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NITROGEN WASTE IN THE EFFLUENT FROM AN INTENSIVE SHRIMP FARM AND THE REMOVAL EFFECTIVENESS OF A WASTWATER TREATMENT SYSTEM INTEGRATING SEWEED PRODUCTION

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ABSTRACT

Environmental concerns and limitations in water sources for aquaculture production are critical factors in the pursuit of wastewater treatment systems for sustainable aquaculture development. In the study, the accumulation of nitrogen components in the effluent from shrimp ponds was examined and the removal efficiency of a wastewater treatment system were evaluated for a commercial production scale. The wastewater treatment system consisted of three steps: (1) sedimentation, (2) sand-filtration, and (3) bioremediation of a high rate algal pond (HRAP), which cultivated *Ulva ohnoi* seaweed as a monoculture. The study showed that the discharged water from intensive shrimp ponds at the end of the production cycle contained high levels of nitrogen components. Compared to the inlet water (from reservoirs), the total nitrogen (TN) concentration in the effluent increased from 0.26 mg/l to 6.23 mg/l and was composed of 25% total particle nitrogen (TPN), 42% total ammonia nitrogen (TAN), 30% dissolved organic nitrogen (DON), and a very low proportion (approximately 3%) of oxidised nitrogen compounds (NO_x). The combined treatment system effectively removed 78% of TN in the wastewater, reducing TN from 6.23 to 1.33 mgN/l. The removal efficiencies of the sedimentation pond, sand-filtration, and HRAP were 47%, 15%, and 16% TN, respectively. While the sedimentation pond mainly cleared the TPN components; the sand-filtration effectively reduced TAN and converted this compound into NO_x due to its function as a habitat for nitrifying bacterial growth, and the treatment step with HRAP significantly removed both TAN and NO_x in the effluent. Therefore, the treatment system integrating seaweed cultivation presented a high efficiency in removing the nitrogen components in the wastewater from intensive shrimp farms.

Keywords: HRAP, nitrogen components, sand-filter, seaweed, sedimentation, wastewater

Thành phần dinh dưỡng nitơ trong nước thải từ hệ thống nuôi tôm thâm canh và hiệu qủa làm sạch môi trường của hệ thống xử lý nước thải có sự kết hợp của sản xuất rong biển

TÓM TẮT

Thiết kế hệ thống xử lý nước thải từ ao nuôi hiệu quả và tái sử dụng nguồn nước sau khi xử lý là yêu cầu cấp thiết, đặc biệt đối với hệ thống nuôi tôm công nghiệp do những mỗi quan tâm tăng lên về ô nhiễm môi trường và giới hạn nguồn nước sạch cho nuôi trồng thủy sản. Nghiên cứu được thực hiện nhằm xem xét sự tích lũy nitrogen trong nước thải từ hệ thống nuôi tôm công nghiệp và đánh giá khả năng làm sạch của hệ thống xử lý nước thải với quy mô xử lý cho trại sản xuất công nghiệp. Hệ thống xử lý nước thải sử dụng trong nghiên cứu gồm ba bước xử lý: (1) xử lý lắng, (2) lọc cát, và (3) khả năng làm sạch sinh học của ao nuôi rong biển hiệu suất cao (HRAP) với quy mô xử lý cho trại sản xuất công nghiệp. Kết quả nghiên cứu cho thấy, nước thải từ ao nuôi tôm thâm canh về cuối chu kỳ nuôi đã có sự tích lũy ở mức độ cao của hầu hết các hợp chất chứa nitơ. So với nước đầu vào (nước ao chứa) giá trị tổng nitơ (TN) đã tăng từ 0,26 mgN/l lên 6,23 mgN/l với thành phần bao gồm: 25% nitơ ở dạng hạt (total particle nitrogen - TPN), 42% ở dạng tổng ammonia (total ammonia nitrogen - TAN), 30% ở dạng nitơ hữu cơ hòa tan (dissolved organic nitrogen - DON) và tỷ lệ rất nhỏ (khoảng 3%) thành phần nitơ đã được oxi hóa (NO_x). Hệ thống xử lý nước thải kết hợp đã loại bỏ được 78% TN trong nước thải, làm giảm lượng từ 6,23 xuống 1,33 mgN/l với khả năng làm sạch của 3 bước xử lý bằng ao lắng, ao lọc cát, và rong biển tương ứng là 47%, 15% và 16% TN. Hệ thống ao lắng cho thấy hiệu quả đặc biệt trong việc làm sạch nhóm hợp chất nitơ dạng hạt (TPN), hệ thống lọc cát có tác dụng chủ yếu lên việc chuyển hóa ammonia sang dạng NO_x nhờ chức năng là nơi cư trú cho nhóm vi khuẩn nitrate hóa, trong khi bước xử lý bằng rong biển cho thấy đặc biệt hiệu quả trong việc làm sạch cả 2 nhóm nitơ vô cơ dạng hòa tan TAN và NO_x. Như vậy, hệ thống xử lý kết hợp rong biển cho thấy hiệu quả rất cao trong việc xử lý các nhóm hợp chất nitơ trong nước thải.

Từ khóa: Ao lắng, ao lọc cát, HRAP, nước thải, thành phần nitơ.

