Nitrate removal and nitrous oxide production from upflow and downflow column woodchip bioreactors

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1 | INTRODUCTION

Nitrate-nitrogen (N) loads to the Gulf of Mexico are considered a major contributor to the gulf hypoxic zone (Rabalais, Turner, & Wiseman, 2002). Efforts to curb losses include improving field N management, changing cropping system or land use, and managing tile drainage effluent with structural practices at the edge of field or in drainage ditches (MPCA, 2013). One structural practice is the woodchip denitrifying bioreactor (WDBR), which removes nitrate-N via dissimilatory denitrification (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010). Flow is typically horizontal through WDBRs designed for tile drainage systems, although vertical flow is common in other water treatment systems (Ilyas & Masih, 2018; Tanner, Sukias, Headley, Yates, & Stott, 2012; Vymazal, 2011). Changing WDBR design to vertical flow could reduce short circuiting (Christianson et al., 2020).

Nitrate-nitrogen removal rate (NRR) of WDBRs is strongly dependent on hydraulic residence time (HRT) and temperature (Hoffman, Larsen, & Kjaergaard, 2019;
Hoover, Bhandari, Soupir, & Moorman, 2016). Bioreactors typically operate with HRTs from 6 to 20 h (Addy et al., 2016), with longer times required during colder springtime conditions (Hoover et al., 2016) when a substantial portion of annual tile drainage flows in the northern U.S. Corn Belt (Jin & Sands, 2003). Corn (Zea mays L.) cobs as a denitrifying medium have been shown to increase NRR compared with woodchips (Cameron & Schipper, 2010), including at low temperatures (2 °C) (Feyereisen et al., 2016). Roser et al. (2018) showed that carbon (C) dosing woodchips with acetate increased nitrate-N load reduction from 5 to 33% at 5 °C and 1.5-h HRT. Shortening HRT by C dosing could reduce WDBR size.

During the denitrification process, nitrous oxide ($N_2O$), a potent greenhouse gas, is produced (Bremner, 1997). Under typical field conditions, i.e., HRT of 8 h, denitrification of $NO_3^-$ to $N_2$ gas is nearly complete and release of $N_2O$ is negligible; however, as HRT is shortened, $N_2O$ releases could increase (Davis, Martin, Moorman, Isenhart, & Soupir, 2019). Additionally, there is no information on the direction of water flow in WDBRs on potential $N_2O$ production. Therefore, a laboratory-scale experiment was designed and conducted to examine flow direction on column bioreactor performance by (a) quantifying nitrate-N removal and NRR and (b) investigating production of $N_2O$ at short HRT.

2 | MATERIALS AND METHODS

2.1 | Bioreactor design and operation

Upflow and downflow columns were packed in triplicate with 22.9 cm of corn cobs (269 ± 8 g dry) at the inlet, followed by 22.9 cm of wood chips (mixed species, primarily hardwood, 13 x 15 x 5 mm; 336 ± 22 g dry), then a 5.1-cm-thick layer of a woven polymeric mat (Bro-Tex), and finally 7.6 cm of lava rock (10- to 60-mm diam.; Vigoro). The purpose of the mat and lava rock were to provide area for biofilm development and space for processing additional C in effluent prior to exiting the system. The outlets of the downflow columns were plumbed to the height of the perforated plate to maintain media saturation. Peristaltic pumps mixed nutrient and potassium acetate solutions (90%:10% nutrient solution/acetate solution; final concentrations: nitrate-N, 20 mg N L$^{-1}$; dissolved P, 0.3 mg P L$^{-1}$; acetate, 93 mg C L$^{-1}$) prior to the inlet of the upflow columns. For the downflow columns, the mixed solutions were pumped onto a perforated plate at the top of the column, which held media in place and distributed the influent. Gravity effected downflow.

Inoculation was achieved by two means. The first was direct mixing of 10 g of oven-dried (48 h, 60 °C) woodchips taken from an operating field bioreactor 19 mo prior (Willmar, MN) with new corn cobs and woodchips during column packing. The second means was soaking (48 h) the column packing materials with effluent from an operating woodchip bioreactor (Blue Earth, MN). Porosity for HRT calculations was determined by draining saturated columns for 24 h.

Water was circulated through the columns (18 d) to detect leaks and clear detritus from the media (Supplemental Figure S1). Flow rates equivalent to a 12-h HRT (4 ml min$^{-1}$) were established at 10 °C and the nutrient solution was introduced for 16 d, after which the acetate solution was added (90%:10% nutrient solution/acetate solution). Flow rates were adjusted to 2-h HRT (23.5 ml min$^{-1}$) during the week (Monday–Friday). Flow rates were reduced to a nominal 12-h HRT on Fridays at 12:00 and acetate additions paused over the weekend because the volume of water needed for the experiment was substantial and weekend oversight of the experiment was limited. Acetate additions and a 2-h HRT were reestablished on Mondays at 13:00 ± 1:00.

