Connecticut Institute of Water Resources
Annual Technical Report
FY 2009

Introduction

The Connecticut Institute of Water Resources is located at the University of Connecticut (UCONN) and reports to the head of the Department of Natural Resources Management and Engineering, in the College of Agriculture and Natural Resources. The current Director is Dr. Glenn Warner, and the Associate Director is Dr. Patricia Bresnahan.

Although located at UCONN, the Institute serves the water resource community throughout the state. It works with all of Connecticut's water resource professionals, managers and academics to resolve state and regional water related problems and to provide a strong connection between water resource managers and the academic community. The foundation for this connection is our Advisory Board, whose composition reflects the main water resource constituency groups in the state. IWR staff also participates on statewide water-related committees whenever possible, enabling our Institute to establish good working relationships with agencies, environmental groups, the water industry and academics. Our seminar series, a long-standing Connecticut IWR tradition, provides a unique opportunity for the water resource professionals and interested members of the public in our small state to gather, be informed, and be come better acquainted.

Research Program Introduction

The USGS 104B program is the financial core of

the CT IWR. The Institute does not receive

discretionary funding from the state or the

university, although it does receive one third of the Associate Director's salary as a match for the administrative portion of our budget.

The majority of our 104B funds are given out as

grants initiated in response to our annual RFP,

with the majority of those funds going to

research projects. When selecting projects for

funding, the Institute considers three main

areas: 1. technical merit, 2. state needs and 3.

CT IWR priorities (use of students, new faculty,

seed money for innovative ideas). In addition to its 104B program, the Institute

conducts externally funded projects.

Development of a new generation of sensitive, fluorescence-based nitrate sensors for use in soil and water

Basic Information

Title:	Development of a new generation of sensitive, fluorescence-based nitrate sensors for use in soil and water
Project Number:	2007CT130B
Start Date:	9/1/2007
End Date:	8/31/2009
Funding Source:	104B
Congressional District:	CT District #2
Research Category:	Water Quality
Focus Category:	Methods, Nitrate Contamination, Nutrients
Descriptors:	
Principal Investigators:	Shawn Christopher Burdette, Zoe G Cardon

Publications

There are no publications.

Title: Development of a new generation of sensitive fluorescence-based nitrate sensors for use in soil and water

Statement of regional or state water problem:

Throughout the world, eutrophication of tributaries, rivers, estuaries, and coastal marine ecosystems by point and non-point sources (e.g. industry, sewage, development, atmospheric deposition and agriculture) is changing these ecosystems' biogeochemical function, ecology, and the human institutions dependent on them [1, 2]. Roughly one-third of the nitrogen (N) reaching Long Island Sound (LIS) is derived from non-point sources, and these non-point sources, and the sinks, transport and processing of N in pathways leading to LIS, are not well understood. Integrating data from 28 water monitoring stations (23 in CT) with land-use/land-cover information, population density, runoff volumes, and other landscape characters, Mullaney et al. (2002) developed a simple linear regression model capable of predicting N-loads and yields in streams as a function of watershed characteristics [3]. This model was used to estimate N-loading in unmonitored watersheds, but unexplained discrepancies were found that indicated unknown but important system drivers were affecting N-loading in both agricultural and forested landscapes [3]. In particular for the current effort, researchers noted that the type of forest cover (e.g. percent deciduous tree cover) significantly affects N-loading [3], suggesting either that the dominant tree types themselves [4], and/or perhaps edaphic factors correlated with those forest communities [5], strongly affect N-yield from forested areas. Recently published data suggest that dominant northeastern forest deciduous and evergreen species are associated with distinct rates of N-cycling and N-retention in the soils supporting them [4, 6]; these distinct N-cycling rates could lead to distinct N-load "signatures" in watersheds that correlate with forest cover.

