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Activated sludge denitrification in marine recirculating aquaculture system effluent using external and internal carbon sources

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Highlights

- FBR is a viable option for end-of-pipe removal of nitrate-N
- Among the five carbon sources tested, acetate sustained the highest N removal rate
- A volume of 33 m³ is needed to remove 39 kg N/d in an acetate-fed reactor
- Using ethanol as a carbon source can cause sulfide production
- Using fish organic waste in N end-of-pipe removal will save operational costs

Abstract

Stringent environmental legislation in Europe, especially in the Baltic Sea area, limits the discharge of nutrients to natural water bodies, limiting the aquaculture production in the region. Therefore, cost-efficient end-of-pipe treatment technologies to reduce nitrogen (N) discharge are required for the sustainable growth of marine land-based RAS. The following study examined the potential of fed batch reactors (FBR) in treating saline RAS effluents, aiming to define optimal operational conditions and evaluate the activated sludge denitrification capacity using external (acetate, propionate and ethanol) and internal carbon sources (RAS fish organic waste (FOW) and RAS fermented fish organic waste (FFOW)). The results show that between the evaluated operation cycle times (2, 4, and 6 h), the highest nitrate/nitrite removal rate was achieved at an operation cycle time of 2 h (corresponding to a hydraulic retention time of 2.5 h) when acetate was used as a carbon source. The specific denitrification rates were 98.7 ± 3.4 mg NO_3^- -N/(h g biomass) and 93.2 ± 13.6 mg NO_x^- -N/(h g biomass), with a resulting volumetric denitrification capacity of 1.20 kg NO_3^- -N/(m³ reactor d). The usage of external and internal carbon sources at an operation cycle time of 4 h demonstrated that acetate had the highest nitrate removal rate (57.6 ± 6.6 mg N/(h g biomass)), followed by propionate (37.5 ± 6.3 mg NO_3^- -N/(h g biomass)), ethanol (25.5 ± 6.0 mg NO_3^- -N/(h g biomass)) and internal carbon sources (7.7 ± 1.6 to 14.1 ± 2.2 mg NO_3^- -N/(h g biomass)). No TAN (Total Ammonia Nitrogen) or PO_4^{3-} accumulation was observed in the effluent when using the external carbon sources, while 0.9 ± 0.5 mg TAN/L and 3.9 ± 1.5 mg PO_4^{3-} -P/L was found in the effluent when using the FOW, and 8.1 ± 0.7 mg TAN/L and 7.3 ± 0.9 mg PO_4^{3-} -P/L when using FFOW. Average sulfide concentrations varied between 0.002 and 0.008 mg S^{2-} /L when using the acetate, propionate and FOW, while using ethanol resulted in the accumulation of sulfide (0.26 ± 0.17 mg S^{2-} /L). Altogether, it was demonstrated that FBR has a great potential for end-of-pipe denitrification in marine land-based RAS, with a reliable operation and a reduced reactor volume as compared to the other available technologies. Using acetate, the required reactor volume is less than half of what is needed for other evaluated carbon sources, due to the higher denitrification rate achieved. Additionally, combined use of both internal and external carbon sources would further reduce the operational carbon cost.

Keywords: carbon source; denitrification; end-of-pipe; fed batch reactor (FBR); marine land-based; recirculating aquaculture system (RAS).

1. Introduction

Environmental control and increased biosecurity concerns have incentivized the construction of land-based recirculation aquaculture systems (RAS) for smolt phases and grow-out cycles of marine fishes (Dalsgaard et al., 2013; Martins et al., 2010). The number of production units with the capacities of 1,000 up to 90,000 tons of fish/y is increasing (Dalsgaard, 2017). However, implementing the European Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD) forces aquaculture industry to reduce nutrient discharge into natural water bodies. Thus, cost-efficient end-of-pipe treatment technologies are needed to reduce nitrogen (N) in saline effluent discharges (Borja et al., 2010; WFD, 2000).

In order to increase water reuse and avoid toxic conditions for fish in RAS, biofilters with nitrifying microbes oxidize ammonium (NH_4^+) into nitrate (NO_3^-), which accumulates and needs to be removed before RAS effluents are discharged into the environment (Hamlin et al., 2008; Suhr et al., 2013; van Rijn et al., 2006). Heterotrophic denitrification, the main NO_3^- removal technology applied in RAS, is a sequential process with four enzymatic steps, including reduction of nitrate to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and finally, to nitrogen gas (N_2) (Henze et al., 1997). Denitrifying bacteria use a wide spectrum of organic carbon sources that can be obtained either commercially (external carbon sources) or from RAS effluent (internal carbon sources). Methanol and ethanol are the common choices of external carbon source for RAS (van Rijn et al., 2006). However, being reactive flammable alcohols, they require special standards for transport, storage, packaging, handling, and disposal (European Parliament and the Council of the European Union, 2008), which causes additional capital costs (Cherchi et al., 2009). Alternative carbon sources, such as volatile fatty acids (VFA) (e.g. acetate, propionate, butyrate), obtained either commercially or produced from the fermentation of fish

waste offer a promising approach for an effective reduction in the operational costs of end-of-pipe treatment (Letelier-Gordo et al., 2017; van Rijn et al., 1995).

