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Biological denitrification in marine aquaculture systems: A multiple electron donor microcosm study

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ABSTRACT

There is a lack of information on denitrification of saline wastewaters, such as those from marine recirculating aquaculture systems (RAS), ion exchange brines and wastewater in areas where sea water is used for toilet flushing. In this study, side-by-side microcosms were used to compare methanol, fish waste (FW), wood chips, elemental sulfur (S^0) and a combination of wood chips and sulfur for saline wastewater denitrification. The highest denitrification rate was obtained with methanol (23.4 g N/(m^3·d)), followed by FW (4.5 g N/(m^3·d)), S^0 (3.5 g N/(m^3·d)), eucalyptus mulch (2.6 g N/(m^3·d)), and eucalyptus mulch with sulfur (2.2 g N/(m^3·d)). Significant differences were observed in denitrification rate for different wood species (pine > oak ≫ eucalyptus) due to differences in readily biodegradable organic carbon released. A pine wood-sulfur heterotrophic-autotrophic denitrification (P-WSHAD) process provided a high denitrification rate (7.2–11.9 g N/(m^3·d)), with lower alkalinity consumption and sulfate generation than sulfur alone.

1. Introduction

Marine recirculating aquaculture systems (RAS) have been developed to minimize land and water use and wastewater discharges caused by rapid expansion of the aquaculture industry (Martins et al., 2010; Christianson et al., 2015). In RAS, nitrification processes, such as moving bed bioreactors (MBBR), are used to transform fish-toxic total ammonia nitrogen (TAN) and nitrite (NO_2^-) to nitrate (NO_3^-) (van Rijn et al., 2006). However, high NO_3^- concentrations have a chronic detrimental effect on marine cultured fish species production, and concentrations less than 75 mg NO_3^--N/L are recommended for fish health (Davidson et al., 2014). The most common method to control NO_3^- in RAS is through water exchanges, which consume large amounts of water, and result in discharges of NO_3^- polluted wastewater to the environment, leading to aquatic ecosystem deterioration (Martins et al., 2010).

Biological denitrification is an effective solution for NO_3^- removal in marine RAS (van Rijn et al., 2006; Simard et al., 2015). The most common RAS denitrification systems are based on heterotrophic metabolism, in which easily biodegradable liquid carbon sources, such as methanol or ethanol, are used as electron donors (Tsukuda et al., 2015). However, careful dosing is required as NO_2^- accumulates when the organic carbon supply is insufficient, while organic substrates are carried over to the effluent when provided in excess of the amount required for denitrification (Hamlin et al., 2008).

Denitrification using fish waste (FW) as an internal organic carbon source offers economic and environmental benefits, owing to the concurrent reduction of NO_3^- and the solid waste stream (Martins et al., 2010; Suhr et al., 2014; Tsukuda et al., 2015). Klas et al. (2006) evaluated distinct phases of NO_3^- removal in a denitrification reactor treating RAS water with FW, and reported that only 4% of the total chemical oxygen demand (COD) in FW was readily biodegradable, while 30% was slowly biodegradable requiring ≥5 days to be utilized.

Wood chips (WC) have gained attention as a biofilter media and carbon source for stormwater and domestic wastewater denitrification applications (Saliling et al., 2007; Lopez-Ponnada et al., 2017). WC media delivered long-term NO_3^- removal (5–15 years), while requiring minimum maintenance (Robertson, 2010). In a recent review, Lopez-Ponnada et al. (2017) reported total nitrogen (TN) removal efficiency was higher with softwood (75.2%) compared with hardwoods (63.0%). In contrast, Cameron and Schipper (2010) reported that mean NO_3^- removal rates were similar for hardwood (3.3–4.4 g N/(m^3·d)) and softwood (3.0–4.9 g N/(m^3·d)) and there was no difference in long-term performance.