Seven days after acetate introduction, the acetate pump failed (Supplemental Figure S1). For the next 7 d, weekend conditions were established: 12-h HRT without acetate addition. The weekday/weekend flow regime was reestablished for the remaining 35 d; data from this period were used for statistical analysis.

2.2 | Sample collection and analysis

Water samples for nutrient analysis were collected on Mondays (12-h HRT) and Thursdays (2-h HRT) from inlets and outlets. Samples for nutrient analysis were filtered (0.45 μm; polyethersulfone), refrigerated (4 °C), and analyzed on Mondays and Thursdays for nitrate-N.
(NO$_2^-$-N+NO$_3^-$-N) and ammonium-N, colorimetrically by flow injection (QuickChem 8500; Lachat).

Samples for dissolved gas analysis were collected Mondays and Thursdays for the final 32 d of the experiment with one 3-ml draw with a disposable syringe (BD: model 309604) through stop cocks (Kimble 420163-0000) plumbed into the inlet and outlet lines. The water was injected into a 20-ml vial previously sealed with a butyl rubber septum and then flushed with helium. Samples were equilibrated (22 ± 1 °C) and analyzed following a minimum of 24 h to allow for equilibrium between the dissolved and headspace N$_2$O concentrations. Samples were analyzed with an automated headspace sampler (Agilent 7694E) plumbed directly to a customized gas chromatographic system (Agilent; HP-5890) with N$_2$O quantified by an electron capture detector (Spokas, Koskinen, Baker, & Reicosky, 2009). Dissolved N$_2$O was then estimated through assuming ideal gas law behavior and using Henry’s law coefficient for N$_2$O.

Flow rates were measured with a bottle, scale, and stop-watch. Loads were calculated by multiplying flow rates by time between flow rate measurements by concentration. Beginning at 33 d after initial addition of acetate, the downflow columns began to overflow as a result of biofilm formation. The overflow was captured and measured. Nitrate-N removal rate (g N m$^{-3}$ d$^{-1}$) was calculated as:

$$\text{NRR} = \left( \frac{\text{NLoad}_{\text{rem}}}{t_i} \right) / \text{Vol}_{\text{med}}$$

where NLoad$_{\text{rem}}$ is nitrate-N load removed between samplings, $t_i$ is time between previous and current samplings, and Vol$_{\text{med}}$ is gross volume of the media. Nitrate-N load reduction (%) was calculated as:

$$\text{NLoadReduction} = \frac{\sum_{i=1}^{n} \text{NLoad}_{\text{rem}}}{\sum_{i=1}^{n} \text{NLoad}_{\text{in}}} \times 100$$

where NLoad$_{\text{rem}}$ is as defined above, $t$ is time between samplings, $n$ is the number of samplings, and NLoad$_{\text{in}}$ is the nitrate-N load at the column inlet between samplings.

Cumulative N$_2$O production, cpN$_2$O, (mg N), was calculated as:

$$\text{cpN}_2\text{O} = \sum_{i=1}^{n} (d\text{N}_2\text{O}_{\text{out}} \times \text{Vol}_i)$$

where $t$ and $n$ are defined as above, dN$_2$O$_{\text{out}}$ is N$_2$O concentration at the outlet, and Vol$_i$ is the volume of effluent since the previous sampling. The relative production of N$_2$O to nitrate-N removed, rN$_2$O (%), was calculated as:

$$r\text{N}_2\text{O} = \frac{\text{cpN}_2\text{O}}{\sum_{i=1}^{n} \text{NLoad}_{\text{rem}} \times 100}$$

where cpN$_2$O, $t$, $n$, and NLoad$_{\text{rem}}$ are as defined above.

Data were analyzed at $P \leq .05$ using the MIXED procedure of SAS v.9.4 (SAS Institute, 2013), with flow direction and nominal HRT as fixed effects, week as a fixed effect and repeated measurement, and replication and interactions with replication as random effects. Outlet dN$_2$O was logarithm base 10 transformed to meet the requirements of normality and common variance. When main effects or interactions for fixed effects were significant, means were compared with pairwise $t$ tests using the PDIF option of the MIXED procedure of SAS.

