Optimizing Hydraulic Retention Times in Denitrifying Woodchip Bioreactors Treating Recirculating Aquaculture System Wastewater

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Abstract

The performance of wood-based denitrifying bioreactors to treat high-nitrate wastewaters from aquaculture systems has not previously been demonstrated. Four pilot-scale woodchip bioreactors (approximately 1:10 scale) were constructed and operated for 268 d to determine the optimal range of design hydraulic retention times (HRTs) for nitrate removal. The bioreactors were operated under HRTs ranging from 6.6 to 55 h with influent nitrate concentrations generally between 20 and 80 mg NO₃-N L⁻¹. These combinations resulted in N removal rates >39 g N m⁻³ d⁻¹, which is greater than previously reported. These high removal rates were due in large part to the relatively high chemical oxygen demand and warm temperature (~19°C) of the wastewater. An optimized design HRT may not be the same based on metrics of N removal rate versus N removal efficiency; longer HRTs demonstrated higher removal efficiencies, and shorter HRTs had higher removal rates. When nitrate influent concentrations were approximately 75 mg NO₃-N L⁻¹ (n = 6 sample events), the shortest HRT (12 h) had the lowest removal efficiency (45%) but a significantly greater removal rate than the two longest HRTs (42 and 55 h), which were N limited. Sulfate reduction was also observed under highly reduced conditions and was exacerbated under prolonged N-limited environments. Balancing the removal rate and removal efficiency for this water chemistry with a design HRT of approximately 24 h would result in a 65% removal efficiency and removal rates of at least 18 g N m⁻³ d⁻¹.

Core Ideas

- Woodchip bioreactor design parameters for aquaculture wastewater were developed.
- This application resulted in the highest N removal rates reported (39 g N m⁻³ d⁻¹).
- Retention times differ for optimized removal efficiency versus removal rate.
- Sulfate reduction intensified under prolonged N-limited environments.

Enhanced-denitrification bioreactors are a farm- or field-scale technology designed to address increasing levels of reactive nitrogen (N) in the environment (Schipper et al., 2010a). Such N pollution from agricultural sources is a causal agent in eutrophication, hypoxia (dead zones), and habitat degradation, resulting in a loss of biodiversity in coastal waters worldwide (Galloway et al., 2003). Over the past 20 yr, wood-based heterotrophic denitrifying bioreactors have been used to mitigate nonpoint source N pollution associated with agricultural tile drainage and groundwater (Robertson and Cherry, 1995; Woli et al., 2010; Christianson et al., 2012; Schmidt and Clark, 2012) as well as N pollution from point sources like greenhouses (Warneke et al., 2011). Other agricultural point sources, in particular land-based recirculating aquaculture systems (RAS), stand to benefit greatly from such relatively simple denitrification technologies (van Rijn et al., 2006).

Fish protein consumption has increased 3.6% per year since 1961, which is double the worldwide population growth of 1.8% per year (WHO, 2015). To meet this growth demand in the face of increasingly stringent restrictions on wild ocean resources, there is a market-based need for the aquaculture industry to expand. As an efficient method for fish production, RAS are highly productive due to their (i) controlled, bio-secure environment, allowing maximized fish production over relatively short periods, and (ii) minimal water use, which makes them viable solutions for areas where water is scarce or in areas near large urban markets where strict water quality criteria exist (Summerfelt and Vinci, 2007; Timmons and Ebeling, 2010; Wheaton and Singh, 1999). However, the minimal water use associated with RAS technology concentrates wastes into (i) a solid waste/wastewater stream associated with solids settling/dewatering and (ii) an effluent stream. The waste/wastewater stream typically contains high concentrations of suspended solids and high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) stemming from concentration and dewatering of fish manure and uneaten fish feed using technologies such as gravity thickening settlers or inclined belt filters (Sharrer et al., 2010). The effluent stream is generated from a small flushing or “exchange”...
flow that is added to the fish production system to help dilute the accumulation of nitrate (NO$_3^-$) in the fish tanks. Within RAS unit processes, a biofilter is generally used to convert organic N and total ammonia N (TAN) to nitrate to avoid accumulation of highly fish-toxic TAN and nitrite (NO$_2^-$) in the culture tanks. Accumulation of nitrate in culture water has not historically been a concern for these systems, with levels as high as 400 mg NO$_3^-$–N L$^{-1}$ considered nontoxic (Timmons and Ebeling, 2010), although new recommendations encourage concentrations of <75 mg NO$_3^-$–N L$^{-1}$ for optimal fish health (Davidson et al., 2014). Both RAS outflow streams (wastewater and effluent) are suitable for application of a denitrification technology, and, compared with many previous applications of wood-based denitrifying bioreactors, treatment of recirculating aquaculture outflows that stem from tightly controlled, often indoor, systems provides an ideal opportunity to avoid variable flow rates and seasonal temperature fluctuations that can affect woodchip bioreactor N-removal performance (Schipper et al., 2010a; Schmidt and Clark, 2013). Although denitrification treatment of RAS wastewater or effluent streams that leave the site as point source discharge is an interesting opportunity for the treatment of point source N pollution, recycling of woodchip bioreactor–treated waters back to fish culture tanks in a tightly controlled RAS is currently not recommended due to biosecurity and other fish health uncertainties.