Ultimately, we would like to explore how patterns of N-cycling, and dynamics of various dissolved N pools, correlate with the patterns and growth of diverse New England forests, but current monitoring methods are inadequate. Though current ecosystems ecological techniques for quantifying net and gross mineralization and nitrification, as well as microbial immobilization, of nitrogen have certainly led to greater understanding of patterns in and mechanisms underlying N-cycling in forested systems, those techniques invariably require destructive harvest of soil prior to assay [7]. Unfortunately, digging up the belowground system under study necessarily leads to severing plant roots that contribute carbon to soil, breaking hyphae of mycorrhizal and saprotrophic fungi, mixing up soil layers, and breaking up soil aggregates that otherwise can have cores hypoxic enough to support denitrification. Ecosystems ecology sorely needs a suite of miniature sensors capable of being deployed to continuously monitor dissolved nitrogen species in soils. Because soil processes are notoriously heterogeneous, ideal would be development of an inexpensive and sensitive enough design to support deployment of a suite of such sensors in multiple locations across a watershed so that networked, continuous pool data could be gathered across the landscape. Ecosystems ecologists have long used stream water concentrations of ions as an integrated measure of terrestrial system output (for example demonstrating that immobilization of otherwise mobile essential nutrients in new aggrading forest biomass after logging leads to dramatic and seasonal decreases in nitrate concentrations in streamwater [8]). But the N processing on the terrestrial landscape itself has been studied destructively, not continuously, and often not *in situ*.

Ecosystems scientists are not alone in needing improved monitoring tools; land managers and environmental engineers also seek sensors that can be deployed in order to detect plumes of contaminants, including nitrate, moving through groundwater. Development of small nitrate sensors is already underway in that context, e.g. at UCLA (see the Center for Embedded Networked Sensing focus on contaminants and terrestrial ecosystems [9]). However, the concentrations of nitrate in a contaminated plume are much higher than background concentrations in forested systems. McDowell showed that nitrate concentrations in soil solution (extracted using zero tension lysimeters, and analyzed in the lab) were between 0 and 1 mg L^{-1} over a time span of ten years in unmanipulated hardwood and pine stands at Harvard Forest [10]. In contrast, in plots experiencing chronic high N deposition (15 g N m^{-2} yr^{-1}) for those same ten years, nitrate concentrations in soil solution ranged between 10 and 28 mg L^{-1} in pine and hardwood stands. In order to have any hope of monitoring, continuously, shifts in pools of nitrate (and potentially, ultimately, other dissolved N species) in soil solution under particular forest tree species, and in streams draining relatively pristine rather than highly polluted watersheds, very sensitive, miniature sensors are needed. Ideally, such a sensor would be inexpensive

and could be coupled into some of the already established protocols for networking sensors like those being developed at CENS. The first step, however, is how to improve sensitivity of sensors to nitrate in low (background or pristine) concentration in freshwaters and in soil solution, and how to package the sensors in a miniaturized form that is relatively inexpensive.

We aim to develop miniature, "turn-on", fluorescence sensors for nitrate that, ultimately, can be deployed in sets in soil and in freshwaters to report nitrate concentrations continuously in background and low contamination ranges – e.g. 0-2 mg per liter. Nitrate sensors for deployment *in situ* are already on the market, for example designed for work in oceans (e.g. UV sensor, Satlantic's ISUS V2) and streams and groundwater (e.g. Hydrolab nitrate ion selective electrodes [11]). Some are already very sensitive. Satlantic, for example, claims sensitivity of 0.007-28 mg nitrate per liter, +/- 0.028 or 10% of the measurement, whichever is larger. But, the sensor is 2 ft long, 4 inches in diameter, and weighs 11 lbs. New amperometry-based nitrate detectors for use in soil and groundwater are becoming more sensitive, but to date their major focal application has been on highly contaminated nitrate plumes in groundwater [9], though the focus is also shifting to detection in more undisturbed, terrestrial ecosystems. It is important to understand the background N-cycling processes occurring in more pristine environments in order to understand the magnitudes and multiple mechanisms of human impacts on that cycling, yet we lack the tools for *in situ* monitoring of pools within the heterogeneous N-cycling pool and flux network.

