR). The main purposes of these modifications are stabilizing microbial density, stabilizing the system as well as improving the quality of the water after treatment; and simplifying system startup. Wastewater from super-intensive shrimp farming with low concentration of organic and ammonium, so FBBR is more suitable (especially in startup process).

This work, therefore, aims to evaluate the influence of ammonium loading rate and salinity on the efficiency of denitrification through aerobic microbiological process using FBBR.

2. MATERIALS AND METHODS

2.1. Experimental system

All experiments were carried out in a laboratory at room temperature. Figure 2 shows diagram of the experimental system.

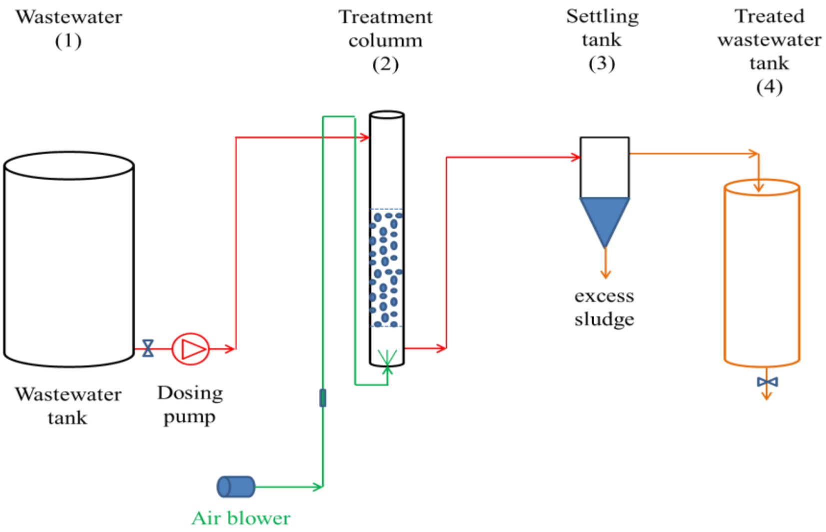

Figure 2. Diagram of experimental system for ammonium treatment through aerobic microbiological process using FBBR.

The experimental column was made of PVC pipe, with height H = 1.5 m and inner diameter d = 13.8 cm. The material for the microbial carrier was lightweight keramzit sized of 8 to 16 mm, volumetric weight of 450 kg/m^3 (Vietnam Vinatap Co.), and had a volume of 7 liters. The microorganisms were obtained from an experimental system of seafood processing wastewater treatment.

2.2. Experimental procedure

The microorganisms were acclimatized and grown using synthetic wastewater (a mixture of sodium chloride, glucose, sodium dihydrogen phosphate, sodium bicarbonate, and ammonium chloride). For complete ammonium nitrification, a minimum alkalinity of 7.14 g $CaCO_3$/g NH_4^+-N was required [27]. Table 2 shows the concentration of substances per liter of water.

Air was supplied through a blower from the bottom-up, during treatment DO was maintained above 5 mg/l, while influent was supplied from the top down with flow a rate of 0.816 l/h. The treated water was allowed time to settle to retain microbiological sludge before being discharged.

Table 2. Concentration of substances in the synthetic wastewater.

No	Substance	Unit	Amount
1.	NaCl	mg/l	10,000; 15,000 and 20,000
2.	Glucose	mg/l	93.75
3.	NH_4Cl	mg/l	19.11; 38.21; 66.88; and 95.54 (ammonium in iffluent were 5; 10; 17.5 and 25 mgN/l, respectively)
4.	NaH_2PO_4	mg/l	3.78
5.	$NaHCO_3$ (as $CaCO_3$)	mg/l	$7.14 \times C_{NH_4^+-N}$

Samples were taken periodically every 03 days for analysis of ammonium, nitrite, and nitrate concentration. When the levels of nitrogen parameters were stable from 2 to 3 times in a row, the input of ammonium load was increased by increasing the ammonium concentration (as N) to the levels of 5, 10, 17.5, and 25 mg/l. At the end of each ammonium loading range, the salinity raised from 10,000 mg/l to 20,000 mg/l. Microorganisms were acclimatized for 05 days before each new round of adjusted ammonium concentration and/or salinity.

