Introduction

Pursuant to the Water Resources Research Act of 1964, the Water Resources Center (WRC) is the federally-authorized and state-designated Water Resources Research Institute (WRRI) for the State of Ohio. The WRC was originally established in 1959 as part of the Engineering Experiment Station, College of Engineering, OSU, and conducted an extensive program of research on water and wastewater treatment processes. The Center continues to be administered through the College of Engineering and has maintained a tradition of placing special emphasis on encouraging and supporting research in the area of physical, chemical, and biological treatment processes for water and wastewater. The mission of WRC is to promote innovative, water-related research in the State of Ohio through research grant competitions, coordination of interdisciplinary research proposals, and educational outreach activities.
Research Program Introduction

Ohio Water Resources Center funded 6 research projects in 2010 and the research and information transfer on 6 projects from previous years was continuing.
### Basic Information

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<tr>
<th><strong>Title:</strong></th>
<th>Competitive Learning to Develop a Biomarker Forecasting Tool for Classifying Recreational Water Quality</th>
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<td>Dominic L Boccelli</td>
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### Publications

Competitive Learning to Develop a Biomarker Forecasting Tool for Classifying Recreational Water Quality

D. L. Boccelli (University of Cincinnati)

1. A progress report containing Problem and Research Objectives, Methodology, and Principal Findings and Significance

Problem and Research Objectives

Recreational users of surface waters can be at risk when there are elevated pathogens in the water. In urban areas, such as Cincinnati, non-point source contamination can occur through increased runoff (due to impervious surface area) and direct discharge of storm water, or combined storm water and sewage, into local surface waters. Indicators such as \textit{E. coli} and fecal coliforms are used as water quality surrogates due to their relative ease of measurement. Unfortunately, complete analysis and data reporting requires, at a minimum, 24 hours, thus limiting the utility of observed data to provide information to the population on water quality aspects in a timely fashion. However, the ability to predict microbial outbreaks in recreational waters would provide engineers, managers, regulators, and public health officials an important tool in disseminating pertinent public safety information in a timely fashion. Data-driven modeling approaches, such as linear regression or artificial neural networks, seek to capture the important forcing factors associated with microbial concentrations within a simple modeling framework. These data-driven models are then used to predict the microbial concentrations, which are then classified with respect to water quality standards. However, these approaches may still suffer from high rates of false-positives and false-negatives regarding classification.

The objective of the proposed study is to develop a Recreation Management Program tool capable of providing water quality classifications to the public regarding the safety of recreational waters. Previous research efforts have focused on quantifying microbial concentrations, prior to classification, using multivariate linear regression or artificial neural networks (used as a “black box” model). The proposed tool utilizes a type of neural network based on self-organizing maps entitled Learning Vector Quantization (LVQ). Rather than estimate the microbial concentration, the tool to be developed will predict the water quality classification directly, thus potentially eliminating the impact of errors in estimating the microbial concentrations. The LVQ approach will be compared to the more “typical” data-driven approaches such as linear regression and neural networks for microbial concentrations with emphasis on comparing the true and false classification rates.

Methodology

The approach for this study has utilized hydrologic and water quality data collected by the Charles River Watershed Association (CWRA) to develop a tool capable of providing a water quality indexing system for recreational water at the Larz Anderson Bridge sampling location. CWRA has collected \textit{E. coli} samples as well as flow and precipitation
data for approximately two recreational seasons (May through October) at multiple locations in the watershed that will be used in model development.

Previous research studies developed models that estimate the microbial indicator concentration first, which is then transformed into a classification. However, these approaches result in measurable false-positive and false-negative rates. Since classification of the water quality is of most importance, the neural network based approach of LVQ is proposed to use the available data to develop a tool that, given the appropriate hydrologic and meteorologic data, will directly produce a classification. This approach removes the reliance on adequate prediction of microbial concentrations.

To adequately compare the performance of the LVQ algorithm to other approaches, equivalent versions of linear regression and artificial neural network (ANN) models based on previous studies will be developed to represent the same data set. For simplicity, the explanatory variables used in the LVQ algorithm will be the same used to develop the linear regression model for the CWRA data (Eleria and Vogel, 2005). However, the dependent variables in the comparative models will be the actual E. coli concentrations with classifications performed after estimation. Comparisons between the different modeling approaches will be made based on the classification characteristics (i.e., true/false positive/negative rates) of each algorithmic approach. Additional studies will focus on exploring important explanatory values to develop the simplest model formulation that adequately represents the range of observed data.

Principal Findings and Significance

The linear regression, ANN, and LVQ modeling approaches have been developed to represent the Larz Anderson Bridge monitoring data using E. coli concentrations as the dependent variable, and the antecedent rainfall during the previous 24- and 168-hours and lag-1 E. coli concentration data used as the independent variables. These independent variables were selected based on previous work performed by Eleria and Vogel (2005). The resulting model classifications were evaluated with respect to the ability of three modeling approaches to satisfy a primary and secondary contact recreation standard (200 and 1000 colony forming units/100 mL of sample).

While there are differences in the classifications from each algorithm, each individual algorithm showed little difference when comparing performance associated with the boating and swimming standards. In fact, all three algorithms performed well with respect to the true negative rates (>92% in all cases; equivalent false positive rates <8%) regardless of the standard.

With respect to the linear regression and ANN approaches, the ANN algorithm performed slightly better than the linear regression. The ANN model produced a true positive rate about 10 percentage points higher than the linear regression model (true positive rates for the linear regression were 45%/50% and for the ANN were 52%/62% for the swimming/boating standards, respectively; false negative rates are equivalent to 100% minus the true positive rate). The LVQ algorithm, however, showed significant
improvements for representing the true positive rates (82%/87% for the swimming/boating standards, respectively).

These preliminary results were followed by research that investigated the importance of the eighteen potential explanatory parameters for classifying the microbial safety of recreational surface waters through input selection techniques (backward stepwise regression for MLR; perturbation analysis for ANN; and variance gain method for LVQ). For the MLR approach, the important factors include the discharge in the river during the current and previous day, the amount of rainfall in the previous week, and the intensity of the storm event were observed to be important. For the ANN approach, the important explanatory variables were intensity of the storm event, average daily net radiation, time since storms greater than 0 and 1.0 inches, and the amount of rainfall in the previous day and week. For the LVQ approach, the important explanatory variables had to be separately investigated for the swimming and boating standards. For both standards, the intensity of storm event, average daily net radiation, and the time since storms greater than 0, 0.2, and 0.5 inches were considered to be important. For the swimming standard, the discharge from five days prior was also important. For the boating standard, the discharge of the current day was also important. For all three approaches the storm intensity was found to be important, and the discharge, rainfall amount, and time since certain rainfalls were found important in varying combinations. The net radiation appears to be important for ANN and LVQ, but not for the MLR.

From the standpoint of classification, throughout the input selection process, the ANN and LVQ approaches consistently outperformed the MLR algorithm. Additionally, the LVQ algorithm performed as good as or better than the ANN algorithm. These results suggest that the LVQ can be a useful algorithmic tool for quickly classifying recreational surface waters relative to the appropriate standard.

2. Publication citations

Publications


3. Number of students supported by the project (MS/PhD) as well as their majors
Srinivas Motamarri (MS in Environmental Engineering) – currently at the US EPA (Athens, GA)

4. Awards or Achievements

The current research performed through the Water Resources Center has resulted in a follow-up proposal submitted to the Metropolitan Sewer District (Cincinnati, OH) to continue this research. The proposal is

"Development of a Recreation Management Tool to Predict Microbial Water Quality for the Metropolitan Sewer District" (2010), PI: D. L. Boccelli, co-PI: S. G. Buchberger, University of Cincinnati ($153,073)
Evaluating Colloid Release from Natural and Model Porous Media

Basic Information

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<td>Principal Investigators:</td>
<td>John Lenhart</td>
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Publications

2. Ye, Q., 2010, Evaluating the role of the secondary energy minimum in colloid deposition and release in saturated porous media MS Thesis, Civil Engineering, The Ohio State University, Columbus, OH
Evaluating Colloid Release from Natural and Model Porous Media

2011 Annual Report

John J. Lenhart, Ph.D.
Dept. of Civil and Env. Eng. and Geod. Science
The Ohio State University
Columbus, OH 43210

Statement of Critical Regional or State Water Problem
The behavior of colloid-sized particles is of significant importance in natural and engineered systems. In natural systems, colloids comprised of singular and aggregated mineral, biological and organic components are ubiquitous in surface and subsurface waters. Due to their propensity to sorb otherwise sparingly soluble contaminants or in the case of biocolloids, inherent risk, their presence in water poses a potential health risk (McDowell-Boyer, Hunt et al. 1986; McCarthy and Zachara 1989; Ryan and Elimelech 1996). Colloidal interactions are also important in water and wastewater treatment, chromatographic separation, oil production, extractive metallurgy, lubrication, coating and cleaning (Osipow 1962; Yao, Habibian et al. 1971; Hiemenz 1986). Consequently, the physical and chemical processes that govern colloid interactions with surfaces have been extensively studied, and significant progress toward identifying processes responsible for colloid deposition has been made (e.g., (Elimelech and O'Melia 1990; Song and Elimelech 1993; Lenhart and Saiers 2002; Lenhart and Saiers 2003; Tufenkji and Elimelech 2005). However, considerable uncertainty remains about the mechanisms that govern colloid interactions under unfavorable conditions, characterized by systems with like-charged surfaces, particularly with regard to mechanisms responsible for reversible deposition and colloid release in porous media (Kretzschmar, Borkovec et al. 1999). Knowledge of the fundamental processes that control the deposition, release and subsequent transport of colloidal particles and associated contaminants is crucial to maintaining the quality of ground water that the nearly five million residents of the Ohio (OhioEPA 2000) rely on for their daily needs.

Research Objective
The objective of this research is to evaluate the extent and kinetics of colloid release in water-saturated porous media under conditions selected to promote unfavorable DLVO interactions. The work will couple laboratory-scale experimental work with mathematical models to test existing theory and approaches to model colloid transport. Results of this research will be used to (1) evaluate the importance of the secondary minimum in reversible colloid deposition in systems with like-charged surfaces, (2) test the influence of system conditions (e.g., porous media, porewater velocity, porewater composition and colloid size) on reversible deposition, and (3) test the rigor of existing approaches that account for non-DLVO deposition (e.g., (Tufenkji and Elimelech 2004) and examine their application to describe colloid release. Such information is needed to accurately predict colloid mobility and appropriately evaluate filtration technologies for their removal from source waters. Two overarching questions drive this research. They are:
To what extent does reversible deposition depend upon the presence of the secondary minimum?

How do system conditions (e.g., grain size, solute composition) influence reversible deposition?

**Methods and Procedures**

The deposition and mobilization of colloids will be evaluated in a series of bench-scale column experiments. Each experiment will consist of two stages, a deposition stage and a mobilization stage. During the deposition stage, a solution comprised of the colloids, suspended in a solution containing simple electrolytes (e.g., 0.01 M NaCl at pH 6.5), will be introduced into the column as a pulse. The concentration of the electrolyte and the valence of the cation in the influent suspension will be varied between experiments and a suite of experimental conditions will be tested in order to prepare columns that have different retained colloid profiles. Colloids deposited in the column during the first stage will be mobilized through a single, or through successive step-changes in the porewater electrolyte in the second stage. At the conclusion of each experiment, and for some experiments at the conclusion of the deposition stage the porous media will be extruded from the column and the profile of retained colloids will be measured.

**Preparation and Characterization of Experimental Materials.** Quartz sand, sieved to provide particles with a nominal size of ca. 250 μm was used as the porous medium in all experiments. As received, the media sand is coated with metal oxides (Fe, Al and Ti) and trace quantities of organics. These impurities were removed because they may influence surface charge characteristics and thus may promote colloid deposition (Litton and Olson 1993). Upon cleaning, the size and surface charge of the media was evaluated via scanning electron microscopy and electrophoretic mobility measurements of native colloids (or streaming potential), respectively. These results were presented in the 2010 Annual Report.

Surfactant-free fluorescent spherical latex particles were used in all experiments as the colloidal phase. Carboxyl-modified surfaces are commonly used and the sizes chosen will depend upon whether deposition within the primary or secondary minima is to be accentuated. For example, Tufenkji and Elimelech (Tufenkji and Elimelech 2005) evaluated the transport of 63, 320 and 3000 nm latex particles through 328 μm soda glass beads as a function of pH and ionic strength. Their results suggest that deposition within the primary minimum is highly unlikely for the 3000 nm particles, but possible for the same particles within secondary minimum. For the 63 nm particles, however, it is expected that conditions favor deposition within the primary minimum. For this work, particles with a nominal size of 40 nm and 500 nm were purchased. Presumably, compared to the 40 nm particles the 500 nm particles should be more amenable to release.

The manufacturer-reported diameter was confirmed by dynamic light scattering. Latex colloid suspensions were prepared by adding aliquots of a concentrated stock to the electrolyte solution to achieve the target colloid concentration. The average zeta-potential of the colloids in the different electrolyte solutions was also determined on the basis of measured electrophoretic mobilities and the tables of Ottewill and Shaw (Ottewill and Shaw 1972) or the Smoluchowski equation (Hunter 1981).

**Column Design.** Experiments on the transport of the latex microspheres follow the methods outlined by Lenhart and Saiers (Lenhart and Saiers 2003). Glass chromatography columns with an internal diameter of 1.0 cm and PTFE end fittings were used to contain the porous media. A
liquid chromatography pump was used to control the flow of colloid and electrolyte solutions. The pump was positioned at the base of the vertically oriented columns and the flow was directed upward. Each experiment used a fresh column prepared by wet-packing methods (Lenhart and Saiers 2003). The column was packed to a predetermined height (15 cm), and vibration was used during packing to minimize air entrapment and the formation of layers. The porosity and pore-volume of each packed column was measured using standard methods.

**Column Experiment Methodology - Stage 1: Colloid Deposition.** Each packed column was pre-equilibrated with the electrolyte solution by pumping approximately 10 – 20 pore volumes of colloid-free electrolyte solution through the column prior to commencing the experiment. Colloid concentrations were monitored in the column effluent during this period to verify that native colloid mobilization is negligible. The equilibration period ceased when the effluent pH matched that of the influent solution. Following column pre-equilibration, a suspension of latex colloids in electrolyte solution was pumped into the column for a specific period of time (3 pore volumes), whereupon the colloid-free electrolyte solution was redirected into the column. Effluent colloid concentrations will be monitored in effluent samples collected with a fraction collector based upon fluorescence intensity. Experiments were conducted for different electrolyte concentrations and porewater pH.

**Column Experiment Methodology - Stage 2: Colloid Mobilization.** Colloid mobilization was induced at the conclusion of the deposition stage, when the effluent colloid concentrations return to baseline levels, by perturbing the composition of the electrolyte solutions to increase repulsive double-layer interactions between the colloid and media surfaces (e.g., by diluting electrolyte concentration). Successive perturbations were examined, depending upon the initial conditions, as will the magnitude of the change in the electrolyte composition.

**Progress Summary**
Work over the past year comprised (1) collecting experimental data evaluating the transport of latex microspheres with a nominal diameter of 40 nm and (2) collecting experimental data evaluating the transport of latex microspheres with a nominal diameter of 500 nm.

**Principal Findings**
A series of column experiments were conducted with the 40 nm and 500 nm particles as a function of ionic strength and for the 500 nm particles, pH. These experiments examined the deposition characteristics and for the conditions with sufficient deposited particles, release was also investigated by systematically altering the solution composition to produce conditions favorable for release by (1) reducing the ionic strength and (2) increasing the pH.