Media-laden reactors are the most popular technology in aquaculture denitrification in brackish or marine water conditions (Balderston and Sieburth, 1976; Grguric et al., 2000; Gutierrez-Wing et al., 2012; Honda et al., 1993). However, channeling, clogging, and increased pressure drops due to the organic matter accumulation are common, when media-laden reactors are used for the denitrification process (Balderston and Sieburth, 1976; Müller-Belecke et al., 2013; Sauthier et al., 1998). This might reduce the effective denitrification capacity of the reactor, requiring frequent back-washing in order to sustain continuous operation. Organic matter accumulation is especially problematic in marine systems, since the anaerobic conditions will promote the production of toxic hydrogen sulfide (H_2S) (Letelier-Gordo et al., 2020). Therefore, alternative solutions, with simple construction and operation, high denitrification rates, and a low footprint, for media-laden denitrification reactors are required in marine land-based facilities.

The use of bacterial flocs or flocculent bacteria (e.g. activated sludge and granules) is globally the most common treatment technology for biological nitrogen (N) and organic matter removal in municipal and industrial wastewater treatment (Henze et al., 1997, 2008; Tchobanoglous et al., 2002). Unlike media-laden reactors, flocculent bacteria live suspended inside the reactors without the need for plastic carrier elements. Depending on the type of flocs developed, high denitrification rates can be achieved (12-14 kg NO_3^- -N/m³ reactor d) (Klapwijk et al., 1981; Letelier-Gordo and Martin Herreros, 2019).

A fed batch reactor (FBR) has a simple fill-and-draw setup (a cycle of fill, react, settle, and discharge) with a small footprint and is easy to construct and operate in e.g. an existing buffer tank. It has a long reliable operation age and a strong tolerance towards variations in the flow and substrate (Strous et al., 1998). In municipal wastewater treatment, FBR has been broadly studied (Wang et al., 2010), but the applicability of FBR for N removal in marine RAS, or in aquaculture in general, is not currently known.

To evaluate the applicability of this technology for RAS, the main objectives of this study were to: 1) define the best reaction time for denitrification using fed batch reactor and 2) measure the activated sludge denitrification capacity using external (acetate, propionate, and ethanol) and internal carbons sources (RAS fish waste and RAS fermented fish waste).

2. Materials and methods

2.1 Experimental setup

The experimental work was divided into two phases. In phase I, the effect of different FBR operational cycle times (OCT; 2, 4, and 6 h) on the denitrification rate was studied, using acetate as a carbon source. In phase II, the denitrification capacity of the FBR was evaluated using different external and internal carbon sources.

2.2 Reactors

The FBR system consisted of six 10 L glass bottles (ISO 4796, PYREX, USA), where the filling and discharge volume was 8 L (80% of volume reactor), and 2 L were left for bacteria settlement (20% of reactor volume) (Fig. 1). Each reactor had a rubber plug with 4 tubes: a substrate inlet, a supernatant outlet, a gas outlet, and a sampling tube. Each reactor had 2 pumps (WT600-2J, LongerPump, China), one for feeding the reactors with the substrate and one for discharging the supernatant (8 L). The reactors were mixed with magnetic stirrers (VS-C10, VWR, USA; C-MAG MS7, IKA, Germany) at a rotation speed of 100 rpm. Stirring was off during the settle and discharge steps.

2.3 FBR operation

Each FBR had an operation cycle including 4 steps: fill, react, settle, and discharge. The different evaluated operational cycles (2, 4 and 6 h) were controlled by three timers (TS-MNN4, E-line, China), two for the aforementioned pumps and one for the stirrers.

2.4 Aquaculture wastewater and formation of activated sludge

In both phases, the FBRs were fed with seawater (35 ppt, 15 ± 2 °C) with a concentration of $50 \text{ mg NO}_3^- \text{-N/L}$ reached by adding NaNO_3 (Sodium nitrate, VWR, USA). Before starting phase I, the denitrifying flocculent sludge was formed by using fish organic waste obtained from swirl separators of an experimental rainbow trout (*Oncorhynchus mykiss*) marine RAS (salinity 25 ppt). The FBRs were each filled with 5 L of organic waste at a COD (Chemical Oxygen Demand) concentration of 10 g/L and 5 L of marine water (salinity of 35 ppt), and were fed with sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$, >99%, GPR RECTAPUR®, VWR, USA) and sodium nitrate (50 mg/L of $\text{NO}_3^- \text{-N}$, 1.5 g of NaNO_3 , >99%, Acros Organics, USA) at a COD: $\text{NO}_3^- \text{-N}$ ratio of 6. The FBRs were operated with an OCT of one day. Once the sludge was conditioned and a clear separation of bacteria with good settling properties was achieved, the bacterial biomass was collected and used to fill the 6 FBRs for phase I evaluation.

