**Modeling and mitigation of denitrification ‘woodchip’ bioreactor phosphorus releases
during treatment of aquaculture wastewater**

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ABSTRACT

Denitrification ‘woodchip’ bioreactors designed to remove nitrate from agricultural waters may either be phosphorus sources or sinks. A 24 d batch test showed woodchip leaching is an important source of phosphorus during bioreactor start-up with a leaching potential of approximately 20-30 mg P per kg wood. The most rapid phosphorus release occurred within the first 24 h regardless of deionized water or aquaculture wastewater matrices; the Elovitch equation generally best modeled this multi-phasic P leaching. Four pilot-scale bioreactors consistently removed total phosphorus from aquaculture wastewater (15-54% removal efficiency). All bioreactors initially exported dissolved reactive phosphorus, although the lower flow rate treatments eventually resulted in dissolved reactive phosphorus removal. Filtered wastewater solids may have contributed more to longer-term P leaching than the woodchips.

Key words: Woodchip bioreactor, water quality, nutrient pollution, leaching, phosphorus

1. Introduction

Severe effects of nutrient-induced eutrophication reverberate upon the cultural, economic and environmental health of water bodies at the local, regional, and global scale (Rabalais et al., 1996; Diaz and Rosenberg, 2008). Conservative estimates of annual economic losses from human-induced eutrophication total over \$2.2 billion in the US (Dodds et al., 2009). Fortunately, a denitrification ‘woodchip’ bioreactor is a passive treatment option highly appropriate for agricultural nitrate (NO_3^-) pollution (Schipper et al., 2010). This technology uses heterotrophic denitrifying bacteria to convert NO_3^- to atmospheric di-nitrogen gas (N_2) in a carbon-filled trench under anaerobic conditions. Wood-based fill is regarded as the most suitable for use in field-scale bioreactors as woodchips are readily available, low cost, allow for easy handling, require

little maintenance and are a source of labile carbon appropriate for long term denitrification (Gibert et al., 2008; Schipper et al., 2010). Woodchip bioreactors are typically designed to operate for 10+ years (USDA NRCS, 2009), though longevity and NO_3^- removal depend on several key factors including temperature, hydraulic retention time (HRT), and microbiology (Christianson et al., 2012). Woodchip bioreactor NO_3^- removal rates range from 2 to 22 grams of N removed per m^3 of bioreactor per day for treatment of agricultural drainage waters, septic and domestic effluents, and several types of wastewaters (Schipper et al., 2010; Christianson et al., 2012). These denitrification bioreactors are promising for many applications, though questions remain about potential negative side effects and tradeoffs (Healy et al., 2015).

Organics and nutrient leaching upon start-up is one of the deleterious effects of woodchip denitrification bioreactors (e.g., dissolved organic carbon (DOC), biochemical and chemical oxygen demand (BOD, COD), ammonium (NH_4^+), total kjeldahl nitrogen (TKN), and phosphorus (P); Gibert et al., 2008; McLaughlan and Al-Mashaqbeh, 2009; Cameron and Schipper, 2010; Healy et al., 2012). Phosphorus leaching from bioreactor fill media is particularly important as many waters that are impaired for nitrogen, and would be suitable to have a bioreactor upstream, may also be P-impaired. There is currently no federal regulatory boundary for total phosphorus (TP) concentrations for US lakes, streams, and estuaries, though the US EPA has recommended 0.038 and 0.076 mg TP/L as limits for lakes/reservoirs and streams/rivers, respectively, in the Midwestern Corn Belt region (USEPA, 2002). With such low P concentrations having ecological significance, it becomes vitally important to better quantify the potential for woodchips in a woodchip bioreactor to leach P to receiving water bodies.

There is evidence of woodchip bioreactors serving as both P sources and sinks. Healy et al. (2012) showed P concentrations in the effluent of a pine woodchip-filled column during a 100

d leaching period were as high as 1.10 mg PO₄-P/L. At a larger scale, Herbstritt (2014) calculated 0.2 g PO₄-P per m³ bioreactor per day was lost from a bioreactor that was approximately one year old, but also concluded "... the benefits of nitrate reduction outweigh this cost." Several lab-scale studies have recently shown wood media can reduce orthophosphate or dissolved reactive P (DRP) concentrations by 5-10% (Goodwin, 2012; Zoski et al., 2013). Warneke et al. (2011) documented P-sink capability at the field-scale by showing TP concentrations decreased over the length of a woodchip bioreactor treating hydroponic greenhouse wastewater on four sampling occasions.