At low ionic strength the transport of both particle types nearly matched that of the conservative tracer, bromide (data not shown), indicating little deposition occurred (Figure 1A and 1B). With increasing ionic strength, the extent of breakthrough decreased as electrostatically repulsive interactions between the particles and sand were reduced. For the 40 nm particles, breakthrough was further reduced and shifted to longer times at an ionic strength of 30 mM, suggestive of behavior similar to solute adsorption (Figure 1A). At an ionic strength of 0.1 M, breakthrough was significantly suppressed and at 0.3 M no breakthrough was observed (data not shown). The behavior of 500 nm particles at elevated ionic strength was similar, with the exception that the delayed breakthrough was not evident (Figure 1B). Results for the 500 nm particles at pH 9.6 (data not shown) were nearly identical to those at 6.5, indicative of colloid deposition to a
homogenous surface devoid of metal oxide impurities. The differences in deposition observed at high ionic strength between the two particle sizes suggests different mechanisms controlled particle behavior.

Pulses of reduced ionic strength solutions were input to the colloid-laden columns in order to investigate particle release. For the 40 nm particles, very little release was observed when reducing the ionic strength from 30 mM to 0.1 mM NaCl, consistent with irreversible deposition of the particles in the primary energy well (Figure 2A). An increase in the solution pH from 6.5 to 9.5 did produce a small pulse of released particles (Figure 2A). While this may reflect surface heterogeneities at the quartz surface, an alternative mechanism could be that this release reflects that colloids were deposited at the secondary energy minimum which decreases in magnitude with decreasing ionic strength. For the 500 nm particles, reductions in ionic strength from 3 mM NaCl to 1 mM NaCl and from 1 mM NaCl to 0.1 mM NaCl both produced noticeable release pulses. Consistent with the larger particle size, this release suggests a more important contribution from the secondary energy minimum.

Figure 1 – Transport of the (A) 40 nm particles and (B) 500 nm particles through water-saturated quartz sand as a function of ionic strength at pH 6.5.

Figure 2 – Release of (A) 40 colloid at initial ionic strength of 30mM and pH 6.5 and (B) release of the 500 nM particles at initial conditions of 3mM and constant pH 6.5±0.1.
Future work consists of (1) correlating deposition and release behavior of the particles to theoretical estimates of the interaction potential for the systems in order to identify mechanisms controlling particle transport and (2) developing or utilize an existing transport model to test the proposed mechanisms.

**Publications**


**Students Supported**
Qing Ye (M.S. student in the Department of Civil and Environmental Engineering and Geodetic Science)

**Awards or Achievements**
None at this time.
References cited
Destruction of Cyanobacterial Toxins in Water with Germicidal UV-254 nm-based Homogeneous and Solar-based Heterogeneous Advanced Oxidation Processes

Basic Information

| Title: | Destruction of Cyanobacterial Toxins in Water with Germicidal UV-254 nm-based Homogeneous and Solar-based Heterogeneous Advanced Oxidation Processes |
| Project Number: | 2009OH119B |
| Start Date: | 3/1/2009 |
| End Date: | 11/30/2010 |
| Funding Source: | 104B |
| Congressional District: | 1 |
| Research Category: | Engineering |
| Focus Category: | Water Quality, Water Supply, Toxic Substances |
| Descriptors: | |
| Principal Investigators: | Dionysios Dionysiou, Dionysios Dionysiou |

Publications

3. He, Xuexiang, Miguel Pelaez, Dionysios D. Dionysiou. Photochemical degradation of microcystin-LR by UV-254 nm and H2O2 (in preparation for submission).
7. Pelaez, Miguel*, Armah A. de la Cruz and Dionysios D. Dionysiou, Nitrogen and Fluorine co-doped


Destruction of Cyanobacterial Toxins in Water with Germicidal UV-254
nm-based Homogeneous and Solar-based Heterogeneous Advanced
Oxidation Processes

(USGS Annual Report FY 2010)

PI: Dionysios D. Dionysiou, Ph.D., Professor

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1. A completion report

The project focused on the destruction of the cyanobacterial toxins by advanced oxidation technologies (AOTs), using both homogeneous and heterogeneous systems. The increasing occurrence of cyanobacterial blooms in surface water resources has become intense around the world. The blooms could not only produce many odor or taste issues, but also result in cases of livestock deaths or human health disorders [1]. Toxic freshwater cyanobacteria such as *Microcystis* could produce cyclic hepatotoxins called microcystins (MCs). They are found to be persistent in aquatic systems [2]. Chronic exposure due to the presence of MCs in drinking water is thought to be a contributing factor in primary liver cancer [3]. Their effect is accumulative and the cancer risk could increase by exposure to even very low levels of the toxins [4]. Cylindrospermopsin (CYN) is a tricyclic guanidine alkaloid and protein synthesis-inhibitor. This toxin is believed to be responsible for a major human poisoning in the “Palm Island mystery disease”. CYN is a hepatotoxin, and beside liver damage, it causes damages to the kidneys, spleen, intestine, thymus, and heart in vertebrates [8-12]. During this period of the project (March 1, 2009 through November 30, 2010), various research activities were conducted to evaluated the degradation of cyanobacterial toxins, such as microcystins and cylindrospermopsin, by solar-based heterogeneous and germicidal UV-254 nm-based homogeneous advanced oxidation processes.


1. Research Objectives

Solar-driven photocatalytic systems, such as titanium dioxide (TiO$_2$), were explored for the destruction of cyanotoxins in water. TiO$_2$ photocatalyst has been considered a key material in the destruction of recalcitrant organic pollutants in water and air as well as for killing pathogenic microorganisms. Recent studies have dealt with the modification of this material towards visible light sensitization via doping of TiO$_2$. A successful approach is the use of non-metal elements, such as C, F, N, S and P, to activate the catalyst under visible light due to either band gap narrowing or the creation of mid gap levels in the TiO$_2$ lattice. The objective was to investigate sol-gel based approaches to developed TiO$_2$ films with enhanced structural and optical properties towards the improvement of photocatalytic activity of TiO$_2$ under visible light irradiation. This is one of the few studies that deal with the synthesis of these nanostructured visible light activated TiO$_2$ photocatalyst using surfactant templating strategies in sol-gel methods. The results and
findings obtained in this study are promising, considering: (i) versatile applications of this method, (ii) immobilization of TiO₂ for more engineered approach and application, and (iii) controllability of the physicochemical properties of TiO₂ at the nano-level for sustainable applications.

2. Methodology and Findings

In one of the studies, we report on the synthesis, characterization and environmental application of immobilized nitrogen and fluorine co-doped TiO₂ (NF-TiO₂) photocatalyst. A fluorosurfactant-based sol-gel approach was employed to enhance the physicochemical properties and photocatalytic activity of NF-TiO₂ under visible and UV light for the degradation of the hepatotoxin microcystin-LR (MC-LR). The films were characterized by XRD, ESEM, TEM, AFM, EPR, micro-Raman, XPS, UV-vis spectroscopy and porosimeter analysis. The results revealed that by modifying the molar ratio of the fluorosurfactant, we could effectively control the physicochemical properties and obtain films with high BET surface area and porosity, small crystallite size and narrow pore size distribution. UV-vis spectroscopy showed an increase in the absorption capacity of NF-TiO₂ in the visible light range compared to reference films. The existence of interstitial nitrogen and substitutional fluorine in the TiO₂ lattice was determined by XPS. Comparative EPR measurements between the co-doped and reference samples identified distinct N spin species in NF-TiO₂, with a high sensitivity to visible light irradiation. The abundance of these paramagnetic centers verifies the formation of localized intra-gap states in TiO₂ and implies synergistic effects between fluorine and nitrogen dopants. Micro-Raman spectroscopy showed the growth of small amounts of brookite concomitantly with the major anatase TiO₂ phase, which could promote the system’s photocatalytic activity through the formation of anatase/brookite heterojunctions. Analysis of the lower frequency E_g anatase Raman mode indicated the occurrence of size effects reflecting phonon confinement in the anatase nanocrystallites as well as deviations from stoichiometry due to structural defects in the co-doped sample. NF-TiO₂ films effectively degraded MC-LR under visible and UV light compared to reference film. Similar MC-LR degradation rates under visible light after three cycles revealed high mechanical stability and no irreversible changes of the film during photocatalysis. This process has the potential of providing environmentally benign routes for drinking water treatment with solar powered photocatalytic systems.

The evaluation of NF-TiO₂ photocatalyst for the degradation of MC-LR under visible light in the presence of different water quality parameters (i.e., natural organic matter, pH, alkalinity and dissolved oxygen) was also investigated. It was found that the degradation
rate of MC-LR was higher under acidic conditions than in alkaline pH due to electrostatic interaction between the positively charge nanoparticle and the negatively charge cyanotoxin. The addition of carbonate to concentrations of 50, 100 and 150 mg CaCO$_3$/L at pH 7.0 reduced the degradation of MC-LR with the highest reduction at a concentration of 150 mg CaCO$_3$/L. The increase of carbonate ions can scavenge the radicals species formed during visible light irradiation of NF-TiO$_2$. In the case of natural organic matter (i.e., fulvic acid and humic acid), the inhibitive effect of fulvic acid, both at 5 and 10 mg/L, was larger than that of the humic acid at neutral pH due to the higher degree of aromaticity of the fulvic acid. Even though there was inhibition in the presence of natural organic matter, degradation of MC-LR was observed at all pH tested with a highest degradation at acidic conditions. In the absence of oxygen (N$_2$ purged), a reduction on the degradation rate of MC-LR was observed while under oxygen saturated conditions (O$_2$ purged); an enhancement on MC-LR rates was obtained. Real water samples from Lake Erie and Florida (St. John’s River) were spiked with MC-LR but no degradation was observed after 5 hrs of visible light irradiation with NF-TiO$_2$. The water quality parameters suggest that the alkaline pH of the water, along with the high alkalinity and TOC values, strongly inhibits the performance of NF-TiO$_2$ under the conditions tested. A manuscript on the results is published in Water Research and a conference proceeding is accepted for the 20th IOA World Congress – 6th IUVA World Congress, May 23-27, 2011, Paris, France (Note appendix A and B for publication details).


1. Research Objectives

Hydrogen peroxide, under the irradiation of UV, produces the generation of nonselective and powerful hydroxyl radicals. The combined UV/H$_2$O$_2$ has some advantages compared to other advanced oxidation processes, e.g., it does not have phase transfer problems and it is a green technology that neither UV nor H$_2$O$_2$ produces harmful residues [5, 6]. In US EPA’s disinfection guidance manual (2006), the 22, 22, and 186 mJ/cm$^2$ UV doses are required for a 4-log inactivation of Cryptosporidium, Giardia and Virus (Adenovirus) respectively [7]. It is thus an advantage if the required UV dose for the destruction of certain amount of microcystin is within the disinfection UV dose. In this part of project, we aim at (i) studying the degradation of MC-LR by UV-254 nm/H$_2$O$_2$, (ii) evaluating the effects of UV dose on the degradation, (iii) investigating the effect of selected important
water quality parameters, and (iv) using real water samples as background solutions to investigate the behavior of the toxins.


Studies have been carried out in a laboratory scale collimated beam system with low pressure germicidal lamps (254 nm) in the presence of hydrogen peroxide. Chemical actinometry methods and radiometer method were used to determine the UV-254 nm fluence. It was found that a 93.9% removal of MC-LR with an initial concentration of 1 mg/L was achieved with a UV dose of 80 mJ/cm² and an initial H₂O₂ concentration of 30 mg/L. The degradation of MC-LR increased with an increase in the UV dose, following a UV-dose-based pseudo-first-order kinetics. Reaction solutions with different pH values were prepared in phosphate buffer. It was suggested that the impact of pH was mild when it was neutral or slightly basic, which means, in natural waters, the required background pH condition could not be as limiting effect as compared to some other technologies, such as the Fenton reagent. The oxidant, hydrogen peroxide, played an important role in the process. At low concentrations, there was a linear relationship between the pseudo-first-order reaction rate constant with the concentration of H₂O₂. After it reached a “threshold” concentration, its scavenger effects became more obvious. The results show that the % removal rate decrease with increase in initial concentration of MC-LR but the initial degradation rate was increasing as the initial MC-LR concentration increased. Alkalinity affected significantly the degradation rates of MC-LR, especially with carbonate alkalinity. Fortunately the pH in real waters is generally neutral in which the alkalinity is mainly bicarbonate alkalinity. When samples with natural organic matter (NOM) and real water samples spiked with MC-LR were used to compared and verify the results, it was clearly shown that the degradation of MC-LR decreased significantly. The impact factors could be the presence of NOM and alkalinity. A manuscript on the results is submitted to Water Research (Note appendix C for publication details).


In the same system, the degradation of the mixtures of microcystins was also investigated by UV/H₂O₂. We found the degradation of the cyanotoxins studied were all a function of UV fluence in both UV direct photolysis system and UV/ H₂O₂ advanced oxidation system, following UV fluence-based pseudo-first-order reaction kinetics, no matter when it was individual cyanotoxin or the toxin mixtures. The addition of H₂O₂ increased
significantly the destruction of the microcystins. The observed behavior of different microcystins towards UV/H$_2$O$_2$ was similar. It was also found that the removal of total microcystins decreased when the initial pH values increased and MC-LR was more vulnerable to the change of pH values than MC-RR. CYN is another category of cyanotoxins. UV-254 nm irradiation had nearly no effect on the degradation of CYN with the UV fluence used here. It seemed to be influenced much more than microcystins by the presence of alkalinity and natural organic matters when tap water was used as background matrix, reducing the removal at 80 mJ/cm$^2$ of UV fluence from 83.7% to 11.2%. A conference proceeding on this result was accepted by the 20th IOA World Congress – 6th IUVA World Congress, May 23-27, 2011, Paris, France (Note appendix D for publication details).

References:
2. Publication citations

I. Journal Articles


II. Proceedings


(2) He, Xuexiang, Armah A. de la Cruz and Dionysios D. Dionysiou, Destruction of cyanotoxins by UV/H$_2$O$_2$ advanced oxidation processes, *Conference proceeding at the 20th IOA World Congress – 6th IUVA World Congress*, May 23-27, 2011, Paris, France.

III. Presentations


(3) Pelaez, Miguel *, Armah A. de la Cruz and Dionysios D. Dionysiou, Nanostructured Non-metal Doped TiO$_2$ for the Degradation of Microcystin-LR under Visible Light. Poster Presentation at the 2$^{nd}$ International Conference from Nanoparticles and Nanomaterials to Nanodevices and Nanosystems (IC4N-2), June 28-July 3, 2009, Rhodes, Greece.


(6) He, Xuexiang, Miguel Pelaez* and Dionysios D. Dionysiou, Degradation of Microcystin-LR by UV/H$_2$O$_2$ Advanced Oxidation Process. Poster Presentation at the 239$^{th}$ American Chemical Society (ACS) National Meeting, Division of Environmental Chemistry, Session on General Posters, March 21-25, 2010, San Francisco, California.


(8) Pelaez, Miguel *, Erick R. Bandala, Jordana Castillo, Patrick S. M. Dunlop, Anthony Byrne and Dionysios D. Dionysiou, NF-co-doped TiO$_2$ for Visible/Solar Treatment and Disinfection of Water Including Applications in Developing


(11) He, Xuexiang, Miguel Pelaez, Christopher D. Williams, Judy A. Westrick, Kevin E. O’Shea, Anastasia Hiskia, Theodoros Triantis, Armah A. de la Cruz and Dionysios D. Dionysiou*. Oral Presentation at the 16$^{th}$ International Conference on Advanced Oxidation Technologies for Treatment of Water, Air and Soil (AOTs-16), November 15-18, 2010, San Diego, California.


3. Students supported by the project

(1) Miguel Pelaez  
Ph.D. student, Environmental Engineering;

(2) Xuexiang He  
Ph.D. student, Environmental Engineering.

4. Brief description of notable awards or achievements resulting from the project

(2) 2010 Graduate Student Award in Environmental Chemistry, Division of Environmental Chemistry, American Chemical Society (to Miguel Pelaez).
(3) 2011 David Eye Scholarship to Xuexiang He. University of Cincinnati.

