

**Ohio Water Resources Center
Annual Technical Report
FY 2014**

Introduction

Pursuant to the Water Resources Research Act of 1964, the Ohio Water Resources Center (WRC) is the federally-authorized and state-designated Water Resources Research Institute for the State of Ohio. The Ohio WRC was originally established at The Ohio State University in 1959, as part of the College of Engineering's Experiment Station. The Ohio WRC continues to be administered through the College of Engineering, in the Department of Civil, Environmental, and Geodetic Engineering.

The Ohio Water Resources Center promotes innovative, water-related research through research grant competitions, coordination of interdisciplinary research proposals, and educational outreach activities. Ohio WRC forges key relationships by connecting researchers and stakeholders. In Ohio – when there is a crisis, such as microcystin toxin detection in drinking water- the Ohio WRC teams up with the Ohio Sea Grant Program, Stone Lab, National Water Quality Research Center, the Lake Erie Research Center and other key organizations and research universities to provide critical support and operational inputs to federal, state and local policy makers, local operators, key professional associations, and civic and educational groups.

Ohio WRC sponsored researchers enable ecologically and socially sound water management by investigating the sources of nutrients and algal blooms in our environment, developing novel methods and technologies to reduce nutrients and other pollutants in water, and characterizing and monitoring the effects of energy development on water resources. By funding researchers early in their careers and developing powerful alliances with partner institutions, Ohio WRC seeds innovative approaches that foster impactful outcomes.

Ohio WRC reaches out to water professionals, educators, and citizens to ensure current and future citizens are water smart. Ohio WRC leaders are active in local and national water research, education and policy organizations such as the Ohio Water Resources Council, Water Management Association of Ohio, National Institutes of Water Resources and University Council on Water Resources.

Research Program Introduction

The Ohio Water Resources Center consistently invests in water related research in the State, growing the number of principal investigators involved in Ohio's water issues, and educating the next generation of water professionals by funding student work on water research projects. Over this past year's reporting period, we sponsored four new projects and administered six ongoing research projects conducted at seven different Ohio universities that totaled \$674,727 in research funding (direct and cost share). The PI's for these projects are seven Assistant Professors, one Research Scientist, one Associate Professor, and one Full Professor. In total, this research helped support nineteen students majoring in environmental engineering, chemical engineering, biochemistry, chemistry, biology, geology, environmental studies, natural resources and other water related fields.

The funded research projects entail studies of important Ohio water resources problems. For example, Dr. Suresh Sharma from Youngstown State University used modelling to assess the impact of water withdrawal for hydraulic fracturing during different stream flow conditions. His project received a no-cost extension until the end of the next fiscal year. Four projects were finalized during this fiscal year, the rest will be continued into next year. These include Dr. Jefferson's (KSU) and Dr. Jaeger's (OSU) projects investigating effects of stream restoration on ecology, geomorphology and water quality; Dr. Toman's (OSU) project on road infrastructure in rural areas and water quality; and Dr. Buffam's and Dr. Chin-Ming's projects on stormwater and nutrients issues.

In summary, Ohio WRC administered 10 research projects this reporting period, 8 of which were funded or co-funded by USGS 104(b) base grants. This resulted in the training of 19 students, 8 manuscripts in development, submitted or accepted in peer-review journals and 27 presentations or posters at local, national or international conferences. In this reporting period our PI's have been able to secure an additional \$395,526 in research awards using data generated with Ohio WRC funding.

Source tracking of Microcystis blooms in Lake Erie and its tributaries

Basic Information

Title:	Source tracking of Microcystis blooms in Lake Erie and its tributaries
Project Number:	2013OH292B
Start Date:	3/1/2013
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	5th
Research Category:	Biological Sciences
Focus Category:	Water Quality, Toxic Substances, None
Descriptors:	
Principal Investigators:	George Shepley Bullerjahn

Publications

1. Bullerjahn, G. S.; Davis, T. W.; Watson, S. B.; Rozmarynowycz, M. J.; McKay, R. M. Linking the genetics, toxicity and physiology of Planktothrix blooms to increased nitrogen and phosphorus concentrations in a eutrophic embayment of Lake Erie, Joint Aquatic Sciences Meeting, Portland OR, USA, May 2014.
2. Davis, T.W., Watson, S.B., Rozmarynowycz, M.J., Ciborowski, J., McKay, R.M., Bullerjahn, G. Molecular and taxonomic characterization of potential microcystin-producing cyanobacteria in Lake St. Clair during a late summer bloom, International Association for Great Lakes Research, Hamilton, ON, May 2014.
3. Davis T.W., Watson, S.B., Schulte, S., Tuttle, T., Wagner, A., Bullerjahn, G. Factors influencing Planktothrix bloom formation in the Lake Erie watershed. Water Management Association of Ohio Annual Meeting, November 2013. Invited Presentation
4. Davis, T.W., Watson, S.B., Rozmarynowycz, M.J., Ciborowski, J., McKay, R.M., Tuttle, T., Bullerjahn, G., submitted. A toxic continuum: Evidence that Lake St. Clair is a source of microcystin-producing cyanobacteria in Lakes Erie and Ontario. PLOS ONE, April 2014.
5. Davis, T.W., Watson, S.B., Rozmarynowycz, M.J., Ciborowski, J., McKay, R.M., Tuttle, T., Bullerjahn, G., (2014) Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. PLOS ONE, 9(9):e106093.

Final Report, Ohio Water Resources Center Project

“Source Tracking of *Microcystis* Blooms in Lake Erie and its Tributaries,” George S. Bullerjahn, PI

Problem and Research Objectives, Methodology, and Principal Findings and Significance:

The objective of this study is to determine the source of the diverse *Microcystis* that comprise the annual Lake Erie cyanobacterial blooms. Whereas some toxic and nontoxic forms may arise within Lake Erie sediments, the diversity of *Microcystis* detected in blooms may reveal genotypes that originate in sites physically removed from the lake. Understanding the origins of toxic and nontoxic forms can inform management strategies in the Great Lakes that may limit the expansion of *Microcystis* blooms in Lake Erie. Our methodology involves sequencing target genes from *Microcystis* populations to determine the toxic genotypes present, and the sites upstream from Lake Erie in which they may originate. Phylogenetic analysis of ribosomal 16S genes, toxin (*mcy*) genes and the N-responsive *ntcA* gene has provided fine-structure analysis of cyanobacterial populations that occur in the Lake Erie drainage basin. One result of our work described below is that while Lake Erie *Microcystis* genotypes are found in abundance upstream in Lake St. Clair, nearshore sites in Lake Erie (Sandusky Bay and Maumee River) are dominated by microcystin-producing *Planktothrix*. This latter finding has led to a future study on *Planktothrix* ecophysiology that has recently been funded by Ohio Sea Grant.

We proposed to monitor *Microcystis* genotypes from the Laurentian Great Lake Basin using archives of DNA from across the Great Lakes and tributaries/connecting channels, together with parallel measurements of nutrients, chlorophyll and physico-chemical parameters. The results will establish the diversity and geographic origins of the bloom-forming genotypes. Analysis of focal cyanobacterial harmful algal bloom (cHAB) species diversity from physically remote sites and Erie blooms has been conducted for two full bloom seasons, 2013 and 2014.

During the summer of 2013 we conducted a spatial and temporal survey of Lake St. Clair (LSC). Three sites around the southeast corner of LSC where the Thames River enters

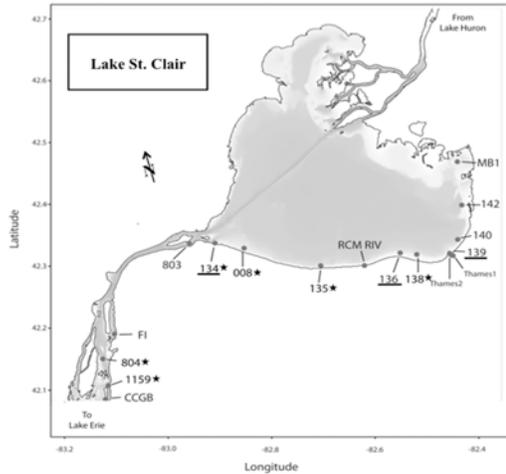


Figure 1: Map of Lake St. Clair indicating sampling sites. The underlined sites were the seasonal monitoring sites; starred sites where DNA was extracted and sequenced for genetic diversity.

LSC were monitored from June through September for CHAB genetic and phytoplankton community composition. In late August, a high biomass event occurred. During this event a spatial survey was conducted from Mitchel's Bay to the mouth of the Detroit River (17 sites; see Figure 1). We collected samples for DNA, dissolved and total nutrients, phytoplankton community composition and toxicity. We targeted the *mcyA* gene, part of the microcystin synthetase gene operon, to investigate the phylogenetics of potential microcystin producers in LSC and the Detroit River. The *mcyA* primers we used amplify the gene segment from *Microcystis*,

Planktothrix and *Anabaena* provided a robust picture of the potential microcystin producers in LSC and helped determine whether those strains were similar to strains found in the lower Great Lakes. We found that *Microcystis* was the predominant microcystin producer and that all toxic *Microcystis* strains found in Lake St. Clair clustered with toxic strains found in samples previously collected from Lakes Erie and Ontario, demonstrating extensive genetic connectivity between the three systems and establishing Lake St. Clair as an important immediate and historical source of toxic *Microcystis* to lakes Erie and Ontario. Also, we found that *Microcystis* blooms in Lake St. Clair can produce microcystin at levels that could negatively affect human health. These data will be presented at the 2014 International Association for Great Lakes Research and have been incorporated into a manuscript submitted to the international peer-reviewed journal PLOS ONE.

Bullerjahn's work is also informed by metagenomic and metatranscriptomic analyses provided through an approved project DOE-Joint Genome Institute ("Metagenomics and metatranscriptomics of the Lake Erie 'dead zone': a seasonal source of greenhouse gases," PIs McKay, Bullerjahn and Bourbonniere). Sampling pelagic Lake Erie and nearshore sites, we currently have metagenomes from Sandusky Bay and metagenomes and metatranscriptome data from the Erie central basin. The N-responsive regulator gene *ntcA* is unique to cyanobacteria, and we have used *ntcA* sequences as a measure of cyanobacterial diversity and relative abundance at these sites. The expression of *ntcA* in the metatranscriptome is also a measure of nitrogen bioavailability, as high levels of *ntcA* mRNA is diagnostic for N-limited cyanobacteria. For example, the recent

availability of the Sandusky Bay metagenome has allowed us to examine total cyanobacterial diversity and diversity of microcystin producers through phylogenetics of *ntcA* and *mcyA*. Regarding total cyanobacterial diversity, toxic *Planktothrix*, not *Microcystis*, dominates in nearshore environments such as Sandusky Bay, with heterocystous cyanobacteria present as minor members of the community. Importantly, the metagenome indicates that the number of *Planktothrix* genotypes is very low, allowing the design of some fairly straightforward experiments to test mechanisms of bloom formation and toxigenicity. Given that Sandusky Bay often experiences N limitation (see below), and that *Planktothrix* is not an N fixer, we speculate that *Planktothrix* is particularly successful at scavenging nitrogen, likely provided from nitrogen fixing taxa present. We hypothesize that N scavenging by *Planktothrix* is responsible for this genus outcompeting *Microcystis* in nearshore waters. Examining the diversity of N fixers by binning all *nifH* reads from the metagenome, only about one-third of the nitrogen fixers are cyanobacteria, the remainder being heterotrophic proteobacteria.

Collectively, these data sets on Erie and Sandusky Bay have allowed the development of RT-PCR primers specific for endemic *Microcystis* and *Planktothrix ntcA*, *mcyA* and cyanobacterial/bacterial *nifH*. qRT-PCR will be employed in field samples and laboratory experiments to assess the nutrient regimes (N speciation/SRP/organic P) that promote *Microcystis* and *Planktothrix* growth and toxigenicity. Additionally, in work stemming from genomic analysis of the nitrogen fixation genes, we are currently measuring nitrogen fixation rates from sediment and planktonic microbes to determine inputs of new N into the bloom sites.

In summary, the funded work targeted sampling at Sandusky Bay, the Maumee River and Lake St. Clair cyanobacteria, comparing the bloom-forming genera at each site using high-throughput DNA sequencing of diagnostic target genes. The results clearly indicated that all toxic *Microcystis* strains found in Lake St. Clair clustered with toxic strains found in samples previously collected from Lakes Erie and Ontario, demonstrating extensive genetic connectivity between the three systems and establishing Lake St. Clair as an important immediate and historical source of toxic *Microcystis* to lakes Erie and Ontario (Figure 1). Furthermore, while Lake Erie *Microcystis* genotypes are found in abundance upstream in Lake St. Clair, nearshore sites in Lake Erie (Sandusky Bay and Maumee River) are dominated by microcystin-producing *Planktothrix*.

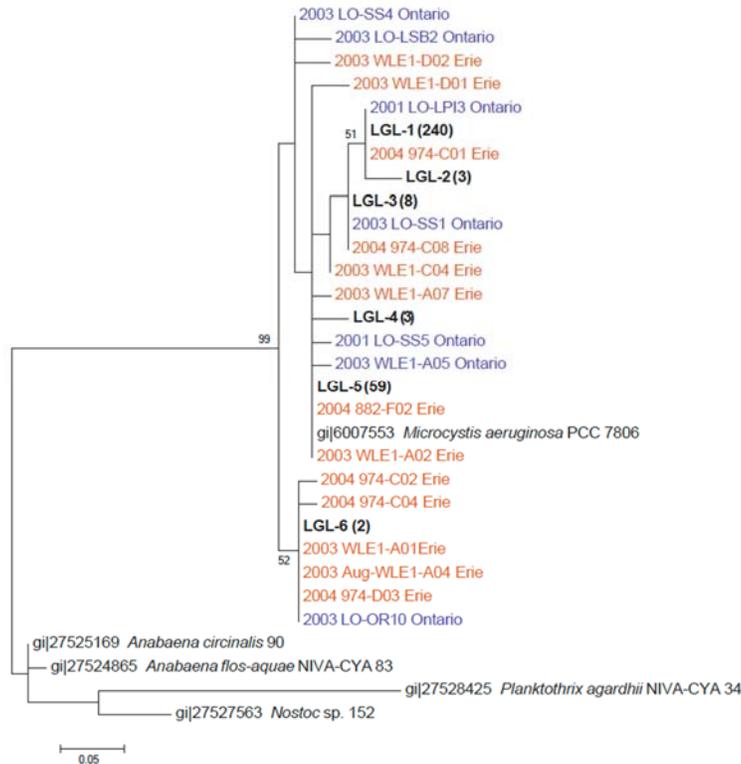


Figure 2. Phylogenetic tree of microcystin synthetase toxin (*mcyA*) gene sequences from Lake St. Clair, compared with environmental sequences from Lakes Erie and Ontario. The Lake St. Clair sequences (LGL) exist as six genotypes that are detected as abundant bloom formers downstream in Lakes Erie (orange) and Ontario (blue).

For further information see the below publications:

Davis, T., S.B. Watson, M.J. Rozmarynowycz, J.J.H. Ciborowski, R.M. McKay and G.S. Bullerjahn. 2014. Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. PLOS ONE 9(9): e106093.

Davis, T.W., G.S. Bullerjahn, T. Tuttle, M. Rozmarynowycz, R.M. McKay and S.B. Watson. 2015. Evaluation of increasing nitrogen and phosphorus concentrations on the toxicity and chlorophyll content during cyanobacterial blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol., in revision.

Rural on-site waste treatment as a source of nutrients to a eutrophic watershed

Basic Information

Title:	Rural on-site waste treatment as a source of nutrients to a eutrophic watershed
Project Number:	2013OH294B
Start Date:	3/1/2013
End Date:	12/31/2014
Funding Source:	104B
Congressional District:	OH-004
Research Category:	Water Quality
Focus Category:	Acid Deposition, Nitrate Contamination, Water Quality
Descriptors:	
Principal Investigators:	Christopher Spiese, Bryan O'Neil Boulanger

Publications

1. Spiese, CE, JM Berry, and B Boulanger. What caffeine tells us about nutrient source apportionment in rural watershed tile drainage. ASCE Environmental and Water Resources Institute Conference Proceeding. (Peer-reviewed & funded by WRRAA).
2. Berry, JM, CE Spiese, LM Streaker, and B Boulanger. Quantification of nitrogen and phosphorus from tile drainage in freshwater systems using caffeine as a human biomarker. Michigan State University Undergraduate Research Symposium, October 5, 2013. Funded by WRRAA, 1 student attending
3. Streaker LM, CE Spiese, and JM Berry. Using sterol biomarkers to trace a eutrophic watershed. Michigan State University Undergraduate Research Symposium, October 5, 2013. Partially funded by WRRAA, 1 student attending
4. Spiese, CE, JM Berry, LM Streaker, B Boulanger, and AG Thayer. Contribution of rural septic systems to nutrient loading in a eutrophic watershed. Water Management Association of Ohio 42nd Annual Meeting, November 13, 2013. Funded by WRRAA, 0 students attending.

2015 Final Progress Report
Ohio Water Resources Center

Title: **Rural on-site waste treatment as a source of nutrients to a eutrophic watershed**

PI: Christopher Spiese, Ohio Northern University

Problem and Research Objectives. This project aimed to determine whether or not rural septic systems contributed to nutrient and microbial loadings in the Blanchard River. The Blanchard River is a tributary to the western Lake Erie Basin and has been heavily impacted by increased phosphorus and nitrogen concentrations. While a number of factors can potentially contribute both nutrients to the river, septic systems have been targeted as an easily regulated source. This project looked at a marker of human waste streams, caffeine, and its relationship with phosphorus and nitrogen concentrations in tile drainage effluent as well as fecal coliform and *E. coli*.

Methodologies. Standards for all chemical parameters (nitrate, ammonia, and phosphorus) were created from certified reference materials or from drug standards (caffeine). Controls, field samples, and standards were determined using at least triplicate replicates. Caffeine was quantified using isotope dilution. Additional details concerning the study site and analytical methodology are presented in the proceeding sections.

Nitrogen. Two forms of dissolved nitrogen were measured: nitrate and ammonia. Both were measured *in situ* simultaneously using an YSI ProPlus meter with ion selective electrodes (ISE) for nitrate and ammonia. This method follows Standard Method (4500-NH₃ D) for ammonia (Standard Methods, 2013) and EPA Method 9210A for nitrate (US EPA, 2007). Standardization was performed using certified standards, and each ISE was calibrated prior to use. Detection limits for nitrate were 0.1 mg N/L.

Phosphorus. Phosphorus was determined using EPA Method 365.1 (US EPA, 1993). Soluble reactive phosphorus (SRP) was determined as molybdenum blue reactive substances present after filtration through a pre-combusted 0.45 μ m glass fiber filter. Total phosphorus was determined using the same method on unfiltered water samples after digestion with sodium persulfate. Absorbance measurements were made on a Shimadzu UV2401-PC UV-Vis

spectrometer at 610 nm using either a 1 cm or 5 cm path length cuvette, depending on absorbance. Method detection limits were 5 µg P/L or lower for all phosphorous measurements. *Caffeine*. Caffeine was determined following the method of Buerge, *et al.* (2003). Briefly, caffeine was extracted from 1 L of water using a solid-phase extraction (SPE) cartridge filled with silica-supported C₁₈. The caffeine was then eluted from the cartridge using methanol and dichloromethane. The methanol portion was re-extracted with dichloromethane and the combined dichloromethane layers were evaporated to dryness under a gentle stream of air. The evaporated sample was then reconstituted in dichloromethane system. Quantitation was achieved using a gas chromatography-mass spectrometry (GC-MS). An internal standard (caffeine-¹⁵N₂) was employed prior to SPE in order to permit accurate quantification. Blank samples were periodically analyzed to control for any potential contamination. Spike recovery studies were carried out for method confirmation. Reported detection limits for this method were approximately 10 ng/L with a reporting limit of 100 ng/L. Previously reported caffeine concentrations observed in a neighboring western Lake Erie watershed were in excess of 100 ng/L (Wu, *et al.*, 2012).

Principal Findings and Significance. At six sites across Putnam County, Ohio, tile drainage water was sampled over the course of a year. Caffeine was found at all of the sites, at varying levels (Fig. 1). Table 1 provides the mean ± standard deviation concentrations for nutrients, suspended sediment, and caffeine observed in each site over the course of the entire research. The observed water temperature at each site ranged from 16.3 to 21.8 °C, the observed pH ranged from 7.5 to 8.0, and chloride ranged from 6.2 to 13.0 mg/L. Temperature, pH, and chloride did not demonstrate any correlation to nutrient levels or caffeine levels observed within the watershed. There were also no correlations observed between caffeine and nitrate, caffeine and ammonium, caffeine and nitrite, or caffeine and total nitrogen. However, caffeine was observed to have a strong negative correlation (Pearson's $r > -0.9$) with total phosphorous (Fig. 2).

Mean ± standard deviation caffeine concentrations across sampling sites ranged from non-detect at the control site (site #4) to 1.2±0.4 µg/L in tile drainage effluents from sites having on-site wastewater systems (site # 1-3, 5, and 6). Mean ± standard deviation concentrations observed for total nitrogen and phosphorous concentrations in all tile drains were 3.5±1.8 mg/L and 0.4±0.07 mg/L, respectively. While the overall standard deviations for all analytes were variable, the observed correlations were maintained during analysis of individual storm events. Therefore, we are confident in the observed trends indicated in the data set when taken for individual sampling events or as grouped means.

Table 1. Mean \pm standard deviation concentrations for nutrients and caffeine observed in each site over the course of the entire research project.

Site number	Ammonia (mg/L)	Nitrate (mg/L)	Total Phosphorous (mg/L)	Suspended Sediment (mg/L)	Caffeine (μ g/L)
1	0.62 \pm 1.0	3.21 \pm 3.87	0.47 \pm 0.4	21 \pm 25	0.45 \pm 0.7
2	0.26 \pm 0.2	2.47 \pm 2.19	0.45 \pm 0.4	8.2 \pm 6.0	0.43 \pm 0.3
3	0.30 \pm 0.5	0.92 \pm 0.72	0.4 \pm 0.3	16 \pm 12	0.82 \pm 1.7
5	0.14 \pm 0.1	5.7 \pm 5.2	0.31 \pm 0.3	10 \pm 14	1.2 \pm 1.4
6	0.48 \pm 0.4	2.1 \pm 1.4	0.5 \pm 0.3	15 \pm 10	0.26 \pm 0.13
control	0.17 \pm 0.04	7.3 \pm 8.6	0.38 \pm 0.3	14 \pm 8.6	BDL

The study results are interesting, because the observed caffeine-total phosphorous correlation indicates that septic effluents are not significant contributors to nutrient loadings within the rural watershed. Additionally, commonalities in nutrient fingerprints (total and speciated phosphorous and nitrogen) in groundwater and tile drainage highlight the complex relationships for nutrient and water quality management in irrigation drainage waters.

The observed trends in nutrient levels from each sampling site and the demonstrated inverse correlation with phosphorous led us to explore groundwater concentrations of the nutrients. Groundwater from a nearby well indicated a mean total phosphorous 0.39 mg P/L. A mean phosphorous tile drain concentration for the entire study was determined to be 0.4 \pm 0.07 mg P/L. Additionally, analysis of fifteen surface water samples from Riley Creek contained an average phosphorous concentration of 0.44 \pm 0.1 mg P/L. Additional groundwater samples are currently being explored to confirm a proposed pathway where property drinking water wells convey nutrient rich groundwater through the OSWT system, which then drains into the Creek via the tile. Therefore, efforts to control OSWT systems will likely not have a measurable effect on nutrient loadings unless groundwater can also be controlled or leach systems become more widely implemented. Evidence for the plausibility of the proposed nutrient pathway is also supplied by the earlier examination of the correlation between nutrients and river discharge.

The lack of a correlation between nitrate and SRP to river discharge points towards a source that is likely not coupled to rainfall. The constant use of wells throughout the region, may explain why phosphorous levels in the watershed have increased as efforts to manage nutrients in runoff have increased.

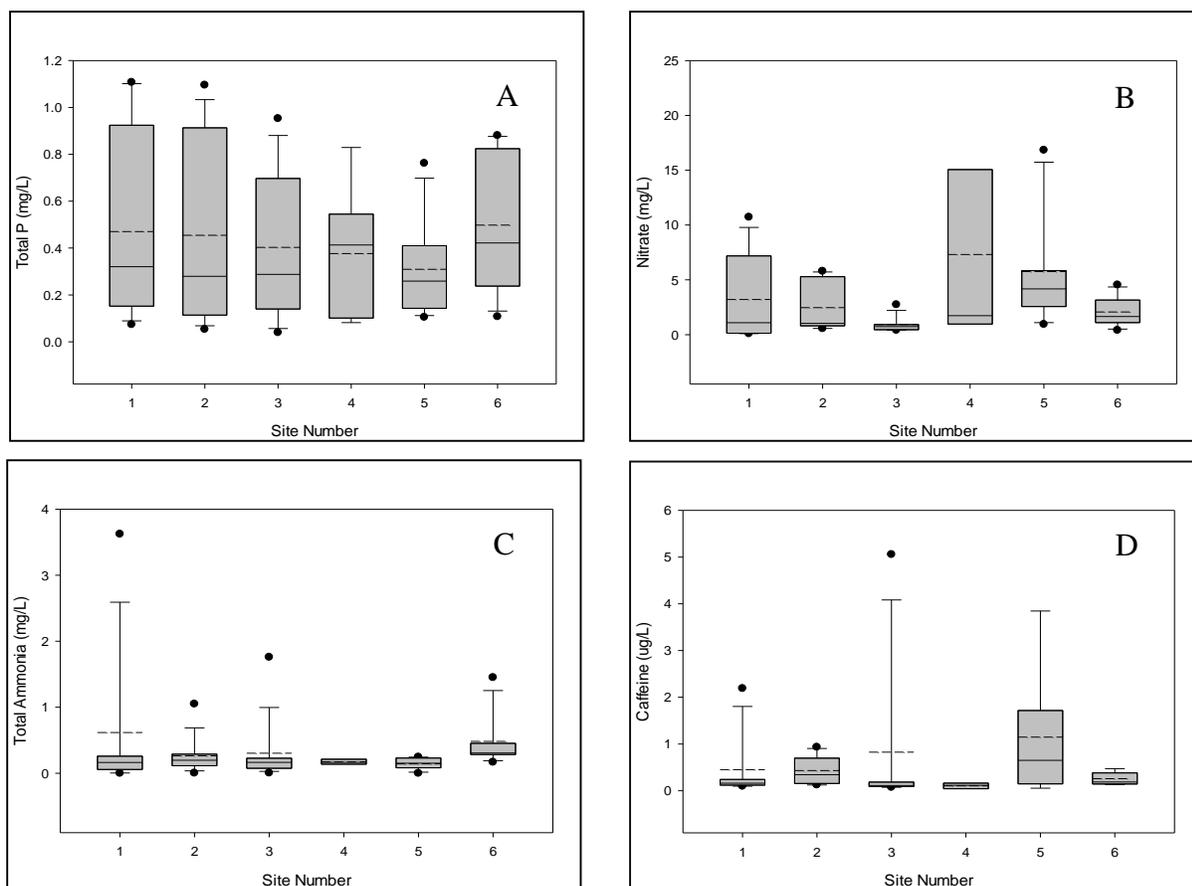
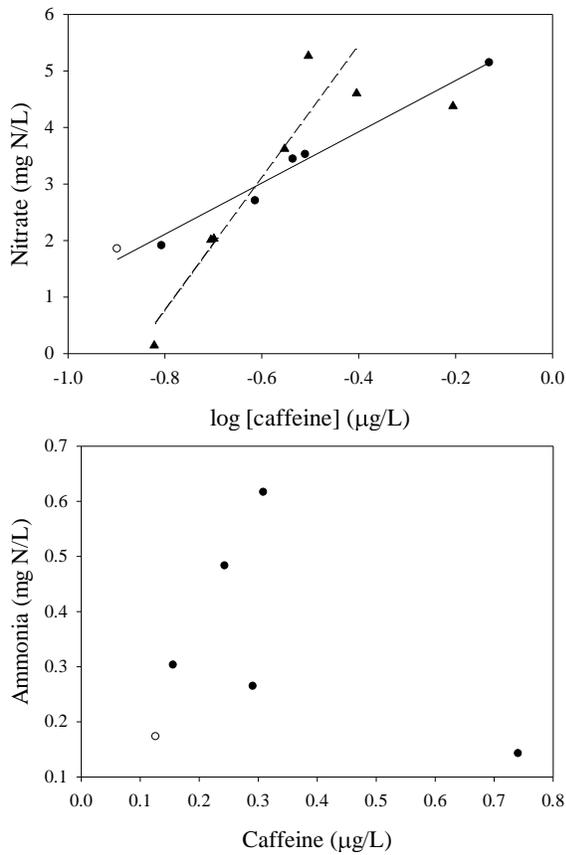
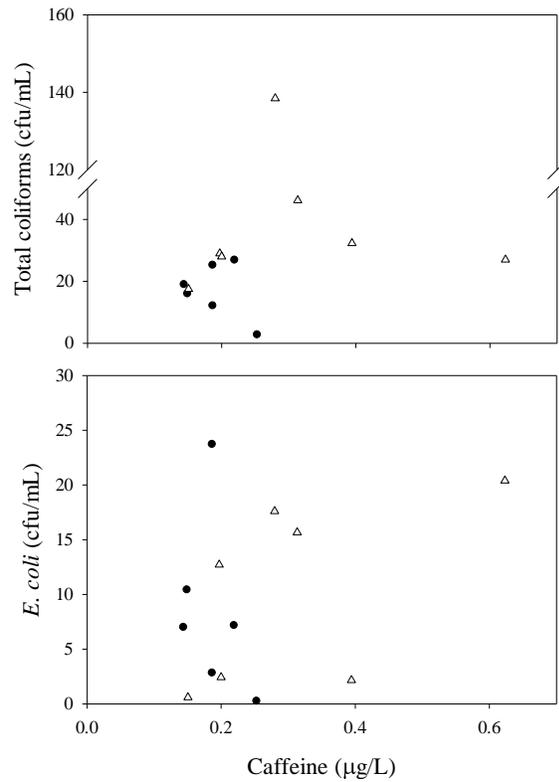
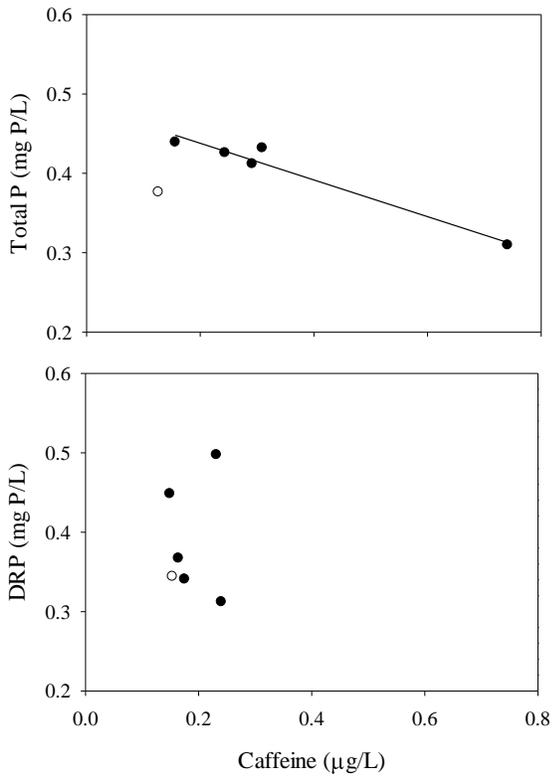


Figure 1. Compiled data for 2013 for A) total phosphorus, B) Nitrate, C) total ammonia, and D) caffeine. The boxes denote 25th through 75th quartiles, with the solid line at the median value and the dashed line at the mean. Error bars denote 95% confidence intervals and points denote outliers.

Initial results are available regarding microbial loadings tied to caffeine concentrations (Fig. 3). At nearly all sites tested, both fecal coliform bacteria and *E. coli* were detected. Both microbe types were correlated with caffeine concentration, indicating a common source. This is particularly true for *E. coli*, where the correlation is statistically significant ($p = 0.015$), and there is precedent for *E. coli* being linked directly to human waste streams (Frenzel and Couvillion 2002).

Taken together, the role of OSWT in loadings of nutrients and bacteria is complex. In this agricultural watershed, OSWT does not contribute significantly to nutrients, but do appear to be a source of bacteria. These results urge caution when making policy decisions related to nutrient reduction by targeting residential OSWT systems.



(left) Figure 2. Relationship between caffeine and total phosphorus, dissolved reactive phosphorus, nitrate, and ammonia. Points denote the mean for each sampling site ($n = 4 - 15$ for caffeine and TP, 2 - 10 for DRP), and the open circle indicates the control site (no OSWT). For TP, the line represents the linear regression for all sites with OSWT present within the drainage area ($[TP] = (-0.2303 \text{ mg P/mg caffeine}) \times [\text{caffeine}] + (0.4839 \text{ mg P/L}), r^2 = 0.957$). Lines denote the best fit for each data set. Putnam County (solid line): $[NO_3^-] = (4.5308 \text{ mg N/L}) \times \log [\text{caffeine}] + 5.7358 \text{ mg N/L}, r^2 = 0.9796$. Hardin County: $[NO_3^-] = (11.714 \text{ mg N/L}) \times \log [\text{caffeine}] + 10.146 \text{ mg N/L}, r^2 = 0.8975$.

(above) Figure 3. Relationship between caffeine and (top) total coliform loading or (bottom) *E. coli* loading at Putnam County sites (circles) and Hardin County sites (triangles). Points denote the mean for each sampling site ($n = 1 - 9$).

References

Buerge IJ, Poiger T, Müller MD & Buser H-R (2003) Caffeine, an anthropogenic marker from wastewater contamination of surface waters. *Env. Sci. Tech.* **37**: 691-700.

Dolan DM & Chapra SC (2012) Great Lakes total phosphorus revisited: 1. Loading analysis and update (1994-2008). *J. Great Lakes Res.* **38**: 730-740.

Frenzel SA & Couvillion CS (2002) Fecal-indicator bacteria in streams along a gradient of residential development. *J. Am. Water Resour. Assn.* **30**: 265-273.

North RL, Guildford SJ, Smith REH, Havens SM & Twiss MR (2007) Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnol. Oceanogr.* **52**: 315-328.

Peeler KA, Opsahl SP & Chanton JP (2006) Tracking anthropogenic inputs using caffeine, indicator bacteria and nutrients in rural freshwater and urban marine systems. *Env. Sci. Tech.* **40**: 7616-7622.

Seiler R, Zaugg SD, Thomas JM & Howcroft DL (1999) Caffeine and pharmaceuticals as indicators of water contamination in wells. *Ground Water* **37**: 405-410.

Standard Methods (2013) Standard Method 4500-NH₃ D. Ammonia-Selective Electrode Method. Standard Methods Online -- Standard Methods for the Examination of Water and Wastewater. <http://standarmethods.org>, last accessed Jan 16, 2014

Starr JL & Sawhney BL (1980) Movement of nitrogen and carbon from a septic system drainfield. *Water Air Soil Pollut.* **13**: 113-123.

US EPA (2013) <http://water.epa.gov/infrastructure/septic/>, last accessed Jan 16, 2014

US EPA (2007) EPA Method 9210A Potentiometric Determination of Nitrate in Aqueous Samples with an Ion-Selective Electrode

US EPA (1993) EPA Method 365.1 Determination of Phosphorous By Semi-Automated Colorimetry

Wu C, Witter JD, Spongberg AL & Czajkowski KP (2012) Occurrence of selected pharmaceuticals in an agricultural landscape, western Lake Erie basin. *Wat. Res.* **43**: 3407-3416.