1. INTRODUCTION

Aquaculture is one of the most rapidly growing food sectors globally (FAO, 2012), with an annual growth rate from 6 - 10% over the last 30 years (Msangi *et al.*, 2013). The contribution of aquaculture to the world aquatic production (e.g. finfish, crustacean, molluscs) reached 43.1% in 2013, up only 30.6% from a decade ago in 2003 (FAO, 2015). Without a doubt, aquaculture will continue playing an important role in the global supply of aquatic products in the future. Therefore, the technologies for sustainable growth, both economically and environmentally, are of increasing interest.

Along with the increase in production, the farming systems have gradually changed from extensive traditional to semi-intensive and intensive culture systems (Anh *et al.*, 2010; Lebel *et al.*, 2010). The shift is due to both increasing demand for aquaculture products and the improvements in culture techniques (Zhong *et al.*, 2011). For intensive shrimp farming, feeds account for more than half of the production cost (Preston *et al.*, 2001; Thakur & Lin, 2003; Sahu *et al.*, 2013). However, shrimp can convert only around 20 - 30% of the total nitrogen and 10 - 15% total phosphorous from the feeds into their biomass (Funge-Smith & Briggs, 1998; Preston *et al.*, 2001; Thakur & Lin, 2003; Khoi & Fotedar, 2011). The major nutrient proportion (more than 70%) is retained in sediment and the water column of the culture system through feed waste, prawn excretion, and faeces production. The resulting environmental impacts from untreated effluent have raised concerns about the ongoing sustainability of shrimp farming, and the treatment of this waste are important for this ends (Jones *et al.*, 2002). Thus, an efficient wastewater treatment system is an urgent requirement to purify the wastewater before discharging into the environment or to reuse the treated water for aquaculture production.

Understanding the nitrogen components of the wastewater is a prerequisite step to designing an efficient water treatment system. Total nitrogen (TN) can be divided into total particle nitrogen (TPN) and total dissolved nitrogen (TDN) which is comprised of dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN). However, there is a large variation in the total nitrogen concentration and the contribution of each nitrogen component in the wastewater in the culture system. Sedimentation ponds have been commonly used in various countries to treat wastewater from intensive shrimp farms (Preston *et al.*, 2001; Castine *et al.*, 2013). Although the sedimentation step can settle the large-sized particles down due to gravitation, the maximum removal efficiency was no more than 60% of the total nitrogen in the wastewater (Preston *et al.*, 2001; Jackson *et al.*, 2003a; Castine *et al.*, 2013). The sedimentation ponds were less effective in removing small particles and dissolved nitrogen fractions in the wastewater. For recycling the treated water and dealing with the stricter regulations on discharging effluent into the natural environment, a combined treatment system is recommended.

This study aimed to examine the build-up of nitrogen compounds in the effluent from an intensive shrimp farm in Australia and evaluate the removal efficiencies of a three step combined wastewater treatment that included sedimentation, sand-filtration, and seaweed purification on nitrogen components in the effluent.

2. MATERIALS AND METHODS

2.1. Study location and shrimp farming system

The wastewater treatment system was set up for a commercial scale at Pacific Reef Fisheries, Queensland, Australia, which is adjacent to the Great Barrier Reef. Sea water from the Coral Sea was pumped into a reservoir before being delivered into the shrimp ponds through earthen channels (Fig. 1).

The shrimp farm is comprised of 68 earthen ponds, one ha each, with an average water depth of 1.4 m. Production is classified as moderately intensive: stocking densities were between 32 and 35 P15/m^2, and four to six electric aerators (2 hp) were operated continually in each pond. At the time of the study, the farm production cycle was unsynchronized (i.e., crop age varied between ponds, and production continued year-round). Each pond completed between one and two production cycles per year. Of these, three ponds, which were stocked at the same time, were selected for water sampling. These ponds were 130 - 135 days into their production cycle with shrimp sizes of 31 - 35 grams/shrimp on average at the first water sampling batch. The tiger shrimps were fed 45% protein pellets using a feeding regime of four times daily. The yield of these ponds at harvest were approximately 4.5 to 5 tones of shrimp per hectare.

Little or no water exchange was done for the first 1 - 2 months after a pond was first filled and stocked. Thereafter, water in each pond was partially exchanged at regular intervals of 7 days with an exchange amount of 20% weekly. No fertilizer was added to the ponds during the study. The trial was monitored for a production cycle of 6 months. The salinity of the shrimp pond effluent over the study period varied from 29 - 32 ppt with an average of 30.7 pp. The temperature was measured continually using automatic temperature measurements and a temperature logger. The lowest temperature was taken at 6am and the highest value was taken at 2 pm. During the study period, the lowest temperature was 19.7 and the highest temperature was 31.8°C. A mean value of 7.74 pH was observed for the shrimp effluent.

Figure 1. Location of the shrimp farm

Source: Google maps

2.2. Wastewater treatment system

The wastewater treatment system was designed with three treatment steps as indicated in the Figure 2, including:

Step 1: The first treatment step was targeted to remove the particulate nitrogen fraction by using sedimentation ponds. The effluent from intensive shrimp ponds was drawn into a sedimentation pond and kept in this pond for 3 to 5 days before going to the next treatment step. The shrimp farm is currently using a sedimentation pond with an area of about 2 ha for a primary treatment of wastewater before discharging into the environment.