5. Appendix List.


**Appendix B**-accepted proceeding. TiO$_2$-based Enhanced Photocatalytic Degradation and Disinfection of Water Under Solar Light Irradiation

**Appendix C**-submitted manuscript. Efficient Removal of Microcystin-LR by UV-C/H$_2$O$_2$ in Synthetic and Natural Water Samples.

**Appendix D**-accepted proceeding. Destruction of cyanotoxins by UV/H$_2$O$_2$ advanced oxidation processes.
Exploring Spatial and Temporal Demand Aggregation on Transport Characteristics in Distribution System Modeling

Basic Information

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Publications

Title: Exploring spatial and temporal demand aggregation on transport characteristics in distribution water modeling

Performed by: Xueyao Yang (MS Student) and Dominic L. Boccelli, Ph.D., University of Cincinnati

Problem and Research Objectives

Drinking water distribution system network models are being increasingly used in applications where understanding the travel path and residence times are important (e.g., contaminant warning system and regulatory sampling design), which require accurate representation of water demands. However, as utilities develop more detailed network models, current assumptions associated with modeling water demands may not adequately reflect the stochastic (random) pattern of water uses typical of water customers (think about the patterns of personal water use) that may impact both travel path and residence time characteristics. The objective of this research was to analyze the potential impacts that temporal averaging of the simulated demands – a value commonly assumed to be 1-hr – had on the underlying hydraulic, transport, and water quality simulations. Dr. Boccelli’s research group utilized a stochastic process model to generate multiple series of random water demands at three different levels of temporal demand aggregation (e.g., 1 hour, 10-minute, and 1-minute). These demands were used to simulate both the hydraulics and water quality associated with a small- and large-scale distribution system. For both network scales, the temporal aggregation scale had little impact on system pressure, yet resulted in areas with increased flow rate variability and directional changes that impacted both travel path and hydraulic residence time. With respect to water quality simulations – performed by assuming a "conservative chemical intrusion" event – increases in demand variability (due to decreasing temporal aggregation) and network scale, and decreasing pipe sizes (correlated with localized water demand) generally resulted in larger impacts on the initial arrival time and travel path (due, in part, to directional changes of the flow) of the chemical compound. In particular, areas that were located between two hydraulic zones appeared more significantly impacted by shifting the area in which the water was blended. An additional risk analysis was performed that suggested an increase in demand variability could impact the potential health risks from exposure to a chemical intrusion event. The results from this study will provide valuable information for understanding current modeling limitations and for improving existing modeling techniques to enhance the industry's ability to investigate multiple water quality applications with increased confidence.

Methodology

The study will be performed by integrating a common distribution system network hydraulic and water quality solver (EPANET) with a computational framework capable of representing stochastic demand behavior (PRPsym). Two different size network models will be utilized to evaluate the impacts of spatial and temporal aggregation of demand on the underlying hydraulic and transport characteristics. Demands will be temporally aggregated at 1-min, 10-min, and 1-
hour time intervals, and spatially aggregated through the use of commercially available algorithms capable of reducing the network model size and redistributing demands. Monte Carlo simulation will be utilized to generate multiple realizations of stochastic demands and used to simulate both the hydraulics and water quality aspects associated with the two models.

The analysis of the hydraulic data will be focused on exploring the impacts of the different scales of demand aggregation on flow rate variability. This information will ultimately be linked with water quality metrics to determine if there is a specific level of underlying flow variability that has a deleterious impact on water quality variability. The water quality analysis will first be focused on evaluating the impact of demand aggregation on the simulated hydraulic residence time, which can be used as a surrogate for water quality. Additional water quality simulations will be performed to assess the potential variability associated with exposure to 1) disinfectant by-products, which are ubiquitous in distribution systems, and 2) a short-duration contamination event, which would be more susceptible to hydraulic uncertainty. All of these simulations will be utilized to understand the potential impacts of various spatial and temporal aggregation scales on different distribution system network modeling objectives. These results will be compiled into a guidance document for the industry, and result in guidance for future research efforts aimed at improving distribution system network modeling.

Principal Findings and Significance

The first part of the research was focused on evaluating the impacts that temporal aggregation of consumptive demands had on pressure and flow rate variability. The results from the hydraulic analysis are qualitatively the same of both the small and large networks. With respect to pressure, decreasing the temporal aggregation from 1-hour to 1-minute time steps had little impact on pressure variability. However, decreasing the temporal aggregation generally increased the flow rate variability with the relative differences being more important as the average flow rate decreased. The decrease in relative demand variability at higher flow rates is expected, by the law of large numbers, as the larger flow rates are typically dependent on many more downstream demands that ultimately average out towards the expected values (i.e., similar to the deterministic case). Also, as the temporal time scale decreases, the probability of a node having no "arrivals" (i.e., no water usage) increases. Thus, between increased variability at the hydraulic edges of the system and an increased probability of no demands, which could increase stagnant flow conditions, we hypothesize that for short-duration water quality events (e.g., (un)intentional intrusion) the decreasing temporal aggregation will increase water quality variability. The increase in water quality variability can impact the use of drinking water distribution system models to perform, for example, system vulnerability and risk assessment.

To evaluate the impacts that temporal aggregation of consumptive demands had on water quality simulations, a conservative tracer signal was simulated by performing a 2-hr or 3-hr "injection" for the small and large network model, respectively. The small model was a more highly skeletonized model (i.e., less spatial detail) and, as such, did not have many nodes with zero consumptive demands (stagnant conditions) and had very few interconnections to impact transport. However, even with such a model, decreasing the temporal scale of the consumptive demand modeling: 1) increased the water quality variability throughout the system; 2) impacted the hydraulic residence times and travel paths (relative to a "deterministic" model); and 3) illustrated that the temporal scale could alter exposure assessments in distribution system analysis.
Similar behaviors were observed for the larger, all-pipes network model. To further study the potential impacts of temporal demand aggregation on hydraulic residence time and transport path, additional analyses on the large-scale network model were performed to investigate: 1) the time to first arrival of the conservative signal at a node, 2) the time required for half of the total mass to reach a node, and 3) the total mass consumed by an individual at a node by using the assumptions of Murray et al (2005). In general, the research team has observed the following results with respect to decreasing temporal aggregation scale: 1) increased time to first arrival and 2) increased time to reach the "half-max" concentration [the exposure assessments are still being performed]. The preliminary analyses of these results suggest that, in general, incorporating demand variability has a greater impact on the metrics of interest than the specific temporal aggregation scale. Additional analysis will be performed to further explore the significance of general demand variability versus the temporal aggregation scale. Regardless of the outcomes, the results will be an important first-step in characterizing the types of variability important within drinking water distribution system modeling.

Publications


Students

Xueyao Yang (MS in Environmental Engineering) – currently at pursuing a Ph.D. at the University of Cincinnati (Cincinnati, OH)

Awards

X. Yang received Best Student Paper Award, and was nominated for Best Paper Award -- top 10 out of approximately 150 papers at the 2010 Water Distribution System Analysis Symposium
MONITORING THE ROLE OF BIOFILM BIOPOLYMERS AGAINST DISINFECTANTS IN WATER DISTRIBUTION SYSTEMS

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<td>Youngwoo Seo</td>
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Publications

1. Z. Xue, Y. Seo, W. Panmanee, and D. J. Hassett, Impact of the Pseudomonas aeruginosa Exopolysaccharide Alginate on Bacterial Inactivation Kinetics by Model Disinfectants, Applied Environmental Microbiology (In Review).

2. Z. Xue, Y. Seo, Susceptibility of biofilm to disinfectants in the presence of disinfectant-demanding substrate, 240th American chemical society national meeting, Boston, USA, August, 2010 (accepted for oral presentation).


8. Xue Zheng and Youngwoo Seo, 2010, Susceptibility of biofilm to disinfectants in the presence of disinfectant-demanding substrate, in 240th American chemical society national Meeting, Boston, MI.

Monitoring the Role of Biofilm Biopolymers against Disinfectants in Water Distribution Systems
Youngwoo (Young) Seo

1. PROBLEM AND RESEARCH OBJECTIVES

Biofilm formations in water distribution systems are ubiquitous. Reports from many water utilities in the US including utilities in Ohio have shown that biofilms survive in water distribution systems despite the continuing presence of disinfectants (Tuovinen and Hsu, 1982; LeChevallier et al. 1996) is great concern about the resistance of biofilms against disinfectants, the inactivation kinetics of biofilms are not well understood, especially compared to the inactivation kinetics of suspended microbial cultures (AWWA, 2007). There is not enough information for water utilities to assess and optimize disinfectant dosage to control biofilms in water distribution systems (AWWA, 2005).

One reason for this could be the complexity of biofilm EPS (Momba et al. 2000; Stewart, 2002; Stewart et al 2002). More than 80% of biofilm is comprised of EPS (Characklis and Marshall, 1990), and it is believed that these structures provide protective barriers for microorganisms (Sibille 1998; Hughes et al. 1998). However, there is still a significant knowledge gap, especially concerning the reaction kinetics of EPS with disinfectants. To date, the role of extracellular polymeric substances (EPS) as a protective barrier against disinfectants has not been quantitatively analyzed, even though the simultaneous interaction between disinfectants and EPS is known to lead to the transport limitation of disinfectants into biofilms. Previous studies mostly focused on retarded or limited transport of disinfectants without considering reactive sites and reaction kinetics of biofilm EPS (Stewart, 2002; Stewart et al. 2002).

The principal research objective in this proposal is to monitor the role of both cell-bond EPS and biofilm EPS as protective barriers against disinfectants in water distribution systems. Physical transports of a model disinfectant and its reaction kinetics in biofilm were quantitatively studied using molecular probes and a chlorine sensitive microsensor. The reaction and disinfection kinetics of EPS was elucidated by 1) characterizing EPS components and their reaction kinetics with a model disinfectant; 2) quantifying EPS and viability of biofilm with fluorescently labeled molecular probes under a model disinfectant; 3) monitoring the transport limitation of a model disinfectant in biofilm. This study provides fundamental and effective biofilm control strategies in water distribution systems supporting research, education and local water utilities.

2. STATEMENT OF RESULTS OR BENEFITS

Water utilities in Ohio have experienced bacteria growth and biofilm formation in water distribution systems (Tuovinen and Hsu, 1982; Craun and Calderon, 2001), even under the presence of residual chlorine. However, the inactivation of biofilm in
drinking water distribution system is not well understood. One reason for our lack of understanding is strongly correlated to the complexity of biofilm EPS and their role in protecting biofilms from disinfectants. In this proposed study, this knowledge gap will be addressed. The results from the proposed study will enable local water utilities to incorporate biofilm control strategies since the results will aid in the development of effective biofilm control methods with disinfectants.

3. MATERIALS/METHODOLOGY

Preparation of Buffer Solutions and Disinfectants

All disinfection experiments were conducted with chlorine demand-free (CDF) buffer (pH=7). CDF buffer was prepared by dissolving 0.54 g of Na₂HPO₄ and 0.88 g of KH₂PO₄ per liter in deionized water. The prepared buffer solution was pre-reacted with chlorine by adding sodium hypochlorite solution and allowed to stand at room temperature for one week, followed by UV light exposure for 48 hours to achieve dechlorination. When the chlorine concentration was lower than 0.01 mg/l, the buffer solution was considered to be chlorine demand free (Engelbrecht et al., 1980).

Chlorine stock solutions were prepared with Clorox bleach (The Clorox Co., Oakland, CA) and concentration were determined by the N, N-diethyl-p-phenylenediamine (PDP) method (Engelbrecht et al., 1980). The chlorine stock solution was diluted to 0.5 mg/l with CDF buffer solution immediately preceding the inactivation experiments. Stock chloramine solution was prepared immediately before each experiment by combining solutions of sodium hypochlorite and ammonium chloride in a 4:1 ratio (chlorine-to-ammonia-nitrogen mass ratio). To obtain the highest monochloramine yield and minimize ammonia volatilization, both solutions were pre-adjusted to a pH of 8.3. Stock chloramine solutions were diluted to a target concentration of 2 mg/l with the CDF buffer. Stock chlorine dioxide solutions were prepared from sodium chlorite (NaClO₂, Selective Micro Technologies, Beverly, MA, USA) (Jang et al., 2006). For inactivation tests, the ClO₂ stock solution was diluted to 0.5 mg/l before each test (Aieta et al., 1986). The concentration of all three disinfectants was selected based on residual disinfectant concentration in water distribution systems (USEPA, 1999) and measured using a DR/2700 spectrophotometer (HACH Company, Loveland, CO, USA).

Batch Experiments of planktonic cells

In this study, three *P. aeruginosa* strains were employed. The first was wild-type strain PAO1 and a well-characterized DNA sequenced strain. Two isogenic mutants of strain PAO1 were also used, (i) *algT(U)* encoding the alternative extracytoplasmic sigma factor AlgT(U) and (ii), *mucA*, encoding a cytoplasmic membrane-bound anti-sigma factor that produces copious quantities of the exopolysaccharide alginate. With these strains in hand, we examined how differences in the relative amount of EPS affected the efficacy of three common disinfectants (chlorine, chloramines, and
chlorine dioxide).

All batch experiments were performed in 250 ml amber glass bottles (Fisher Scientific, Itasca, IL) at room temperature for planktonic cells. Three amber-glass bottles were used as parallel reactors. The first bottle, containing bacterial suspension and the CDF buffer solution without disinfectant, served as a control reactor. The other two bottles contained only bacterial suspension and disinfectant solution. Experiment setup is shown in Fig 1. Microbial inactivation tests with disinfectants were performed and disinfectant decay and bacteria survival were measured simultaneously. Enumerations of viable microbial cells were performed using the heterotrophic plate count method. Serial dilutions were conducted in CDF buffer solution containing Na2S2O3 (1 mmol/l final concentration) to quench residual disinfectants, followed by spreading 0.1 ml aliquots onto R2A media plates (Difco Laboratories, Detroit, MI). All plates were incubated at 37°C for 24 hours prior to enumeration of colony forming units (CFU).

**Figure 1: Batch experiment setup**

**Biofilm experiment**

Two carboys were used as medium feeding and chlorine supply reservoirs respectively. A 0.02 strength LB broth was used as a medium to create nutrient limiting growth conditions mimicking low-carbon environment as in drinking water distribution systems. All feeds to reactors were delivered using a multichannel peristaltic pump (ISMATEC, Glattbrugg, Switzerland) and silicone tubing (Masterflex, Vernon Hills, IL). The flow cell system is shown in Fig. 2. Flow cells, tubing and solutions were sterilized at the start of each experiment. Operation and sampling of the flow cells followed aseptic technique throughout the experiments.
Biofilms were grown in continuous-culture flow cells (channel dimensions, 1.6 by 12.7 by 47.5 mm; flow rate, 0.2 ml/min) at room temperature. The flow cell contained a standard glass microscope slide on one side and a glass cover slip on the other side. Flow rate of the flow cells simulated laminar flow with an average flow velocity of 0.16 mm/s throughout each experiment. Under this flow condition, a residence time that improved biofilm formation was achieved. Channels were inoculated with bacterial suspension and incubated statically for 1 h at room temperature for initial bacterial attachment. After 1 hour, flow rate was gradually increased to 0.2 ml/min. For each experiment, the two channels in one flow cell were operated in parallel under identical conditions, the only exception being that one channel received chlorine and the other served as a non-chlorinated control.

**Bacteria Cell Staining**

The LIVE/DEAD Bacterial Viability Kit (BacLight, InVitrogen) was applied to estimate both viable and total counts of bacteria in disinfectant treated samples. The BacLight LIVE/DEAD stain is composed of two nucleic acid-binding stains: SYTO 9E and propidium iodide (PI). SYTO 9E penetrates all bacterial membranes and stains the cells green, while PI only penetrates cells with damaged membranes, while the combination of the two stains stoichiometrically produces red fluorescing cells. Total (red and green) and viable (green) cells can hence be counted simultaneously (Boulos et al., 1999). Stained solution was filtered through black polycarbonate filters for fluorescence microscopic imaging. Fluorescent images were observed at 480/500 nm for SYTO 9 and 488/617 nm for PI, respectively. For fluorescent stained cell counting, an Olympus fluorescent microscope with an 100X oil immersion objective and a TCS SP5 multi-photon laser scanning confocal microscope (Leica Microsystems) were used. Images were processed by CellCounter (Heracle Software), CellAnalyst (AssaySoft, Inc.) and COMSTAT.