2.3. Analytical equipment and methods

Table 3. Analytical parameters, methods and equipment.

No.	Parameters	Analytical methods	Equipment
1.	pH	SMEWW 4500-H^+ B	HI 9811-5, Hana
2.	DO	SMEWW 4500-O G	EUTECH DO 450, Thermo Scientific
3.	TDS	SMEWW 2520 B	HI 9811-5, Hana
4.	Ammonium	SMEWW 4500-NH_3 F	Model DR3900, HACH
5.	Nitrite	SMEWW 4500-NO_2^- B	
6.	Nitrate	SMEWW 4500-NO_3^- B	

The analytical parameters, methods and equipment used in this experiment were presented in Table 3.

3. RESULTS AND DISCUSSION

Test results of ammonium, nitrite and nitrate concentrations at ammonium loading rates of 0.014; 0.028; 0.049 and 0.070 kg/m^3/d (input ammonium concentrations are 5, 10, 17.5 and 25 mgN/l, respectively) at salinity of 10, 15 and 20 are shown in Figures 3, 4 and 5.

Under the above of ammonium loads, Figure 3 shows that at 10 ‰ salinity, the ammonium treatment efficiencies were 98 - 99; 97.7 - 98.8; 96.8 - 98.7 and 95.7 - 98.0 percent, respectively (ammonium in effluent were 0.05 - 0.1; 0.12 - 0.23; 0.23 - 0.56 and 0.51 - 1.07 mgN/l, respectively). Figure 4 shows that at salinity of 15 ‰, the ammonium removal efficiencies were 95.8 - 96; 92.0 - 94.8; 92.3 - 93.1 and 66.8 - 68.8 percent, respectively (effluent ammonium concentrations were 0.2 - 0.4; 0.55 - 0.8; 1.2 - 1.35 and 7.8 - 8.3 mgN/l, respectively). Figure 5 shows that at salinity of 20 ‰, the ammonium removal efficiencies were 92 - 96; 87.0 - 89.0; 70.9 - 69.1 and 59.6 - 66.0 percent, respectively (effluent ammonium concentrations were 0.2 - 0.4; 1.1 - 1.3; 5.1 - 5.4 and 8.5 - 10.1 mgN/l, respectively).

Despite ammonium treatment efficiency decreasing with increasing ammonium load and salinity, the ammonium load treated still increased reaching 0.028; 0.049, and 0.070 kg/m^3/d at the salinity of 20 ‰. A number of previous studies show that, at similar loading and ammonium concentrations, the volumetric load has reached 1 to 1.3 kg/m^3.d in freshwater [14, 28].

It was observed that nitrite concentration increased at the early stage at the salinity of 10 %, with the highest value reaching 2.7 mgN/l at the input ammonium loading rates of 0.070 kg/m^3/d (ammonium concentration of 25 mgN/l) (Figure 3). However, this value gradually decreased at the end of the period, even when the salinity was increased to 15 ‰ and 20 ‰, the nitrite concentration in the effluent was only 0.06 mgN/l (Figure 4) and 0.016 mgN/l (Figure 5). Reid and Arnold [29] used a biological filter tank to treat water circulation for 2 shrimp farming ditches, the results showed that when increasing of salinity of the water from 20 to 35 %, with DO of about 4.2 to 8 mg/l, pH about 7.2 to 8, then NO_2^- was always less than 0.2 mgN/l.