### 3 RESULTS AND DISCUSSION

Cumulative nitrate-N load reduction over the 35-d experiment was not significantly different between the upflow and downflow columns, 21.3 and 27.5%, respectively (Table 1; $P = .13$; Supplemental Figure S2). Across the flow direction treatments, a greater percentage of nitrate-N was removed at 12-h HRT (35.1%) than at 2-h HRT (22.2%). The value for the 12-h HRT is identical to the findings of Hoover et al. (2016), 36 ± 4%, for laboratory columns with woodchips at the same HRT, temperature (10 °C), and inlet nitrate-N concentration. Feyereisen et al. (2016) tested columns with woodchips followed by corn cobs at 1.5 and 15.5 °C and reported nitrate-N removal of 15 and 62%, respectively, which brackets the current findings. No ammonium concentrations were above detection limit (0.005 mg N L$^{-1}$) for downflow samples and only a few were above detection limit for upflow samples (data not shown), suggesting that nitrate-N removal was primarily by denitrification and not dissimilatory nitrate reduction to ammonium.

There were no significant differences in rN$_2$O or dN$_2$O between upflow and downflow treatments across HRTs, although variability tended to be lower for the downflow treatment, particularly at 2-h HRT (Table 1; Figure 1). Across flow directions, rN$_2$O was not significantly different ($P = .14$) between 2- and 12-h HRT with high variability; means (SE) were 0.15 (0.09) and 0.39 (0.06)%, respectively. Dissolved N$_2$O concentrations at the outlet, averaged across HRTs and weeks, were significantly greater for 12-h than for 2-h HRTs ($P = .01$); back-transformed means were 23.2 and 5.3 µg N L$^{-1}$, respectively. There were significant dN$_2$O differences among weeks with dN$_2$O declining until the third week and then stabilizing (Figure 1). Dissolved N$_2$O was greater for upflow through Week 3;
TABLE 1 Cumulative nitrate-N load reduction, relative N\textsubscript{2}O production (rN\textsubscript{2}O), dissolved N\textsubscript{2}O outlet concentrations (dN\textsubscript{2}O), and nitrate-N removal rate (NRR) for bioreactor columns operated in two flow directions at two hydraulic residence times (HRT)

| HRT (h)  | Flow direction       | Cumulative NO\textsubscript{3}-N load reduction, % | rN\textsubscript{2}O, % | dN\textsubscript{2}O, μg N L\textsuperscript{-1} | NRR, g N m\textsuperscript{-3} d\textsuperscript{-1} |
|----------|----------------------|-----------------------------------------------|-----------------|----------------|-----------------|
|          | Up and Down          | Up                                            | Down            | Up and Down    | Up and Down    |
| 2 & 12\textsuperscript{a} | 21.3 (2.2) A\textsuperscript{b} | 27.5 (2.4) A       | 0.28 (0.12) A    | 16.7 (16.8) A        |
| 2        | 19.1 (2.0)\textsuperscript{d} | 25.4 (2.4)        | 0.22 (0.12)     | 6.4 (4.1)       |
| 12       | 32.2 (3.7)           | 38.1 (5.6)        | 0.49 (0.23)     | 27.1 (22.2)     |
| 2        | 22.2 (2.0) a         | 35.1 (3.3) b      | 0.15 (0.06) a   | 5.3 (2.4) a      |
| 12       | 35.1 (3.3) b         | 0.39 (0.13) a     | 0.39 (0.13) a   | 23.2 (13.5) b    |

a Values in column “Up and Down” represent mean (SE) across flow directions (n = 6).

b Values in rows with “2 & 12” in HRT column represent mean (SE) of treatment columns (n = 3) throughout the experiment across HRTs.

Means (SE) within a row for each variable followed by the same uppercase letter are not significantly different at P \leq 0.05; means within a column for each variable followed by the same lowercase letter are not significantly different at P \leq 0.05.

Means (SE) within a row and without a letter were not compared statistically because the ANOVA p value for the interaction between HRT and flow direction was not significant at P \leq 0.05.

dN\textsubscript{2}O for the final two weeks was not significantly different between flow directions (Figure 1).

Feyereisen et al. (2016) reported average rN\textsubscript{2}O of 0.92% across 1.5 and 15.5 °C with dN\textsubscript{2}O from 2 to 164 μg N L\textsuperscript{-1}. In another column experiment, Feyereisen, Christianson, Moorman, Venterea, and Coulter (2017) found that rN\textsubscript{2}O averaged across treatments of corn cobs and corn cobs followed by a layer of plastic biofilm carrier was 0.3 and 1.6% at 15.5 and 1.5 °C, respectively. Davis et al. (2019) measured dN\textsubscript{2}O and N\textsubscript{2}O emissions from the surface of uncapped field pilot-scale WDBRs at 2-, 8-, and 16-h HRTs. Dissolved N\textsubscript{2}O comprised >97% of N\textsubscript{2}O fluxes, with total ratios of dN\textsubscript{2}O-to-NO\textsubscript{3}\textsuperscript{-} removed of 5.19, 0.35, and 0.52%, for 2-, 8-, and 16-h HRTs.