**EPS Extraction**
All extraction procedures were performed on three separate 50 ml samples from an initial 200 ml bacterial culture. The modified EDTA extraction method described by Brown and Lester (1980) was employed (Brown et al., 1980). In this method, 100 ml of 2% EDTA (tetrasodium salt) was added to 100 ml of each culture suspension and shaken for 3 h at 4°C. High speed vortex was applied both at the initial mixing of EDTA and culture suspension and after 3 h interaction. The samples were then centrifuged at 14,000 x g for 20 min at 4°C. The supernatant was analyzed to quantify EPS composition.

**EPS Characterization**

Total protein and polysaccharide was measured using standard colorimetric techniques. Protein concentrations were determined using the modified Lowry Protein Assay Kit (Pierce Biotechnology, Rockford, IL) with bovine serum albumin (BSA) as standard. Polysaccharide concentration was measured using the phenol-sulfuric acid method using glucose as standard (Jost Wingender, 1999).

**Principle Findings and Significance**

Both chlorine and chlorine dioxide are very effective disinfectants. However, these two disinfectants were heavily consumed during the initial period of inactivation. Limited by such short inactivation times and high reactivity, no significant disinfectant residual differences were observed. Chloramine was found to be a slow-acting disinfectant for the three strains. Thirty minutes of chloramine exposure were required to achieve 99% inactivation for the three strains in this study. The \textit{mucA} mutant consumed the greatest amount of chloramine, while the \textit{algT(U)} mutant had comparably high levels of residual chloramine. For chloramine inactivation, disinfectant residual is in inverse proportion to cell bound EPS amount. In other words, the more EPS bound to bacterial cells the more disinfectants were consumed. The result of the chloramine consumption study indicated that cell-bound EPS interacted with disinfectant during the inactivation process and part of the disinfectant consumption could be attributed to cell bound EPS. The transport limitation of disinfectant in biofilm was also monitored by microelectrode.

The results in this study indicate that the higher EPS production yields higher survival ratio and viability rate, which was confirmed by both heterogeneity plate counting and Live/Dead staining results. The \textit{mucA22} mutant with higher EPS production had a proportionally greater survival ratio and viability rate and more variation in protein and polysaccharide functional groups by interaction with disinfectants. The \textit{mucA22} mutant also had a prolonged lag time when interacting with the less reactive disinfectant chloramine.

For low disinfectants concentrations as used in water distribution system, key factor of chlorination bactericidal is not extensive membrane damage but functional
group deformation in bacteria membrane, which lead to membrane permeabilization. The acidic polysaccharide alginate, representative component in EPS of P. aeruginosa, has strong deformation after inactivation, which confirms that cell-bound EPS have high reactivity with the disinfectant used in this study. The combined results support that cell bound EPS consume disinfectant, retard bacterial membrane permeabilization, and thus decrease the susceptibility of bacteria.

REFERENCES


American Water Works Association (2003). Assessment of chlorine and chloramine residual decay in the distribution system, AWWARF, Denver, CO.


A Hydraulic Modeling Framework for Producing Urban Flooding Maps in Zanesville, Ohio

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Publications

2. Lant, Jeremiah, Michael Durand, and Doug Alsdorf, 2009, Hydrodynamic modeling of the Muskingum River near Zanesville, Ohio, paper presented at the Water Management Association of Ohio 2009 meeting, 4-5 November, Columbus, Ohio.
FINAL REPORT TO USGS OHIO WATER RESOURCES CENTER

REPORT PERIOD: 1 APRIL 2010 – 31 MARCH 2011

Grant Title:  A Hydraulic Modeling Framework for Producing Urban Flooding Maps in Zanesville, Ohio

Investigators:  Michael Durand (PI) and Konstantinos Andreadis (Co-PI)

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PROBLEM AND RESEARCH OBJECTIVES

This project examines the flooding dynamics along the Muskingum River near the city of Zanesville, Ohio. Simulating various peak flood events using a hydrodynamic model will provide Muskingum County engineers with valuable information regarding inundated areas, extent, and effect on local communities for different flood events. The impact of various Muskingum River flood events, including the 100 year flood, on the urban environment in Zanesville, Ohio was studied. The project provides a useful hydraulic modeling framework that produces urban flooding maps for the city of Zanesville. These maps show how water surface elevations and water depths vary spatially and temporally, and will provide a more detailed picture of how flood waves move in urban environments. A hydrodynamic model called LISFLOOD-FP is used to simulate river flow and flooding. LISFLOOD-FP is a finite-difference flood inundation model that can accurately model 1D channel flow along with 2D floodplain flow. LISFLOOD-FP is a well-established hydrodynamic model that has been proven to properly simulate flood inundation for fluvial, coastal, and urban events.

The Federal Emergency Management Agency, FEMA, conducts flood insurance studies to identify a community’s flood risk. The flood risk study is based upon statistical data for river flow, storm tides, hydrologic and hydraulic analyses, and rainfall and topographic surveys. The FEMA maps only provide a one-time snapshot of a flood, and do not describe the full extent of the flood event including the spatial and temporal variability of various flood events. Questions, such as the changes in flood inundation extent with time for the city of Zanesville, cannot be fully explored using the FEMA maps. It has been shown that accurate mapping of urban flooding events must take hydraulic connectivity and mass conservation into account. In other words, extending potential flood elevations along lines of equal elevation given a river elevation, the so-called “Planar GIS method,” may be inadequate for characterizing urban flooding. An
alternative approach involves the simulation of hydraulic processes, which would control flooding and inundation patterns in downtown Zanesville given the FEMA 100 year Muskingum River main stem water surface elevation. Such an approach provides the framework, not only for producing dynamic maps of different frequency flood events for the city of Zanesville, but also evaluating the impacts of adding and/or removing structures or changing land use on urban flooding.

PERSONNEL
This work was done by Mr. Jeremiah Lant, and makes up his Master of Science thesis under Prof. Doug Alsdorf in the Geodetic Science division of the School of Earth Sciences. Mr. Lant defended his M.S. thesis on 24 May, 2011, and is currently finalizing the thesis document to be filed with the University.

METHODOLOGY
The objective of this research project was to create an urban flood study using a 2D hydrodynamic model, LISFLOOD-FP, for the city of Zanesville, Ohio. Flood inundation on a floodplain is controlled by the overlying topography and friction. Such flow is spatially complex, especially in the urban environment, with varying patterns of water velocity and depth that are two dimensional in space and dynamic in time. The creation of flood maps of water surface elevation and depth that provide a dynamic picture of flood inundation in the urban environment require a two dimensional hydrodynamic model. The LISFLOOD-FP hydrodynamic model is a two dimensional storage cell hydrodynamic model based on a finite difference scheme that can accurately simulate floodplain inundation in urban environments. The purpose of the LISFLOOD-FP code is to help improve understanding of flood hydraulics, flood inundation prediction, and flood risk assessment.

A 5 meter DEM derived from a 2.5 foot LIDAR dataset from the Ohio Geographically Referenced Information Program, OGRIP, was used. For an urban flooding scenario, building heights for the city of Zanesville, acquired from the Muskingum County engineers’ office, were added onto the high resolution bare earth DEM using the same techniques to change the channel cell values. Muskingum River cross-sections are from 1934 Army Corps of Engineer maps. These maps were acquired from the Ohio Department of Natural Resources. Average bed elevations at each data cross-section were computed within the river channels. From the Federal Insurance Study (FIS) conducted for the city of Zanesville, FEMA defined the 100 year flood discharge along the Muskingum River near the city of Zanesville, Ohio to be 68,000 cubic feet per second, and the 100 year flood discharge along the Licking River below the Dillon Dam to be 7,200 cubic feet per second. To show the spatial and temporal evolution of a possible 100 year flood for Zanesville, hydrographs from the USGS for the Muskingum River and Licking River with peaks nearest the FEMA defined 100 year flood discharge were found and used. These hydrographs consisted of a twelve day flood discharge. Each hydrograph was linearly scaled to match the respective FEMA peak.
PRINCIPAL FINDINGS AND SIGNIFICANCE

The study produced a model framework that yields dynamic urban flood maps of Zanesville. The framework was built around simulating a FEMA-defined 100 year flood. Modeling efforts demonstrate similar flood profiles and water surface elevations when compared to FEMA (Figure 1). The flood maps show how a 100 year flood wave evolves over time.

A comparison study was made between 1D HEC-RAS and 2D LISFLOOD-FP models. Results have shown that both models produce comparable water surface elevations within the river channel and on the floodplain (Figure 2). With the model framework built, simulations of other flood events can be completed. Since the heart of Zanesville is fairly protected from the 100 year flood, an investigation into the amount of discharge needed to reach downtown Zanesville was conducted. It was found that a massive flood wave, over 100,000 cfs, might be needed to inundate the entire downtown area of Zanesville (Figure 3). A flowrate of this magnitude is approximately 50% greater than the 100 year flood utilized in the FEMA study. Although highly improbable, the dynamic mapping of this flood event provides a deeper understanding of how flood waters could move in the urban environment. A flood event case with data from USGS Streamstats was completed to analyze how a flood might impact Zanesville without the influence of any flood control structures. Without such flood control structures, Zanesville would be seriously inundated if a 100 year flood occurred.

The model framework built in this study allows the sensitivity of climate change and urbanization on the FEMA 100 year water surface elevations and extent to be analyzed. A follow-on study could, ideally, use this framework to further explore changing flooding patterns in an urban environment due to dam sedimentation, land use and land cover change, climate change, and urbanization. This study of urban flooding on the Muskingum River also represents an opportunity to more fully understand the performance of the upcoming Surface Water Ocean Topography Mission, SWOT (http://swot.jpl.nasa.gov), over modest sized rivers in an urban environment. The SWOT satellite will have the capability of measuring temporal and spatial changes in water surface elevations and inundated areas for fresh water bodies around the world. From these measurements, depth and discharge along much of the Muskingum River can be extracted and used in hydrodynamic models like LISFLOOD-FP. Better knowledge of discharge on the Muskingum River will provide a valuable insight into how floods travel through the floodplain and affect the urban environment.

CONCLUSION

Mr. Lant has completed the construction of the hydraulic framework for testing flooding scenarios in Zanesville. Several example scenarios were presented here. The work formed the bulk of Mr. Lant’s M.S. thesis, and was presented at four conferences, including ASCE and AWRA. We plan to submit a full-length journal manuscript on this work in the upcoming months.
Figure 1. LISFLOOD-FP flood extent shows good agreement with FEMA 100 year flood extent (red). Water surface elevation values are in meters.

Figure 2. Comparison of water surface profiles.
Figure 3. Water depth map of increasing FEMA flood peak by 50%. Water depths are in meters. The city of Zanesville is inundated with water depths ranging from 0.1 meters to 1.5 meters.

MASTER OF SCIENCE THESIS

CONFERENCE PRESENTATIONS
Lant, Jeremiah, Michael Durand, and Doug Alsdorf, 2009, Hydrodynamic modeling of the Muskingum River near Zanesville, Ohio, paper presented at the Water Management Association of Ohio 2009 meeting, 4-5 November, Columbus, Ohio.


Nitrogen Removal by Microbial-Mediated Processes Under Hypoxic Conditions in Lake Erie

Basic Information

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<td>Principal Investigators:</td>
<td>Xiaozhen Mou, Darren Bade, Robert Heath, Laura Leff</td>
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Publications

1. Lu, Xinxin, (in progress), The Relative Importance of Denitrification and Anammox in Freshwater Lakes and Coastal Marine Environments, Ph.D. Department of Biological Sciences, Kent State University, Kent OH
NITROGEN REMOVAL BY MICROBIAL-MEDIATED PROCESSES UNDER HYPOXIC CONDITIONS IN LAKE ERIE

Xiaozhen Mou, Darren Bade, Laura Leff and Robert Heath

Department of Biological Sciences, Kent State University, Kent OH 44242

1 Problem and Research Objectives

Lake Erie is facing a critical problem: eutrophication is no longer effectively controlled by solely limiting P loading. In the past, it was shown that P availability limited algal growth, particularly blooms of cyanobacteria (blue-green algae) in Lake Erie. This was evident with the low N: P ratio in lake waters and diminished phytoplankton blooms following declines in P-loading mandated by the Great Lakes Water Quality Agreement (GLWQA 1978, 1987). However, large scale phytoplankton blooms have recurred in Lake Erie in recent years with increasing frequency and extent. Given that P-centered management is still enforced and local P-loading has not significantly changed in Lake Erie (Dolan and McGunagle, 2005) (USEPA Great Lakes Monitoring, http://www.epa.gov/glmpo/monitoring/limnology), the unexpected return of undesirable phytoplankton blooms clearly indicates that the nutrient status of Lake Erie may no longer be solely P-limited. The cause of this shift is not clear, but it is clear that to control eutrophication, new management strategies must be developed. Phytoplankton of Lake Erie being co-limited by both P and N have been repeatedly reported or implicated (Wilhelm et al., 2003) (Guildford et al., 2005) (Hill et al., 2006) (Moon and Carrick, 2007) (North et al., 2007). The indicated limitation of N to phytoplankton may be attributed to an unexpected decrease of N in Lake Erie. The N concentration in Lake Erie has dropped from 0.26 mg/L in 2005 to 0.18 mg/L in 2008 (USEPA Great Lakes Monitoring, http://www.epa.gov/glmpo/monitoring/limnology). This is exceptional to a generally increased pattern observed in the other four Great Lakes and is extremely surprising. These multiple lines of evidence suggesting N becoming co-imitated with P, clearly indicate that N is actively removed through internal processes within Lake Erie ecosystems.

Two processes leading to N removal are currently known: denitrification and anaerobic ammonium oxidation (anammox). Both reactions convert reactive nitrogen species to inert dinitrogen gas (N₂), which is only available to nitrogen fixers. Anammox and denitrification are performed exclusively under anaerobic conditions by microorganisms. Suitable low-oxygen conditions are commonly found in Lake Erie, especially in the central and western basins, with increasing frequency, duration and extent (Edwards et al., 2005). Anammox and denitrification, however, follow distinct reaction routes to produce N₂, meaning that factors that influence their importance and rates are undoubtedly different. Despite the potential importance to ecosystem function,
knowledge on denitrification and anammox activity and bacteria in Lake Erie or the Great Lakes in general is extremely limited.

Our proposal aims to study denitrification and anammox, the two processes that may lead to the lost of fixed nitrogen in Lake Erie. Effective management of Lake Erie and the other Great Lakes requires knowledge on the nutrient status and its consequences. This project responds to these two needs. Specifically, we will provide data to advance our understanding of the current nutrient status, which has apparently shifted from historic P limitation to more P and N co-limitation. We evaluated whether or not microbially-mediated N loss could drive this shift and identify responsive microorganisms. In addition to the growth and structure of phytoplankton community, N dynamics affect the formation and diminishment of hypoxia through close interactions between anammox, nitrification and denitrification. Therefore, acquired data from this project would also aid management decisions to address hypoxia in Lake Erie water. Research on freshwater anammox is limited and virtually absent in Lake Erie. This project attempted to fill this gap and expand our understanding on N dynamics in the Great Lakes and other freshwater systems.

2 Methodology

Sample Collection and Processing.

Samples were taken from three sites in Lake Erie along a transect from Sandusky bay to the central basin on Sept 13th, 2010 (Figure 1), one in the Sandusky Bay (SB), one in the Sandusky Sub-Basin (SS) and one in the Central Basin (CB). At each site, three individual samples were collected each from the sediment and its overlaying water. Sediment samples were collected using a sediment grab sampler and subsurface layer (2-4 cm) were collected into whirl-pak bags, obvious air was squeezed out when seal the bags. Sediment overlaying water samples were collected by direct pumping water using a peristaltic pump. Samples were further processed differently according based on their use.

