Technology Transfer of Bioreactor Operations and Conservation Drainage Placement

Principal investigator: Roger Wolf

Project timeframe: 9/15/2011 – 9/19/2015

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Project Deliverables:

1. Bi-Annual update to participating producers on monitoring results.
2. Bioreactor operations guide to optimize nitrate reduction based on temperature, flow rate, and nitrate concentrations and to minimize contaminants.
3. Documentation of levels of N_2O production from recommended bioreactor management procedures.
4. Toolkit to determine which form of conservation drainage best fits which landscape position.
5. Training sessions in each state for a total of three sessions to transfer technology to 300 producers, land managers, drainage contractors, and NRCS personnel.
6. 1,500 operational guidelines and 1,500 conservation drainage toolkits distributed to producers, NRCS, staff, land managers, and drainage contractors with opportunities for further distribution through ISA EPS's website.
7. Interim standards developed by 2014 in Illinois and Minnesota and moving the Iowa standard towards finalization.

Contents

Executive Summary

The project entitled "Technology Transfer of Bioreactor Operations and Conservation Drainage Placement" was a result of an announcement for program funding through the United States Department of Agriculture Natural Resources Conservation Services (USDA-NRCS) through the Conservation Innovation Grants (CIG) program. The project fell within the Mississippi River Basin funding category and was aimed to address water management, specifically to demonstrate treatment effectiveness and efficiency of innovative practices, including bioreactors, for nitrogen contaminants in runoff or drainage water.

The goals of the project were to increase the awareness and knowledge of producers, watershed coordinators, NRCS personnel, and drainage contractors in terms of conservation drainage practice options and placement, and to determine the best way to manage a bioreactor in order to maximize denitrification while minimizing potential contaminant production. Failure to recognize appropriate practice placement could mean the failure of a particular installation or reduction in the practice cost effectiveness. A toolkit to guide recommendations based on site location and contributing areas is needed to aid the decision making process. At the beginning of the project there was also a knowledge gap of the best management practices for bioreactors. Bioreactors need to be managed to minimize potential contaminants stemming from the denitrification process. The project objectives included:

1. Determine management guidelines for denitrifying bioreactors to maximize their ability to reduce nitrogen discharge and minimize their production of environmentally undesirable by-products.
2. Quantify the export of undesirable byproducts, including methyl mercury, dissolved organic carbon, and nitrous oxide, from the bioreactors under a variety of operating conditions.
3. Enlarge the general knowledge base concerning denitrifying bioreactors for use by producers, NRCS personnel, land managers, and drainage contractors.
4. Facilitate widespread implementation of bioreactors in the three project states by developing and disseminating fact sheets, installation and management guides, instructional conferences, and webinars.
5. Develop interim EQIP standards in Minnesota and Illinois and solidify the existing interim standard (Interim Conservation Practice Standard, Denitrifying Bioreactor, Code 747) in Iowa.

The project was granted a one year no cost extension from its original close date of 9/15/14 to 9/19/15 due to droughty conditions that limited sampling events in 2012 and 2013. The limited sampling led to incomplete data sets and unspent funds. Extending the grant into 2015 allowed for an additional year of data collection.

Objective 1

It was determined that a zone of influence from the designed inlet head of the bioreactor should be mapped out to show the potential affected drainage areas in the field. For example, a two foot inlet head design for a bioreactor would raise the drainage base by two feet creating a zone of influence until two feet of surface elevation is gained from the placement of the upper control structure. The size of the zone of influence impacts how the owner of the bioreactor should manage the stop logs. Stop logs from the upper control structure may need to be removed in the spring to ensure seed bed preparation,

planting, fertilization, and spraying can take place in a timely fashion. Stop logs should be removed in a similar fashion to drainage water management recommendations (10-14 days prior to field operations), and promptly replaced following operations. It probably is not necessary to remove all stop logs, but this should be left to the discretion of the owner as it will impact drainage.

Periodic monitoring for nitrate concentrations, alkalinity, and flow or depth of flow over stop logs should be conducted to provide feedback to the bioreactor manager for stop log placement in the lower control structure. If the amount of nitrate-nitrogen removed is less than 30%, more stop logs should be put in place to ensure adequate hydraulic retention time. Once adequate nitrate removal is realized the manager should, at a minimum, visually inspect the flow within the bioreactor control structures. If tile flow appears to be coming to a stop or there is a strong sulfur odor emitting from the lower control structure, all stop logs should be removed from the lower control structure to prevent excessive organic carbon discharge and methylation of mercury.

Objective 2

Data collected for methylmercury indicated that production was minimal even under conditions which favored production. Results indicate that, as long as water is allowed to drain out of the bioreactor, methylmercury production should not be a concern. Organic carbon, either dissolved or total, is released with bioreactors, however levels are not excessive as long as water is not allowed to become stagnant within the bioreactors for extensive periods of time. Nitrous oxide is the result of incomplete denitrification. Bioreactors should be periodically monitored for alkalinity generated and compared to the predicted amount of alkalinity generation for the levels of nitrate reduction occurring. If measured/predicted alkalinity is less than 0.80, nitrous oxide production is possible, and additional stop logs should be put in place ensure more time for complete denitrification.

Objective 3

During the CIG project period of 2011-2015, partners were able to reach out to approximately 4,275 farmers, landowners, agency staff, scientists, and contractors. Outreach was accomplished through field days, meetings, webinars, and presentations at scientific conferences.

Objective 4

Bioreactor implementation is beginning to scale up from the demonstration phase to an implementation phase. The states of Iowa, Illinois, and Minnesota have included bioreactors in their state nutrient reduction strategies. As a result, many individual watershed plans call for bioreactor and other conservation drainage practices.

