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Heterotrophic denitrification of aquaculture effluent using fluidized sand biofilters

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ABSTRACT

The ability to consistently and cost-effectively reduce nitrate-nitrogen loads in effluent from recirculating aquaculture systems would enhance the industry's environmental stewardship and allow improved facility proximity to large markets in sensitive watersheds. Heterotrophic denitrification technologies specifically employing organic carbon found in aquaculture system waste offer a unique synergy for treatment of land-based, closed-containment production outflows. For space-efficient fluidized sand biofilters to be used as such denitrification reactors, system parameters (e.g., influent dissolved oxygen and carbon to nitrogen ratios, C:N) must be evaluated to most effectively use an endogenous carbon source. The objectives of this work were to quantify nitrate removal under a range of C:Ns and to explore the biofilter bacterial community using three replicated fluidized sand biofilters (height 3.9 m, diameter 0.31 m; fluidized sand volume plus biofilm volume of 0.206 m^3) operated at a hydraulic retention time of 15 min and a hydraulic loading rate of 188 L/min m^2 at The Conservation Fund Freshwater Institute in Shepherdstown, West Virginia, USA. Nitrate reduction was consistently observed during the biofilter study period (26.9 ± 0.9% removal efficiency; 402 ± 14 g NO_3-N/(m^3 biofilter d)) although nitrite-N and total ammonium nitrogen concentrations slightly increased (11 and 13% increases, respectively). Nitrate removal efficiency was correlated with carbonaceous oxygen demand to nitrate ratios ($R^2 > 0.70$). Nitrate removal rates during the study period were moderately negatively correlated with influent dissolved oxygen concentration indicating it may be possible the biofilter hydraulic retention time was too short to provide optimized nitrate removal. It is reasonable to assume that the efficiency of nitrate removal across the fluidized sand biofilters could be substantially increased, as long as organic carbon was not limiting, by increasing biofilter bed depths (to 6–10 m), and thus hydraulic retention time. These findings provide a low-cost yet effective technology to remove nitrate-nitrogen from effluent waters of land-based closed-containment aquaculture systems.

1. Introduction

The annual U.S. seafood trade deficit of greater than $10 billion provides a unique market niche for sustainably produced seafood products (NOAA, 2012). Land-based, recirculating aquaculture systems (RAS) allow intensive production of quality fish protein in close proximity to large markets while minimizing water footprints and environmental concerns (Timmons and Ebeling, 2010; Wheaton and Singh, 1999). In the design of such systems, removal of solids, total ammonium nitrogen (TAN), and nitrite (NO_2^-) are critical to allow recirculation of water to fish culture tanks (Summerfelt and Vinci, 2009). Although nitrification unit processes transform fish-toxic TAN and NO_2^- to nitrate (NO_3^-), this nitrogenous waste, which is typically not considered a key contaminant within recirculated waters, presents concerns of its own. Nitrogen, often in the NO_3^- form, is now being targeted by many local and national groups as a primary water quality contaminant due to a growing number of nitrogen-induced marine hypoxic zones and other water quality environmental problems (Diaz and Rosenburg, 2008; Turner and Rabalais, 1994; USEPA, 2006).

The ability to consistently and confidently reduce waste products from land-based RAS, specifically by treating NO_3^- effluent loads, could provide a number of distinct economic and environmental benefits (van Rijn, 2013). While RAS facilities are ideally located near urban areas with strong market demands for sustainably produced protein, these populous locales often have already

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placed significant pressures upon their watersheds (e.g., pollutant loads from urban storm water, municipal waste water treatment plants, and industrial discharges). Essentially eliminating nitrogenous discharges in RAS effluent waters could allow improved proximity to these urban markets. Such efforts will also further the public perception of this industry as a sustainable, environmentally friendly option amongst urban consumers; economically, the value of such public opinion is very important. Additionally, if NO_3^- concentrations in effluent waters could be dependably and cost-effectively reduced, reuse of such water could be increased to even more fully close the RAS water-use cycle. Although NO_3^- has traditionally not been considered a critical fish toxicant, recent evidence warns against NO_3^- accumulation in fish culture tanks especially in low exchange reuse systems (Davidson et al., 2011, 2014).

Biological denitrification, the microbially mediated reduction of NO_3^- to various oxides of nitrogen and eventually nonreactive dinitrogen (N_2), is one of the most effective and cost efficient methods to remove NO_3^- from wastewater (Metcalf Eddy, 2003; van Rijn, 1996). Most denitrification technologies utilize heterotrophic denitrification, where an organic carbon (C) source fuels facultative anaerobic denitrifying bacteria. Heterotrophic denitrifying bacteria have a higher growth rate than autotrophic bacteria (Metcalf Eddy, 2003), and are the most abundant type of denitrifiers found in the natural environment (van Rijn et al., 2006). Controls on the microbial denitrifier community, denitrification rate, and denitrification end products include NO_3^- and C availability, oxygen, pH, and temperature (Wallenstein et al., 2006). Carbon consumption in denitrification systems is due to (1) oxygen utilization, (2) NO_3^- conversion, and (3) cell growth (Hamlin et al., 2008), with high influent dissolved oxygen (DO) concentrations capable of not only inhibiting facultative denitrifiers from utilizing NO_3^- as the final electron acceptor in their respiratory electron transport chain, but also of reducing the C available for denitrifiers through aerobic consumption (Klas et al., 2006a,b). This C availability, often discussed using the ratio between influent carbonaceous oxygen demand and NO_3^- (i.e., chemical oxygen demand to NO_3^-, COD:NO_3-N, or carbonaceous biochemical oxygen demand to NO_3^-, $cBOD_5$:NO_3-N), is critical for heterotrophic denitrification, because insufficient C can lead to incomplete denitrification and undesirable NO_2^- production. On the other hand, an oversupply of C relative to N (i.e., excessive reducing conditions) can facilitate the reduction of sulfate to sulfide, a highly fish-toxic compound, in zones of complete N removal (Cytryn et al., 2005; Neori and Mendola, 2012). High COD:NO_3-N and anoxic conditions can also bring about dissimilatory nitrate reduction to ammonium (DNRA), where ammonium (NH_4^+), rather than N_2, is produced (Hamlin et al., 2008; van Rijn et al., 2006). van Rijn et al. (2006) reported optimum COD:NO_3-N ratios were 3:1–6:1 for readily available C sources to reduce NO_3^- to elemental N.