While there are gaps in understanding of woodchip P release under saturated, anaerobic conditions necessary inside a woodchip bioreactor, stream ecology literature helps shed light on vascular plant breakdown in aqueous environments. This generally occurs in three phases: (1) a rapid loss through leaching of soluble organic and inorganic materials; (2) a period of microbial decomposition and conditioning; and (3) mechanical and invertebrate fragmentation (Webster and Benson, 1986). France et al. (1997) reported a slower rate of TP leaching from woody debris compared to leaves (100% in 7 versus 2 weeks, respectively), and Díez et al. (2002) also showed that initial P loss due to leaching (40-80% leached, tree species dependent) occurred over the first 6 weeks of immersion, although this eventually stabilized with no further losses. Woody media placed in a woodchip bioreactor contains a finite mass of P with the P content of wood generally spanning 40 to 200 mg P/kg dry weight (or, 0.004 to 0.02%) with branches or bark extending this range up to roughly 300 to 800 mg P/kg (Showalter and Morrell, 2002; Leite et al., 2011; Bruckman et al., 2013). Young trees (i.e., < 1.0 y) have relatively greater N and P contents than older trees (Leite et al., 2011), and conifers are thought to contain greater P concentrations than hardwoods of the same age grown under similar growing conditions

(Ovington and Madgwick, 1958). Wood decomposition rates differ between species, specifically, between deciduous and coniferous (i.e., hardwood and softwood, respectively; Díez et al., 2002), with deciduous woodchips decomposing more rapidly (Melillo et al., 1983).

The objectives of this work were to quantify woodchip bioreactor start-up P flushing losses and to evaluate different operational approaches (i.e., different flow rates that, in practice, translate to different design retention times) to minimize P losses from bioreactors treating aquaculture wastewater. The magnitude and duration of P leaching from woodchip bioreactors treating aquaculture wastewater was evaluated using (1) bench-scale batch tests and (2) four pilot-scale woodchip bioreactors each operated under a different flow regime.

2. Materials and Methods

2.1 Batch Testing

Bench-scale batch tests were performed at The Conservation Fund's Freshwater Institute (Shepherdstown, WV, USA) from 12 August to 05 September 2014 to evaluate woodchip leachable P and assess interactions of leached P with aquaculture wastewater versus a control (deionized water; Table 1). Loosely following batch test methods from Gibert et al. (2008), seventy acid-washed glass jars (Ball™, 0.95 L) were each filled with fifty grams of air-dried woodchips. The "3-inch" hardwood chips were purchased from a local mulch plant (Lowe's Products, Shepherdstown, WV; classified as simply a "hardwood blend" by the supplier), and had a dry bulk density of 217 kg/m³ and a total porosity of 70%. The woodchips had an interpolated particle diameter of approximately 1.2 cm at 50% of the cumulative distribution (D_{50} or median diameter) with an initial nutrient content of 0.211% N, 0.014% P, and 46.17% C, and fiber content of 14% lignin, 15% hemicellulose, and 62% cellulose (Agri-Analysis Labs, Leola,

PA). Woodchip moisture content was determined by drying a subset of woodchips at 103°C until a constant weight was reached.

Thirty-five jars were filled with 500 mL high-nitrate (55 mg NO₃-N/L; Table 1) aquaculture wastewater, previously described in Ebeling et al. (2003), which was the supernatant from two gravity thickening settlers receiving waste biosolids from salmonid fish production systems at the Freshwater Institute research campus. This supernatant was dosed with sodium nitrate to simulate an approximate range of realistic NO₃-N concentrations at a commercial recirculating aquaculture system (RAS) fish farm. The remaining 35 jars were filled with 500 mL deionized water (Barnstead E-Pure with D50228 cartridges) to evaluate maximum leaching potential with the relatively greater osmotic gradient versus the treatment wastewater.

The jars were inverted once after filling ($t = 0$ h), and placed in darkness at room temperature (20°C) for up to 24 days with jars harvested at predetermined intervals ($t = 0, 20$ min, 40 min, 60 min, 2 h, 4 h, 8 h, 16 h, 24 h, 36 h, 48 h, 72 h, 6 d, 9 d, 13 d, 17 d, and 24 d; jars in duplicate, $n = 2$). For each sampling event, dissolved oxygen (DO) and pH were measured in the jars (Hach HQ40d handheld meter with LDO101 probe and PHC101 probe), after which the liquid from the jars was decanted and filtered using coarse filters (10 micron) to remove woodchip particles from the water samples. Approximately 50 mL from each sample was filtered immediately with a 0.45 micron filter and analyzed within 48 h for DRP (Hach, 2003; Hach Method 8048). The remainder of the sample volume was collected in acid-washed bottles acidified with sulfuric acid and refrigerated up to 28 days before analysis following standard methods (Hach, 2003; APHA, 2005; TP: Hach Method 8190, 10127; TN: Standard Methods 10071; NO₃-N: Hach Method 8171; COD: Hach Method 8000). Note the use of the initial 10 micron filter meant the TP, total nitrogen (TN) and COD, concentrations reported were coarse

139 filtered values. Following the $t = 0$ h, 24 h, 9 d and 24 d sample events, the woodchips were air-
140 dried, stored at -10°C , and analyzed for nutrient content (Agri Analysis, Leola, PA).