Significance: Statement of results or benefits

Fluorescent sensors are an attractive target for developing a next generation of nitrate detection systems, because fluorescence methodologies are more sensitive, and easier to apply than current technologies. Assistant professor of chemistry Burdette specializes in the chemical synthesis and the principles of photochemistry relevant to sensor construction. Funding from CTIWR for 2 years that is earmarked to support work on developing a fluorescence-based nitrate sensor, and would provide the necessary seed money to initiate the molecular design of a sensing system. Cardon can guide the preliminary tests of sensitivity in real samples from the field, and analysis of comparable data from other detection methods (e.g. anion exchange membrane and buried resin bag-estimates, as well as KCl extraction-estimates of nitrate pools in soil [7]). With a proof-of-concept sensor in hand, more funding (e.g. from NSF's Bioengineering and Environmental Systems program) will be sought to advance the technology beyond molecular design toward the miniaturization and field-readiness of continuous sensing systems. Because Cardon serves on the national SAMSI program steering committee for development of mathematical and statistical analysis of sensor network data [12], she already has the necessary contacts to help bring the established technology rapidly to an interested community nationwide. Cardon has already collaborated with John Mullaney at USGS and Paul Stacey at DEP in CT writing grant proposals (to NSF) to further explore nitrogen processing in Connecticut's watersheds and Long Island Sound. The links from the sensor development proposed here to CT government agencies thus promises to be a natural progression.

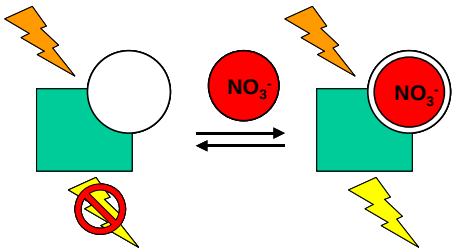

Figure 1. Representation of a typical fluorescent sensor. Whether sensor refers to a small molecule, a polymer or a device, the signaling action involves fluorescence emission (yellow bolt) that occurs only in the presence of analyte binding (NO_3^-) when the sensor is exposed to excitation light (orange bolt).

Nature, scope, and objectives of the project

Fluorescent Sensors (Fig. 1). Fluorescent sensors have been vital in identifying the cellular functions of metal ions [13], as well as the impact of environmental contaminants like mercury [14] and polychlorinated biphenyls [15]. With appropriate molecular design, fluorescent sensors are capable of making sensitive measurements using signals that are easy to monitor [16]. Since these chemical tools are typically constructed using modular approaches, simple structural modifications can be made to adapt the sensor for a variety of different applications and circumstances. Analyte concentrations from sub-pM (equivalent of 0.1 μg/L of nitrate) to saturated solutions can be accurately measured. In addition to these advantages, fluorescent sensors

are particularly amenable for monitoring environmental analytes because the imaging reagents and instrumental techniques are non-evasive and simple to employ. One distinct improvement over conventional techniques for measuring nitrate concentrations is that sensors are typically inexpensive and can be designed to be either disposable or reusable. As a result, an array of many individual sensors can be distributed easily over a large area and monitored by one research worker using an inexpensive, portable fluorescence spectrometer. While methodologies for constructing some types of fluorescent sensors are straightforward, designing useful sensors for anions presents a significant challenge. Although fluorescent anion sensors exist, very few systems have been reported for nitrate [17].

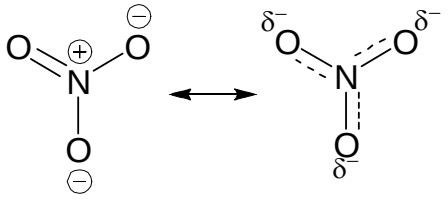

Figure 2. The structure of the nitrate anion. The structure on the right shows the composite resonance structure the best describes the chemistry and structure. The molecule is trigonal planar (120° O-N-O angles) and has a negative charge distributed evenly over the 3 oxygen atoms.

Nitrate chemistry. The most challenging obstacle to overcome when constructing a nitrate sensor is finding a receptor to bind what is essentially a non-coordinating anion (Fig. 2). Nitrate is a weak base that does not form covalent bonds with metal cations or protons readily, a behavior that is consistent with it being the conjugate base of a strong acid (nitric acid). In the majority of nitrate complexes characterized crystallographically to date, the nitrate group is located several angstroms away from other atoms, hence the nomenclature “non-coordinating” [18]. Unlike metal cations that can form strong covalent bonds with receptors containing electron-donating atoms like oxygen or nitrogen, anion receptors usually rely on noncovalent interactions like hydrogen bonding or electrostatic interactions for chelation [19]. Noncovalent interactions are weak forces, so it is counterintuitive that a receptor using these interactions can bind an analyte tightly; however, if a receptor takes advantage of several noncovalent interactions simultaneously, a reasonably high affinity interaction can be established.