Step 2: The next treatment step, sand filters (SF), was designed to aid the sedimentation pond in the capture of particulates of all size fractions, mainly suspended and fine solid. SF functions also included improved water clarity to *Ulva* growth in the next treatment step, and was also expected to be a bio filter media for the inhabitation and development of the bacteria community to convert organic nitrogen to inorganic forms and among inorganic forms. There are two parallel 120 m length sand-filters constructed at the farm, (SF1: Area = 2,897 m^2; SF2: Area = 3,460 m^2) and both contain sand media at a depth of 0.5 m . During the study period, only sand-filter 1 (2,897 m^2) was online. The water depth of the sand-filter was 0.4 - 0.6 m, which was the optimal values reported by the farm (unpublished document). Sand filters were supplied at their maximum flow rates (19 - 23 L/s) and drained at a rate that allowed for maintenance of water levels.

The performance of the sand-filter can reduce over time as the results of filamentous algae and microalgae at the SF surface, particularly at lower water levels. These issues can be detected by periodic measurement of maximum SF filtration rate (discharge valve 100% open and assuming pipework sizing is not the flow restriction) which indicates the need for maintenance and swapping of the SF (preventative and proactive maintenance). Maintenance of sand-filters can be carried out manually or using machines.

Step 3: The last step of treatment aimed to clean up the dissolved inorganic nitrogen components (DIN) using seaweed. The wastewater after the sand-filter treatment step was drawn into a High Rate Algal Pond (HRAP) at a controled flow rate. In the study, *Ulva ohnoi* seaweed was cultivated as a monoculture in the HRAP with a dimension of 150 × 10 m (LxW) and a water depth of 50 cm at the centre point to uptake the DIN compounds. The stocking density was maintained at 3 - 4 gram fresh weight/l (FW/l), which was the suitable stocking density reported by the farm (unpublished report). Typically, the *Ulva* more than doubled its mass in 1 week, therefore, seaweed biomass was havested weekly to keep a constant density in the system over time. Paddlewheel rpm settings were fixed and sufficiently high to ensure algae suspension regardless of water depth and algae density. No additional nutrients or additives were added for maintenance of the *Ulva* stock into the SF or HRAP system during the study period. Water exchange rate was at 300% x 24h through the HRAP. During the study, the treated water after going through the HRAP step was discharged into the environment and wasn't used for recycling.

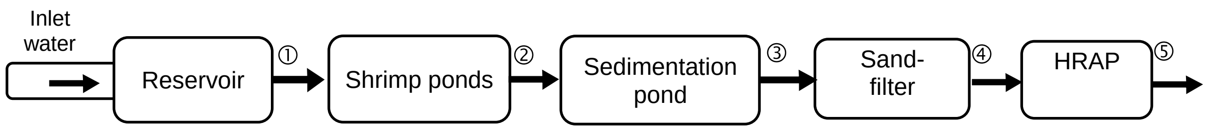

Figure 2. The water flow across the farm and the treatment steps used in the study

Note: The inlet water from the ocean was pumped into a reservoir before being delivering to the culture ponds. The effluent from the shrimp ponds was sent through three treatment steps including sedimentation ponds, sand-filer bed, and high rate algal ponds (HRAP)

Figure 3. Sand-filter bed used in the study

Note: The size of the sand filter bed was 120 m x 24.5 m (L x W) containing sand media at a depth of 0.5 m

Figure 4. High rate algal pond (HRAP) culturing *Ulva ohnoi* as an monoculture

Note: The size of the HRAP was 100 m x 10 m (LxW) with 0.5 m being the deepest point at the center

2.3. Water sampling site and analysis

The water samples were collected from five sources across the farm as indicated in Figure 2 to evaluate the changes in the concentrations of water environmental factors and removal efficiencies of each treatment step. Five sampling batches were conducted during the period from April to May, 2016. For each sampling batch, three samples from each water source were collected with the sampling schedule indicated in Table 1. Water samples from the reservoir and shrimp ponds were collected on the days of water exchange for the shrimp ponds. The discharged water from the shrimp ponds was drawn into the sedimentation pond and left for 3 days of settling. Thus, the samples from the settlement ponds, SF, and HRAP were taken 3 days thereafter. After sampling, the water samples were preserved at approximately 4°C (using ice boxes) and analysed within 24 hours. The type of nitrogen variables and the methods for analysing and calculating of each of the factors are presented in Table 2.

