Short Hydraulic Residence Times with Corn Cob-Woodchip Flow Columns Using Labile Carbon Addition



August 2020

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Table of Contents

Acknowledgments	3
Executive Summary	4
Project Objectives	5
Publication Note	5
Background	6
Description of Work Performed	6
Run 1	7
Run 2	11
Conclusions	12
Future Needs/Plans	12
References	13
Appendix	18

List of Tables

Table 1	Cumulative nitrate-N load reduction, nitrate-N removal rate, relative N ₂ O production (rN_2O) , and dissolved N ₂ O outlet concentration (dN_2O) for bioreactor columns operated in two flow directions at two hydraulic residence times (HRT) for Run 1	ction 8
Appendix	Average outlet concentrations for Run 1 for ammonium-N, dissolved reactive phosphorus, total phosphorus, and dissolved organic carbon	18
List of Fig	ures	
Figure 1	Schematic of a denitrifying bioreactor	15
Figure 2	Pictures of water supply tanks, pumps, and columns	15
Figure 3	Diagrams of upflow and downflow columns	16
Figure 4	Pictures of extracellular polymeric substance (EPS)	17
Figure 5	Pictures of extracellular polymeric substance (EPS)	17

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the funding for this project provided by the Agricultural Utilization Research Institute (AURI). Working spaces for the various experimental setups were provided by the Horticultural Science Department of the University of Minnesota. We acknowledge Todd Schumacher, Marta Roser, Lizzy Selvik, Olivia Richter, Martin DuSaire, and Sam Okkema for their technical assistance.

EXECUTIVE SUMMARY

Purpose

Nitrogen (N) losses in the form of nitrate from tile-drained row crop agriculture in the Mississippi River Basin contribute to ecological challenges in the Gulf of Minnesota. One approach to reducing nitrate-N losses is to treat drainage water with a denitrifying bioreactor. The typical bioreactor routes drainage water through a bed of woodchips; hydraulic retention times (HRT) are on the order of a third to half a day. The purpose of the reported research was to test whether carbon (C) additions to a combination of woodchips and corn cobs could increase the rate of nitrate-N removal in vertically oriented laboratory columns. Reduced HRT and vertical orientation, which provides a shorter hydraulic flow path, would permit more compact designs that could be situated in or near drainage ditches.

Major Points

It is possible to improve the rate of Nitrate-N removal with the addition of readily available carbon (C).
 The Nitrate-N removal rate for the treatment with C additions and 2-h HRT was 2.6-fold

that of the treatment without C addition and 12-h HRT.

- It is possible to shorten Hydraulic retention times (HRT) to two hours without increasing greenhouse gas emissions.
 In contrast to expectation, the treatment with 2-hr HRT and C addition did not increase emissions of nitrous oxide (N₂O), a greenhouse gas. Without C additions, others have reported a significant increase in N₂O emissions for 2-h vs. 8- or 16-h HRT.
- Formation of extracellular polymeric substance (EPS) during C addition inhibits flow. Bio-clogging during the first experiment prevented planned experiments at 1- and 0.5-h HRT. An attempt to reduce bio-clogging by introducing the C in the middle of the bioreactor column actually increased the severity of bio-clogging.
- Nitrate-N removal performance is similar between vertical upflow and downflow designs.

There were no significant differences in cumulative nitrate-N load removal or nitrate-N removal rate between upflow and downflow directions, although performance tended to be numerically better for the downflow design.

Key Recommendations

• Finish analysis of results from field pilot trial using the system with downflow and C addition.

Field trials at the University of Minnesota Southwest Research and Outreach Center near Lamberton, Minnesota, have been conducted for a couple of seasons. Placing results from those trials alongside the results of this research study offers a perspective on how well the laboratory work did or did not mimic field results.

Pursue a more fundamental understanding of EPS (extracellular polymeric substance) formation and how to avoid its production.
 Some progress has been realized in the field pilot bioreactors by reducing the amount of C added, but there is some loss of nitrate-N removal efficiency in doing so. Other potential avenues for investigation include the use of enzymes to disrupt microbial

communication and formation of EPS and the use of microfluidics to determine whether precise timing and location of C addition would diminish EPS formation.

Project Objectives and Hypothesis

• Quantify nitrate-N removal rates of the experimental system for HRTs of 0.5 to 2 hours at one fixed rate of acetate addition and a temperature of 10°C (50°F).

