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Optimization and evaluation of a bottom substrate denitrification tank for nitrate removal from a recirculating aquaculture system

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Abstract

A bottom substrate denitrification tank for a recirculating aquaculture system was developed. The laboratory scale denitrification tank was an 8 L tank (0.04 m^2 tank surface area), packed to a depth of 5 cm with a bottom substrate for natural denitrifying bacteria. An aquarium pump was used for gentle water mixing in the tank; the dissolved oxygen in the water was maintained in aerobic conditions (e.g. > 2 mg/L) while anoxic conditions predominated only at the bottom substrate layer. The results showed that, among the four substrates tested (soil, sand, pumice stone and vermiculite), pumice was the most preferable material. Comparing carbon supplementation using methanol and molasses, methanol was chosen as the carbon source because it provided a higher denitrification rate than molasses. When methanol was applied at the optimal COD:N ratio of 5:1, a nitrate removal rate of 4591 ± 133 mg-N/m^2 tank bottom area/day was achieved. Finally, nitrate removal using an 80 L denitrification tank was evaluated with a 610 L recirculating tilapia culture system. Nitrate treatment was performed by batch transferring high nitrate water from the nitrification tank into the denitrification tank and mixing with methanol at a COD:N ratio of 5:1. The results from five batches of nitrate treatment revealed that nitrate was successfully removed from water without the accumulation of nitrite and ammonia. The average nitrate removal efficiency was 85.17% and the average denitrification rate of the denitrification tank was 6311 ± 945 mg-N/m^2 tank bottom area/day or 126 ± 18 mg-N/L of pumice packing volume/day.

Key words: denitrification; nitrate removal; methanol; pumice stone; recirculating aquaculture system

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Introduction

The advantages of recirculating aquaculture systems (RAS) over traditional aquaculture ponds with water exchange is that RAS provide higher production yield, better disease control and lower water consumption and wastewater discharge (Crab et al., 2007). With aquaculture systems, nitrogenous waste (i.e. ammonia and nitrite) derived from high protein feeding must be properly treated as these compounds are toxic to aquatic animals. The most efficient nitrogen treatment in RAS is the nitrification process in which ammonia and nitrite are converted to nitrate. In earthen ponds, nitrate can be eliminated by the natural denitrification process taking place in the pond bottom. On the other hand, accumulation of nitrate is common in recirculating systems without a bottom sediment such as aquariums and indoor tanks which have only nitrification biofilters. Although the toxicity of nitrate is lower than that of ammonia or nitrite, most aquaculture practices recommended that nitrate in an aquaculture pond must be kept below 50 mg-N/L to avoid sub-lethal toxicity. Apart from water exchange, the anaerobic denitrification process is among the most efficient nitrate treatment processes in aquaculture (Menasveta et al., 2001; Lee et al., 2000; Singer et al., 2008). Biological denitrification is an efficient process for nitrogen removal from wastewater in which heterotrophic bacteria under anaerobic conditions convert nitrate-N and nitrite-N into nitrogen gas (van Rijn et al., 2006). Unfortunately, most nitrate removal technologies are under research and are not yet practically used in commercial aquaculture systems. This is due to the high cost of construction with sophisticated equipment, high operating costs and complex operating skills.

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The efficiency of the denitrification process depends on a number of factors such as temperature, pH, dissolved oxygen (DO), nitrite concentration (Rivett et al., 2008) and the amount of the organic carbon substrate (Mokhayeri et al., 2008; Magram, 2010). This study was an attempt to develop a simplified denitrification tank using a bottom substrate for nitrate treatment in aquaculture systems. The most important characteristic of the denitrification tank is that anoxic denitrification takes place only in the bottom substrate while aerobic conditions are maintained in the water column. The concept of a denitrification tank is therefore easy to apply for nitrate removal with a minimal risk of hydrogen sulfide production in the anoxic bottom substrate.

The experiments consisted of two phases. The first phase evaluated suitable substrates for the bottom denitrification process and the optimal organic carbon sources on nitrate removal in the denitrification tank. The second phase was an evaluation of the denitrification tank for nitrate treatment in a closed recirculating tilapia culture system over 99 days of operation.