For woodchip bioreactor treatment of aquaculture wastewater to work successfully, several key design and operational issues need to be addressed. Hydraulic retention time (HRT) (bioreactor pore volume divided by flow rate) is a primary reactor design parameter, and engineering-based designs are essential for preventing water from passing too quickly through a system that minimizes nitrate removal (i.e., HRTs too short) or too slowly (i.e., HRTs too long), which wastes the carbon investment as other reduction processes become active (e.g., sulfate reduction and associated mercury methylation, methanogenesis) (Robertson and Metkle, 2009; Shih et al., 2011). Reported nitrate removal across HRTs varies widely due to differing environmental conditions and bioreactor system design. Influent nitrate concentrations of 10 to 30 mg NO$_3^-$–N L$^{-1}$ can be reduced by approximately 50% with woodchip bioreactor HRTs ranging from 6 to 30 h, a relatively wide range at groundwater temperatures that generally range from approximately 10 to 15°C (Christianson et al., 2013; Moorman et al., 2015). Within this span of influent concentrations, Woli et al. (2010) reported that <3 h of HRT resulted in 33% reduction at a field site, and Chun et al. (2009) found that 100% of nitrate was removed at an HRT as low as 19 h (both woodchip bioreactors treating groundwater). Higher influent concentrations tested in lab settings show that much greater HRTs may be required. Bock et al. (2015) observed only a 13% concentration reduction from influent of 35 mg NO$_3^-$–N L$^{-1}$ at 18 h with a woodchip-only control column at 22°C, and Greenan et al. (2009) reported that 50% reduction from an initial concentration of 50 mg NO$_3^-$–N L$^{-1}$ required 2.8 d of HRT in a woodchip-packed column at 10°C in an incubation chamber.

There has been no woodchip bioreactor HRT model calibrated specifically for the high nitrate concentrations and other parameters (e.g., COD, dissolved oxygen, temperature) associated with recirculated aquaculture wastewater. This particular water chemistry may help encourage denitrification along with steady flows and constant temperature but may also lead to excessive bacterial growth and clogging. Wood-based treatment of aquaculture wastewater has been trialed at a small scale by Saliling et al. (2007), although the woodchips were intended to primarily serve as a colonization surface area and methanol was added to fuel denitrification. Their simulated wastewater initially ranged from 232 to 800 mg COD L$^{-1}$ and from 50 to 200 mg NO$_3^-$–N L$^{-1}$, and the methanol-enhanced systems achieved >95% removal efficiency and removal rates as high as 1365 g N removed m$^{-3}$ d$^{-1}$. Other relevant previous applications of woodchip bioreactors include treatment of greenhouse wastewater with nitrate concentrations between 200 and 300 mg NO$_3^-$–N L$^{-1}$ (Schipper et al., 2010b) and of septic effluent with BOD concentrations as high as 150 mg BOD L$^{-1}$ (Robertson et al., 2005). Nitrex treatment of septic effluent in Canada has used a design HRT of 1 to 10 d, with N removal typically >90% (Robertson et al., 2005). The primary objective of this work was to evaluate woodchip denitrifying bioreactor N-removal performance under varying HRTs given influent water chemistries associated with RAS wastewater. This will help calibrate woodchip bioreactor design models specifically for recirculated aquaculture wastewater.