Based on previous findings, such as Davis et al. (2019), and the temperature and step-based nature of denitrification wherein the last step to be mediated is from N\textsubscript{2}O to

FIGURE 1 Time plot of dissolved N\textsubscript{2}O concentration
N₂ (Wallenstein, Myrold, Firestone, & Voytek, 2006), we expected N₂O production for the 2-h HRT to increase. In this respect, our findings were unexpected. Apparently, the addition of readily available C via acetate addition provided ample electron donor capacity to maintain nearly complete denitrification (Hanaki, Hong, & Matsuo, 1992). Although not significantly different, the suggestion of lower N₂O production for downflow could be a result of gas diffusion gradient counter to water flow direction (Bruun, Hoffmann, & Kjaergaard, 2017) or additional aeration at the tops of the downflow columns, which were open to atmosphere (Pijuan et al., 2014). The lower variability in the downflow columns is most evident in the standard errors in rN₂O (Table 1).

For NRR, neither the interaction of flow direction × HRT (P = 0.36) nor the main effect of flow direction (P = .16) were significant (Table 1). Averaged across flow directions, NRR at 2-h HRT was greater than at 12-h HRT, 30.1 vs. 11.8 g N m⁻³ d⁻¹, respectively (Table 1). Averaged across flow directions, NRR at 12 h was slightly greater than that reported by Hoover et al. (2016), 6.9 ± 0.3 g N m⁻³ d⁻¹, and again bracketed by values from Feyereisen et al. (2016) at 1.5 and 15.5 °C, 6.8 and 29.3 g N m⁻³ d⁻¹, respectively. Two factors explain the 2.6-fold increase in NRR at the shorter HRT. First, as input loading into a WDBR is increased by greater flow rate, NRR tends to increase (Greenan, Moorman, Parkin, Kaspar, & Jaynes, 2009; Pluer, Geohring, Steenhuis, & Walter, 2016). Second, the addition of readily available C increases electron donor availability for denitrification (Lemaire et al., 2006). Roser et al. (2018) reported a 2.4- and 3.1-fold increase in NRR with C dosing and woodchips at 12-h HRT at 5 °C.

Addition of C poses the challenge of bioclogging of woodchip bioreactors (Anderson, Jang, Venterea, Feyereisen, & Ishii, 2020). Given the limited gravity head gradient driving flow, the downflow columns were susceptible to bioclogging at the ratio of C/N of this experiment (Fig. S3). The issue of bioclogging in denitrification bioreactors has been noted by others (Inês, Soares, Braester, Belkin, & Abeliovich, 1991) and remains an issue to be solved. Solutions may include reducing C/N or adjusting the location of C delivery. However, the benefits in dramatically increasing NRR at high flow and low temperatures continue to be worth further study.

4 | CONCLUSIONS

The overall purpose of this laboratory experiment was to evaluate the influence of vertical column flow direction on bioreactor performance. There was no significant difference observed in nitrate-N removal rates as a function of column flow direction. Additionally, the data collected here confirm a lack of significant difference in N₂O production potentials, although the downflow direction did result in numerically lower values. Production of N₂O was reduced with C additions at short HRTs, although individual effects of C addition and HRT were uncertain from this experiment. A necessary consideration for downflow bioreactors is the microbial clogging of water flow. This biofilm production must be further evaluated prior to adding supplemental C in downflow field bioreactors.

AUTHOR CONTRIBUTIONS

STATEMENT

Conceptualization, GWF, KAS, JSS, DJM, and AZR; Methodology, GWF, KAS, JSS, and JAC; Validation, GWF, KAS, and JAC; Formal Analysis, JAC, KAS, and GWF; Investigation, GWF, KAS, JSS, DJM, AZR, and JAC; Resources, JSS, KAS, and GWF; Data Curation, GWF, KAS, and JAC; Writing–Original Draft Preparation, GWF, KAS, and JAC; Writing–Review & Editing, GWF, KAS, DJM, AZR, and JAC; Visualization, GWF, KAS, and JAC; Supervision, GWF, KAS, and JSS; Project Administration, JSS; Funding Acquisition, JSS.

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DATA AVAILABILITY STATEMENT

The data used for the analyses herein are available at Feyereisen (2020), https://doi.org/10.5061/dryad.hqbzkh1d2.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**SUPPORTING INFORMATION**
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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