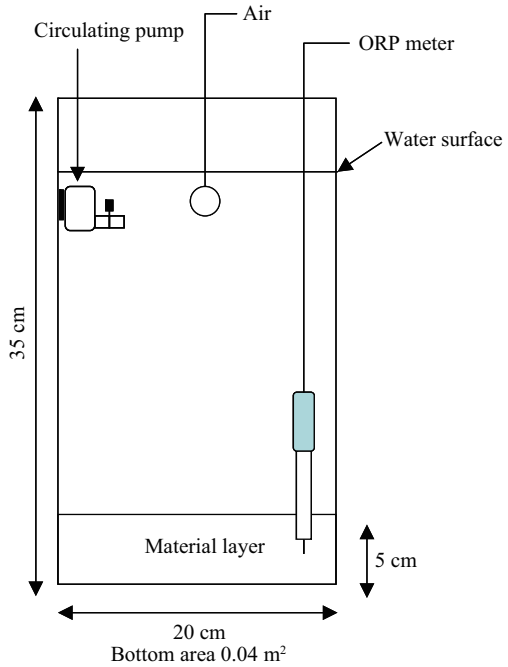

Fig. 1 Side view illustration of the denitrification tank used.

1 Materials and methods

1.1 Denitrification tank

The denitrification tank was a glass aquarium tank ($20 \times 20 \times 35$ cm^3, 0.04 m^2 tank bottom area), packed to a depth of 5 cm with a bottom substrate for natural bacteria. The denitrification tank was filled with 8 L of fresh water containing 100 mg-N/L $NaNO_3$. The external carbon supplement was provided by the addition of methanol. The COD:N ratio was assigned as g COD per g nitrate-N of which 1 g methanol is equivalent to 1.5 g COD. In order to improve water mixing in the denitrification tank, a small aquarium pump was provided in all tanks for gentle internal water circulation. Although anoxic conditions predominated in the bottom substrate layer, which is suitable for anaerobic denitrification, dissolved oxygen in the water column of the tank was kept at greater than 2 mg/L using a diffusive air stone located near the water surface (**Fig. 1**). This was to prevent the risk of hydrogen sulfide production during anoxic treatment as hydrogen sulfide is highly toxic to aquatic animals. With these procedures, using a concept modified from Kutako et al. (2009), the denitrification tank in this study was simultaneously operated under two conditions, i.e. aerobic conditions in the water column and anoxic conditions in the bottom substrate layer. The oxidation-reduction potential (ORP) in the substrate layer was measured daily using an ORP probe (HI3230, Hanna Instruments, USA) placed at a depth of 2.5 cm in the bottom media layer. All experiments were conducted with three replicate tanks.

1.2 Experimental procedures

1.2.1 Effect of packing materials on nitrate removal in the denitrification tank

Four substrates, i.e. natural soil (control), sand, pumice stone and vermiculite, were chosen as the bottom substrates. The particle sizes of the sand, pumice stone and vermiculite were 1–3 mm. Natural soil with 33.69% organic carbon content was assigned as the control since the natural denitrification process in earthen ponds occurs in anoxic bottom soil. In this case, sand was chosen as it is commonly used as a bottom substrate in aquaria and in water treatment tanks (Toonen and Wee, 2005). Pumice stone with a porous structure was chosen as a low-cost packing material for bacterial immobilization (Pazarlioğlu and Telefoncu, 2005; Keskin et al., 2011). Vermiculite has been reported as a substrate for denitrification in anaerobic reactors (Kida et al., 1990), wetland and hydroponic wastewater treatment experiments (Vaillant et al., 2003; Sánchez et al., 2011). All substrate materials were washed with tap water and dried in the oven before used. After drying, the sand, pumice stone and vermiculite were sieved with a nylon screen to obtain a particle size of 1–3 mm. All substrates were packed in the bottom of the glass tank at a thickness of 5 cm. The initial nitrate concentration in the artificial wastewater was 100 mg-N/L. Methanol was used as the external carbon source at a COD:nitrate-N ratio of 5:1 (1.5 g COD = 1 g methanol). During the experiment, when the nitrate concentration fell below 5 mg-N/L, water was exchanged with fresh water containing 100 mg-N/L nitrate. The nitrate removal experiment was repeated at least three times and the average nitrate removal rate was calculated.