Hypothesis 1: The nitrate-N removal rate will diminish with shorter HRTs but still be greater than for woodchips.

• Confirm the effectiveness of a downflow vs. an upflow design.

Hypothesis 2: Nitrate-N removal rates will be equivalent for columns of upflow and downflow designs.

Publication Note

The results from Run 1 have been published as a peer-reviewed scientific journal article in *Agricultural and Environmental Letters*: <u>"Nitrate removal and nitrous oxide</u> <u>production from upflow and downflow column woodchip bioreactors."</u> (Feyereisen et al., 2020). <u>Nitrate removal and nitrous oxide production from upflow and downflow</u> <u>column woodchip bioreactors - Feyereisen - 2020 - Agricultural & amp; Environmental</u> <u>Letters - Wiley Online Library</u>

BACKGROUND

Woodchip denitrifying bioreactors (Fig. 1) can reduce nitrate-N concentrations from agricultural tile effluent. The rate of removal is dependent on temperature and hydraulic residence time (HRT), that is the time water spends in the bioreactor bed. Recommended HRT is >3h (USDA-NRCS, 2015) but typical design HRTs are 8h and longer times are required to support effective nitrate-N removal under cooler temperatures (Hoover et al., 2016). It is important to design bioreactor beds large enough to accommodate the longer HRTs. However, increasing the rate of nitrate-N removal would allow a decrease in bed size, thus reducing the cost of construction, or would support processing more water in the same footprint, again improving the economics of removing nitrate-N. Also, shortening HRTs may lead to bioreactor solutions in or near drainage ditches, where flow is great and multiple-hour HRTs are unrealistic.

Laboratory column experiments have shown that nitrate-N removal rates for corn cobs are greater than for woodchips (Feyereisen et al., 2016) and that nitrate-N removal rates for woodchips at a 1.5-h HRT can be enhanced by an order of magnitude (10X) through the addition of acetate, which provides a readily available source of carbon (C) for denitrifying microbes (Roser et al., 2018). Following these results, novel pilot-scale bioreactors were developed to test the concept of short HRTs with C additions in the field (Strock et al., 2017). These pilot bioreactors were designed for use in or near drainage ditches and were based on vertical downflow of tile effluent. The research work reported herein represents testing of the same material combinations as the field pilot bioreactors. The overall goals were to quantify nitrate-N removal rates for short HRTs while comparing the performance of vertical upflow versus vertical downflow in a controlled laboratory setting.

DESCRIPTION OF WORK PERFORMED

A system was designed and constructed in a temperature-controlled chamber in the greenhouse basement at the University of Minnesota – St. Paul (Fig. 2) for the purpose of pumping a synthetic water solution through six PVC column bioreactors (4-in. diameter by 19 in. long), three upflow and three downflows. Chemicals were added to reverse osmosis water to reflect nitrate-N and dissolved phosphorus concentrations of agricultural drainage water in Minnesota, resulting in a synthetic nutrient solution. The water solution was tempered in tanks within the chamber at the experimental temperature of 50°F (10°C).

The researchers conducted two experiments: Run 1 and Run 2. Run 1 lasted 10 weeks. Acetate solution was pumped with the synthetic nutrient solution at a 2-h HRT from Monday mornings until noon Fridays. At noon Friday, the flow rate was reduced to 12-h HRT to reduce water needs and weekend oversight. Collection of water samples occurred on Mondays (9:30 a.m.), Wednesdays (12:30 p.m.), and Thursdays (9:30 a.m.), from the outlets, mid-ports (between the corn cob and woodchip media layers), and inlets, for dissolved gas and nutrient analysis. Water quality parameters – dissolved oxygen concentration (DO), pH, and electrical conductivity (EC), were measured with a handheld multiparameter sonde (YSI, Model: Professional Plus, Yellow Springs, OH). Equipment failure precluded measurements during the final three weeks of Run 1.

During the experiment, the bacteria produced excessive biofilm, known as extracellular polymeric substance (EPS), partially clogging the columns and causing water to flow over the top of the downflow columns. The project team anticipated this problem would be more acute

with the planned shorter HRTs (1h and 0.5h), so the initial design changed for Run 2. In an attempt to minimize the production of EPS, the columns were replumbed and the acetate solution was injected into the mid-ports of the columns, between the corn cob and woodchip sections. Scientists reasoned that the anaerobic conditions at the mid-port location would prevent aerobes from producing EPS. Run 2 was conducted at 2-h HRT for 6 weeks with water and gas sampling and analyses conducted as for Run 1.