2.5 Phase I: Defining optimal Operational Cycle Time

To investigate the optimal FBR reaction time, three different OCTs (2, 4, and 6 h) were tested (Table 1). Each reactor was evaluated twice on the condition ($n=4$ for each OCT) with one week of acclimatization time between the operational cycles. The wastewater was prepared as described in the section 2.4, and COD/ $\text{NO}_3^- \text{-N}$ ratios in all treatments were maintained at 6 (300 mg COD/L and $50 \text{ mg NO}_3^- \text{-N/L}$), which has been described as adequate for the denitrification process (Van Rijn et al., 2006; Letelier-Gordo et al., 2015). One hour prior to the new daily cycle, the carbon source and nitrate were added into a fully mixed holding tank (Figure 1).

2.5.1 Phase II: Effect of carbon source on denitrification rate

Based on the results obtained during phase I, the operational cycle was set to 4 h, consisting of 4 steps: fill (15 min), react (180 min), settle (30 min), and discharge (15 min), in order to compare the denitrification capacity using different carbon sources. The external carbon sources used were acetate (sodium acetate, $\text{C}_2\text{H}_3\text{NaO}_2$, >99%, VWR, USA), propionate (sodium propionate, $\text{C}_3\text{H}_5\text{NaO}_2$, >99%,

Alfa Aesar, USA), and ethanol (C_2H_5OH , >70%, VWR, USA), and the internal carbon sources used were fish organic waste (FOW) and fermented fish organic waste (FFOW) maintaining COD/NO₃-N ratio at 6. Fish organic waste was collected in swirl separators of a RAS rearing rainbow trout (*Oncorhynchus mykiss*) fed 1% of their biomass/day (EFICO ENVIRO 920 – 4.5 mm, BioMar, Denmark). Any un-eaten pellets were removed from the fish waste prior to collection. The FFOw was prepared by fermenting the FOW for 4 days at room temperature (15 ± 2 °C) in a 40 L open tank.

2.6 Sample collection and analysis

Samples for water quality in phase I were taken according to Table 1. In phase II, the sampling was done every 30 min following OCT of 4 h (Table 1). All samples were taken during the reaction step of the FBR.

For total chemical oxygen demand (TCOD) analysis, 100 mL of FBR sludge was sampled and preserved with 1% v/v sulfuric acid (4 mol/L H₂SO₄, Merck Millipore, Germany). For VFAs, ethanol, and soluble chemical oxygen demand (sCOD), FBR sludge samples were centrifuged at 4,500 rpm at 5°C for 15 min and filtered through 0.45 µm and 0.20 µm syringe filters (Filtropur S, SARSTEDT, Germany). Filtered samples for sCOD, VFAs, and ethanol were subsequently preserved by adding 1% v/v sulfuric acid. All the samples were maintained at 4°C until analysis. Dissolved sulfide samples were precipitated and fixed as zinc sulfide (ZnS) using a 10% zinc acetate solution, and the absorbance of the produced methylene blue was measured immediately at 665 nm with a spectrophotometer (Beckman DU 530, USA) as in Letelier-Gordo et al., (2020).

2.7 Analytical methods

VFAs were determined using 881 Compact IC Pro (Metrohm, Switzerland) with 887 Professional UV/VIS Detector (Metrohm, Switzerland). The mobile phase lasted 1h with a flow rate of 0.25 ml/min at 35°C. The system was able to detect concentration ranges from 1 to 100 mg/L for acetate, propionate, formate, butyrate, and valerate at 8 × dilution. Ethanol concentration was determined using a

“Megazyme Ethanol Assay Procedure” (K-ETOH 01/14, Megazyme International Ireland Ltd, Ireland) and measured at 340 nm using a HACH Lange spectrophotometer (DR2800, HACH Lange, Germany). Nitrate (NO_3^-), nitrite (NO_2^-), and orthophosphate (PO_4^{3-}) were determined by 930 Compact IC Flex system (Metrohm, Switzerland) with 887 Professional UV/VIS Detector (Metrohm, Switzerland). The mobile phase lasted 30 min with a flow rate of 0.9 ml/min at 45°C. Total ammonia nitrogen (TAN) was analyzed according to Danish standard method (DS 224, 1975). Oxygen and pH were monitored in the influent tank with Hach HQ40d equipment (Hach Lange GmbH, Düsseldorf, Germany). TCOD and sCOD were measured by digestion vials (LCK 314, 514, and 1414, Hach Lange, Germany). The particulate COD (xCOD, calculated as TCOD - sCOD) was used as a proxy for activated sludge biomass (Henze et al., 2008).