For nutrient analysis, one liter of water was filtered through GF/F filter and collected in autoclaved media bottles before immediately stored on ice or at 4°C. For bacterial cell counting, 1.8 ml whole water was mixed with 0.2 ml freshly made paraformaldehyde (final concentration 2% wt/vol). For DNA-based molecular analyses, 500 ml of whole water was filtered through 0.2 µm-pore-size membrane filters. Cells collected on the filters were transferred into a 15 ml sterilized Eppendorff tube and stored on ice. For ^15N-incubation analysis, 250 ml of whole water was collected in acid-washed BOD glass bottles with 2- to 3-fold overflow. When capping the glass bottles, care was taken to avoid head space or bubbles in the glass bottles.

All samples were stored on ice on site and transported (2-hr) back to the lab and processed immediately.

Anammox and denitrification potential measurements.
15N tracer incubation analysis was performed to measure anammox and
denitrification potentials, following a procedure developed previously (Kuypers et al.,
2005) with minor modifications. Briefly, 250 ml of whole water samples were mixed
individually with three isotopic 5 µmol of Na15NO3, 2.5 mol of 15NH4Cl or 5 µmol
Na15NO3 and 2.5 mol of 14NH4Cl. The amended water was used to fill up a set of
exetainer tubes and flushed for 15 min with helium. One ml of water was taken from the
tubes at the end of the flush and replaced with 1 ml of helium. All tubes were incubated
in the dark at in situ temperature for a total 7 days with the sampling time at 0, 48 h and
7 days of incubation. At each sampling time, 9 exetainer tubes (3 each from the three
kinds of amendments) were sacrificed by replacing 1 ml of water with 1 ml of helium gas
followed by ZnCl2 treatment to stop the total biological activity. Sacrificed sampled tubes
were stored at 4°C until headspace gas analysis. Sediment samples were made to
slurry by mixing with the same volume of sediment overlying water and then processed
the same as the water samples. Production of 15N15N from denitrification and 15N14N
from anammox in the head space was measured by by gas chromatography isotope
ratio mass spectrum (GC-IR-MS) at the UC Davis Stable Isotope Facility.

DNA extraction and gene analyses.

DNA was extracted from sediment and water samples using MoBio PowerSoil
DNA extraction kits (MoBio Laboratory Inc., Carlsbad CA). Anammox-specific 16S rRNA
genes were amplified using a sequential PCR protocol described previously (Tal et al.,
2005). Briefly, Planctomycetales-specific 16S rRNA genes were amplified from
extracted DNA using Pla46 forward primer (Neef et al., 1998) and universal bacterial
reverse primer (Delong et al., 1989) (Britschgi and Giovannoni, 1991). The PCR
amplicons then served as templates for the second anammox specific PCR using Pla46
forward and Amx1240 reverse primers (Britschgi and Giovannoni, 1991). Anammox-
specific 16S rRNA genes were also amplified using a second set of primers
Brod541F/1260R (Junier et al., 2010).

Gene amplicons were used to construct clone libraries. Clones with inserts of
correct PCR amplicons were sequenced for phylogenetic identification. General primers
for 16S rRNA genes were also amplified by Fam-labeled 27F and1522R primers
(Delong et al., 1989) and subjected to terminal restriction length polymorphism (T-
RFLP).

Denitrifying-specific nosZ genes were quantitatively PCR amplified using a
primer set described previously (Scala and Kerkhof, 1998). To access the diversity of
denitrifying bacteria, nosZ genes were also subjected to quantitative PCR analysis
(qPCR).

Clone library sequencing and T-RFLP analyses were outsourced to the Plant-
Microbe Genetics Facility at the Ohio State University.

Nutrient analysis
To describe the physical, chemical and biological properties of the abiotic factors at the time of sampling, a series of variables were measured following standard methods. Temperature, dissolved oxygen concentration, and conductivity were measured using a Hydrolab H2O multi-datasonde at the time of sampling. Concentrations of N compounds were measured following standard flow injection protocols (APHA, 1999). Nitrate/nitrite concentrations were determined by the cadmium reduction method using Lachat 8000 QuikChem Analyzer and measured directly using an Ion Chromatography Dionex system. NH4+ was measured fluorometrically (Holmes et al., 1999). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were measured using a Shimadzu TOC/TN analyzer by combustion oxidation/infrared detection and combustion chemilluminescence detection methods, respectively. Soluble reactive phosphorus (SRP) was determined following the standard colorimetric molybdenum blue method using flow injection protocols (APHA, 1999). Bacterial abundance was measured by flow cytometric analysis using 1µm-diameter yellow-green beads as the internal standard.

**Statistical Analysis**

A suite of statistic analyses were performed to identify which biotic and/or abiotic factors may affect N2 production using a software package that has been specifically designed for community ecology and environmental science (Primer v5, Plymouth Marine Laboratory, Plymouth, United Kingdom). A similarity matrix of bacterial 16S rRNA gene T-RFLP data was calculated based on Bray-Curtis similarity. Nonmetric multiple displacement analysis (MDS) of T-RFLP pattern was performed within the Primer 5 software package.

3 Principle Findings and Significance
The bottom water at all three sampling sites were well oxygenated, although DO concentration was the lowest at CB (Figure 2). The depth profiles of temperature and dissolved oxygen (DO) also indicated that the water in SB and CB were well mixed, while the water at SS had slight stratification.

Bacterial cell number was the highest in the SB site, which was in accordance with its high organic and inorganic nutrient supply. Bacterial community structure of the sediment and sediment overlaying water samples at the three sites were measured based on 16S rRNA genes using a community fingerprinting method, namely T-RFLP. MDS analysis of T-RFLP data grouped all sediment samples together and away for those of the sediment overlaying water samples, indicating distinct bacterial community structures between these sample types (Figure 3). Sediment samples had more T-RF than the water samples, indicating a higher diversity. The distribution pattern also indicated that sediment overlaying water samples from the three sites were composted differently. The taxa associated with T-RFs of 222 bp and 290 bp accounted for over 27% and 15% for sediment overlaying water at the SS and CB sites, respectively, but

Table 1. Average measurements of bacterial cell abundance and nutrient supplies in the sediment overlaying water samples. Standard deviation for each parameter measured was less than 10% of the average values.

<table>
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<th>Sample</th>
<th>Bact. Abun. (x10^6 cells/ml)</th>
<th>Nitrate (µmol/L)</th>
<th>Ammonium (µmol/L)</th>
<th>TOC (mg/L)</th>
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<td>CB-BW</td>
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<td>18.14</td>
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<td>2.47</td>
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Table 2. Anammox-specific 16S rRNA genes analysis.

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<td></td>
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<td>PLA-46F/1037R, nested Amx 368F/820R</td>
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were only 8% and 6% in the SB sites, respectively (Figure 4). Community structures in the sediment samples, on the other hand, were quite similar to each other. Further statistical analyses, such as ANOSIM, were planned to identify the taxa that contributed significantly to the grouping pattern of the T-RFLP data.

The denitrifying and anammox potential were measured for the bottom water and sediment samples using both genetic and biogeochemical methods. Diagnostic genes for denitrification, i.e., nosZ genes, and anammox, i.e., anammox-specific 16S rRNA genes, were analyzed for the sediment and overlaying water samples (Table 2). Denitrifiers have varying functional capability, only those that carry nosZ genes are capable of reduce nitrogen to dinitrogen gas. The nosZ genes were amplified from all sediment and their overlaying water samples. But their copy numbers in the original samples were different. Water samples in general have very low copies of the nosZ genes, which were all significantly lower than their corresponding sediment samples (Figure 5). The process of anammox is less studied than denitrification. Functional genes of anammox have been identified recently in an anammox bacterial strain, however, its ubiquity among anammox bacteria was less clear. Studies have indicated that anammox capability were restricted within a distinct subset of Planctomycetes. Currently, genetic identification of anammox is mainly using 16S rRNA gene primers to target this subset. In this study, anammox 16S rRNA genes were analyzed by direct and nested PCR of primer sets developed previously (REF). Except for the sediment overlaying water samples in SB, anammox 16S rRNA genes were amplified from all the other sediment and overlaying water samples by both or either of the approaches. The gene amplicons were further cloned and prepared for upcoming sequencing analysis.

Dinitrogen production potentials in the sediment overlaying water samples through anammox and denitrification were measured using the $^{15}$N incubation assay (Figure 6). Anammox and denitrification produce N$_2$ gas through distinct pathways. The two atoms of N are both from oxidized N (Nitrate/nitrite), while, for anammox, one atom is nitrate/nitrite and the other is from ammonium. Trace amount of $^{15}$N-nitrate and/or $^{15}$N-ammonium were added to the samples and the production of $^{30}$N through denitrification and $^{29}$N through anammox were measured following different duration of incubation. Consumption of total nitrate, ammonium and DOC were also tracked. Our results showed positive anammox potential in the sediment overlaying water of the SB and CB sites, but not the SS site. At the mean time, denitrification potential was measured in the SS and CB sites, but not the SB site. Overall, N$_2$ production the sites was attributed mostly to denitrification in the sites within Lake Erie (i.e., SS and CB) and to anammox in the site within the Sandusky Bay.
Our study represented one of the first investigations on anammox and their relative importance in nitrogen loss through production of $N_2$ in Lake Erie and the Laurentian Great Lakes in general. Anammox potential was measured in the sediment overlaying water samples at the CB site, where the oxygen was the lowest among the three sites. To a larger extent, anammox potential was also measured at SB within the Sandusky Bay, a small coastal water reservoir semi-isolated from the main body of the Lake Erie. This was somewhat surprising since the SB site was well oxygenated throughout the water column. However, the site is also known for its eutrophic condition, and cyanobacterial blooms are frequently developed throughout the summer. We hypothesize that during cyanobacteria blooms, microzones of hypoxia can be developed to incubate anammox bacteria. Further investigation is needed to test this hypothesis and verify our finding in this project.

5 Publication citations

Dissertations

Lu, Xinxin, (in progress), The Relative Importance of Denitrification and Anammox in Freshwater Lakes and Coastal Marine Environments, Ph.D. Department of Biological Sciences, Kent State University, Kent OH

Conference Proceedings
Lu, Xinxin; Darren Bade, Laura Leff, Robert Heath and Xiaozhen Mou, 2011, Denitrification Is More Important Than Anammox In Microbially Mediated N Removal In Lake Erie, International Association for Great Lakes Research (IAGLR) 54th Annual Conference on Great Lakes Research, Duluth, Minnesota (Abstract accepted).


5 Student Supported

Lu, Xinxin, PhD, Major: Ecology

6 Awards or Achievements

None.

References:


THE INFLUENCE OF NATURAL ORGANIC MATTER ON BIOFILM GROWTH, CHLORINE EFFICACY, AND BY-PRODUCT FORMATION IN WATER DISTRIBUTION SYSTEMS

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Publications

2. Xue Zheng and Youngwoo Seo, Quantitative Analysis of Biofilm Susceptibility against a Model Disinfectant, Water Research (In preparation)
5. Wang, Zhikang and Youngwoo Seo; 2011, Affect of Phenotypic Variation on Biosorption of Natural Organic Matter (NOM) Under Simulated Drinking Water Distribution System Conditions, Borchardt Conference, University of Michigan, Ann Arbor, MI, USA.
7. Xue, Zheng, Cyndee Gruden, and Youngwoo Seo; 2011, Efficacy of model disinfectant on biofilm and biofilm detachment, American Water Works Association Water Quality Technology Conference, Phoenix, Arizona, USA. (Submitted)
1. PROBLEM AND RESEARCH OBJECTIVES

In water supply, treatment, and distribution systems natural organic matter (NOM) creates various problems as it is highly reactive and is not fully remediated by conventional water treatment procedures. Consequently, NOM creates two major problems in water supply, yield, and quality. First, NOM can be utilized by bacterial biofilm (consortium of bacteria attached with surfaces) as a carbon and nutrient source, thus leading to subsequent deterioration of water system infrastructure (biocorrosion and biofouling), as well as deterioration of water quality in water treatment, distribution and storage systems (Lechevallier, et al., 1991). Second, NOM is highly reactive with chlorine-based disinfectants and creates unwanted disinfection by products (DBPs), which can potentially cause cancer in humans with long term exposure.

Biofilm formation and bacterial regrowth are dependent upon complex interactions between drinking water characteristics, as well as engineering and operational parameters (LeChevallier, et al., 1996). The efficiency of residual disinfectants is critical for the reduction of total bacterial cell counts in bulk water and incidences of biological contamination in drinking water. However, the role of natural organic matter on biofilm disinfection and viability of detached biofilm has not been well studied. Specifically, the impact of NOM interaction with both biofilm and detached biofilm clusters still remains elusive.

The objective of this study is to examine the role of dissolved NOM on biofilm growth and susceptibility to model disinfectants. Strains from an opportunistic pathogen, *Pseudomonas aeruginosa* (both wild type and mutant strains) with different extracellular polymeric substance (EPS) secretion capabilities were used to investigate (i) NOM interaction (adsorption) with biofilm, (ii) the impact of the presence of natural organic matter on the viability of both attached and detached biofilm.

2. STATEMENT OF RESULTS OR BENEFITS

In water distribution systems, biofilm survival has been reported despite the regulatory presence of residual disinfectants. Natural organic matter (NOM), which is ubiquitous in drinking water systems, contributes to biofilm growth as a carbon source as well as increasing disinfectant demand. In this study, strains from an opportunistic pathogen, *Pseudomonas aeruginosa* (both wild type and mutant strains) with different extracellular polymeric substance (EPS) secretion capabilities were
used to cultivate single species biofilms. Biofilms were grown in a continuous flow system under low nutrient condition simulating the drinking water distribution system. Post chlorine disinfection, biofilm was visualized using a confocal laser scanning microscope (CLSM) followed by image analysis software to quantify biofilm EPS content and spatial distribution of viability. The survival rate of detached cells from PAO1 biofilm was analyzed by flow cytometry to differentiate live, dead and membrane compromised cells considering the presence or absence of NOM- materials known to consume residual . Both biofilm and detached cluster viability were confirmed utilizing the plate count method.

Results
The following results were obtained from this study.
1) All tested cultures showed lower biosorption capacities compared to the counterparts without divalent ions (Huh?). In the presence of divalent ions, biosorption of NOM is proportional to the amount of capsulated EPS on bacteria culture.

2) The amount of EPS produced is positively related to biofilm viability in both the presence and absence of NOM. Resistance to disinfection was significantly enhanced in EPS overproduction biofilm compared to EPS deficient strains. Due to chemical reactions between NOM and residual disinfectants in the bulk solution, the presence of NOM improved detached biofilm resistance to chlorine residuals.

Benefit
Presence of NOM and biofilm formation greatly increases biological and chemical instability in the drinking water supply, which will decrease water yield and result in increased operational costs. Presence of NOM and biofilm also requires higher disinfectant doses to comply with Environmental Protection Agency regulations. From this study, the minimum required dose of chlorine to control both bacterial biofilm and detached biofilm clusters was elucidated. The results from the proposed study will enable local water utilities to incorporate biofilm control strategies by effectively utilizing disinfectant with consideration of biofilm / NOM prevalence.

3. MATERIALS/METHODOLOGY

*Pseudomonas aeruginosa* strain PAO1 and two mutant strains *algT* (inhibited alginate EPS production) and *mucA* (overproduction of alginate EPS) were used in this experiment (Figure 1). All strains were grown in one-tenth strength LB broth at 37°C with mixing and then harvested during the late-exponential phase. The bacterial suspensions were prepared by centrifugation at 2,000 × g for 15 min, allowing for minimal removal of cell-bound EPS. The cells were diluted in chlorine demand free (CDF) buffer as a bacterial suspension.
Solution preparation

A 0.02 strength LB broth was used as a biofilm growth medium to create nutrient limited growth conditions mimicking low-carbon drinking water environment. For the biofilm cultivation experiments with NOM, filtered (0.45µm) Suwannee River NOM (SR-NOM) was added to the medium, resulting in a final NOM concentration of 2 ± 0.2 mg/L (Croue, et al., 1999). Chlorine solutions were prepared by adding Clorox bleach (The Clorox Co., Oakland, CA) to autoclaved dionized water. The free chlorine concentration was determined by the N, N-diethyl-p-phenylenediamine (DPD) method.