RUN 1

Overview and Objectives

The information below in the Run 1 section pertains to the 10-week experiment wherein acetate was mixed with the inlet water and flowed through columns in the up versus down direction in a temperature-controlled chamber (10°C) at a 2-h HRT. The objectives of Run 1 were to examine flow direction on column bioreactor performance by (i) quantifying nitrate-N removal and nitrate-N removal rate (NRR), and (ii) investigating the production of N₂O at short HRT.

Materials and Methods

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, 13 x 15 x 5 mm; 336±22 g dry), then a 5.1-cm thick layer of a woven polymeric mat (Brotex[®], Bro-Tex, St. Paul, MN), and finally 7.6 cm of lava rock (10- to 60-mm dia.; Vigoro). The outlets of the downflow columns were plumbed to the height of the perforated plate to maintain media saturation. Peristaltic pumps pushed synthetic water (nitrate-N, 22.2 mg N L⁻¹; dissolved P, 0.33 mg P L⁻¹) and acetate (potassium acetate, 103 mg C L⁻¹) solutions up through the upflow columns. For the downflow columns, the mixed solution was pumped onto a perforated plate at the top of the column, which held media in place and distributed the influent, and gravity affected downflow.

Inoculation was achieved by two means. The first was direct mixing of 10 g of oven-dried (48 hours, 60°C) woodchips taken from an operating field bioreactor 19 months prior (Willmar, MN) with new corn cobs and woodchips during column packing. The second means was soaking (48 h) the column packing with effluent from an operating field woodchip bioreactor (Blue Earth, MN). Water was circulated through the columns (18 d) to detect leaks and clear detritus from the media. The team established flow rates equivalent to a 12-hour HRT (4 mL min⁻¹) and introduced the synthetic nutrient solution for 16 d after which the acetate solution was added (90%:10% synthetic nutrient: acetate solution). Flow rates were adjusted to 2-hour HRT (23.5 mL min⁻¹) during the week (Monday through Friday). Because the volume of water needed for the experiment was substantial and weekend oversight of the experiment was limited, flow rates were reduced to a nominal 12-h HRT on Fridays at 12:00, and the acetate additions paused over the weekend. Water and acetate additions were reestablished on Mondays at 13:00±1:00. Seven days after acetate introduction, the acetate pump failed. For the next seven days, weekend conditions were established: 12-hour 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.

Sample Collection and Analysis

Water samples for nutrient analysis were collected on Mondays and Thursdays from the inlets and outlets. Samples for nutrient analysis were filtered (0.45 μ m; polyethersulfone), refrigerated (4°C), and analyzed on Mondays and Thursdays for nitrate-N (NO₂⁻-N+NO₃⁻-N) (QuikChem[®] Method 10-107-04-1-A, High Range - 0.2 to 20 mg N/L as NO₃⁻ or NO₂⁻) and ammonium-N (Method 10-107-06-2-A, 0.1 to 5.00 mg N/L as NH₃) colorimetrically by flow injection (QuickChem[®] 8500; Lachat, Loveland, CO).

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 ($22\pm1^{\circ}$ C) and analyzed following a minimum of 24 h to allow for equilibrium between the dissolved and headspace nitrous oxide (N₂O) concentrations. Samples were analyzed with an automated headspace sampler (Agilent 7694E; Santa Clara, CA) plumbed directly to a customized gas chromatographic system (Agilent; HP-5890; Santa Clara, CA), which has been described previously (Spokas et al., 2009). Dissolved N₂O was then estimated by assuming ideal gas law behavior and utilizing Henry's law coefficient for N₂O of 2.4 x 10-4 mol m⁻³ Pa⁻¹.

Flow rates were measured with a bottle, scale, and stopwatch. Loads were calculated by multiplying flow rates by the time between flow rate measurements by concentration. Beginning at 33 d after the initial addition of acetate, the downflow columns began to overflow as a result of extracellular polymeric substance (EPS) formation. The overflow was captured, measured, and subtracted from inflow for load removal calculations. Nitrate-N removal rate (g N m⁻³ d⁻¹) was calculated by dividing nitrate-N load removed between samplings by the delta time by the gross volume of the media.