Objective 5

Each state has used an interim denitrifying bioreactor standard during the lifetime of the CIG. Bioreactors have a draft national standard in place that will be used going forward.

Through this CIG, bioreactors were shown to continue to be an efficient way to remove nitrate-nitrogen from subsurface drainage water prior to reaching surface waters. When monitored, bioreactors can be

managed to prevent possible contaminants stemming from the denitrification process from being produced in excessive levels.

Introduction

The Iowa Soybean Association partnered with the Minnesota Department of Agriculture and the University of Illinois to accelerate farmer awareness and implementation of denitrifying bioreactors and other conservation drainage systems in the Upper Mississippi River Basin. The project titled "Technology Transfer of Bioreactor Operations" was funded by a USDA-NRCS CIG. The goals of the project were to increase the awareness and knowledge of producers, watershed coordinators, NRCS personnel, and drainage contractors in terms of conservation drainage practice options and placement, and to determine the best way to manage a bioreactor in order to maximize denitrification while minimizing potential contaminant production. The awarded CIG also allowed partners to conduct and participate in outreach events to transfer lessons learned about bioreactor management and design to farmers, watershed coordinators, NRCS personnel, and drainage contractors. Table 1 displays the partnerships management team to carry out the work of the project.

Table 1 Project Management

Name, Title, Organization	Degrees, Experience, Credentials	Project Role
Roger Wolf, Director, Environmental Programs and Services (EPS), Iowa Soybean Association (ISA)	BS-Geography (Environmental Mgmt), University of Iowa, Creator and Director of ISA's EPS; Executive Director of ACWA	Program Administrator: oversees contract and financial management, project evaluation, reporting
Keegan Kult, Environmental Scientist, Environmental Programs and Services, Iowa Soybean Association	B.S.- Forestry, Iowa State University; M.S. - Environmental Science, Iowa State University; Manager of ISA's Bioreactor Demonstration Program	Project Manager: Lead in coordinating efforts among partners and states. Responsible for monitoring of bioreactors in IA as well as data analysis.
Mark Dittrich, Planner, Minnesota Department of Agriculture	B.S. – Agricultural Economics, University of Wisconsin-Madison; M.S. – Land Resources Program, Gaylord Nelson Inst. for Env. Studies, Univ. of Wisconsin-Madison	Minnesota Project leader and coordinator. Provide day-to-day leadership among Univ. of Minnesota, contractors, farmers, and local org.
Dr. Richard Cooke, Associate Professor, Agricultural and Biological Engineering, University of Illinois	B.S. - Agricultural Engineering, University of the West Indies St. Augustine, Trinidad; M.S. – Agricultural Engineering, University of Guelph, Ontario Canada; Ph.D. – Agricultural Engineering, Virginia Polytechnic Institute; Leads research in optimizing subsurface drainage system design and investigating preferential flow paths in sludge amended soils.	Illinois Project Manager: Lead in coordinating efforts in Illinois, including developing sampling and outreach efforts.
Dr. Robert Hudson, Associate Professor, Natural Resources and Environmental Sciences, University of Illinois	B.S. – Chemistry/Chemical Engineering, University of California, Santa Barbara; Ph.D. - Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts. Current research focuses on modeling biogeochemical process dynamics, including fate and	Mercury Analysis Expert: Develop mercury sampling and analysis protocols for the monitoring of total mercury and methyl mercury stemming from the bioreactor

	transport of metals in the environment, trace metal-phytoplankton interaction, and global cycles of carbon and mercury.	

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The project has obtained a 1:1 match with the CIG funding through a combination of cash and in-kind match. ISA, MDA, and UI have all provided cash match. The cash match is greater than the 50:50 cash to in-kind required by the grant. The in-kind match was obtained from Agri-Drain Corporation through a 20% discount in equipment purchases. In-kind match was also accounted for from meeting participants' time attending outreach events as outlined in the grant agreement.

Background

Bioreactors have been identified as an effective tool to help reduce the nitrate-N load to downstream water users and the Gulf of Mexico. Since they are a relatively new tool, bioreactors have yet to be implemented on a wide scale. In order for this to happen, the technology – optimal reactor designs, criteria for placement, and operating procedures – must be transferred from research entities to producers via NRCS personnel, watershed coordinators, and drainage contractors who advise producers in the landscape. It is also important that farmers or bioreactor managers are made aware of how to minimize the discharge of environmentally undesirable byproducts. The main byproducts of concern are extensive export of dissolved organic carbon, methylmercury, and nitrous oxide.

The intended direct positive environmental impact that could be realized from the project is the reduction of nitrate-N load being delivered by subsurface drainage while minimizing possible contaminants stemming from the denitrification process. Social impacts include increased awareness to producers on the water quality impacts of artificial drainage and an increased awareness of solutions to water quality problems at various landscape positions. The project has stimulated dialogue among producers, drainage contractors, watershed coordinators, NRCS personnel, and research institutions.

Addressing nitrate-N load losses at the edge of the field through conservation drainage practices generally comes as an expense to the farmer without the potential for agronomic benefits. Since there is limited public funding available for conservation, it is important to ensure that the practices which come as an expense to the producers operate in the most cost effective manner possible.

Methods

Sites

Table 2 displays the ten bioreactors monitored by the partners as part of the CIG. Bioreactor installation dates ranged from 2007 (Deland South, Illinois) to 2012 (Granite Falls, Minnesota). The Illinois bioreactors had the smallest contributing areas, with the Monticello site being the largest Illinois site at only 15 acres. The Iowa and Minnesota monitored bioreactors were more typical field scale implementations. Minnesota collected data from a bioreactor with the largest contributing area of 200 acres.

Table 2 CIG monitored bioreactors.