141 Following methods from McLaughlan and Al-Mashaqbeh (2009) who modelled DOC
142 release from woodchips in similar batch tests (Gérard-Marchant et al., 2005), TP and DRP
143 releases were modelled using first order, second order, power law function, and Elovitch
144 equation relationships (Sigma Plot 13.0). The first and second order models, respectively, were:

$$D(t) = M_0[1 - e^{-t/\tau_1}] \quad (1)$$

$$D(t) = M_0 \left[\frac{t}{(t+\tau_2)} \right] \quad (2)$$

147 In both, $D(t)$ was the cumulative solute release at a given time (t), M_0 was a fitted steady-
148 state soluble solute concentration, and τ_1 and τ_2 were fitted characteristic times (d). In Gérard-
149 Marchant et al. (2005), M_0 was the initial water-extractable P content of the manure, and in
150 McLaughlan and Al-Mashaqbeh (2009), M_0 was the DOC concentration after an initial round of
151 leaching that ranged from 80-220 h. While this parameter previously has been determined
152 through measurement, McLaughlan and Al-Mashaqbeh (2009) also evaluated the first and
153 second order models by fitting this M_0 parameter, so here, it was fitted. The characteristic times
154 indicated the speed at which the cumulative eluted concentration reached the greatest value;
155 larger τ values indicate slower solute releases. The power law function was:

$$D(t) = At^b \quad (3)$$

157 where A ($\text{mg}/(\text{L-d})$) and B (dimensionless) were fitted parameters. The Elovitch equation, which
158 best represented woodchip DOC leaching (McLaughlan and Al-Mashaqbeh, 2009), was:

$$D(t) = \left(\frac{1}{\beta} \right) \ln(1 + \alpha\beta t) \quad (4)$$

160 where α (mg/L) and β ($\text{mg}/(\text{L-d})$) were fitted parameters.

A subset of jars was used in “re-flooding” tests to determine woodchip P leaching potential after cycles of wetting/drying (i.e., “fill and drain” cycles). The four jars harvested at $t = 24$ h were allowed to air dry for six days in darkness after their initial water sample harvest. On $t = 7$ d (i.e., initial 24 h of flooding + 6 d of drying), the jars were refilled with 500 mL of either deionized control water ($n = 2$ jars) or high-nitrate treatment water ($n = 2$ jars; initial range of 55-77 mg NO₃-N/L in the treatment solution), inverted once, and allowed to equilibrate for 24 h before water sample collection which consisted of the entire solution volume. A sample of the treatment wastewater used to fill the jars each week was retained for analysis to provide the “initial” nutrient concentrations given the variability of nutrients in the continuously produced wastewater at this aquaculture facility. These tests continued for 29 d, with samples collected four times ($t = 8$ d, 15d, 22d, and 29 d where $t=0$ was the start of both the batch test and re-flood test, i.e., the tests were started simultaneously) with the same four jars. All batch tests were performed at 23°C.

2.2 Pilot-scale Denitrification Bioreactors

Four pilot-scale woodchip bioreactors (3.8 x 0.76 x 0.76 m; 1:10 scale surface foot print; 1.76 m³ saturated volume based on outlet standpipe height of 0.61 m) were constructed from plywood and operated May 2014 through February 2015 in The Conservation Fund’s Freshwater Institute greenhouse (Shepherdstown, WV, USA). These systems previously have been described by Lepine et al. (2015). The bioreactors were lined with 20 mil pond lining (Just Liners, Inc., Bogota, TN; where “mil” is the standard production specification for liner thickness and 1 mil = 0.025 mm) and filled with woodchips from the same production batch as described above.