**Materials and Methods**

**Bioreactor Experimental Design**

Four pilot-scale woodchip bioreactors (3.8 × 0.76 × 0.76 m; ~1:10 scale based on surface footprint; i.e., the depth dimension was not 1:10 scale) were constructed at The Conservation Fund’s Freshwater Institute to evaluate N removal across a range of HRTs (Fig. 1). Testing was completed in two phases to capture a full range of potential design HRTs. During the Establishment Period (Days 0–162), the four bioreactors were operated at approximately 12, 24, 42, and 55 h HRT (1.68 ± 0.06, 0.85 ± 0.07, 0.47 ± 0.06, 0.36 ± 0.07 L min$^{-1}$, respectively; mean ± SD). The Loading Test Period (Days 169–268) allowed further refining of the evaluation, with operation at 6.6, 12, 20, and 29 h HRT (3.22 ± 0.35, 1.72 ± 0.24, 1.04 ± 0.07, and 0.71 ± 0.07 L min$^{-1}$, respectively).

The aboveground bioreactors were constructed of plywood and lumber bracing and lined with plastic pond liner (JPL-24, Just Liners). Inlet and outlet manifolds (5.1 and 10.2 cm diameter PVC, respectively) with custom-drilled holes (4.0 and 5.5 cm diameter, respectively) and wrapped in plastic mesh (Pentair N1020, 1.3-cm hole size) spanned the width of each bioreactor at the base of each system. The bioreactors were filled with woodchips to a depth of 76 cm, and the 61-cm saturation level was maintained by a static standpipe outside the bioreactors that routed water through the base of each bioreactor’s downstream wall (4-cm Uniseal fitting) to an effluent collection tank. The locally sourced woodchips were classified by the vendor as “a 3 inch, hardwood blend” (Lowe Products) (no additional species information available) and had an interpolated particle diameter of approximately 1.2 cm at 50% of the cumulative distribution (median diameter). Woodchip porosity was 70 ± 0.7% following methods from Im and Mann (2007), and dry-basis bulk density was 217 ± 11 kg m$^{-3}$ (means ± SD). One pore volume based on the bioreactor saturated volume was 1240 L (3.81 × 0.76 ×
Aquaculture wastewater was generated on-site by the production of rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar). Waste was concentrated by a microscreen drum filter and pumped into a series of gravity thickening settlers (i.e., settling cones). Overflow liquid from the settling cones was directed into a supernatant holding tank, which was then pumped to a mixing tank used to feed the four bioreactors (Fig. 1). The waste treatment system was previously described by Tsukuda et al. (2015). In the mixing tank, the supernatant from the settled fish waste was dosed with a concentrated sodium nitrate solution to simulate nitrate concentrations experienced at RAS facilities. The typical expected range is 60 to 80 mg NO₃⁻N L⁻¹, although concentrations can be highly variable due to specific facility-based practices and flushing rates (van Rijn et al., 2006). Some week-to-week nitrate concentration variation (e.g., ±10 mg N L⁻¹) in the supernatant wastewater was expected due to changes in fish growth and water flushing requirements at this production aquaculture facility; however, several equipment and personnel oversights (e.g., dosing pump dial malfunction, dosing calculations done in error) resulted in more variability than was initially expected. Once dosed, the mixing tank solution was circulated into four individual treatment tanks of defined volume, from where it was pumped into each bioreactor (Model# 6-CIA 115V, Little Giant Pump) over a period of less than 5 min on an electronically controlled schedule (i.e., during the Establishment Period, each pump kicked on one time per hour for generally about 3 min to pump each treatment’s predefined volume; pumps kicked on two times per hour during the Loading Test). Because only sodium nitrate was added to the mixing tank solution and because this solution was fairly quickly circulated to the bioreactor upstream wall in each bioreactor to provide access for in situ measurements. 