State	Site	Year Installed	Contributing Area, Acres	Dimensions, ft
Minnesota	Windom	2009	58	75 x 10
Minnesota	Granite Falls†	2012	20	75 x 10
Minnesota	Grand Meadows‡	2011	200	285 x 8
Minnesota	Morris	2012	20	60 x 20
Iowa	Greene	2008	47	50 x 25
Iowa	Hamilton	2009	50	100 x 10
Iowa	LWFC	2011	45	126 x 31
Illinois	Deland North	2009	7	56 x 10
Illinois	Deland South	2007	3	93 x 10
Illinois	Monitcello (Amenia)	2008	15	40 x 10

† Four chambered control structure
‡Treat all- no bypass

Bioreactors were to be monitored for nitrate-N reductions, dissolved organic carbon (DOC) or total organic carbon (TOC), nitrous oxide generation, and methylmercury generation. Originally, the bioreactors were to be monitored during the 2012 and 2013 seasons. However, the drought, which spanned from 2011 to the spring of 2013, throughout Midwest severely limited sampling opportunities. The University of Illinois had a graduate student dedicated to the project who was able to sample during the 2013 season. Iowa was able to sustain sampling into 2015. Minnesota elected to design a pump test to ensure tile flow and adequate sampling opportunities in the fall of 2013 and again in the fall of 2015. Minnesota wanted to create ideal conditions for methylmercury production and did this by creating low flow and high temperature conditions in four bioreactors. Water was pumped through the bioreactors for six days at a flow rate to create a 24 hour retention time.

Anions

Iowa bioreactors were sampled for anions 2 – 3 times per month, generally beginning early spring and ending in late summer to correspond with the period of greatest tile flow. Stop log adjustments were made based on observed inlet flows and recent water quality data feedback. Data loggers were

deployed in both control structures to determine continuous flow rates. A sample collecting apparatus was lowered into the control structure and rinsed three times with the water to be sampled before transferring to a sample bottle. Following collection, the samples were placed in a cooler with ice and delivered to ISA's state certified lab on the same day. Samples for nitrate-N, nitrite-N, and sulfate were refrigerated at 0-4°C and analyzed within 48 hours of sample collection. These parameters were measured using EPA method 300.0 (Pfaff 1993). Laboratory quality assurance/quality control procedures included blanks, fortified samples (spikes), replicates, and known concentration samples, all analyzed with each analytical batch.

Nitrous Oxide

Nitrous oxide samples were collected in evacuated 20 ml glass vials capped with rubber septa. Triplicate samples were collected at each site. Vials were prepared by flushing with helium three times followed by a three minute evacuation period. Capped-evacuated vials were then treated with 0.3 ml of an 80 percent $ZnCl_2$ solution. In the field, samples were collected by slowly submerging a sample bottle in the control structure to prevent turbulent flow. Water was transferred from the bottle to the vials using 10 ml syringes. Syringes were rinsed once with sample water and then filled to capacity past the 10 ml mark. Air bubbles were forced out and sample excess of 10 ml was discharged. The syringe needle was then inserted into the rubber septa and the sample was injected into the vial. Vials were stored on ice in a cooler and transported to the lab where they were stored in a refrigerator until analyzed.

Headspace concentrations for nitrous oxide were determined with a gas chromatograph equipped with an electron capture detector methanizer-flame ionization detector (SRI Instruments, model 8610 "Greenehouse Gas" System). Samples were analyzed within 72 hours of collection.

Alkalinity

Alkalinity was quantified by titration with acid to pH 4.5 and reported as mg L^{-1} $CaCO_3$ using Standard Methods procedure 2320B (APHA, 2005). During this study, bicarbonate concentrations were calculated in a subset of samples from pH and total alkalinity to ensure influent and effluent alkalinity were in this form. Inorganic carbon was then calculated from total alkalinity assuming a factor 2 correction for charge difference between carbonate and bicarbonate (Jones and Schilling, 2013).

Total Organic Carbon

Samples were preserved with hydrochloric acid, refrigerated at 0-4°C and analyzed within 30 days of sample collection. TOC was quantified using Standard Methods procedure 5310B (APHA, 2005), high temperature combustion followed by infrared detection of CO_2.

Mercury

Before going to collect samples in the field, new 500-mL PETG sample bottles (Nalgene™) were double-bagged in re-sealable plastic bags in the NRES clean lab. In addition, a custom-built, acid-washed device for collecting samples from bioreactor control structures was sealed in a large plastic bag in the clean lab. The device comprises a flexible ¼"-ID tube of approximately 3-m length and sections of ¾"-ID PVC pipe that serves to hold the flexible tube in a fixed position after assembly in the field. The tube has a short (30-40 cm long) section of C-Flex tubing that was in contact with the rollers of the peristaltic pump

head (Cole-Parmer). Samples were pumped through the Teflon/C-flex tubing into new PETG bottles. A different device was used to sample each bioreactor. The sample collector wore clean nitrile gloves while handling the sample collection device and sample bottles.

After collection using clean methods, samples are preserved by filtration and acidification (0.4% HCl) in a laminar flow bench in the clean lab. We employed pre-baked quartz fiber filters held in a USGS-style, vacuum chamber/filtration apparatus to remove particulate matter from the sample. Samples were stored in 250-mL bottles, either new PETG or borosilicate baked at 425 °C, at 4 °C for most of the holding time.

The method used to pre-concentrate mercury, thiourea-catalyzed solid phase extraction (SPE), remains the same as described in Vermillion and Hudson (2007) except that a commercially-available thiol resin, Silia-MetS Thiol (Silicycle, Montreal, Canada) was used.