Table 1. Sampling schedule across the farm for the study. Five sampling batches were conducted for all water sources. Water samples from the reservoir and shrimp ponds were collected on the days of water exchange for the shrimp ponds. The samples from settlement ponds, SF, and HRAP were taken 3 days thereafter

Water sources	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Reservoir	10/4 am	17/4 am	24/4 am	03/5 am	10/5 am
Shrimp pond	10/4 am	17/4 am	24/4 am	03/5 am	10/5 am
Settlement pond	13/4 am	20/4 am	27/4 am	06/5 am	13/5 am
SF	13/4 am	20/4 am	27/4 am	06/5 am	13/5 am
HRAP	13/4 pm	20/4 pm	27/4 pm	06/5 am	13/5 am

Table 2. Analysing methods, practical quantification limits (PQL) and units of nitrogen components included in the research

Parameters	Analysing methods	PQL	Units
Total nitrogen - TN	APHA 4500 N C	0.1	mg/L as N
Total dissolved nitrogen - TDN	APHA 4500 N C	0.1	mg/L as N
Dissolved organic nitrogen - DON	TDN - DIN	0.1	mg/L as N
Dissolved inorganic nitrogen - DIN	DIN = TAN + NO_x	0.1	mg/L as N
Total ammonia nitrogen - TAN	USEPA 103,104,129	0.02	mg/L as N
Oxidised nitrogen - NO_x	APHA 4500 NO_3 I	0.01	mg/L as N
Nitrite - N	TM_NO_2	0.015	mg/L as N

2.4. Data analysis

For the nitrogen water samples, the mean (n = 5) values of each water quality variable were plotted for each of the five water sources. One-way ANOVA and Tukey's post-hoc comparisons were used to compare the differences in the nitrogen content among the water sources. The data analysis presented the key nitrogen variables of total nitrogen (TN), total ammonia nitrogen (TAN), dissolved organic nitrogen (DON), total particulate nitrogen (TPN), and oxidized nitrogen (NO_x).

3. RESULTS AND DISCUSSIONS

3.1. Nitrogen waste in shrimp effluent

The effluent from shrimp ponds significantly increased ($P < 0.05$) in the content of all nitrogen components (Table 3) except for oxidised nitrogen (NO_x) compounds compared to the reservoir water. The mean total nitogen (TN) concentration increased 27 times from 0.26 to 6.23 mgN/l compared to the reservoir water. Of these, the TAN content was more than 100 times higher than in the reservoir water, rising from 0.02 to 2.63 mgN/l. The dissolved organic nitrogen (DON) level increased from 0.20 to 1.85 mgN/l, which was 9 times higher than in the reservoir water. Total particle nitrogen fraction (TPN) and oxidised nitrogen (NO_x) levels increased from 0.03 to 1.53 mgN/l and from 0.01 to 0.19 mgN/l, respectively, in the shrimp discharge water. During the sampling period, 25% of the nitrogen in the shrimp pond effluent was TPN, 42% was TAN, and 30% was DON. The NO_x component contributed only a small proportion (3%) to the TN (Figure 5).

There was a clear accumulation of waste nitrogen in the effluent from shrimp ponds at the end of production cycle (around 130 - 160 days of culture). In the present study, the TN content in the prawn effluent was in the same range of the farming system in Thailand (6.5 mgN/l) (Thakur & Lin, 2003) and higher than reported for several prawn farms (2 - 3 mgN/l) in Australia (Preston *et al.*, 2001; Jackson *et al.*, 2003b). The present results, however, showed that the dissolved nitrogen component (TDN) formed the dominant nitrogen fraction (76%) in the effluent, while Jackson *et al.* (2003b) reported predominantly particulate nitrogen (TPN) in the prawn effluent from other culture systems. The variations in the content and contribution of the different nutrient wastes in the shrimp ponds across the culture systems was not surprising, and was related to the differences in stocking densities and farming management practices, such as feeding strategies, food quality, and water exchange regimes (Thakur & Lin, 2003; Martin *et al.*, 2010). The higher the stocking density used, the higher the amount of waste generated (Martin *et al.*, 1998).

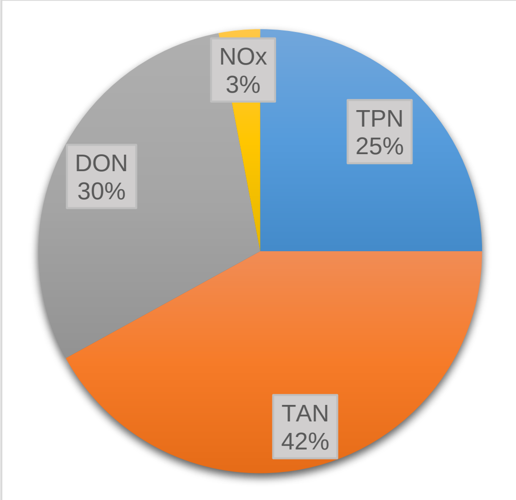

Figure 5. Comparative pie chart of nitrogen components in the wastewater from an intensive prawn farm

Note: The total nitrogen (TN) was comprised of total particulate nitrogen (TPN), total ammonia nitrogen (TAN), dissolved organic nitrogen (DON), and oxidised nitrogen (NO_x)

Table 3. Changes in the total nitrogen (TN) load and the main nitrogen components, including total ammonia nitrogen (TAN), total dissolved organic nitrogen (DON), total particulate nitrogen (TPN), and dissolved nitrogen (NO_x), and total phosphorous (TP) of the water samples after each treatment step