1.2.2 Effect of organic carbon sources on the denitrification process in the denitrification tank

The denitrification tank was packed with pumice stone as the bottom substrate and the experimental procedure was performed according to the previous experiment (Section 1.2.1). The experiment consisted of a control (without carbon addition), treatment-1 with methanol addition at a COD:nitrate-N ratio of 5:1 and treatment-2 with molasses addition at a COD:N ratio of 5:1. The physical characteristics of molasses derived from sugar processing was a dark brown slurry containing 993.55 ± 3.58 g/L COD, 11.46 ± 0.42 g/L TKN (total Kjeldahl nitrogen) and pH 6.7.

1.2.3 Effect of the C/N ratio

A denitrification tank with pumice stone was used in this experiment. The carbon supplement was applied by adding methanol at various COD:nitrate-N ratios, including 3:1, 4:1, 5:1 and 6:1; the initial nitrate concentration was 100 mg-N/L. The experiment consisted of a control without carbon addition and treatments with methanol addition. Nitrate removal rate was determined from at least three repeated nitrate removal trials.

1.2.4 Nitrate removal from a laboratory scale recirculating fish culture system using the denitrification tank

Nitrate removal from a recirculating aquaculture system was performed using the denitrification tank under laboratory conditions. The fish (tilapia) culture experiment was carried out at the Center of Excellence for Marine Biotechnology, Department of Marine Science, Chulalongkorn University, Thailand. The initial fish density was 0.3 kg/m^3 and the culture duration was 3 months. Fish were fed with a commercial feed at 5% fish weight per day. The RAS, 450 L in water volume, was incorporated with a 160 L tank containing fibrous nitrification biofilter material (Biocord^{TM}) for ammonia treatment. Hence, with nitrification treatment, nitrate accumulated in the water along with fish cultivation. The denitrification tank was attached to the nitrification tank and the experiment was operated under a recirculating scheme without water exchange from an external source except for water addition to compensate for evaporation and water sampling. The major water treatment process was hence based on inorganic nitrogen removal by a combination of nitrification and denitrification processes.

The denitrification tank procedure followed the optimized conditions from the previous experiment, but scaled up to a plastic tank 80 cm in diameter, packed with pumice stone at a depth of 5 cm. The tank surface area was 0.5 m^2. The denitrification tank working water volume was 80 L. At the beginning, the nitrate removal efficiency of the denitrification tank was evaluated by adding synthetic wastewater containing 100 mg-N/L KNO_3. When the denitrification process occurred in the tank and nitrate concentrations decreased to lower than 5 mg-N/L, water was then replaced with fresh synthetic wastewater. Nitrate removal was repeated three times and the average nitrate removal rate was calculated.

During fish culture in the RAS, water containing a high nitrate concentration (e.g. more than 50 mg nitrate-N/L) from the RAS was applied to the denitrification tank. Nitrate removal was undertaken in batch mode in which water in the treatment tank was returned back to the fish culture system when the nitrate concentration decreased to lower than 5 mg-N/L.

1.3 Water analysis

Water samples were filtered through 0.45 μm Whatman GF/C filter papers and kept refrigerated prior to analysis. Collected samples were analyzed for ammonia-N, nitrite-N, nitrate-N, alkalinity and residual methanol. Ammonia and nitrite concentrations were analyzed using a colorimetric method. The nitrate concentration was measured with a spectrophotometric screening method (APHA, 1992), alkalinity was measured using a test kit (Aquatic Animal Medicine Division, Department of Veterinary Medicine, Chulalongkorn University, Thailand) and residual methanol in the water from the denitrification tank on the last day was analyzed by gas chromatography (GC-2010, Shimadzu, Japan). Other parameters, such as pH, dissolved oxygen and temperature were measured using a pH meter (HI98240, Hanna Instruments, USA), DO meter (HI964400, Hanna Instruments, USA) and thermometer, respectively.

2 Results and discussion

2.1 Effect of packing materials on nitrate removal in the denitrification tank

The denitrification tanks were packed with various substrates including soil (2.8 kg/tank), sand (3.5 kg/tank), pumice stone (1.0 kg/tank) and vermiculite (0.5 kg/tank), all providing a similar bottom substrate layer depth of 5 cm. The results from the one-month experiments with a nitrate concentration of 100 mg-N/L and methanol supplementation revealed that natural soil (control), sand and pumice stone could successfully remove nitrate via a denitrification process. As shown in **Fig. 2**, the nitrate concentration decreased from 100 mg-N/L to below 5 mg-N/L within 4.80 ± 0.51 and 5.03 ± 0.82 days for soil and pumice stone, respectively. A longer treatment period of 12.70 ± 0.58 days was required for the sand tank. The nitrate removal experiments were repeated several times. The denitrification tank with vermiculite, on the other hand, could not perform nitrate removal as the nitrate concentration remained at 60 mg-N/L until the end of the experiment.