Another challenge in anion receptor design is the ability to discriminate between other anions. In particular for nitrate found in the environment, anions like chloride (Cl^-), sulfate (SO_4^{2-}), and phosphate (PO_4^{3-}) could interfere with measurements of nitrate concentration if the receptor lacks selectivity. In order to enhance selectivity for nitrate, the receptor can take advantage of the coordination number and charge density. Nitrate is a trigonal planar anion, as opposed to primary oxoanions competitors like PO_4^{3-} and SO_4^{2-} (tetrahedral), and anions like hydroxide (HO-) and halides (F^-, Cl^-, Br^-, I^-) with simple geometrical shapes. Nitrate is monoanionic, with the charge distributed evenly over the 3 oxygen atoms, making them the primary targets for noncovalent interactions with receptors. The monoanionic, trigonal planar structure of nitrate is an uncommon structural motif for anions found in soil and water. Therefore, receptors that can only accommodate a trigonal anionic guest will provide the desired binding selectivity.

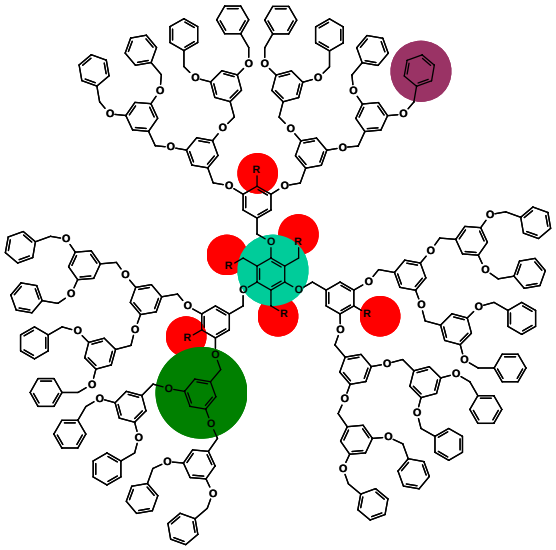

Figure 3. The basic structure of a dendrimer, shown is a 3rd generation poly(benzyl ether). The generation number refers to the number of repeating building blocks (green) between the core (blue) and the periphery (purple). The structure of the repeats and periphery groups determine the physical properties of the dendrimer, and can be changed to attain the desired features. Dendrimers of generation 1-5 are straightforward to prepare. In addition, functional groups such as nitrate receptors or fluorophores (red, “R” groups) can be incorporated at the core, or with in the branching units as needed. For use in water, the peripheral groups will be water soluble (polyethylene glycol) for soil-based systems, hydrophobic organic groups will be utilized.

Dendrimers. Dendrimers are globular polymers frequently prepared using conventional organic synthetic methodologies (Fig. 3). When convergent synthetic methods are employed [20], dendrimers have uniform size, shape and molecular weight, a property reminiscent of enzyme structures [21, 22]. Because of their unique properties, dendrimers are attractive targets for a variety of applications in catalysis [23], medicine

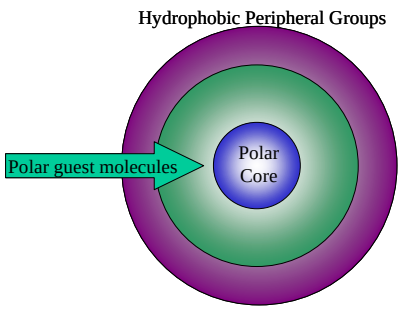

Figure 4. Dendrimers as concentrators. In more hydrophobic environments like soil, a concentrator effect will help to transport nitrate inside the sensor.