Nutrients	Reservoir	Prawn discharge	Sedimentation	Sand-filter	HRAP
TN (mg l^{-1})	0.25 ± 0.067[a]	6.23 ± 0.471[b]	3.30 ± 0.306[ab]	2.40 ± 0.203[a]	1.33 ± 0.203[a]
TAN (mg l^{-1})	0.02 ± 0.003[a]	2.64 ± 0.202[b]	1.82 ± 0.442[ab]	0.74 ± 0.186[a]	0.17 ± 0.094[a]
DON (mg l^{-1})	0.20 ± 0.089[a]	1.85 ± 0.146[b]	0.62 ± 0.111[a]	0.69 ± 0.124[a]	0.64 ± 0.124[a]
TPN (mg l^{-1})	0.03 ± 0.010[a]	1.53 ± 0.030[b]	0.73 ± 0.090[a]	0.33 ± 0.088[a]	0.21 ± 0.046[a]
NO_x (mg l^{-1})	0.01 ± 0.003[a]	0.19 ± 0.031[ab]	0.14 ± 0.066[ab]	0.65 ± 0.097[b]	.31 ± 0.079[ab]

Note: Data are shown as mean ± SE for each variable. The values with different letters (a or b) in the same row indicate significant differences (Tukey's HSD, $P < 0.05$)

Regarding the specific nitrogen fractions, present results showed the dominance of nitrogen (TAN) among the TDN forms contributing 55% of the TN in the shrimp pond effluent. A number of previous studies also reported the dominance of TAN in shrimp farming systems (Fvnge-Smith & Briggs, 1994) while others reported the major contribution of DON (Jackson *et al.*, 2003b; Molnar *et al.*, 2013). In a culture system, TAN is produced by microbial remineralization processes within the sediment and by direct excretion by prawns (Burford &Williams, 2001; Burford *et al.*, 2003), while the high level of DON has been suggested to be as a result of leaching from commercial feed (Burford & Williams, 2001). A very low concentration of oxidised nitrogen (NO_x) in the wastewater corroborated with the previous findings in prawn farming systems (Jackson *et al.*, 2003b; Thakur & Lin, 2003; Molnar *et al.*, 2013). The low concentrations of NO_x was a result of the low nitrification rate, which is one of the major limitation of intensive farming systems as it is difficult to design prawn ponds to maximize natural microbial processes (Hargreaves, 1998). In these situations, the nitrification process of oxidizing ammonia to nitrate is usually limited by aerobic heterotrophs and other chemoautotrophic bacteria, which have higher affinities for oxygen, outcompeting nitrifying bacteria for oxygen (Nizzoli *et al.*, 2006). Variations in the contribution of different nitrogen forms, particulate versus dissolved, and organic versus inorganic, highlight the importance of having multiple steps in the treatment process to treat aquaculture wastewater effectively.

3.2. Treatment of sedimentation pond

The sedimentation pond significantly reduced TN concentration compared to the shrimp effluent water, with significant reductions in total particulate nitrogen (TPN) and dissolved organic nitrogen (DON) (Fig. 8 & 10; $P < 0.05$). The sedimentation decreased TN content by 47% from 6.2 to 3.3 mgN/l on average. The main effects were reductions in TPN from 1.5 to 0.7 mgN/l, and DON from 1.85 to 0.62 mgN/l. In addition, the sedimentation step also reduced the TAN content by 31% from 2.6 to 1.8 mgN/l although the NO_x concentration of the sedimentation water was essentially the same as the shrimp discharge water. The nitrogen composition of the sedimentation water was 55% TAN, 22% TPN, 19% DON, and 4% NO_x (Fig. 6, 7, 8, 9, & 10).

The present results confirm the effectiveness of sedimentation ponds in reducing the particulate nitrogen load (TPN). The TPN removal efficiencies by sedimentation (52% reduction) was within the range of that reported for other shrimp farming systems (<60%) in Thailand and Australia (Preston *et al.*, 2001; Jackson *et al.*, 2003a). According to Castine *et al.* (2013), the particulate fractions which settle in sedimentation ponds due to gravitation range in size from 1 to 100 µm. However, the initial reduction of particulate material in the sedimentation pond eventually leads to the mineralization of organic nitrogen and the release of dissolved nitrogen into the water column from the settled particles (Jackson *et al.*, 2003a) due to the activities of microbial communities (Castine *et al.*, 2012). Therefore, the levels of TAN and DON are generally increased in sedimentation water over time (Preston *et al.*, 2001; Jackson *et al.*, 2003a; Erler *et al.*, 2007). A number of factors may influence the removal effectiveness of sedimentation ponds, including effluent composition, residence time, pond design, and pond management (Preston *et al.*, 2001). These could be the causes of the wide fluctuations in the contents of nitrogen components of sedimentation water samples as shown in the present study. In fact, the sedimentation ponds need to frequently removed sediments to improve their removal effectiveness.

3.3. Treatment by sand filter and *Ulva* seaweed

Sand filter and HRAP further decreased the total nitrogen (TN) concentration by 32% from 3.3 to 1.33 mgN/l, of which sand-filter contributed 15% and HRAP contributed 16% to the reduction. The main removal efficiency of sand-filtration reduced 41% of the TAN by converting the nitrogen into oxidised nitrogen (NO_x) resulting in an increase in NO_x levels 4.5 times from 0.14 to 0.65 mgN/l (due to nitrification). The conversion processes are based on the presence of both heterotrophic and nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) in the sand-filter (Prochaska & Zouboulis, 2003). However, nitrifying bacteria are generally dominant in the sand-filter, leading to slower ammonification compared to nitrification (Prochaska & Zouboulis, 2003). In addition, the treatment by sand-filteration also removed 26% of the TPN component in the shrimp effluent.