Aquaculture wastewater was pumped once per hour from the aquaculture wastewater supernatant tank described above into a rectangular mixing tank (1 in Fig. 1; 0.60 m x 0.91m x 0.91m), and dosed directly with a concentrated sodium nitrate solution (2) using a peristaltic pump (3; Masterflex pump, Cole Parmer, Vernon Hills, IL). This mixed treatment water was pumped to the four treatment vessels (Fig. 1; 4-7), located directly before the bioreactors; each vessel received a specified volume based on the desired HRT (i.e., 22, 28, 51, and 101 L each hour for 55, 42, 24, and 12 h HRT treatments, respectively). Those defined volumes were pumped one time per h (i.e., pulsed flow) to their respective bioreactors (Fig. 1; 8-11; Franklin Electric, Little GiantTM), through five cm PVC influent manifolds (Fig.1; 12-15). A 10 cm outlet manifold (Fig.1; 16-19) inside each bioreactor connected through the bioreactor downstream wall to a 0.61 m standpipe outside each bioreactor which directed outflow into a common sump (Fig.1; 20). Water quality samples were collected at the treatment water mixing tank (Fig.1; 1-combined influent source) and four outlet points (Fig 1; 20- four effluent pipes in the effluent sump), and analysis was consistent with the batch test (Hach, 2003; APHA, 2005). Wastewater temperature ranged from 14°C to 24°C over the experiment.

The four flow treatments were designed to study P leaching under a range of hydraulic retention times (HRT 12, 22, 42 and 55 h). Hydraulic retention time is a design parameter describing the average length of time solution remains in a reactor (Tchobanoglous et al., 2003):

$$\theta = \frac{V_r \cdot \rho}{Q} = \frac{\text{Pore Volume}}{Q} \quad (5)$$

where θ was the mean hydraulic retention time (hours), Q was the reactor flow rate, V_r was the saturated volume of the reactor (3.81 x 0.76 x 0.61 m), and ρ was woodchip porosity (70%). These design HRTs were selected to maximize the potential effects between treatments, while also being representative of HRTs reported for this technology (Christianson et al., 2012). An

evaluation of HRT impacts on N removal from this experiment has been presented previously (Lepine et al., 2015). In general, N removal rates > 18 g N removed per m^3 bioreactor per day were observed, and shorter hydraulic retention times (i.e., higher flow rates) led to greater removal rates by avoiding N-limitation. It was thought the flow treatments would result in different masses of P eluted and would transition from start-up P leaching to P removal at different times.

Technical difficulties at bioreactor startup resulted in the need to drain each bioreactor's saturated volume after the first full day of woodchip immersion (i.e., a static HRT of 28 h). Samples of this initial drained effluent were collected, and contained DRP concentrations of 6.96, 7.90, 8.06 and 7.60 mg DRP /L for the bioreactors that would eventually be operated at 12, 24, 42, and 55 h HRT, respectively (though all four bioreactors had been filled and sat for 28 h in this case). While this delay and subsequent draining were not intentional, this resulting "Flush Test" allowed nearly direction comparison between the pilot-scale bioreactors and the batch test sample collected at $t = 24$ h.

3. Results and Discussion

3.1 Batch Testing

Over the 24 d batch test, DRP and TP leaching from the woodchips occurred most rapidly within the first 24 h for both the control water and wastewater (Fig. 2). Less than 20 min was required to reach environmentally significant leached concentrations of at least 0.1 mg TP/L for the control; the wastewater treatment water exceeded this concentration before being added to the woodchips (initial wastewater: 2.48 mg TP/L; Table 1). Though these tests were only approximately half the length of those reported in the stream ecology literature (e.g., France et al.

(1997) assigned wood a conservative leaching period of 7 weeks), this clearly demonstrates the potential for rapid P losses upon woodchip bioreactor start-up.

First and second order models over-predicted P releases early in the test and under-predicted later on, which was similar to the DOC modeling results of McLaughlan and Mashaqbeh (2009) (Fig. 2). The Elovitch equation was a slightly better performing model for three of the four P relationships (all R^2 's > 0.90; Table 2), whereas the power law was slightly better than the Elovitch model for representing DRP release into wastewater (R^2 = 0.83). Chemical oxygen demand releases into deionized water were modeled very similarly by the power law and Elovitch equation (R^2 = 0.94 for both; Fig. 2d and Table 2), but the power law was a better predictor of COD release into wastewater than the Elovitch equation (R^2 of 0.94 vs 0.84, respectively; Fig. 2c and Table 2). McLaughlan and Al-Mashaqbeh (2009) surmised that early, rapid woodchip DOC leaching (i.e., within 24 h) was due to film diffusion which later transitioned to releases more driven by intra-particle diffusion. Rapid P leaching within the first 24 h was similar to this, suggesting the same mechanisms may be at play. The predictive models generally fit the deionized water data better than the wastewater (Table 2), which was not surprising as microbial activity supplied by the wastewater likely complicated P cycling.