Calculation of the nitrate removal rate (denitrification rate) showed that the average nitrate removal rates were 5383 ± 506, 2586 ± 169, and 3905 ± 95 mg-N/m^2 tank

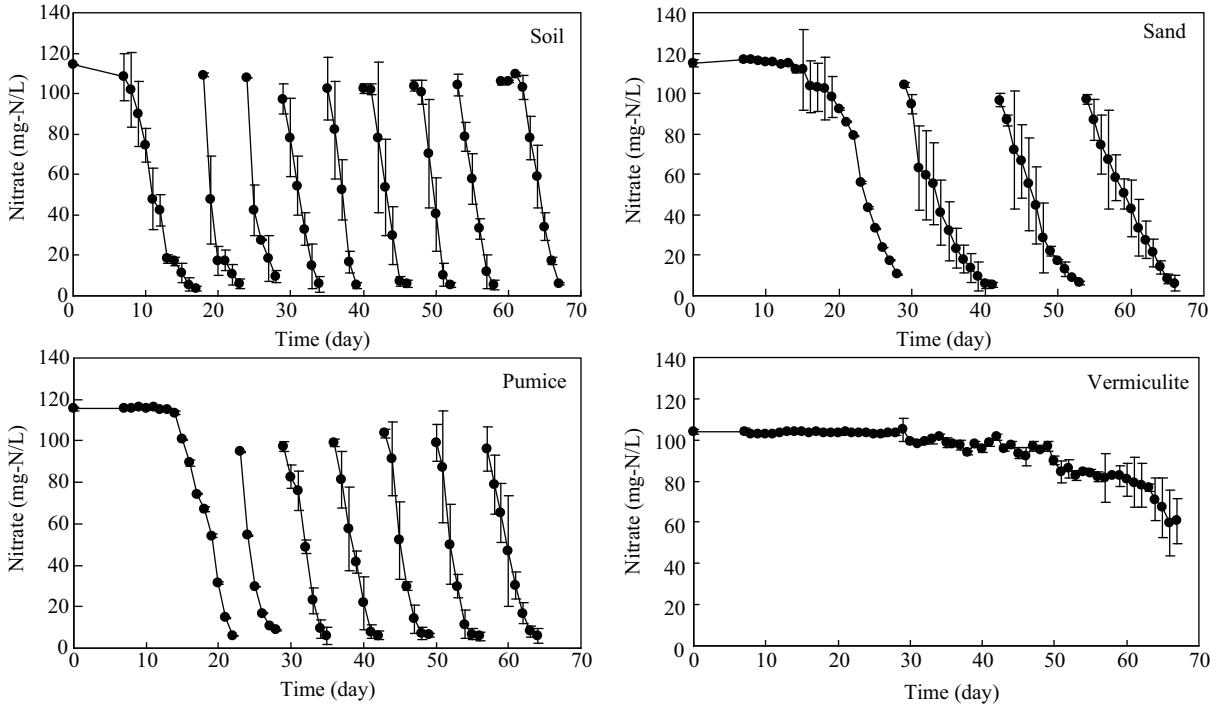

Fig. 2 Nitrate removal in denitrification tanks packed with soil (control) and treatment tanks with sand, pumice stone and vermiculite. The experiments were repeated by water exchange when the nitrate concentration fell below 10 mg-N/L.

bottom area/day for soil, sand and pumice, respectively. Denitrification was not successfully initiated in the vermiculite tank as the vermiculite particles were apparently light and loosely settled at the bottom. This enhanced oxygen dispersion in the vermiculite layer and impaired the anoxic conditions. ORP measurements in the bottom material layer revealed that the average ORP values in soil, sand and pumice stone were –283 ± 61, –209 ± 14, and –230 ± 9 mV, respectively. These ORP values confirmed the anoxic denitrification process in the bottom layer since a complete denitrification process results in an ORP less than –200 mV (Sillen, 1965). A dramatic decrease in the ORP value in the soil layer was found from day 59–61 of the experiment in which the ORP was lower than –350 mV (–420 to –375 mV). An ORP value below –400 mV indicates sulfate reduction (Sillen, 1965) accompanied with the bad odor of hydrogen sulfide. Since hydrogen sulfide is highly toxic to aquatic animals, this condition must be avoided, so methanol addition had to be stopped until the ORP value increased to more than –200 mV.