Nitrate-N load reduction over the experiment, beginning with acetate addition, was calculated for each column as nitrate-N load in minus nitrate-N loadout, divided by the nitrate-N load in. Cumulative N₂O production, cpN_2O , was calculated by multiplying the outlet dN_2O concentration by volume of effluent since the previous sampling. The relative production of N₂O to nitrate-N removed, rN_2O , was calculated by dividing nitrate-N load removed by cpN_2O and expressed as a percentage.

Data were analyzed at $P \le 0.05$ using the MIXED procedure of SAS v.9.4 (SAS Institute Inc., Cary, NC), 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_2O 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 PDIFF option of the MIXED procedure of SAS.

Results and Discussion

Cumulative nitrate-N load reduction over the 35-d experiment was insignificant between the upflow and downflow columns, 21.3 and 27.5%, respectively (Table 1; P = 0.13). Across the flow direction treatments, a greater percentage of nitrate-N was removed at 12- than 2-h HRT, 35.1 vs. 22.2%, respectively. 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, 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, a range that brackets the current findings. The fact that no ammonium concentrations were above the detection limit (0.005 mg N L⁻¹) for downflow samples and only a few for upflow samples (data not shown) suggests that nitrate-N removal was primarily by denitrification and not dissimilatory nitrate reduction to ammonium (DNRA).

The NRR was insignificant between upflow and downflow at both at 2- and 12-h HRTs, (Table 1). Averaged across flow direction, NRR at 2-h was greater than at 12-h HRT, 30.1 vs. 11.8 g N m⁻³d⁻¹, respectively (Table 1). Averaged across flow direction, NRR at 12 h was slightly greater than that reported by Hoover et al. (2016), 6.9±0.3 g N m⁻³d⁻¹, and again bracketed by values from Feyereisen et al. (2016) for lower and higher temperatures, 6.8 and 29.3 g N m⁻³d⁻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 a greater flow rate, NRR tends to increase (Greenan et al., 2009; Pluer et al., 2016). Second, the addition of readily available carbon (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.

HRT	Flow Direction			
	Up and Down†	Up	Down	
(h)				
	Cumulative NO ₃ -N load reduction, %			
2, 12‡		21.3 (2.2) A§	27.5 (2.4) A	
2		19.1 (2.0) ¶	25.4 (2.4)	
12		32.2 (3.7)	38.1 (5.6)	
2	22.2 (2.0) a			
12	35.1 (3.3) b			
	NRR, g N m ⁻³ d ⁻¹			
2		25.8 (5.5)	34.5 (7.5)	
12		10.7 (2.8)	12.8 (4.9)	
2	30.1 (4.9) a			
12	11.8 (2.8) b			

Table 1. Cumulative nitrate-N load reduction, nitrate-N removal rate (NRR), relative N₂O production (rN_2O), and dissolved N₂O outlet concentrations (dN_2O) for bioreactor columns operated in two flow directions at two hydraulic residence times (HRT).

	rN ₂ O, %		
2, 12		0.28 (0.12) A	0.12 (0.02) A
2		0.22 (0.12)	0.08 (0.02)
12		0.49 (0.23)	0.29 (0.13)
2	0.15 (0.06) a		
12	0.39 (0.13) a		
	d N ₂ O, μg N L ⁻¹		
2, 12		16.7 (16.8) A	11.7 (12.0) A
2		6.4 (4.1)	4.2 (2.5)
12		27.1 (22.2)	19.3 (15.9)
2	5.3 (2.4) b		
12	23.2 (13.5) a		

+ Values in column "Up and Down" represent mean (s.e.) across flow directions (n=6).

‡ Values in rows with "2, 12" in the HRT column represent mean (s.e.) across HRTs (n=3).

§ Means (s.e.) within a row for each variable followed by the same uppercase letter are not significantly different at $P \le 0.05$; means within a column for each variable followed by the same lower-case letter are not significantly different at $P \le 0.05$.

¶ Means (s.e.) 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 $P \le 0.05$.

There were no significant differences in rN_2O or dN_2O between upflow and downflow treatments across HRTs, although variability tended to be lower for the downflow treatment, particularly at 2-h HRT (Table 1). Across flow direction treatments, rN_2O was insignificant (P = 0.14) between 2- and 12-h HRT with high variability; means (s.e.) were 0.15 (0.09) and 0.39 (0.06)%, respectively. Dissolved N₂O concentrations at the outlet, averaged across HRTs and weeks, were significantly greater for 12-h than 2-h HRTs (P = 0.01); means, back-transformed from logarithm base 10, were 23.2 and 5.3 µg N L⁻¹, respectively. There were significant dN_2O differences among weeks with dN_2O declining until the third week, then stabilizing (data not shown). Dissolved N₂O was greater for upflow through week three; dN_2O for the final two weeks was insignificant between flow directions (data not shown).