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Initially, water quality sample collection timing was based on flow through the reactors to normalize organic flushing parameters (e.g., COD) by cumulative pore volume eluted by each HRT treatment. Thus, for the first 47 d, not all four bioreactors had effluent samples collected at the same time. Beyond this day, influent and effluent samples were collected concurrently weekly. All collected samples were analyzed on-site for total nitrogen (TN), TAN, NO₃⁻N, NO₂⁻N, sulfate, sulfide, COD, alkalinity, and pH following standard methods (APHA, 2005; Hach, 2003). Oxidation reduction potential (ORP), dissolved oxygen (DO), and temperature were measured in the sampling wells at least twice weekly (Hach HQ 40-d meter with ORP redox probes calibrated every 90 d).

Nitrate removal rates (g N removed m⁻³ bioreactor d⁻¹) were calculated from the cumulative mass of NO₃⁻N removed between two sample dates divided by the entire bioreactor volume (length × width × depth of woodchips; 2.21 m³) and the difference between the two sample dates (which is equivalent to the difference in inflow and outflow NO₃⁻N concentrations times the bioreactor flow rate divided by the total bioreactor volume). Alkalinity production and COD removal rates were calculated similarly. Nitrogen removal efficiency was calculated by dividing the difference of the inflow and outflow loads by the inflow load. One-way ANOVA was used to assess significant differences between HRT treatments (α = 0.05).

Results and Discussion

Nitrate Removal

Nitrate removal occurred in all bioreactors over the course of the experiment, with HRT means ranging from 6.6 to 55 h (Fig. 2a). The initial influent nitrate concentration of 33.9 ± 7.37 mg NO₃⁻N L⁻¹ (mean ± SD) over the first 50 d was generally reduced to <3.0 mg NO₃⁻N L⁻¹ in all four bioreactors due to flushing of easily degradable carbon as evidenced by high effluent COD concentrations (Fig. 2c). The effluent contained >300 mg COD L⁻¹ during first 28 d but was reduced to generally <80 mg COD L⁻¹ and to values lower than the influent after Day 92 (influent mean ± SD, 83 ± 21 mg COD L⁻¹; Day 92–268; n = 26). After this flushing period and when a higher N mass loading was applied (i.e., influent nitrate increased to consistently between 71 and 78 mg NO₃⁻N L⁻¹ between days 113 and 146), a relatively more steady-state measurement period was observed where the bioreactors operating under the two shortest HRTs did not continue to achieve complete nitrate removal (Fig. 2a).

Analyzed across the Establishment Period’s Measurement Period (n = 6 sample events), the 12-h HRT treatment had the
lowest removal efficiency (45%) (Table 1). However, because this bioreactor received the highest hydraulic and N loading rate, it also resulted in a removal rate that was significantly greater than the two longest HRT treatments (39 g N m\(^{-3}\) d\(^{-1}\)) (Table 1). The 24-h HRT treatment also demonstrated a significantly greater removal rate than the longest HRT treatment (32 g N m\(^{-3}\) d\(^{-1}\)) (Table 1). This Measurement Period evaluation (i.e., Table 1) was only performed during the end of the Establishment phase because this period provided the longest record of consistent influent nitrate concentrations. Removal rates were generally greater than the highest reported rate of 22 g N m\(^{-3}\) d\(^{-1}\) (David et al., 2015; Schipper et al., 2010a) (Table 1) due to the high influent nitrate loads combined with HRTs sufficient to facilitate anoxic conditions (i.e., not too short), high COD loading in the wastewater, and warm water temperatures (mean ± SD, 18.9 ± 1.4°C; \(n = 6\)). Warneke et al. (2011) reported a maximum removal rate of 11.2 g N m\(^{-3}\) d\(^{-1}\) for treatment of greenhouse wastewater with influent nitrate concentrations of 100 to 250 mg NO\(_3\)–N L\(^{-1}\) at comparable water temperatures (15.5–23.7°C). The 99% nitrate removal efficiency in the 42- and 55-h HRT treatments indicated that denitrification was N limited. 

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**Fig. 2.** Influent and effluent NO\(_3\)–N (a), total ammonia N (b), NO\(_2\)–N (c), total N (d), and chemical oxygen demand (e) concentrations from pilot-scale woodchip bioreactors. Bioreactor hydraulic retention times in hours by testing period are indicated in the legend.
and resulted in an excess of reducing conditions and sulfate reduction (i.e., a hydrogen sulfide "rotten egg" smell).