The method used to analyze dissolved monomethyl mercury (MMHg) used at the University of Illinois evolved in very significant ways over the period of the project. Before the project began, this lab had been using a slightly modified version of the original mercury thiourea complex ion chromatography (HgTU-IC-CVAFS) procedure developed by Shade and Hudson (2003). By the time we began analyzing samples for this project, we had vastly improved the post-column chemistry of the method and switched to Hg detection by ICPMS, but still retained the original mobile phase chemistry, which defines the separation between Hg species achievable using the system. By the end, we had thoroughly revised the separation chemistry as well. The latest version of the method has been described in detail in our just-published paper (Olsen et al., 2015).

Quality assurance during sample collection primarily requires avoidance of contamination. This is shown by i) occasionally processing field blanks and ii) regularly comparing inlet and outlet samples for individual bioreactors. When detectable in samples, the MMHg and THg data are nearly always lower in the inlets, which is consistent with the inlet samples being groundwater. Field blanks were always at or near detection limits.

As in sample collection, the main QA concern in sample processing is avoidance of contamination. To test whether filtration added Hg to the samples, one or two blanks (samples of high-purity deionized water) were processed in every batch of samples filtered. The blanks also served as a test for Hg added via the acid used in preservation. Process blanks for methylmercury were always negligible.

As in sample processing, DI water blanks were processed with every batch of samples pre-concentrated to check for contamination. In addition, MMHg standards were processed as well. For batches of samples to be analyzed without isotope dilution, a known amount of MMHg was added to DI water, acidified and processed like a regular sample. Our later samples were analyzed with isotope dilution, i.e., a known amount of ^{198}Hg- or ^{199}Hg-enriched methylmercury standard was added to every sample and analyzed by ICP-MS (see Olsen et al., 2015).

The main aspect of QA/QC during analytical runs is accounting for drift in instrumental sensitivity. When analyzing samples without isotope dilution, several calibration standards were analyzed throughout the run to ensure that sensitivity had not drifted outside of the acceptable range (10%). When using isotope dilution, the internal standard accounts for instrumental drift.

Results

Nitrate-Nitrogen reduction

Concentration Reduction

Average influent nitrate-N concentrations at the Iowa bioreactor sites during the 2012-2015 collection periods ranged from a low of 15.10 mg/L at LWFC to a high of 25.36 mg/L at the Greene bioreactor. The Illinois sites had a narrow range of average influent nitrate-N concentration with 22.09 mg/L at the Monticello site being the lowest and 23.18 mg/L at Deland North being the highest during the 2013 sampling season. The average concentration reduction percentage was 40%, 61%, and 57% at the Greene, Hamilton, and LWFC bioreactors, respectively. The average concentration reduction at the Illinois sites were 12%, 46%, and 51% at the Deland North, Deland South, and Monticello sites, respectively. The Deland North bioreactor was the only bioreactor that did not meet the 30% nitrate-N reduction criteria outlined in the NRCS bioreactor standard during the sampling period.

Table 3 Illinois (2013) and Iowa (2012-2015) bioreactor nitrate-N concentration reduction.

Bioreactor	Samples	Average nitrate-N influent, mg/l	Average nitrate-N effluent, mg/l	Concentration reduction, %
Greene (IA)	46	25.36	15.24	40
Hamilton (IA)	36	22.82	8.88	61
LWFC (IA)	39	15.10	6.51	57
Deland North (IL)	12	23.18	20.40	12
Deland South (IL)	11	22.74	12.18	46
Monticello (IL)	21	22.09	10.90	51

Load Reduction

Table 4 displays the load reductions at the Greene bioreactor from 2009-2015. The nitrate-N load reductions ranged from 25 kg in 2015 to 206 kg in 2009 as reflected in the actual load column. The potential load column describes how much nitrate-N would have reached the receiving water body had the bioreactor not been in place. Potential load varied with the amount of tile flow due to precipitation patterns. The performance of the Greene bioreactor has declined significantly since installation in 2008. Load reductions ranged from 34–43% from 2009–2012, but have since declined to 1–13%. There is significant visible subsidence over the bioreactor which was noticed in the spring of 2014. It is thought that the severe drought which spanned from 2011 through the spring of 2013 greatly reduced the amount of carbon available for denitrification as the bioreactor went unsaturated for long stretches leading to aerobic conditions. In addition to the drought and the age of the bioreactor leading to the decline in performance, approximately 30 acres of additional pattern tile drainage was added to the main which the Greene bioreactor services in the fall of 2014. The Greene bioreactor was already undersized, as it sits on a twelve inch line, but since it has a surface intake, it was designed to only treat an eight inch tile system. It was designed for an 8 inch system, since an 8 inch main would have provided sufficient drainage capacity for the site, and the 12 inch line was only used to accommodate the surface intake. It was thought that allowing the lower nitrate water stemming from the surface intake to bypass would be a reasonable way to keep expenses down. The site also had limited space available, restricting

the bioreactor length to only 50 feet or a 2 hour hydraulic retention time. The Greene bioreactor would have benefitted from additional length.

Table 4 Greene bioreactor annual performance (March – July).

Year	Potential nitrate-N load, kg	Actual nitrate-N load, kg	Nitrate-N load removed, kg
2009	585	379	206
2010	367	231	136
2011	251	144	107
2012	94	62	32
2013	1109	976	133
2014	244	212	32
2015	2035	2010	25
Total	**4685**	**4014**	**671**

Table 5 displays the nitrate-N load reduction performance for the Hamilton bioreactor from 2010 – 2015. Data from 2011 were omitted due to lack of quality flow data. Potential loads ranged from 31 kg in 2015 to 185 kg of nitrate-N in 2014. The bioreactor removed the largest amount of nitrate-N in its first full year of operation in 2010, with 109 kg removed. The lowest amount of nitrate-N removed was in 2015, with only 8 kg removed. The highest percentage of load removed was in 2009 at 76%, and 2014 saw the lowest percentage of load removed at 12%. The bioreactor has averaged a 40% load reduction. Similar to the Greene bioreactor, there was visual subsidence occurring over the bed of woodchips beginning in the spring of 2014. However; the Hamilton bioreactor appears capable of still performing.