Assessment of a Novel Application of Biochar to Improve Runoff Water Quality from Vegetated Roofs

Basic Information

Title:	Assessment of a Novel Application of Biochar to Improve Runoff Water Quality from Vegetated Roofs
Project Number:	2013OH297B
Start Date:	3/1/2013
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	1
Research Category:	Water Quality
Focus Category:	Hydrogeochemistry, Nutrients, Water Quality
Descriptors:	
Principal Investigators:	Ishi Buffam, Dominic L Boccelli

Publications

1. *Hochwalt, P. and I. Buffam. April 2014. "Can biochar increase nutrient binding and water holding capacity in vegetated roof growing medium?" University of Cincinnati 2014 Undergraduate Research Symposium, Cincinnati OH, USA (poster presentation).
2. *Divelbiss, D., P. Hochwalt, M. Mitchell, D. Boccelli, and I. Buffam. May 2014. "Black Is The New Green - Enhancing Green Roof Performance With Novel Substrate". Confluence Water Technology Innovation Cluster, 2014 Water Symposium, Covington, KY, USA (oral presentation).
3. Wright, P., P. Hochwalt and I. Buffam. April 2014. "A batch study of the sorption kinetic properties of biochar amendment to vegetated roof growing medium" University of Cincinnati 2014 Undergraduate Research Symposium, Cincinnati OH, USA (poster presentation).
4. Hochwalt, P., M.E. Mitchell, D. Boccelli, D. Divelbiss, and I. Buffam. November 2014. Biochar enhances water and nitrogen retention in green roof substrate. Cities Alive: 12th annual Green Roofs and Walls Conference, Nashville, TN, USA (poster presented by I. Buffam)
5. Buffam, I. and M.E. Mitchell. 2015. Nutrient cycling in green roof ecosystems. Chapter 5 in R. Sutton, ed. Green Roof Ecosystems, Springer, New York (May 2015; Research from this project is mentioned in the paper, though not the focus of the entire chapter)

Progress Report 2014-15

Assessment of a Novel Application of Biochar to Improve Runoff Water Quality from Vegetated Roofs

*Project G101643/G200355: 3/1/2013 – 2/28/2016 (including no-cost extension)
This progress report includes results from the entire project period.*

Ishi Buffam and Dominic L. Boccelli
University of Cincinnati

Table of Contents

1. SUMMARY	2
2. PROBLEM AND RESEARCH OBJECTIVES.....	3
3. COLUMN STUDIES ON BIOCHAR-AMENDED VEGETATED ROOF SUBSTRATE (2013-2014)	4
3.1 METHODOLOGY	4
3.2 PRINCIPAL FINDINGS	6
4. BATCH EXPERIMENTS TO DETERMINE SORPTION KINETICS (SPRING 2014)	8
4.1 METHODOLOGY	8
4.2 PRINCIPAL FINDINGS	8
5. EFFECT OF BIOCHAR PRETREATMENT ON WATER HOLDING CAPACITY (SPRING 2015).....	10
5.1 METHODOLOGY	10
5.2 PRINCIPAL FINDINGS	10
6. EFFECT OF BIOCHAR-AMENDED SUBSTRATE ON HYDRODYNAMICS IN PLOTS (SPRING 2015)	11
6.1 METHODOLOGY	11
6.2 PRINCIPAL FINDINGS	12
7. SIGNIFICANCE	14
8. PUBLICATION CITATIONS.....	15
9. STUDENTS SUPPORTED BY THE PROJECT	16
10. PROFESSIONAL PLACEMENT OF STUDENTS.....	16
11. NOTABLE AWARDS AND ACHIEVEMENTS	17
12. REFERENCES	18

1. Summary

Vegetated (green) roofs – a type of urban green infrastructure – have been demonstrated to improve stormwater retention and provide energy savings, but can also serve as a source of inorganic nutrients nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) through runoff. An early study using biochar – a type of activated carbon – within the green roof substrate (soil mix) suggested that an amended soil mix could improve the effluent water quality from vegetated roofs. The *overarching objective of this project* is to improve our understanding of the water quality benefits associated with the use of a biochar-amended substrate mix within vegetated roof technology. Our *central hypothesis* is that biochar can enhance the ability of vegetated roofs, which can already reduce the quantity of stormwater runoff, to decrease nutrient loading by binding nutrients as the runoff passes through the amended green roof substrate. In the first year of the project (2013-14), using column experiments in the lab we demonstrated that the incorporation of biochar substantially increased the water holding capacity of the substrate, reduced and delayed the efflux of NH_4^+ and slightly delayed the passage of NO_3^- , but had little effect on PO_4^{3-} . We also carried out batch experiments of the dynamics of sorption kinetics, which suggest a two-phase sorption mechanism onto biochar, the slower process taking several days to reach equilibrium. In the most recent phase of the project (2014-2015), our research group carried out a pilot study on green roof test plots and developed a method for making continuous measurements of evapotranspiration on the plots. Our results so far indicate that biochar has excellent potential as a low-cost amendment to green roof substrate to improve downstream surface water quality. Our ongoing research in the coming year includes measuring the effect of biochar on nutrient retention, water retention, and plant vitality in green roof plots (research of a new MS student in Biological Sciences), as well as constructing a hydraulic/water quality model parameterized with the results from the lab and field experiments (research of an MS student in Environmental Engineering).

2. Problem and Research Objectives

A significant issue many urban centers face is the direct discharge of untreated sewage into receiving waters due to overburdened and antiquated combined sanitary and stormwater sewers. While conventional grey infrastructure approaches to mitigating combined sewer overflows (CSOs) tend to be disruptive and costly, the use of urban green infrastructure (UGI) – generally defined as implementations (often vegetated) to reduce stormwater surface runoff by increasing infiltration and evapotranspiration – can mitigate overflow events and the associated deleterious impacts, while contributing other co-benefits. Green infrastructure techniques have been gaining traction for reducing urban stormwater runoff and improving water quality (e.g., NYC Department of Environmental Protection, 2011), and are one of the best management practices for CSO reduction recommended as part of the integrated green infrastructure program by Cincinnati’s Metropolitan Sewer District (www.projectgroundwork.org). Vegetated (green) roofs – a type of UGI – are becoming increasingly popular with for example >20% coverage of the flat roof area in cities like Stuttgart, Germany. These installations are expected to continue to proliferate in the near future with stated goals of 20% coverage of large buildings in Washington, D.C. by the year 2025 (Deutsch et al. 2005) and 50-70% coverage of city owned buildings in Toronto and Portland (Carter and Laurie 2008). Vegetated roofs have been demonstrated to improve stormwater retention (Bliss et al, 2009; Getter et al, 2007; Carter and Rasmussen, 2006, Mentens et al, 2006; Van Woert et al, 2005) and provide energy savings (van Woert et al, 2005), but also serve as a source of organic carbon, nutrients, and metals through runoff (Berndtsson et al., 2010). There is concern that the water quality benefits of green roofs related to reduced CSO events, may be offset by the direct contribution of organic carbon and nutrients in runoff from these systems. Runoff from vegetated roofs often contains very high concentrations of nutrients, particularly phosphorus but also organic carbon and sometimes inorganic nitrogen (Berndtsson et al. 2009; Berndtsson et al. 2010; Oberndorfer et al. 2007; Buffam and Mitchell 2015; Gregoire and Clausen 2010). Thus, there is a need for further study into the potential water quality benefits and, potential, negative impacts associated with nutrient release. These are at high enough levels to contribute to eutrophication in downstream waterways, and to date no clear solution to this ecosystem disservice has been found and tested.

A novel potential solution to this problem has been identified: the integration of biochar into the vegetated roof substrate. Biochar is the term given to biomass, such as wood, which has undergone pyrolysis to generate a carbon-rich product. The production of biochar is similar to the process which creates charcoal but is distinct in that the end product is meant to be used as a soil amendment. The purpose of this soil amendment is to increase soil productivity, sequester carbon, and filter percolating water (Lehmann, 2009). Adding biochar to soils has improved the ability of the soil to absorb phosphorous (Lehmann, 2002; Beaton, 1960; Downie, 2007), absorb metals by increasing cation exchange rate (Lehmann, 2009), and increase water holding capacity (Piccolo, 1996).

Biochar is a proven technology to improve water quality but it has not been extensively challenged in the treatment of green roof effluent. The multifaceted claims of biochar, specifically, improved soil fertility, carbon sequestration, and improved effluent water quality (Lehmann, 2009), suggest the technology could reduce threats to ecosystems receiving runoff, create cost savings due to reduced green roof maintenance through nutrient retention, and increase effectiveness of green roofs to retain water. Evaluation of this application is necessary

to determine the true effectiveness of this possible game changing technology for green roofs. One previous study (Beck et al., 2011) carried out in Portland, OR has shown that biochar is capable of improving effluent water quality (i.e. phosphorous, nitrate, organic carbon) and reducing runoff volume in green roofs. However, this study did not examine the temporal dynamics of sorption, nor the changes in performance by varying biochar concentrations in the media, instead using a fixed proportion of 7% biochar.

The *overarching objective of this project* is to improve our understanding of the water quality benefits associated with the utilization of a biochar-amended soil mix within vegetated roof technology. Our *central hypothesis* is that biochar can enhance the ability of vegetated roofs to improve water quality by binding nutrients (N and P) as the runoff passes through the amended green roof medium. While vegetated roof technology has been demonstrated to reduce rainfall runoff, additional research has demonstrated a potential degradation in the effluent water quality (Oberndorfer et al. 2007; Berndtsson et al. 2010). Recent research has shown a net leaching of dissolved nitrogen and exceptionally high levels of inorganic phosphorous in green roof runoff, both from full-scale green roofs and small-scale experimental plots (Buffam and Mitchell 2015). The use of biochar – an inexpensive activated carbon – is expected to improve the ability of vegetated roofs to retain nutrients. The *rationale* for understanding the water quality impacts of a biochar-amended soil medium is to evaluate the benefits for use within vegetated roofs as part of an integrated stormwater management plan, which would benefit designers and planners in assessing the potential impact to water quality conditions within a regional design setting.

Our project has three associated objectives: 1) evaluating the abiotic capabilities for nutrient and stormwater runoff retention due to enhanced sorption properties of biochar-amended soil medium via column reactors; 2) evaluating biotic and abiotic capabilities for nutrient and stormwater runoff retention due to enhanced sorption properties of a biochar-amended soil medium in vegetated green roof plots; and 3) developing a computational model for representing the hydraulic and water quality performance of vegetated roofs with and without biochar. In the first year of the project we carried out the first objective, while the second and third objectives were begun during year two, and will be completed in the coming year.

3. Column Studies on biochar-amended vegetated roof substrate (2013-2014)

3.1 Methodology

3.1.1 Column experiment design. We designed and carried out column studies in order to determine the nutrient holding capacity and water-holding capacity of biochar-amended vegetated roof substrate. Fixed bed column reactors (7 cm diameter, Figure 1) were packed with four different treatments in duplicate of combined biochar and commercial green roof media (with biochar proportions of 0%, 2%, 7%, and 14% of total weight) at 10 cm of media depth to conform to common extensive green roof construction. Biochar samples used were derived from a wood-based feedstock from chips or grounds, 3 mm minus, >70% carbon sorption >8% butane ash up to 23% but with low buffering at 500°C. The growing medium used was a proprietary aggregate based extensive blend from Tremco Roofing Inc, (Cincinnati, OH), sieved through a 2 mm sieve.

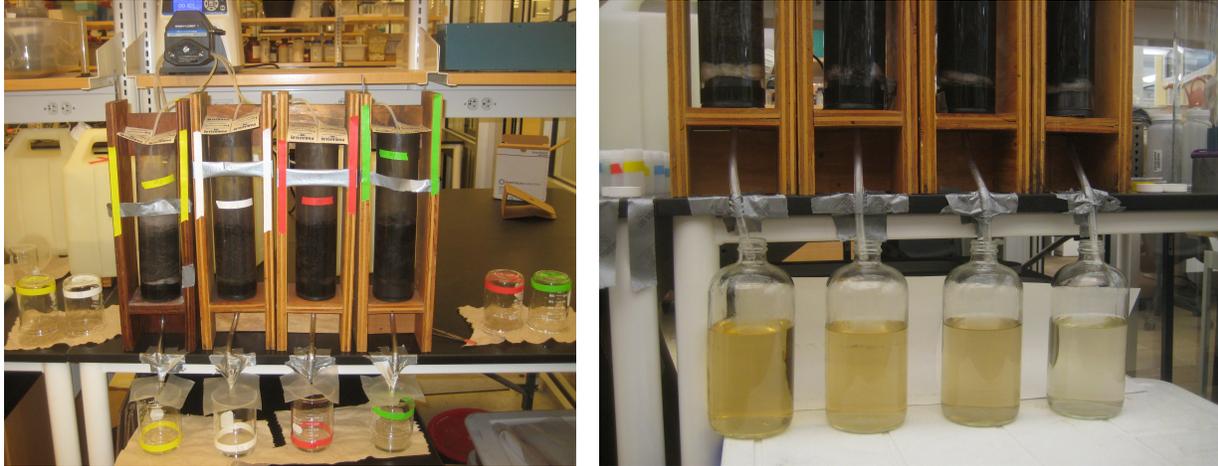


Figure 1. Left panel: Column setup showing pump, four columns varying in % biochar integrated into substrate, and beakers collecting continuous runoff water samples. **Right panel:** Collection bottles showing, from left to right, runoff water from 0%, 2%, 7%, & 14% by mass biochar/ substrate mixtures. Note the decreasing brown color for the high biochar mixtures, indicating removal of dissolved humics.

The columns were flushed during 24 hours with 1 year’s worth of artificial rain water (ARW), equivalent to 1 meter depth, immediately prior to the beginning of the experiment. This was done so that the experiment would not be dominated by the initial release of nutrients from the substrate and would instead behave as a more established green roof. The concentrations of compounds in the ARW were determined by using the NADP precipitation averages from 2007 to present for the Oxford, Ohio station (OH09) (<http://nadp.sws.uiuc.edu/NTN/ntnData.aspx>). The flow rate was then reduced to 1cm/h to simulate a heavy, yet realistic, rain event. This treatment was continued for 24 hours, followed by a “heavy nutrient” mix containing ARW enriched with approximately 3 mg/L each of NH₄-N, NO₃-N, and PO₄-P. This heavy nutrient mix was added at the same flow rate for 48 hours. This was determined to be long enough to observe the breakthrough of nutrients. This was followed by a 48h flush out using ARW. Thus, the total experiment length was 120 hours.

3.1.2 Water Quality Collection and Analysis. Water samples were collected at 1 hour intervals throughout the entire experiment, and enough samples analyzed to adequately construct the breakthrough curves (in practice, typically one sample every 4 hours). We collected a total of 120 per treatment (n=4) per trial (n=2) for a total of 960 samples. Column effluent and batch samples were collected in acid-washed high-density polyethylene containers, filtered at 0.45 μm, frozen to preserve and subsequently analyzed for the concentrations of the inorganic nutrients ammonium, nitrate and phosphate. Colorimetric techniques were used at a microplate scale (Ringuet et al. 2010) for nitrate (Doane and Horwath, 2003), ammonium (Weatherburn, 1967), and phosphate (Lajtha et al., 1999). Initial pilot study samples for the concentration of metals common in the urban environment including copper (Cu) and zinc (Zn), revealed that the green roof substrate was a sink for these metals when loading occurred at a level to be expected in urban environments. Even in the absence of biochar, effluent concentrations of these metals were below the detection limit (Atomic Absorption spectroscopy). Therefore, detailed breakthrough curves were not generated for these elements, and focus was rather placed on inorganic nutrients, which have been shown to be a pervasive issue in green roof effluent.

3.2 Principal Findings

In summary, we found that the incorporation of biochar into vegetated roof substrate substantially reduced and delayed the efflux of NH_4^+ and slightly reduced and delayed the passage of NO_3^- , but had little effect on PO_4^{3-} (Figure 2). Biochar also increased the water-holding capacity of the substrate (Figure 3), which has important implications for the stormwater runoff reduction potential of green roofs. When averaged over the entire 5-day experiment, the volume-averaged mean concentrations (directly proportional to total flux) were reduced in the high-biochar treatment by up to 75% for ammonium and 17% for nitrate, while all columns were a slight net source of phosphate regardless of biochar amendment (Figure 4).

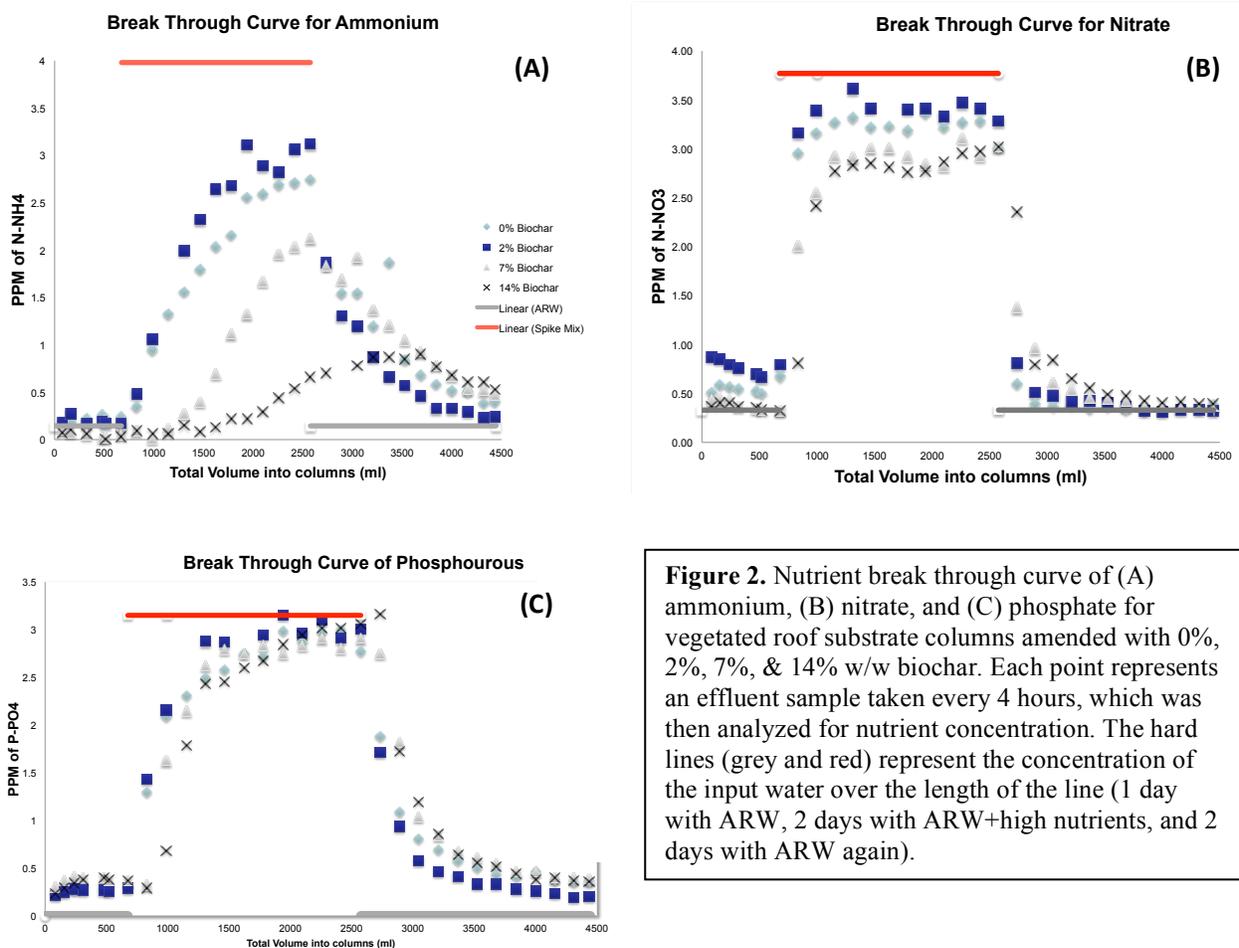


Figure 2. Nutrient break through curve of (A) ammonium, (B) nitrate, and (C) phosphate for vegetated roof substrate columns amended with 0%, 2%, 7%, & 14% w/w biochar. Each point represents an effluent sample taken every 4 hours, which was then analyzed for nutrient concentration. The hard lines (grey and red) represent the concentration of the input water over the length of the line (1 day with ARW, 2 days with ARW+high nutrients, and 2 days with ARW again).

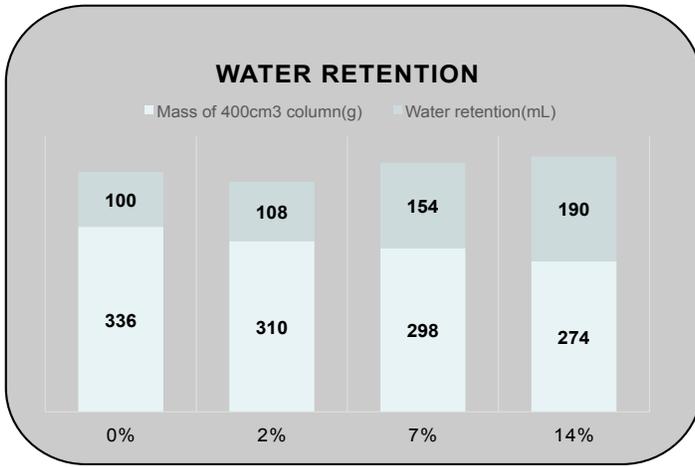


Figure 3. The water holding capacity was measured by the wet weight of the columns at the finish of the experiment. Increasing biochar had the effect of increasing water retention, with the 14% biochar treatment nearly doubling the water retention relative to the biochar-free control – this in spite of a lower initial mass for the high biochar column.

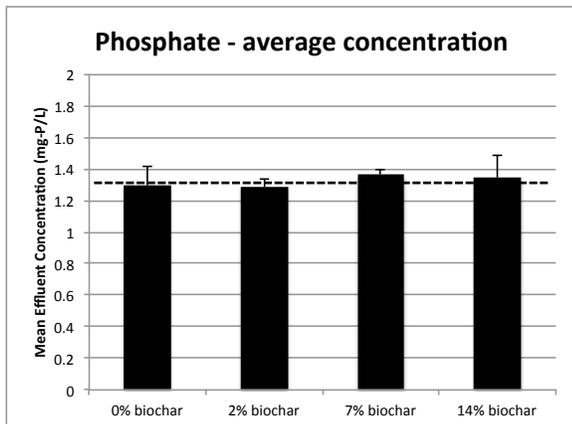
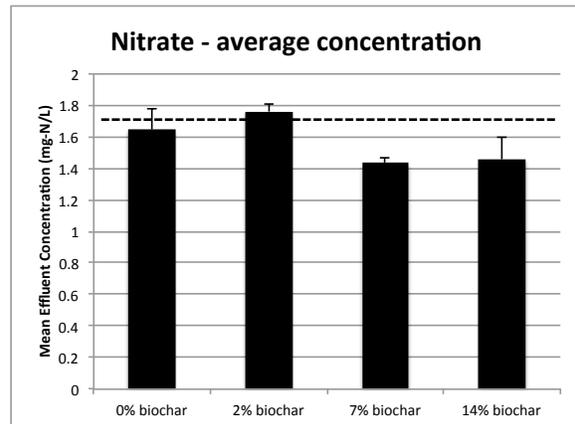
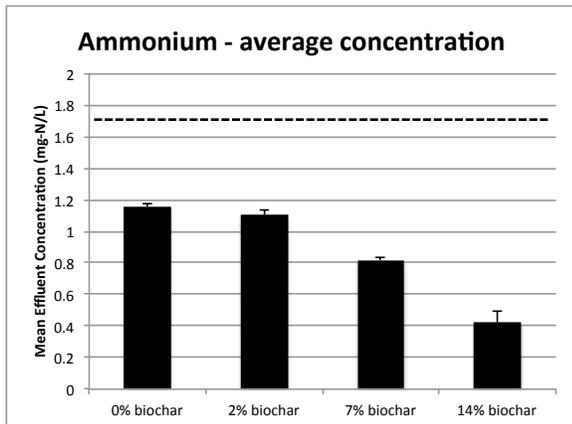


Figure 4. Volume-averaged mean effluent nutrient concentrations for the entire 5-day experiment, for columns varying in biochar % integrated into green roof substrate. Error bars represent standard error of the mean for duplicate trials. The horizontal dotted lines represent the volume-averaged influent “precipitation” concentration in the experiment. Substrate alone resulted in a 34% reduction in NH₄-N, with higher biochar resulting in a reduction of up to 76% of NH₄-N. NO₃-N fluxes were not affected by substrate alone, but the higher biochar treatments resulted in a 17% decrease in NO₃. PO₄-P fluxes were not affected significantly by either substrate alone, or biochar amendments; though all columns were a slight source of PO₄.

4. Batch experiments to determine sorption kinetics (Spring 2014)

4.1 Methodology

Pilot batch studies were carried out to determine the sorption kinetics for nutrients in vegetated roof substrate with and without biochar. Batch experiments were run with a known ratio of biochar to growing medium in solutions with a known concentration of three nutrients - ammonium, nitrate, and phosphate. The solutions contained either artificial rainwater (ARW) containing nutrients at levels observed in local precipitation, or ARW + 20 mg/L of NH₄-N, NO₃-N, and PO₄-P. Samples were taken periodically and analyzed for nutrient concentration.

Two sample levels were prepared per treatment, 0% biochar and 14% biochar, measured by mass. The 0% contained no biochar and 1.00 g of growing medium. The 14% contained 0.14 g biochar and 0.86 g growing medium. The mixtures were placed in 50 mL centrifuge tubes along with 40 mL of either ARW or the 20 ppm nutrient spike, depending on the treatment. Five centrifuge tubes were used per sample per treatment (20 total). Once the treatment water was added to the mixtures, the tubes were immediately placed on a shaker table shaking 100 rpm at 25°C. The table was covered to ensure no light penetrated the tubes to control for any photocatalytic activity that may ensue. Samples were taken at five time points- 10 min, 30 min, 1hr, 24 hr, and 96 hr- and vacuum filtered through a 0.45 µl filter then immediately frozen for further analysis. Nutrient concentrations of the effluent were determined using a spectrophotometer (Figure 5) as described in *Section 3.1.2* above.

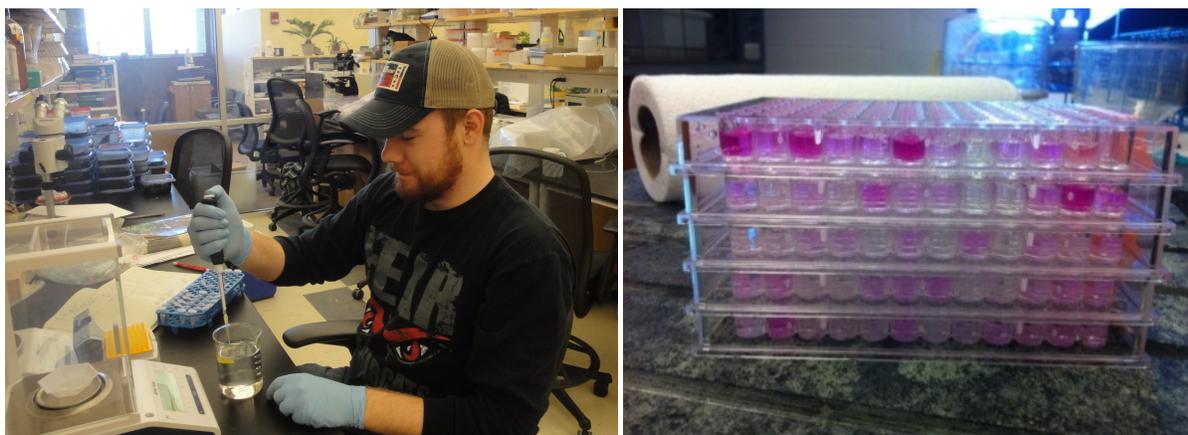


Figure 5. Left panel: Undergraduate research assistant Pat Wright pipetting out standards for use in nutrient analysis. **Right panel:** Side view of five 96-well microplates showing color development used in nitrate analysis.

4.2 Principal Findings

The objective of the batch study was to determine the kinetics and equilibrium concentrations for standard and biochar (14%)-amended substrate, subjected to two different initial nutrient concentrations in solution (ARW and 20 ppm). For the small-scale batch study, the hypothesis was that that for ammonium, nitrate, and phosphate, the concentration of nutrients in the effluent will be reduced with a higher ratio of biochar to growing medium. This was corroborated for ammonium (Figure 6), low concentrations of nitrate, and high concentrations of phosphate but rejected for low concentrations of phosphate because final concentrations were higher than initial values (data not shown). The 14% biochar amendment was quite effective at binding and

reducing concentrations of ammonium from ARW- though equilibrium was not reached after 96 hours as well as reducing concentrations from a 20 ppm spike and coming to equilibrium in 1 hour (Figure 6). This conclusion is congruent to Yao et al. (2011) finding in that biochar does have the ability to bind contaminants from water. This is also supported by the effect biochar had in reducing nitrate concentrations from ARW (data not shown). With phosphate, the opposite occurred and phosphate was leached out of the substrate into the ARW, as was seen by Buccola (2008). However, when initial phosphate concentrations are increased to 20 ppm, biochar did show the capability to bind phosphate with equilibrium reached at 1 hour (data not shown).

In the batch study, the biochar appeared to come to an initial equilibrium with the water for several hours then followed by a later decrease in concentration. This may indicate that multiple equilibria exist that could include binding sites on the surface of the biochar as well as deeper within the particle. This would entail a fast and slow cycle, where the slow cycle is the binding of nutrients to the surface sites, and the slow cycle is the nutrients diffusing further into the biochar particles to inner binding sites. This would seem plausible because the diffusion process through solids takes longer than surface binding and would account for the ~23 hour period of equilibrium. As a consequence of the complicated kinetics, estimated time to equilibrium varied substantially among treatments (Table 1).

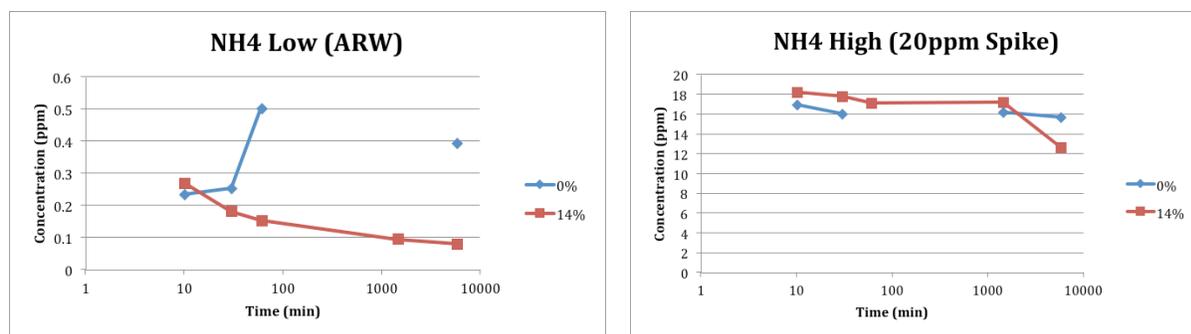


Figure 6. Left panel: The addition of 14% biochar to substrate decreases the concentration of NH_4 in ARW while substrate alone is a source of NH_4 . **Right panel:** For the high ammonium spike solution, both substrate alone and substrate + 14% biochar result in a gradual decrease in ammonium over time, presumably due to sorption. A fast initial drop (within 1 hour) followed by continued gradual decline in both experiments suggests multiple mechanisms of sorption. Missing points represent missing analytical samples in this pilot study.

Table 1. Time of equilibrium reached for each analyte and treatment, in the batch kinetic study.

Analyte	0% ARW	14% ARW	0% 20ppm	14% 20ppm
NH_4	≈ 2 hrs	N/A	30 min	>24 hr
NO_3	1 hr	>24 hr	N/A	N/A
PO_4	N/A	24 hr	>30 min	1 hr

5. Effect of biochar pretreatment on water holding capacity (Spring 2015)

5.1 Methodology

We designed and carried out a column study of the water retention capacity of green roof substrate vs. biochar of differing particle size distributions. Fixed bed column reactors (7 cm diameter, Figure 1) were packed with four different treatments, at 5cm depth: commercial green roof substrate, and three different treatments with 100% biochar, differing only in the size distribution of the biochar particles. One column was filled with raw biochar including some larger pieces, one was filled with biochar that had been sifted to have particles smaller than 2mm and one with biochar that had been blended in a blender on high for 3 bursts of 15 seconds. All columns were treated with a 2.5 cm/hour rainfall by using a Cole Palmer Masterflex L/S vacuum pump for approximately 3.5 hours. Biochar samples used were derived from a wood-based feedstock from chips or grounds, 3 mm minus, >70% carbon sorption >8% butane ash up to 23% but with low buffering at 500°C. The growing medium used was a proprietary aggregate based extensive blend from Tremco Roofing Inc, (Cincinnati, OH), sieved through a 2 mm sieve.



Figure 7. Column setup showing pump, four columns comparing the water retention capacity of green roof substrate (far left) vs. biochar varying in the particle size distribution. Note the brown color of the water coming from the substrate only column, indicating high dissolved humic material content.

5.2 Principal Findings

Biochar is much less dense than green roof substrate, but has a higher water holding capacity (Figure 8). We found that when we scaled the amount of water retained to the total mass of the material, biochar held about three times as much water as the vegetated roof substrate, and the three types were relatively equal to one another in their water holding capacity (Figure 8). With pure biochar mixes, when saturated 70+/-3% of the weight of the columns was water, while the remaining 30% of the weight was biochar. In contrast, saturated vegetated roof substrate was 25% water and 75% substrate by mass. We also found that blending resulted in fine particles of

biochar which packed into the column more densely, resulting in a density of 0.29 g/ cm³ when dry, and 1.01 g/ cm³ when fully saturated. Vegetated roof substrate had a dry density of 1.00 g/ cm³, and a wet density of 1.33 g/ cm³. Raw and sifted biochar were lighter still, with densities of 0.14-0.18 g/cm³, and wet density of 0.40-0.69 g/ cm³. As a consequences of these differences, the overall mass of the 192 cm³ (5 cm depth) columns varied substantially among the treatments. This has implications for construction of full-scale green roofs, which ideally have a high moisture holding capacity but are relatively lightweight. We chose to continue using raw biochar for future plot-scale experiments, since the pretreatment of the biochar (sifting, blending) was labor intensive and did not substantially affect the moisture-holding capacity per unit mass.

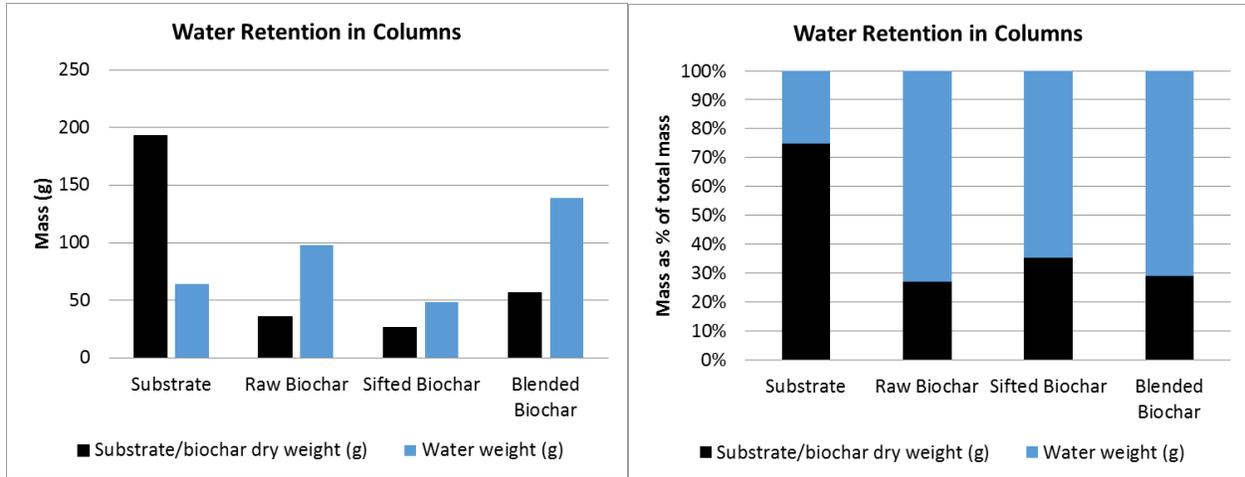


Figure 8. Substrate/biochar mass and water retention in 192 cm³ substrate or biochar (raw, sifted, blended) columns. (A) Mass in grams; (B) Mass as a % of total. Although blending and sifting affected biochar density, the water holding capacity as a proportion of the total weight was essentially identical for all of the biochar columns, which all held much more water per unit weight than did the substrate alone.

6. Effect of biochar-amended substrate on hydrodynamics in plots (Spring 2015)

6.1 Methodology

We carried out a pilot study using small-scale green roof test plots (Figure 9). The purpose of the pilot study was to develop a method for continuous measurement of evapotranspiration on green roof test plots, as well as determine the differences among evaporation rates among three plots with differing proportions of biochar incorporated into the substrate. Three small (30.5 cm x 61 cm) green roof test plots were constructed. Each plot was comprised of two Eco-Roof green roof trays, with one placed on top of the other. The substrate was held by the top tray and the bottom was empty and lined with 6 millimeter UV resistant polyethylene greenhouse sheeting (Greenhouse Mega Store). This bottom tray served the purpose of making the setup leak resistant and included a spigot with plastic tubing which allowed for easy collection of runoff. The outflow was collected in 10 liter HDPE carboys. The top tray was lined with a single layer of geotextile and held the Tremco extensive green roof growing media being used as a substrate mix and two plots also included biochar from Bluegrass Biochar, identical to that used in the experiments described in Sections 4 and 5 above. The substrate mix in the top tray reached a uniform depth of 8cm throughout the tray. Once two trays were fitted together and lined with

plastic and textile they were positioned at a 4% slope atop of an Adam CPW Plus-35 scale, linked to the Adam DU program on a PC, which logged masses for all plots at a 10 minute interval. This made it possible for us to view water loss due to evaporation in real time.



Figure 9. Caitlin Shaw overseeing plot and scale setup for continuous recording of weight changes due to evaporation from biochar-amended vegetated roof substrate.

Once the empty plots were secured atop their individual Adam CPW Plus-35 scale with data collection using the ADAM DU program, each of the three plots was filled with a substrate mix of 0, 5 or 10% biochar by mass. All plots were treated with 4 liters of tap water using a watering can at a slow pace (approximately equivalent to a 1" rainfall). The mass of the plots when water finished dripping is the saturated weight and was used to calculate water holding capacity. We then incubated the plots undisturbed for 11 days in the lab with ambient light and temperature, during which time the change in weight was recorded every 10 minutes as an indication of evaporation rate. After 11 days we oven dried each plot at 70 degrees Celsius for 48 hours. The weight after being oven dried provided us with a dry weight. The dry weight was subtracted from the saturated weight to yield the water holding capacity of each plot.