To quantify the mass of P leached over time solely from the woodchips, the background TP and DRP concentrations in the initial deionized control (0.04 and 0.04 mg P/L, respectively; Table 1) and the initial wastewater (2.48 and 2.37 mg P/L, respectively) were subtracted from each sample value (i.e., the leached P concentrations were normalized for P initially present in the two water types). As an estimate of a “worst-case” leaching scenario, the greatest TP mass leached based on the highest normalized eluted P concentration was 1.2 mg in the control and 2.6 mg in the wastewater treatment jars, which corresponded to 26.9 and 30.6 mg TP leached per kg

of dry wt. woodchip, respectively, a metric which allows these batch tests to be put in context of other woodchip-based evaluations.

The P release trends were notably similar between the control and wastewater with strong, positive Pearson correlation coefficients (r) when the normalized deionized and normalized wastewater P concentrations were regressed against each other (TP: $r=0.885$, $R^2=0.78$; DRP: $r=0.778$, $R^2=0.61$). Pearson coefficient values greater than 0.7 are typically considered a strong correlation, between 0.3 and 0.7 is considered moderately correlated, and less than 0.3 is a poor correlation. Results of the comparison indicated an increase in concentration for one variable is correlated to an increase in the second variable, or more plainly, that the increases in concentrations were predominantly a result of the P leaching from chips and not from the solution used.

Woodchip P content generally declined during the batch test, which mirrored the initial increase in aqueous P concentrations (Fig. 3; samples at $t = 0$ h, 1 d, 9 d, and 24 d). The average estimated woodchip P mass in the control jars at $t = 0$ was 2.02 mg P (i.e., $t = 0$ P content of 0.0046% times the average woodchip dry weight of 44g). The mean P remaining in the chips by the end of the 24 day experiment was 0.97 mg based on a P content of 0.0022% (Fig. 3 control jars). Thus, over the 500 mL batch solution, 2.11 mg P/L was leached (i.e., 2.02 mg P minus 0.97 mg P, divided by the 500 mL volume). This was similar to the control solution leached P concentrations beyond approximately $t = 5$ d (Fig. 2), verifying the P balance in these jars.

3.1.1 Re-flooding Cycles

Phosphorus leaching was observed primarily during the first “flooding” event of the re-flooding test, with TP and DRP concentrations generally decreasing with each subsequent fill and drain

cycle (Fig. 4). The deionized water mean TP and DRP concentrations remained below approximately 0.20 mg P/L following the first cycle (Fig. 4b). However, these never fell below recommended values above which eutrophication is possible within freshwater bodies (e.g., 0.038 mg TP/L in US Midwestern lakes; USEPA, 2002), and net contribution of DRP and TP continued to occur compared to initial deionized values. The TP and DRP concentrations in the wastewater varied slightly between refill cycles, in part due to the variability of P in the continuously produced wastewater at this aquaculture facility. Nevertheless, only the first flooding cycle (TP) and first and second cycles (DRP) resulted P concentrations eluted from the woodchips that exceeded the initial wastewater concentrations (Fig. 4a). By the final flooding cycle, although the TP and DRP concentrations were still elevated above USEPA recommendations, these values were reduced to 0.99 mg TP/L and 0.60 mg DRP/L which represented 46% and 56% concentration reductions from the initial wastewater, respectively. Within context of the batch test where the jars were continuously saturated for 29 d, the re-flooding test showed the woodchips had the capacity to remove P from this wastewater given an initial leaching flush and subsequent cycles of drying.

3.2 Pilot-scale Woodchip Bioreactors

Bioreactor influent wastewater TP concentrations were generally higher than effluent concentrations regardless of HRT treatment during the first 165 d of pilot-scale bioreactor operation (Fig. 5a). However, there was an initial DRP flushing period for all four bioreactors (Fig. 5b), and the transition from DRP leaching to consistent removal was seen earliest in the 55 h retention time reactor at approximately d 47 (19 cumulative pore volumes or 23.6 m³ treated; first sample event after axis break in Fig. 5b). Transition to consistent DRP removal occurred by d 92 (55 cumulative pore volumes) for the 42 h retention time bioreactor neglecting one

remaining P contribution event from this bioreactor on d 155. Dissolved reactive P removal/loss was mixed for the 24 h treatment with effluent values usually close to influent, and no DRP removal occurred in the shortest retention time treatment. This variation in the time required to transition from DRP leaching to removal between treatments was consistent with findings from Schipper et al. (2010), who noted the duration and magnitude of leached organics from a woodchip bioreactor are partially dependent on the hydraulic retention time. Regarding cumulative pore volumes required to move through the leaching phase, extrapolation from Healy et al. (2012) showed approximately 20 pore volumes were required to flush COD concentrations to below influent levels for pine woodchips (300 d divided by a 13 to 17 d retention time). This was consistent with the longest retention time treatment here (19 pore volumes required), but the other treatments required additional pore volumes.