Biofilm cultivation in flow cell system

Single-species biofilms were grown in continuous-culture flow cells (channel dimensions, 1.6 by 12.7 by 47.5 mm; flow rate, 0.2 ml/min) at room temperature. The flow cell contains a standard glass microscope slide on one side and a glass cover slip on the other side. Flow rate of the flow cells simulated the laminar flow with an average flow velocity of 0.16 mm/s throughout each experiment. Under this flow condition, a residence time that enhanced biofilm formation was achieved. Two carboys were used as medium feeding and chlorine supply reservoirs respectively. All feeds to reactors were delivered using a multichannel peristaltic pump (ISMATEC, Glattbrugg, Switzerland) and silicone tubing (Masterflex, Vernon Hills, IL). Flow cells, tubing and solutions were sterile at the initiation of each experiment. Operation and sampling of the flow cells followed aseptic technique throughout the experiments. The flow cell channels were inoculated with bacterial suspension and incubated without flow for 2 hours at room temperature for initial bacterial attachment. After 2 hours, flow rate was gradually increased to 0.2 ml/min. For each experiment, the two
channels in one flow cell were operated in parallel under identical conditions, although one channel received chlorine and the other served as a non-chlorinated control. Chlorine concentration was maintained at 0.5 mg/L at the flow cell inlet throughout the disinfection process. Flow cell effluent was collected every 30 minutes for 2 hours in total and quenched with 0.1 M sodium thiosulfate before further analysis.

**Figure 2 – Flow cell system set up**

**Confocal Laser Scanning Microscopy and Image Analysis**

The biofilm on the cover slides was visualized by fluorescent staining with BacLight LIVE/DEAD bacterial viability staining kit (Molecular Probes Inc.) to differentiate live and dead cells. Extracellular polysaccharide in the biofilm formed by *P. aeruginosa* was visualized with Alexa 633 conjugated concanavalin A (ConA-Alexa 633). Biofilms were visualized with a Leica confocal laser scanning microscope (CLSM) equipped with a 63X oil immersed objective and a 20X objective. The CLSM images were further processed with mathematical analysis to determine total biomass, EPS content, and surface characteristics using image analysis. For each culture strain, experiments were repeated at least three times. All image analysis was based on at least 5 images of one sample.

**Flow cytometry analysis**

The total cell amount and viability distribution from the detached biofilm was quantified as a function of fluorescence intensity measured with flow cytometry. Samples were run at high speed and approximately 10,000 events were taken for each measurement. Data were acquired in log mode by a FACScalibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed using CELLQUEST software (BD Biosciences, San Jose, CA). The flow cytometer was equipped with an argon laser set
at 15 mV and turned to an excitation wavelength of 488 nm. Propidium iodide (PI) and SYTO9 (InVitrogen, Carlsbad, CA) were used in combination as membrane compromised cells and intact cells marker respectively. Cell concentration was determined by adding microsphere standard (InVitrogen, Carlsbad, CA) and adjusted to a concentration of ~10^6 cells/ml. On the basis of negative and positive controls, the analysis using flow cytometry was performed by making a comparison plot between the PI and the SYTO9 fluorescence to quantify the cellular viability.

**NOM adsorption tests**

Seven concentrations (5 mg/L, 7.5 mg/L, 10 mg/L, 12.5 mg/L, 15 mg/L, 17.5 mg/L, 20 mg/L) of absorbate solutions were diluted from three NOM stock solutions (SR NOM, SR humic acid standard, SR fulvic acid standard) (100 mg/L). Adsorption isotherms were conducted in triplicate for each culture variant with methods mimicking kinetic tests. Tests were conducted with and without divalent ions at a fixed pH. The isotherm equilibrium time was selected as 5 h based upon the results from kinetic experiments.

3. **PRINCIPLE FINDINGS AND SIGNIFICANCE**

**Principle Finding**

1) Our results indicate that in the presence of divalent ions, all tested cultures exhibit higher biosorption capacities compared to the counterparts without divalent ions. The possible mechanisms are: i) the existence of divalent ions could compress the electrical double layers which reduce the repulsive force between cultures and NOM. ii) Functional groups in EPS, microbial cell membrane and NOM play a significant role, possibly bridging of biomolecules by divalent ions.

In water distribution systems,

2) The biofilm CLSM image analysis reveals that alginate EPS production has an effect on biofilm viability. The biofilm viability is found to be positively related to its EPS content. The EPS overproducing *mucA* biofilm shows comparatively high resistance to chlorine, when compared to the wild type and EPS deficient strains. The presence of NOM did not significantly affect the viability of biofilm due to insignificant interaction between NOM and biofilm in the absence of divalent ions. However, the presence of NOM enhances the detached cell viable rate, which would lead to bacteria regrowth or reattachment in distribution system.

**Significance**

1) Sorption mechanism of NOM on biofilm was elucidated under relevant conditions in water distribution system.

2) From this study, the required dose of chlorine to control both bacterial biofilm and detached biofilm clusters was elucidated. The results from the proposed study will enable local water utilities to incorporate biofilm control strategies by effectively utilizing disinfectant with consideration of biofilm / NOM prevalence.
Publication

Journal Paper

Xue Zheng and Youngwoo Seo, Quantitative Analysis of Biofilm Susceptibility against a Model Disinfectant, Water Research (In Preparation)

Conference papers and presentations

Wang, Zhikang, Zheng Xue, and Youngwoo Seo; 2010, Influence of Bacterial Extracellular Polymeric Substances (EPS) on Biosorption of Natural Organic Matter in Water Distribution System, 240th American chemical society national meeting, Boston, MA, USA.

Wang, Zhikang and Youngwoo Seo; 2011, Affect of Phenotypic Variation on Biosorption of Natural Organic Matter (NOM) Under Simulated Drinking Water Distribution System Conditions, Borchardt Conference, University of Michigan, Ann Arbor, MI, USA.

Xue, Zheng, and Youngwoo Seo; 2011, Factors Modulating Biological Stability of Water in a Model Distribution System, Association of Environmental Engineering and Science Professors Education and Research Conference, Tampa, USA (Submitted).

Xue, Zheng, Cyndee Gruden, and Youngwoo Seo; 2011, Efficacy of model disinfectant on biofilm and biofilm detachment, American Water Works Association Water Quality Technology Conference, Phoenix, Arizona, USA. (Submitted)

Wang, Zhikang and Youngwoo Seo; 2011, Biosorption of Natural Organic Matter in Water Distribution System, American Water Works Association Water Quality Technology Conference, Phoenix, Arizona, USA. (Submitted)
References


The Environmental Fate and Transport of Denatonium Benzoate

Basic Information

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Publications

There are no publications.
A progress report containing Problem and Research Objectives, Research Methodology, and Principal Findings and Significance

Statement of regional or state water problem

H.B. 96 was introduced during the 128th General Assembly of the Ohio legislature. This bill specifies that “engine coolant or antifreeze sold in Ohio that contains more than 10% ethylene glycol and that is manufactured after December 1, 2008 must include a bittering agent to render the engine coolant or antifreeze palatable”. This legislation parallels H.R. 615, ‘Antifreeze Bittering Act of 2009’ which, if passed, will amend the Federal Hazardous Substances Act and require that the bittering agent denatonium benzoate (DB) be added to engine coolant and antifreeze to make it undesirable to humans and pets. Although, well-intentioned, no published data exists pertaining to potential negative environmental effects of this highly water-soluble compound. This lack of information, was highlighted by Tom Bonacquisti, director of water quality and production for Fairfax, VA Water and representative of The American Water Works Association who stated that “little is known about the environmental fate and transport of DB...” and who pointed “...out that small amounts of this material could render drinking water supplies bitter and unpalatable” due to the extreme bitterness of DB”. Given the lack of reliable empirical data pertaining to DB environmental behavior and the potential for DB to be released into the environment, a project evaluating DB behavior in the sub-surface environment is timely. At present, there are no known reports of problems associated with DB, however, if Ohio’s legislation and the federal legislation are enacted into law, unintended consequences could arise. A well-known example of unforeseen effects associated with attempts to achieve a greater good was the addition of methyl tert-butyl ether (MTBE) to automobile gasoline formulations to mitigate air pollution; MTBE is now known to have contaminated water bodies around the country rendering them unpalatable at minute concentrations of MTBE. Thus, with the move toward extensive use of DB as an antifreeze additive seemingly imminent, negative environmental impacts associated with DB must be thoroughly investigated before widespread introduction. The proposed project will help to articulate the environmental behavior of DB in the sub-surface environment.

Research Objective

The overall focus of this project was to evaluate the hypothesis that the environmental fate and transport of denatonium benzoate (DB) is governed by sorptive interactions with natural surfaces, dissolved aqueous species, and solution conditions. DB is an aversive agent added to many consumer products to discourage intentional use and to minimize (if not prevent) accidental ingestion by animals or humans. The motivation behind this study was the lack of information pertaining to the environmental fate and behavior of this compound. The information is necessary given the fact that certain federal legislators have proposed laws mandating the addition of this agent to anti-freeze sold in the United States.
The specific objectives of the study were to:

a. Characterize the sorption behavior of the denatonium cation to a variety of clay surfaces
b. Delineate the role of solution chemistry and temperature in the partitioning of the denatonium cation between aqueous and solid phases.
c. Determine the sorption mechanism of the denatonium cation to clay surfaces

**Research Methodology**

Sorption experiments using clay minerals and whole soils will be conducted using specific size fractions for each clay. To evaluate sorption characteristics of clay minerals, the well-established batch test method will be utilized according to Organisation for Economic Co-operation and Development guidelines. In the experiment, a measured amount of sorbent material (solid) is suspended in a sorbate solution and rotated end-over-end. In our batch experiments, initial DB (sorbate) concentrations ranging from 10 ppb to 100 ppm (in 100 mM CaCl$_2$) from pH = 1 to pH = 10 will be performed. The temporal evolution of aqueous sorbate concentration is evaluated up to equilibrium or near-equilibrium conditions. Following the reaction, the system will be centrifuged and aqueous-phase DB concentrations determined via high-pressure liquid chromatography. The amount sorbed can be directly measured on the sorbent or calculated using mass-balance relationships between the initial aqueous sorbate amount and the final sorbate masses. Based on preliminary measurements, our team determined that significant container adsorption does not occur. Thus, we will measure concentrations using mass-balance relationships.

**Principal Findings and Significance**

Figure 1 shows room temperature, pH dependent (pH 6.5 and 8.5) denatonium benzoate sorption to kaolinite clay. The data were obtained over an initial aqueous concentration range of 0 – 100 ppm. As initially suspected, the clay exhibited an affinity for denatonium benzoate. The data also suggest that pH has a negligible impact on DB sorption at the values tested. (The values shown were chosen to approximate a slightly acidic to slightly alkaline environment.) Given the fact that silanol and aluminol groups are protonated at the pH values investigated and the structure of denatonium benzoate, it is not surprising that the sorption behavior should be similar under both solution conditions.

Temperature dependent sorption experiments (not shown) at two initial DB concentrations (25 ppm and 50 ppm) did not indicate that temperature significantly impacted the sorptive behavior of the kaolinite over the temperature range chosen (25, 45, and 55°C) but did indicate an endothermic sorption process at initial concentrations of 25 and 50 ppm at pH 6.5 as shown in figure 2.
The results taken with data room temperature (25°C) data (not shown) for hectorite and montmorillonite clay minerals (at various pH values) suggest that the clay identity could impact the transport of denatonium benzoate in the subsurface environment. The results from varied pH experiments with hectorite and montmorillonite clays also indicate that the solution chemistry impact the ability of denatonium benzoate to sorb to clay mineral surfaces.

The partitioning of denatonium benzoate between clay mineral surfaces and water was evaluated using multiple types of clay sorbents (components of natural soils). Generally, one can expect intentional (or unintentional) releases of this aversive agent to result in sorption to mineral components of natural soils. pH-dependent sorption isotherms showed that solution chemistry plays a role in the sorption behavior of denatonium benzoate to each mineral studied. Finally, the sorption process for kaolinite was endothermic at 25 ppm and 50 ppm initial concentrations of DB. The implications of the observed behavior in clay minerals are that the mobility of this bittering agent in the subsurface environment is likely to be controlled in soils by interactions with these clay minerals. Thus, more detailed knowledge on the interactions with whole soils should be undertaken in an effort to more accurately characterize the ability of this material to migrate through soil environments. This work is currently being undertaken in this laboratory.

**Publications**
None at this time.

**Students Supported**
Yun Yun Zhou, (M.S. Student, Department of Chemistry), Taylor Pair (B.S. Student, Department of Chemistry)

**Notable Awards or Achievements**
None at this time.
State-of the-art membrane characterization toward biofouling control and improved membrane performance

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Publications

1. Amr Zaky, Isabel Escobar, and Cyndee Gruden. “A designed characterization technique towards understanding fouling mechanisms of cellulose acetate ultrafiltration membranes,” September 2010, Ohio Section AWWA Annual Conference.
4. Zaky, Amr, 2011, Characterization of Ultrafiltration Membranes and Effect of Biofouling on their Water Treatment Performance, “PhD Dissertation”, Department of Civil Engineering, University of Toledo, Toledo, Ohio, 86.
PROJECT TITLE: State-of-the-art membrane characterization toward biofouling control and improved membrane performance

PROJECT TEAM. Cyndee L. Gruden, Isabel Escobar, Amr Zaky (PhD student), Amir Motlagh (MS Student)

Department of Civil Engineering and Department of Chemical and Environmental Engineering, University of Toledo, Toledo, OH.

PROBLEM.
To address emerging water quality concerns, membrane separation technologies have been expanded, resulting in the continuous reduction of their cost and rapid extension of their application possibilities. Moreover, numerous case studies suggest that the cost of new pressure-driven filtration plants are expected to be comparable with the available treatment processes in the near future (Escobar et al, 2005; Hilal et al.; 2005; Zhou et al., 2002). Microfiltration (MF) and ultrafiltration (UF) membranes can be used to meet the turbidity and disinfection requirements of surface water treatments rules. These membranes do not remove disinfection byproducts precursors, but they can reduce DBPs formation by reducing the disinfectant dose requirements. Moreover, MF and UF membranes have shown their ability to effectively remove microbial contaminants such as Cryptosporidium and Giardia and viruses (EPA, 2001). Despite the remarkable advantages of membrane separation technologies, many researchers agree the drastic reduction of water flow due to membrane fouling and the high cost of membrane replacement is considered a significant barrier to widespread use of membranes (Al-Ahmed et al., 2000; Escobar et al., 2005; Bos et al., 1999).

The main types of fouling mechanisms are inorganic fouling (including scaling, particulate and colloidal fouling), organic fouling, and biological fouling (biofouling). However, membrane biofouling is inherently more complicated than other membrane fouling phenomena because it accompanies other fouling mechanisms. This is due to the capabilities of microorganisms to adapt their growth rate, multiply, and relocate. This guarantees their survival even if they were 99.99% removed from the feed stream (Flemming et al., 1997; Ivnitsky et al., 2005). Apparently, the tangential forces of membrane flux, transport bacteria to the membrane surface causing bio-film formation, thus reducing the permeate flux (Sablani et al., 2001). Therefore, the first step in bio-film formation is the initial attachment of bacteria cells to the membrane surface. The significant factors that affect cell attachment are the membrane surface properties which affect the speed and strength of the attachment (Pasmore et al., 2001).

RESEARCH OBJECTIVES.

In this research, we used emerging materials characterization techniques to quantify membrane surface properties toward predicting biofouling and improving membrane performance.