6.2 Principal Findings

Note, given the exploratory nature of this preliminary study which was primarily devoted to methods development, we only conducted a single replication of each treatment. Therefore, no statistical difference can be inferred until further tests can be completed.

1. The plot with 10% biochar substrate held the most water (Figure 10), retaining 3.29 liters (22.1 % v/v) despite having the lowest mass The 5% biochar plot retained 3.02 liters (20.3 % v/v) and the control (non-amended) substrate retained the least with 2.89 liters (19.4% v/v).

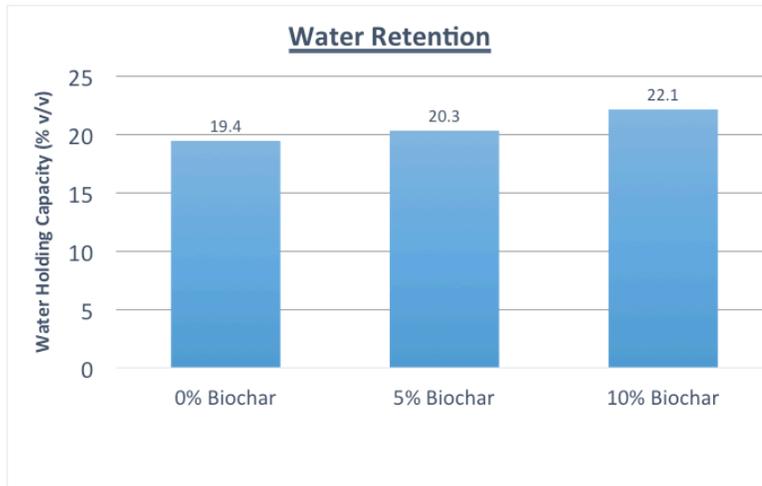


Figure 10. Water holding capacity as a percentage of total volume, for three vegetated roof plots with substrate and amended with either 0%, 5%, or 10% biochar by mass. All three plots were the same volume; the high biochar plots were lighter due to the low density of biochar.

- The plot with 10% biochar substrate had the fastest average rate of evaporation, losing 7.20 ml/hour on average over 11 days (Figure 11). The non-amended substrate and the plot with 5% are almost even averaging a loss of 6.73 ml/hour and 6.74 ml/hour respectively.

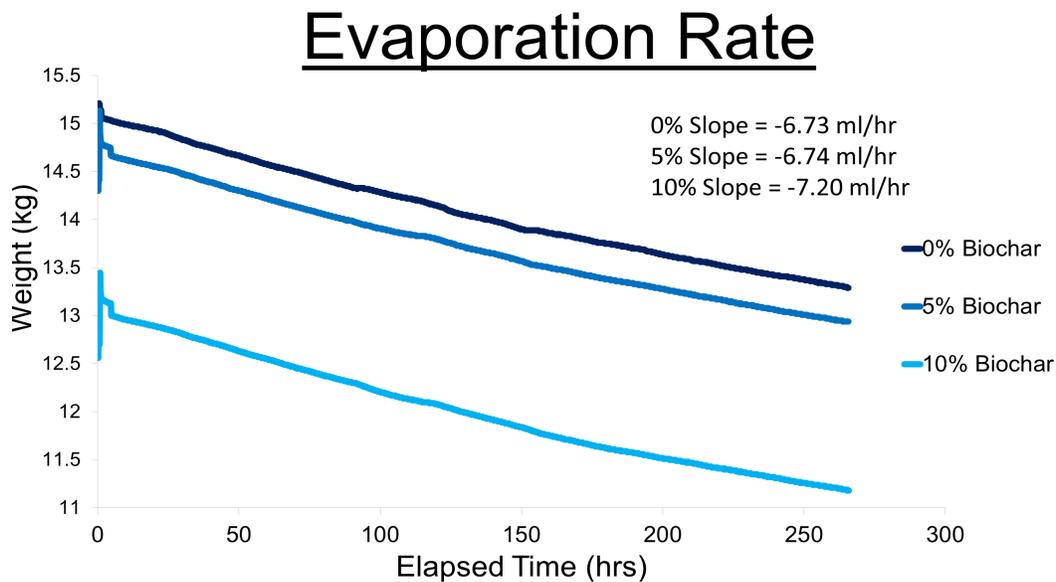


Figure 11. Weight change of each green roof plot (vegetated roof substrate alone, 5% biochar mix, 10% biochar mix) during 11 day incubation period. Evaporation rates were similar for all three treatments.

Our results demonstrate that biochar positively impacts the water holding capacity and that evaporation rates were not strongly affected by the use of biochar. Note, the experimental plots

were tested indoors in climate controlled (air conditioning/heat) were the average temperature was approximately 20 degrees Celsius. These conditions are much different than the normal outdoor conditions a green roof would typically experience. Variability of the evaporation rate will occur as a function of precipitation input, wind speed, wind direction, sunlight exposure, ambient relative humidity and temperature for outdoor applications.

Biochar increased the effectiveness of the green roof substrate by increasing the substrate's water retention capacity, but no significant impact on the rate of evaporation or the length of time the substrate can retain water could be inferred. All three plots (with identical volume) lost water to evaporation at very similar rates and retained water for approximately the same length of time. In another study, non-vegetated biochar amended plots retained 2-7% more water than the control plots, but the volume of water retention actually decreased by 1-3% when the plots include vegetation (Beck et al., 2011). Our study saw an increase in the volume of water retained, consistently, upon addition of biochar.

The main purpose of this study was development of a method for continuous measurement and recording of changes in plot weight, to get at evaporation and ultimately evapotranspiration rates. This succeeded and a working method was developed, but given the exploratory nature of this preliminary study, we conducted just a single replication of each treatment. Therefore, no statistical difference can be inferred until further tests can be completed. The scales are fieldwork scales and were covered in water resistant contact paper for our study. This will make it possible for the setup to be taken outside for ambient environmental exposure during subsequent experimental trials. In order to have more certainty and to better evaluate biochar's ability to retain water more replications would be required.

Future studies will examine the effects of biochar on plant vitality, particularly if biochar has an effect on the transpiration rate of plants by increasing water availability. Further plot studies must be conducted in order to continue to evaluate different combinations of substrates that could enhance green roof effectiveness and performance. We predict, due to biochar's ability to retain water, the plots with increasing biochar will produce the densest vegetation because plants will not need to compete for water, and possibly nutrients, as much as in other plots. Based upon the increase biochar can provide to water retention, designers of vegetative roofs should give serious consideration to the amending substrate with biochar. Its ability to increase the water retention of substrate would increase the effectiveness of the roof and may reduce the frequency of when the vegetation experiences water stress.

7. Significance

The integration of biochar is a potential breakthrough in reducing water quality degradation by green roof runoff, but very little is known about the sensitivity to variation in the proportion of the biochar amendment, or the dynamics of sorption kinetics or equilibria. Our project has demonstrated that a biochar amendment substantially decreases ammonium leaching from green roof substrate, by up to 75% for the high biochar (14% w/w) treatment. The high biochar treatment also doubled water holding capacity of the substrate, a finding with great significance for green roof design for stormwater runoff reduction. This is of particular note because on a per-mass basis, biochar is no more expensive than typical commercially available green roof substrate mixes. The patterns of breakthrough curves also give insight into likely physicochemical mechanisms of nutrient binding. Specifically, the inflections in the curve

suggest a dual-layer sorption mechanism for the biochar for ammonium and phosphate, with initial surficial sorption occurring within a few hours followed by a slow sorption process taking a few days, perhaps limited by diffusion into the interior of biochar particles. Follow-up work using different sorption breakthrough models and isotherms are underway, to further explore the sorption/desorption dynamics.

This study evaluates a low-cost option for improving the effluent water quality of vegetated roof technology, which is becoming increasingly more important as part of green-engineered solutions for stormwater management. The research demonstrates the water quality improvements associated with a biochar-amended green roof, but will also result in a modeling component that can be used within an integrated assessment framework both within and beyond the Ohio River Valley. As a result, the positive impact of this project will be a significant step forward in developing a more integrated infrastructure solution for storm water management by illustrating the potential impacts of biochar-amended vegetated roofs on CSO and nutrient management in urban environments.

8. Publication Citations

Journal Articles:

Buffam, I. and M.E. Mitchell. 2015. Nutrient cycling in green roof ecosystems. Chapter 5 in R. Sutton, ed. *Green Roof Ecosystems*, Springer, New York (May 2015; Research from this project is mentioned in the paper, though not the focus of the entire chapter).

Presentations at Conferences (March 2014 – Feb 2015)

*Indicates student presenter

*Hochwalt, P. and I. Buffam. April 2014. “Can biochar increase nutrient binding and water holding capacity in vegetated roof growing medium?” *University of Cincinnati 2014 Undergraduate Research Symposium, Cincinnati OH, USA (poster presentation)*.

*Wright, P., P. Hochwalt and I. Buffam. April 2014. “A batch study of the sorption kinetic properties of biochar amendment to vegetated roof growing medium” *University of Cincinnati 2014 Undergraduate Research Symposium, Cincinnati OH, USA (poster presentation)*.

*Divelbiss, D., P. Hochwalt, M. Mitchell, D. Boccelli, and I. Buffam. May 2014. “Black Is The New Green - Enhancing Green Roof Performance With Novel Substrate”. *Confluence Water Technology Innovation Cluster, 2014 Water Symposium, Covington, KY, USA (oral presentation)*.

Hochwalt, P., M.E. Mitchell, D. Boccelli, D. Divelbiss, and I. Buffam. November 2014. Biochar enhances water and nitrogen retention in green roof substrate. *Cities Alive: 12th annual Green Roofs and Walls Conference, Nashville, TN, USA (poster presented by I. Buffam)*.

12. References

- Beaton, J.D., H.B. Peterson, and N. Bauer. (1960). "Some aspects of phosphate adsorption to charcoal." *Soil Science Society of America Proceedings*. 24:340-346.
- Beck, D.A, G.R. Johnson, G.A. Spolek. (2011) "Amending greenroof soil with biochar to affect runoff water quantity and quality" *Ecological Engineering* 159:2111-2118.
- Berndtsson, J.C., Bengtsson, L., and Jinno, K. (2009) "Runoff water quality from intensive and extensive vegetated roofs" *Ecological Engineering* 35: 369-380.
- Berndtsson JC (2010) Green roof performance towards management of runoff water quantity and quality: A review. *Ecological Engineering*, 36, 351-360.
- Bliss, D. J., Neufeld, R. D., and Ries, R. J. (2009) "Storm Water Runoff Mitigation Using a Green Roof" *Environmental Engineering Science*, 26 (2), 407-417.
- Buccola, N. 2008. "A laboratory comparison of green roof runoff." A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Civil and Environmental Engineering, Portland State University, Portland, OR.
- Buffam, I. and M.E. Mitchell. 2015. Nutrient cycling in green roof ecosystems. Chapter 5 in R. Sutton, ed. *Green Roof Ecosystems*, Springer, New York.
- Carter, T. L. and Rasmussen, T. C. (2006) "Hydrologic Behavior of Vegetated Roofs" *Journal of the American Water Resources Association*, 42 (5) 1261-1274.
- Carter, T.; Laurie, F., Establishing green roof infrastructure through environmental policy instruments. *Environmental Management* **2008**, 42, 151-164.
- Deutsch, B.; Whitlow, H.; Sullivan, M.; Saveneau, A. *Re-Greening Washington, DC: A Green Roof Vision Based on Quantifying Storm Water and Air Quality Benefits*; 2005; p 15.
- Doane, T.A., and W.R. Horwath. (2003) "Spectrophotometric determination of nitrate with a single reagent" *Analytical Letters* 36: 2713-2722.
- Downie, A., V.C. Lima, G. Seehann, J. Donath, V.R. Monntoia, and T. Schwarz. (2007) "Retention properties of wood residues and their potential for soil amelioration." *Wood Science and Technology*. 41:169-189.
- Getter, K. L., Rowe, D. B., Andersen, J. A. (2007) "Quantifying the Effect of Slope on Extensive Green Roof Stormwater Retention" *Ecological Engineering*, 31 (4), 225-231.
- Gregoire, B. G. and Clausen, J. C. (2011) "Effect of a modular extensive green roof on stormwater runoff and water quality" *Ecological Engineering*, 37, 963-969.

Lajtha, K., Driscoll C.T., Jarell W.M., and Elliott E.T. (1999) "Soil phosphorus: characterization and total element analysis" Pages 115-142 in G.P. Robertson, D.C. Coleman, C.S. Bledsoe, and P. Sollins, editors. *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, New York.

Lehmann, J. and S. Joseph. (2009) *Biochar for environmental management: Science and technology*. Stirling, VA: Earthscan.

Lehmann, J., J.P. da Silva Jr., M. Rondon, M.S. Carvo, J. Greenwood, T. Nehls, C. Stiener, and B. Glaser. (2002) "Slash-and-char – a feasible alternative for soil fertility management in the central Amazon?" *Proceedings of the 17th World Congress of Soil Science*. Bangkok, Thailand. Paper no 449.

Mentens, J., Raes, D., and Hermy, M. (2006) "Green Roofs as a Tool for Solving the Rainwater Runoff Problem in the Urbanized 21st Century" *Landscape and Urban Planning*, 77 (3), 217-226.

NYC Department of Environmental Protection. (2011) "NYC Green Infrastructure Plan: A Sustainable Strategy for Clean Waterways", 154 pp.

Oberndorfer, E., Lundholm, J., Bass, B., Coffman, R., Doshi, H., Dunnett, N., Gaffin, S., Kohler, M., Liu, K. K. Y., and Rowe, B. (2007) "Green roofs as urban ecosystems: Ecological structures, functions, and services" *Bioscience*, 57, 823-833.

Piccolo, A., G. Pietramellara, and J.S.C Mbagwu. (1996) "Effects of coal-derived humic substances on water retention and structural stability of Mediterranean soils." *Soil Use and Management*. 12:209-213.

Ringuet, S., Sassano, L., & Johnson, Z.I. (2010) A suite of microplate reader-based colorimetric methods to quantify ammonium, nitrate, orthophosphate, and silicate concentrations for aquatic nutrient monitoring. *Journal of Environmental Monitoring*, **13**, 370-376.

Van Woert, N. D., Rowe, D. B., Andersen, J. A., Rugh, C. L., Fernandez, R. T., and Xiao, L. (2005) "Green Roof Stormwater Retention: Effects of Roof Surface, Slope and Media Depth" *Journal of Environmental Quality*, 34 (3), 1036-1044.

Weatherburn, M.W. (1967) "Phenol-hypochlorite reaction for determination of ammonia" *Analytical Chemistry* 39: 971-974.

Yao; Gao; Inyang; Zimmerman; Cao; Yang. 2011. "Removal of phosphates from aqueous solutions by biochar derived from anaerobically digested sugar beet tailings." *Journal of Hazardous Materials*. 190: 501-507.

Characterizing the influence of surface chemistry and morphology on biofilm formation of ceramic membranes in wastewater treatment

Basic Information

Title:	Characterizing the influence of surface chemistry and morphology on biofilm formation of ceramic membranes in wastewater treatment
Project Number:	2013OH300B
Start Date:	3/1/2013
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	OH-12
Research Category:	Engineering
Focus Category:	Treatment, Acid Deposition, Water Quality
Descriptors:	
Principal Investigators:	Paula J Mouser, Hendrik Verweij, Linda Kay Weavers

Publications

1. Krinks, J.K., Weavers, L.K., and Mouser, P.J., 2014, Effectiveness of Ultrasound to Clean Biologically-Fouled Membranes. Poster presentation at Water Management Association of Ohio: 43rd annual conference. November 18-19, 2014, Columbus, Ohio.
2. Krinks, J.K., Weavers, L.K., and Mouser, P.J., 2014, Recovery of biologically-fouled ceramic membranes using ultrasonic cleaning. Oral presentation, In: American Chemical Society: 250th national conference. August 16-20, 2014, Boston, Massachusetts.

Title: Characterizing the influence of surface chemistry and morphology on biofilm formation of ceramic membranes in wastewater treatment

Ohio Water Resources Center: Final Project Report

PI: Paula J. Mouser, **co-PI:** Linda Weavers, Hendrik Verweij

Abstract

The commercial use of membranes for water and wastewater treatment has been steadily growing for decades, and is now considered a cost-effective alternative for new treatment facilities when compared to conventional treatment systems. The state of Ohio alone has dozens of large-scale water and wastewater treatment facilities utilizing membranes; many of which have begun operation within the last ten years. Although there are advantages to utilizing membranes, their major disadvantage is their susceptibility to fouling – and particularly biological fouling (biofouling). Effective methods of mitigating biofouling are therefore critical for efficient operation and longevity of membranes. This study investigates ultrasonic cleaning of ceramic microfiltration membranes fouled by municipal membrane bioreactor wastewater. Our study gives specific attention to the composition of extracellular polymeric substances (EPS) on fouled membranes and the influences of membrane surface charge by altering the pH of ultrasonic cleaning solution. Experimental results indicate ultrasonic cleaning outperforms conventional cleaning methods (rinsing and air scouring) with post-sonication flux measured as over 30% greater than post-conventional cleaning flux. Characterization of EPS indicated proteins were effectively removed via sonication, whereas polysaccharides were more persistent foulants. Differences in pH affected both the surface charge of the membrane and the structural characteristics of the biological foulants, verified with Fourier transform infrared spectroscopy. The highest recovery of membrane flux occurred at the pH closest to the isoelectric point of the ceramic membrane, indicating that minimizing the membrane surface charge may be a key parameter for optimizing ultrasonic cleaning.

Methodology and Materials

Wastewater: Domestic wastewater was collected from an MBR facility in the City of Delphos, Ohio. The wastewater was analyzed for various properties, including concentrations of proteins, polysaccharides, total suspended and volatile solids, pH, and molecular structure characteristics of the solids at varying pH using a Fourier transform infrared (FT-IR) spectrometer.

Membrane Fouling: Ceramic microfiltration (0.2 μm) membranes constructed of alpha-alumina were purchased and used for fouling and cleaning experiments. Filter cells were constructed in dead-end filtration mode and wastewater was delivered using a variable speed peristaltic pump. Permeate was collected on an electronic balance and mass was continuously recorded to calculate flux and gauge the rate and extent of fouling. Following significant decline in flux ($>80\%$ below initial flux) the membranes were removed from the filter cells for further analysis or cleaning. A photo of the experimental equipment used for the filtration tests is shown in Figure 1.

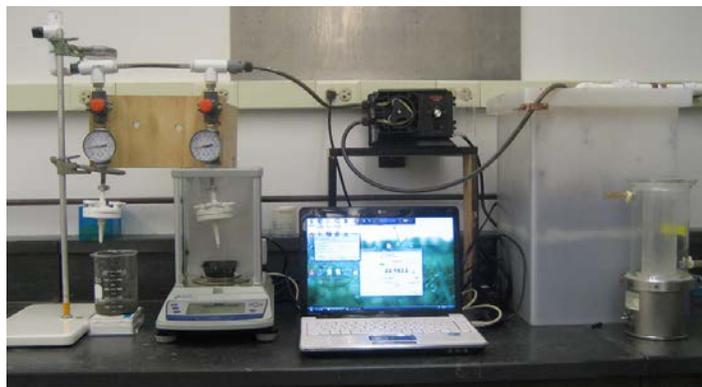


Figure 1. Experimental apparatus used for fouling tests.

Membrane Cleaning: Off-line cleaning of fouled membranes was performed via one of the following strategies: 1) conventional cleaning by rinsing with MBR wastewater and light air scouring in the aerated tank; 2) ultrasonic cleaning in pH 4 solution; 3) ultrasonic cleaning in pH 7 solution; and 4) ultrasonic cleaning in pH 9 solution. Following cleaning, membranes were either returned to the wastewater filtration cell for flux measurement or underwent fluorescent staining and confocal laser scanning microscope (CLSM) imaging for EPS quantification. Ultrasonic cleaning equipment consisted of a glass reaction vessel affixed to an ultrasonic transducer. The reaction vessel was filled with pH-adjusted solution and membranes were submerged and sonicated at 620 kHz at 2.7 watts per cm^2 for 30 seconds.

Fluorescent Staining and CLSM Imaging: A quadruple staining procedure coupled with CLSM imaging was performed on fouled and cleaned membranes to identify relative quantities and spatial characteristics of EPS. The employed fluorescent stains included FITC for proteins, concanavalin A for α -D-glucopyranose polysaccharides, calcofluor white for β -D-glucopyranose polysaccharides, and STYO 63 for nucleic acids. An Olympus FV1000 CLSM (Ohio State University, Campus Microscopy and Imaging Facility) was used to observe EPS at a minimum of five random locations for each membrane analyzed. Confocal image slices were analyzed with specialty software to quantify the abundance of the four individual EPS components.

Findings

Fouled membranes were subjected to fluorescent staining and CLSM imaging to identify the abundance of α - and β -D linked glucopyranose polysaccharides, proteins, and nucleic acids. From these CLSM images (example in Figure 2), a vertical profile numerically representing the percent abundance of the four EPS constituents within the fouling layer above the membrane surface was generated, which is shown in Figure 3. Percent abundance refers to the percent of pixels within the CLSM image detecting the respective fluorescent stain.

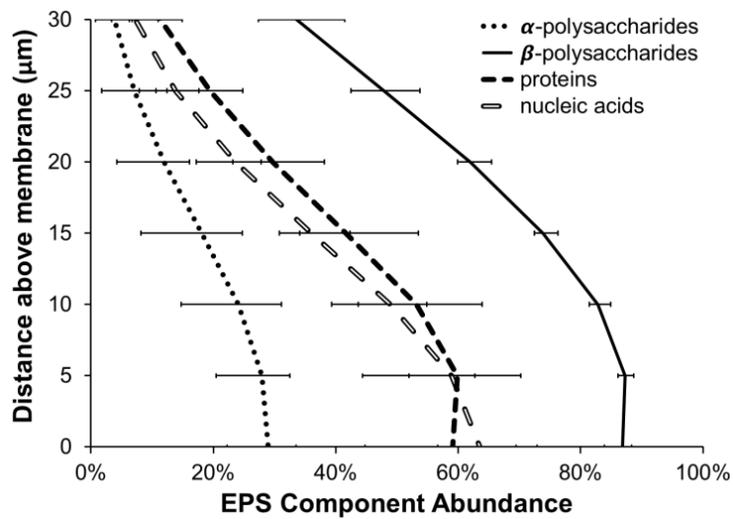
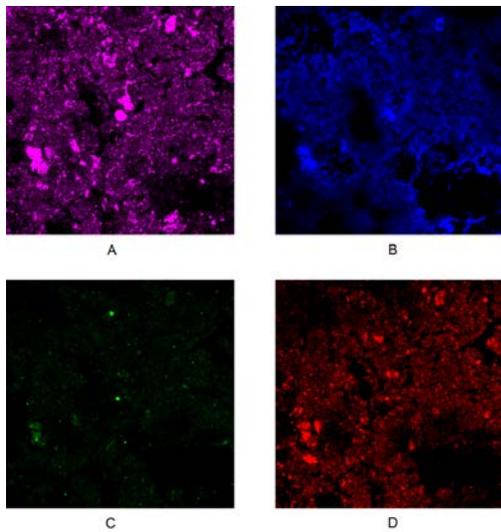


Figure 2. CLSM image of EPS taken at the surface of a fouled membrane; Channels are (A) β -D-glucopyranose polysaccharides, (B) proteins, (C) α -D-glucopyranose polysaccharides, and (D) nucleic acids.

Figure 3. Vertical profile of EPS composition above the surface of a fouled membrane.

Fouled membranes were cleaned by either conventional methods or sonication with solution pH of 4, 7 or 9. Cleaned membranes were returned to the wastewater filtration cell for measurement of initial flux to quantify recovery. The results of the initial recovery for each cleaning are provided in Figure 4. Figure 4 also provides a relative distribution of the four EPS components observed on the membrane surface with respect to each other. Using the CLSM images of fluorescent stains, vertical depth profiles of EPS foulants were developed for each cleaning regimen, which are shown in Figure 5. The profiles concluded highly effective removal of proteins by sonication. Different locations on the surface of cleaned membranes did not contain repeatable quantities of foulants. Therefore, to create a normalized distribution for all observed images, the four EPS constituents were multiplied by a factor which resulted in the most abundant EPS component accounting for approximately 8% to 10% of the total image resolution. These depth profiles therefore do not yield quantifiable measures of EPS foulants after cleaning, but rather qualitative information on their relative abundance and distribution.

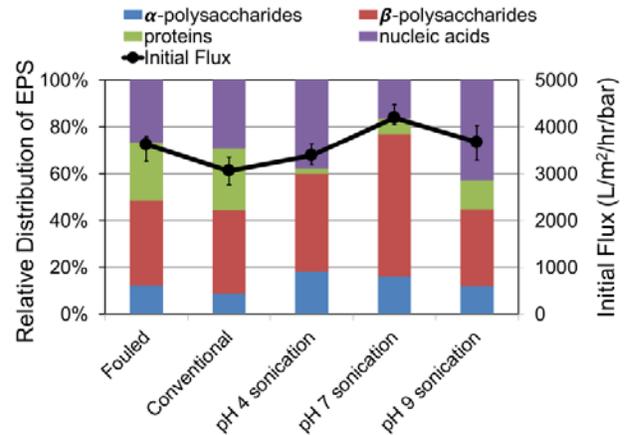


Figure 4. EPS distribution on fouled membrane surfaces and initial flux of cleaned membranes. Note 'fouled' flux refers to the initial flux of a virgin membrane (prior to fouling).

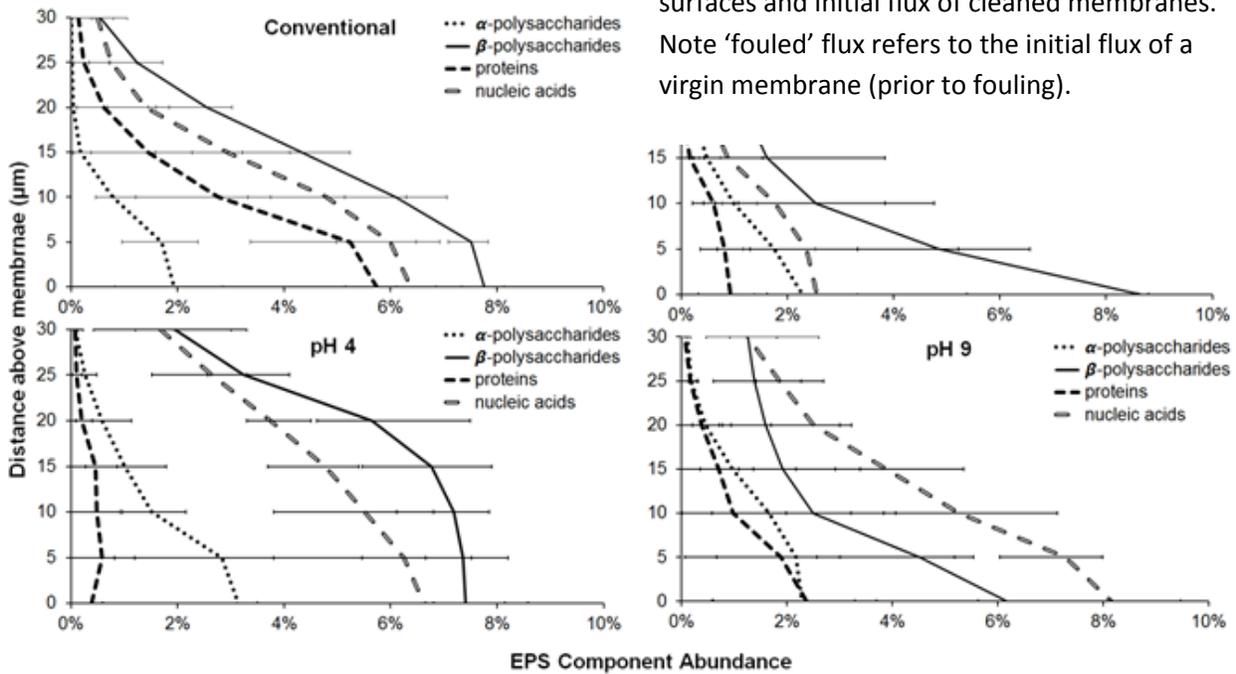


Figure 5. Vertical profiles of EPS composition above surface of cleaned membranes.

Significance of Findings

We have demonstrated the application of ultrasound to be an effective method of recovering flux across ceramic membranes fouled by municipal MBR wastewater. Compared to conventional cleaning, the use of ultrasound results in differing distributions of EPS at the membrane surface and higher flux recovery. We observed that the pH of the ultrasonic cleaning solution plays a role in EPS removal selectivity, which can be attributed to a combination of altering the membrane surface charge and structural characteristics of the foulants. Ultrasonic cleaning near the isoelectric point of the membrane (pH 7) yielded the highest recovery of flux, suggesting minimizing the electrostatic charge interactions between the membrane and foulants may be an optimum cleaning condition. Regarding large-scale applicability of ultrasonic cleaning systems for MBRs, it is not considered viable to install transducers in aeration tanks adjacent to the membranes. Rather, such an application is better suited for a small cleaning system in which individual membranes could be removed from service, cleaned via sonication, and returned to the aeration tank.

High-performance biologically inspired membranes for water treatment

Basic Information

Title:	High-performance biologically inspired membranes for water treatment
Project Number:	2013OH4350
Start Date:	5/1/2013
End Date:	2/28/2015
Funding Source:	Other
Congressional District:	9th
Research Category:	Engineering
Focus Category:	Treatment, Water Quality, Waste Water
Descriptors:	Membrane separations; water treatment; biomimetic
Principal Investigators:	Isabel Escobar

Publications

1. Wagh P. and I.C. Escobar (2013). "Synthesis of Biomimetic Polybenzimidazole Nanofiltration Membranes," 2013 AIChE Annual Meeting, San Francisco, CA, November 3-8, 2013.
2. Escobar (2014). "Development of Biomimetic Nanofiltration Membranes Using Aquaporins," 2014 NAMS Annual Meeting, Houston, TX, May 31-June 4, 2014.

Title. High-performance biologically inspired membranes for water treatment

PI. Isabel C. Escobar, The University of Toledo.

Problem and Research Objectives, Methodology, and Principal Findings and Significance

Problem:

Water is an essential element in all separation processes involved in any application. Water is required in varying degrees of purity depending on the application for which it is used. Membrane separations play an important role in different industrial applications related to water, energy, pharmaceutical and life sciences. They provide an alternative to conventional separation processes to obtain cost effective and high quality water [1-3]. However, there are some drawbacks involved with membrane separation processes; for example, desalination membranes provide very low water flux values. In addition to this, limited lifetime of membranes, insufficient pollutant rejection, further treatment of concentrates, and chemical resistance of membranes are some of the problems that we have to face while dealing with membrane separations [2-5]. Therefore, even though membrane separations are now established processes for water treatment, there is still need to develop new membranes that would avoid these issues with membranes.

Aquaporin is a bidirectional water channel protein present in cells, and it regulates the flow of water in and out of cells. Aquaporin has, therefore, a potential to make high water flux biomimetic membranes. Water passes through porous structures as a single unbroken column of molecules with hydrogen bonds between two adjacent water molecules and can carry protons with it. However, this flow of protons along with water is prevented while passing through aquaporins. Aquaporins have pores which have constriction in the middle and they become wide at the opening. So it causes high dielectric barrier for charged entities like protons or other ions

while allowing penetration of neutral solutes. Amide and carbonyl groups in the Asparagine-Proline-Alanine motif that is present facing out in the pore walls in aquaporin molecule make hydrogen bond with oxygen atom causing reorientation of water molecule and it becomes perpendicular to the axis. Therefore the two hydrogen atoms can't make H-bond with adjacent water molecules. All other groups present on the pore-walls are hydrophobic leaving the two hydrogen atoms without hydrogen bonding partners and giving energy nearly equal to 3 kcal.[6-9] so water can pass through with minimal energy barrier. Figure 1 shows a schematic representation explaining the mechanisms for blocking proton permeation in aquaporin. The diagram illustrates how partial charges from helix dipoles restrict orientation of water molecules [6].

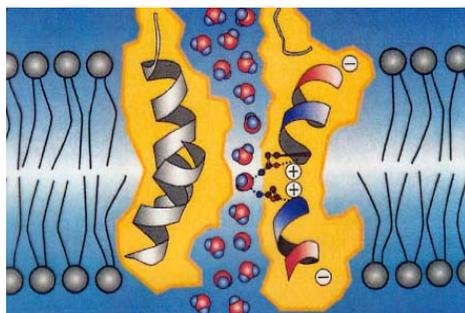


Figure 1. Aquaporin structure [6].

For this research project, AqpZ was used, which is a water channel protein found in *Escherichia coli*, since it is inexpensive and can be expressed in large quantities. AqpZ is robust under different reducing conditions and at low temperatures [10, 11]. The overarching hypothesis is that by incorporating AqpZ into the membrane, water permeability can be improved while maintaining or improving ion rejection. Successful formulation could lead to the formation of biomimetic membranes with high selectivity and high water flux. However, challenges involved in the incorporation of the AqpZ protein in membranes limit their applicability. One of them is to

attach aquaporin to the membrane without chemically altering or damaging the aquaporin during the binding to the membrane. The second challenge is to design and prepare an assembly that allows biomimetic membranes with aquaporins to sustain hydraulic water pressure gradients without losing their integrity and performance [10]. To address these, in this study, aquaporins were treated with gum arabic (GA), a polysaccharide used previously to disperse carbon nanotubes to PVA without causing any change in their structure or without affecting the stability of the final assembly [12]. GA was hypothesized to act as an intermediate layer to enhance the interfacial interaction between selective layer and the substrate making the assembly stronger. Aqp-GA was then dispersed in an amphiphilic polyvinyl alcohol carrying alkyl side chains (PVA-alkyl) matrix. PVA-alkyl is amphiphilic in nature with high hydrophilicity of PVA and hydrophobicity of long alkyl side chains. It has good film forming properties and outstanding physical and chemical stability [13]. It is proposed to be an excellent material to support aquaporins. Thus, by attaching PVA-alkyl matrix with Aqp-GA dispersed in it to the hydrophilized PBI membranes, the assembly was made mechanically stronger and it was proposed to withstand hydraulic water pressure gradients.

Research Objective:

The objective of the project was to make a new class of biomimetic nanofiltration membranes made of unaltered aquaporin dispersed in a membrane selective layer and capable of operation under high hydraulic pressure. Unaltered AqpZ can be dispersed in an amphiphilic PVA-alkyl matrix. The PVA-alkyl with embedded aquaporins will be used as the nanofiltration membrane active layer (Figure 2).

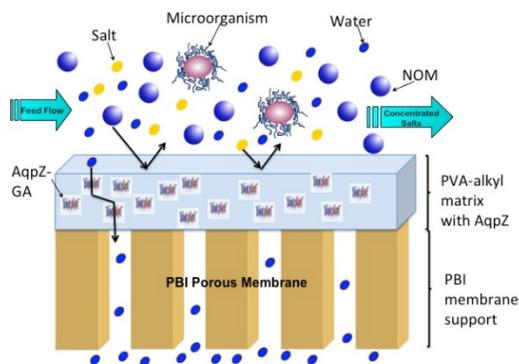


Figure 2. Schematic of biomimetic membranes.

Methodology:

PBI membranes casting:

The dope polymer used to cast the backbone of the membranes was polybenzimidazole (PBI). PBI is stable polymer, which has robust mechanical strength with thermal stability for a wide range of high temperature applications and it also provides chemical stability over a wide range of pH. PBI membranes are hydrophobic [14, 15]. They are strong but brittle [16-18]. The structure of PBI molecule is shown Figure 3. Within the imidazole group of PBI, the heterocycle has two nitrogen atoms. One is attached to the hydrogen atom as a site to form hydrogen bonds while other nitrogen has a lone pair, which can act as a proton acceptor.

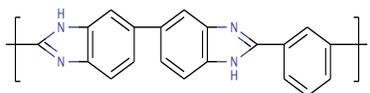


Figure 3. PBI molecule structure.

The solvent used to make the dope solution was N, N-Dimethylacetamide. Commercially-available 26% w/w dope solution, which contains 26% PBI polymer, 72% N, N-

Dimethylacetamide (DMAc) and 2% Lithium chloride (LiCl) was used and obtained from PBI Performance Products, inc. (Charlotte, NC). LiCl served the function of pore former and it prevented PBI polymer from phasing out of the solution [17, 19]. Hence, it imparts long shelf life to the solution. The dope solution was diluted to 21-23% PBI by adding solvent to it and the solution was sealed with parafilm in order to prevent the air bubbles from being trapped inside the solution affecting its homogeneity. Because of very high viscosity of the solution, the solution was kept in the sonicator and degassed for 2 days in order to ensure complete mixing of the solvent and the solute and make the solution homogeneous. After sonication, the solution was allowed to come to room temperature and then the solution was ready to make the membranes. The membranes were cast using phase inversion process. Phase inversion is the phenomenon whereby the phases of a liquid-liquid dispersions interchange such that the dispersed phase spontaneously inverts to become the continuous phase [18, 20]. The non-solvent phase that is used in this process is water.