In general, denitrification is often seen in the bottom substrate such as the sand substrate of shrimp culture tanks (Sellars et al., 2005) and aquaria (Toonen and Wee, 2005). However, anoxic sulfate reduction to hydrogen sulfide imposes a substantially higher risk to the health of cultured animals than nitrate. The design of the denitrification treatment system proposed in this study minimized the chance of sulfate reduction by maintaining a high dissolved oxygen content in the water column. Of the four materials tested as the substrate in the denitrification tank, pumice stone was the best alternative for the denitrification substrate. Although the nitrate removal rate with pumice was slightly lower than that of natural soil, the advantages of using pumice stone are its high nitrate removal rate and low risk of hydrogen sulfide production. Moreover, pumice is a lightweight material so it can be easily transferred, transported and cleaned for use in a large denitrification tank.

2.2 Effect of organic carbon sources

Molasses has been popularly used as the carbon source to enhance microbial growth in aquaculture ponds, especially in Thailand. Molasses has also been used as the carbon source for denitrification filters in aquaculture (Hamlin et al., 2008). **Figure 3** shows that methanol and molasses at a COD:nitrate-N ratio of 5:1 could be used as the carbon source for denitrifying bacteria in the bottom pumice stone layer of the denitrification tank. The results illustrate that the nitrate concentration in the denitrification tank with methanol supplementation decreased from 100 mg-N/L to 6.19 ± 0.57 mg-N/L in 6 days, while molasses supplementation demonstrated lower efficiency as the final nitrate concentration was 28.82 ± 4.06 mg-N/L and the water color in the tank changed to dark brown (the color of molasses). The average denitrification rate of methanol supplementation was 4531 ± 186 mg-N/m^2 tank bottom area/day. Nitrite, the intermediate product of the denitrification process, was found in all denitrification tanks, but the concentration was lower than that of nitrate. The lower nitrite concentration with methanol treatment indicated the completeness of the denitrification process (Chiu and Chung, 2003; Saliling et al., 2007). Moreover,

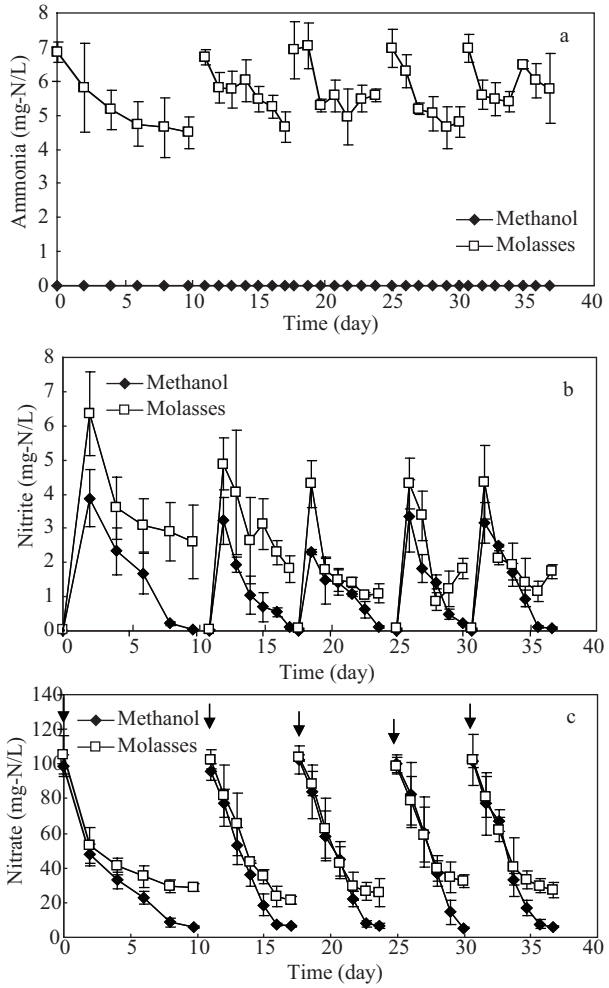

Fig. 3 Variation of ammonia (a), nitrite (b) and nitrate (c) during the carbon sources experiment. Arrows indicate methanol/molasses addition.