Feyereisen et al. (2016) reported an average rN_2O of 0.92% across 1.5 and 15.5°C temperatures. The dN_2O values listed in that study ranged from 2 to 164 µg N L⁻¹. In another column experiment, Feyereisen et al. (2017) found that rN_2O 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_2O and N_2O emissions from the surface of uncapped field pilot-scale WDBRs at 2-, 8-, and 16-h HRTs. Dissolved N₂O comprised >97% of N₂O fluxes, with total ratios of dN_2O -to- NO_3^- removed of 5.19, 0.35, and 0.52%, for 2-, 8-, and 16-h HRTs. Elgood et al. (2010) reported dN_2O of 6.4 µg N L⁻¹ at the outlet of a stream-bed denitrifying bioreactor and a rN_2O of 0.6% over one year of monitoring. Based on previous findings, e.g., Davis et al. (2019), and the temperature and step-based nature of denitrification wherein the last step mediated is from N₂O to N₂ (Lemaire et al., 2006), expectations were that N₂O production for the 2-h HRT would increase. In this respect, our findings are unexpected. Apparently, the addition of readily available C via acetate addition provided ample electron donor capacity to maintain nearly complete denitrification. Although insignificant, the suggestion of lower N₂O production for downflow could be a result of gas diffusion gradient counter to the water flow direction (Bruun et al., 2017) or additional aeration at the tops of the downflow columns, which were open to the atmosphere (Pijuan et al., 2014). The lower variability in the downflow columns is most evident in the standard deviations in rN_2O (Table 1).

The addition of C poses the challenge of bio-clogging of woodchip bioreactors (Anderson et al., 2020). The downflow columns were susceptible to bio-clogging during this experiment given the limited gravity head gradient driving flow. The issue of bio-clogging in denitrification bioreactors has been noted by others (Ines et al., 1991; Feyereisen et al., 2018) and remains an issue to be solved. However, the benefits in dramatically increasing NNR at high flow and low temperatures continue to be worth further study.

Conclusions

The overall purpose of the Run 1 experiment was to evaluate the nitrate-N removal performance of vertical column bioreactors at short HRT in both upflow and downflow directions. The addition of C at the bioreactor inlet at 2-h HRT increased nitrate-N removal rate 2.6-fold over conditions of no added C and 12-h HRT. There was no significant difference observed in the overall removal rate as a function of the column flow direction. Additionally, the data collected here also confirm a lack of significant difference in N₂O production potentials, although the downflow direction did result in numerically lower production potentials. N₂O production was reduced with C additions at short HRTs, an unexpected and useful finding. A necessary consideration for downflow bioreactors is the microbial clogging of water flow through the treatment columns. This biofilm production must be further evaluated prior to implementing carbon dosing in downflow field bioreactors.

RUN 2

Overview and Objectives

Since EPS formation began to interfere with flow in the downflow columns toward the end of Run 1, the original project objective to evaluate performance at shorter HRTs (higher flow rates) was revised. In an effort to reduce the negative effects of EPS, the columns were replumbed to deliver additional C in the form of acetate to the mid-port of the columns. The thinking for this was that the low dissolved oxygen status of the water at the mid-port would prevent the additional C from being used by aerobic bacteria to produce EPS. Thus, the objectives of Run 2 were to (i) determine if EPS production could be minimized, and (ii) quantify nitrate-N removal, NRR, and N₂O production for upflow and downflow columns at 10°C and 2-h HRT.

Materials and Methods

The operation of the columns was identical to that in Run 1 except that the acetate solution was not mixed with the synthetic nutrient solution prior to the inlet. In order to keep HRT in the inlet half of the column closer to that of Run 1, concentrations of the synthetic nutrient and acetate solutions were adjusted such that 95% of flow was a synthetic nutrient solution and 5% was from the acetate. The C concentration of the combination of synthetic nutrient solution plus acetate solution remained the same as in Run 1.