Table 5 Hamilton bioreactor annual performance (March – July).

Year	Potential nitrate-N load, kg	Actual nitrate-N load, kg	Nitrate-N load removed, kg
2010	143	34	109
2012	40	21	19
2013	162	96	66
2014	185	162	23
2015	31	23	8
Total	**561**	**336**	**225**

Table 6 display the nitrate-N load reduction performance for the LWFC bioreactor from 2012–2015. Potential nitrate-N load ranged from 36 kg in 2012 to 626 kg in 2014. The amount of nitrate-N load removed ranged from 12 kg in 2012 to a maximum of 159 kg in 2014. The percentage load removed ranged from 20-33% and had an overall average of 24%. Even though 2013 and 2014 had the lowest percentage of load reduction, the most nitrate-N load was removed in those two years due to the volume of flow.

Table 6 LWFC annual bioreactor performance (March - July).

Year	Potential nitrate-N load, kg	Actual nitrate-N load, kg	Nitrate-N load removed, kg
2012	36	24	12
2013	424	341	83
2014	626	467	159
2015†	63	42	21
Total	**1149**	**874**	**275**

† Transducer error limited loading data to after June 10, 2015.

Dissolved Organic Carbon/Total Organic Carbon

Tables 7-9 display the pump test dissolved and total organic carbon (DOC and TOC) results at the Minnesota sites. The Grand Meadow and Granite Falls sites had minimal production of DOC/TOC. The Grand Meadow site's outlet DOC average was 2.68 mg/L higher than the inlet average, whereas the Granite Falls outlet DOC average was only 0.3 mg/L higher.

Table 7 Grand Meadows bioreactor pump test organic carbon results.

	DOC, mg/L		TOC, mg/L	
GMD Site	Inlet	Outlet	Inlet	Outlet
Max	5.61	6.17	5.96	6.49
Average	1.62	4.30	2.27	4.75
Median	1.23	3.99	1.90	4.46
StDev	1.66	1.32	1.54	1.22
Min	0.42	2.26	1.15	2.86

Table 8 Granite Falls bioreactor pump test organic carbon results.

	DOC, mg/L		TOC, mg/L	
GRF Site	Inlet	Outlet	Inlet	Outlet
Max	5.47	5.99	5.08	5.64
Average	4.59	4.89	4.13	4.45
Median	4.48	4.67	4.01	4.21
StDev	0.56	0.66	0.60	0.71
Min	4.10	4.21	3.60	3.72

The Windom bioreactor had stagnant flow conditions prior to the pump test, which resulted in higher outputs of DOC/TOC. The maximum DOC/TOC levels seen in the outlet were 67.9 mg/L and 73.7 mg/L respectively. The average increase of DOC/TOC from the inlet to the outlet were 5.6 mg/L and 5.5 mg/L respectively.

Table 9 Windom bioreactor pump test organic carbon results.

WND Site †	DOC, mg/L		TOC, mg/L	
	Inlet	Outlet	Inlet	Outlet
Max	14.5	67.9	16.1	73.7
Average	9.6	14.8	10.9	16.4
Median	9.0	9.8	10.2	11.0
StDev	2.2	16.8	2.3	18.1
Min	7.2	7.7	8.2	8.8

†Stagnant flow

Table 10 displays the median and 25th and 75th quartiles for dissolved organic carbon and total organic carbon of the influent and effluent bioreactor samples. The median dissolved organic carbon values for the Illinois bioreactor effluent were 1.56 mg/L at Deland North, 1.76 mg/L at Deland South, and 6.56 mg/L at Monticello. The median total organic carbon values for the Iowa bioreactor effluent were 6.1 mg/L at LWFC, 4.34 mg/L at Hamilton, and 3.37 mg/L at Greene. For comparison, prairie streams often have TOC values in the 5 – 10 mg/L range, while forested streams are in the 10 – 20 mg/L range. Even looking at the 75th percentiles, only Monticello and LWFC exceeded the expected ranges for natural stream conditions. Organic carbon values were higher in low flow situations, especially when tile flow is ending in the summer. Stop logs should be removed from the lower control structure when tile flow is about to cease in the summer to prevent extended retention time within the bioreactor and dissolved organic carbon/total organic carbon generation.

Table 10 Organic carbon production in the Illinois and Iowa bioreactors.

Site	Samples	Median	25%	75%
Deland North In†	7	0.96	0.88	1.44
Deland North Out†	7	1.56	1.40	2.62
Deland South In†	10	1.27	1.06	1.74
Deland South Out†	10	1.92	1.57	4.65
Monticello In†	12	1.76	1.17	1.91
Monticello Out†	12	6.56	3.43	36.20
LWFC In††	24	2.6	2.4	3.28
LWFC Out††	23	6.1	4.7	11.80
Hamilton In††	19	3.16	2.74	3.98
Hamilton Out††	19	4.34	3.84	5.60
Greene In††	23	2.90	2.70	3.20
Greene Out††	23	3.37	2.91	3.81

† Dissolved organic carbon

†† Total organic carbon

Methylmercury

The major portion of the Iowa samples (24) appeared to have experienced an error in processing of the sample to prepare for the Hg-thiourea complex ion chromatography analytical method at the end of the grant period. The batch of samples all came back at or just above the 0.02 ng/L detection limit for methylmercury, which is unlikely. The isotopically-labeled internal standard and the labeled MeHg were recovered making it likely that there was a sample processing error and not an analytical method error. Results below are from batch runs in 2013 in which methylmercury and a low charge species of mercury were grouped together in the chromatography, meaning that the reported values are slightly higher than the true concentration of methylmercury. For more information on the mercury speciation, please refer the Hudson and Cooke (2015) report.