A casting knife, or doctor's blade (Paul N Gardner Co, U.S. Pat 4869200, Pompano Beach, FL.) was used to make flat sheet membranes. A clean glass mirror was used as a surface, which provides optimum hydrophobicity to the membranes and helps for detachment of polymer films during phase inversion [20]. The solution was placed in an even line on the surface and the casting knife was used to push the solution across the glass surface to make a thin film. The thickness of the membranes was kept between 150 μ m and 200 μ m. A water coagulation bath was used to induce phase inversion subsequent pore formation within the membranes. Once the phase inversion had taken place, the membrane came out of the surface of the water. The membrane was thoroughly washed with water and kept in a 50/50 glycerol-DI water solution. Glycerol was added to DI water due to possibility of water evaporation because PBI can become brittle and

susceptible to breakage [16]. The membranes were kept in the solution at least one day before they were analyzed.

Surface activation of membranes:

Polybenzimidazole membrane surface was needed to activate for further modifications with PVA-alkyl and additions of Aqp-GA to the PVA-alkyl matrix. Activation was achieved by way of reaction of a highly reactive chlorine atom, from 4-chloromethyl benzoic acid (CMBA) purchased from Sigma-Aldrich (USA), with the secondary amine group in the imidazole ring of repeat unit in PBI backbone (Figure 3). CMBA adds a carboxylic group to the surface, which serves two purposes: 1) to impart negative charge on the membrane surface, and 2) to act as a platform for subsequent functionalization of the membrane. It is important to note that there are two secondary amine sites in PBI molecule, so after the reaction, carboxylic groups are added on both sites on the molecule. For simplicity, the reaction at only one site is shown (Figure 4).

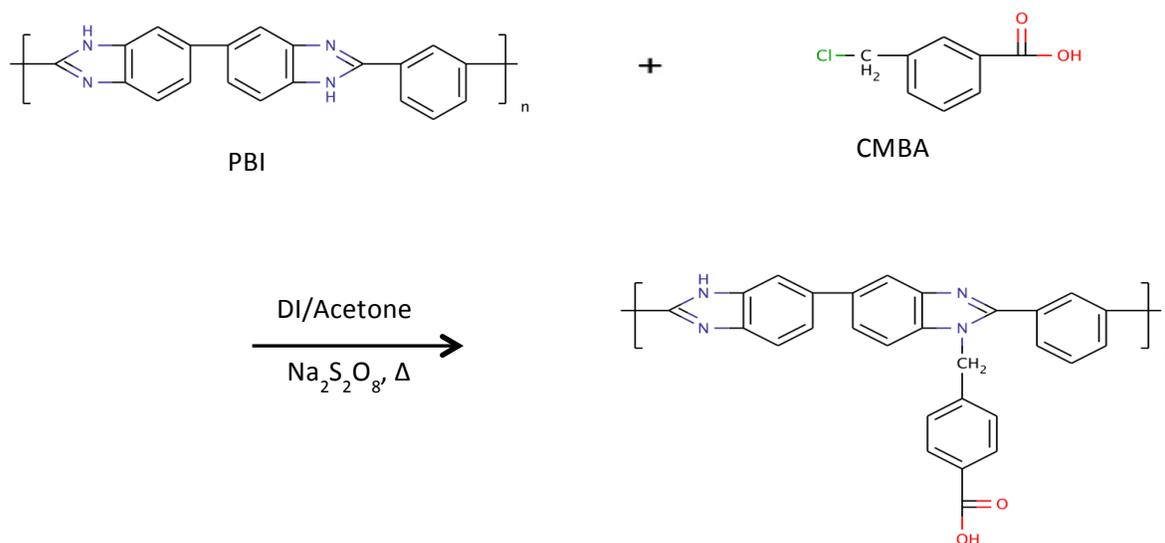


Figure 4. CMBA modification of membrane.

Modification was performed according to previous studies [16]. For the reaction, 1 wt% solution of sodium persulphate in water was prepared. Sodium persulphate was used as a free radical initiator for the reaction. 200 ml DI water were taken in a 500mL beaker with a stir bar. 2.02g sodium persulphate was added to the water, and the solution was stirred on hot plate. The temperature was set at 40°C. Two membranes were added to the solution, which were kept fully submerged and were not stuck under the stir bar. In a second beaker, 0.5 wt% solution of CMBA in acetone was prepared. 0.788 g CMBA was added to 200 mL acetone and was stirred until all CMBA was dissolved. Then acetone/CMBA solution was slowly added to the beaker on a hot plate while stirring, and then covered. This was done in order to prevent the precipitation of CMBA as it is insoluble in water. The final solution was a 50/50 mixture. The temperature of the solution was kept at 40°C and it was stirred for 24 hours. The temperature was chosen to keep all the reactants in solution and prevent the evaporation of CMBA. Once the reaction was finished,

the membranes were washed with copious amounts of DI water to remove excess sodium persulphate and placed in glycerol/water bath as soon as possible.

Preparation of PVA-alkyl:

PVA-alkyl is polyvinyl alcohol carrying long alkyl side chains. It is amphiphilic as it has both hydrophilic and hydrophobic elements present in it. PVA has hydrophilic properties and long alkyl chains that account for the hydrophobicity of the molecule. It has good film forming properties and outstanding physical and chemical stability. Using PVA as skin layer provides the resulting membranes with high water permeation rate, good antifouling nature, and excellent integrity in acidic and alkaline and remarkable resistance to abrasion [13, 21]. PVA-alkyl was prepared in the lab in two steps [22, 23], the preparation of (1) carboxy-methyl PVA followed by (2) PVA-alkyl.

1) Preparation of carboxy-methyl PVA (PVA-COOH):

The preparation of carboxy-methyl PVA (PVA-COOH) followed literature methods [24, 25], summarized here. Initially, 50mL water were taken in a 100 mL beaker, 1g PVA (Acros Organics, USA) was added to it, and the solution was kept at 70°C. The mixture was stirred for 1 hour continuously to prevent PVA from sticking to the bottom of the beaker. The stirring was continued until PVA was completely dissolved in water. The solution was transferred to a 500mL beaker, and 50g sodium monochloroacetate (Fisher Scientific, Pittsburgh PA) were added to it. The solution was then covered with aluminum foil and was incubated at 4°C for 24 hours. After that, 42mL water were taken in a 100mL beaker, 42g sodium hydroxide (Fisher

Scientific, Pittsburgh PA) were added, and the mixture was stirred until sodium hydroxide was completely dissolved.

NaOH/water solution was added to the incubated solution of PVA and sodium monochloroacetate in water, and it was kept while stirring at room temperature for 24 hours. Then, it was neutralized using hydrochloric acid. For that, a 6M solution of hydrochloric acid (Fisher Scientific, Pittsburgh PA) in water was prepared in a separate beaker with pH being continuously monitored. This neutralized solution was dialyzed against deionized water. The molecular weight cut-off for the dialysis was chosen as high as possible, which was 12-14 kDa in order to maximize dialysis rate. The dialysis tubing (Fisher Scientific, Pittsburgh PA) used was soaked in water for 3 hours in order to open it and fill it with the solution. It was then sealed with dialysis locking membrane clamps. A 2000mL beaker with a stir bar in it was filled with DI water to be used as a dialysate. It was kept stirring and the water was changed after every 4-5 hours to make sure that the driving force for the dialysis is high. The procedure was continued for 3 days, and the remaining solution in the tube was taken out and stored in another beaker. Then, the stored solution after dialysis was deionized using ion-exchange resins. DOWEX 1X8 (Acros Organics, USA) was used for negatively charged ions and DOWEX 50WX8 (Acros Organics, USA) was used for positively charged ions. The output solution after the ion exchange was lyophilized using freeze dryer. For that, the solution was kept in centrifuge tubes and was allowed to freeze dry for 3 days. PVA-COOH was obtained as a white solid after freeze-drying. The weight of the product was 0.45 g and the yield was 39%. The overall reaction is shown in Figure 5.

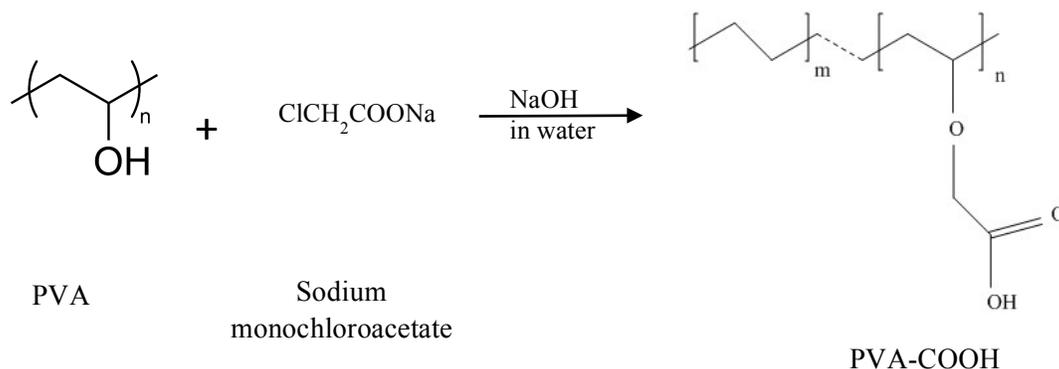


Figure 5. PVA-COOH synthesis.

2) Preparation of PVA-alkyl:

The second step of making PVA-alkyl was synthesis of hexadecanal. Chemicals used for the preparation were celite, pyridinium chlorochromate and 1-hexadecanol. Dichloromethane was used as the solvent. 11.2mL dichloromethane were taken in a 50mL beaker while stirring, and 0.95g celite, 0.95g pyridinium chlorochromate and 0.5 g 1-hexadecanol were added to the beaker under a fume hood. The solution was sealed with aluminum foil and stirred for 6 days at room temperature. After that, the reaction mixture was diluted by adding 40mL diethyl ether. Florisil columns were used to remove excess celite, pyridinium chlorochromate and 1-hexadecanol. Then the mixture was evaporated, and hexadecanal was obtained as a white solid.

PVA-COOH obtained after first reaction was dissolved in DMSO. Hexadecanal and 200 μ m of 12M hydrochloric acid were added to the solution, which was maintained at 70°C and kept stirring for 25 hours. The reaction mixture was extracted with diethyl ether. The mixture was then neutralized with 1M sodium hydroxide. The neutralized solution was dialyzed against DI water using dialysis tubing of 12-14kDa following the same procedure as described previously dialysis. The solution was then desalted with ion exchange resins previously

mentioned and lyophilized using a freeze dryer for 3 days. PVA-alkyl was obtained as a white solid with yield of 49% at a final weight of the product was 0.22g. The overall reaction for the second step of the process is shown in Figure 6.

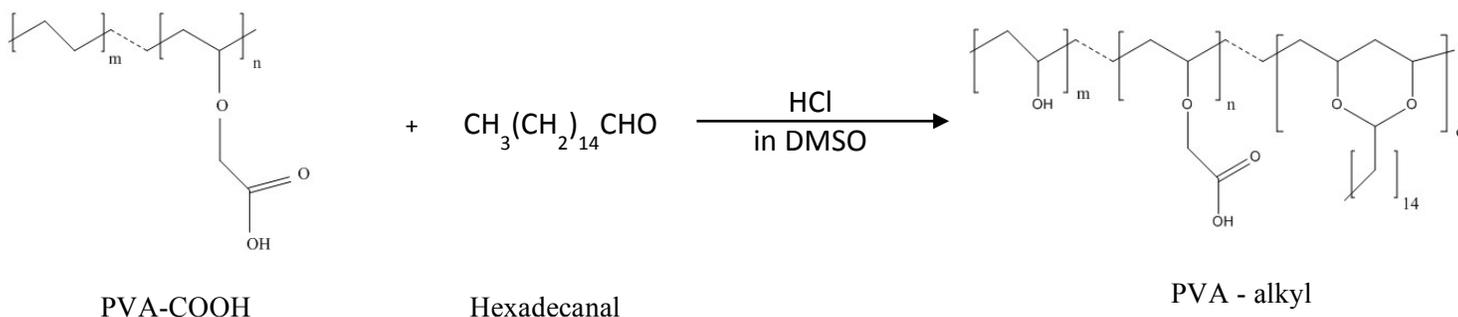


Figure 6. PVA-alkyl synthesis.

AqpZ expression:

Aquaporin Z (AqpZ) is a bacterial aquaporin from *Escherichia coli*, which shows high degree of homology to its mammalian counterparts and can be easily expressed from the bacteria cell. Furthermore, it is highly stable and resists denaturing due to heat, detergent, voltage, and pH changes [8, 10]. This makes the protein suitable for commercial use.

For the expression of AqpZ, a cell culture was prepared in LB media at 37°C and either used immediately or stored at -80°C. SDS-PAGE was performed with NuPAGE Novex 12% Bis-Tris gels in MES SDS running buffer (Invitrogen Life Technologies, Carlsbad, CA). The calculated monomer mass of hexahistidine-tagged AqpZ was 27 kDa but on NuPAGE Novex Bis-Tris gel the AqpZ proteins run at a lower electrophoretic mass of approximately 18kDa, as shown in Figure 7.

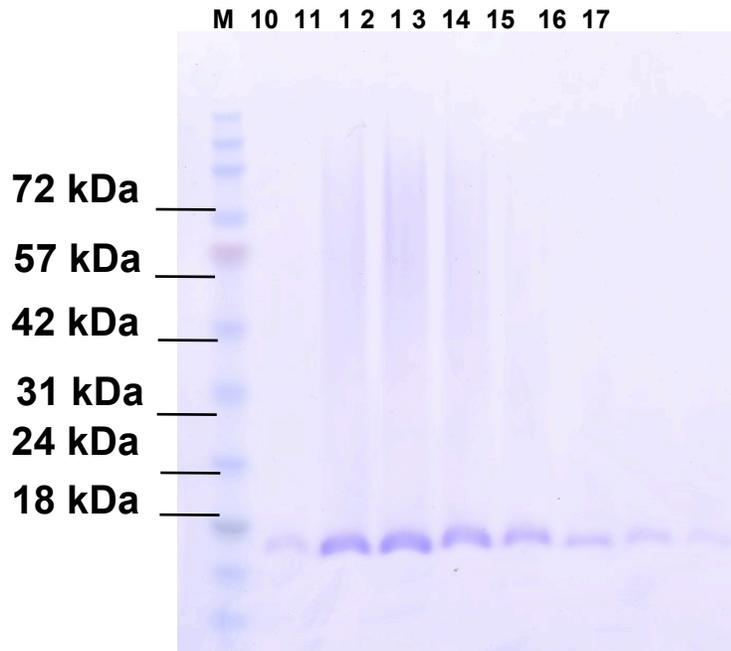


Figure 7. SDS- PAGE data showing expression of AqpZ at 18 kDa.

Treatment of AqpZ with gum arabic:

A 15wt. % solution of gum arabic (Fisher Scientific, USA) in water was prepared. AqpZ was added to this solution and sonicated under mild conditions for 4 hours in order to disperse it in the gum arabic solution. Gum arabic has been used previously to disperse carbon nanotubes to PVA without either shortening the nanotubes or disrupting their structures. Hence, it was proposed to protect the structure of aquaporins. The weight ratio of AqpZ to gum arabic was kept 1:4. The mixture was kept stirring at room temperature for 72 hours and then heated slowly to remove water [12, 26]. Gum arabic-treated AqpZ were then added to de-ionized water and sonicated for 30 minutes. The solution with gum arabic-treated AqpZ was physically dispersed into the PVA-alkyl solution. Aquaporin is bidirectional water channel protein; hence the orientation of the protein doesn't change the water flux through the protein [27].

Surface modification of PBI membrane using Aqp-PVA-alkyl:

Aqp-PVA-alkyl was attached to the membrane using carbodiimide chemistry [16]. Carbodiimide chemistry was used for the reaction between carboxylic group and a nucleophile. The nucleophile that was used in this case was the hydroxide group present in PVA-alkyl molecule. For the reaction, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDCH) and N-hydroxysuccinimide (NHS), both purchased from Sigma-Aldrich were used. The reactions were performed in 2-(N-morpholino) ethanesulfonic acid (MES) buffer (Sigma-Aldrich, USA).

400mL water were taken in a 500mL beaker and 7.85g MES buffer was added to it. The solution was stirred until MES was completely dissolved in water. 11.75g NaCl were added to the solution and stirred until dissolved. The solution was titrated to pH 6 using NaOH. 0.23g NHS and 0.153g EDCH were added to the solution and stirred well. CMBA modified PBI membranes were added to the solution, which was stirred for 15 minutes. After 15 minutes, the reaction mixture was titrated to pH 7 using NaOH. PVA-alkyl was added to the solution and it was kept stirring for 24 hours. After 24 hours, the membranes were taken out of the solution and rinsed well with DI water and stored in a beaker filled with DI water. The membranes were stored for 24 hours before using it for analysis. The overall reaction is shown in Figure 8.

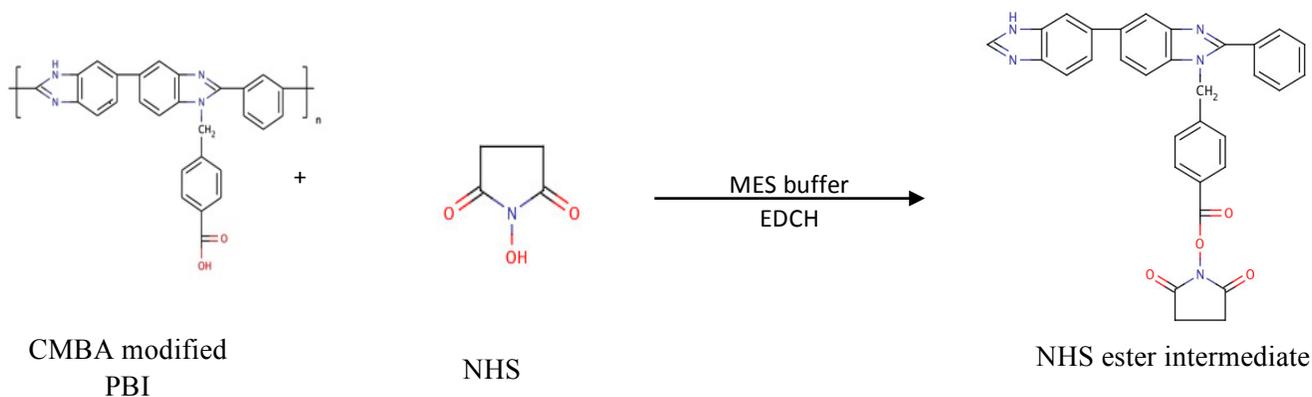


Figure 8(a)

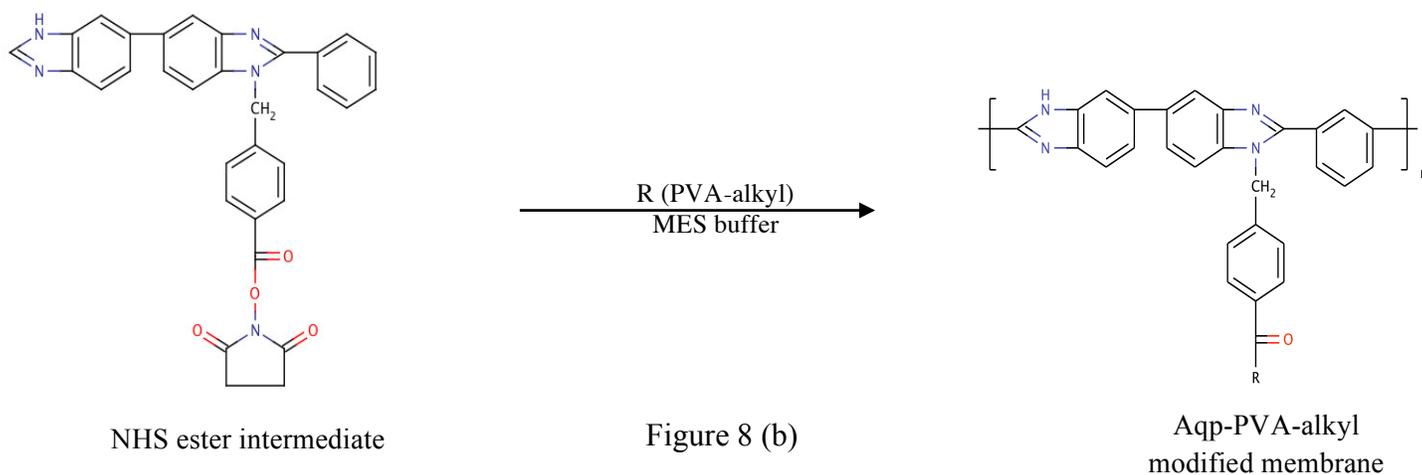


Figure 8 (b)

Figure 8 (a) and (b). Carbodiimide membrane activation chemistry.

Membrane characterization:

1. Fourier Transform Infrared Spectroscopy (FTIR):

Vibrational spectrum is a characteristic of every molecule and is considered a unique property of that molecule [28]. FTIR was used to determine the chemistry of the proposed reactions and modifications on membrane surface. FTIR was used in ATR mode to study the

chemical nature of the membranes. Digilab UMA 600 FT-IT microscope was used for all the analysis of membrane samples performed in this study.

2. Contact angle measurements:

Contact angle is defined as the measure of wettability of a surface. Cam-Plus Micro contact angle meter (Tantec Inc., Schaumburg, IL) was used for the contact angle measurement of all the membrane samples in this study. A small drop of water was placed on the membrane surface and resultant angle of the droplet to the surface was measured as shown in Figure 13. The higher the hydrophobicity of the membrane, the higher the contact angle is.

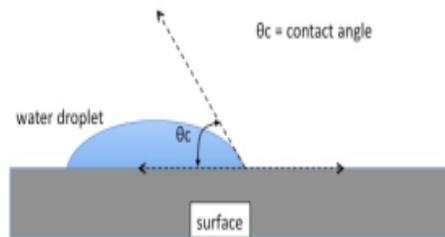


Figure 9. Contact angle measurement [29].

3. Flux analysis:

Dead end filtration was used to monitor the flux decline of both PBI and PVA-alkyl-AqpZ membranes. Filtration experiments were performed using Amicon filtration cell (Amicon Stirred Cell 8010 – 10 ml). Using a constant membrane surface area of 4.1 cm^2 , the time to collect a 2-ml permeate sample was measured for each feed and flux was calculated. A constant pressure of 70 psi and continuous stirring was applied in all tests. Flux values were calculated as

L/m²-hr and plotted against the total time of filtration. Membrane samples were cut into circular pieces of area 4.1 cm² and supported by a WhatmanTM filter paper (110 mmø). Each membrane was precompact with DI water for 7-8 hours until a stable flux was reached. Precompaction was followed by filtration of protein solutions of 10 ppm each of bovine serum albumin (BSA) and lipase protein in water. The same filtration cell was used for protein filtration under the same conditions of pressure and stirring. Protein rejections were calculated using UV-VIS spectrometer (Varian Inc. Cary 50, Agilent Technologies, Santa Clara, CA) according to following equation (1):

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100\% \quad (1)$$

Salt rejection was tested using five solutions of different concentrations of sodium chloride and calcium chloride in DI water: 3.4, 10, 20, 35 and 100 mM solutions. Solutions were run through the 10 ml dead-end cell under the same conditions as used for the pure water permeability testing. Continuous stirring was applied inside the cell to prevent salt build-up on the membrane surface. Two mL permeate samples were collected for each feed. Salt rejections were calculated using conductivity meter. The apparent solute rejection R (%) was calculated using equation (1).

After each feed water filtration, the membrane was backwashed for 1 hour with DI water and filter paper support was changed. The flux recovery of the membrane was measured after backwash.

Results and Discussion:

Chemical analysis:

Figure 10 shows the FTIR analysis of PBI, CMBA and PVA-alkyl-AqpZ membranes, and Table 1 identifies all peaks. FTIR showed peaks at $\sim 1050\text{ cm}^{-1}$ for C-O stretch, $\sim 1200\text{ cm}^{-1}$ for alkyl substituted ether, which is observed after PVA-alkyl is attached to CMBA-modified PBI molecule using carbodiimide chemistry (Figure 11). A peak at 1660 cm^{-1} corresponds to C=O stretch that is present in the final molecule due to addition of CMBA. FTIR spectrum of PBI molecule also shows a peak at 1650 cm^{-1} , which is owing to the presence of secondary amine group present in PBI. Peak 4 corresponds to O-H stretch, which is present in CMBA-modified PBI molecule as well as PVA-alkyl-AqpZ-modified molecule (Figure 11). Peak 5 in the analysis is due to long alkyl chains that are present in the PVA-alkyl-AqpZ-modified molecule [28]. A very broad peak around 3400 cm^{-1} is observed in all the membrane samples, which is associate with N-H stretching [30]. Bonds corresponding to different functional groups are indicated in Figure 11.

Table 1: Functional groups and corresponding wave number in IR spectra.

Number	Functional group	Wave number (cm ⁻¹)
1	C-O	1057
2	C-O (ether)	~ 1200
3	C=O	1670-1820
4	OH	~ 2670
5	C-H	~ 2850
6	>N-H	1550-1650

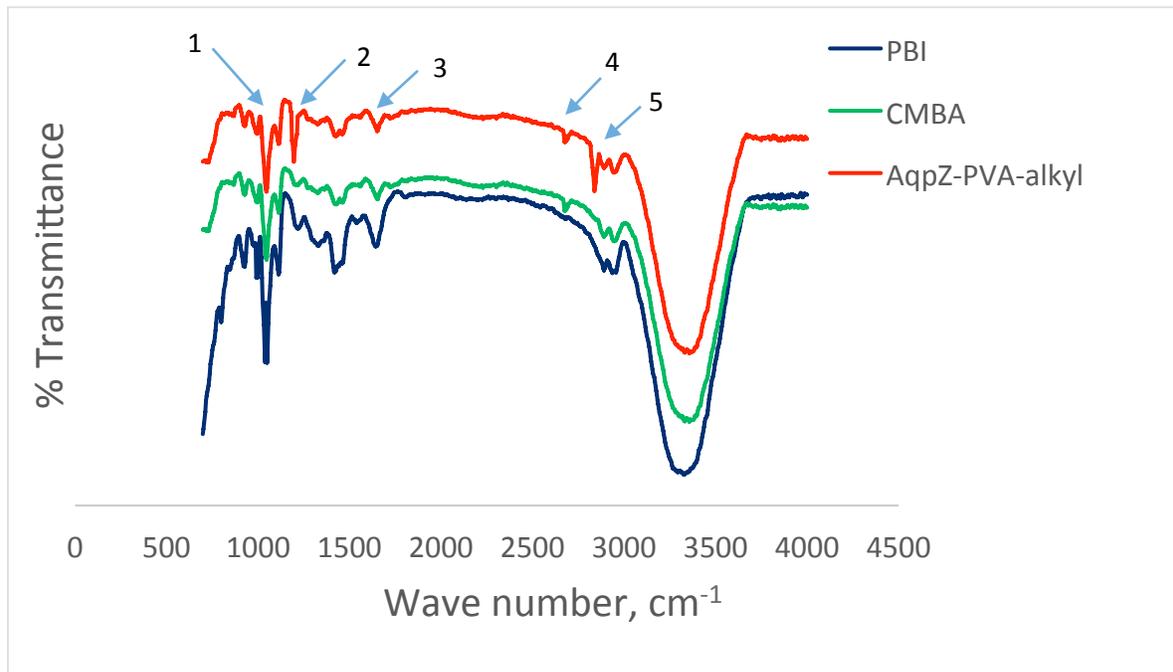


Figure 10. FTIR analysis of unmodified, CMBA modified and PVA-alkyl modified membranes.

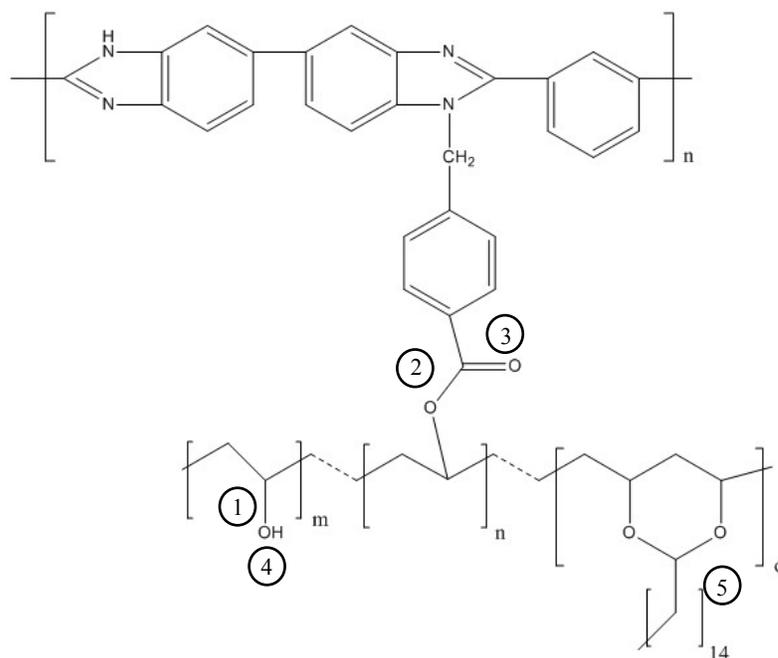


Figure 11. AqpZ-PVA-alkyl modified PBI molecule.

Hydrophobicity:

Contact angle was used as a measure of hydrophobicity, and results are shown in Figure 12. CMBA modified membranes were found to be more hydrophilic than PBI membranes [16, 18]. This was most likely due to addition of a -COOH bond in the modified molecule and its increased ability to form hydrogen bonds because of the presence of oxygen with a lone pair. However, the change is not statistically significant because of the overlap of standard deviations. After the addition of PVA-alkyl to the membranes, the contact angle decreased further showing a significant increase in the hydrophilicity of the membrane. This was most likely due to high hydrophilicity of PVA [13]. After AqpZ was added to the membrane, there was no significant difference between its contact angle and that of the PVA-alkyl membranes, which is likely because the aquaporins that were added to PVA-alkyl matrix were embedded inside the matrix

and not on the surface of the membrane. This might be the reason of almost similar values of the contact angles of PVA-alkyl modified and Aqp-PVA-alkyl modified membrane surfaces.

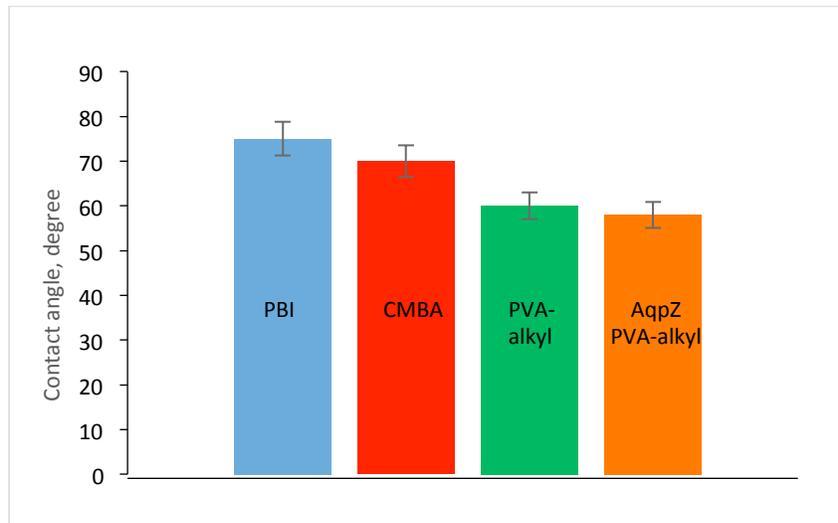


Figure 12 Hydrophobicity via contact angle

Flux analysis:

1) Permeability:

Before comparing observations between PBI and PVA-alkyl-AqpZ (or AqpZ-modified) membranes, it is important to separate the effects of the addition of PVA-alkyl only and PVA-alkyl-AqpZ to the membrane. Experiments were conducted in parallel comparing PBI, PVA-alkyl and PVA-alkyl-AqpZ membranes using BSA and lipase solution filtration. For PBI membranes, the pure water flux was 7.90 L/m²-h (LMH), the initial flux for BSA filtration was 5.77 LMH, the recovery after BSA filtration (i.e. after DI backwash) was 5.47 LMH (or a 69% flux recovery, as compared to the initial pure water flux), and the final flux after lipase filtration was 3.82 LMH. BSA and lipase rejections were approximately 86% and 84%, respectively. After

the addition of PVA-alkyl (no aquaporins), the pure water flux was lower at 6.0 LMH, which is likely due to the extra resistance added by the addition of PVA-alkyl to the surface of the membrane. The initial flux for BSA filtration was 4.36 LMH, the recovery after BSA filtration was 4.80 LMH (or an 81% flux recovery), and the final flux after lipase filtration was 4.13 LMH. BSA and lipase rejections were approximately 90% and 87%, respectively. While flux values for the PVA-alkyl membranes were not as high as for the PBI membranes, it is clear that the flux decline during filtration was improved and the final flux after the 36-hour filtration period was higher. This was associated with the increase in hydrophilicity of PVA-alkyl (Figure 12). Lastly, after the addition of PVA-alkyl with embedded aquaporins (PVA-alkyl-AqpZ), the initial pure water flux was lower at 5.32 LMH, which again is likely due to the extra resistance. The initial flux for BSA filtration was 5.0 LMH, the recovery after BSA filtration was 5.0 LMH (or a complete recovery of the filtration flux, or 96% flux recovery as compared to the initial flux), and the final flux after lipase filtration was 4.43 LMH. BSA and lipase rejections were approximately 91% and 87%, respectively. Therefore, it is hypothesized that the addition of aquaporins to the membrane led to a more consistent flux value and a nearly complete recovery of flux back to its initial pure water flux after cleaning.

More in-depth flux comparisons were then conducted between PBI and PVA-alkyl-AqpZ membranes for a period of 140 hours with protein solutions and salt solutions. These flux analyses are shown in Figures 13 and 14. For unmodified PBI membranes, initial flux values observed were higher than for the PVA-alkyl-AqpZ membranes due to extra increased thickness, and hence resistance, associated with the addition of PVA-alkyl to the membrane matrix. However, the flux decline observed was higher in unmodified membrane than modified membrane.

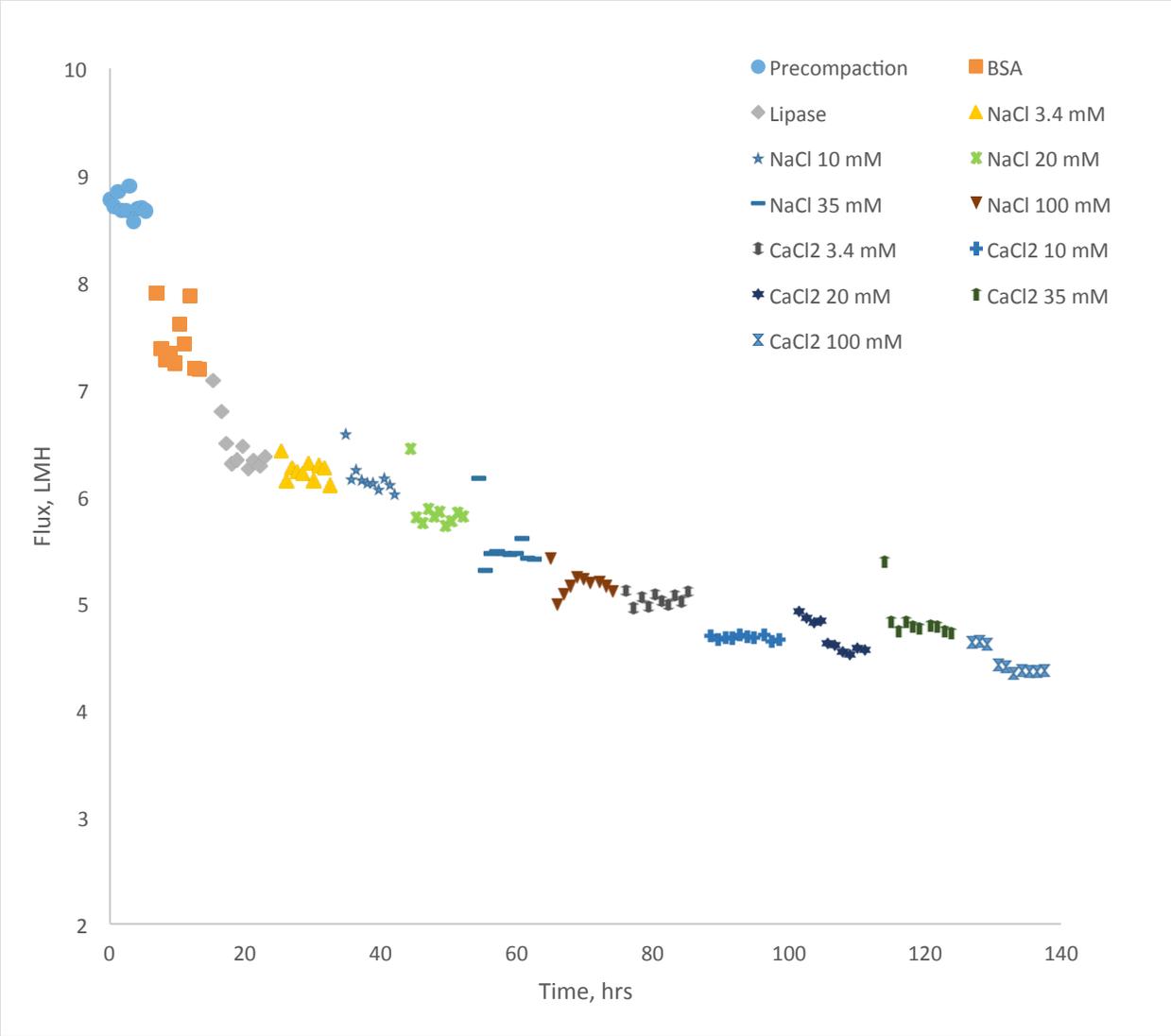


Figure 13. Flux analysis of unmodified PBI membranes.