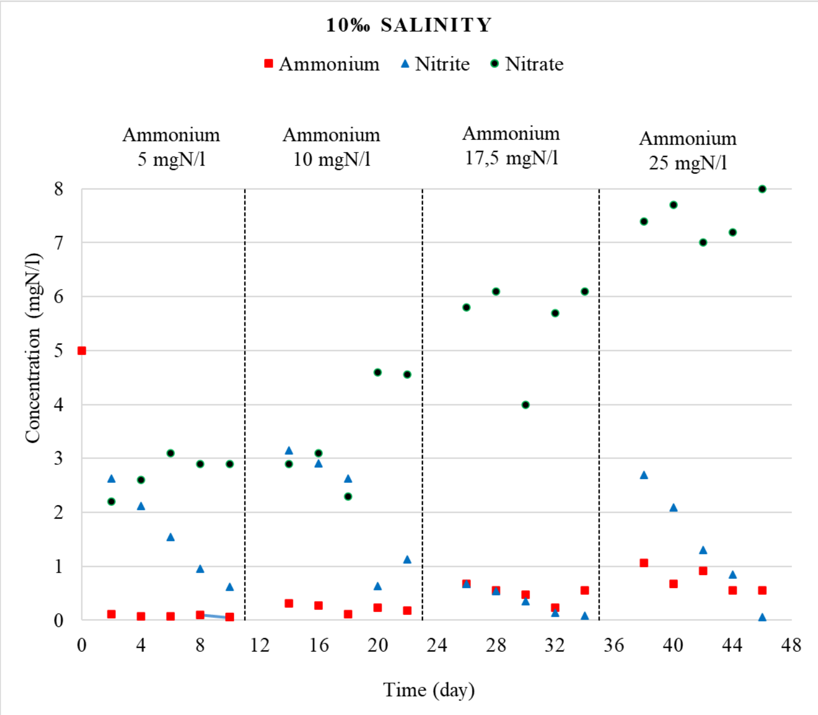

Figure 3. Effluent ammonium, nitrite and nitrate concentration at the salinity of 10 ‰.

On the other hand, nitrate concentration in the treated water was found to be increasing gradually as the ammonium concentration and treatment time increased. The behavior of nitrite and nitrate increased at the beginning of each stage and nitrite decreased afterward could be attributed to the fact that during nitrification, 80 % of the energy (generated from the oxidation of ammonium to nitrite) was used to form CO_2 and only 2 – 11 % of it was used for biomass synthesis. This explains why the biomass production efficiency of nitrification is small. The maximum biomass yield of nitrifying bacteria was about 0.1 - 0.15 g/g NH_4^+-N [30].

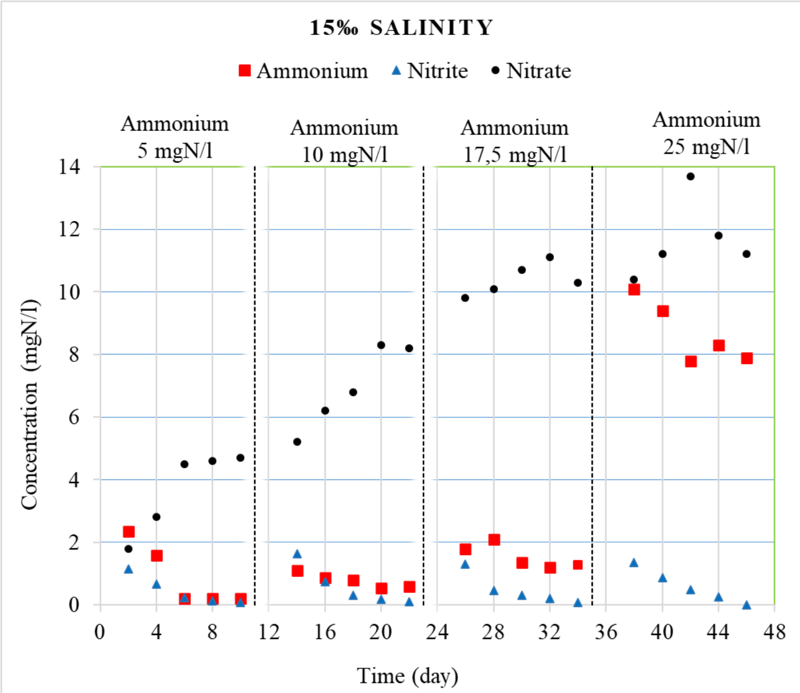

Figure 4. Effluent ammonium, nitrite and nitrate concentration at the salinity of 15 ‰.