Nitrate reduction represents the first step of denitrification (nitrate removal), while the reduction of NO_x ($\text{NO}_3^- + \text{NO}_2^-$) includes the further nitrite reduction step of denitrification. Specific denitrification rates (SDNR; mg NO_x /(h g biomass)) were calculated by nitrate and nitrite reduction rates (the linear parts of the curve versus time) standardized by the biomass of activated sludge (xCOD) Eq. (1). Volumetric denitrification capacity (VDN; kg NO_x /(m^3 reactor d)) was calculated by multiplying the SDNR by the activated sludge biomass in reactor Eq. (2).

$$\text{Eq. (1) } SDNR = \frac{NOx \text{ influent (mg/L)} - NOx \text{ effluent (mg/L)}}{\text{time (h)} \times \text{Activated sludge biomass (g/L)}}$$

$$\text{Eq. (2) } VDN = SDNR * \text{bacteria in reactor (g/m}^3) \times 24 \text{ h/d} \times 10^6 \text{ mg/kg}$$

2.8 Data analysis

The differences in the specific denitrification rates within different operational times and carbon sources were examined using one-way ANOVA analysis followed by Tukey-Kramer multi comparison of means test with 95% family-wise confidence level. The statistical analysis was done with R (version 3.5.1; R Core Team 2016). All values are presented as the mean \pm standard deviation (SD).

3 Results and discussion

3.1 Operational conditions in FBR (phase I)

The OCT significantly affected both nitrate (ANOVA, $F_{2,9} = 46.8$, $P < 0.001$) and nitrite ($F_{2,9} = 25.2$, $P < 0.001$) removal rates, when the activated sludge was fed with acetate. The highest denitrification rates (98.7 ± 3.4 mg NO_3^- -N/(h g biomass); 93.2 ± 13.6 mg NO_x^- -N/(h g biomass)) were obtained on 2 h cycle conditions. Denitrification rates decreased as the operational time increased to 6 h (Fig. 2), but there was no significant difference in NO_x^- removal rates (pairwise comparisons, $P > 0.05$) between 4 h and 6 h cycle tests. Due to the periodical batch characteristic of FBR, the volumetric denitrification rates in 2, 4, and 6 h cycles resulted in 1.2, 0.8, and 0.4 kg N/(m³ reactor volume d), respectively. Even though an OCT of 2 h resulted in the highest N mass removed on a daily basis, 4 h OCT was chosen as the operating cycle condition for phase II to allow different carbon sources to have adequate reaction time for denitrification (Elefsiniotis et al., 2004; Xu, 1996).

3.2 Effects of carbon source on denitrification rates (Phase II)

The removal rates of nitrate and nitrite varied significantly with the carbon source added (ANOVA, nitrate: $F_{4,15} = 62.6$, $P < 0.001$; nitrite: $F_{4,15} = 84.0$, $P < 0.001$; Fig. 3). Using acetate as the carbon source resulted in the highest denitrification rates (57.6 ± 6.6 mg NO_3^- -N/(h g biomass) and 50.5 ± 7.8 mg NO_x^- -N/(h g biomass)), followed by propionate (37.6 ± 6.3 mg NO_3^- -N/(h g biomass) and 28.5 ± 2.7 mg NO_x^- -N/(h g biomass); Fig. 3). With ethanol, the nitrate removal rate was even lower (25.5 ± 6.0 mg NO_3^- -N/(h g biomass)), being approximately half of the one obtained with acetate. Nitrite removal rates, however, were not significantly different between ethanol (21.4 ± 6.2 mg NO_x^- -N/(h g biomass)) and propionate (Fig. 3). The nitrate and nitrite removal rates obtained using FFOW were 14.1 ± 2.2 NO_3^- -N/(h g biomass) and 12.6 ± 2.0 mg NO_x^- -N/(h g biomass), both rates being approximately two times higher than the ones obtained using FOW (7.7 ± 1.6 and 6.4 ± 0.5 mg N/(h g biomass), respectively) (Fig. 3).

The highest denitrification rate obtained with acetate can be explained by it forming Acetyl-CoA, which can be directly used by the bacteria in the tricarboxylic acid cycle (TCA) to generate energy, whereas the biodegradation pathway of propionate is more complicated, including a series of enzymatic carboxylations and enzymatic epimerizations (Xu, 1996). Furthermore, ethanol requires an extra step of acidification in the mitochondria slowing down the whole process. It can, however, be utilized in multiple alternative pathways in TCA, explaining why propionate was only 3.7% better than ethanol despite the extra acidification step needed (Fig. 3). The immediate response to ethanol was probably due to the presence of alcohol dehydrogenase enzymes, converting ethanol into acetate and further into Acetyl-CoA. Therefore, acetate is a better candidate for faster denitrification rates.

3.3 Effluent quality

To estimate the overall potential FBR discharge, we measured the concentrations of TAN and phosphate (PO_4^{3-}) in the reactor effluents. No TAN or PO_4^{3-} accumulation was observed in the effluent when using the external carbon sources. However, 0.9 ± 0.5 mg TAN/L and 3.9 ± 1.5 mg PO_4^{3-} -P/L were found in the effluent when using FOW, and 8.1 ± 0.7 mg TAN/L and 7.3 ± 0.9 mg PO_4^{3-} -P/L when using FFOW.