The HRAP, which was cultivating *Ulva ohnoi,* proved to be effective in the removal of inorganic nitrogen including the reduction of TAN from 0.74 to 0.17 mgN/l, and the reduction of NO_x from 0.65 to 0.31 mgN/l. Noticeably, HRAP showed a high capacity in the removal of NO_2 which is a toxic compound to culture animals, reducing it from 0.46 to 0.20 mgN/l. The DON content, however remained largely unchanged compared to the sedimentation water. Therefore, after the three treatment steps, the treated water contained 1.33 mgN/l TN with the final relative contribution of 48% DON, 23% NO_x, 16% TPN, and 13% TAN. Compared to the initial shrimp effluent, 79% of the TN content was removed in the final treated water, in which 94% TAN, 86% TPN, and 65% DON was reduced. Compared to the reservoir water, no significant difference in the contents of nitrogen compounds in the treated water was shown despite being five times higher. This could be due to the considerable variation between sampling batches (see standard errors -SE in the Figure 6, 7, 8, 9, &10).

The combination of sand-filteration and *Ulva* seaweed proved to be an effective complementary treatment option for the further reduction of total nitrogen (TN), particularly TAN and TPN in the wastewater. The sand-filter was designed for further removal of all sized nitrogen components, mainly suspended and fine solids to complement the sedimentation pond (Castine *et al.*, 2013). Compared to the sedimentation water, the major changes in the sand-filtered water were the reductions of 47% TAN and 26% TPN, along with the 4.5 times increase in NO_x content (due to nitrification). The high DON concentration in the sand-filtered water could be due to the outcompeting of nitrifying bacteria compared to the heterotrophic bacteria leading to limitations of ammonification as explained above.

The final treatment step by HRAP with *Ulva* seaweed was effective in removing TAN and NO_x, with a higher removal efficiency for TAN. The high removal efficiencies of seaweed, particularly *Ulva,* for dissolved inorganic nitrogen (both TAN and NO_x) have been extensively reported (da Silva Copertino *et al.*, 2009; Khoi & Fotedar, 2011; Aníbal *et al.*, 2014; Rabiei *et al.*, 2014). In this case, the higher removal efficiencies for TAN compared to NO_x may be due to a higher preference of *Ulva* species for ammonia than nitrate (da Silva Copertino *et al.*, 2009). These results highlighted the key role of seaweed and the suitable combination of treatment steps in the purification of nitrogen waste in the effluent from shrimp farms.

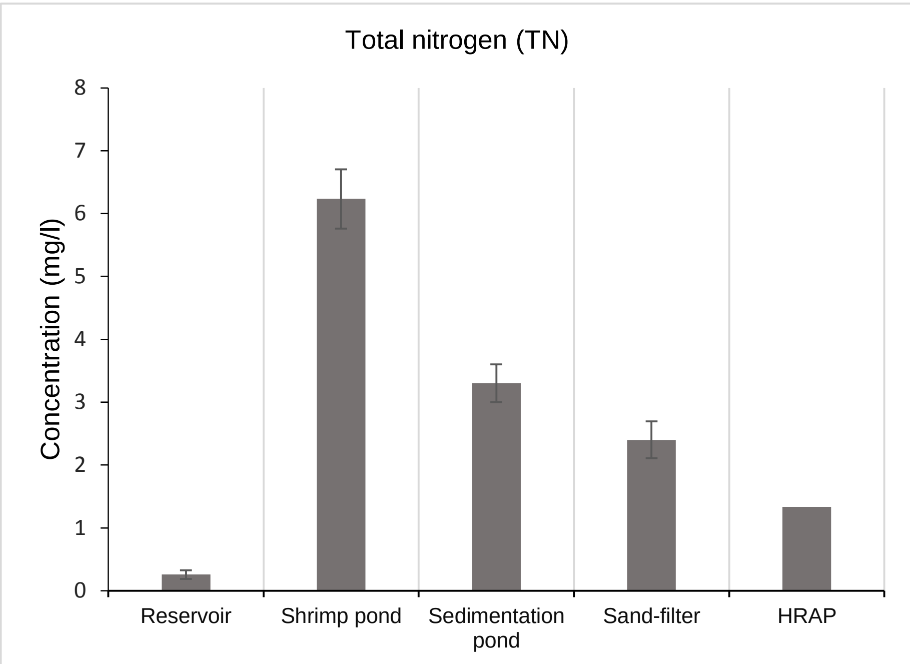

Figure 6. The changes in total nitrogen (TN) concentration in the water samples across the farm