In water, ammonium, and ammonia exist together, the amount of ammonia in the water depends however on the pH level as can be seen in Figure 6. Following the FAO (2015) [31], ammonia is toxic to fish at levels above 0.02 mg/l. Therefore, the ammonium concentration in the water needs to be kept low. Nitrogen is an essential nutrient for all organisms, being part of important molecules such as proteins, nucleic acids, adenosine phosphates, pyridine nucleotides, and pigments [32]. However, in super-intensive shrimp culture, shrimps excrete nitrogen through urination and excretion, uneaten food and the decomposition of dead shrimps also contribute to nitrogen waste in aquaculture systems [32, 33]. It was observed that, under the above ammonium loads, ammonium concentration in water treated was below 10 mg/l, with pH in the effluent is about 7.5, and ammonia (NH_3) concentrations were below 0.02 mg/l all. However, if the pH value is 8, the maximum ammonia (NH_3) concentrations were: 0.01, 0.023, 0.056 and 0.107 mg/l, respectively (at a salinity of 10 ‰); 0.04, 0.08, 0.135 and 0.83, respectively (at 15 ‰ salinity); 0.04, 0.13, 0.54 and 1.01, respectively (at 20‰ salinity). Therefore, to limit the formation of NH_3 in water, it is necessary to maximize the nitrification efficiency and maintain a pH of around 7.5. This is also the required pH value for shrimp culture water.

Nitrite (NO_2^-) is formed at the intermediate step in the nitrification process and toxic to fish at levels above 2.0 mg/L [31]. Both un-ionized ammonia and nitrite are toxic to shrimp at low concentrations. *L. vannamei*, exhibited a 96 h LC50 (median lethal concentration) of 24.39 mg/L ammonia with a salinity at 15 ppt, 8.05 pH, and a temperature of 23 °C. The 96 h LC50 for nitrite in *L. vannamei* is 76.5 mg/L at 15 ppt salinity, with a water temperature at 18 °C and the pH at 8.02 [34].

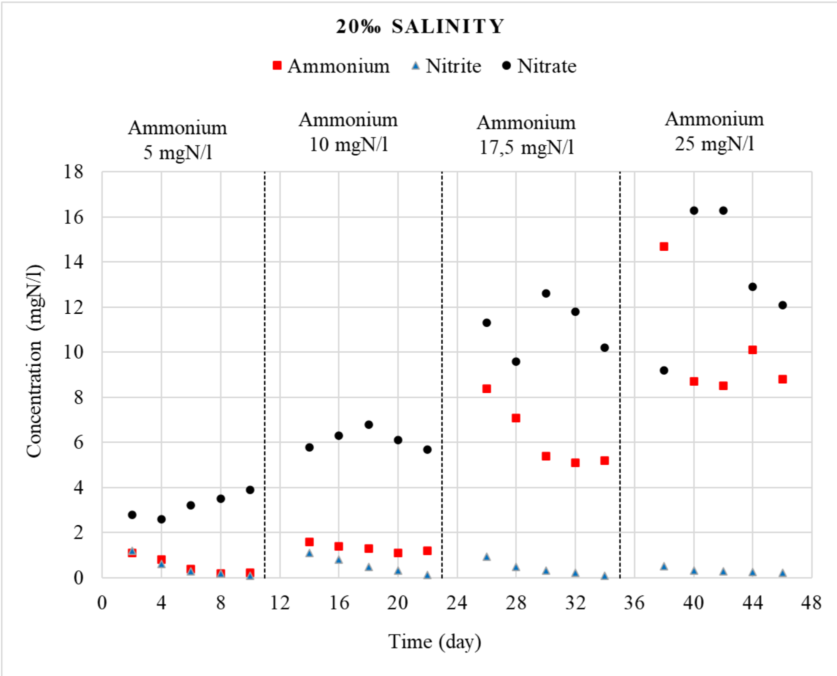

Figure 5. Effluent ammonium, nitrite and nitrate concentration at the salinity of 20 ‰.