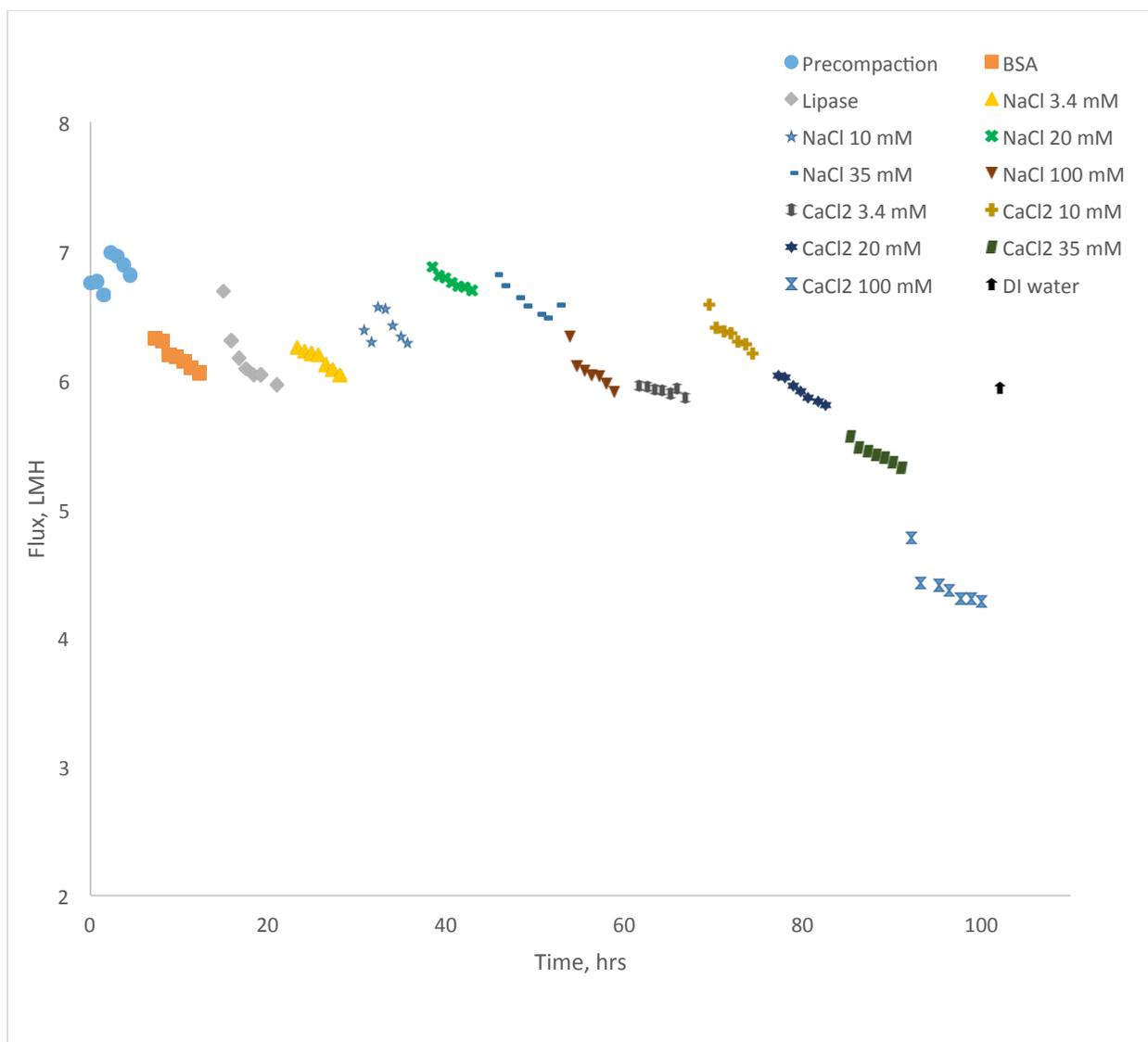


Figure 14. Flux analysis of AqpZ modified PBI membranes.

For PBI membranes, initial pure water flux was 8.77 LMH, initial flux for BSA filtration was 7.89 LMH and that for lipase filtration was 6.79 LMH. The flux recovery obtained after BSA filtration was 7.07 LMH (or an 80% flux recovery as compared to the initial pure water flux). Final flux after lipase filtration was 6.37 LMH and flux recovery after DI backwash was 6.41 LMH (a 73% of initial water flux). Rejections for protein filtrations are shown in Table 2.

BSA and lipase rejections for unmodified PBI membranes were 84% and 83%, respectively, and these were not significantly different from our initial PBI studies. For PVA-alkyl-AqpZ membranes, initial pure water flux was 6.74 LMH, which again was likely due to the additional resistance to flow from the addition of PVA-alkyl to the surface of PBI membranes. Initial flux for BSA solution for these membranes was 6.32 LMH and for lipase solution was 6.30 LMH. The flux recovery after BSA filtration was 6.68 LMH (or a 99% as compared to initial pure water flux). The final flux after lipase filtration was 5.96 LMH, and flux recovery after that was 6.24 LMH (a 92% of initial water flux). Rejection values for both BSA and lipase solutions increased for PVA-alkyl-AqpZ membranes. For BSA solution, an 88% and for lipase, an 86% rejection was observed. This might be due to addition of AqpZ in the PVA-alkyl matrix since aquaporins prevent the flow of protein molecules through the aquaporin structure. Any protein going through the membrane would be moving through the membrane regular pores.

Table2: Rejection and flux recoveries for protein filtrations

	PBI membranes	AqpZ-PVA-alkyl membranes
BSA rejection	84% ± 1%	88% ± 1%
Lipase rejection	83% ± 1%	86% ± 1%
Flux recovery	61.11%	87.98%

Filtrations with protein solutions were followed by filtration with solutions of NaCl and CaCl₂ in water. Five different concentrations of salt solutions were chosen for filtration studies of both unmodified and modified membranes. For unmodified PBI membranes, initial flux for 3.4 mM NaCl feed solution was 6.14 LMH and final flux was 6.10 LMH, with a flux recovery of 6.58 LMH (75%). For PVA-alkyl-AqpZ membranes, the initial flux for the same NaCl solution

was 6.22 LMH, the final flux was 6.03 LMH and flux recovery was 6.38 LMH (94.6%). A 10 mM NaCl solution was used as feed after DI water backwash. For PBI membranes, the initial flux was 6.16 LMH, the final flux was 6.02 LMH and flux recovery was 6.44 LMH (73.43%), while for PVA-alkyl-AqpZ membranes, the initial flux was 6.30 LMH, the final flux was 6.29 LMH and flux recovery was 6.87 LMH (100%). When the NaCl solution concentration was increased to 20 mM, the unmodified PBI membranes showed an initial flux 5.80 LMH, final flux was 5.81 LMH and flux recovery was 6.15 LMH (70%). For PVA-alkyl-AqpZ membranes, initial flux was 6.80 LMH, final flux was 6.69 LMH and flux recovery obtained was 6.81 LMH (100%). Upon another increase in the NaCl feed solution concentration to 35 mM, the initial flux for the PBI membranes was 5.30 LMH, the final flux was 5.41, and flux recovery was 5.41 LMH (61.7%). Again, for PVA-alkyl-AqpZ membranes, flux values remained not significantly different from previous solutions with an initial flux of 6.73 LMH, final flux of 6.47 LMH and flux recovery of 6.33 LMH (93%). For 100 mM NaCl feed solution, PBI membranes showed an initial flux of 4.98 LMH, a final flux of 5.10 LMH and a flux recovery of 5.60 LMH (63.8% flux). For this this concentration of NaCl, the permeability of PVA-alkyl-AqpZ membranes decreased slightly to an initial flux was 6.10 LMH, a final flux was 5.90 LMH and flux recovery of 5.95 LMH (88.3%). While flux declines during filtration were lower and flux recoveries higher with the PVA-alkyl-AqpZ membranes, flux values after the final recovery were approximately the same as PBI membranes; however, the greatest advantage of the PVA-alkyl-AqpZ membranes seems to be with respect to sodium chloride rejection, as shown in Figure 15. AqpZ-PVA-alkyl membranes showed higher rejections for the solutions as compared to PBI membranes. Unmodified PBI membranes showed 19% rejection during filtration of the 3.4 mM NaCl solution, and as the NaCl concentration went up to 100 mM, the rejection decreased to

5.3%. On the other hand, PVA-alkyl-AqpZ membranes showed a much better rejection of 73.5% for 3.4 mM feed solution of NaCl and 36% for 100 mM NaCl.

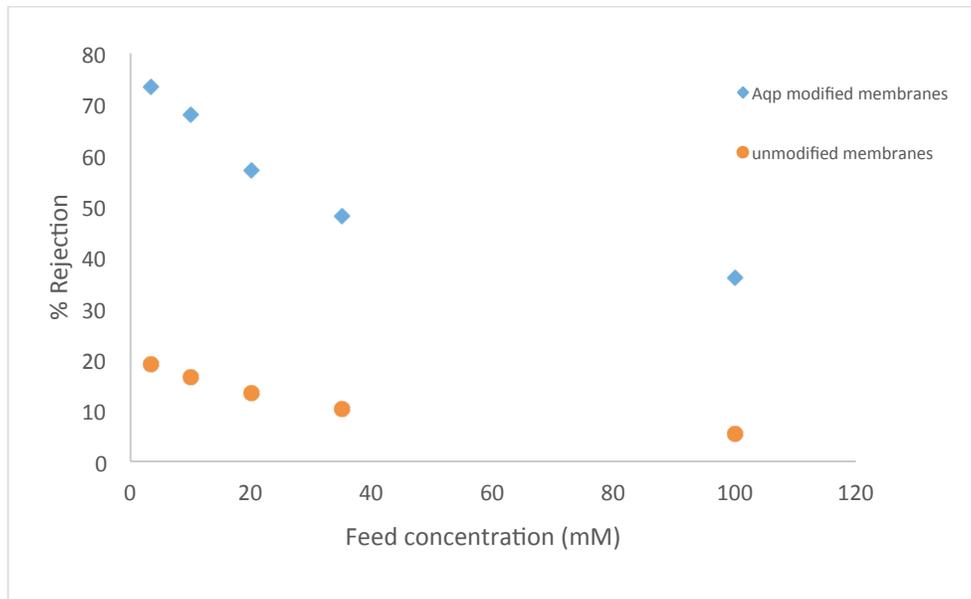


Figure 15. Sodium chloride filtration rejection.

After NaCl filtration and the final flux recovery, CaCl₂ solutions of five different concentrations were filtered through the membranes (flux declines and recoveries are shown in Figures 13 and 14). For a 3.4 mM CaCl₂ feed solution, PBI membrane had an initial flux of 4.95 LMH, a final flux of 5.10 LMH and a flux recovery of 6.33 LMH (72.2%). PVA-alkyl-AqpZ membrane displayed an initial flux of 5.94 LMH, a final flux of 5.85 LMH and flux recovery of 6.58 LMH (97.6%) for the same solution. This was followed by filtration of 10 mM CaCl₂ solution. For PBI membranes, the initial flux was 4.69 LMH, final flux of 4.65 LMH and flux recovery of 5.97 LMH (68%), while for PVA-alkyl-AqpZ membranes, these were 6.40 LMH, 6.20 LMH and 6.03 LMH (89.5%), respectively. After that, the concentration of the feed solution was increased again to 20 mM CaCl₂. PBI membranes gave initial flux of 4.92 LMH, final flux of 4.56 LMH and flux recovery of 6.02 LMH (68.6%), while PVA-alkyl-AqpZ membranes

displayed 6.01, 5.80 LMH and 5.56 LMH (82.5%), respectively. For a feed solution of 35 mM CaCl_2 solution, the initial flux for PBI membrane was 5.39 LMH, final flux was 4.71 LMH and flux recovery was 5.76 LMH (65.7%). For the same solution, PVA-alkyl-AqpZ membranes had an initial flux of 5.47 LMH, final flux of 5.31 LMH and flux recovery of 4.77 LMH (70.8%). When feed concentration was increased to 100 mM of CaCl_2 , the initial flux for PBI membranes was 4.63 LMH, the final flux of 4.36 LMH and flux recovery of 5.36 LMH (61.1%), while PVA-alkyl-AqpZ membranes had an initial flux of 4.42 LMH, final flux of 4.28 LMH and a flux recovery of 5.93 LMH (88%). Figure 16 shows CaCl_2 salt rejections. For CaCl_2 salt solutions of low concentration (3.4 mM), the maximum salt rejection observed for PBI membranes was 24.3% while for PVA-alkyl-AqpZ membranes was 76.2%. As the concentration of CaCl_2 feed solution was increased to 100 mM, the rejection for PBI membranes dropped to 8%, while that for PVA-alkyl-AqpZ membranes was 39.6%. Therefore, as with NaCl filtrations, the final rejection of salts by aquaporin membranes remained significantly higher than for PBI membranes.

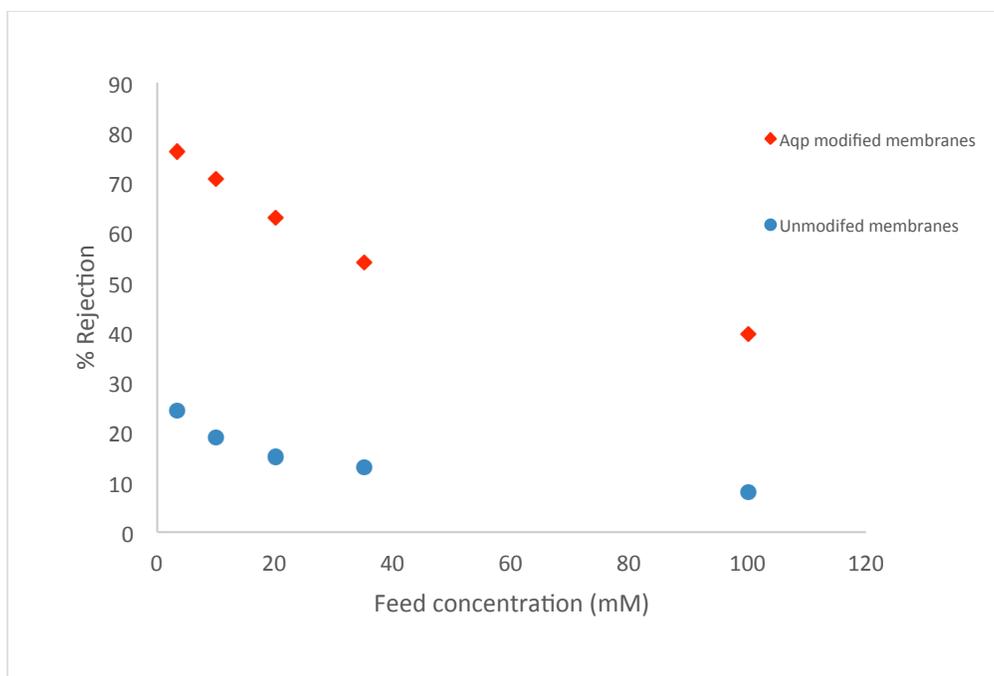


Figure 16. Calcium chloride filtration rejection.

Discussion:

While the initial water flux values of PVA-alkyl-AqpZ membranes were lower than unmodified PBI membrane, the flux decline was lower when using PVA-alkyl-AqpZ membranes, and final flux after 140 hours of flux experiment was higher for PVA-alkyl-AqpZ membrane as compared to PBI membrane. Therefore, it is believed that the presence of aquaporins helped to increase and maintain the water flux through the membranes, and the addition also led to a better flux recoveries. Aquaporins are bidirectional, hence during backwash, aquaporins might have helped in the increased water flux through the membrane and more removal of reversible fouling resulting in better flux recovery of the membrane. The embedment of AqpZ in the modified membranes is hypothesized to have facilitated water transport through the membrane and brought about an increase in the water flux, which agrees

with previous studies [31, 32]. Furthermore, the increase in the hydrophilicity of the membrane after modification (Figure 12) could also be associated with the increase in the water being transported across the membrane [33]. Aquaporins selectively filter water molecules through their porous structures while preventing the passage of ions and other solutes (Figure 1). Thus they form channels for water molecules to pass through them and in turn increase the water flux and the flux recovery of the PVA-alkyl-AqpZ membrane.

It is hypothesized that the presence of aquaporins in the PVA-alkyl-AqpZ membranes acted as water channels and prevented the flow of ions and salt solutions that flow along with water molecules. This helped in the increase in water flux and rejection of salt solutions of different concentrations. Previously, Chung et al. [32] prepared Aqp-modified cellulose acetate membranes functionalized with methacrylate end groups. Aqp was introduced into the membranes using vesicle rupture of triblock (ABA) copolymer vesicles and UV polymerization. Maximum salt rejection obtained with NaCl salt solution of concentration 3.4 mM was 32%. Increase in water flux was observed with an increase in Aqp:ABA ratio and thus improved mechanical stability and selective separation. The biomimetic membranes from the study presented here, which were modified with PVA-alkyl embedded with AqpZ, produced higher rejections for higher concentrations of NaCl solutions and higher flux recoveries as compared to literature studies. It is hypothesized that the reason is that PVA-alkyl with the gum arabic treated aquaporins prevented the aquaporins from flattening during pressure-induced filtration.

Significance:

Aquaporins have received worldwide attention due to their potential to significantly improve water flux across synthetic membranes. However, under high pressure, aquaporins can

get chemically altered and not function properly resulting into failure of the assembly of synthetic membrane and the protein. In this study, aquaporins were protected with gum arabic and dispersed into a PVA-alkyl layer, which was crosslinked to a synthetic PBI membrane backbone using carbodiimide chemistry. Membranes modified with aquaporins showed higher water fluxes, flux recoveries and selectivities as compared to unmodified synthetic membranes and may offer ultimate breakthrough for low energy desalination.

References:

1. Van der Bruggen, B., M. Mänttari, and M. Nyström, *Drawbacks of applying nanofiltration and how to avoid them: A review*. Separation and Purification Technology, 2008. **63**(2): p. 251-263.
2. Brandhuber, P. and G. Amy, *Alternative methods for membrane filtration of arsenic from drinking water*. Desalination, 1998. **117**(1-3): p. 1-10.
3. Waypa, J.J., M. Elimelech, and J.G. Hering, *Arsenic removal by ro and nf membranes*. Journal American Water Works Association, 1997. **89**(10): p. 102-114.
4. Urase, T., J.-i. Oh, and K. Yamamoto, *Effect of ph on rejection of different species of arsenic by nanofiltration*. Desalination, 1998. **117**(1-3): p. 11-18.
5. Xia, S., B. Dong, Q. Zhang, B. Xu, N. Gao, and C. Causseranda, *Study of arsenic removal by nanofiltration and its application in china*. Desalination, 2007. **204**(1-3): p. 374-379.
6. Murata, K., K. Mitsuoka, T. Hirai, T. Walz, P. Agre, J.B. Heymann, A. Engel, and Y. Fujiyoshi, *Structural determinants of water permeation through aquaporin-1*. Nature, 2000. **407**(6804): p. 599-605.
7. Borgnia, M., S. Nielsen, A. Engel, and P. Agre, *Cellular and molecular biology of the aquaporin water channels*, in *Annual review of biochemistry*, C.C. Richardson, Editor 1999, Annual Reviews Inc. {a}, P.O. Box 10139, 4139 El Camino Way, Palo Alto, California 94306, USA. p. 425-458.
8. Borgnia, M.J., D. Kozono, G. Calamita, P.C. Maloney, and P. Agre, *Functional reconstitution and characterization of aqpz, the e-coli water channel protein*. Journal of Molecular Biology, 1999. **291**(5): p. 1169-1179.
9. Heo, J., F. Meng, and S.Z. Hua, *Contribution of aquaporins to cellular water transport observed by a microfluidic cell volume sensor*. Analytical Chemistry, 2008. **80**(18): p. 6974-6980.
10. Kaufman, Y., A. Berman, and V. Freger, *Supported lipid bilayer membranes for water purification by reverse osmosis*. Langmuir, 2010. **26**(10): p. 7388-7395.
11. Li, X.A., J.J. Lenhart, and H.W. Walker, *Dissolution-accompanied aggregation kinetics of silver nanoparticles*. Langmuir, 2010. **26**(22): p. 16690-16698.

12. Ni, W.J., B. Wang, H.P. Wang, and Y.M. Zhang, *Fabrication and properties of carbon nanotube and poly(vinyl alcohol) composites*. Journal of Macromolecular Science Part B-Physics, 2006. **45**(4): p. 659-664.
13. Shang, Y. and Y. Peng, *Uf membrane of pva modified with tdi*. Desalination, 2008. **221**(1-3): p. 324-330.
14. Vogel, H. and C.S. Marvel, *Polybenzimidazoles, new thermally stable polymers (reprinted from journal of polymer science, vol 50, pg 511-539, 1961)*. Journal of Polymer Science Part a-Polymer Chemistry, 1996. **34**(7): p. 1125-1153.
15. Chung, T.S., *A critical review of polybenzimidazoles: Historical development and future r&d*. Journal of Macromolecular Science-Reviews in Macromolecular Chemistry and Physics, 1997. **C37**(2): p. 277-301.
16. Flanagan, M.F. and I.C. Escobar, *Novel charged and hydrophilized polybenzimidazole (pbi) membranes for forward osmosis*. Journal of Membrane Science, 2013. **434**: p. 85-92.
17. Wang, K.Y. and T.-S. Chung, *Polybenzimidazole nanofiltration hollow fiber for cephalixin separation*. AIChE Journal, 2006. **52**(4): p. 1363-1377.
18. Hausman, R., B. Digan, I.C. Escobar, M. Coleman, and T.-S. Chung, *Functionalization of polybenzimidazole membranes to impart negative charge and hydrophilicity*. Journal of Membrane Science, 2010. **363**(1-2): p. 195-203.
19. Ma, Y.L., J.S. Wainright, M.H. Litt, and R.F. Savinell, *Conductivity of pbi membranes for high-temperature polymer electrolyte fuel cells*. Journal of the Electrochemical Society, 2004. **151**(1): p. A8-A16.
20. Aerts, P., I. Genné, R. Leysen, P.A. Jacobs, and I.F.J. Vankelecom, *The role of the nature of the casting substrate on the properties of membranes prepared via immersion precipitation*. Journal of Membrane Science, 2006. **283**(1-2): p. 320-327.
21. Li, N., Z.Z. Liu, and S.G. Xu, *Dynamically formed poly (vinyl alcohol) ultrafiltration membranes with good anti-fouling characteristics*. Journal of Membrane Science, 2000. **169**(1): p. 17-28.
22. Totani, T., Y. Teramura, and H. Iwata, *Immobilization of urokinase on the islet surface by amphiphilic poly(vinyl alcohol) that carries alkyl side chains*. Biomaterials, 2008. **29**(19): p. 2878-83.
23. Gung, B.W., H.D. Dickson, S. Seggerson, and K. Bluhm, *A short synthesis of an acetylenic alcohol from the sponge cribrochalina vasculum*. Synthetic Communications, 2002. **32**(17): p. 2733-2740.
24. Hosono, M.S.S.K.R.M.-H.Y., *Polyelectrolyte complex prepared from carboxymethylated and aminoacetalized derivatives of poly(vinyl) alcohol*. Journal of Applied Polymer Science, 1977. **21**: p. 2125-2134.
25. Dickson, H., B.W. Gung, S. Seggerson, and K. Bluhm, *A short synthesis of an acetylenic alcohol from the sponge cribrochalina vasculum*. Abstracts of Papers American Chemical Society, 2002. **223**(1-2): p. 356.
26. Bandyopadhyaya, R., E. Nativ-Roth, O. Regev, and R. Yerushalmi-Rozen, *Stabilization of individual carbon nanotubes in aqueous solutions*. Nano Letters, 2002. **2**(1): p. 25-28.
27. Hite, R.K., Z. Li, and T. Walz, *Principles of membrane protein interactions with annular lipids deduced from aquaporin-0 2d crystals*. EMBO J, 2010. **29**(10): p. 1652-8.

28. J, C., *Interpretation of infrared spectra, a practical approach*, in *Encyclopedia of Analytical chemistry*, R. A. Meyers, Editor 20002006, John Wiley and Sons Ltd.: Chichester. p. 10815-10837.
29. Xu, B.L., D.W. Liu, G.Y. Xu, X.L. Zhang, and L.C. Bi, *A measurement method for contact angle based on hough transformation*. *Measurement*, 2013. **46**(3): p. 1109-1114.
30. Musto, P., F.E. Karasz, and W.J. Macknight, *Fourier-transform infrared-spectroscopy on the thermooxidative degradation of polybenzimidazole and of a polybenzimidazole polyetherimide blend*. *Polymer*, 1993. **34**(14): p. 2934-2945.
31. Wang, M.Q., Z.N. Wang, X.D. Wang, S.Z. Wang, W.D. Ding, and C.J. Gao, *Layer-by-layer assembly of aquaporin z-incorporated biomimetic membranes for water purification*. *Environmental Science & Technology*, 2015. **49**(6): p. 3761-3768.
32. Zhong, P.S., T.-S. Chung, K. Jeyaseelan, and A. Armugam, *Aquaporin-embedded biomimetic membranes for nanofiltration*. *Journal of Membrane Science*, 2012. **407-408**: p. 27-33.
33. Gullinkala, T. and I. Escobar, *Study of the hydrophilic-enhanced ultrafiltration membrane*. *Environmental Progress*, 2008. **27**(2): p. 210-217.

SEPARATION OF PHOSPHORUS- AND NITROGEN-NUTRIENTS FROM AGRICULTURAL DEGRADED WATERS USING PERVIOUS FILTER MATERIAL DEVELOPED FROM INDUSTRIAL BY-PRODUCTS

Basic Information

Title:	SEPARATION OF PHOSPHORUS- AND NITROGEN-NUTRIENTS FROM AGRICULTURAL DEGRADED WATERS USING PERVIOUS FILTER MATERIAL DEVELOPED FROM INDUSTRIAL BY-PRODUCTS
Project Number:	2013OH4360
Start Date:	9/1/2013
End Date:	2/28/2015
Funding Source:	Other
Congressional District:	OH-12
Research Category:	Engineering
Focus Category:	Treatment, Agriculture, Water Quality
Descriptors:	Industrial by-rproducts, filter material, nutrients
Principal Investigators:	Linda Kay Weavers, ChinMin Cheng

Publications

There are no publications.

SEPARATION OF PHOSPHORUS- AND NITROGEN-NUTRIENTS FROM AGRICULTURAL DEGRADED
WATERS USING PERVIOUS FILTER MATERIAL DEVELOPED FROM INDUSTRIAL BY-PRODUCTS

Progress Report

Submitted to:

Ohio Water Resources Center

Submitted by:

Principal Investigator:

Linda Weavers, Ph.D., P.E., BCEE.

Professor

Department of Civil, Environmental, and Geodetic Engineering

e-mail: weavers.1@osu.edu; Telephone: 614-292-8263; Fax: 614-292-3780

Co-PI:

Chin-Min Cheng, Ph.D., P.E.

Research Associate II-Engineer

Department of Civil, Environmental, and Geodetic Engineering

ABSTRACT

End-of-tail filtration has been suggested as a more aggressive and effective approach to reduce losses of nutrients from crop lands compared to current best management practices (BMPs) focusing on source reduction and minimizing transportation. A number of industrial by-products, e.g., coal combustion by-products and bauxite leaching residual, have been proven chemically effective in trapping P- and/or N-nutrients, and therefore, are potential low-cost nutrient sorbents for the end-of-tail filtration approach. However, the application of these industrial by-products as the filtration media is limited due to unfavorable hydraulic properties, as well as unknown associated environmental impacts. In this proposed study, pervious filter materials owning both reactivity to nutrients and adequate hydraulic properties are developed using fly ash, stabilized FGD materials, and bauxite leaching residual as the feedstock. By modifying the composition of these industrial by-products, the pervious materials are expected to have selective nutrient-sequestering capabilities, which can be used to separate and recycle phosphorus- and nitrogen-nutrients from agricultural drainage waters (ADWs). This study is carried out in three tasks to (1) investigate the adsorption efficiency and service lifetime of selected pervious materials with synthetic ADW; (2) evaluate the physical and chemical integrity of the pervious materials before and after service; and (3) study the interactions between the prepared filter materials and emerging pollutants commonly found in ADW (e.g., estrone). The goal of this study is to demonstrate the feasibility of applying a low-cost and environmentally-sustainable approach to ADW handling and treatment. This alternative to current BMPs is able to convert agricultural and industrial wastes to value-added products containing concentrated and specific nutrients. Currently, the project is still on going. Results obtained from this study will be used to develop a competitive proposal for external funding.

1. Introduction

Eutrophication of water bodies, a result of release of excessive phosphorous (P) and nitrogen (N) from soil to drainages¹, has been an increasing environmental issue in the US, especially in the Midwest, northeast, and Gulf coast area where the watersheds of major freshwater bodies involve rapid growth and intensification of crop and livestock farming². Not only eutrophication posts unpleasant aesthetic characteristics to water bodies, accumulation of toxic, volatile chemicals produced by algae can cause neurological damage in people and animals being exposed to them. Consequently, eutrophication of water resources results in losses of biodiversity, as well as their amenities and services³. For example, the recent outbreaks of Cyanobacteria, or blue-green algae, in the Grand Lake at St. Mary's area in Ohio has led to state officials to issue water contact and fish consumption advisories.

The major cause of many eutrophication incidents can be directly correlated to fertilizer application⁴. To prevent accumulation of nutrients in surface waters, reduction of nutrients present in the agricultural degraded waters (ADW, i.e., livestock wastewater overflow, subsurface drainages, and surface runoffs from cropland) is perceived as necessary approach⁵. Although many best management practices (BMPs) focusing on source reduction and minimizing transportation have been implemented to reduce losses of nutrients from crop lands, these approaches have shown no control on dissolved phosphorus losses^{6,7}, which is the most readily available form of phosphorus to aquatic organisms⁸. Instead, end-of-tail filtration has been suggested as a more aggressive and effective approach⁶. However, the application is limited. Ideal filter materials, i.e., material with both favorable nutrient-sequestering capability and hydraulic property, have yet been identified⁹.

In this study, low-cost pervious sorption materials prepared from a self-geopolymerization process using agricultural wastes and industrial by-products are tested for their potential as an alternative to current BMPs. The self-geopolymerization process enchains agricultural wastes with chemically-effective, nutrient-sorbing industrial by-products (e.g., coal ash, flue gas desulfurization materials, and bauxite residual) and forms pervious materials. By modifying the composition, the pervious materials are expected to have selective sorption capabilities to nitrogen (N-) and phosphorus (P-) nutrients with adjustable hydraulic properties, which can be used to separate and recycle nutrients from ADWs.

2. Objectives

In this study, a geopolymerization procedure is developed to convert coal combustion by-products (i.e., fly ash and flue gas desulfurization (FGD) material) and alkaline bauxite leaching

residual (bauxite red mud) to pervious filter materials. The materials are tested in a bench-scale setting for their effectiveness and capacity on removing nutrients from simulated agricultural drainage waters. The specific objectives of this proposed project are to:

- (1) Assess the performance of the industrial by-product-derived pervious filter materials with respect to their nutrient removal efficiencies, service lifetime, and hydraulic properties;
- (2) Evaluate the chemical and physical integrity of the materials; and
- (3) Study the interactions between the prepared filter materials and other pollutants contained in ADWs (i.e., estrogens).

3. Materials and Method

The work of this proposed study is divided into three tasks. In summary, the first task focuses on preparing and characterizing the pervious filter materials. At least three sets of P-type (i.e., materials selectively adsorb P-nutrients) and N-type (i.e., materials adsorbed nitrate and/or other N-nutrients) are prepared. In the second task, a series of column experiments are setup to (1) evaluate the adsorption efficiency and capacity of the selected pervious materials with a simulated ADW and (2) study the interactions between estrogens and filtration materials. In addition, the physical and chemical integrities of the pervious filter material during and after service are evaluated. The release of metals and metaloids (e.g., mercury, arsenic, selenium, thallium, and boron), as well as sulfate, from the filter materials during filtration are monitored. In addition, surface characterization techniques, such as X-ray diffraction (XRD) and scanning electron microscopy (SEM), are applied to investigate the transformations of mineral composition and surface morphology before and after the filtration materials are exhausted.

Pervious Filter Material Preparation and Characterization

Coal combustion by-products (i.e., fly ash and stabilized FGD materials) and bauxite leaching residue (i.e., red mud) are used in the preparation of the nutrient-selective pervious filtration materials (Figure 1). Two different types of pervious filtration materials (i.e., P- and N-types) are prepared using a method modified from Cheng et al.¹⁰ and Jin¹¹. Class F fly ash and sulfite-rich stabilized FGD material provided by coal combustion power plants located in eastern Ohio are used to prepare the phosphorous-capture (P-type) filtration materials. Quick lime

(Carmeus USA, Pittsburg, PA), CaO, is added to provide required alkalinity. The nitrogen-capture materials are prepared from red mud, fly ash, and stabilized FGD material. No quick lime is used in the preparation of N-type filter materials. The bauxite red mud provided by a bauxite processing plant located at southeast Texas is oven-dried before use. In one batch, manganese oxide (MnO₂) is also added in the preparation of N-type material. Woodchip is used in the preparation of both N and P-type filter mixtures to modify the hydraulic properties. The prepared mixtures are then cured in a humidity chamber.

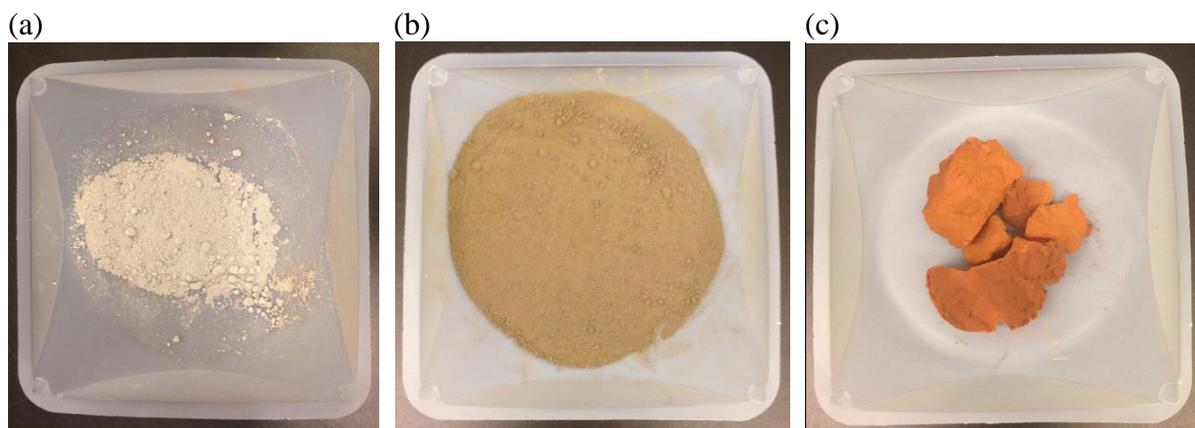


Figure 1. (a) Stabilized FGD material, (b) fly ash, and (c) bauxite red mud used in the preparation of pervious filtration materials.

The cured filter materials are tested for their chemical (i.e., elemental and mineral compositions), physical (density and surface morphology), and engineering (i.e., permeability (k) and/or hydroconductivity (K)) properties as per standard testing protocols. Details on the chemical and physical characterizations of the filter materials are described in the “*Physical and Chemical properties Integrity Evaluation*” section.

Bench-Scale Column Test

A series of column tests are carried out to measure the adsorption capacity and efficiency of prepared pervious materials for P- and N-nutrients with a simulated ADW. In addition to the prepared filter materials, two reference columns, packed separately with granular activated carbon (GAC) and top soil from the OSU’s Waterman Farm Complex, are also included in the column study. A control column, i.e., without packing medium, is included to evaluate the adsorption of nutrients and compounds on the experimental apparatus.

The setup of the column test is illustrated in Figure 2. The ADW used in the column test is synthesized based on formula listed in Table 1. In addition to the constituents listed in the table, one estrogen, e.g., estrone (E1) or 17 α -Estradiol (17 α -E2), commonly found in dairy wastewater¹² is added in selected experimental batches. A peristaltic pump delivers the synthetic ADW to the inlet of a series of two vertically-oriented columns at a constant feed rate (Figure 2). The ADW sequentially passes through the column containing P-type filter material (P-type column) and then the N-Type column. For a given set of filter materials, the column test is carried out under a saturation condition demonstrated in Figure 2.