Note: Data are shown as mean ± SE

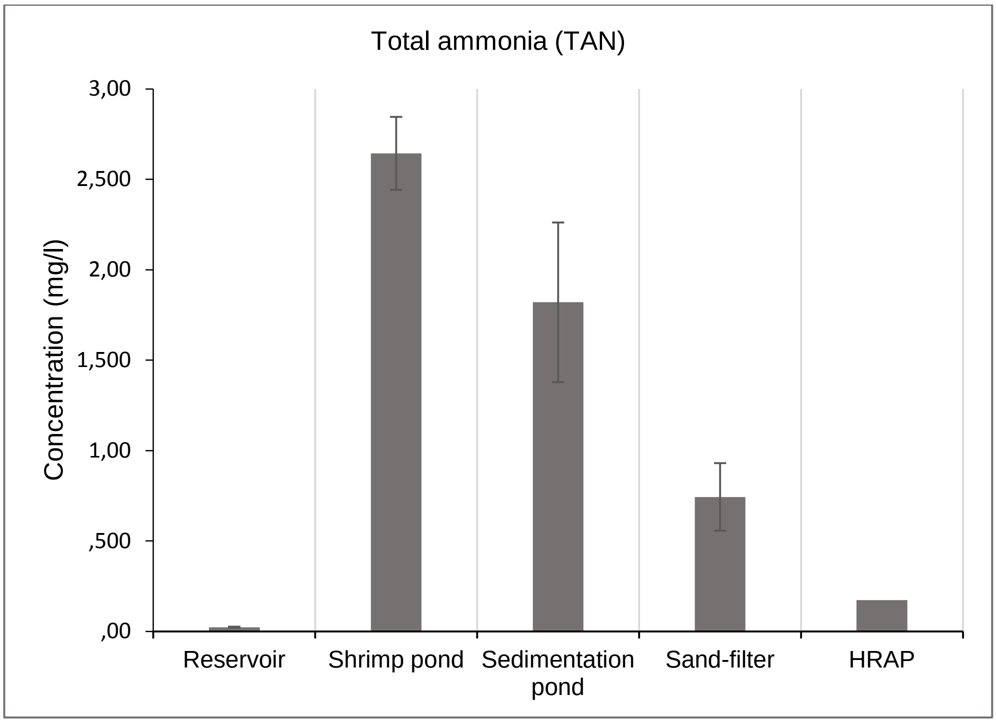

Figure 7. The changes in total ammonia nitrogen (TAN) concentration in the water samples across the farm

Note: Data are shown as mean ± SE

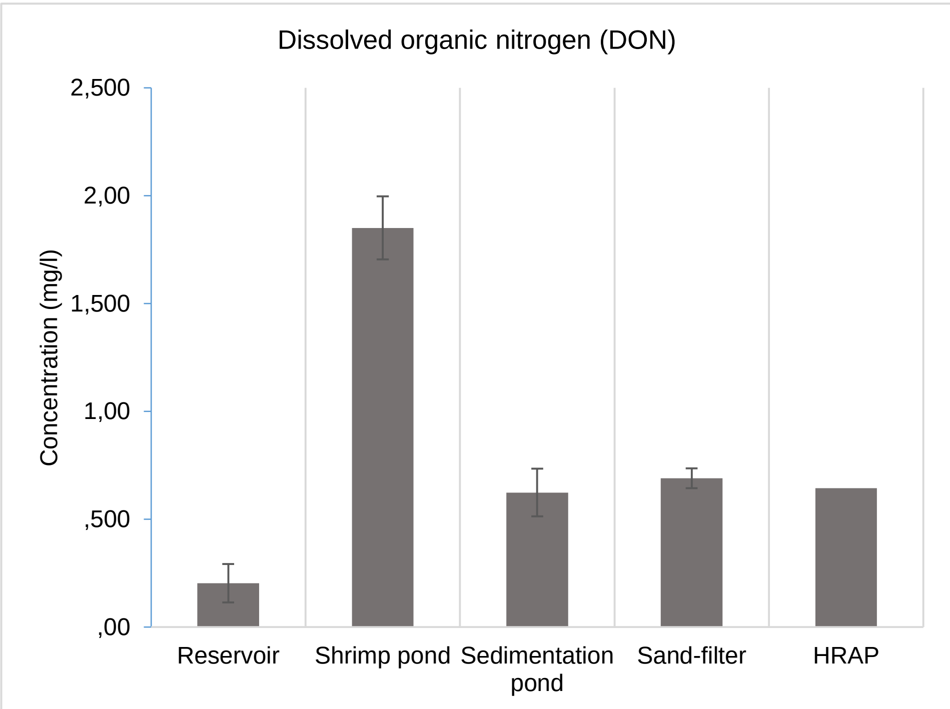

Figure 8. The changes in dissolved organic nitrogen (DON) concentration in the water samples across the farm