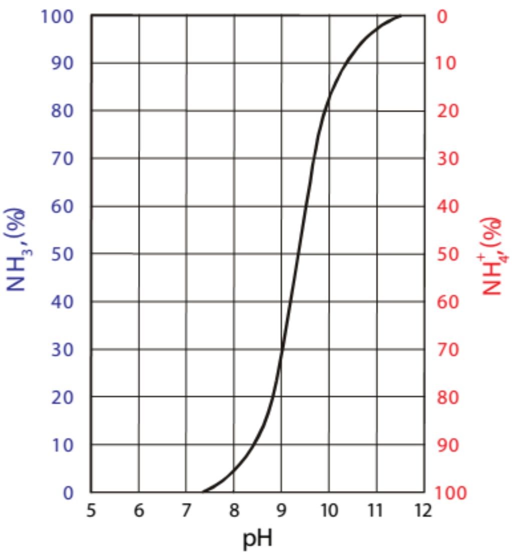

Figure 6. The equilibrium between ammonia (NH_3) and ammonium (NH_4^+) at 20 °C [31].

The requirement for shrimp culture water is that the NO_2 concentration is lower than 0.35 mg/l. Therefore, nitrification and denitrification are very important processes in the treatment of shrimp aquaculture wastewater, so that ammonia and nitrite do not accumulate in recirculating aquaculture systems. Ammonia and nitrite become mineralized through nitrification into nitrate compound and then nitrate becomes volatilized through denitrification and into nitrogen gas. The experimental results also showed that, with all load levels, the nitrite concentration after treatment was quite low and could meet the standard of shrimp culture water. The results also showed that the nitrification process can be used with the above load levels to treat wastewater from super-intensive shrimp farming for reuse. However, it is necessary to control the pH value at an appropriate level as well as balance other essential minerals for normal shrimp growth.

4. CONCLUSION

The fixed bed biofilm reactor (FBBR) is an effective process for the treatment of ammonium in wastewater with high salinity. With ammonium loading rate of 0.014; 0.028; 0.049 and 0.070 kg/m^3/d: at salinity of 10 ‰ the ammonium removal efficiency was 98 - 99; 97.7 - 98.8; 96.8 - 98.7 and 95.7 - 98.0 percent, respectively; at salinity of 15 ‰ the ammonium removal efficiency was 95.8 - 96.0, 94.5 - 92.0, 93.1 - 92.3 and 66.8 - 68.8 percent, respectively; and at salinity of 20 ‰ the ammonium removal efficiency was 92.0 - 96.0, 87.0 - 89.0, 69.1 - 70.9 and 59.6 - 66.0 percent, respectively. This experiment shows that the influence of salinity on ammonium treatment efficiency was very significant. At ammonium loading rate of 0.014; 0.028; 0.049 and 0.070 kg/m^3/d, average efficiency of ammonium treatment decreased by 2.6, 5.5, 5.0 and 29.0 percent, respectively. When the salinity increased to 20 ‰, the efficiency of treatment decreased quite sharply, averaging 4.5, 10.3, 27.7 and 34.0 percent respectively, compared to the salinity of 10 ‰. Nitrification on a fixed bed biofilm reactor system can be used to treat ammonium in super-intensive shrimp wastewater for reuse purposes when other factors are considered.

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CRediT authorship contribution statement. Tran Manh Hai: Conceived and planned the experiments, design and manufacture test system, collected, wrote the manuscript. Nguyen Hoai Chau: Conceived and planned the experiments; provided critical feedback and helped shape the research, analysis and manuscript. Nguyen Trieu Duong and Nguyen Truong Quan: design and manufacture test system. Nguyen Thanh Tung and Nguyen Cam Tu: experimental parameters analysis.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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