Elemental sulfur (S^0), which is a non-toxic by-product of petroleum refining, is a low cost electron donor for autotrophic denitrification

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(Christianson et al., 2015). Advantages of sulfur oxidizing denitrification (SOD) include elimination of carry-over of organic carbon and lower excess biomass production than heterotrophic denitrification (Christianson et al., 2015). A disadvantage of SOD is alkalinity consumption (4.57 mg $CaCO_3$/mg NO_3^--N; Batchelor and Lawrence 1978), necessitating addition of a pH buffer material such as oyster shells (Sengupta et al., 2007). In addition, sulfate (SO_4^{2-}) production (7.54 mg SO_4^{2-} per mg NO_3^--N reduced; Batchelor and Lawrence 1978) could potentially negatively affect fish health in RAS; however, we were unable to find any research this topic.

A mixotrophic process combining heterotrophic and SOD is a potential strategy to limit SO_4^{2-} production (Sahinkaya and Kilic, 2014; Sahinkaya et al., 2011). In addition, alkalinity generated by heterotrophic denitrification (3.57 mg $CaCO_3$/mg NO_3^--N) can compensate for alkalinity consumption by SOD (Oh et al., 2001; Rodriguez-Gonzalez, 2017). Krayzelova et al. (2014) reported a high NO_3^- removal efficiency (90%) with reduced SO_4^{2-} production by including scrap tire chips in an SOD column. Li et al. (2016) evaluated the denitrification performance of wood-sulfur heterotrophic-autotrophic denitrification (WSHAD) microcosms and reported a higher denitrification rate (0.055 h^{-1} to 0.066 h^{-1}) than SOD alone (0.010 h^{-1} to 0.013 h^{-1}). Limited studies have been conducted for WSHAD in RAS, and the impact of different wood species on denitrification in marine systems has not been investigated.

Different electron donors have been investigated in freshwater aquaculture (Hamlin et al., 2008), wastewater (Saliling et al., 2007), groundwater microcosms (Fowdar et al., 2015) and drinking water (Sahinkaya et al., 2011). However, no prior study has evaluated denitrification performance using different electron donors in side-by-side trials for treatment of saline wastewater. High salinity can affect denitrification performance by preventing microorganisms from maintaining their osmotic pressure balance, giving rise to bacterial plasmolysis (Lay et al., 2010).

In this study, side-by-side denitrification microcosm experiments were set up to compare the denitrification capacity of different electron donors for fully nitrified marine RAS water. Specific objectives were to investigate: i) the effect of different electron donors (methanol, WC, FW, S^0, and a mix of WC and S^0) on NO_3^- removal from marine water; ii) the influence of different wood species (pine, eucalyptus and oak) on denitrification performance; and iii) the effect of wood species (pine and eucalyptus) and alkalinity addition (oyster shells) on WSHAD performance.

2. Materials and methods

Three experimental phases were set up in this study (Table 1 and Supplementary material): 1) Phase I was a screening study (no duplicates) to compare denitrification performance of methanol, FW, WC, SOD and WSHAD; 2) Phase II investigated the influence of wood species on denitrification performance; 3) Phase III investigated the effect of different wood species on WSHAD performance and the effect of oyster shell on the WSHAD performance.

2.1. Synthetic marine RAS water

Synthetic marine RAS water was prepared by adding 15 g/L Instant Ocean Sea Salt (Instant Ocean®), 0.607 g/L sodium nitrate ($NaNO_3$) and 0.044 g/L potassium dihydrogen phosphate (KH_2PO_4) to tap water. This resulted in a solution with a salinity of 15 ppt, NO_3^- concentration of 97.2 ± 1.8 mg NO_3^--N/L and phosphorus concentration of 10.0 ± 0.5 mg PO_4^{3-}-P/L, which are typical values for land-based marine RAS (Boxman et al., 2015). According to the manufacture's information, Instant Ocean contains major, minor and trace elements, and is free of NO_3^- and phosphate. The COD in the synthetic marine RAS water was 9 ± 2 mg/L.