Table 1. Composition of synthetic dairy wastewater used in this study

Component	Amount (mg/L)
Urea	115.7
NH ₄ Cl	250.0
Na ₂ PO ₄ ·12H ₂ O	385.7
KHCO ₃	257.1
NaHCO ₃	668.6
MgSO ₄ ·7H ₂ O	257.1
FeSO ₄ ·7H ₂ O	10.3
MnSO ₄ ·H ₂ O	10.3
CaCl ₂ ·6H ₂ O	15.4

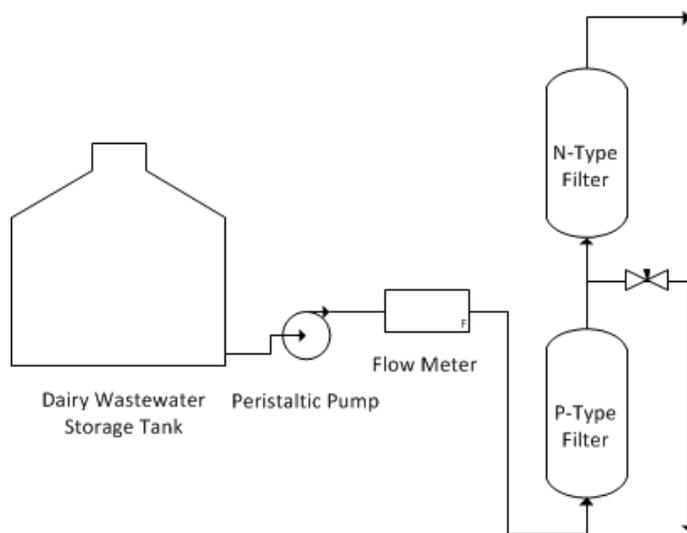


Figure 2. Setup of bench-scale column test

Effluent samples are collected periodically from the outlets of P-type and N-type columns for a list of chemical analyses shown in Table 2. After collection, sample is immediately separated into four sub-samples. The first sub-sample is for pH, conductivity, and redox potential measurements. In the selected batches when estrogen is included in the synthetic ADW, an aliquot of the first subsample is filtered with 1.2µm glass fiber and concentrated by solid-phase extraction for estrogen analysis. Any compounds remained on the sample collection bottle or filter is desorbed by rinsing the bottle and filter with methanol. The concentrated sample is analyzed using a high-performance reverse-phase liquid chromatography tandem electrospray ionization mass spectrometry (HPLC/MS/MS). Deuterated internal standards is added to the samples to correct the interferences caused by the matrix of the sample.

The second sub-sample is filtered and analyzed for alkalinity, total dissolved solids, Cl⁻, SO₄⁻², PO₄⁻³, total Kjeldahl nitrogen, ammonia, and NO₃⁻. The third sub-sample is preserved with 5% HNO₃ and analyzed for “total” elements in the solution. The final sub-sample is filtered through a 0.45-µm syringe filter and preserved with 5% HNO₃ before being analyzed for “dissolved” elements.

Table 2. List of monitoring parameters and respective analytical methods for aqueous samples

Subsample	Parameter	Detection Methods	Instruments	Locations
Subsample I	Conductivity	AWWA Sec. 2510	Thermo Orion 1234	<i>in-situ</i>
	pH		Thermo Orion 1234	<i>in-situ</i>
	Redox Potential		Thermo Orion 1234	<i>in-situ</i>
	Estrogen ^c	HPLC/MS/MS	Micromass Q-TOF II	CCIC ^b
Subsample II	Alkalinity	AWWA Sec. 2310	-	CEGE EER Lab/ OARDC STAR Lab
	Total dissolved solid	AWWA Sec. 2540	-	
	Chloride (Cl)	AWWA Sec. 4110C	Dionex 2100	
	Sulfate (SO ₄ ⁻²)	AWWA Sec. 4110C	Dionex 2100	
	Phosphate(PO ₄ ⁻³)	AWWA Sec. 4110C	Dionex 2100	
	Nitrate (NO ₃ ⁻)	AWWA Sec. 4110C	Dionex 2100	
	Ammonia (NH ₄ ⁺)	AWWA Sec. 4110C	Dionex 2100	
Total Kjeldahl Method	AWWA Sec. 4500 N _{org}	-		
Subsample III/ Subsample IV	Mercury (Hg)	CVAFS	Varian CVAAs,	
	Selected Elements ^a	AWWA Sec. 3120B	Varian VISTA-AX	
	Arsenic (As)/ Thallium(Tl)	AWWA Sec. 3120B	Varian GFAAs, Varian 880Z	
	Selenium (Se)	AWWA Sec. 3120B	Varian GFAAs, Varian 880Z	

^a Aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), phosphorous (P), sodium (Na), silver (Ag), zinc (Zn).

^b Campus Chemical Instrument Center at The Ohio State University

^c On selected experimental batches

Chemical and Physical Integrity Evaluations

The exhausted filter materials are preserved using liquid nitrogen and freeze-dried before being analyzed for the mineral and chemical compositions, surface morphology, and forms of adsorbed phosphorus by the methods listed in Table 35. The mineral compositions and morphology of the selected N- and P- type filters materials before and after service are characterized using X-ray diffraction (XRD) and scanning electronic microscopy (SEM), respectively. A Bruker D8 Advance X-ray diffractometer or equivalent is used to identify the mineral composition. The mineral patterns in the diffractograms are matched using the DIFFRACplus EVA software with ICDD Power Diffraction File (PDF2+) database. The complete elemental composition analysis is measured with the assistance of the digestion procedure described in EPA method 3052. A reference coal fly ash, 1633b, provided by the National Institute of Standards and Technology (NIST), is included for analytical quality control. A list of the analyses performed on the materials can be seen in Table 4.

The release potential of trace elements from filter materials before and after service will also be characterized. Standard protocols, i.e., EPA Standard Method 1311, Toxicity Leaching Characteristic Procedure (TCLP), the EPA Standard Method 1312, Synthetic Precipitation Leaching Procedure (SPLP), are used.

Table 3. Physical, mineral, and chemical analyses for selected pervious filter materials

	Method	Instrument	Location
Permeability	ASTM D4525-08		CEGE Soil Lab
Hydraulic Conductivity	ASTM D7100-06		
Morphology	Scanning Electron microscopy	Hitachi S-3000 SEM	OSU Nanotech West Lab
Mineral Composition	X-ray Diffraction	Bruker D8 Advance X-ray diffractometer	SENR Soil Lab ^c
Selected Elements ^a	ASTM D-6357	Milestone Microwave Digestor/ Varian VISTA-AX	CEGE EER Lab ^b
Mercury	ASTM D-6414	Varian CVAAs, Varian 880Z	CEGE EER Lab
Selenium	ASTM D-4606	Varian CVAAs, Varian 880Z	CEGE EER Lab
Arsenic, Thallium	ASTM D-3683	Varian GFAAs, Varian 880Z	CEGE EER Lab

^a aluminum (Al), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), phosphorous (P), sodium (Na), sulfur (S), and zinc (Zn).

^b Environmental Engineering Research Laboratory at Department of Civil, Environmental, and Geodetic Engineering of The Ohio State University

^c Soil Lab at School of Environment and Natural Resources of The Ohio State University

4. Current Progress and Tasks to be completed

Characterizations of Industrial By-products

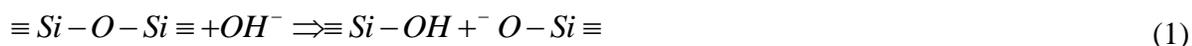
The chemical compositions of fly ash, stabilized FGD material, and bauxite red mud are first characterized and the results are summarized in Table 4. As shown in the table, calcium (Ca) and sulfur (S) are the two most abundant elements in the stabilized FGD material, which is associated with the presence of hannebachite ($\text{CaSO}_3 \cdot 0.5\text{H}_2\text{O}$), portlandite ($\text{Ca}(\text{OH})_2$), and enttringite ($\text{Ca}_6\text{Al}_2(\text{SO}_4)_3(\text{OH})_{12} \cdot 26\text{H}_2\text{O}$) in the material. The X-ray diffractogram and mineral composition of stabilized FGD material can be seen in Figure 3. Iron (Fe), aluminum (Al), sulfur (S), and silicon (Si) are the major elements in fly ash. Based on XRD analysis, the fly ash used in this study is comprised of amorphous glass, aluminum silicates (e.g., mullite), and iron oxides (hematite, magnetite, and maghemite). Bauxite red mud is consisted of Al, Fe, and Ca. The X-ray diffractograms of fly ash and red mud are not shown.

By properly coalescing fly ash, stabilized FGD material, and red mud under high alkaline environment, fly ash acts as an inorganic polymer binder to enchain active ingredients through a geopolymerization process. After being alkali-activated, the Si-O-Si or Al-O-Si bonds in fly ash and stabilized FGD material are disassociated and subsequently form network-like crystalline and/or amorphous alkaline aluminosilicates with structural framework similar to zeolite¹³. In a previous project, it has been demonstrated that a geotextile material derived from the geopolymerization process with a mixture of fly ash and stabilized FGD material, has effective phosphorus sorption capability by forming Ca- and Fe-precipitates^{10,14,15}. However, the fly ash/stabilized FGD material mixture did not show observable effect on nitrate mitigation¹⁰.

The addition of bauxite red mud is to enhance the nitrogen-nutrients adsorption capability of the fly ash/FGD mixture. Bauxite red mud contains minerals, e.g., iron (III) (hydr)oxides and hydrous aluminum oxides, that have high affinities for nitrate¹⁶. As a result, the material has been shown to be an effective nutrient sorbent¹⁷. Cengeloglu et al¹⁷ used original and acid-treated bauxite red mud to remove nitrate from aqueous solution and reported 70% and over 90% of removal, respectively. They found the alkaline property of bauxite red mud hindered the adsorption performance.

In this study, bauxite red mud is used as the sole alkalinity source in the geopolymerization process, which might promote the nitrate adsorption capacity. During geopolymerization, the OH^- ions from bauxite red mud is consumed (eq. 1) and redistribute the electron density around the silicon atom in fly ash, which weaken the strength of Si-O-Si bond¹⁸

and progress the polymerization process. The reaction neutralizes the negative surface charge of red mud particles, and therefore, might promote the nitrate sorption.



Preparation of P- and N-type pervious filtration

A series of P- and N-type pervious filtration materials have been prepared based on the formulas listed in Tables 5 and 6. Currently, the prepared materials are undergoing a 21-day curing process. The images of two selected prepared materials can be seen in Figure 4. The hydraulic property of the filtration materials are adjusted by the addition of woodchip. Two different sizes of woodchip, i.e., <2.3mm and 2.3-3.6mm, are used. The addition of woodchip creates larger capillary routes for water to pass through. During the geopolymerization process, active ingredients are coated on the surface of woodchip, which allows the nutrients in ADW to react with the active ingredients while passing through the void space.

Table 4. Chemical compositions of fly ash, stabilized FGD material and bauxite red mud used in this study

		Fly Ash	Stabilized FGD material	Red Mud
Phosphorus	P	531	177	1054
Potassium	K	2986	1307	310
Calcium	Ca	9836	172906	33055
Magnesium	Mg	1528	10026	227
sulfur	S	11827	85746	2867
Aluminum	Al	27050	9705	62817
Boron	B	531	313	<3
Copper	Cu	42	<0.4	<0.8
Iron	Fe	59824	18855	240960
Manganese	Mn	85	73	139
Molybdenum	Mo	22	<13	<0.5
Sodium	Na	18851	5296	32412
Zinc	Zn	109	40	22
Arsenic	As	143	36	28
Barium	Ba	177	137	61
Beryllium	Be	<0.18	<0.11	<0.18
Cadmium	Cd	2	6	5
Cobalt	Co	23	4	15
Chromium	Cr	74	25	1397
Lithium	Li	167	106	55
Nickel	Ni	48	7	6
Lead	Pb	28	8	46
Antimony	Sb	<1.5	17	<1.5
Selenium	Se	20	18	1
Silicon	Si	4771	1481	184
Strontium	Sr	229	212	117
Thallium	Tl	129	38	871
Vanadium	V	2	<1.1	<0.6
Mercury	Hg	NA	0.318	NA

NA:Not Available
Unit: mg/kg

Table 5. Formulas of Prepared P-type Filtration Materials

	P-Control	P-type I	P-type II	P-type III
Fly Ash	10.0	10.0	10.0	10.0
Stabilized FGD material	6.0	6.0	6.0	6.0
Quick Lime (CaO)	1.2	1.2	1.2	1.2
Deionized Water	10.5	10.5	10.5	10.5
Wood Chip (<2.3 mm)	0	2.5	5.0	0
Wood Chip (2.3-3.6 mm)	0	0	0	2.5

Unit: g

Table 6. Formulas of Prepared N-type Filtration Materials

	N-Control	N-type I	N-type II	N-type III
Fly Ash	10.0	10.0	10.0	10.0
Stabilized FGD material	6.0	6.0	6.0	6.0
Red Mud (dried weight)	8	8	8	8
Deionized Water	10.5	10.5	10.5	10.5
Wood Chip (<2.3 mm)	0	2.5	5.0	2.5
MnO ₂	0	0	0	2.0

Unit: g

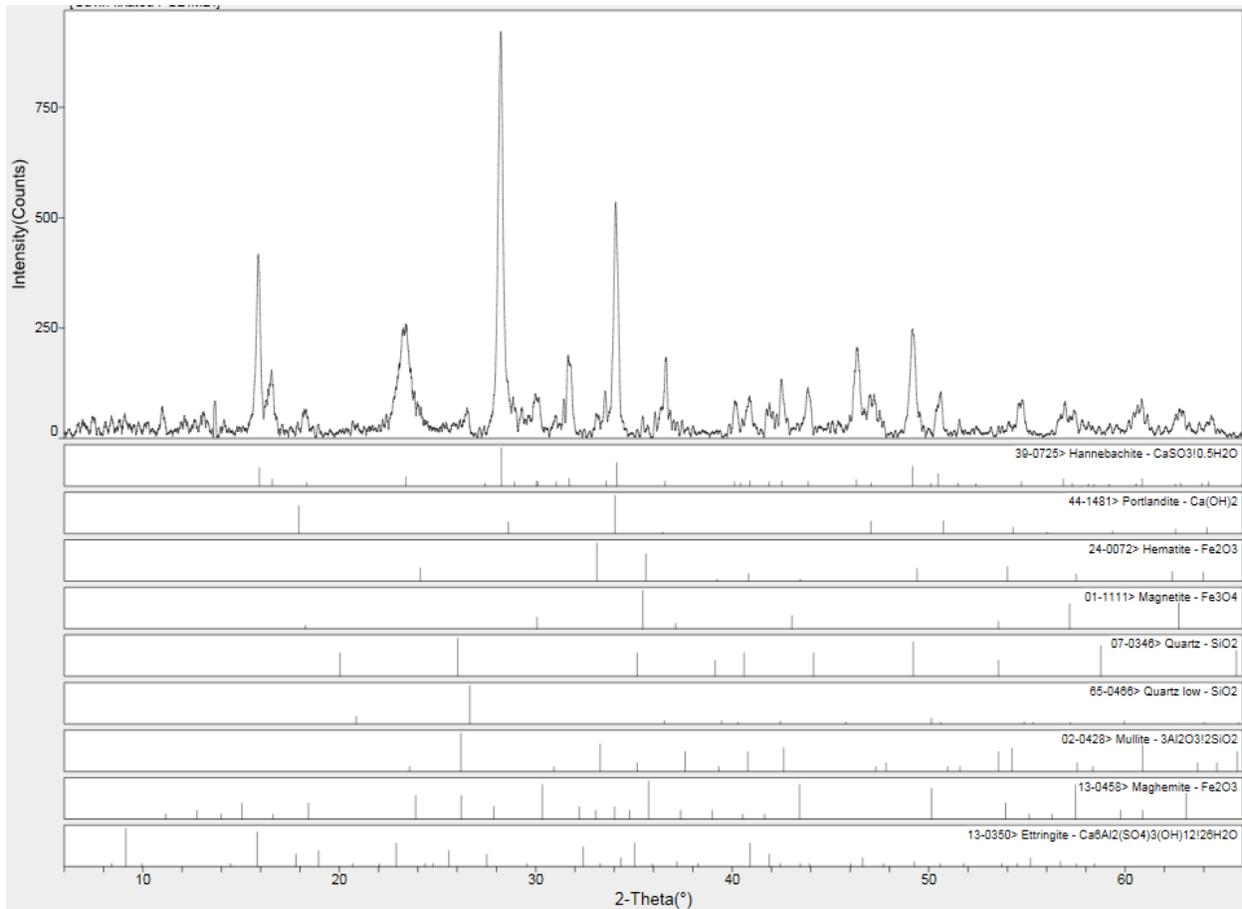


Figure 3. Mineral composition of stabilized FGD material

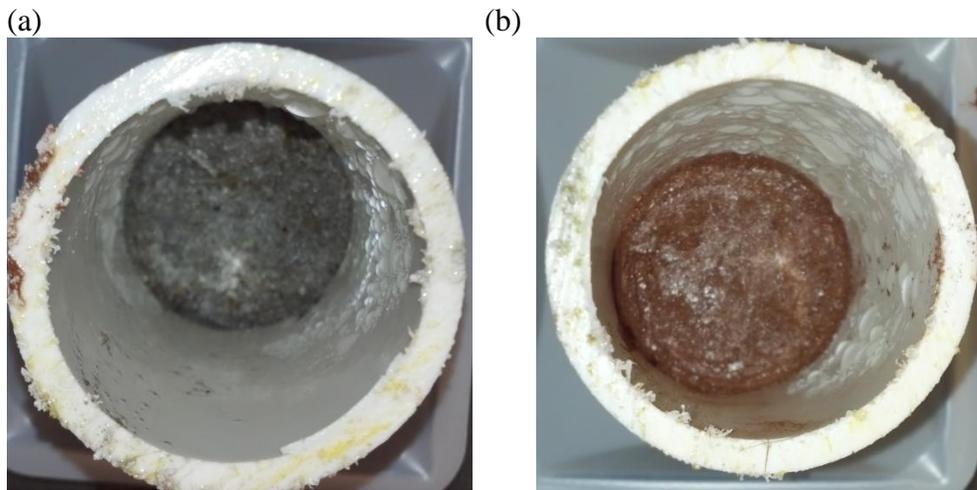


Figure 4. Prepared Pervious filtration materials. (a) P-type and (b) N-type.

These two types (i.e., P- and N-types) of pervious materials are expected to have selective sorption capacity, which can be used to sequentially separate and recover soluble phosphorous and nitrogen in agricultural drainage waters. In practice, two different pervious filter materials can be used in series. The dissolved phosphorous is expected to be selectively retained in the first pervious material (P-type) containing only fly ash and FGD material while allowing nitrate to pass through. Nitrate is captured in the second pervious material (N-Type) containing bauxite red mud, fly ash, and stabilized FGD material.

Adsorption Capacities

The nutrient adsorption capacities of P- and N-type materials were evaluated using the materials prepared from the formulas listed in Tables 5 and 6 for the P-Control and N-Control materials. For either type of the material, the adsorption experiment was carried out by adding six different amounts of the prepared solid, ranging from 0 to 1 gram, into six separate 125-mL HDPE bottles. Each bottle contains 100mL of either 250 mg/L of phosphate or 100 mg/L of nitrate solution. The bottles were then mixing by a tumbler for 24 hours at a rotating speed of 18 rpm. After mixing, the solution collected from each bottle was filtrated with 0.45mm filter and analyzed for NO_3^- or PO_4^{-3} .

The equilibrium concentrations of phosphate and nitrate in the solution after mixing as a function of material dosage are shown in Figure 5. As shown in the figure, over 97% of phosphate was removed by the P-type material with a solid-to liquid (L/S) ratio of 100. With the same L/S ratio, nearly 4% of nitrate was adsorbed by the N-type material.

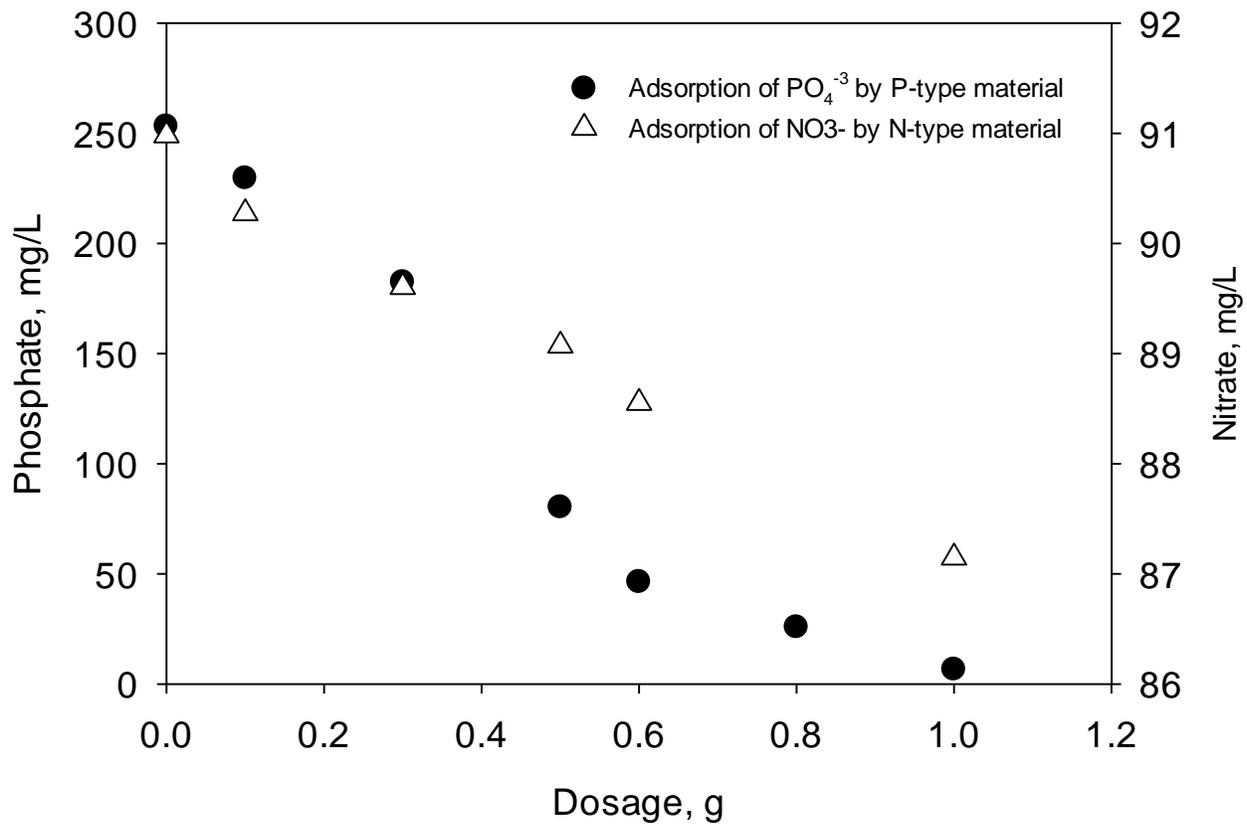


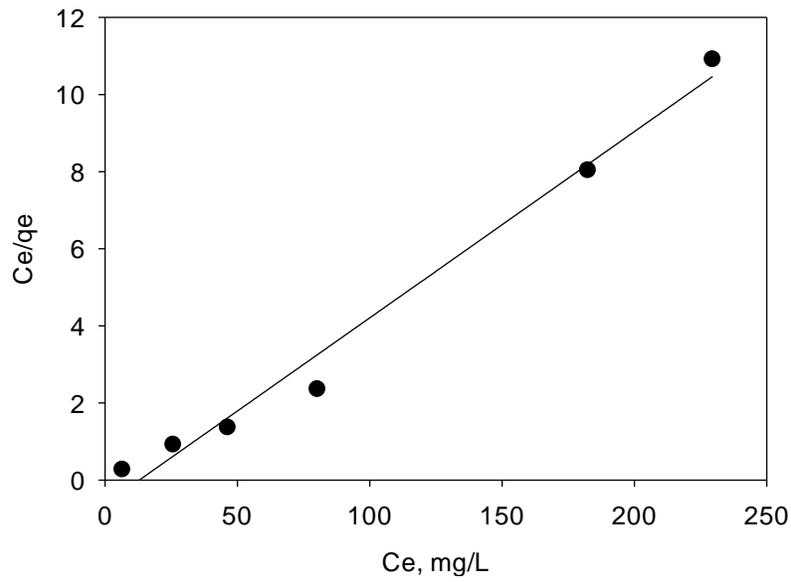
Figure 5. The equilibrium concentrations of phosphate and nitrate in the solution as a function of material dosage

The adsorption isotherms of phosphate on P-type material and nitrate on N-type material are illustrated in Figure 6. As shown in the figure, the adsorption isotherms of phosphate and nitrate can be expressed as Langmuir isotherm. The Langmuir isotherm equation is written as

$$\frac{C_e}{q_e} = \frac{1}{K \cdot Q_a^0} + \frac{C_e}{Q_a^0} \quad \text{Eq. 1}$$

where q_e is mass of material adsorbed (at equilibrium) per mass of adsorbent; Q_a^0 represents the maximum adsorption capacity (monolayer coverage); C_e is the equilibrium concentration in solution when amount adsorbed equals q_e ; K is constant (L/mg).

(a)



(b)

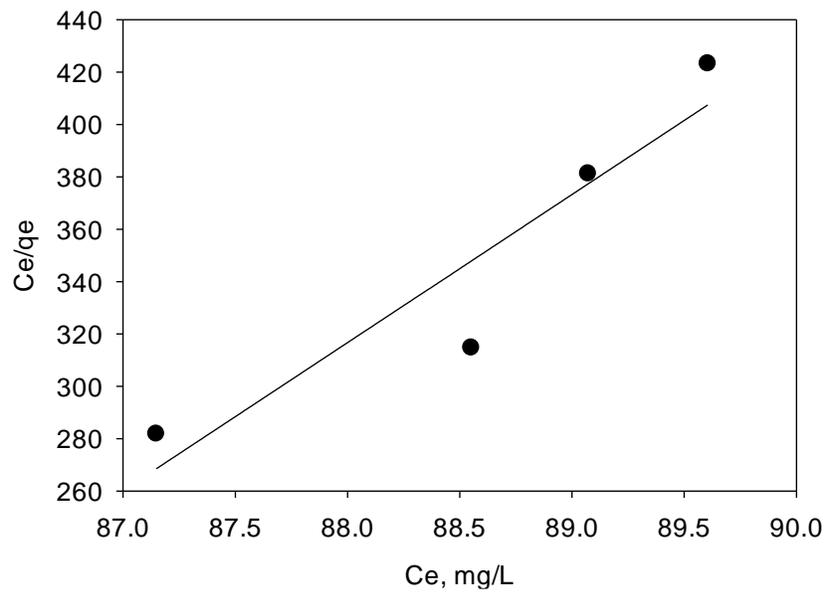


Figure 6. Langmuir isotherms for (a) phosphate and (b) nitrate

It is estimated that the maximum phosphate adsorption capacity of P-type material is 20.7 mg/g. For the N-type material, the adsorption capacity was approximately 0.18 mg/g, which is much less than the expected adsorption capacity.

Tasks to be completed

The bench scale column test described in the “*Materials and Methods*” section is to be carried out soon after the curing of pervious filtration materials is completed. In addition, the integrities of physical and chemical properties of the pervious materials after adsorption will be evaluated.

Despite the great potential for the proposed filtration application, the major concern of reutilizing these by-products is the release of trace elements contained in the materials after being contacted with water. Cheng *et al.*^{Error! Bookmark not defined.} investigated the water quality impacts associated with using stabilized FGD material as a low permeability liner for a swine manure storage pond. Based on five-year worth of field monitoring data, the concentrations of arsenic (As), boron (B), chromium (Cr), copper (Cu), and zinc (Zn) were consistently found lower in the water passing through the liner than the water collected from the pond. Other trace elements, such as Cd, Se, and Hg were often below the analytical detection limits. Ruyter *et al.*¹⁹ investigated the red mud accident occurred on October 4th 2010 in Ajka, Hungary by testing the plant toxicity and trace element availability with mixtures of red mud and non-contaminated soil. They observed the concentrations of trace elements in the leachate of red mud were either non-detectable or less than 20µg/L. In addition, Peters and Basta^{Error! Bookmark not defined.} added bauxite red mud directly to soil to reduce the bioavailable phosphorus. No excessive soil pH and increases of soil salinity, extractable Al, or heavy metals in soils were found in their study. Based on available field data, the application of coal combustion by-products and bauxite red mud has not been suggested to post adverse impacts on the environments.

However, to comprehend the overall benefits of reusing these by-products, it is vital to understand the leaching properties of the prepared pervious materials under different application scenarios.

Expected Outcomes and Significances

The outcome of this study is expected to provide:

- (1) Initial feasibility evaluation of a potential beneficial utilization for by-products produced from coal combustion and aluminum production processes
- (2) Insights regarding the interaction between nutrients and an agricultural emerging pollutant (i.e., estrogen) of FA zeolite-like material and the properties of biopolymers, and

- (3) Results to be transferred in forms of peer-reviewed publications and conferences, and be based upon in preparing competitive proposal for external funding.

The advantage of using selective sorption materials in the filtration approach is the potential to recycle and reutilize nutrients and industrial by-products, which promotes agricultural production to be in accord with the principles of sustainability. FGD gypsum and stabilized FGD material have shown to improve the yield of crops by providing necessary elements (e.g., calcium), changing soil physical properties, and increasing water infiltration and storage when they are applied as soil amendments^{20,21}. Hylander et al.²², used different filter materials (i.e. limestone, Polonite®, and sand) to capture soluble phosphorus and evaluated the subsequent suitability for plant production. They observed some of recycled phosphorus achieved 76% of the yield increased by commercially available P-fertilizer. As demand for food increases, which results in more land to be used for agricultural purpose and a requirement for increased crop yields, the fertilizer demand have been projected to increase faster than world population²³. With foreseeable increase in demand and depletion in reserve, use of recycled nutrients rather than a raw material is important step toward sustainable agricultural development. Currently, the majority of phosphate rock from mining goes into artificial fertilizer production²⁴. It estimates that sources of high-grade phosphate ore deposits could disappear within the next 100 years at current use rates²⁵.

5. References

- ¹ Alexander, R. B., Smith, R. A., Schwarz, G.E., Boyer, E.W., Nolan, J.V., Brakebill, J.W. (2008) Differences in phosphorous and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. *Environ. Sci. Technol.*, 42, 822-830.
- ² Mueller, D.K., Helsel, D.R., Nutrients in the Nation's Waters--Too Much of a Good Thing?. U.S. Geological Survey Circular 1136, <http://pubs.usgs.gov/circ/circ1136/circ1136.html#PUBS>, accessed 2/4/2011.
- ³ Smith, V.H., Tilman, G.D., Nekola, J.C. (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, *Environ. Poll.*, 100, 179-796.
- ⁴ Smil, V. *Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food*. The MIT Press, 2001, Cambridge, U.K.
- ⁵ Sharpley A, Foy B, Withers P (2000) Practical and innovative measures for the control of agricultural phosphorus losses to water: An overview. *J. Environ. Qual.*, 29, 1-9.
- ⁶ Kleinman P.J.A., Sharpley, A.N., Buda, A. R., McDowell, R.W., Allen, A.L. (2011) Soil controls of phosphorus in runoff: Management barriers and opportunities. *Can. J. Soil Sci.* 91, 329-338.

- ⁷ Sharpley A., Kleinman, P., and Weld J. (2010) Assessment of best management practices to minimize the runoff of manure-borne phosphorous in the United States, *New Zealand J. Agric. Res.*, 47, 461-477.
- ⁸ Sonzogni, W.C., Chapra, S.G., Armstrong, D.E., Logan, L.T. (1982) Bioavailability of phosphorus inputs to lakes. *J. Environ. Qual.*, 11, 555-563.
- ⁹ King, k.W., McDonald, J., Moore, J.F., Agrawal, S.G., Fischer, E.N., Balogh, J.C. (2010) Nutrient and pesticide removal from laboratory-simulated tile drainage discharge, *Trans. ASABE*, 53, 769-777.
- ¹⁰ Cheng, C.-M., Tu, W., Behrad, Z., Tarunjit B., Wolfe, W., Walker, H. (2007) Beneficial Reuse of FGD Material in the Construction of Low Permeability Liners: Impacts on Inorganic Water Quality Constituents, *J. Environ. Eng.*, 133, 523-531.
- ¹¹ Jin, N. fly ash Applicability in Pervious Concrete, Master Thesis, The Ohio State University, Columbus, OH 2010.
- ¹² Gall, H. E., Sassman S.A., Lee, L.S., and Jafvert, C.T., Hormone discharges from a Midwest tile-drained agroecosystem receiving animal wastes. *Environ. Sci. Tech.*, 2011, 45, 8755-8764.
- ¹³ Buchwald A., Dombrowski, k., and Weil, M. Influence of geopolymer binder composition on conversion reactions at thermal treatment, in *Developments in porous, Biological and Geopolymer Ceramics: A collection of Papers Presented at the 31st international conference on advanced Ceramics and Composites*, eds. Brito, M., Case, E., Kriven, W.M., Ceramic Engineering and Science Proceedings Volume 28. 257-271. Florida 2007,
- ¹⁴ Allred, B. (2010) Laboratory batch test evaluation of five filter materials for removal of nutrients and pesticides from drainage waters. *Transactions of the ASABE*, 53, 39-54.
- ¹⁵ Bryant, R.B., Buda A.R., Kkeinman, P.J.A., Church, C.D., Saporite, L.S., Folmar, G.J., Bose, S., Allen, A. (2012) Using fluegas desulfurization gypsum to remove dissolved phosphorus from agricultural drainage waters. *J. Environ. Qual.* 41, 664-671.
- ¹⁶ Yao, W., Millero, F.J. (1996) Adsorption of phosphate on manganese dioxide in seawater, *Environ. Sci. Technol*, 30, 536-541.
- ¹⁷ Cengeloglu, Y., Tor, A., Ersoz, M., Arslan, G. (2006) Removal of nitrate from aqueous solution by using red mud. *Sep. Purif. Technol*, 51, 374-378.
- ¹⁸ Duxson P, Lukey G.C., Separovic F. van Deventer J.S.J., (2005) Effect of alkali cations on aluminium incorporation in geopolymeric gels, *Ind. Eng. Chem. Res.*, 44, 832-839.
- ¹⁹ Ruters, S., Merten, J., Vassilieva, E., Dehandschutter, B., Poffijn, A., Smolders, E. (2010) The red mud accident in Ajka (Hungary): plant toxicity and trace metal bioavailability in red mud contaminated soil. *Environ. Sci. Technol.*, 45, 1616–1622.
- ²⁰ Chen L., Dick W., Nelson S., (2001) Flue Gas Desulfurization by-product additions to acid soil: Alfalfa productivity and environmental quality, *Environ. Poll.*, 114, 161-168.
- ²¹ Punshon T., Adriano D.C., Weber, J.T. (2001) Effect of flue gas desulfurization residue on plant establishment and soil and leachate quality, *J. Environ. Qual.*, 30, 1071-1080.
- ²² Hylander, L.D., Kietlinska, A., Renman, G., Siman, G. (2006) Phosphorus retention in filter materials for wastewater treatment and its subsequent suitability for plant production. *Biores. Technol.*, 97, 914-921.
- ²³ Haar, A. The Reuse of Phosphorus. Eureau Position Paper EU2-04-SL09, 2005; http://eureau.org/sites/eureau.org/files/documents/2005.02.21_recovery_of_phosphorus_from_sludge.pdf. Accessed 10/6/2012.

- ²⁴ Jasinski S.M., Phosphate rock, 2006 Minerals Yearbook. United States Geological Survey, 2007, http://minerals.usgs.gov/minerals/pubs/commodity/phosphate_rock/myb1-2006-phosp.pdf, accessed 10/2/2012.
- ²⁵ Christen, K. (2007) Closing the phosphorus loop, *Environ. Sci. Technol.*, 41, 2078.