All four treatments resulted in cumulative TP removal with 15, 31, 54, and 54% removal efficiencies for bioreactors 1 through 4 that had retention times of 12 to 55 h, respectively (Table 3). However, due to the relatively lower loading of the highest retention time treatment (55 h), the P removal rate was slightly lower for bioreactor 4 (1.2 and 1.3 vs 0.9 g TP removed/d; Table 3). No cumulative DRP mass loading reduction occurred for the two shortest retention time treatments (-28, -4, +28, and +35% removal for bioreactors 1 through 4 operating at 12, 24, 42, and 55 h, respectively; Table 3). Clearly there were important loading rate impacts for P removal. The high retention time treatments (i.e., low flow rates, low loading) had the greatest P removal efficiencies, but a slightly shorter retention time might help optimize nutrient removal on a mass basis. For example, the 42 vs 55 h treatments had removal rates of 1.3 vs 0.9 g TP/d and 0.4 and 0.3 g DRP/d. These results must ultimately be weighed with the importance of retention time for N removal. Shorter retention times can maximize N removal rates as long as

the retention time is not so short as to preclude development of anaerobic conditions (Lepine et al., 2015). While TP removal via sorption or settling of particulates is not dependent on the formation of anaerobic conditions, the importance of retention time in this case may be related to the woodchip/wastewater contact time.

Regressing the mg TP/L removed (i.e., influent TP concentration minus effluent TP concentration) by the influent TP concentration resulted in a statistically significant positive linear correlation (R^2 : 0.29; $p < 0.0001$; data not shown), whereas the same analysis for DRP did not result in a significant relationship (regressing mg DRP/L removed vs. influent DRP concentration; R^2 : 0.006; $p = 0.24$; data not shown). In other words, at relatively higher TP influent concentrations, greater TP removal occurred, but the same was not true for DRP. This is supporting evidence that TP removal in this wastewater application was comprised in large part by removal of particulate P.

Comparing the four pilot-bioreactor treatments based on cumulative pore volumes (or, flow treated), the bioreactor with the highest flow rate (12 h retention time) removed the lowest cumulative TP mass (Fig. 6a), though this treatment's TP removal was comparable to the other flow treatments when evaluated across the study period (i.e., Table 3: 202 g TP removed at d 165). Similar to the time-based cumulative results (i.e., Table 3), the shortest retention time treatment reactor consistently exported DRP, whereas the two longest retention treatments removed DRP (Fig. 6b). This further indicates there may be an important design retention time impact to mitigate P flushing from woodchip bioreactors. For example, after treatment of 60 pore volumes (or, 74.3 m³ wastewater treated), bioreactor 1 operating at a 12 h retention had removed approximately 65 g TP (on d 31), while bioreactor 4, which was operating at a 55 h retention, removed approximately 132 g TP (d 144). A slower flow rate provided increased TP and DRP

removal, although this type of design will require a longer time period or a larger reactor volume to treat a given wastewater volume.

3.3 Batch-scale versus pilot-scale bioreactor flush test comparison

The Flush test at the start of pilot-scale bioreactor operation (i.e., flooding of the bioreactors for ≈ 28 h, then draining) allowed a nearly equal comparison of the P leached per mass of woodchip between the batch test at the 24 h sample event (wastewater matrix) and the pilot reactors at a 28hr ‘static’ retention time. The pilot-bioreactor Flush test P concentrations were higher than the batch test P concentrations due to the difference in the woodchip-to-water ratio in the two tests (Table 1 “Effluent”). That is, the woodchips in the Batch Test were able to leach P into a relatively greater volume of water over 24 h compared to the saturated woodchips in the 28 h pilot Flush test. Nevertheless, the mg P leached/kg woodchip should theoretically be similar between the Batch and Flush tests, as the Flush test acted as a scaled-up “batch test”. Indeed, the TP leached per kg woodchip was not statistically different between the three treatments (17.1, 16.0, and 12.4 mg TP/kg chip for the batch test deionized control, batch test wastewater treatment, and pilot Flush test, respectively; $p = 0.729$; Table 1). However, the DRP leached per chip from the pilot bioreactors during the Flush test (16.9 mg DRP/kg chip) was significantly greater than that from the Batch Test’s deionized control (12.4 mg DRP/kg chip) and wastewater treatment (8.35 mg DRP/kg chip) ($p = 0.002$; Table 1).