Note: Data are shown as mean ± SE

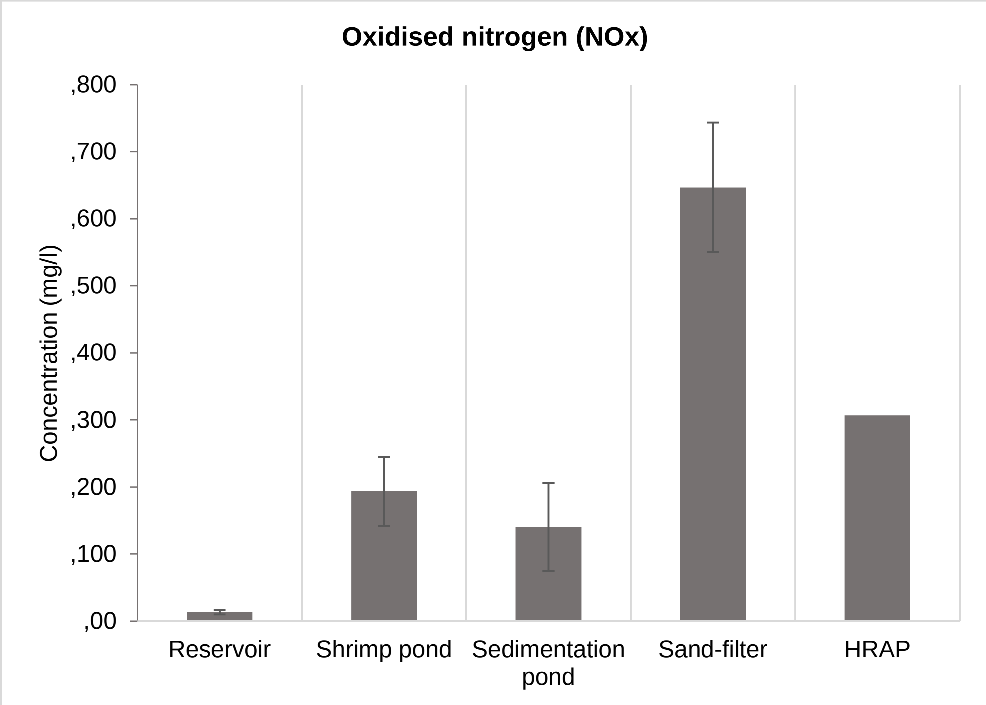

Figure 9. The changes in the oxidized nitrogen (NO_x) concentration in the water samples across the farm

Note: Data are shown as mean ± SE

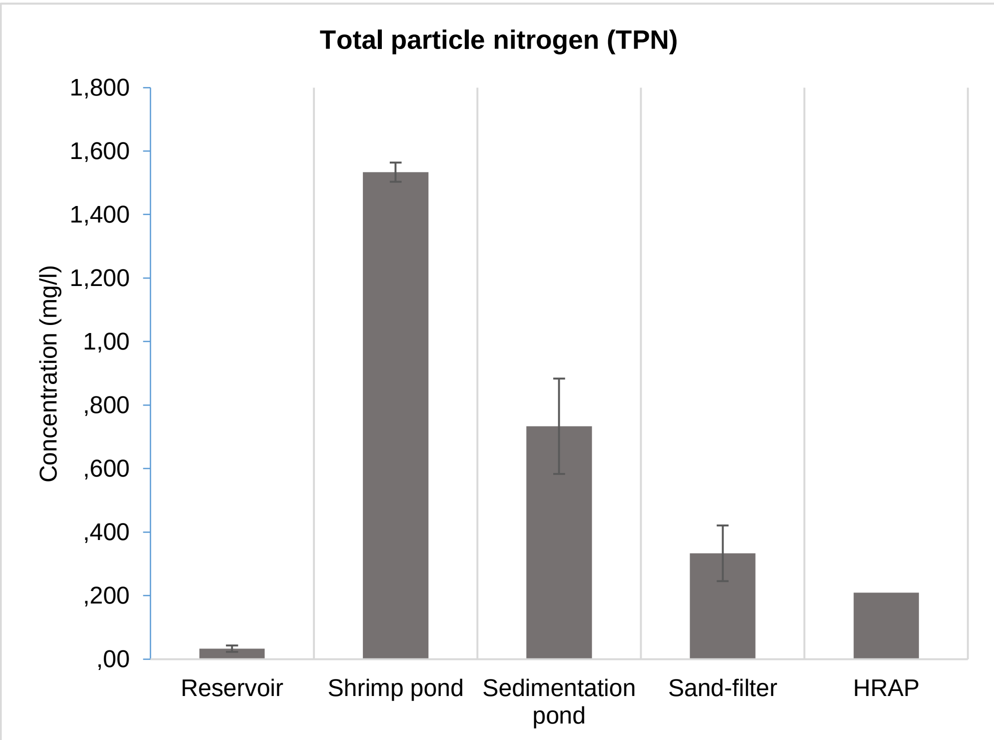

Figure 10. The changes in the total particle nitrogen (TPN) concentration in the water samples across the farm

Note: Data are shown as mean ± SE values for the content of each water source

4. CONCLUSION

The effluent from intensive shrimp ponds accumulated high levels of most nitrogen compounds, particularly total ammonia nitrogen (TAN) and dissolved organic nitrogen (DON). A treatment system consisting of seaweed purification was highly efficient in the removal of nitrogen from waste water (reduced TN by nearly 80%). Compared to the reservoir water, the content of waste nutrients in the new treated water showed no significant differences. However, the TN content in the new treated water was 5-times higher than that in the reservoir water indicating room for improvement. To improve this, the operation of treatment systems should be upgraded in order to reduce the DON component by converting it into inorganic nitrogen forms, and reduce the flowing rate through the sand-filter and the final HRAP step to allow the effective removal of dissolved inorganic compounds.

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