



Nitrogen removal from a recirculating aquaculture system using a pumice bottom substrate nitrification-denitrification tank



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ARTICLE INFO

Article history:

Received 9 September 2015

Received in revised form 16 June 2016

Accepted 20 June 2016

Available online 4 July 2016

Keywords:

Denitrification

Nitrification

Nitrogen treatment

Recirculating aquaculture systems

Pumice bottom substrate tank

ABSTRACT

This research investigated the efficiency of a pumice stone biofilter tank for nitrogen removal from a recirculating aquaculture system. The pumice bottom substrate nitrification-denitrification tank was a glass tank packed with a 5 cm depth of pumice stone (approximately 3 mm in diameter) at the bottom. It was found that the pumice stone could perform as a nitrification biofilter under aerobic conditions. When applying methanol as the external carbon source at a COD:N ratio of 5:1 and then covering the tank with a plastic sheet to reduce gas exchange, pumice stone could remove nitrate through denitrification. Thereafter, nitrification and denitrification treatments using the pumice tank were applied to a 100 L moderate density (10 kg m⁻³) recirculating aquaculture system (RAS) under laboratory conditions. The RAS consisted of a 100 L tilapia culture tank connected to a 100 L pumice tank packed with a 5 cm layer of pumice stone. It was found that the nitrification treatment performed by the pumice tank could control ammonia and nitrite concentrations within the required safety range throughout the 121-day culture period. When nitrate accumulated to approximately 50 mg-N L⁻¹ in water, water recirculation was paused, after which batch denitrification treatment was performed by adding methanol at COD:N of 5:1 in the pumice tank. With nitrification-denitrification treatment, ammonia and nitrite concentrations were below 1 mg-N L⁻¹ and nitrate was kept below 50 mg-N L⁻¹ while nitrate in the control tank was as high as 352.47 ± 9.67 mg-N L⁻¹. Moreover, the pumice bottom substrate tank with methanol supplement had no negative effect on growth and survival of fish in the recirculating system.

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1. Introduction

Recirculating aquaculture systems (RAS) have been developed to serve sustainable, environmentally friendly aquaculture. Nitrification is the major treatment process in RAS to remove ammonia and nitrite, which are toxic to aquatic animals. With aerobic nitrification, ammonium is converted to nitrite and finally to the lower toxic nitrate; thus, the water can be reused for a longer period (Gutierrez-Wing and Malone 2006; Crab et al., 2007; van Rijn 2013). The accumulation of nitrate derived from nitrification treatment is a common problem in most RAS. The use of anaerobic denitrification to remove nitrate, in contrast, is not yet widely applied in commercial RAS due to its level of efficiency, complexity and cost

(Menasvata et al., 2001; Singer et al., 2008; Christianson et al., 2015; Zhu et al., 2015).

In an outdoor earthen pond, the major portion of nitrogen waste is treated naturally by nitrification and denitrification processes that take place in the bottom sediment (Kutako et al., 2009). If fish density is within the carrying capacity of the natural processes in the pond (fish density < 1 kg m⁻³), nitrogen can be completely converted to nitrogen gas and finally discarded. Hence, ammonia, nitrite and nitrate in earthen ponds are usually found in low concentrations. Indoor RAS, on the other hand, require an effective waste treatment system to maintain good water quality. One of the bottle necks of RAS technology remains construction cost. Typical RAS are developed to serve high density aquatic animal cultivation due to the economy of scale. With its low cost, moderate fish density (approximately 10 kg m⁻³) RAS with appropriate water treatment technology is required by small and medium size fish farms, including fish broodstock cultures, fish disease quarantine tanks and fish larviculture, but the technology is not yet widely used.

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In a previous experiment with pumice bottom substrate denitrification tank (Pungrasmi et al., 2013), nitrate was successfully removed from a laboratory scale RAS. The denitrification process in the pumice layer was enhanced by external carbon (methanol) addition. Furthermore, preliminary results from this study illustrated nitrification activity in the pumice substrate after an appropriate acclimation period. Hence, it is possible to combine nitrification and denitrification treatments in a single treatment tank. The study on nitrification and denitrification process (SND) in a moving bed biofilm reactor by Chu and Wang (2011) had a problem with oxygen control for simultaneously aerobic (nitrification) and anaerobic (denitrification) processes in the reactor. On the other hand, while a sequencing batch reactor (SBR) could successfully remove nitrogen by nitrification and denitrification reactions in a single reactor (Kern and Boopathy 2012), SBR operation appeared to be only suitable for a low volume of wastewater. It was also quite complicated to operate with the RAS under farm conditions. Moreover, as nitrification is the major nitrogen treatment process in the RAS, a continuous denitrification treatment is not necessary as the denitrification is needed only when nitrate reaches the control level (e.g. 50 mg-NL^{-1}). Therefore, this present study aimed at evaluating the possibility of using pumice bottom substrate for ammonia removal by nitrification treatment and nitrate removal by denitrification treatment from a recirculating aquaculture system. The experiment consisted of two phases. The first phase was an evaluation of nitrification and denitrification activities of pumice stone while the second phase evaluated the use of a pumice bottom substrate tank for nitrogen removal from recirculation in a Tilapia culture tank during an operating period of 121 days.