The 2012 methylmercury sampling in Iowa occurred near the end of the tile flow season when the highest amount of methylation was expected. Figure 1 displays the methylmercury generated in the Iowa bioreactors during the sampling occasion in 2012. LWFC generated the most methylmercury (+low charge mercury species) at 0.204 ng/L. The flow at the time of sampling indicated that the retention time was calculated to be 71 hours, which is almost considered stagnant flow. The Hamilton bioreactor generated 0.027 ng/L of methylmercury at a 38 hour HRT. The Greene bioreactor generated 0.050 ng/L of methylmercury at a 20 hour HRT.

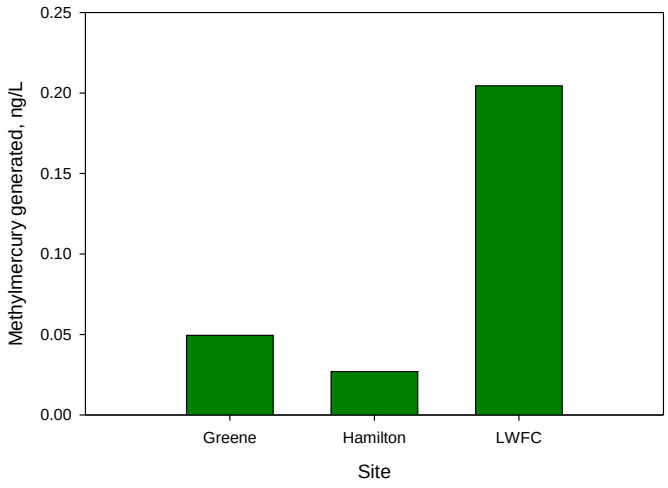

Figure 1 2012 Iowa mercury sampling.

Figure 2 displays the methylmercury generated in the Illinois bioreactor during the 2013 sampling events. The largest amount of methylmercury generated was on 5/14/13 at the Deland North site and was 0.0253 mg/L. The Deland North site averaged 0.008 ng/L of methylmercury +low charge mercury species generation. The Deland South averaged 0.002 ng/L of methylmercury +low charge mercury species generation. The Monticello site had the highest average methylmercury +low charge mercury species average generation at 0.011 ng/L.

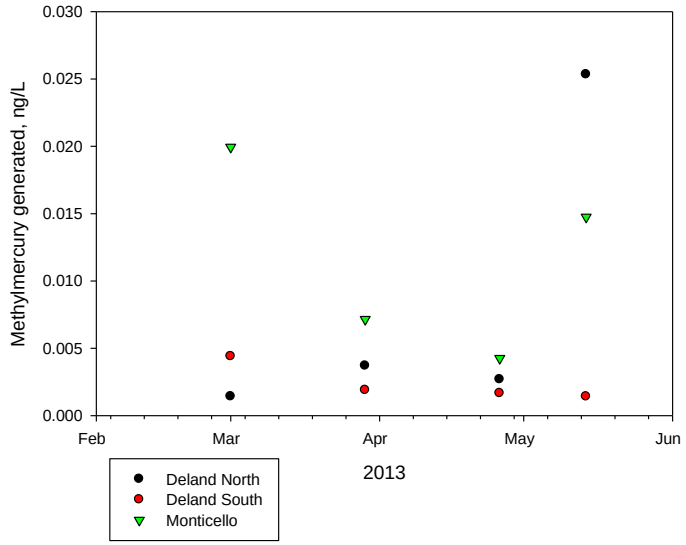

Figure 2 2013 Illinois methylmercury sampling.

Minnesota was able to create conditions suitable for production of methylmercury at each site during the pump test. All redox potentials at the bioreactor outlet were less than -100 mV, except for the Morris site (-84.7 mV). Dissolved oxygen concentrations were below 0.20 mg/L. Temperatures were above 16°C at all of the sites but the Morris site, which was at 11.8°C. The Grand Meadow (GMD) site generated the largest average methylmercury production at 0.398 ng/L, with the Morris (MRS) site generating the second highest amount at 0.104 ng/L. The Windom (WND) and Granite Falls (GRF) sites both experienced consumption of methylmercury as the outlets had lesser amounts of methylmercury compared to their respective inlets.

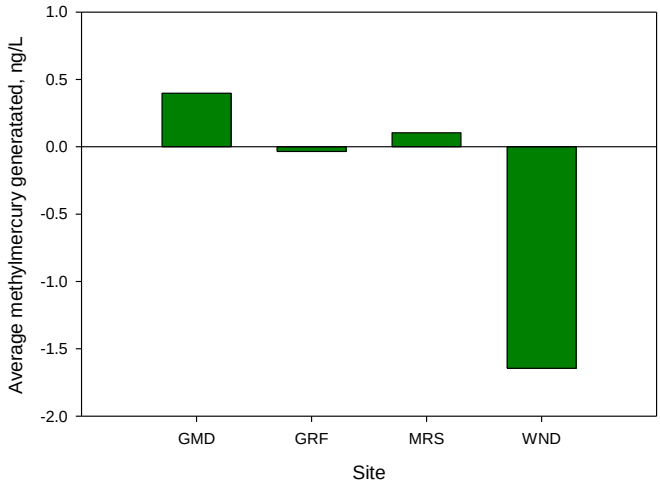

Figure 3 2013 Minnesota methylmercury sampling.

Nitrous Oxide

The pumped tests in Minnesota, which emulated a 24 hour retention time, detected minimal amounts of nitrous oxide production. There was actually consumption of nitrous oxide in the Grand Meadow and

Granite Falls sites. There was production in the Morris and Windom sites at 0.002 mg/L and 0.028 mg/L, respectively. The nitrous oxide production at Morris and Windom accounted for 1.5% and 7.5% of the nitrate reduction.