Results and Discussion

Two of the three downflow columns began to overflow four days after the initiation of acetate. After nine days and continuing to the end of Run 2, overflow was half or more of the total flow for one of the columns and after 23 and 38 days, the same was true for the second and third columns, respectively. Flow was increasingly restricted by the formation of EPS (Fig. 4). Although EPS also formed in the upflow columns (Fig. 5), pump pressure delivered to the inlet of the upflow columns continued to force water through the columns throughout the duration of Run 2.

The formation of EPS is caused by excess carbon in the system and lack of nitrogen. So, when the flow of nitrate-N-rich inlet water was restricted in the downflow columns, carbon from the acetate became more highly concentrated and nitrate-N became more limiting, further accelerating EPS formation. Those involved with this project considered the Run 2 experiment a failure or "success of a different kind." Until discovery of a way to prevent flow blockage by EPS, dosing bioreactors with carbon at the levels, location, and flow rates tried appears to not work.

CONCLUSIONS – ENTIRE EXPERIMENT

The nitrate-N removal rates for the experimental system (with C addition) were 2.6-fold that of standard conditions (no C addition) at 2-h HRT and 10°C (50°F). Researchers were unable to fully address Hypothesis 1, comparison of HRTs at 2- to 0.5-h, because EPS caused biofouling in the 2-h HRT experiment (Run 1). Hypothesis 2 was shown to be true: the upflow and downflow designs had equivalent nitrate-N removal rates and cumulative nitrate-N load reduction.

Shortening HRT in denitrifying bioreactors below the recommended minimum, e.g., 3 h (NRCS, 2015), typically increases the production of N₂O, a potent greenhouse gas, which is seen as a negative tradeoff: improved water quality for degraded air quality. The results of this experiment indicate that with C additions HRTs can be shortened to 2h without increasing N₂O production. This finding is unexpected and is perhaps the most important of this research.

FUTURE NEEDS/PLANS

Field trials of downflow pilot bioreactors of the same material configuration as the downflow columns in this experiment are being conducted at the University of Minnesota's Southwest Research and Outreach Center near Lamberton. Results from the first two years of trials are being analyzed and prepared for peer-reviewed publication.

Researchers continue to seek solutions to the problem of EPS formation in denitrifying bioreactors when readily available C is added. Some success toward this end has been achieved with the pilot bioreactors at Lamberton by reducing the C concentration of the dosing solution. This reduces the improvement in nitrate-N removal rate, but a workable balance between removal rate and EPS formation has been achieved. Another potential approach being investigated is the use of enzymes that interfere with EPS production, known as quorum quenching, by interfering with bacterial communication.

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Figure 1. Schematic of a denitrifying bioreactor designed to reduce the nitrate-nitrogen concentration of agricultural drainage water.



Figure 2. (L) Water supply tanks and peristaltic pumps. The acetate solution supply tank is shown tucked under the pumps and near the floor at the right-hand side of the picture. It is rectangular in shape and covered with aluminum to retard algal growth. (R) Two of the six experimental columns. The column on the left is the downflow design while the column on the right is the upflow design.



Figure 3. Bioreactor column schematics: (a) Upflow design; (b) Downflow design.



Figure 4. Examples of extracellular polymeric substance (EPS) that formed within downflow columns on: (L) corn cobs, (R) woodchips.



Figure 5. Extracellular polymeric substance (EPS) that formed on the woven polymeric mat at the outlet of one of the upflow columns.

Appendix

Average outlet concentrations of water quality parameters during Run 1: ammonium-N (NH4-N), dissolved reactive phosphorus (DRP), total phosphorus (TP), and dissolved organic carbon (DOC). There were three upflow columns and three downflow columns.

HRT	Flow Direction				
	Up & Down	Up	Down		
(h)					
	NH	NH4-N concentration out, mg N L ⁻¹			
2, 12		0.007	0.016		
2		0.003	0.003		
12		0.011	0.029		
2	0.003				
12	0.020				
	D	DRP concentration out mg L ⁻¹			
2, 12		0.194	0.174		
2		0.176	0.154		
12		0.213	0.194		
2	0.165				
12	0.203				
	,	TP concentration out mg L ⁻¹			
2.12		0.253	0.233		
2		0.224	0.220		
12		0.282	0.245		
2	0.222				
12	0.263				

HRT	Flow Direction		
	Up & Down	Up	Down
(h)			
	DOC concentration out, mg L ⁻¹		
2, 12	2.57	3.85	
2	1.92	2.61	
12	3.21	5.10	
2			2.27
12			4.16