Deeper investigation into the wastewater used for the pilot Flush test showed a relatively smaller percentage of the initial wastewater TP load was in the dissolved form compared to the wastewater used in the batch test (Table 1 “Influent”: 52% of 5.00 mg TP/L occurred as DRP vs. 96% of 2.48 mg TP/L as DRP, respectively). It follows that the pilot-scale bioreactors received a

much higher fraction of solids-bound P during start-up than the Batch Test reactors. This inconsistency may be attributed to the variability in the wastewater on the dates the Batch and Flush tests were initiated. Nevertheless, it was likely the particulate P in the Flush pilot-bioreactor influent wastewater was essentially removed from the flow via woodchip filtration. This fraction of the TP load remained in the bioreactor to become further mineralized. The relatively higher DRP concentration as a percent of TP concentration exiting the pilot bioreactors at the conclusion of the Flush test (88% of 8.70 mg TP/L occurred as DRP; Table 1) compared to that entering the bioreactors (52% of 5.00 mg TP/L as DRP; Table 1) was strong supporting evidence of this filtration and mineralization. Assuming conservation of P mass inside the bioreactor for the one pore volume during the Flush Test, 3.0 g particulate P entered the bioreactors in the wastewater, whereas only 1.3 g were eluted (i.e., based on the difference between TP and DRP concentrations times one pore volume of 1240 L). This confounding factor of wastewater-associated particulate P, and its suspected conversion over the test to DRP (which was then eluted) likely explained why the mg DRP per kg dry woodchip was nearly twice for the pilot Flush Test compared to the Batch Test (Table 1). Additional supporting evidence for this contribution of filtered particulate P load is the extremely high solids removal across the bioreactors based on TSS (> 90% TSS removal across all bioreactors and sample dates; data not shown). Sharrer et al. (2010) similarly showed settled aquaculture wastewater solids degraded and mineralized within a geotextile bag filter, releasing DRP over time and further corroborating the findings here. Woodchip bioreactor treatment of this type of wastewater will likely require a pre-treatment step for solids removal.

4. Conclusion

Four pilot-scale bioreactors consistently removed TP from aquaculture wastewater with a maximum removal efficiency of 54%. All bioreactors initially exported DRP, although the lower flow rate treatments eventually resulted in DRP removal. Wastewater solids may have contributed more to longer-term P leaching than the woodchips. Nevertheless, woodchips are an important source of P exiting bioreactors during start-up with a leaching potential of approximately 20-30 mg P per kg wood (over 24 d; $\approx$ 10-20 mg P/kg wood over 24 h). Woodchip P leaching was multi-phasic with the most rapid release in the first 24 h regardless of the water matrix, and was best modeled using the Elovitch equation.

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Fig. 1. Schematic of the pilot-scale woodchip bioreactor setup: 1: treatment water mixing tank; 2: sodium nitrate dosing tank; 3: Masterflex peristaltic pump; 4 through 7: 12 h, 24 h, 42 h and 55 h treatment water vessels; 8-11: treatment tank pumps; 12-15: inlet manifolds; 16-19: outlet manifolds; 20: effluent sump.

Fig. 2. Total phosphorus (a), dissolved reactive phosphorus (b), and chemical oxygen demand (c and d) concentrations eluted from woodchips into either deionized water (control; panels a, b, d) or wastewater (treatment; panels a, b, c) over a 24-day batch test.

Fig. 3. Woodchip total phosphorus content (% P) at four sampling dates during the 24 d batch test; points jittered slightly to show error bars; initial P content of fresh chips was $0.014\% \pm 0.0006\%$ (mean $\pm$ SD, $n=2$) where the $t = 0$ sample event was chips that had been submerged for less than 3 min.; $n = 2$.

Fig. 4. Total phosphorus (TP) and dissolved reactive phosphorus (DRP) concentrations for five 24 h re-flood events each six days apart ($n = 2$) for wastewater (a) and the deionized control (b); initial deionized control values were assumed to be the same as the Batch Test control initial sample (Table 1).

Fig. 5. Influent and effluent TP (a) and DRP (b) concentrations for four plot-scale bioreactors with hydraulic retention time of each bioreactor indicated in the legend; note modified x-axis to

507 more clearly show that water samples were collected more frequently from d 0 to 40, then
508 weekly.

509

510 **Fig. 6.** Cumulative total phosphorus (a) and dissolved reactive phosphorus (b) mass (g) removed
511 (or eluted, negative values) from four pilot-scale woodchip bioreactors operating at different
512 retention times shown by cumulative pore volumes.