The wastewater influent COD:NO₃⁻–N ratio averaged 1.26:1 during the measurement period (range, 0.86:1–1.66:1; n = 6). The optimal range for denitrification is 3:1 to 6:1 (van Rijn et al., 2006); thus, the COD in the wastewater was not sufficient alone to completely fuel denitrification. This COD load accounted for roughly a maximum of 42% of the COD required for denitrification (or 1.26 divided by 3.0, assuming this to be the optimal range). Extrapolating this, if the wastewater COD load was fueling at most 42% of observed N removal rates, the woodchips themselves would have accounted for removal rates of at least 22.6, 18.6, 14.4, and 10.4 g N m⁻³ d⁻¹ for bioreactors 1, 2, 3, and 4, respectively (i.e., removal rates in Table 1 multiplied by 1 minus 0.42). These "corrected" rates are more consistent with previously reported N removal rates for woodchip bioreactors.

### Additional N Species and Water Chemistry Parameters

Total N generally mirrored nitrate in the influent and effluent because nitrate was the primary N species present due to the supplemental dosing system (fraction of TN occurring as nitrate in the influent [mean ± SD], 91 ± 26%; n = 66) (Fig. 2a, d). During the initial phase, when nitrate was frequently removed to below 3 mg NO₃⁻–N L⁻¹, effluent TN was mainly composed of TAN and some organic N. An initial flush of ammonium has similarly been reported by Healy et al. (2015). Total ammonium N concentrations in the effluent were elevated above the influent throughout the experiment for all treatments (Fig. 2b). High removal of total suspended solids across all bioreactors (generally >90%; data not shown) may have led to waste-associated N cycling within the bioreactors and may account for a portion of the elevated effluent TAN levels. An increase in TAN production was observed coincident with a spike in influent nitrate loading—N L⁻¹, effluent TN was mainly composed of TAN and some organic N. An initial flush of ammonium has similarly been reported by Healy et al. (2015). Total ammonium N concentrations in the effluent were elevated above the influent throughout the experiment for all treatments (Fig. 2b). High removal of total suspended solids across all bioreactors (generally >90%; data not shown) may have led to waste-associated N cycling within the bioreactors and may account for a portion of the elevated effluent TAN levels. An increase in TAN production was observed coincident with a spike in influent nitrate loading (Days 50–75). After oxygen depletion occurs within a system, nitrate is reduced to either molecular nitrogen (i.e., denitrification) or ammonium (i.e., dissimilatory reduction of nitrate to ammonium [DRNA]), depending on the environmental conditions and bacteria present. This may have been an indication of DRNA because (i) nitrate was nearly completely removed during this time and (ii) the available carbon relative to nitrate was still likely relatively high during this initial time period, a condition thought to favor this process over denitrification (Tiedje, 1994). Nitrite production was observed coincident with the incomplete nitrate reduction during the measurement period in the 12- and 24-h HRT treatments as well as in the three longest HRT treatments toward the end of the experiment (Fig. 2a, c). Regardless of internal TAN and NO₃⁻–N cycling, overall reductions in TN were consistently achieved across all bioreactors.

The water temperature of these greenhouse-run experiments significantly declined from 14.6 to 23.5°C during the Establishment Period to 12.6 to 16.2°C during the Loading test (t test: p < 0.001) (Fig. 3a). Bioreactor effluent generally contained <1 mg DO L⁻¹, which was greatly reduced from the influent (5.7–9.1 mg DO L⁻¹) (Fig. 3b). Oxidation reduction potentials of less than −300 mV measured within 30 cm of the bioreactor outlets indicated that conditions were extremely reduced in all the bioreactors during the early stages of the experiment (Fig. 3c). Influent ORP was recorded 25 times during the Establishment Period and averaged −24 mV (range, −133 to 45 mV; data not shown). As influent nitrate loading was gradually increased and the HRTs were reduced for the loading test, ORPs more targeted for denitrification (i.e., +50 to −50; Gerardi, 2007) were observed near the outlet of each bioreactor. The two longest HRT treatments (20 and 29 h) of the loading test period showed the least reduced and most oxygenated conditions (i.e., the most positive ORPs and DOs); this was an unexplained observation and was contrary to the observed greatest nitrate concentration reductions in these bioreactors during this time.