Table 11 Minnesota bioreactor average nitrous oxide levels during pump test. Standard deviations in parenthesis.

Site	GMD	GRF	MRS	WND
Average Inlet N_2O,mg/L	0.399 (0.021)	0.406 (0.018)	0.328 (0.014)	0.247 (0.091)
Average Outlet N_2O, mg/L	0.393 (0.014)	0.377 (0.027)	0.330 (0.026)	0.275 (0.014)
Difference, mg/L	-0.006	-0.029	0.002	0.028

Table 12 displays the ratio of measured alkalinity to that predicted by equation 1, if all the nitrate-N concentration decline was due to complete conversion to N_2. The ratio is theoretically 1.0 if all the nitrate concentration reduction is due to complete denitrification, assuming no off-gassing. If N_2O is the endpoint, the ratio would theoretically be 20% lower or 0.80 (Jones and Kult, 2016). In six of the seven sample pairs where N_2O was generated, the ratio was ≤0.79, with the exception of UWFC on July 16, 2015. In the four sample pairs where N_2O was not generated, the ratio was ≥0.98.

$$4NO_3^- + 5CH_2O \rightarrow 2N_2(g) + 5HCO_3^- + H^+ + 2H_2O \quad (1)$$

Table 12 Influent and effluent nitrous oxide concentrations, percent nitrate reduction and inorganic carbon ratios in Iowa Soybean Association studied bioreactors. The final column is the ratio of measured effluent inorganic carbon concentration compared to the concentrations predicted by the stoichiometry of Eq. 1, if N_2 was the only product of denitrification.

Bioreactor (Date)	N_2O influent (mg L^{-1})	N_2O effluent (mg L^{-1})	% Nitrate-N reduction	effluent IC measured/predicted
Greene (7/6/15)	0.17	0.98	31	0.48
Greene (7/22/15)	0.03	1.38	62	0.60
Hamilton (7/16/15)	0.05	0.00	97	1.08
Hamilton (7/22/15)	0.02	0.00	99	0.98
LWFC (7/7/15)	0.03	0.45	60	0.75
LWFC (7/16/15)	0.01	0.00	96	1.80
LWFC (7/22/15)	0.01	0.00	96	1.94
UWFC (7/7/15)	0.23	0.91	33	0.79
UWFC (7/16/15)	0.11	0.95	31	1.08
LEC2 (7/7/15)	0.03	0.91	51	0.76
LEC2 (7/16/15)	0.01	0.84	83	0.56

Tables 13, 14, and 15 display the annual median values of measured to predicted inorganic carbon change and nitrate-N concentration reduction. Looking at the NRCS standard recommendation of a 30% load reduction of water passing through the bioreactor (concentration reduction), there were four occurrences where the annual median concentration reduction fell at or below the 30% recommendation. In three out of the four occasions where the median nitrate-N concentration reductions were at or below the 30% level, the measured/predicted inorganic carbon change fell below 0.80. The exception was the Hamilton bioreactor in 2015, which had a 30% median nitrate-N reduction value with a 0.82 measured/predicted IC change. The two lowest measured/predicted IC change ratios

were at the Greene bioreactor in 2013 and 2014 at 0.48 and 0.67, respectively. Those also represented the lowest annual median nitrate-N concentration reduction at 23%.

Table 13 Annual median values of measured to predicted inorganic carbon change and nitrate-N concentration reduction at the Greene bioreactor.

	Greene Bioreactor Median Values	
Date	Measured/Predicted IC Change	Nitrate-N Concentration Reduction
2012	0.93	80%
2013	0.48	23%
2014	0.67	23%
2015	0.65	30%

Table 14 Annual median values of measured to predicted inorganic carbon change and nitrate-N concentration reduction at the Hamilton bioreactor.

	Hamilton Bioreactor Median Values	
Date	Measured/Predicted IC Change	Nitrate-N Concentration Reduction
2012	0.89	87%
2013	0.81	62%
2014	0.81	68%
2015	0.82	30%

Table 15 Annual median values of measured to predicted inorganic carbon change and nitrate-N concentration reduction at the LWFC bioreactor.

	LWFC Bioreactor Median Values	
Date	Measured/Predicted IC Change	Nitrate-N Concentration Reduction
2013	0.92	82%
2014	0.87	87%
2015	0.86	60%

Bioreactors were variable in terms of operating above or below the 0.80 threshold of measured/predicted alkalinity. The Hamilton and LWFC bioreactors operated above the 0.80 threshold a majority of the time at 57% (17 out 30 samples) and 69% (20 out of 29 samples), respectively. The Greene bioreactor, which had inadequate retention time design and is nearing the end of its life cycle, only performed above the 0.80 threshold in 46% (19 out of 41 samples) of the readings. Properly designed bioreactors were operating under conditions which would reduce indirect nitrous oxide production a majority of the time.

Outreach

From August 2013 through September 2014 Mark Dittrich, Michelle Natarajan, and Andry Ranaivoson from the Minnesota Department of Agriculture and the University of Minnesota, planned, coordinated,

and presented at industry meetings, workshops, symposiums, conferences and field days attended by over 2200 people at 30 events. Dr. Richard Cooke presented to nearly 1300 participants for a total of over 1500 participant hours from 2012-2015. Participant members included farmers, contractors, crop consultants and environmental scientists. Keegan Kult presented to 775 participants, which included farmers, contractors, agency staff, scientists, and policymakers over the life of the CIG. The cumulative participation hours attending outreach events from the Iowa partners was nearly 1200 hours.