Table 1
Experimental phases and materials added in each microcosm.

Phase	Microcosms	Electron donor	Inoculum
Phase I	Methanol	0.336 mL methanol	Plastic carriers
	FW	200 mL fish waste	IMTA Sand
	WC	10 g eucalyptus mulch	IMTA Sand
	SOD	10 g elemental sulfur + 4 g crushed oyster shells	IMTA Sand
	WSHAD	5 g wood chips + 5 g elemental sulfur	IMTA Sand
Phase II	P-WC	10 g pine wood chips	IMTA Sand
	E-WC	10 g eucalyptus wood chips	IMTA Sand
	O-WC	10 g oak wood chips	IMTA Sand
Phase III	P-WSHAD	5 g pine wood chips + 5 g elemental sulfur	Pilot RAS SOD reactor
	PO-WHSAD	5 g pine wood chips + 5 g elemental sulfur + 2 g oyster shell	Pilot RAS SOD reactor
	E-WSHAD	5 g eucalyptus wood chips + 5 g elemental sulfur	Pilot RAS SOD reactor
	EO-WSHAD	5 g eucalyptus wood chips + 5 g elemental sulfur + 2 g oyster shell	Pilot RAS SOD reactor

2.2. Electron donors and inoculum

A summary of the materials used in each experimental phase is provided in Table 1. Methanol (> 99.9%) was purchased from Fisher Science (Fisher Science, USA). Elemental sulfur pellets (4.0–6.0 mm) were obtained from Southern Ag in Palmetto, Florida. Crushed oyster shells, an alkalinity source for SOD, were obtained from Myco Supply (Pittsburgh, Pennsylvania) and sieved to a size of 1.0–2.0 mm. FW was collected from a 0.085 m^3 drop filter (Aquaculture Systems Technologies, L.L.C, New Orleans, LA) for solids removal in a marine RAS containing marine broodstock fish (*Centropomus undecimalis*) at Mote Aquaculture Research Park (MAP; Rhody et al., 2014). Different WC species were chosen based on their local availability in Florida (United States) and prior performance for denitrification (Lopez-Ponnada et al., 2017). Natural eucalyptus mulch (100% Florida-Grown Eucalyptus) was used in Phase I. The mulch was obtained from Scotts Company LLC (Marysville, Ohio, USA); however, no additional wood species information for this eucalyptus was available. In Phases II and III eastern white pine (P-WC; *Pinus strobus*; soft wood), eucalyptus (E-WC; *Eucalyptus camaldulensis*; hardwood) and red oak (O-WC; *Quercus rubra*; hardwood), were obtained from a specialty lumber supplier in Tampa, Florida. To maintain uniformity, the WCs were cut into blocks of approximately 4–6 mm (L) × 4–6 mm (W) × 2–4 mm (D).

Different sources of inoculum were used to have an appropriately acclimated microbial community for the different electron donors tested (Table 1). Plastic carriers (AMBTM media, EEC, Blue Bell, PA, USA) were obtained from a methanol-fed denitrification reactor in the MAP red drum RAS described above. Sand was collected from a partially submerged denitrification filter in a marine integrated multi-trophic aquaculture (IMTA) system described by Boxman et al. (2015). Inoculum used in WSHAD microcosms was biomass from a SOD reactor in a pilot-scale marine RAS set up in the USF laboratory. Regardless of the inoculum source, all microcosms were inoculated with 500 ± 21 mg VSS/L (1422 ± 27 mg TSS/L) except for un-inoculated controls.

2.3. Experimental setup and operation

The experiments were carried out in the Environmental Engineering Laboratory at the University of South Florida (USF), Tampa. Microcosms were set up in 1 L glass bottles containing 800 mL of synthetic RAS water. To maintain anoxic conditions, bottles were purged with nitrogen gas for 5 min to remove oxygen after the addition of all materials (excluding methanol). For the methanol microcosm,