Already the initial concentrations of TAN and PO_4^{3-} were high in the FFOW reactors (8.9 ± 0.8 mg TAN/L; 7.6 ± 0.5 mg PO_4^{3-} -P/L), which can be explained by the accumulation during the initial fermentation process (Conroy and Couturier, 2010; Letelier-Gordo et al., 2015). Both TAN and PO_4^{3-} were slightly reduced in the FFOW reactors (-0.8 ± 0.4 mg TAN/L; -0.3 ± 0.4 mg PO_4^{3-} -P/L), probably due to the consumption by the growing microbial biomass. Indeed, both TAN and PO_4^{3-} are essential for microbial growth, and a proportional replacement of external carbon sources with FFOW may enhance the bacterial yield and community diversity without promoting TAN or PO_4^{3-} production.

The sulfide production in the FBRs was measured, since the high concentration of SO_4^{2-} in seawater increases the probability of H_2S production during denitrification, in particular if the more favorable electron acceptors, nitrate and nitrite, are depleted. There was no significant difference in the influent sulfide concentrations ($P > 0.1$) between carbon sources applied, the average being between 0.005 to

0.007 mg S²⁻/L for external carbon groups, 0.017 mg S²⁻/L for FFOW, and 0.012 mg S²⁻/L for FOW. Sulfide accumulation (0.26±0.17 mg S²⁻/L) was observed using ethanol, while for the other C-sources, fluctuations with no specific pattern were observed. The average sulfide concentration ranged from 0.002 to 0.008 mg S²⁻/L in acetate, propionate, and FOW reactors. Previously, sulfide has been found to accumulate after 5 h in a denitrifying upflow sludge blanket reactor and after 10 h in batch experiments (van der Hoek et al., 1988), indicating that the 3 h FBR reaction time used in this study is still short enough to suppress the activity of sulfate reducing bacteria.

The effluent contained some COD, the amount depending on the treatment. The highest effluent COD concentrations were found in the effluents from acetate (106.7±47.3 mg/L) and ethanol (67.4±1.9 mg/L) reactors, while the effluent from propionate had intermediate COD concentration (25.1±50.2 mg/L), and the ones from FOW and FFOW had low COD concentrations (5.4±4.8 mg/L and 2.2±0.4 mg/L). In the experimental design, we followed the theoretical COD/NO₃-N ratio of denitrification (Van Rijn et al., 2006; Letelier-Gordo et al., 2015) when dosing carbon source. However, the calculated stoichiometric COD/NO₃-N ratios are 3.7 for acetate, 4.2 for ethanol, and 4.9 for propionate (Burghate and Ingole, 2010; Elefsiniotis and Wareham, 2007), explaining the amount of excess COD in the effluent.

3.4 Applying FBRs at a marine-land based RAS

Traditional media-based denitrification systems have been well studied for aquaculture wastewater treatment (Table 2), while less information exists on the application of activated sludge FBR to treat aquaculture effluents. Hamlin et al. (2008) obtained a maximum denitrification rate of 0.67–0.68 kg N/(m³ media d) in commercial scale upflow biofilters when using both methanol and acetate. Dupla et al. (2006) and Labelle et al. (2005) obtained volumetric denitrification rates of 2.7 and 1.77 kg N/(m³ media d), respectively, using moving-bed bioreactors (MBBR). However, a low media filling rate, ranging from 25% to 53%, was needed to ensure the free movement of media in these reactors, resulting in a compromise regarding system volume. Fixed bed systems have a better denitrification efficiency

than moving bed systems (Sauthier et al., 1998), although sludge clogging and sulfide accumulation occur more frequently. The volumetric denitrification capacity of FBR obtained with acetate in this study aligns with reported values for media-laden reactors (Table 2).

Previously, in a salt water activated type reactor employing fish organic waste as carbon source, an average volumetric denitrification rate of 120-150 g N/(m³ reactor d) was reached at a solid retention time of 11 days and a reported hydraulic retention time between 12 to 16 h (Klas et al., 2006), the controlling of solid retention being the most complicated operation task in the system. In the present experiment a similar volumetric denitrification rate was obtained of 173 g N/(m³ reactor d) at a hydraulic retention time of 5 h using 4-day fermented fish waste. No complications existed in the operation of the FBR as the biomass is self-regulated through the fill and discharge steps, and fermentation of the fish waste is carried out in another tank (i.e., the reservoir of the backwash of drum filters). All in all, FBR shows to be a competitive alternative to media-laden reactors found in the literature (Table 2), adding long-term operational reliability without backwash (Strous et al., 1998). The operational mode of FBRs entails them being a more suitable configuration for end-of-pipe denitrification (e.g. using the backwash of the drum filters as internal carbon source and/or supplementing with external carbon sources) than for in-line denitrification.