Exogenous C sources such as methanol or ethanol are provided to fuel denitrification in tertiary denitrification technologies (Hamlin et al., 2008; Müller-Belecke et al., 2013), but because such external sources can be expensive, there is interest in denitrification systems where an endogenous source supplies the electron donor (Conroy and Couturier, 2010; Klas et al., 2006a,b; van Rijn, 1996). Backwashed RAS solids hold potential to be utilized as an endogenous C source in these designs because biosolids dewatering methods produce a supernatant containing high levels of organic C (Sharrer et al., 2010; Summerfelt and Vinci, 2009; van Rijn, 1996). Such use of a waste stream provides the additional benefit of overall reduction of this load though C utilization (Klas et al., 2006a,b; Phillips and Love, 1998). However, the biological availability of the C products is critical for NO_3^- removal in denitrification technologies with microbial digestibility of aquaculture waste solids impacted by parameters including pH, salinity, temperature, loading factors, and chemical composition (Mirzoyan et al., 2010; Neori and Mendola, 2012). Klas et al. (2006a) noted the 5–35 μm particle size typical of RAS solids (e.g., "fines" are 1–100 μm; Summerfelt and Vinci, 2010) means the C must be enzymatically degraded into smaller molecules capable of penetrating cellular membranes before utilization (Aboutboul et al., 1995; Suhr et al., 2013). In the anaerobic digestion of waste solids, the first step (i.e., hydrolysis) involves degradation of complex organic matter such as carbohydrates and proteins to soluble organics, which are correspondingly degraded into volatile fatty acids (VFAs) (i.e., via fermentation) that can be utilized for denitrification (Mirzoyan et al., 2010). Not only is hydrolysis the limiting step for VFA formation (Conroy and Couturier, 2010), but this step is also typically slower than denitrification meaning the ready availability of C products may be the limiting step in the use of endogenous organic matter for heterotrophic denitrification (Klas et al., 2006a,b). Klas et al. (2006a) documented distinct phases of N removal in a continuously stirred denitrification reactor treating RAS waste, each characterized by the biodegradability of the organic matter being utilized; only 4% of the total COD was readily biodegradable, 50% was considered to have medium biodegradability, and 30% was slowly biodegradable requiring >5 days to be utilized. While hydrolysis/fermentation products of aquaculture waste can be used for denitrification, these processes can also solubilize TAN and phosphorus meaning additional treatment may be necessary for these by-products (Conroy and Couturier, 2010). These hydrolysis/fermentation products of aquaculture waste are also released into the supernatant and filtrate of gravity thickening settlers and geotextile bag filters, respectively (Sharrer et al., 2010).

In addition to biochemical properties of the aquaculture solids and associated supernatant, system design influences N removal in denitrification biofilters. Fluidized beds, which are widely used for nitrification in RAS (Summerfelt, 2006), present a novel option for denitrification treatment. The very high specific surface area provided within a fluidized biofilter could offer sufficient surface area for aerobic organisms to produce anoxic conditions while also allowing supplemental area for heterotrophic denitrifier colonization. In previous studies using an endogenous C source with a fluidized sand denitrification biofilter to treat water in a RAS, a sedimentation basin prior to the denitrification reactor was included to allow partial digestion of the waste (Arbiv and van Rijn, 1995; Gelfand et al., 2003; Shnel et al., 2002). This two-stage system proved successful for not only partial hydrolyzing and providing fermentation pretreatment of the organic matter, but also for actually providing denitrification in the pre-biofilter basin. With most of the NO_3^- removal occurring in the sedimentation basin (i.e., biofilter removal was only 10% that of the basin at 173 and 1689 g N removed per day, respectively; Shnel et al., 2002), the biofilter's predominant function was to remove sulfide before water was recirculated back to the culture tanks (Gelfand et al., 2003). Although Shnel et al. (2002) concluded the fluidized biofilter was not a necessary component of the denitrification system, extrapolated volumetric N removal rates were nevertheless much higher for the fluidized biofilter than for the basin (1330 vs. 140 g NO_3-N/(m^3 d), respectively).

Clearly denitrification technologies for RAS effluent could reduce NO_3^- loads to surface waters while additionally reducing the waste stream, minimizing NO_2^- concentrations, and supplementing buffering capacity through alkalinity production (van Rijn et al., 2006). Although fluidized sand biofilters for denitrification are a viable aquaculture technology (e.g., Gelfand et al., 2003; Shnel et al., 2002), the importance of the carbon to nitrogen ratio (C:N) has not been evaluated for fluidized biofilter designs treating aquaculture outflows with use of a cost-saving endogenous C source. The objectives of this work were to quantify NO_3^- removal under a range of C:Ns when using fluidized-sand biofilters to treat an