Discussion

Bioreactors were shown to be cost effective measures to remove nitrogen from tile systems before entering surface waters. Properly sized bioreactors were removing 20-76% of the potential nitrate-N load volume from the field tile. The Greene bioreactor, which was undersized in terms of retention time, was even shown to be capable of removing 34-43% of the annual nitrate-N load prior to the 2012 drought, which seemed to have shortened its life expectancy. Organic carbon discharge from the bioreactors were typically at levels already seen in prairie and forested streams. High dissolved organic carbon or total organic carbon was seen when flows were extremely low in the bioreactors when the tile was almost done flowing for the season. This can be remedied by removing the stop logs in the lower control structure, typically mid-July, to allow the water to flow through faster and to prevent stagnation. Methylmercury production was found to be minimal even though conditions were created that were ideal for the methylation of mercury during the pump tests conducted by Minnesota. The largest average amount of methylmercury generated was 0.398 ng/L, which is less than what can be found in wetlands. Alkalinity data can be used to determine if nitrous oxide production may be occurring within the bioreactor. If measured/predicted alkalinity ratios are <0.80, additional stop logs should be put in place to create higher retention times to allow for complete denitrification to occur. Annual nitrate-N concentration reduction data did show that if the NRCS standard of minimum 30% reduction in nitrate-N within the bioreactor was met, that the measured/predicted alkalinity ratio was greater than 0.80. The three Iowa bioreactors combined were performing above the 0.80 threshold 56% of the time. This indicates that bioreactors are reducing indirect emissions of nitrous oxide a majority of the time. The Greene bioreactor was below the 0.80 threshold a higher percentage of time than any other bioreactor, but it also had inadequate retention times due to being under designed in terms of length (2hr HRT). Designed retention times of 4 -6 hours appears to be adequate to prevent nitrous oxide production a majority of the time. If measured alkalinity is still less than 80% of the predicted alkalinity after additional stop logs have been put in place to increase retention time, the wood chips may need to be replaced within the bioreactor as it is likely they have reached their potential life expectancy.

Since this CIG was funded, the partners have been able to conduct significant amounts of outreach in the Midwest. Each of the states involved in this CIG have included bioreactors to some extent in their state nutrient reduction strategies. The conservation drainage guide that was created as a result of the CIG is in the process of being distributed and is available at http://www.iasoybeans.com/environment/pdf/EPS15_conservationDrainageBrochureFinal_WEB.pdf . Roughly 1000 copies have been request by nine drainage industry partners involved in the Agricultural Drainage Management Coalition.

Recommendations

- Monitoring should be conducted on the bioreactors to guide management of stop logs, since bioreactors don't always perform as designed in terms of expected flow rates. At a minimum, bioreactors should be sampled every two weeks during initial flow in the spring and be analyzed for nitrates and alkalinity. Tweaks to the lower control structure stop log settings should be made until adequate amounts of nitrate removal have been realized.
- Checks need to be in place to ensure that bioreactors are being actively managed. Removing stop logs from the upper control structure for extended periods of time could greatly reduce the life expectancy. The life expectancy would be shortened as the bioreactor would become aerobic which increases the decomposition rate of the wood chips. The extended drought from 2011-2013 appeared to reduce the performance of the Iowa bioreactors installed prior to the drought (Greene and Hamilton).
- Stop logs in the lower control structure should be removed when it is expected that tile flow will cease to prevent excessive organic carbon discharge and methylation of mercury. This is typically mid-July.
- Years with lower percent load reduction actually often removed the largest nitrogen loads. Thought should be given to installing bioreactors on larger tile systems to decrease the amount of time that the bioreactor is operating under nitrate-limited conditions. The bioreactor should still be designed for near 4 hour retention times to lower the chances of nitrous oxide production, but the widths should be restricted (<30 feet) to prevent dead zones within the bioreactor. An additional option to design bioreactors based on amount of potential nitrogen removed should be thought about in addition to bioreactors that treat 15% of peak flow.
- A zone of influence should be mapped out in the design documents of the bioreactor, based upon tile gradient and designed stop log placement in the upper control structure. For example, if the bioreactor is designed with two feet of inlet head, the zone of influence should be mapped back until two feet of ground elevation is gained from where the upper control structure is placed. The magnitude of the zone of influence will impact how the producer should manage the stop logs.
- Continued research should be conducted on bioreactor material and additives to increase the rates of denitrification. Wood chips have been proven to provide enough denitrification to be cost effective compared to other denitrifying practices, but gains can still be made.

Conclusions

The levels of nitrate reduction and potential contaminant production found in this project align well with the drafted standard design criteria of treating 15% peak flow with a minimum three hour retention time. Reducing the peak flow treated from 20% to 15% will decrease the width of designed bioreactors which limits the likelihood of dead zones being created within the bioreactor. Adequate retention time within the bioreactor is needed to drive denitrification to completion instead of stopping at nitrous oxide. Consideration should be given to additionally design bioreactors on larger tile systems (200-300 acres) and base the size on potential pounds on nitrate removed instead of percentage of peak flow treated. Larger tile systems will have longer periods of sustained flow, which shortens the window that the bioreactors are operating under nitrate-limited conditions (conditions suitable for

methylmercury production). The longer periods of sustained flow also means that the wood chips are saturated for longer periods which means a greater proportion of the carbon will be used for denitrification instead of aerobic digestion. Bioreactors will perform more efficiently if they are actively monitored to tweak stop log placement in the lower control structures. We understand that it is not realistic for bioreactor owners to conduct weekly monitoring, but initial samples during the start of each growing season should be taken to ensure there is an adequate amount of denitrification occurring. Bioreactors should also be inspected when tile flow is expected to cease for the year. At this time, the bottom stop log should be removed from the lower control structure. Removing the bottom stop log in mid-July should usually suffice.

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