Based on the results, using external carbon sources for denitrification is more efficient than using internal carbon sources, since the obtained denitrification rates are higher, meaning that smaller reactor volumes are needed. For example, a salmon RAS feeding 1 ton per day and a FCR of 1.1 with an average protein content in feed of 45% will have a maximum NO₃⁻-N production of 39 kg N/day (Dalsgaard and Pedersen, 2016). To remove such amount of nitrate, the working volume of FBR is expected to be 33 m³ when using acetate and 127 m³ when using ethanol, assuming that the activated biomass is similar to the one obtained in this experiment (1 g/L). Since acetate requires a smaller reactor, the expense in construction is reduced. Furthermore, being a non-alcohol substrate, acetate is safer to handle and store, making it a more practical alternative than ethanol in treating aquaculture wastewater. Utilizing the FOW and FFOW requires a larger reactor (226-424 m³). However, they are free carbon sources, being

attractive alternatives cost-wise in long-term use, and thus, should be considered to be used either solely or combined with external carbon sources for a cost-efficient removal of nitrogen at the end-of-pipe treatment. Moreover, using fish waste as a carbon source will allow the simultaneous removal of organic matter and nitrate from the discharge (Suhr et al., 2015, 2013; Tal et al., 2009).

4. Conclusions

This study demonstrates that FBR could be a viable option for end-of-pipe N removal in marine land-based RAS. Among the five carbon sources tested, acetate had the highest N removal rate, resulting in a smaller reactor volume needed. Furthermore, no accumulation of TAN, PO_4^{3-} , or S^{2-} was observed when using external carbon sources, except a pronounced sulfide production found when using ethanol. Using fish organic waste for N removal will save operational costs, becoming a cost-efficient option in aquaculture denitrification, but the accumulation of TAN and PO_4^{3-} in the effluent should be prevented. Altogether, the results obtained demonstrate that FBR is a suitable technology for end-of-pipe treatment in marine land-based RAS, due to the simple setup, easy maintenance, low operational costs, small footprint, and a reliable N removal. However, it should be further combined with a solid removal process to remove possible excess carbon before discharging into the environment.

Author statement

Carlos O. Letelier-Gordo: Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Xiaoyu Huang: Investigation, Writing - Original Draft, Sanni L. Aalto: Formal analysis, Writing - Review & Editing, Visualization, Per Bovbjerg Pedersen: Resources, Funding acquisition

Declaration of interests

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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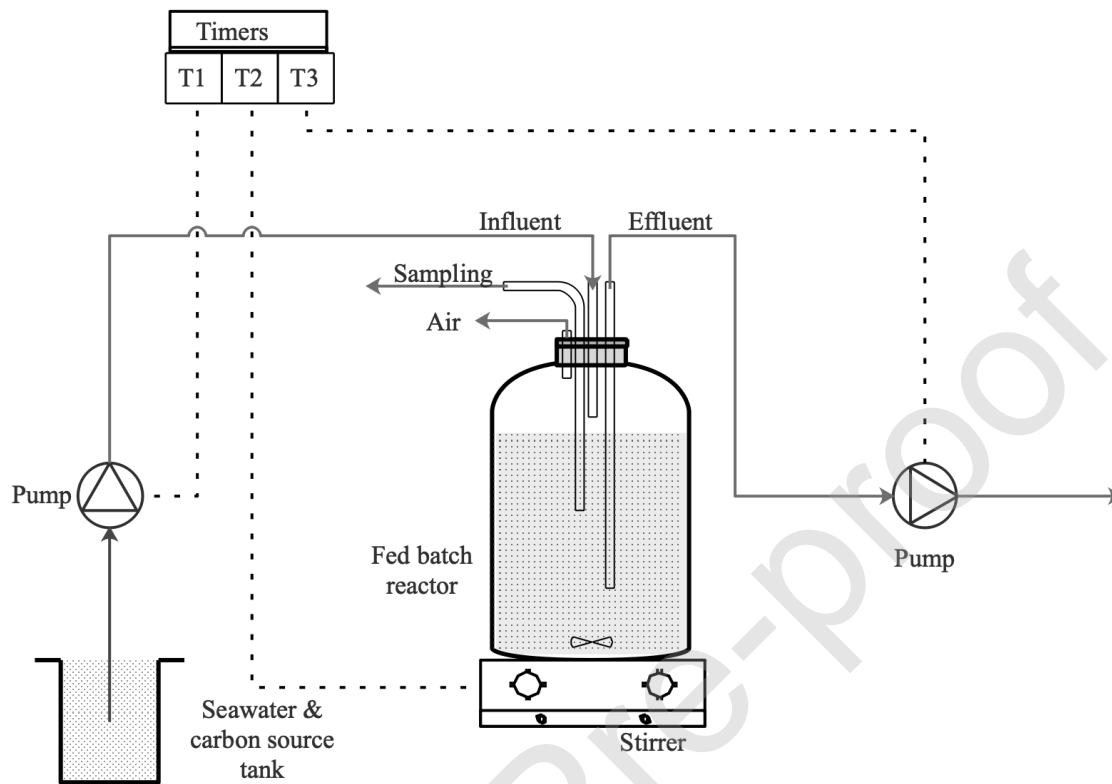


Figure 1: Set-up of the fed batch reactor (FBR) used for the trials.