a high concentration of ammonia with an average of 5.09 ± 0.55 mg-N/L was found in the denitrification tank with molasses supplementation as a result of the high initial TKN content in molasses (11.46 ± 0.42 g/L). A similar high amount of ammonia derived from molasses addition was also reported by Hamlin et al. (2008). As the practical safe concentration of ammonia in aquaculture tanks is only 0.5 mg-N/L, this ammonia concentration was not acceptable for aquaculture purposes. Furthermore, it was found that molasses addition incurred a high risk of sulfate reduction in the pumice stone layer. This was indicated by the lower ORP values between –200 to –342 mV, while the ORP values with methanol treatment were between –161 to –258 mV. The production of hydrogen sulfide would result in a lower pH (pH 6.8) and alkalinity (110 mg as $CaCO_3$/L), which are not the optimal conditions to the complete the denitrification process (Lee et al., 2000). Hence, methanol was therefore chosen as the carbon source for further experiments.

2.3 Effect of the C/N ratio

The results in **Fig. 4** illustrate that average denitrification rates increased from 2505 ± 300 to 3684 ± 181, 4591 ± 133 and 7300 ± 797 mg-N/m^2 tank bottom area/day, respectively with an increase in the COD:N ratio from 3:1 to 4:1, 5:1 and 6:1. Nitrite and ammonia analysis showed a low concentration of both nitrogenous compounds in comparison with nitrate (data not shown), therefore it could be assumed that the denitrification process in all treatments was complete without intermediate compound residues. In general, excess methanol must be avoided in an aquaculture system. Kaviraj et al. (2004) reported that a methanol concentration greater than 47.49 mg/L (equivalent to 71.23 mg-COD/L) affects the growth and maturation of aquatic animals while a high concentration of methanol e.g. 1527.60 mg/L (2291 mg-COD/L) can induce acute toxic effects in fish. With this experiment, although the 6:1 ratio provided the highest denitrification rate, the ratio of 5:1 was finally chosen for further experiments because excess methanol (as COD) was detected in the outlet water of the denitrification tank with a 6:1 ratio. The results of the COD analysis showed that the average COD of the 6:1 ratio treatment was 63.01 ± 15.55 mg/L while the average COD for the 5:1 treatment was only 0.50 ± 0.10 mg/L.

2.4 Nitrate removal from a recirculating fish culture system using a denitrification tank

The recirculating aquaculture system included a fish (tilapia) tank connected to a nitrification tank. Water from the nitrification tank was withdrawn for nitrate treatment in the denitrification tank containing pumice stone. The experimental period was 99 days without water exchange. At the end of the culture period, the average fish weight increased from 0.44 to 5.86 g and the final density was 3.57 kg/m^3.

Water quality parameters in the denitrification tank during the experiment are shown in **Table 1**. It was noted that the influent water was retrieved from the nitrification biofilter tank, which resembled the water in the fish tank. It was found that, after 50 days of fish culture, the nitrate concentration had increased to a concentration of

Table 1 Average water quality parameters in the denitrification tank attached to the recirculating tilapia culture system

	Influent water[a]	Effluent water
Ammonia (mg-N/L)	0.17 ± 0.08	0.14 ± 0.09
Nitrite (mg-N/L)	0.03 ± 0.01	0.02 ± 0.01
Nitrate (mg-N/L)	45.77 ± 8.33	6.62 ± 0.70
Alkalinity (mg/L as $CaCO_3$)	165 ± 69.19	395 ± 19
pH	7.02 ± 0.34	7.93 ± 0.41
ORP[b] in pumice layer (mV)	–189.68 ± 22.27	
ORP[b] in water column (mV)	122.34 ± 16.04	

[a] Influent water was collected from the nitrification tank; [b] ORP was the average from day 51–99.