2. Material and methods

2.1. Determination of ammonia and nitrate removal rate of pumice stone

Pumice stone used in this study was porous volcanic rock imported from Lombok, Indonesia. The pumice, approximately 3 mm in diameter, was washed in tap water and dried in an oven prior to use. Acclimation of nitrifying bacteria on pumice stone was performed by submerging it in a 100 L water plastic tank with 15 mg-NL^{-1} of ammonium chloride. Aquaculture feed (frog diets) containing 40% protein at a concentration equivalent to 1.5 mg-NL^{-1} was added in the acclimating tank to provide additional nutrients and vitamins to the nitrifying bacteria (Sesuk et al., 2009).

Continual aeration was applied to maintain dissolved oxygen at higher than 4 mgL^{-1} , and alkalinity was kept between 100 and 150 mgL^{-1} as CaCO_3 by adding sodium bicarbonate. This acclimation was operated for at least 45 days to ensure nitrification of bacteria. Changes in inorganic nitrogen compounds, i.e. ammonia, nitrite and nitrate, were regularly monitored, and ammonium chloride was added when ammonium concentration reduced to lower than 1 mg-NL^{-1} . After acclimation, nitrification of the pumice stone was evaluated by packing 2000 mL in volume of pumice stone in a $20 \times 20 \times 35 \text{ cm}$ glass tank with continuous aeration. Five liters of synthetic wastewater containing 1 mg-NL^{-1} ammonium chloride and 150 mgL^{-1} alkalinity were added to the tank. Reduction of ammonium concentration due to nitrification was monitored every hour until an ammonium concentration was undetectable. The previous study mentioned earlier (Pungrasmi et al., 2013) showed that pumice stone in a denitrification tank with methanol supplement had a high capability of nitrate removal by denitrification. With this study, denitrification activity of pumice stone was measured by packing a 5 cm layer of pumice stone in a $20 \times 20 \text{ cm}^2$ glass tank (Fig. 1) filled with 8 L of synthetic wastewater containing 100 mg-

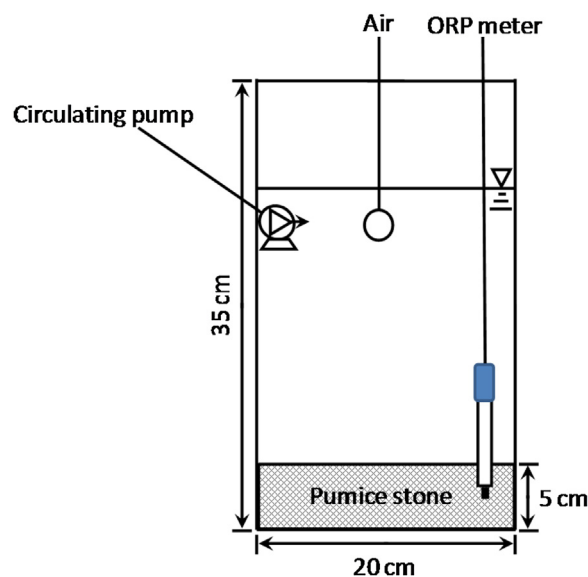


Fig. 1. Instrumental set-up for combined nitrification-denitrification reactor. Gentle water movement in the reactor is generated by small circulating pump located near the water surface. Aeration is provided by airstone during nitrification treatment.

NL^{-1} potassium nitrate. To accelerate the denitrification process, an organic carbon supplement was provided by an addition of methanol at a COD:Nitrate-N ratio of 5:1. A small aquarium pump located near the water surface was used to circulate the water in the tank. With this setup, water in the upper layer still contained a moderate to low concentration of oxygen, whereas the anaerobic condition occurred only in the pumice layer. This condition allowed the simultaneous treatment of nitrification on the surface of the bottom substrate and denitrification below the surface of the substrate layer. Oxidation-Reduction Potential (ORP) in water and pumice stone (2.5 cm depth) was measured daily using an ORP probe. In addition, water samples were taken daily for nitrate analysis in order to monitor the nitrate removal rate.

2.2. Evaluation of simultaneous nitrification and denitrification activities of a pumice stone reactor

The experiment was conducted with 8 L glass reactor tanks packed with 5 cm of pumice stone and approximately 23 cm water depth as described in the previous experiments. To simulate the operating conditions for simultaneous nitrification-denitrification, 20 mg-NL^{-1} of ammonium chloride was added to the reactors as an ammonia supply. Thereafter, methanol at a COD:Nitrate-N ratio of 5:1 was added into the reactors to serve as an external carbon source, and the reactor tanks were covered with a plastic sheet to reduce gas exchange. This condition provided low oxygen with high organic carbon concentration in the tank, even though it is known that organic carbon (e.g. methanol) and low oxygen can inhibit nitrification process. The experiment was then conducted with two treatments. The first treatment (treatment-1) was the alternation of ammonia and methanol addition. With this treatment, only ammonia was added into the pumice reactor at the beginning. Total ammonia nitrogen (TAN) and nitrate in the water were monitored until ammonia depleted and nitrate was at the highest concentration. Then, methanol at the COD:N ratio of 5:1 was added to stimulate the denitrification process. The second treatment (treatment-2), on the other hand, was operated with ammonia added to the methanol in the pumice reactor. When nitrate accumulation reached the maximum concentration, water circulation was turned off and methanol at the COD:N ratio of 5:1 was added to accelerate nitrate removal. When nitrate was

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