Specifically, our objectives were to:
• Determine the contribution of membrane surface roughness on its biofouling behavior.

• Assess the correlation between the membrane surface charge and its morphology and their contribution to biofouling behavior.

• Study the effect of membranes’ hydrophobicity/hydrophilicity on both biofouling and membrane filtration performance.

• Improve existing approaches to quantitatively characterize membrane biofouling.

METHODOLOGY

Membranes. CAUF membranes, with a molecular cut-off weight of 20,000 Daltons (General Electric Water and Process Technologies, Minnetonka, MN), were used and stored in deionized water (DI) at 5°C with regular water replacement.

Crossflow membrane filtration unit. Crossflow experiments were performed on membrane sheets with an area of 138.7 cm². The feed water was pumped from the feed water reservoir to the cell membrane (Osmonics Sepa CF, Minnetonka, MN). Both concentrate and permeate were recycled to the reservoir. Crossflow filtration was performed for 53 hours at a constant pressure of 172.36 kPa (25 psi) and water temperature of 26°C. Fouling experiments were performed to provide a basic understanding of the influence of physical and chemical operating conditions on biofouling behavior of the tested membranes. The permeate flux \( J \) and final permeate flux \( J_f \) were measured during and at the end of the filtration experiment, respectively. The final permeate flux was reported with respect to initial flux \( J_o \). Flux experiments were performed using tap water and synthetic water.

Membrane biofouling analysis. Crossflow experiments were performed on membrane sheets, as control samples, with tap water discharged from The University of Toledo, Ohio. Since the key goal of this study is biofouling, a synthetic water composition was designed to support microbial growth. The synthetic water contained sodium acetate trihydrate (130.14 ppm), sodium thiosulfate (65.07 ppm), and buffering in the form of 0.050 mM NaHCO₃, 0.010 mM CaCl₂, 0.200 mM NaCl, 0.094 mM NH₄Cl, 0.045 mM KH₂PO₄, and 0.060 mM MgSO₄ all added to tap water. After incubation for 5 hours, *Pseudomonas fluorescens Migula* (ATCC # 12842) culture was added to the feed reservoir to achieve a cell concentration of \( 10^3 \) cells/mL. To insure a fresh single colony, *Pseudomonas fluorescens Migula*, pre-grown on LB broth for 24 hours, was grown on LB agar for 18-22 hours. A minimum of three crossflow filtration experiments were performed on both tap and synthetic water compositions.

Surface roughness and feature height analysis. Surface morphology of clean and fouled membranes was evaluated using atomic force microscopy (AFM). The Multi-mode Nanoscope IIIa scanning probe microscopy (Santa Barbara, CA) at The University of Toledo, Ohio was operated in ambient conditions. The membrane surface was imaged
in tapping mode (TM) using silicon nitride cantilevers with integrated pyramidal tips (radius < 10 nm, force constant of 20-70 N/m, and resonance frequency in the range of 300 kHz). AFM scans were performed on clean and fouled membranes from the permeate outlet (A2) and the flow inlet (A1) regions, as shown in Figure 1.

The software SPIP version 4.7.4.0 was used to calculate surface roughness and skewness in order to evaluate the change in the membrane morphology under different fouling conditions.

**Figure 1: Fouled membranes sheets were subdivided at three regions: A1 (flow inlet), A2 (permeate outlet), and A3 (concentrate outlet). AFM and biofilm intensity analysis were performed at regions A1 and A2.**

**Biofilm surface area coverage and intensity analysis.** The surface area coverage and intensity of the formed biofilm on membrane sheets were calculated using the software Image J 1.41. After the crossflow experiments were performed, membrane sheets (14.6 cm x 9.5 cm) were incubated at 26 °C for an hour. Then images were captured using a digital scanner (Visioneer 4800 USB with 600 x 1200 dpi optical resolution).

**PRINCIPLE FINDINGS**

**Membrane biofouling experiments.** While the flux decline ((1 - \( J_f/J_0 \)) * 100), of membranes filtered using tap water ranged from 5.0 to 27.4 percent, it ranged from 10.0 to 35.0 percent when using synthetic water.

**Biofilm surface area coverage and intensity analysis.** Table 2 shows the total biofilm surface area coverage calculated from regions A1, A2, and A3 on fouled membranes filtered using tap water (\( J_f/J_0 = 73.0 \% , 76.8 \% , 90.9 \% , \) and \( 95.0 \% \) ) and synthetic water (\( J_f/J_0 = 65.0 \% , 86.7 \% , \) and \( 90.0 \% \) ). At high flux declines (\( J_f/J_0 = 73.0 \% \) and 76.8 % for tap water and \( J_f/J_0 = 65.0 \% \) for synthetic water), the biofilm surface area coverage on fouled membranes using tap water [46.4 to 63.1 %] was lower when using synthetic water (75.4 %).
Table 1: Percent of biofilm surface area coverage by region (A1, A2, and A3) and total biofilm surface area coverage calculated on fouled membranes filtered using tap and synthetic water.

<table>
<thead>
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<th>Fouled membranes using</th>
<th>Percent of biofilm surface area coverage by region</th>
<th>Percent of the total biofilm surface area coverage</th>
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<tr>
<td>Tap water ( J_f/J_o = 73.0% )</td>
<td>17.4 11.5 17.4</td>
<td>46.4</td>
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<tr>
<td>Tap water ( J_f/J_o = 76.8% )</td>
<td>25.7 10.8 26.6</td>
<td>63.1</td>
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<tr>
<td>Tap water ( J_f/J_o = 90.9% )</td>
<td>39.2 9.7 37.3</td>
<td>86.2</td>
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<tr>
<td>Tap water ( J_f/J_o = 95.0% )</td>
<td>39.4 10.7 41.0</td>
<td>91.1</td>
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<tr>
<td>Synthetic water ( J_f/J_o = 65.0% )</td>
<td>29.5 15.4 30.5</td>
<td>75.4</td>
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<td>Synthetic water ( J_f/J_o = 86.7% )</td>
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<td>29.5 14.0 31.5</td>
<td>74.9</td>
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On the other hand, at low flux declines \( J_f/J_o = 90.9 \% \) and \( 95.0 \% \) for tap water and \( J_f/J_o = 86.7 \% \) and \( 90.0 \% \) for synthetic water), the biofilm surface area coverage on fouled membranes using tap water [86.2 to 91.1 %] was higher than when using synthetic water [75.5 to 75.9 %]. However, in all cases, at the permeate outlet, the biofilm surface area coverage when using tap water was lower [9.7 to 11.5 %] than when using synthetic water [14.0 to 15.4 %].

In addition, the intensity of the biofilm, presumably related to biofilm thickness, was calculated on fouled membranes at the permeate outlet and flow inlet based on the difference in the grey value (pixel amplitude) of captured images. The grey value ranges from level 0 (represents the black color trend toward presence of biofilm) and level 256 (represents the white color trend toward absence of biofilm). Figures 2 is an example of the pixel amplitude profile of clean and fouled membranes at permeate outlet and flow inlet.

In the permeate outlet regions (A2), it was lower than in the flow inlet regions (A1) indicating higher biofilm intensities. This is likely due to the difference in the hydrodynamic shear force applied during crossflow filtration; lower fouling concentration profile in regions of higher shear forces. In addition, the biofilm intensity of fouled membranes using synthetic water was always observed to be higher than when using tap water. Moreover, as the normalized flux values decreased from [86.7 to 90.9 %] to [65.0 to 76.8 %], the pixel amplitude decreases ± 20 units indicating more biofouling.
Figure 2: Estimate biofilm intensity profile on fouled membranes filtered using tap water ($J_f/J_0 = 76.8\%$) and synthetic water ($J_f/J_0 = 65.0\%$) using pixel amplitude on clean membrane as a reference profile. The reduction in the pixel amplitude corresponds to an increase in the biofilm intensity.

Assessment of biofouling behavior using atomic force microscopy (AFM)
AFM imaging, in the tapping mode (TM), and the corresponding feature height distribution of a clean membrane. As expected, the manufactured clean membrane was relatively smooth ($S_q = 6.2\$ nm) and uniform ($S_d = -0.2\$ nm). The calculated roughness and skewness of clean and fouled membranes are summarized in Table 2. The roughness values of fouled membranes filtered using tap water ($J_f/J_0 = 76.8\%$ and 90.9\%) at the permeate outlet ($S_q [110.1 \text{ to } 117.4\text{ nm}]$) was higher than the values at the flow inlet ($S_q [49.5 \text{ to } 73.7\text{ nm}]$). On the other hand, the difference in roughness of fouled membranes, filtered using synthetic water ($J_f/J_0 = 65.0\%$ and 86.7\%), between their permeate outlet and flow inlet values were not significant and had an average of 89.5 nm.
Table 2: Calculated roughness and skewness of clean and fouled membranes.

<table>
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<th>Samples filtered synthetic water</th>
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<tr>
<td></td>
<td>Normalized flux = 76.80%</td>
<td>Normalized flux = 90.90%</td>
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<td></td>
<td>Permeate outlet</td>
<td>Flow inlet</td>
<td>Permeate outlet</td>
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<tr>
<td>$S_q (nm)$</td>
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<td>49.5</td>
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<td>$S_{sk}$</td>
<td>0.5</td>
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<td>-0.2</td>
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<tr>
<td></td>
<td>Normalized flux = 65.00 %</td>
<td>Normalized flux = 86.70%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeate outlet</td>
<td>Flow inlet</td>
<td>Permeate outlet</td>
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<tr>
<td>$S_q (nm)$</td>
<td>89.8</td>
<td>83.8</td>
<td>95.3</td>
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<tr>
<td>$S_{sk}$</td>
<td>0.2</td>
<td>-0.7</td>
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Based on this analysis, it can be concluded that the distribution of the biofilm was less uniform across membrane sheets fouled using tap water as compared to synthetic water. The tap water data is shown in Figure 3. This agrees with the biofilm surface area coverage and intensity analysis; at the permeate outlet, the biofilm surface area coverage when using tap water was lower [9.7 to 11.5 %] than when using synthetic water [14.0 to 15.4%]. Surface skewness ($S_{sk}$), measures surface texture uniformity, was calculated for scanned samples by indicating the asymmetry of the height distribution data about its mean. When $S_{sk} = 0$, the height distribution fits a Gaussian distribution, when $S_{sk} < 0$, it indicates bearing surfaces with valleys and when $S_{sk} > 0$, it indicates flat surfaces with peaks. However, regardless of the skewness sign, it can be directly correlated to the uniformity of biofilm. It was observed that the biofilm is closer to uniformity ($S_{sk} = 0$) on fouled membranes using synthetic water (Figure 6) than when using tap water. This was valid for all cases except at the permeate outlet of the fouled membrane using synthetic water with $J/J_f = 86.7$ percent. The difference between the biofilm distribution, at the permeate outlet and at the flow inlet for membranes fouled using tap water was less uniform ($\Delta S_{sk} [1.3 to 1.7]$) than on membranes fouled using synthetic water ($\Delta S_{sk} [-0.7 to -0.9]$). This indicates that bacterial cells are more likely to deposit and form a consolidated biofilm on the membrane surface near the permeate outlet region with lower shear rates. Biofilms grow to a thickness that allows it to extend to zones of greater velocity and more turbulent flow. This extension, from the permeate outlet to the flow inlet, was found to be higher when membranes were filtered using synthetic water than for membranes filtered with tap water.
SIGNIFICANCE AND IMPACTS OF RESEARCH ACTIVITIES

UF membranes provide a promising technology for purification of macromolecular and colloidal species in solutions (EPA, 2005; Goosen et al., 2004; Hilal et al., 2005). However, membrane fouling still remains a significant challenge that affects flux, operation, and performance of membranes (Lee et al., 2004). Moreover, the role of membrane chemical heterogeneity and morphology in biofouling research has received little attention. This is due to the limitations in techniques available to study surface chemical heterogeneity with its correlation to the membranes’ morphology (Brant et al., 2002, 2006). The degree to which organic fouling contributes to biofouling is still poorly understood.
understood. Through understanding the complex dynamics of the membrane separation processes, we can develop a better system of membrane filtration. This system will provide a better overall productivity through fewer membrane backwash cycles, longer operating runs, longer membrane lifetimes, higher fluxes, and lower capital cost.

CONFERENCE PRESENTATIONS

Amr Zaky, Isabel Escobar, and Cyndee Gruden. “A designed characterization technique towards understanding fouling mechanisms of cellulose acetate ultrafiltration membranes,” September 2010, Ohio Section AWWA Annual Conference.


DISSERTATION

Zaky, Amr, 2011, Characterization of Ultrafiltration Membranes and Effect of Biofouling on their Water Treatment Performance, “PhD Dissertation”, Department of Civil Engineering, University of Toledo, Toledo, Ohio, 86.

PEER REVIEWED MANUSCRIPTS


BOOK CHAPTER


STUDENTS SUPPORTED BY THE PROJECT

2 students were supported. 1 MS student and 1 PhD student both in the Department of Civil Engineering.
Ozonation as a clean technology for environment and food industry: impact on quality of processing and waste-water

Basic Information

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Publication

Ozonation as a clean technology for environment and food industry: improving the quality of processing and wastewater

2011 Annual Report

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1. Statement of regional or State water problem

While fresh produce provides quality nutrition, it is one of the major sources for transmitting pathogens, such as enterohemorrhagic Escherichia coli and Salmonella spp. Thus, it can cause serious foodborne illness outbreaks because it is usually consumed without cooking. Post harvest practices commonly include a rinse step in which the fresh produce is sanitized using tap water supplemented with 50 to 200 ppm of free chlorine (Riley et al. 1983; Klockow and Keener, 2009). This washing is a critical process in preparing fresh produce for retail market. Chlorination is a convenient treatment, but even these high levels of chlorine have shown to be only marginally effective at reducing the level of E. coli O157:H7 on lettuce (Mead et al., 1999). Additionally, chlorination is not effective against noroviruses and other pathogens associated with fresh produce (Beuchart et al., 1999). It is well known that chlorination generates carcinogenic disinfection byproducts (DBP), such as trihalomethane (THM) (Wei et al., 1985), therefore using high level of chlorine for fresh produce washing may have adverse effects on human health. Chlorination, an average concentration of ~200 ppm used in fresh produce industry, is not favored by organic food processors. Chlorination of fresh produce also poses undesirable environmental impact on receiving water body when the washing water with high chlorine is discharged, thus it often requires neutralization, which causes additional cost. Furthermore, chlorine washing generates wastewater containing high levels of biological oxygen demand (BOD). Due to these environment and human health risks, several European countries banned the use of chlorine from organic food production (Kim et al., 1999; Rogers 2004; Selma et al., 2007; Olmez et al., 2009).

Other than chlorine, ozone, chlorine dioxide, UV irradiation and natural antimicrobial compounds are used in food industry. Among these, ozone is the most suitable disinfectant because it is a highly effective oxidant and is much more reactive than chlorine (Kim et al., 2003) and it does not generate carcinogenic THMs. With the increasing availability and consumption of ‘ready-to-eat’ or ‘pre-packaged’ products, there has been an increased awareness about methods ensuring the quality of products during, and to the end of, the ‘best-before’ date (Hassenberg et al., 2007). Most information on ozone disinfection and its trial in the food industry are mostly about its effect on food quality and safety (Wei et al., 2007). However, very little is known about
the effectiveness of ozone treatment on the quality of washing water, and eventually the quality of wastewater. Regulators, such as Department of Environmental Quality (DEQ) and US EPA would be interested in the outcome of this study because the use of ozone can reduce water consumption and improve wastewater quality, thus it can benefit the environment while the fresh produce processing and consumption are on the rise. The ozone disinfection treatment can be applied to other food industries which require plenty of water consumption. Using ozonation in food industry can improve product safety, extend product shelf-life, and save water usage. As demand for organic food increases, ozone treatment would be one of the best available disinfection processes in this food industry.