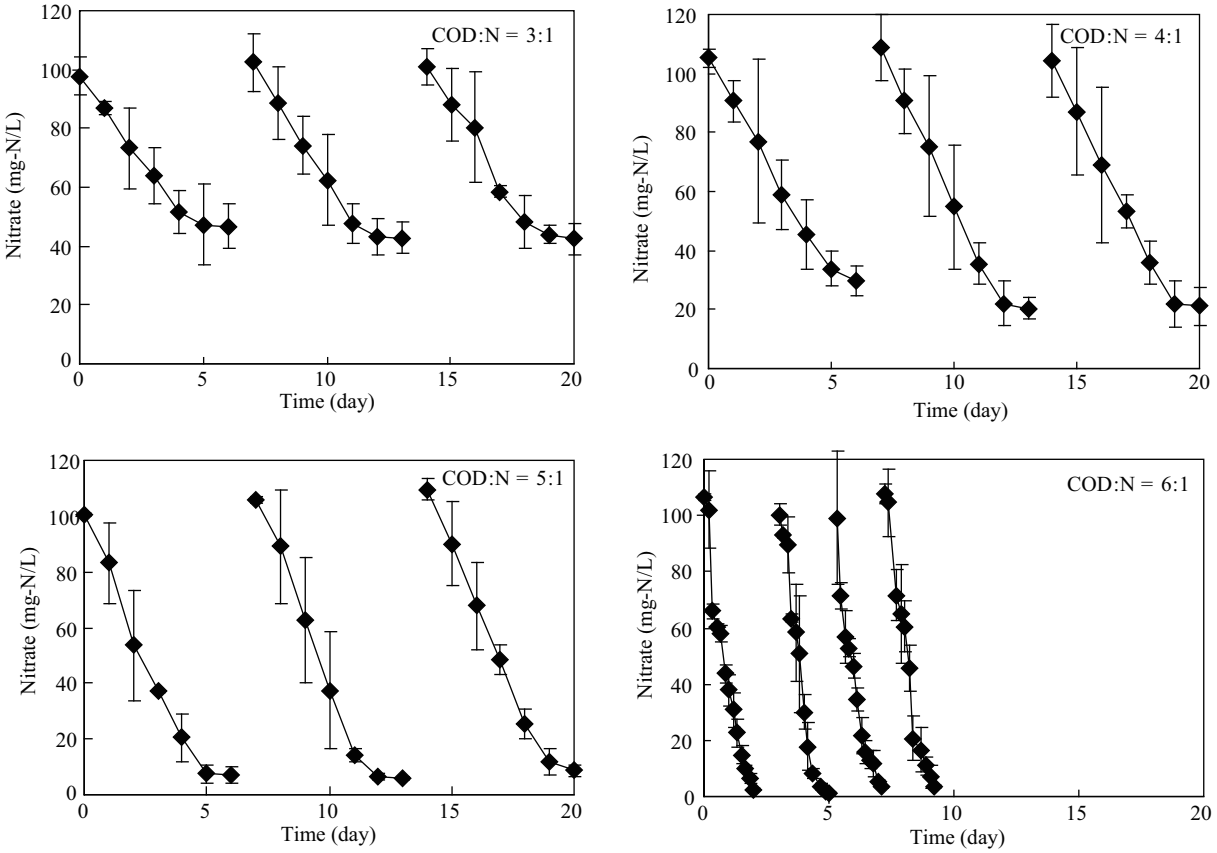

Fig. 4 Nitrate removal by the denitrification tank packed with pumice stone and supplemented with methanol at COD:N ratios from 3:1 to 6:1.