513

List of Tables

Table 1

Initial/influent water quality parameters for the deionized water (control), aquaculture wastewater used in the Batch Test (effluent values for the 24 h sample), and pilot-scale bioreactor Flush Test (28 h retention time) along with the effluent and normalized effluent concentrations and associated total phosphorus (TP), dissolved reactive phosphorus (DRP), and chemical oxygen demand (COD) leached per dry weight of woodchips at 24 to 28 h; Batch $n = 2$, Pilot Flush test $n = 4$.

	Experiment	Initial/ Influent mg/L	Effluent ^a mg/L	Normalized effluent ^b mg/L	mg P or COD leached per kg dry woodchip ^c ---
TP	Batch - Control	0.04	1.55 (0.12)	1.51	17.1 (1.4) a
	Batch - Wastewater	2.48	3.89 (0.40)	1.41	16.0 (4.6) a
	Pilot Flush	5.00	8.70 (2.65)	3.70	12.4 (8.9) a
DRP	Batch - Control	0.04	1.13 (0.00)	1.09	12.4 (0.0) b
	Batch - Wastewater	2.37	3.11 (0.04)	0.74	8.35 (0.4) b
	Pilot Flush	2.60	7.63 (0.49)	5.03	16.9 (1.6) a
TN	Batch - Control	0.65	1.40 (0.0)	0.75	---
	Batch - Wastewater	66.3	48.6 (6.01)	^d	---
	Pilot Flush	84.6	13.0 (0.91)	^d	---
NO ₃ -N	Batch - Control	0.3	0.10 (0.00)	^d	---
	Batch - Wastewater	55	24.4 (1.56)	^d	---
	Pilot Flush	21	1.05 (1.10)	^d	---
COD	Batch - Control	5.5	336 (63)	330	3,760 (715)
	Batch - Wastewater	49.5	262 (8.8)	212	2,410 (100)
	Pilot Flush	256	1380 (39)	1121	3,770 (129)

^a Mean (SD); Batch effluent concentrations were for the $t = 24$ h sample and Pilot Flush effluent concentrations represent a 28 h HRT

^b Subtracted influent concentrations from effluent concentrations to indicate woodchip contribution

^c Mean (SD) based on normalized P concentrations; Means followed by the same letter for TP or for DRP are not significantly different at $\alpha = 0.05$ (one way ANOVA)

^d Indicates water quality improvement, i.e., nutrient removal

Table 2

Total and dissolved reactive phosphorus and chemical oxygen demand release models from woodchip batch tests with either aquaculture wastewater or deionized water.

	----- DRP -----		----- TP -----		----- COD -----	
	Wastewater	Deionized Water	Wastewater	Deionized Water	Wastewater	Deionized Water
<i>First order model</i>						
M_o (mg P/L)	3.38	1.50	4.18	1.99	803	633
τ_1 (d)	0.015	0.329	0.011	0.133	4.9	2.0
R^2	0.34	0.75	0.31	0.73	0.74	0.87
p	0.017	<0.0001	0.025	<0.0001	<0.0001	<0.0001
<i>Second order model</i>						
M_o (mg P/L)	3.50	1.58	4.33	2.12	917	703
τ_2 (d)	0.010	0.204	0.007	0.108	3.61	1.48
R^2	0.50	0.85	0.58	0.84	0.78	0.91
p	0.002	<0.0001	0.0006	<0.0001	<0.0001	<0.0001
<i>Power law function model</i>						
A (mg/(L-d))	3.21	1.02	4.05	1.51	281	286
B (dimensionless)	0.083	0.208	0.064	0.166	0.35	0.30
R^2	0.83	0.93	0.89	0.88	0.94	0.94
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<i>Elovitch equation model</i>						
α (mg/L)	135310	41.35	2938203	137.8	665	1306
β (mg/(L-d))	4.03	4.68	3.97	3.85	0.0057	0.0079
R^2	0.80	0.96	0.90	0.92	0.84	0.94
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

535 **Table 3**

536 Total Phosphorus and Dissolved Reactive Phosphorous mass balance and removal metrics for
 537 four woodchip bioreactors over a 165 d period.

	----- Total Phosphorus -----				Dissolved Reactive Phosphorus			
Bioreactor No.	1	2	3	4	1	2	3	4
Hyd. Retention Time (h)	12	24	42	55	12	24	42	55
P influent load (g)	1310	696	402	277	764	405	234	163
P effluent load (g)	1108	482	185	128	976	423	168	106
P load removed (g)	202	214	216	149	-213	-18	66	57
Removal Efficiency (%)	15	31	54	54	-28	-4	28	35
g P removed per d	1.2	1.3	1.3	0.9	-1.3	-0.1	0.4	0.3

538

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6