2. Research objectives
The objective of this research was to evaluate the efficiency of ozonated water in deactivating microbial contamination in fresh produce and the water quality in the course of recycling the washing water and final wastewater. We used Bacillus spores as a disinfection efficiency surrogate of parasites. Bacillus spores are known to be much more resistant to disinfection than vegetative cells. Specific aims are (1) to compare the efficiency of microbial reduction (Bacillus subtilis spores) using chlorine and ozone; and (2) to estimate the washing water quality after chlorination and ozonation (chemical oxygen demand (COD), total suspended solids (TSS)). For these, lettuce was used as a representative leafy green. Most of previous studies only investigated microbial reduction, particularly vegetative cells, using ozone or chlorine in deactivating various microbes. Hardly any study was undertaken to track the water quality change following the consecutive recycling washes by simulating a real fresh produce industry setting. Therefore, the results of this research can be used for understanding the effectiveness of ozone washing in deactivating more resistant microbial contaminants and the water quality of recycled water during washing and the final wastewater, which can fill knowledge gaps.

3. Methodology
3.1 Ozone treatment system construction
Customized washing system for fresh produce was constructed that can do both ozone and chlorine washing. A 3/4 horsepower centrifugal pump, with ozone safe seal’s was chosen to ensure longevity of the system. High density polyethylene was used for the tanks, along with ozone safe materials for the piping and connections. A U-Channel seal was used to prevent ozone leaking from the processing tanks, and along with a vacuum pump (1/4 hp) creating a driving force for the ozone through the ozone destructor unit. Valves and flow controller were attached in between chambers, so that we can control the water flow and separation/connection between the water reservoir and the produce treatment chamber. The whole operation was set up and operated in a chemical hood.

3.2 Bacillus subtilis spore preparation
TSA supplemented with yeast extract (TSAYE) and MnSO₄ was used to grow spores.
The cultures were harvested after 10 days incubation and examined by phase-contrast microscopy before the harvest to make sure >95% population was sporulated. Sterile plastic L-shaped spreaders were used when collecting spores. Cultures were washed with cold sterile deionized water and centrifuged (repeated for 4 times). Then, the solutions were heat-treated at 80°C to kill vegetative cells and then treated with lysozyme. Lastly, spores were re-suspended in sterile deionized water and stored at 4°C until use.

**3.3 Spore inoculation and sampling method**
Lettuce was purchased from a supermarket in Columbus, Ohio on the day of the experiment. Lettuce leaves were separated out and then were inoculated with *B. subtilis* spores. Initial lettuce samples were taken with (t= 0 min) and without (natural background) spore-spiking in order to establish a baseline that can be compared with the spore levels after disinfection treatments. From each batch of experiment, 5 pieces of lettuce were collected in duplicate mode (10 leaves total). The collected lettuce was homogenized with peptone water and the surviving spores were measured with plate count method using TSA.

**3.4 Ozone treatment**
After drying the lettuce in the biosafety cabinet, the spore-contaminated lettuce samples were added into the produce treatment chamber (the valve between the produce treatment chamber and the water reservoir was being closed at first) and ozone generation was run in the reservoir tank for 10 minutes before the real washing was started. In this system, the 10min ozone purging was an optimal time to obtain the desired ozone concentration in the water reservoir tank. At each washing run, 300 grams of lettuce and 1.5 grams of dirt were added to mimic the soil debris attaching to the newly harvested lettuce that brings in turbidity in the washing water. After ten minute of washing, both valves were closed and then lettuce samples were retrieved. Water samples were taken from each tank during this time as well. Water
characteristics (TSS, COD, and \(O_3\) concentration) and surviving \(B.\ subtilis\) spores were determined at each time interval. Five sequential ozone treatments were performed by removing the spore-contaminated lettuce after each run and also adding new lettuce before the next run in order to mimic the reuse of water in fresh produce industry.

3.5 Chlorine treatment
For comparison, chlorine treatment of lettuce was conducted. Hypochlorite was added into the reservoir tank to generate an initial concentration of chlorine \(~100\text{mg/L}\). Unlike continuous addition of ozone into the system, chlorine was added once at the beginning stage and the water was circulated through the entire system with a pump to make sure that the chlorine was distributed evenly. Then, spore-inoculated lettuce was added into the system. The treatment procedures (treatment time, water flow rate, collection of lettuce and water), the measurements of surviving spores and water quality parameters were the same as the ozone treatment.

4. Major findings
4.1 Efficiency of Spore reduction
Under our experimental setups, ozone and chlorine treatments achieved similar log reductions of \(B.\ subtilis\) spores during the 5 repeated washings (ozone 0.95 - 2.08 log CFU/g-lettuce reduction; chlorine 0.86 - 1.61 log CFU/g-lettuce). The concentration of ozone and chlorine was 2ppm and 100ppm, respectively. In order to compare their disinfection efficiency accurately, \(C\) (concentration) x \(t\) (time) values were calculated. The \(C\cdot t\) values of ozone washing were all below 18 mg/L\(\cdot\)min. In contrast, those of chlorine washing were all above 900 mg/L\(\cdot\)min, which is more than 50 times higher than the ones calculated from the ozone treatments (Table 1). This indicates that ozone is far more effective than chlorine in \(Bacillus\) spore disinfection. The food quality, both color and texture of lettuce, remained intact after both treatments.

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4.2 Water quality
Overall, COD continuously increased in repeated washing of lettuce. After the final runs of treatments, the average COD in the ozone treatments was 14mg/ml and the one in the chlorine treatments was 22mg/ml. This implies that ozone disintegrated more solids than chlorine. The TSS of both treatments increased from 1-2mg/L (initial) to 3-4mg/L (final), so it seems that both chlorine and ozone treatments did not
generate much solid particles. The levels of remaining spores in the repeated washing water were also determined. In the chlorine treatments, the concentration of spores never decreased, but increased ~1.5 log at the end of the 5th run. On the contrary, the level of spores in the ozone treatment obviously declined, and after the 4th run, they were not detected in both reservoir and treatment tanks. Thus, the microbial quality of the wastewater after ozonation was much safer than the one after chlorination, which will decrease the cost for wastewater treatment.

**Figure 1.** The change of TSS levels during the ozone washing and chlorine washing during the repeated cycles for removing *B. subtilis* spores.

**Figure 2.** The change of COD levels during the ozone washing and chlorine washing during the repeated cycles for removing *B. subtilis* spores.

**Figure 3.** Remaining *B. subtilis* spores in the wastewater after ozone (ozone concentration <2mg/L) and chlorine (chlorine concentration 100mg/L) treatments.

**5. Significance**

The results show that ozone treatment of spore-contaminated lettuce is more effective than chlorine in both deactivation of *B. subtilis* and water quality. These findings suggest that multiple washes with ozonated water was efficient against microbes and reducing the organic matter in the water. Thus, it shows the promising use of
ozonation as a clean technology for leafy greens together with recycling practices, improved wastewater quality.

6. Publications

7. The number of students supported by the project
- James S. Rosenblum (PhD student, Division of Environmental Sciences, College of Public Health, The Ohio State University)
- Kaedra Wetzel (MPH student, Division of Environmental Sciences, College of Public Health, The Ohio State University)

8. References


A comparative sustainability analysis of water management options in buildings

Basic Information

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Publications

A Comparative Sustainability Analysis of Water Management Options in Buildings

Project PI: Defne Apul, The University of Toledo

May 16, 2011

1. Project Description

Problem Description and Research Objectives
Nationwide and in Ohio, both the drinking water and wastewater infrastructures are continuously degrading and in urgent need of upgrades. ASCE’s report card grades for these infrastructures have consistently gotten worse over the years and were D- in 2009 (ASCE 2011). A federal report (WIN, 2000) has estimated a funding gap of $23 billion a year between current investments and the investments that will be needed in the next 20 years to meet mandates of Clean Water Act and Safe Drinking Water Act. WIN (2000) estimated that this huge problem cannot be addressed by simplified solutions, such as increased water and wastewater service rates or operating efficiencies since doubling or tripling service costs would leave at least 25% of Americans unable to afford these services. Innovative technical solutions for the water infrastructure are necessary to avoid an upcoming possible crisis. This project was based on the assumption that integrated management of water in buildings might provide one of the innovative solutions to the problem. The goal of this project was to evaluate the sustainability of different water management scenarios in buildings. Technologies considered were: rainwater harvesting, composting toilets, standard toilets, and high efficiency toilets.

Methodology
This project resulted in two publications and a manuscript in review:


In Apul (2010), a literature review was conducted to compile a list of ecological design principles. Then, these principles were organized within themes and implication of each theme on water infrastructure engineering was explored.

In Anand and Apul (2010) and West et al (in review) life cycle assessment was used to analyze different water and sanitation scenarios for the University of Toledo Engineering Complex and Crossings Dormitory buildings, respectively. Building specific data were collected from the facilities department of the University of Toledo. Life cycle assessment process and input output based databases were used for the life cycle inventory. Life cycle cost, energy, and greenhouse gas emissions and pay back periods were calculated.
**Principal Findings and Significance**

Apul (2010) developed a vision for sustainable water infrastructure design. She organized the 99-item list of ecological design principles in three themes: (1) human dimension, (2) learning from nature (biomimicry), and (3) integrating nature. Biomimicry concept was further divided into six sub-themes; (1) complex system properties, (2) energy source, (3) scale, (4) mass and energy flows, (5) structure, and function, and (6) diversity and cooperation. The implications of these concepts on water infrastructure design suggested that the water infrastructure should be conceptualized in a more holistic way by not only considering water supply, treatment, and storm water management services but also integrating into the design problem other provisioning, regulating, cultural, and supporting ecosystem services. A decentralized approach for this integration and innovation in adaptive design were found to be necessary to develop resilient, and energy efficient water infrastructures. This work is significant because to my knowledge, it was the first study to compile ecological design principles and interpret them in the context of water infrastructure engineering towards developing a vision for sustainable infrastructure design.

Anand and Apul (2010) found that for the Engineering Complex at University of Toledo, use of harvested rainwater in toilets was a viable option only if low-flush toilets (1.2 gallons per flush or less) were used. They also found that composting toilets had low life cycle cost, energy, and emissions compared to standard toilets, low flush toilets, toilets flushed with rainwater, and high efficiency toilets flushed with rainwater. Finally, they found that the pay back periods for any one of the technologies were highest when analyzed in terms of cost, lower for energy, and lowest for greenhouse gas emissions. This work is significant because, to my knowledge, it is the first paper to compare different toilet technologies using a life cycle assessment and life cycle costing approach.

West et al. (in review) found that rainwater harvesting was not a viable option for the Crossing Building. The cost, energy, and greenhouse gas emissions required to produce rainwater tank were too high to be recovered in the life cycle of the building. This work is significant because, it was the first life cycle assessment study to evaluate the rainwater harvesting and its end use for irrigation or toilet flushing in new and existing buildings.

**References:**


Water Infrastructure Network (WIN), 2000, Clean and safe water for the 21st century: A renewed national commitment to water and wastewater infrastructure
2. Publications Resulting from This Project


West, Hannah, Chirjiv Anand and Defne Apul, in review, Life cycle based evaluation of rainwater use in toilets and for irrigation, Journal of Building and Environment


3. The number of students supported by the project

Two civil engineering students and one environmental sciences student have worked on this project. Chirjiv Anand is the only student that formally received funds from the project. Other students were either paid using other funds or were not paid due to internship, class assignments.

Chirjiv Anand is a PhD student in civil engineering. She is too early in her program to produce a dissertation.

Hannah West started working on this project as an undergraduate student in civil engineering. She later on completed her MS degree on the same project and graduated in Spring 2011.

4. Brief description of notable awards or achievements resulting from the project, if you have any

A new model, EEAST, was developed for sustainability evaluation of buildings’ water and sanitation infrastructures. EEAST stands for Economic and Environmental Analysis of Sanitation Technologies. EEAST is available for download at: http://utwatersustainability.wikispaces.com/

Hannah West won first place in the undergraduate research category at the Sigma Xi Research Symposium of Toledo in October 2009.


5. Presentations

This project was presented in 17 different local, regional, and national meetings. Some of these presentations are available for download from: http://utwatersustainability.wikispaces.com/

2. Apul, D. and Anand, C. Path towards a sustainability water infrastructure includes finding and evaluating the alternatives to using potable water to flush toilets. Chemistry Department, University of Toledo, OH, April 2009


4. West, H. and Apul, D.S. Documenting the Connection Between Water and Energy in Buildings: A Comparative Case Study on Environmental Footprint of Sending Rainwater to Sewers, Using Rainwater to Flush Toilets and to Irrigate, Posters at Capitol event for undergraduate students, April 2009, Columbus, OH


7. West, H., Robinson, L., and Apul, D.S. A Comparative Sustainability Analysis of Water Management Options for the Collier Building Addition on Health Science Campus of University of Toledo, presented by undergraduate student H. West at Air and Waste Management Association’s 102nd Annual Conference and Exposition, Detroit, MI, June 16-19, 2009


17. West, H. and Apul, D.S. Life cycle analysis of rainwater harvesting at University Dormitory, Engineering Sustainability 2011: Innovation and the Triple Bottom Line, Pittsburgh, PA, April 2011
Information Transfer Program Introduction

The Ohio Water Resources Center (WRC), at Ohio State University, conducted a number of activities to transfer water related information to a wide range of state, federal, county, and municipal agencies, to the private sector, academic community, students, and to private citizens throughout Ohio. Specific activities included: 1) Preparation of information for the web site of the Ohio Water Resources Center and maintenance of the web site 2) Administration of a Special Water and Wastewater Treatment Grants Competition funded through the Ohio Water Development Authority - administration of the 104(B) In-State Competition and the 104(G) National Competitive Grants Program - encouraged investigators of projects funded through the Ohio WRC to develop publications in peer-reviewed journals and other outlets 3) Continued administrative support for the Water Management Association of Ohio (WMAO) and associated WMAO meetings, conferences, and division activities 4) Support for Ohio Water Education Program, especially Project WET (Water Education for Teachers) 5) Participation of both directors in the WiE (Women in Engineering) GROW program which focuses on introducing 8th grade girls to careers in various areas of engineering, including water resources and environmental engineering 6) Responding to questions from the public regarding water resources issues in the state of Ohio 7) Assisting in organizing Ohio American Water Works Association (AWWA) South East district section meeting at Ohio State University to facilitate transfer of research results to the water treatment profession 8) Advising the newly started WEF/AWWA student chapter at Ohio State University
USGS Summer Intern Program

None.
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Notable Awards and Achievements

Project 2009OH89B "Monitoring the role of biofilm polymers against disinfectants in water distribution systems": Second place winner in student paper competition at the 2010 Ohio Section AWWA Annual Conference.

Project 2008OH64B "Competitive Learning to Develop a Biomarker Forecasting Tool for Classifying Recreational Water Quality" has resulted in a follow-up proposal submitted to the Metropolitan Sewer District (Cincinnati, OH) to continue this research. The proposal is "Development of a Recreation Management Tool to Predict Microbial Water Quality for the Metropolitan Sewer District" (2010), PI: D. L. Boccelli, co-PI: S. G. Buchberger, University of Cincinnati ($153,073)

Project 2009OH135B "Exploring Spatial and Temporal Demand Aggregation on Transport Characteristics in Distribution System Modeling": X. Yang received Best Student Paper Award, and was nominated for Best Paper Award -- top 10 out of approximately 150 papers at the 2010 Water Distribution System Analysis Symposium


Project 2010OH249O "A comparative sustainability analysis of water management options in buildings": (1) A new model, EEAST, was developed for sustainability evaluation of buildings’ water and sanitation infrastructures. EEAST stands for Economic and Environmental Analysis of Sanitation Technologies. EEAST is available for download at: http://utwatersustainability.wikispaces.com/ (2) Hannah West won first place in the undergraduate research category at the Sigma Xi Research Symposium of Toledo in October 2009. (3) Hannah West won the 2009 Ohio EPA Environmental Scholarship for her research related to rainwater harvesting in buildings. (http://ohio.gov/news/2009/07/#072309)