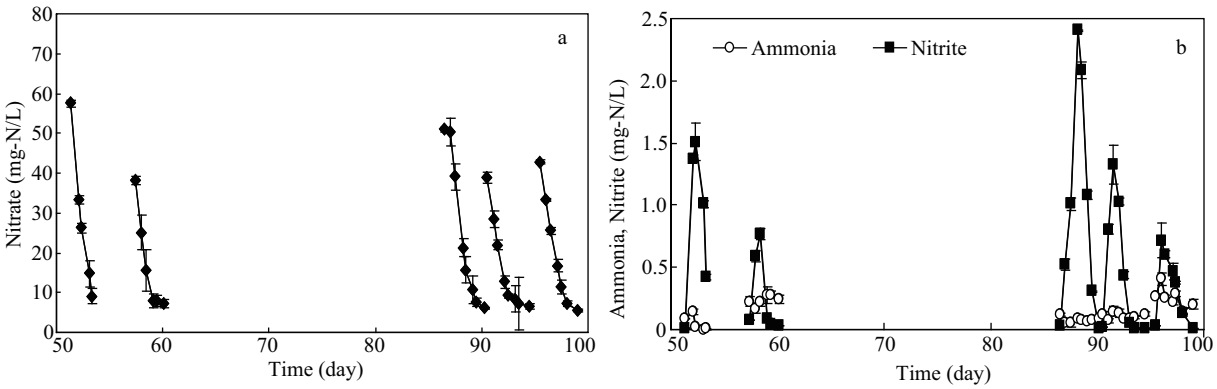

Fig. 5 Nitrate (a), ammonia and nitrite (b) in the 80 L denitrification tanks during five batches of nitrate removal from 610 L of the recirculating tilapia culture system.

56 mg-N/L (**Fig. 5**). Nitrate treatment was performed by transferring high nitrate water from the nitrification tank to the denitrification tank and mixing the water with methanol at a COD:N ratio of 5:1. After nitrate removal, water from the denitrification tank was transferred back to the nitrification tank. The results from five batches of nitrate treatment (**Fig. 5**) reveal that nitrate was successfully removed from the water without the accumulation of nitrite and ammonia. The average nitrate removal efficiency was 85.17% and the average denitrification rate of the denitrification tank was 6311 ± 945 mg-N/m^2 tank bottom area/day or 126 ± 18 mg-N/L of pumice packing volume/day.

The average ammonia concentration in denitrification tanks was low throughout the experiment (0.14 ± 0.09 mg-N/L), as shown in **Fig. 5b**. The average nitrite concentration in the effluent water (**Table 1** and **Fig. 5b**) was lower than 0.1 mg-N/L. As the concentration of nitrite was substantially lower compared to nitrate, it could be concluded that the denitrification process in the tanks was complete. In fact, nitrite in the effluent must be avoided because nitrite is toxic to aquatic animals, leading to methemoglobinemia or brown blood disease in fish (Boyd and Tucker, 1998). The residual methanol in the water of the denitrification tank on the final day was 5.16 ± 0.17

mg/L. Although the residual methanol was still low, a high concentration of methanol must be avoided since it could affect the growth, maturity index and fecundity of fish.

As illustrated in **Table 1**, ORP, pH and alkalinity can be used as process control parameters for nitrate removal in a denitrification tank. The ORP in the bottom pumice layer, between –120 to –230 mV, indicated that nitrate and nitrite reduction occurred and the denitrification process was rather complete. This was due to the fact that if there was substantial sulfate reduction in pumice layer, the ORP would decrease to below –300 or –400 mV, followed by hydrogen sulfide production (Sillen, 1965; Menasveta et al., 2001). The production of hydrogen sulfide would result in lower pH and alkalinity. The denitrification process, on the other hand, increases the pH and regenerates alkalinity (Kim and Bae, 2000; Ghafari et al., 2010). The increase in pH and alkalinity in **Table 1** also confirmed the occurrence of the denitrification process. In recirculating aquaculture systems, the aerobic nitrification process leads to an alkalinity loss and a resulting pH decline in the water. Alkalinity supplements such as sodium bicarbonate are generally used to stabilize pH and alkalinity. The anaerobic denitrification process, in contrast, returns alkalinity to the water, hence it reduces the need for alkalinity supplementation in nitrification biofilters.

3 Conclusions

This study illustrates the possibility of a nitrate treatment system for small-scale closed recirculating aquaculture systems using a denitrification tank. Of the four bottom packing materials tested, i.e. natural soil, sand, pumice stone and vermiculite, pumice stone was chosen as it provided a high denitrification rate with a low risk of sulfate reduction. The use of pumice stone as the filtration material at the bottom of the denitrification tank coupled with methanol as the carbon source accelerated the nitrate removal rate to 6311 ± 945 mg-N/m^2 tank bottom area/day or 126 ± 18 mg-N/L of pumice packing volume/day. The denitrification tank coupled with the tilapia RAS showed good performance in terms of nitrate removal throughout the 99-day experimental period with a nitrate removal efficiency of 85.17%. It was found that the reused water from the denitrification tank imposed no harm on the fish and water exchange could be kept at a minimal rate.

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