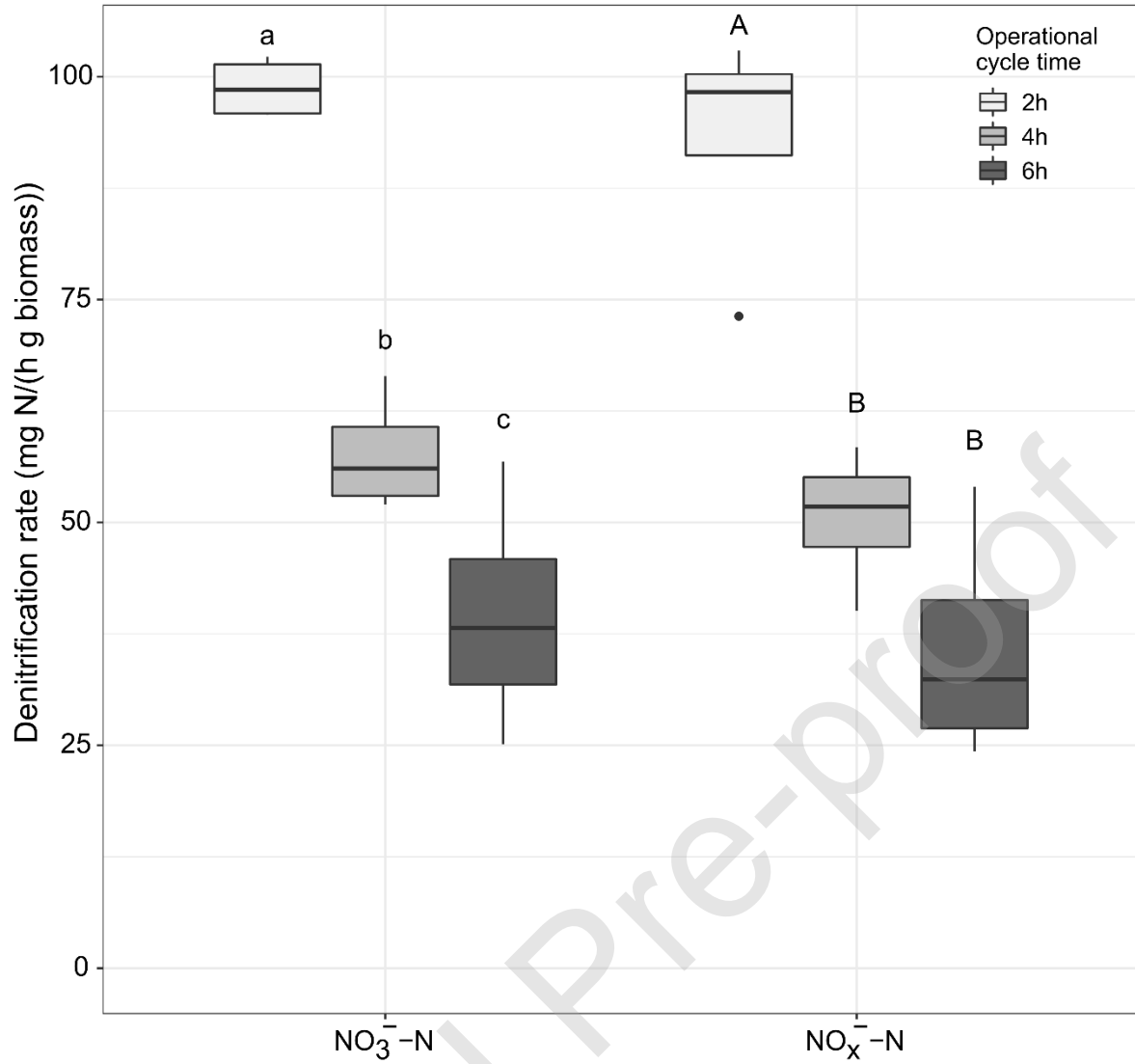


Figure 2. The effects of operational cycle time on denitrification rate ($\text{mg N}/(\text{h g biomass})$) using acetate ($n=4$). Letters indicate significant differences between groups.