Scenario Analysis for the Impact of Hydraulic Fracturing on Stream Low Flows and Water Supplies: A Case Study of Muskingum Watershed in Eastern Ohio

Basic Information

Title:	Scenario Analysis for the Impact of Hydraulic Fracturing on Stream Low Flows and Water Supplies: A Case Study of Muskingum Watershed in Eastern Ohio
Project Number:	2014OH312B
Start Date:	3/1/2014
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	OH-013
Research Category:	Climate and Hydrologic Processes
Focus Category:	Hydrology, Drought, Water Use
Descriptors:	None
Principal Investigators:	Suresh Sharma

Publications

1. Aashish Shrestha, Suresh Sharma, Scott C. Martin, and Colleen McLean (2014) Scenario Analysis for the Impact of Hydraulic Fracturing on Stream Low Flows in Various Climatic Conditions: A Case Study of Muskingum Watershed in Eastern Ohio (Sept 10-12, ORBC symposium, 2014), Poster
2. Aashish Shrestha, Suresh Sharma, Scott C. Martin, and Colleen McLean Bryan E. Kelly (2014) Scenario Analysis for the Impact of Hydraulic Fracturing on Stream Low Flows in Various Climatic Conditions: A Case Study of Muskingum Watershed in Eastern Ohio (Nov 18-19, WMAO conference, 2014), Poster

Progress Report, Ohio Water Resources Center Project

Title: “Scenario Analysis for the Impact of Hydraulic Fracturing on Stream Low Flows and Water Supplies: A Case Study of Muskingum Watershed in Eastern Ohio,”

PI: Suresh Sharma

1. Problem:

A significant amount of energy has been produced in United States for residential and industrial use from oil and natural gas (USEIA, 2011a). Natural gas production is expected to increase by nearly 30% over the next 25 years (Cooley and Donnelly, 2012), in response to the growing demand for energy to meet domestic needs, and to support economic development. In this context, the hydraulic fracturing is being utilized in association with oil and gas production in the Marcellus and Utica shale formations, ranging from the southern tier of New York through Pennsylvania, West Virginia, western Maryland, and eastern Ohio. Hydraulic fracturing (fracking) is a process to produce oil and natural gas by injecting water mixed with chemical additives into a geologic formation. Depending upon the type of gas production, water use per well is estimated to range from 65,000 gallons for methane production to 13 million gallons for shale gas production (GWPC and all consulting, 2009).

The majority of permitting decisions through the National Pollution Discharge Elimination System (NPDES) are based on hydrologic low flow conditions such as 7Q10, 7Q2, 4B3, etc. These criteria are developed based on the statistical analysis of long term historical stream flows records without anticipating water withdrawal for hydraulic fracking. This raises serious questions as to whether the permit conditions developed for low flows period are adequate to protect water quality in the current situation of hydraulic fracking. If unexpected water withdrawals cause low flow condition (e.g. 7Q10) to be overestimated, then water quality protection provided by NPDES permits could be inadequate. Since the underestimated hydrologic/biologic conditions for permitting threatens the water quality protection and overestimated conditions leads to uneconomical treatment in waste water facilities, proper estimation of these conditions is essential. In addition, reservoirs used for water supply purposes will be at critical stage during low flow (drought) periods. As water withdrawal for hydraulic fracking increases, the water supply may be inadequate to meet the needs of other users. Hence, water use due to hydraulic fracking not only reduces the assimilating capacities of the stream for pollutants, but also affects in water availability for water supply purposes.

2. Research Objectives:

The impact of water withdrawal may not be significant at the state level but it may have relatively greater impact in specific tributaries. Therefore, this research is investigating the water use for hydraulic fracturing at various spatial scales such as watershed, sub-watershed and county level. In addition, analysis will be conducted to evaluate the impact at various temporal scales such as seasonal, monthly and daily. More specifically, there are two major objectives of this study; 1) to analyze the potential impact of water withdrawals for hydraulic fracking on stream low flows, and 2) to analyze the water availability in a water supply reservoirs of Muskingum watershed under various water acquisition scenarios associated with

hydraulic fracking and population projection in future. The Objective 1 included thorough review of the existing watershed models with their potential capabilities and limitations, including issues and challenges in order to conduct simulation study under hydraulic fracking conditions.

3. Methodology:

Objective 1: Long term historical stream flows data will be utilized for the computation of low flows (7Q10). Model simulation will be utilized to compute low flows at various points of watershed locations in case the USGS gage is not available. For this, various watershed models were compared and calibrated and validated using USGS stream flows records. The model performance was be evaluated through widely used statistical measures such as Nash-Sutcliffe efficiency, R-square and percent bias. Using SWAT model, low flows were analyzed using the period before hydraulic fracturing which is called baseline condition. In the next step, we analyzed 7Q10 low flows utilizing the same period of data records but including the current level of hydraulic fracking, which is called current conditions. We evaluated the impact of hydraulic fracturing in low flows from the differences in current condition and baseline condition.

Objective 2: In order to develop realistic scenarios, it is required to have actual information of water acquisition, well injection and waste water recycle. Therefore, a thorough data collection related with hydraulic fracking, population growth and possible hydrologic drought was conducted. In the next step, plausible future scenarios were generated based on water demand for current trend of population growth. Statistical downscaling model (SDSM) was used to generate thirty years of plausible future daily weather series. The generated data was incorporated in the SWAT model to examine the level of impact due to fracking at various scales. Environmental flow criteria were analyzed, using plausible set of generated climate data over 30 years period based on historical climate, on sixteen similar subbasins of Tuscarawas watershed.

4. Principal Findings:

The state of art of existing watershed models has been presented to conduct simulation study due to water withdrawal associated with hydraulic fracking. The capabilities of widely adopted 7 watershed models (HSPF, MIKESHE, SWAT, WARMF, APEX, WAM and HEC-HMS) to incorporate fracking was systematically reviewed and documented with proper citation. Our study does not warrant the above-mentioned models only to incorporate fracking or simulation study as there are numerous watershed models available.

Our flow analysis using SWAT model depicted the consistent increasing drilling trend in Muskingum watershed. The result showed that the water withdrawal during low flow period (August through November) was about 43% of the total water withdrawal. Model was used to quantify the effect of these withdrawals over this period. 32 subbasins were affected by fracking, which had drainage area less than 140 km². The case study revealed that the impact of water withdrawal is significant flow period, and this effect is significant particularly in small order streams. Figure 1 presented both the monthly mean and seven days monthly minimum

flows in 8 subbasins. Interestingly, major impacts were observed in first order streams. Additionally, baseflow variation during low flow period suggests that ground water is dominant component for the discharge into most of rivers during this period.

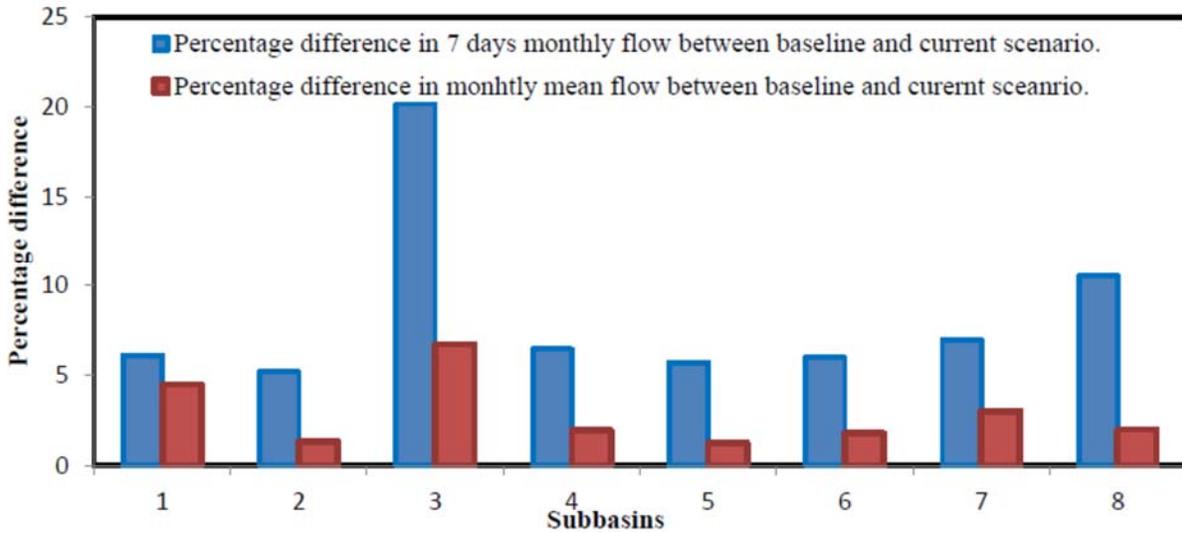


Figure 1. Percentage differences of 7 day minimum monthly flow and monthly mean between baseline and current fracking scenario on 8 affected for current period subbasins.

The percentage difference in 7Q10 between baseline and future scenarios was analyzed with increased size of the drainage area. A relatively smaller difference was detected when analysis was conducted in a large drainage area. The analysis was also conducted to see the effect in the flow duration curve, as it is one of the important statistics to quantify hydrologic regimes (Kim et al. 2009). Ninety-five percentile flow exceedance was considered as the threshold for the extreme low flows, which indicates a very stressful drought period as streams drop to very low level. Similarly, seventy-five percentile flow exceedance was considered as low flows, which is the dominant low flow condition sustained by the ground discharge into the streams. Subbasin with drainage area 920 km² was selected to analyze the flow duration curve between current and future scenario for 30 year period (Figure 2). The results showed that the extreme flow was not affected in this drainage area. However, the low flow was affected as the alteration was noticed below 85% flow exceedance.

Proper development of complete database of hydraulic fracking information is needed for the use of current watershed models to deal with the complex interactions of hydraulic fracking with watershed characteristics.

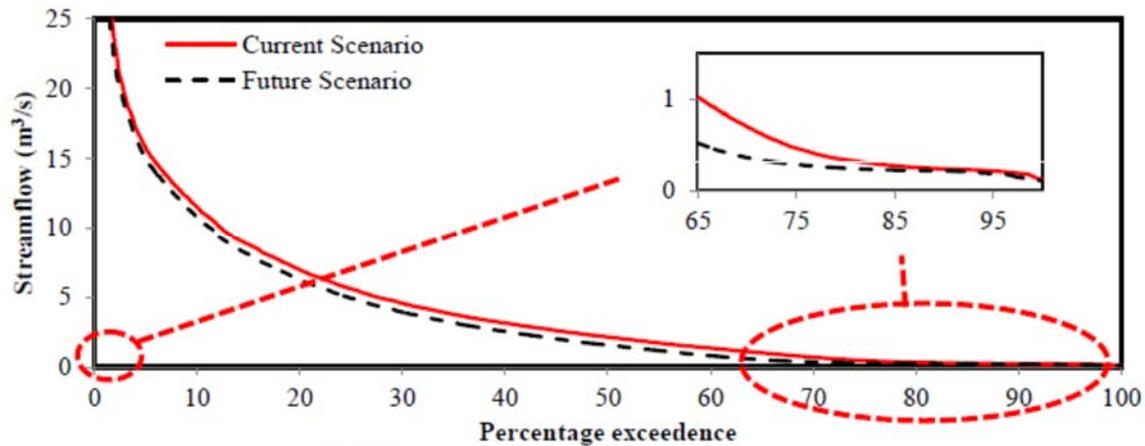


Figure 2. Flow duration curve for current and future scenario calculated over a 30 year period.

5. Significance:

Simulated flows in ungauged locations under the current fracking situation were used to assess the potential impact of water withdrawal for hydraulic fracking on water resources both at spatial and temporal scales. The study suggested that the impact was more significant during low flow than average flow or peak flow period. 7 days minimum flows showed some variation when compared with the 7 days minimum flow without fracking indicating that proper regulations of drilling activities are needed during the low flow period. Such flow alteration may bring the daily flow below the environmental flow limits, which may eventually threaten the water resources sustainability. Furthermore, the future scenario was modeled using 30 years of generated climate data based on historical precipitation to SWAT model. SDSM was used to generate future precipitation based on the occurrence of 25 percentile dry precipitation in past 30 years (1961- 1990). Seven day minimum monthly flows showed large variability when compared with and without fracking, indicating that flow alteration during low flow period will be more critical than average flow or peak flow period. The difference was also noticed on flow duration curves and base flow time series implying the clear impact of fracking during low flow period. The dramatic alteration in the flow system during daily or monthly low flows might drop below the environmental flow limits, causing concern for water quantity, water quality, and thus, aquatic life.

Surface water quality and ecosystem health with shale energy development

Basic Information

Title:	Surface water quality and ecosystem health with shale energy development
Project Number:	2014OH316B
Start Date:	3/1/2014
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	Ohio Congressional District 15
Research Category:	Water Quality
Focus Category:	Water Quality, Surface Water, Sediments
Descriptors:	None
Principal Investigators:	Elizabeth Myers Toman, Jiyoung Lee

Publications

1. B. A. Silliman (student), E.M. Toman. "Quantification of Gravel Rural Road Sediment Production." Poster Presentation. Annual Meeting of the American Geophysical Union, San Francisco, California. December 15-19, 2014.
2. C. R. Sheban (student) E.M. Toman, J. Lee. "Surface Water Quality and Microbial Communities in Three Ohio Watersheds." Poster Presentation. Denman Undergraduate Research Forum, The Ohio State University, Columbus, Ohio. March 25, 2015.
3. E.M. Toman. "Unpaved Rural Roads and Stream Water Quality." Invited speaker, Water Luncheon Seminar presented by The Water Management Association of Ohio and The Ohio Water Resources Center. April 15, 2015.

Surface water quality and ecosystem health with shale energy development

Elizabeth Myers Toman, School of Environment and Natural Resources

Jiyoung Lee, Division of Environmental Health Sciences & Dept. of Food Science & Technology

The Ohio State University

Issue

The development of shale energy in Ohio and throughout the world has dramatically increased in recent years without full understanding of the impacts to surface water quality and, in turn, total ecosystem health. Shale energy exploration and extraction activities, including the construction and traffic use of access roads and well pads, can produce sediment that may runoff to surface waterbodies. Sediments carry metals, chemicals, and nutrients that when released may disrupt aquatic microbial communities and encourage the growth of toxic algae. Besides the disturbance to aquatic ecosystems, increased sediments and associated algae, especially blue green algae, may affect the health of terrestrial systems as humans and animals interact with surface waters.

Research Objectives

The overall goal of this research is to characterize the impacts of shale development to surface waters and ecosystem health. We will accomplish this with two main objectives:

- 1) Determine the changes to stream water quality with development of shale energy within a watershed in eastern Ohio, and
- 2) Evaluate the response of microbial communities to environmental stresses, such as changes in sediment loading and water quality.

Methodology

Beginning in June 2014, we installed equipment in perennial streams at the mouth of two small watersheds on property owned and managed by The Ohio State University, OARDC in Noble County Ohio. This equipment continuously measures and records water quality parameters including temperature, dissolved oxygen, conductivity, pH, and turbidity. We surveyed the stream channel at each gauging site and installed equipment that continuously measures flow velocity and depth and uses the channel dimensions to determine stream flow. All collected data are sent via cellular modem to a publically accessible website for viewing or download.

Beginning in March 2014 we took monthly grab samples at each site that were transported to an EPA certified lab and analyzed for total suspended solids (TSS). From June to October 2014, additional grab samples from the streams were analyzed for nutrients and microbes.

Principal findings and significance

This research was designed with the intent of comparing water quality measures and stream microbial communities in paired watersheds before, during and after shale energy development in one of the watersheds (treatment watershed). The timeline for drilling at the study site has been postponed. Although this development is a disappointment to the researchers, we feel confident that we have measured and recorded baseline data regarding water quality parameters and microbial communities in these watersheds and have the infrastructure ready to begin monitoring activities if or when shale energy development at the site begins. From our analysis of the data collected for this research we have:

- Developed a stage to discharge relationship for the streams

- Developed a relationship between turbidity and TSS for the streams
- Identified bacteria and other microbial communities present in the streams
- Determined a positive relationship between stream turbidity and *E. coli* counts
- Related suspended sediment concentration and volume of sediment produced by the watershed to the volume of sediment that could be contributed to the streams by the existing road network

These findings provide a foundation for future work at this site regarding water quality, microbial communities and land use.

Characterizing stream restoration's water quality improvement potential through hyporheic exchange enhancement

Basic Information

Title:	Characterizing stream restoration's water quality improvement potential through hyporheic exchange enhancement
Project Number:	2014OH318B
Start Date:	3/1/2014
End Date:	5/31/2015
Funding Source:	104B
Congressional District:	13
Research Category:	Water Quality
Focus Category:	Water Quality, Surface Water, Geomorphological Processes
Descriptors:	None
Principal Investigators:	Anne Jefferson

Publications

1. Baker, S.B., & Jefferson, A. Development of hyporheic exchange and nutrient uptake following stream restoration. Consortium of Universities for the Advancement of Hydrologic Sciences International Biennial Meeting, Shepherdstown, WV. August, 2014. (poster)
2. Baker, S.B., & Jefferson, A. Development of hyporheic exchange and subsurface processes following stream restoration. Geological Society of America Annual Conference, Vancouver, B.C. October, 2014. (poster)
3. Baker, S.B., & Jefferson, A. Development of hyporheic exchange and subsurface processes following stream restoration. Kent State Water Research Symposium, Kent, OH. October, 2014. (poster)
4. Baker, S.B., & Jefferson, A. Development of hyporheic exchange and subsurface processes following stream restoration. Department of Geology Colloquium. Kent State University, Kent, OH. January, 2015. (oral presentation)
5. Baker, S.B., & Jefferson, A. Development of hyporheic exchange and subsurface processes following stream restoration. Upper Midwest Stream Restoration Symposium, Dubuque, IA. February, 2015. (poster)

Characterizing stream restoration's water quality improvement potential through hyporheic exchange enhancement

Principal Investigator:

Anne Jefferson, Department of Geology, Kent State University

Problem and Research Objectives

Restoration of streams degraded by urban or agricultural runoff is a multi-million dollar industry in the state of Ohio (Mecklenberg and Fay, 2011). Restoration ultimately seeks to return streams to their pre-disturbance physical and biological conditions, though pragmatic goals include improving geomorphic stability, habitat diversity, stormwater management, and water quality. Despite these goals, restoration often falls short of significant biological improvements, as quantified by post-restoration fish and macroinvertebrate monitoring. However, these biotic metrics may be limited by other watershed factors or by lack of connected habitat from which recolonization could occur (Spanhoff et al., 2007; Bond and Lake, 2003). Instead, direct measurements of physiochemical processes may be more useful indicators of a stream restoration project's long-term potential to improve water quality and facilitate ecosystem services. The way the restored reach moves and stores water and changes the water chemistry is what sets the template for the biotic communities to return and populate the stream successfully in the long term. Unfortunately, the focus on macrobiology as a monitoring tool means that data and scientific understanding are limited with respect to how stream restoration alters underlying physiochemical and ecosystem processes.

The research undertaken focuses on a specific question: ***How does stream restoration affect hyporheic flow over time and does this improve water quality?*** This question addresses multiple levels of stream function (Harman *et al.*, 2012), but focuses on one of the most important processes affecting stream water chemistry: *hyporheic exchange*. Hyporheic exchange moves water into, through, and out of the streambed sediment matrix, in the zone of stream water and groundwater mixing known as the hyporheic zone (Bencala, 2006). Hyporheic flowpaths allow for stream water to participate with the streambed substrate in biogeochemical cycling of nutrients and pollutants, buffering stream water temperature, and supporting important benthic microhabitats for invertebrates and fish. Thus, reestablishment of hyporheic exchange is critical to overall restoration success (Lawrence et al., 2013).

Hyporheic exchange is controlled by pressure gradients generated by streamflow over and around geomorphic structures and by the hydraulic conductivity of the streambed sediment (Buffington, 2009). Thus, restoration alters both of the controls on hyporheic exchange, by reshaping the channel bed and by changing the size or compaction of stream sediments (Boulton, 2007). Further, post-restoration flushing of fine sediments into or out of the streambed and geomorphic structures may alter hydraulic conductivity and therefore hyporheic exchange (Figure 1, Nowinski, 2011), even in the absence of visible changes in stream morphology. Yet, there is limited research on how restoration affects hyporheic exchange, and there is no research on how hydraulic conductivity and

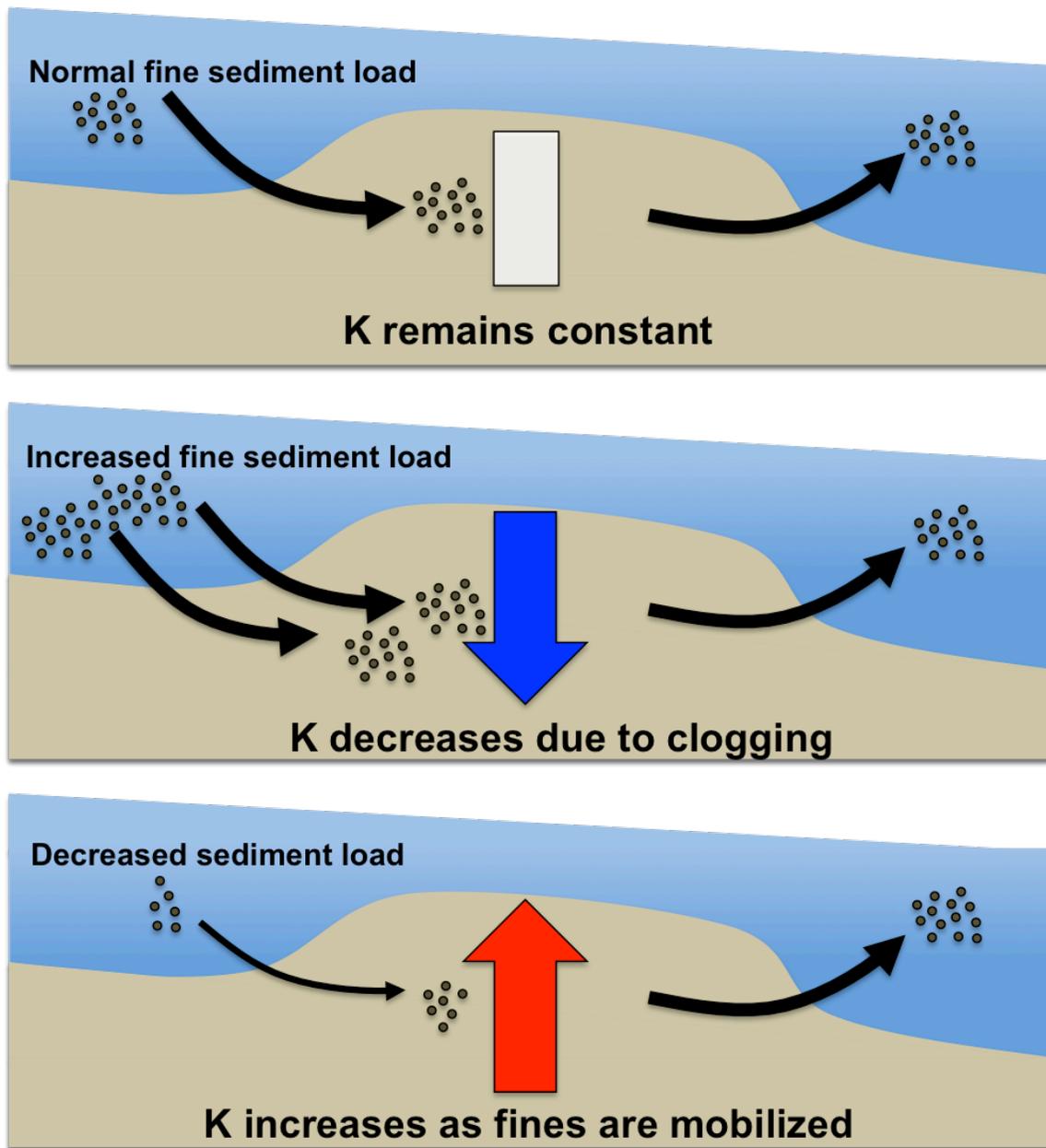


Figure 1. The fine sediment load in a stream can affect streambed hydraulic conductivity. If fine sediment is in balance with the energy to mobilize it within and from the streambed, no change is likely. If the load is increased, flowpaths may become blocked and reduce permeability. If the load is less than what the stream can move then the flow of water may winnow out fine material from the bed and increase hydraulic conductivity.

hyporheic exchange evolve following stream restoration. However, research on these topics has significant potential to inform and improve stream restoration practices, since restoration designers and construction crews can control or influence many of the factors that set the template for hyporheic exchange.

If stream restoration is to be successful it is important that hyporheic flowpaths are generated by the constructed channel form and sustained over the project lifetime.

Despite the significance of hyporheic exchange and the associated biogeochemical cycling, these processes are not specifically designed for nor monitored following completion of a project. Without an understanding of the how these processes function in a restored stream, restorations will continue to lack ability to fully develop to an ecologically beneficial state.

The objectives of the project are to:

- (1) evaluate changes in hyporheic exchange over time following stream restoration; and
- (2) assess the effects of physical on nutrient uptake and water quality in restored stream reaches

Methodology

Study Sites

Kelsey Creek at Kennedy Park in Cuyahoga Falls, OH has an 8.36 km² suburban watershed with 18% forested area (Figure 1). The stream was restored in August 2013 to address head cutting and bank erosion propagated upstream after a low head dam was removed adjacent to Munroe Falls Ave. Biohabitats, Inc. conducted the restoration, building floodplains and long pools and riffles, intended to mitigate stormwater flows. The construction used two layers of large cobbles in the riffles to ensure geomorphic stability, while the rest of the material used was sourced from the banks. Willow stakes and a sterile cover of rye grass was planted after construction was completed in 2013. Native plants, shrubs, trees, and seedmix were all planted during the fall of 2014.

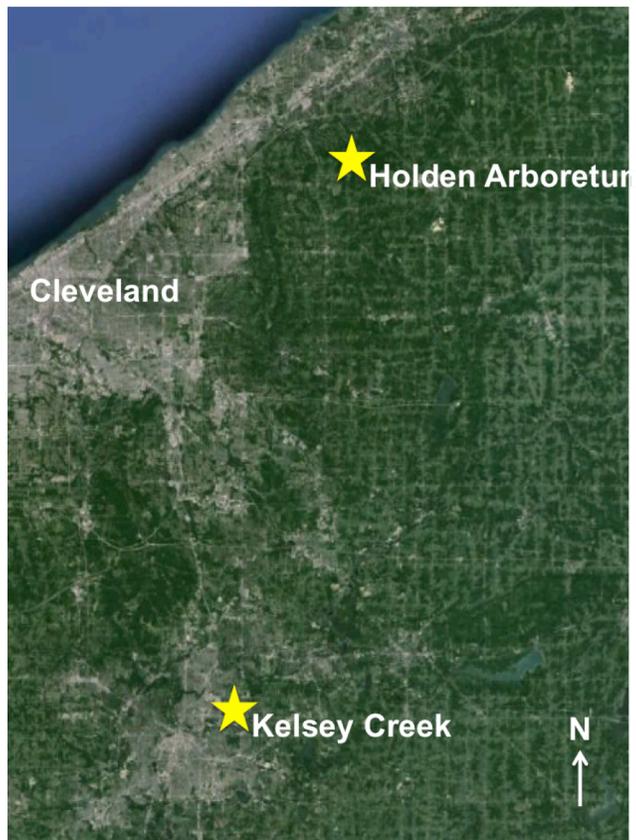


Figure 2. Map of field sites in northeast Ohio.

An unnamed tributary to Pierson Creek at **Holden Arboretum** near Kirtland, OH was restored in two sections (October 2013 and April 2014) at the outflow of Heath Pond to accommodate a new outlet (Figure 1). The restoration implemented boulders for geomorphic stability and externally sourced bank run material for the streambed. The

stream is ephemeral, flowing when the pond overtops a constructed lip. The drainage is 0.39 km² and is 58% forested. Freeze coring of the sediment found that the streambed is predominantly clay and silt, with some gravel near the surface.

Hydraulic head and hydraulic conductivity

Piezometers were installed at Holden Arboretum in three stream reaches, one restored in April 2014, one restored in October 2013, and one which was unrestored. At each reach, 12 piezometers were arrayed in a longitudinal transect over a riffle, with the upstream and downstream most positions in a pool, and some lateral positions within the riffle. At Kelsey Creek, piezometers were removed after each set of measurements was completed, and then reinstalled in the same position every six months to make ensuing measurements.

Piezometers were constructed from 1 meter long 1¼" inner diameter schedule 40 PVC pipe with a rounded end cap, screened with 80+ 1.5mm holes over the bottom 15 cm including through the sides of the end cap. Piezometers were then installed in longitudinal arrays through riffle structures, driven to a depth of 30 cm with a small sledge hammer. This placed the screened interval at 15-30 cm below the top of the streambed. A peristaltic pump was used to develop piezometers and evacuate any infilling sediment from the pipe. Hydraulic head was measured with an electric tape to find the depth from the top of the pipe to water inside the piezometer and to the stream surface outside.

Hydraulic conductivity was determined using the Hvorslev slug test method. HOBO Water Level Loggers (Onset Computer Corporation, Bourne, MA) were placed in the bottom of a piezometer to measure pressure at the bottom of the pipe and water was rapidly poured into the top of the piezometer to raise the water level suddenly and then allow for recovery. Atmospheric pressure was measured with an additional HOBO hung in the air, and used in the HOBOWare Barometric Pressure Assistant function to correct pressure during slug tests and determine depth of water over time.

Water Chemistry

Water samples were collected from the upstream end of each study area, transitions between restored and unrestored reaches, and at the downstream end of each study area. Samples were collected using 50 mL syringes which were first rinsed three times with stream water. The collected volume was filtered through a glass fiber filter and into a 50 mL Falcon tube. In situ measurements of pH, conductivity, water temperature, and dissolved oxygen were made concurrent with water grab sampling, using a YSI Professional Plus multiparamter sonde (Yellow Springs, OH). Samples were also collected from the hyporheic zone by drawing water from piezometers. For these samples, rinses of the syringe with sample water was conducted when available volumes allowed. All samples were chilled on ice in coolers during collection in the field and then frozen for storage.

For laboratory analyses, water samples were thawed and immediately prepared for analyses. One milliliter from each sample was diluted 1:10 with 2% nitric acid and analyzed on a Perkin-Elmer ICP-OES 8000 for calcium, potassium, magnesium, manganese, sodium, nickel, and zinc. Samples were also analyzed on a Dionex ICS-2100 chromatography system for chloride, fluoride, nitrate, nitrite, sulfate, bromide, and phosphate, at both full strength and at 1:10 dilution with deionized water in order to bring some constituents into a readable range.

Freeze Coring and Sediment Cation Extraction

Streambed sediments were sampled using freeze coring methods. A ¾" galvanized steel pipe with an end cap was driven 30 cm vertically into the streambed, 5-15 cm from each piezometer location, at a position as close to directly downstream as possible. In some instances the presence of cobbles required that the core be taken to the side of the piezometer. Once the pipe was in position, dry ice pellets were dropped into the pipe and isopropyl alcohol was added. After 5-20 minutes the saturated stream sediments around the pipe had frozen and the core was lifted out. A hammer and chisel were used to break the sediment and ice away from the pipe and subdivide it into the upper portion from 0-15 cm depth, and the lower portion from 15-30 cm depth. The frozen sediment was stored in plastic Ziploc bags, allowed to melt, and dried in an oven at 105°C. Aggregates were broken apart gently using a mortar and pestle to separate grains without altering them. Sediment was then analyzed for grain size using a Retch Camsizer P4 Video Particle Size Analyzer (Haan, Germany) with a detection range of 30-30,000 microns in 50 bins distributed logarithmically. Binned data was interpolated linearly to derive percentiles of the sediment size distribution for each sample.

Sediment from freeze cores was ground in a SPEX Sample Prep 8000M Mixer/Mill (Metuchen, NJ) for 5 minutes to create a powder. Approximately 2.5 g of milled soil was weighed and put in preweighed 50 mL centrifuge tubes. 25 mL of 0.1 M BaCl₂-NaCl₂ was added and the tubes were placed on a rotator for 30 min at 180 rpm. Samples were then centrifuged for 30 min at 4000x g and the supernatant was collected in a new tube, diluted at 1:10 with 10% HNO₃ and analyzed on an ICP-OES for Ca, Na, Mg, K, Fe, and Mn.

Principal Findings

Hyporheic exchange

During the study period (June-November 2014) at Holden Arboretum, the overall variability in hydraulic conductivity (K) across each reach did not change significantly (Figure 3), but K at most individual piezometer positions did change over time (Figure 4). Generally, piezometers in pools had a decrease in K, while those in riffles had an increase in K. This likely due to the steeper channel slope in the riffles allowing sediment mobilization, which is then deposited in the more

gently sloping pool areas. These two patterns approximately balance each other out within a given reach, as sediment mobilized from one area, increasing K , is then deposited shortly down gradient, decreasing K . The lack of change in the overall variability substantiates this at Holden Arboretum, showing no reach-wide shift over time (Figure 3), regardless of restoration age. Hydraulic head measurements showed very small gradients suggesting localized upwelling and downwelling was not strong. In this stream, hyporheic exchange may be limited by lack of well-defined geomorphic structures that promote downwelling and upwelling rather than by clogging of bed sediments.

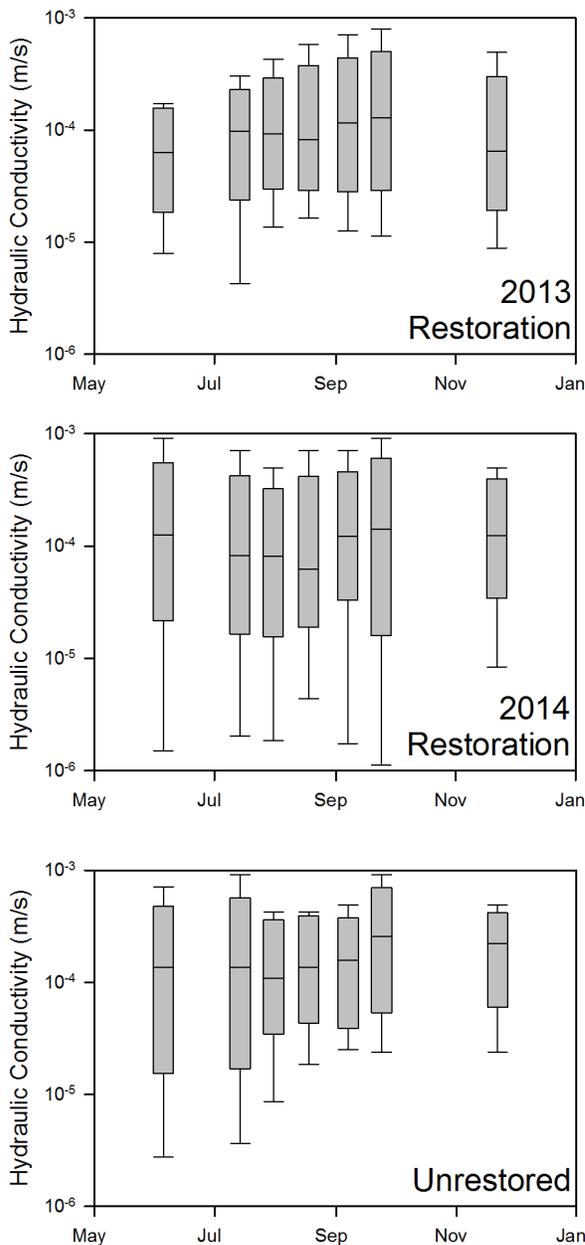
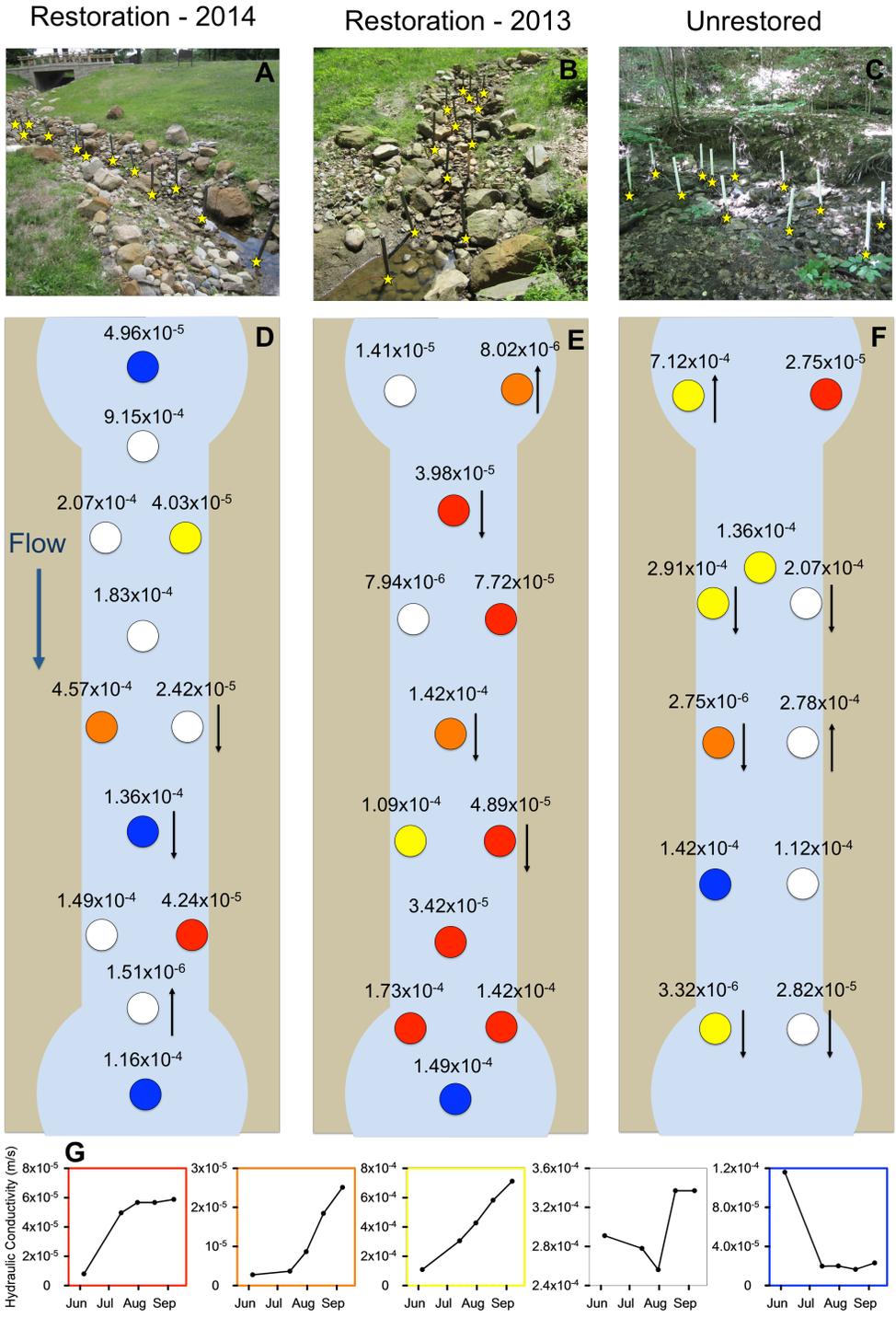


Figure 3. Distribution of hydraulic conductivities for all measured points at each stream reach through time.