Heterotrophic denitrification produces alkalinity, and here alkalinity production increased with increasing HRT, thereby also increasing nitrate removal, across the entire study (Fig. 3d). Influent alkalinity was inherently high due to the limestone karst-sourced spring water used in the aquaculture growth systems. Alkalinity production rates across the bioreactors were significantly different between treatments during the measurement period (Table 1). Theoretical alkalinity production rates based on the stoichiometric ratio of 3.57 mg alkalinity (as CaCO₃) produced for each milligram of NO₃⁻–N reduced (Thobanoglous et al., 2003; van Rijn et al., 2006) were within 10% of the observed production rates (Table 1). However, sulfate reduction also produces alkalinity (reduction of 1 mol SO₄²⁻ yields 100 mg CaCO₃) (van Rijn et al., 2006), and this may account for some of the difference in effluent alkalinity concentrations between treatments. The dip in effluent alkalinity on Day 78 coincided with a decrease in influent nitrate loading, leading to complete nitrate and sulfate removal and thus limited alkalinity production.

### Table 1.Nitrate-N and chemical oxygen demand removal and alkalinity as CaCO₃ production from four hydraulic retention times during the Measurement Period

<table>
<thead>
<tr>
<th>HRT†</th>
<th>NO₃⁻–N removal efficiency</th>
<th>NO₃⁻–N removal rate</th>
<th>Observed alkalinity production rate</th>
<th>Theoretical alkalinity production rate‡</th>
<th>Chemical oxygen demand removal rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td></td>
<td>g N m⁻³ d⁻¹</td>
<td>g CaCO₃ m⁻³ d⁻¹</td>
<td>g CaCO₃ m⁻³ d⁻¹</td>
<td>g COD m⁻³ d⁻¹</td>
</tr>
<tr>
<td>12 ± 0.4</td>
<td>45 (9)b§</td>
<td>39 (11)a</td>
<td>152 (28)a</td>
<td>139</td>
<td>66 (33)a</td>
</tr>
<tr>
<td>24 ± 1.9</td>
<td>71 (8)ab</td>
<td>32 (5)ab</td>
<td>110 (24)b</td>
<td>115</td>
<td>31 (16)ab</td>
</tr>
<tr>
<td>42 ± 4.1</td>
<td>99 (0.5)a</td>
<td>–§</td>
<td>85 (18)bc</td>
<td>89</td>
<td>12 (8.0)b</td>
</tr>
<tr>
<td>55 ± 3.6</td>
<td>99 (0.9)a</td>
<td>–§</td>
<td>61 (14)c</td>
<td>64</td>
<td>8.0 (5.1)b</td>
</tr>
</tbody>
</table>

† Hydraulic retention time. Values are average (± SD) for each treatment across the entire Establishment Period.
‡ Based on 3.57 mg alkalinity as CaCO₃ produced for each mg NO₃⁻–N reduced.
§ Results are mean (SD). Means with the same letter are not significantly different (α = 0.05; n = 6 sample events).
§ Nitrate removal rates were N limited and were thus excluded from the table. For reference, these mean rates were 25 (3)bc and 18(2)c g N m⁻³ d⁻¹ for the 42- and 55-h HRT bioreactors, respectively.
The wastewater influent was generally within the optimum denitrification pH range of approximately 7 to 9 (influent range, 7.22–7.96; mean ± SD, 7.76 ± 0.15) (Lu et al., 2014). Bioreactor effluent pH was initially lower likely due to flushed organics (Fig. 3e). Slower flow rate treatments exhibited the lowest effluent pH for longer periods, potentially because this start-up flushing took more time and therefore there may have been some additional processing of organic acids within these high HRT bioreactors.