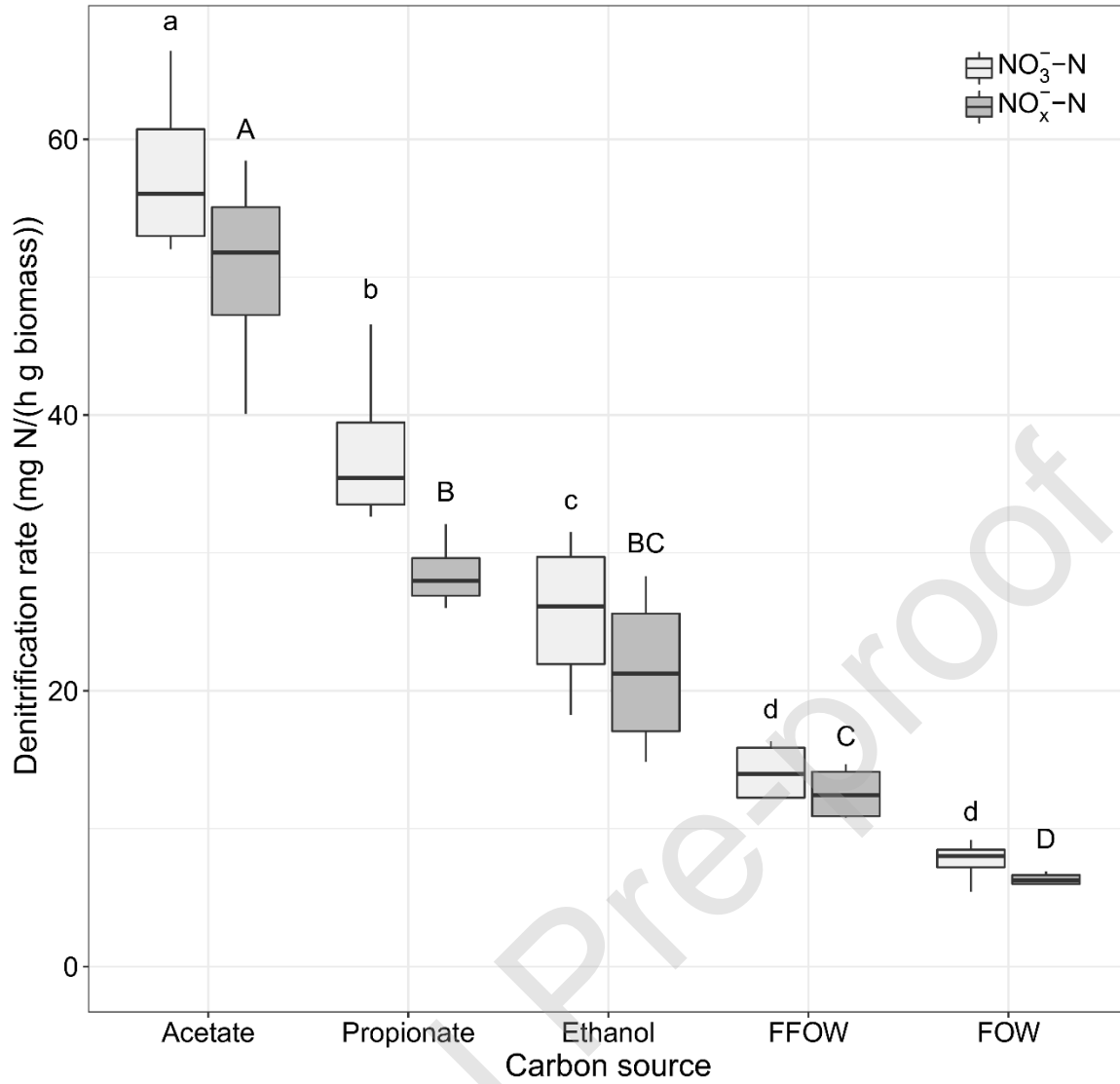


Figure 3: Denitrification rates (mg N/(h g biomass)) with different carbon sources: acetate, propionate, ethanol, fermented fish organic waste (FFOW) and fish organic waste (FOW) (n=4). Letters indicate significant differences between groups.

Table 1. Reaction time, hydraulic retention time, number of daily cycles and sampling intervals during different operational cycle times of FBRs tested in phase I.

Operational cycle time (OCT) (h)	Reaction time (h)	Hydraulic retention time (HRT) (h)	Sampling interval (min)	Number of samples
2	1	2.5	15	5
4	3	5.0	30	7
6	5	7.5	60	6

Table 2. Denitrification capacities reported for different bioreactors.

Reactor	Salinity	Carbon source	Volumetric denitrification capacity	Media filling (%)	Ref.
FBR	Seawater	Acetate	1.2 kg NO _x / (m ³ reactor d)	-	a
Upflow Biofilter	Freshwater	Methanol/acetate	0.7 kg NO _x / (m ³ media d)	53	b
MBBR ¹	Seawater	Methanol	2.7 kg NO _x / (m ³ media d)	30	c
MBBR	Seawater	Methanol	1.7 kg NO _x / (m ³ media d)	25	d
Fixed bed	Seawater	Ethanol	2.4 kg NO _x / (m ³ media d)	100	e
UASB ²	Seawater	Acetate	14.9 kg NO _x / (m ³ reactor d)	-	f

¹ MBBR: moving bed biofilm reactor, ² UASB: upflow anoxic sludge bed, ^a This study, ^b Hamlin et al., 2008, ^c Dupla et al., 2006, ^d Labelle et al., 2005, ^e Sauthier et al., 1998, ^f Letelier-Gordo and Martin Herreros, 2019.