At three reaches (A, B, C) at Holden Arboretum, 12 piezometers were installed (screened at a depth of 15-30 cm) to monitor hydraulic head and measure hydraulic conductivity with slug tests. Representative patterns in hydraulic conductivity change from June 5th 2014 to November 22nd 2014 (G) are indicated by colored circles at each piezometer position (D, E, F). Initial hydraulic conductivity is noted next to each (m/s) and arrows indicate upwelling or downwelling where hydraulic head was not neutral.

- ↑ Positive hydraulic head
- ↓ Negative hydraulic head
- ★ Piezometer position

Figure 4. Change in hydraulic conductivity over time for each piezometer at Holden Arboretum.

Hydraulic conductivity measurements at Kelsey Creek reveal a different trend. The mean K for each set of measurements decreases over time as does the lowest quartile, particularly for the November 2014 data (Figure 5). Hydraulic gradients at Kelsey Creek were also typically not strong. The overall trend toward a less permeable streambed at this site suggests that hyporheic flowpaths are being blocked and restricted by infilling of the sediment matrix with fine material. This may be due to sediment input from the stream reach directly upstream of the restored reach, which suffers from severe bank erosion as well. It is therefore likely that the hydraulic conductivity in the riffle structures of the restored reach will continue to decrease until the bank erosion upstream is stabilized. This may require additional restoration.

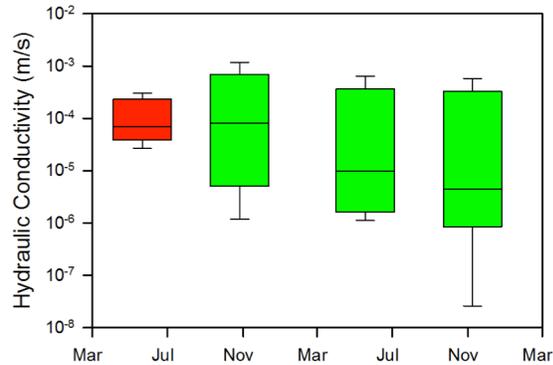


Figure 5. Hydraulic conductivity distribution at Kelsey Creek before (red) and after (green) the August 2013 restoration.

Water Quality

No significant changes to water quality were observed across the restored reaches. Water grab samples were analyzed for anions including nitrate, nitrite, chloride, phosphate, fluoride, bromide, and sulfate, and cations including calcium, magnesium, manganese, iron, nickel, and sodium. The chemistry from the top of the restored reaches at both Holden Arboretum and Kelsey Creek did not vary significantly from that at the downstream end of the reaches and thus the restorations are judged to have no major impact on the chemical quality of the water in the channel. The only notable change to water quality was a decrease in temperature across each reach at Holden Arboretum. This is largely due to elevated water temperature in the pond, which then cools off as it flows through the restored reaches. Such decreases in water temperature could result from hyporheic exchange, or from increased shading of the stream reaches relative to the pond.

Pore water collected from piezometers at Kelsey Creek revealed one important trend. Manganese concentrations were greatest in the head or upstream end of riffle 2 and 3, and in the middle of riffle 1, in each case as much as ten times higher than the surface water (Figure 6). Over the length of the each riffle, these high manganese levels dropped off, returning to levels similar to the surface water by the end of each riffle. Concentrations were also high at positions near the side of the channel. These results suggest that redox chemistry is active within the constructed riffles in the restoration and is likely caused by dissolved oxygen gradients along flowpaths through these structures. Nutrient levels did not change significantly within riffle sediments though this may be due to low initial concentrations. These restoration structures may instead have more impact on dissolved metal loads in

the stream, serving as a source for redox species like manganese. This may change over time as manganese is depleted from the hyporheic zone and other biogeochemical processes become more important.

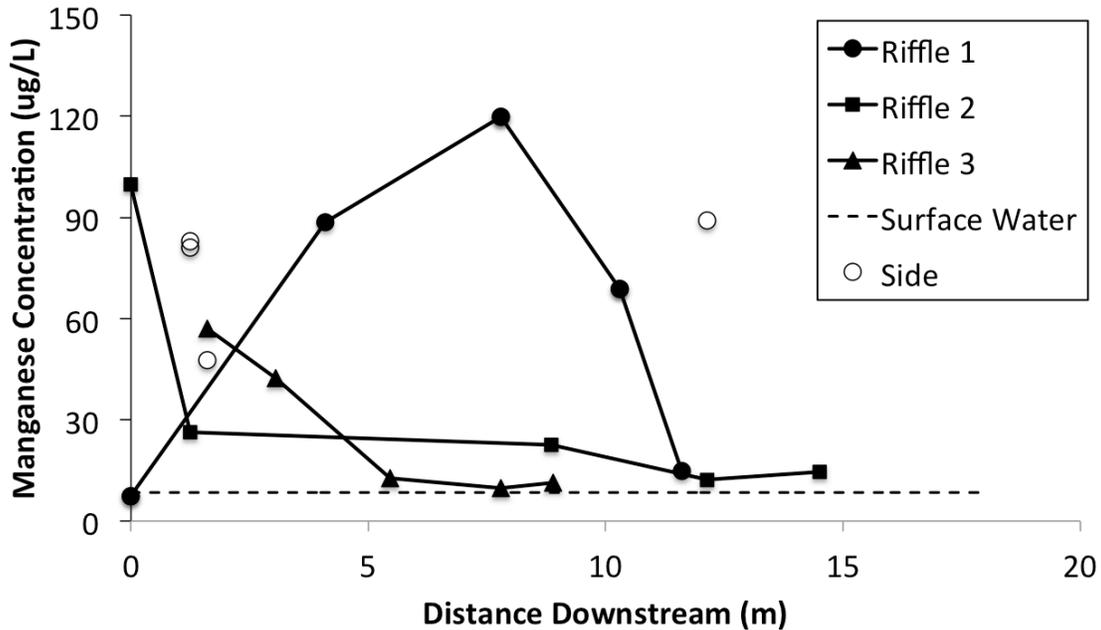


Figure 6. Manganese concentrations from pore water 15-30 cm below the streambed surface at Kelsey Creek.

On-going analyses

Freeze coring demonstrated a range of sediment sizes and sorting as well as colors indicative of redoximorphic features (Figure 7). Grain size analysis, sediment cation extractions and nutrient uptake measurements have been completed and analysis of these data will inform the hydraulic conductivity and porewater chemistry interpretations. Beyond the original scope of the grant, salt and dye tracer tests were conducted in the study reaches and 2-D model simulations will be completed later in 2015. These data and simulations will help generalize the findings.

Upstream → Downstream

Riffle 1



Riffle 2



Riffle 3



Figure 7. Freeze cores from riffles at Kelsey Creek. Sediment was sampled to a depth of 30 cm adjacent to each piezometer.

Significance

Overall this study has discovered a dynamic environment in the hyporheic zone of restored streams, with changing hydraulic conductivity and strong chemical gradients. In the stream where sediment inputs were restricted by the upstream dam (Holden Arboretum), hydraulic conductivity did not change at the reach scale over a 5 month period. Changes at individual points in the streambed, however, were substantial both in restored and unrestored reaches. In the stream where sediment inputs were unrestricted, and likely substantial, hydraulic conductivity at the reach scale declined over 15 months following restoration. Despite these dynamics, neither restored reach effected a change in surface water chemistry, as measured by baseflow grab samples analyzed for nitrate and other anions.

Hyporheic exchange was not significant enough to modify the water quality signal resulting from upstream land use and geology. This could be either be the result of insufficient hydraulic conductivity; the observed weak upwelling and downwelling; or short restored reach length. The observed weak upwelling and downwelling at both sites is notable because it belies claims that the geomorphic structures built during stream restoration are sufficient to generate substantial hyporheic exchange. Hydraulic conductivity was relatively high at Holden Arboretum and initially post-restoration at Kelsey Creek, so limiting hydraulic conductivity is unlikely for those sites and timepoints. However, the lowered hydraulic conductivity at Kelsey Creek by November 2014 may further impede already limited hyporheic exchange at this site. Finally, the short reach lengths in this study, which are typical of stream restorations, limit the residence time of water in the reach and the probability that water will spend sufficient time in the hyporheic zone to undergo biogeochemical processes such as denitrification.

While the study was limited to two sites and approximately one year of data, the results suggest that stream restoration practices may not induce sufficient hyporheic exchange to improve downstream water quality. Further, for reaches with fine sediment inputs, hyporheic exchange may become limited over time post-restoration.

References

- Bencala, K. E. (2006). Hyporheic exchange flows. *Encyclopedia of hydrological sciences*, John Wiley & Sons, Ltd.
- Bond, N. R., & Lake, P. S. (2003). Local habitat restoration in streams: Constraints on the effectiveness of restoration for stream biota. *Ecological Management & Restoration*, 4(3), 193-198.
- Boulton, A. J. (2007). Hyporheic rehabilitation in rivers: Restoring vertical connectivity. *Freshwater Biology*, 52(4), 632-650.

- Buffington, J. M., & Tonina, D. (2009). Hyporheic exchange in mountain rivers II: Effects of channel morphology on mechanics, scales, and rates of exchange. *Geography Compass*, 3(3), 1038-1062.
- Harman, W., R. Starr, M. Carter, K. Tweedy, M. Clemmons, K. Suggs, C. Miller. 2012. *A Function-Based Framework for Stream Assessment and Restoration Projects*. US Environmental Protection Agency, Office of Wetlands, Oceans, and Watersheds, Washington, DC EPA 843-K-12-006.
- Lawrence, J. E., Skold, M. E., Hussain, F. A., Silverman, D. R., Resh, V. H., Sedlak, D. L., et al. (2013). Hyporheic zone in urban streams: A review and opportunities for enhancing water quality and improving aquatic habitat by active management. *Environmental Engineering Science*, 30(8), 480.
- Mecklenberg, D. A., & Fay, L. A. (2011). *A functional assessment of stream restoration in Ohio* (Technical Report, Ohio Department of Natural Resources).
- Nowinski, J. D., Cardenas, M. B., & Lightbody, A. F. (2011). Evolution of hydraulic conductivity in the floodplain of a meandering river due to hyporheic transport of fine materials. *Geophysical Research Letters*, 38(1), - L01401.

Linked geomorphic and ecological responses to river restoration: Influence of dam removal on river channel structure and fish assemblages

Basic Information

Title:	Linked geomorphic and ecological responses to river restoration: Influence of dam removal on river channel structure and fish assemblages
Project Number:	2014OH327B
Start Date:	4/1/2014
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	15
Research Category:	Biological Sciences
Focus Category:	Ecology, Geomorphological Processes, Hydrology
Descriptors:	None
Principal Investigators:	Kristin L Jaeger, Mazeika Sullivan

Publications

1. Dorobek, A.C. and S.M.P. Sullivan. Fish community dynamics in the short-term following lowhead dam removal in an urban river system. Oral. Ohio Biodiversity Conservation Partnership Annual Meeting, Feb. 2015, OSU 4-H Center, Columbus, OH. Oral Presentation
2. Vent, D. and S.M.P. Sullivan. Restoration of sensitive and rare fish in altered landscapes. Poster. Ohio Biodiversity Conservation Partnership Annual Meeting, Feb. 2015, OSU 4-H Center, Columbus, OH.

Linked geomorphic and ecological responses to river restoration: Influence of dam removal on river channel structure and fish assemblages

Progress report for period March 1, 2014 through February 28, 2015

PI: Dr. Kristin Jaeger, School of Environment and Natural Resources, The Ohio State University, Wooster, OH

Co-PI: Dr. Mazeika Sullivan, School of Environment and Natural Resources, The Ohio State University, Columbus, OH

Problem and Research Objectives

Over half of the large rivers in the world are affected by dams (Nilsson et al. 2005). As of 1999, 75,000 dams existed in the continental United States (Graf 1999). Ohio alone contains approximately 4,800 dams, the majority of which are lowhead (<4 m height) dams. As these small and lowhead dams age or their upstream reservoirs fill with sediment thus limiting their ability to store water, their removal is becoming an increasingly popular restoration method to reestablish connectivity of upstream and downstream streamflow, sediment regimes, and movement of organisms (Poff and Hart 2002). Yet despite the increasing trend towards dam removal there is an alarming lack of data relative to the ecological impacts of small dams and dam removal (Hart et al. 2002, Stanley and Doyle 2002, 2003). Dam removal results in upstream and downstream changes to both the channel morphology (the physical shape of channel) and the streamflow velocity regime, with subsequent consequences on the aquatic ecosystem including fish assemblages. However, the particular character of geomorphic change associated with dam removal and its subsequent influence on ecosystem processes remain poorly resolved. Thus, there is an urgent need to evaluate ecosystem consequences of dam removal (Gangloff 2013). In particular, fish communities represent an important component of aquatic ecosystems and play important social and economic roles in Ohio. Recreational fishing is a major revenue generator within the state. Therefore, how fish assemblages respond to dam removal reflects a critical knowledge gap in the burgeoning dam removal and river restoration research.

The removal of the 77 year-old “5th Avenue” lowhead dam (145m long, 2.5m high) on the Olentangy River in Columbus, OH presents an opportunity to investigate linked ecological-geomorphic consequences of dam removal as they relate to fish assemblage structure. The removal of the dam is part of the Lower Olentangy River Restoration project to restore the river channel to free-flowing conditions found upstream and downstream of the dam, reestablish floodplain features and vegetation, and develop pockets of wetlands. Because the restoration project is designed to accelerate long-term river change associated with dam removal (Figure 1), it creates an extraordinary scientific opportunity to monitor and investigate changes in linked ecological-geomorphic processes, particularly within the context of evaluating changes in the fish assemblage over time as well as overall evaluation of the function of a re-created river system.

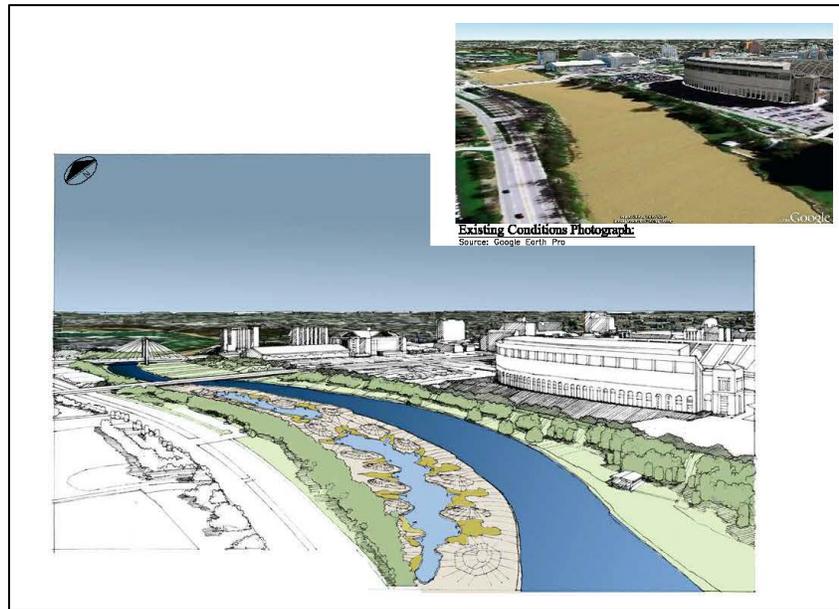


Figure 1. Proposed river corridor upstream of dam ~2 years after dam removal. Downstream of dam will not be actively restored. Dam was removed in August-September 2012.

Research Objectives

The overarching research objective is to investigate linked hydrogeomorphic and ecological short-term response in a river following dam removal with a focus on fish community assemblages. We will test 1) that the character of downstream geomorphic-ecological response is driven by the reservoir sediment composition, and 2) that upstream response is driven by changes in streamflow velocity regimes. In addition, we will also evaluate differences between restored and unrestored upstream reaches.

Methods

Study Design: The study will follow a BACI (before-after, control-impact design) (Stewart-Oaten et al. 1986, Downes et al. 2002). Through ongoing research in the Olentangy-Scioto River system (2010-present), Co-PI Sullivan has “before” data relative to fish community assemblages from river reaches upstream and downstream of the 5th Avenue Dam, as well as at “control” sites. Pre-dam removal geomorphic data (Stantec, Columbus, OH) are available for use in this project. The control sites represent an upstream (impounded) reach of an intact lowhead dam of comparable size and age in the same study river system. Therefore, the proposed work will focus on the “after” component of the design, and will include data collection at study reaches upstream (two, one in unrestored and one in restored section) and downstream (one) of the former 5th Avenue Dam, and one upstream of the intact “control” dam ($n = 5$). Study reaches are ~300m long. Sampling will occur at approximately 3-month intervals for a total of 4 sampling campaigns over the course of the year.

Geomorphic Data Collection: Changes in geomorphic complexity will be quantitatively evaluated using repeated fine-resolution (0.5 m spacing array) bathymetry surveys in a grid array within each of the upstream and downstream reaches using an Acoustic Doppler Profiler (ADP) to characterize variability in streambed elevation and quantify pool density. Pre-dam removal bathymetry exists, which serves as a baseline channel condition. In addition, fine-resolution, 3-dimensional velocity measurements using the ADP taken in a grid array within a stream reach

will quantify variability in local streamflow velocity. Finally, sieve analysis of bulk sediment samples will characterize grain size distribution of reservoir sediments to determine the relative contribution of fine and coarse sediments. Sediment grain size analysis will be completed through sieve sampling in the Soil Characterization lab at the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, where PI Jaeger is based.

Biological Data Collection: Fish will be sampled using a combination of standard boat- and backpack electrofishing (e.g., Brousseau et al. 2005, Kautza and Sullivan 2012) protocols. Individuals will be identified to species, weighed (mg) and measured (mm). Species will be classified by ecological and life-history traits following Frimpong and Angermeier (2009).

Data analysis: We will use general linear models (GLM) and multivariate regression to relate continuous variable descriptors of hydrogeomorphic change (e.g., spatial variability in streamflow velocity, streambed bathymetry, pool and bedform spacing) to measures of changes in fish assemblages. We will use non-metric multidimensional scaling (NMDS) to quantify changes in community composition among treatments. NMDS is an ordination technique that reduces multiple variables to a few graphically-displayed interpretable dimensions (James and McCulloch 1990). Following our BACI design, we will use t-test and ANOVA based approaches to test for spatial and temporal differences in our response variables (Stewart-Oaten et al. 1986, Downes et al. 2002, Meyer and Sullivan 2013). Analysis of similarity (ANOSIM) will be used to compare before-after fish assemblage compositions (Quinn and Keough 2002).

Principal Findings

Hydrogeomorphic component

Hydrogeomorphic sampling was conducted in April, June, and September 2014, and included surveys of all study reaches using the ADP and streambed substrate sampling. The current data set include a total of five (5) hydrogeomorphic sample periods.

In the short term, dam removal has decreased cross-sectional area in the former impoundment, flow velocity has increased and become more varied, and the channel has incised into the reservoir sediment generally flushing finer sediments in the unrestored portion of the river as evidenced by increased diameters in the distribution of fine sediments (e.g., < 10 mm). Substantial channel reconfiguration in the restored reach has resulted in temporary introduction of fine sediment as a consequence of in-channel construction activities (Sept 2013). Downstream reaches do not appear to have experienced significant aggradation or changes in sediment grainsize distributions.

Ecological component

Ecological sampling was conducted prior to and after dam removal during the summer and early fall. Upstream fish assemblage (at restored reach) composition shifted significantly and was accompanied by a significant decrease in species richness and diversity. These changes represented changes in the relative abundance of taxa within different feeding guilds. Specifically, reductions in species richness and diversity at the upstream reaches were accompanied by the loss of large-bodied omnivorous species. Between year 1 and year 2 post-dam removal, diversity increased significantly at the upstream restored and downstream reaches. Species richness increased significantly at the upstream restored reach and showed an increasing trend at the upstream

unrestored and downstream reach. Shifts in fish assemblages as the upstream restored and downstream reaches were accompanied by a substantial increase in insectivorous species including an increase in darter (*Etheostoma* spp.) species.

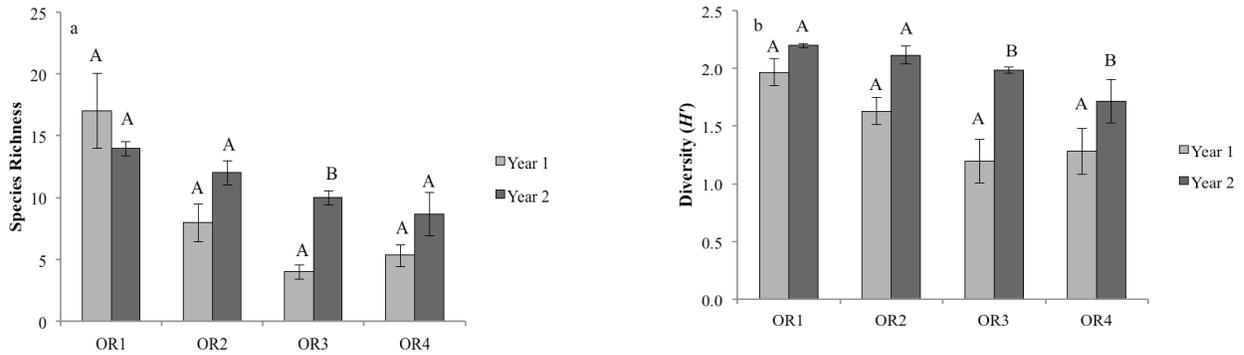


Figure 2. Fish assemblage (a) species richness and (b) diversity (H') in years 1 & 2 following dam removal of the Olentangy River study reaches. OR1 is the upstream of an existing dam control reach; OR2 is the upstream of the removed dam, unmanipulated experimental reach; OR3 is the upstream of the removed dam, restored experimental reach; and OR 4 is the downstream of the removed dam experimental reach. Significant differences based on t -tests are indicated by different letters ($p < 0.05$). Error bars represent ± 1 SE from the mean. From Dorobek, Sullivan, and Kautza (In press).

Significance

This project quantifies linked geomorphic and ecological response to removal of a lowhead dam with respect to fish community assemblages. The geomorphic influence of dam removal on fish community assemblages builds on documented community shifts in aquatic biota following dam removal.

Literature Cited

- Brousseau, C., R. Randall, and M. Clark. 2005. Protocol for boat electrofishing in nearshore areas of the lower Great Lakes: transect and point survey methods for collecting fish and habitat data, 1988 to 2002. *Canadian Manuscript Report of Fisheries and Aquatic Sciences* **2702**.
- Downes, B. J., L. A. Marmuta, P. G. Fairweather, D. P. Faith, M. J. Keough, P. S. Lake, B. D. Maptson, and G. P. Quinn. 2002. *Monitoring ecological impacts: concepts and practice in flowing waters*. Cambridge University Press, Cambridge, UK.
- Frimpong, E. A. and P. L. Angermeier. 2009. FishTraits: A Database of Ecological and Life-history Traits of Freshwater Fishes of the United States. *Fisheries* **34**:487-495.
- Gangloff, M. M. 2013. Taxonomic and ecological tradeoffs associated with small dam removals. *Aquatic Conservation-Marine and Freshwater Ecosystems* **23**:475-480.
- Graf, W. L. 1999. Dam nation: A geographic census of American dams and their large-scale hydrologic impacts. *Water Resources Research* **35**:1305-1311.
- Hart, D. D., T. E. Johnson, K. L. Bushaw-Newton, R. J. Horwitz, A. T. Bednarek, D. F. Charles, D. A. Kreeger, and D. J. Velinsky. 2002. Dam removal: Challenges and opportunities for ecological research and river restoration. *Bioscience* **52**:669-681.
- James, F. C. and C. E. McCulloch. 1990. Multivariate-analysis in ecology and systematics - Panacea or Pandora's box. *Annual Review of Ecology and Systematics* **21**:129-166.
- Kautza, A. and S. M. P. Sullivan. 2012. Using a process-based catchment-scale model for enhancing field-based stream assessments and predicting stream fish assemblages. *Aquatic Conservation-Marine and Freshwater Ecosystems* **22**:511-525.
- Meyer, L. A. and S. M. P. Sullivan. 2013. Bright lights, big city: influences of ecological light pollution on reciprocal stream-riparian invertebrate fluxes. *Ecological Applications* **23**:1322-1330.
- Poff, N. L. and D. D. Hart. 2002. How Dams Vary and Why It Matters for the Emerging Science of Dam Removal: An ecological classification of dams is needed to characterize how the tremendous variation in the size, operational mode, age, and number of dams in a river basin influences the potential for restoring regulated rivers via dam removal. *BioScience* **52**:659-668.
- Quinn, G. P. and M. J. Keough. 2002. *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, UK.
- Stanley, E. H. and M. W. Doyle. 2002. A geomorphic perspective on nutrient retention following dam removal. *Bioscience* **52**:693-701.
- Stanley, E. H. and M. W. Doyle. 2003. Trading off: the ecological removal effects of dam removal. *Frontiers in Ecology and the Environment* **1**:15-22.
- Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. ENVIRONMENTAL-IMPACT ASSESSMENT - PSEUDOREPLICATION IN TIME. *Ecology* **67**:929-940.

Information Transfer Program Introduction

The Ohio WRC conducted a number of activities to transfer water related information to a wide range of state, federal, county, and municipal agencies, to the academic community, students, children, and to private citizens throughout Ohio. The Ohio WRC mainly conducted information transfer by 1) promoting center activities, researchers, and research projects via newsletters, the Ohio WRC website, email correspondence, brochures, booths at conferences, personal meetings with water professionals and agencies representatives; 2) organizing, sponsoring, and participating in workshops, seminars, guest lectures and conferences; 3) serving and volunteering on various water organizations and their advisory boards such as the Water Management Association of Ohio, Ohio Water Resources Council and Friends of Lower Olentangy Watershed NGO. Specific activities included:

1) Promoting Ohio WRC research, results of projects, and investigators a)Preparation of Ohio WRC website content (wrc.osu.edu), website updates of events and news, and general maintenance of website. We had over 2,000 website hits, the majority of which came from new visitors. b)Preparing one page summaries of research projects, including the importance of the research topic for the State, relevant outcomes and results, and investigator background. These summaries were distributed to our Advisory Board members and other stakeholders. c)Publishing research project summaries in the Ohio Water Table, a quarterly newsletter published by the Water Management Association of Ohio (WMAO). During the reporting period, the highlighted researchers and projects were: Dr. Mouser's project #2011OH205B, Dr. Senko's project 2012OH250B, Dr. Bullerjahn's project 2013OH292B and Dr. Spiese's project 2013OH294B. This newsletter is distributed to about 575 people and organizations in Ohio in the water resources field from private sector (33%), universities (8%), nonprofit/citizens (17%) and federal, state and local government agencies (42%) d)Preparing and publishing an Ohio WRC postcard, brochure, and poster highlighting the annual activities and projects of the Ohio WRC. These are distributed at various events and presented at the Ohio WMAO conference. Responding to questions from public regarding water resources issues in the State of Ohio. e)Maintaining and updating statewide database of investigators in Ohio universities with research interests related to water. Currently, the database contains around 250 researchers from 15 different Ohio Universities. f)Meeting with the Ohio WRC Advisory Board Members – twice a year - discussing Center direction, requests for proposals, current research and results dissemination, and expanding the Ohio WRC's impact. g)Meeting with the Ohio Congress and Senate members office staff to discuss Ohio WRC activities, research results, and their impact for the State.

2)Organizing and sponsoring information transfer events a)Co-organized quarterly Ohio WRC-WMAO luncheon seminars, which includes assisting with luncheon administration and securing speakers. This past year the four luncheons were attended by approximately 120 water professionals from government, academia, NGOs and industry. The speakers and topics in this reporting period were: Gaj Sivandran (OSU): "Assessing the Impact of Increased Climate Variability and Land Use Change on the Water and Nutrient Budget of the Upper Walnut Creek: Downscaling Climate Models to Drive Watershed Models"; James Noel (NOAA): "Ohio River Basin Climate Change Project"; Xiaozhen Mou (Kent State University): "Removing microcystins from our water, a study to identify toxin-degrading bacteria in Ohio lakes"; MaryLynn Lodor (Cincinnati Metropolitan Sewer District): "Project Groundwork and updates towards an integrated watershed solution: the Lick Run Valley Conveyance System". b)Sponsored Water Management Association of Ohio (WMAO) conference titled: "VALUING WATER: Exploring the interactions between people, markets, and water." The conference is attracting increasing amount of academic researchers, including Ohio WRC researchers, based on our promotion of the conference. We also helped with selecting the student candidate for WMAO award, talked to students during the "Careers in Water Resources" session, and set up a booth at the conference to discuss Center activities. c)Sponsored Environmental Professional Network seminar on October 14th, 2014 titled: "Ohio's Water Resources and Citizens at Risk – Ag-related Practices and Policies to Prevent Harmful Algal Blooms, Post-Toledo." This seminar included a panel discussion of two areas: "The

Information Transfer Program Introduction

Science: Practices, technology and equipment to reduce run-off” and “The Policy: Laws, rules and engagement/outreach.” d)Guest lecture for the “Seminar on Sustainability” at the Ohio State University, talking about water and sustainability with a focus on urban water infrastructure and treatment. The seminar is attended by approximately 25 engineering undergraduate students each semester. e)Organizing and leading a 25 minute, hands-on workshop for 3rd grade students on principles of buoyancy in the 2014 Central Columbus Children’s Water Festival f)Leading water related activities during Life in the City summer camp in Columbus, near the Nationwide Children Hospital for children in grades K-8. g)Participating in BGSU workshop – “Global Solutions to Regional Problems – Collecting Global Expertise to Address the Problem of Harmful Algal Blooms Workshop,” discussing current science and identification of research gaps, will lead to development of workshop paper.

3)Serving in multiple water organizations a)Serving on Water Management Association of Ohio (WMAO) board as a Director of Research and Data Management. In this role, we focus on promoting water resources research in the State, and attend bimonthly meetings. b)Member of WMAO student awards committee – evaluating student proposals and deciding the best candidate for the award. c)National Institute of Water Resources Regional Representatives of the Great Lakes Region. d)Participating in quarterly meetings of the Ohio Water Resources Council meetings, forum for collaboration and coordination among state agencies – helping with strategic planning of the Council for FY2015 – 2018 e)Meetings with representatives of Ohio EPA, drinking and groundwater division – discussing plans how academic researchers can help State agencies during emergency events f)Lead Board of Regents “Producing of Safe Drinking Water” research group – response to State microcystin emergency. g)Stakeholders in the newly developed Global Water Institute Field-to-Faucet initiative at OSU, helping to formulate strategy, research capabilities and needs in solving the harmful algal bloom issue in Ohio and globally. Leading regional group of scientists in application for NSF-STC grant. h)Serving on Friend of Lower Olentangy Watershed (FLOW) NGO Science committee, helping organize events, write outreach and education proposals. i)Leader of OSU Shale Water Management interdisciplinary network that successfully, together with other partners, was awarded a DOE-NETL \$6,000,000 grant to establish shale gas development research laboratory in Ohio. j)Part of OSU Discovery Themes effort – institution wide strategic planning efforts for the University in teaching, research and engagement.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	8	0	0	0	8
Masters	9	0	0	0	9
Ph.D.	1	0	0	1	2
Post-Doc.	0	0	0	0	0
Total	18	0	0	1	19

Notable Awards and Achievements

2013OH297B Mark Mitchell (Graduate assistant) – 2014, Research featured in UC Graduate School Annual Report

2013OH297B Paul Hochwalt (Undergraduate Student) – 2014 University of Cincinnati STEM Excellence in Research Award, Biology: This award included a \$500 honorarium and was awarded based on Paul's research funded by this project.

2013OH297B Ishi Buffam (PI) – 2014 University-Wide Nominee, Simons Investigator in Mathematical Modeling of Living Systems

2014OH312B Graduate student, Mr. Aashish Shrestha was ranked first position under student poster competition categories both in ORBC symposium and WMAO conference. Altogether, Mr. Shrestha received two first awards for this research.

2013OH300B Work that is closely related to this project is continuing through a \$200,000 grant awarded by the Ohio Water Development Authority to PI Linda Weavers in the Department of Civil, Environmental and Geodetic Engineering (CEGE), and co-PIs Paula Mouser (CEGE) and Henk Verweij in Material Sciences at Ohio State.

2013OH292B Ohio Board of Regents, R/HAB-2-BOR. \$250,000. "HAB Detection, mapping and warning network: Sandusky Bay." Funded for period 3/1/15 – 8/31/16. With R.M.L. McKay (co-PI) and subawards to Kent State and OSU Stone Lab. \$102,656 direct costs to BGSU.

2014OH316B Houston Advanced Research Center (HARC) provided \$14,036 through their Environmentally Friendly Drilling program to aid in the collection of road related measurements towards this project.

Publications from Prior Years

1. 2012OH250B ("Microbial Modulation of Acidic Coal Mine Drainage Chemistry: Implications for Passive Treatment of Minewater") - Articles in Refereed Scientific Journals - Justin S. Brantner, and John M. Senko. 2014. "Response to Soil-Associated Microbial Communities to Intrusion of Coal-Mine Derived Acid Mine Drainage", *Environmental Science and Technology*, 48(15): 8556-8563
2. 2011OH205B ("The Constructed Wetland Dilemma: Nitrogen Removal at the Expense of Methane Generation?") - Articles in Refereed Scientific Journals - Michael R. Brooker, Gil Bohrer, and Paula J. Mouser. 2014. "Variations in potential CH₄ flux and CO₂ respiration from freshwater wetland sediments that differ by microsite location, depth and temperature", *Ecological Engineering*, 72: 84-94
3. 2012OH259B ("Green-House-Gas budget of constructed wetlands: Understanding the sources to maximize benefits") - Articles in Refereed Scientific Journals - Timothy H. Morin, Gil Bohrer, R.P.d.M. Frasson, Liel Naor-Azreli, S.Mesi, Kyle C.Stefanik, and Karina V. R. Schäfer. 2014. "Environmental drivers of methane fluxes from an urban temperate wetland park", *Journal of Geophysical Research: Biogeosciences*, 119(11): 2188-2208
4. 2012OH259B ("Green-House-Gas budget of constructed wetlands: Understanding the sources to maximize benefits") - Articles in Refereed Scientific Journals - Timothy H. Morin, Gil Bohrer, Liel Naor-Azrieli, S. Mesi, William T. Kenny, William J. Mitsch, Karina V.R. Schäfer. 2014. "The seasonal and diurnal dynamics of methane flux at a created urban wetland", *Ecological Engineering*, 72: 74-83
5. 2012OH311O ("An Integrated Framework for Response Actions for a Drinking Water Distribution Security Network") - Articles in Refereed Scientific Journals - Xueyao Yang, and Dominic L. Boccelli. 2014. "Simulation Study to Evaluate Temporal Aggregation and Variability of Stochastic Water Demands on Distribution System Hydraulics and Transport", *Journal of Water Resources Planning and Management*, 140(8), DOI: 10.1061/(ASCE)WR.1943-5452.0000359
6. 2010OH249O ("A comparative sustainability analysis of water management options in buildings") - Articles in Refereed Scientific Journals - Chirjiv K. Anand, and Defne Apul. 2014. "Composting Toilets as a Sustainable Alternative to Urban Sanitation – A Review", *Waste Management*, 34: 329-343
7. 2012OH262B ("Discriminating Biotic and Abiotic Arsenic Release Processes under Highly Reduced Ground Water Conditions") - Dissertations - Stuckman, Mengling, 2014, *Biotic Arsenic Mobilization in Natural and Anthropogenic Systems from Redox Transformations of Arsenic, Iron and Sulfur*, PhD Thesis, Environmental Science, Environmental Sciences Graduate Program, Ohio State University, Columbus, OH, 306 Pages
8. 2012OH250B ("Microbial Modulation of Acidic Coal Mine Drainage Chemistry: Implications for Passive Treatment of Minewater") - Dissertations - Haake, Zachary, 2014, *Biogeochemical Gradients within an Acid Mine Drainage-Derived Iron Mound, North Lima, Ohio*, M.S. Dissertation, Geology, University of Akron, Akron, OH, 43 pages