**Loading and Temperature Impacts on N Removal**

Data from across all periods showed increased N removal rates at increased N influent loading (Fig. 4a, b; data from only Day 113 onward to avoid carbon flushing effects). That is, at lower HRTs (i.e., higher flow rates and greater hydraulic loading; Fig. 4a) and at higher influent N concentrations at a consistent HRT (Fig. 4b), N removal rates increased. The exponential decay relationship between HRT and N removal rate indicated there may be a “change-point” HRT for a given water chemistry and temperature where each additional unit decrease of HRT merited a relatively greater gain in removal rate (Fig. 4a). For example, these change points occurred at HRTs of 13.4 and 14.5 h for the Establishment Period and the Loading Test Period, respectively (Fig. 4a). These were the HRTs at which the rate of change, or derivative, of the regression (Table 2) was approximately −1.0, indicating that a one-unit decrease in HRT gained a one-unit increase in removal rate. This type of relationship also showed there was no additional notable removal rate benefit of increased HRTs beyond these given change points. An exponential relationship between HRT and N removal rate has previously been demonstrated by Healy et al. (2015). For design purposes, this indicated the optimal HRT for this type of wastewater was less than approximately 15 h to maximize the mass of N removed per volume of bioreactor. However, this HRT only resulted in <60% N removal efficiency (Fig. 4c). Using a longer design HRT
would be slightly less efficient in terms of bioreactor volume (i.e., g N removed per m³), but if a bioreactor must achieve a 90% removal efficiency to meet effluent water quality permit criteria, for example, a longer design HRT would be necessary. In this case, 75 and 90% removal efficiencies required design HRTs of at least 33 and 48 h, respectively (Table 2). Selection of a 24-h design HRT would result in a 65% modeled removal efficiency and modeled removal rates of at least 18 g N m⁻³ d⁻¹; this example of design parameter selection allows a compromise between these two N-removal performance metrics by not falling too far to the right of the change point in Fig. 4a while still providing notable (e.g., >50%) N treatment efficiency in Fig. 4b.

When N loading was increased via changes in influent nitrate concentration rather than flow rate manipulation (i.e., Fig. 4b rather than Fig. 4a), significant relationships showed removal rates increased at increasing influent N concentrations (Table 2). For these data spanning a relatively consistent range of 10.2 to 12.9 h HRT, this indicates that first-order kinetics existed. It is thought denitrification within woodchip bioreactors follows Michaelis–Menten kinetics (Schipper et al., 2010a), but both zero-order (Robertson, 2010; Schmidt and Clark, 2013) and first-order kinetics have been reported (Christianson et al., 2012; Chun et al., 2009). Modeling both the Establishment Period and the Loading Test Period together resulted in a reasonably strong correlation (R² = 0.46; p < 0.0001) (dashed line in Fig. 4b).

Removal rates tended to be lower for the Loading Test versus the Establishment Period (e.g., Fig. 4a, b), which may have been caused by differences in water temperature (mean ± SD, 19.5 ± 1.8 and 13.8 ± 1.0°C for the Establishment Period and the Loading Period, respectively). However, when data were sorted to analyze across a relatively consistent HRT (10.2–12.6 h) and influent nitrate concentration (64–79 mg NO₃-N L⁻¹), removal rates were not significantly different between the two periods (p = 0.272) (Fig. 4d). Nevertheless, there was a significant relationship across the selected dataset (Fig. 4d) (R² = 0.25; p = 0.0431; removal rate = 18.47 × e^{-0.047 × Temperature}), which allowed calculation of a Q₁₀ value of 1.6, or the removal rate increased by a factor of 1.6 for every 10°C increase. This is similar to previous Q₁₀ values of 1.6 and 2.0 reported by Cameron and Schipper (2010) and Warneke et al. (2011), respectively, but lower than those reported by Van Driel et al. (2006) and Schmidt and Clark (2013) (i.e., >2.7).

### Sulfate Reduction

Significant sulfate reduction and sulfide production occurred for the two bioreactors operating with the longest HRTs during nearly the entire experiment (Fig. 5a, b). The highest effluent sulfide concentrations of 13,230 to 27,760 μg S L⁻¹ occurred early in the experiment, when effluent COD concentrations still indicated the occurrence of flushing particularly from the long HRT treatments and when water temperatures were relatively high (i.e., Days 57–71, effluent 51–205 mg COD L⁻¹, 21.0 ± 1.4°C [mean ± SD]). Samples before these dates were not analyzed for sulfide. A spike of 208 mg NO₃-N L⁻¹ used to induce non-nitrate limited conditions in all four bioreactors on Day 155 resulted in a notable decrease in both sulfate reduction and sulfide production. Sulfate reduction was not notably observed toward the end of the Loading Test Period when no systems were nitrate limited.

### Table 2. Regression relationships from Fig. 4 for hydraulic retention time (Fig. 4a, c) and influent nitrate N concentration (Fig. 4b) modeled against N removal rate and removal efficiency for pilot-scale bioreactors treating aquaculture wastewater. Nitrogen-limited points are not included in regression analysis.

<table>
<thead>
<tr>
<th>Establishment Period (14.6–23.5°C)</th>
<th>Loading test period (12.6–16.2°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regression</strong></td>
<td></td>
</tr>
<tr>
<td>HRT†</td>
<td></td>
</tr>
<tr>
<td>removal rate = 34.8 + (15,270,000 × e^{-1.25 × HRT})</td>
<td>removal rate = 17.3 + (111.2 × e^{-0.22 × HRT})</td>
</tr>
<tr>
<td>removal efficiency = 33.5 + 1.23 × HRT</td>
<td>removal efficiency = 39.9 + 1.05 × HRT</td>
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<tr>
<td>Influent NO₃–N concentration</td>
<td></td>
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<tr>
<td>removal rate = -109 + 2.01 × influent nitrate concentration</td>
<td>removal rate = 5.9 + 0.41 × influent nitrate concentration</td>
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<tr>
<td>Regression</td>
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<tr>
<td>R²</td>
<td>p value</td>
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<tr>
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<td>0.072</td>
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<tr>
<td>0.36</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

† Hydraulic retention time.
Sulfate reduction was clearly related to both N limitation and highly reduced conditions in excess of −300 mV ORPs (Fig. 6a, b). The longer HRT treatments (Fig. 6a, green square and blue upright triangle symbols) exhibited the most sulfate reduction as these bioreactors more consistently achieved 100% nitrate removal. Shih et al. (2011) recommended bioreactors be operated to maintain no less than 0.5 mg NO$_3$–N L$^{-1}$ in the outflow to minimize the potential for sulfate reduction and associated mercury methylation. Interestingly, the bioreactor operating under the shortest HRT achieved 100% nitrate removal during several sample events (Fig. 6a, red circles on right side of graph), but less notable sulfate reduction occurred. This may indicate that sulfate reduction becomes exacerbated under prolonged N-limiting conditions. Sulfide production was more evident when sulfate reduction occurred to a greater extent (Fig. 6c). Sulfate production was also observed, primarily in the shortest HRT treatment (i.e., negative sulfate reduction in Fig. 6a and b, primarily red circles). Although sulfate reduction in woodchip bioreactors operating under highly reduced conditions is a well-established phenomenon, this signifies additional potential for internal sulfur cycling when N removal is not complete under short HRTs. Nevertheless, in this case, these concentration increases were typically small (Fig. 5).
Conclusions
Pilot-scale woodchip bioreactor treatment of RAS wastewater resulted in some of the highest N removal rates reported to date (≥39 g N removed m⁻¹ d⁻¹) due to high nitrate and COD loading inherent to this water, relatively warm water temperatures, and consistent flow rates. This application of woodchip bioreactors provides the opportunity for a more tightly controlled design compared with treatment of nonpoint source nitrate flows, such as agricultural drainage.

Manipulating HRTs to span 6.6 to 55 h demonstrated that the optimal HRT to maximize N removal rate may not be the same as the optimal HRT to maximize N removal efficiency. Balancing these two removal metrics at a design HRT of approximately 24 h would result in a 65% removal efficiency and removal rates of at least 18 g N m⁻³ d⁻¹. Longer HRT designs may maximize removal efficiency but may also result in sulfate reduction/sulfide production under highly reduced, N-limited conditions. Sulfate reduction was observed in the bioreactors under such highly reduced conditions and was exacerbated under prolonged N-limited environments. Heterotrophic denitrification, as well as sulfate reduction, resulted in notable alkalinity production. Overall, woodchip bioreactor denitrification treatment of relatively high COD RAS wastewater is a useful application of this simple water treatment technology.

Acknowledgments
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References