[24] and sensing [25]. Several properties of dendrimers make them attractive scaffolds for environmental nitrate sensors. Dendrimers can be prepared with robust chemical linkages that will resist degradation by microrganisms and variations in acidity/basicity in soil and water. Dendrimers also provide encapsulation, and hence protection, for the nitrate receptors and fluorophores that are susceptible to decomposition reactions under the conditions the sensors will be deployed. Most importantly, however, dendrimers can act as "molecule concentrators" (Fig. 4) [26]. When mobility of an analyte like nitrate is lower (like in soil before reaching water), the ability of the dendrimer to concentrate a substrate will amplify the resulting fluorescent signal. The ability of a dendrimer to act as a concentrator, as well as many other physical properties, is dictated by its chemical structure. Dendrimers have three important structural components, the core, the branching groups (polymer repeat units) and the peripheral groups, that can be varied to tune the properties of the resulting macromolecule. As shown in Figure 4, a dendrimer with a hydrophobic periphery and a polar interior will be predisposed to concentrate a charged molecule like nitrate from nonaqueous sources.

Specific Aims

1. Construct nitrate receptors for incorporation into dendritic fluorescent sensors
2. Verify the ability of dendrimers to concentrate nitrate anions
3. Investigate the strategy of using accumulation of anionic charge inside a dendrimer to modulate the emission intensity of polarity sensitive fluorophores
4. Investigate the viability of displacing a negatively charge fluorescence quencher at the core of a fluorescent dendrimer as a detection strategy
5. Investigate the strategy of using charge-induced swelling and contracting of dendrimers to induce fluorescence resonance energy transfer
6. Demonstrate proof-of-concept nitrate sensing with dendrimers in prepared solutions and environmental water samples
7. Devise methodology to attach/adsorb dendritic sensors onto glass surfaces for nitrate sensing in soil samples

Methodology

General sensor design strategy: dendrimer structure and nitrate receptors. The majority of the existing sensors for anions (e.g. F^-, Cl^-, Br^-, PO_4^{3-}, CO_3^{2-}) rely on either photoinduced electron-transfer (PET), which requires the formation of covalent bonds with the receptor, or electronic energy transfer (EET), a "turn-off" mechanism which requires an analyte with an electronic structure that triggers fluorescence quenching, as the signaling mechanism [17]. Neither of these common strategies is applicative to the disparate requirements of sensing nitrate. An alternative approach to anion sensing is to couple an accumulation of negative charge with a change in the fluorescence intensity. In aqueous solution, nitrate can freely flow into the dendrimer, and be trapped by the receptors; however, in soil nitrate may be less modile with respect to entering the dendrimer. In order to concentrate anions, dendritic molecules will be utilized as concentrators. As a consequence of dendrimers possessing hydrophobic peripheries and polar cores, a concentration gradient is established between the interior of the macromolecule and the exterior that provides the driving force to amass nitrate. All three sensor strategies described below will take advantage of

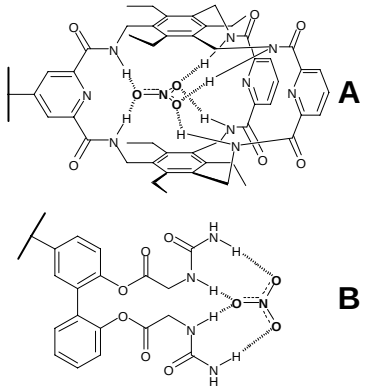

Figure 5. Examples of nitrate receptors. Both cryptate (A) and "tweezer" type ligands selectively bind nitrate (illustrated) in the presence of other anions. The dendrimer attachment site is shown as the squiggly line on the left of each receptor.

the concentrator effect to sequester nitrate in soil. For sensing in water, the hydrophobic peripheral groups will be substituted for with water-soluble groups like short polyethylene glycol (PEG). In addition to concentrator effects, the interior of the dendrimer will be functionalized with molecules capable of binding nitrate to provide anion selectivity and trapping. Several receptors have successfully been applied to nitrate binding (Fig. 5). Both cryptate [27] and "tweezer" type ligands [28] possess nitrate selectivity, because of the common feature that the binding cavity orients hydrogen-bonding interactions in a trigonal planar coordination sphere. These molecules will be the primary candidates for incorporation into the sensors in this initial phase of the research project. Design of new and improved nitrate receptors will not be a major component of CTIWR funded research; however, future proposals and projects will address any limitations of these systems.

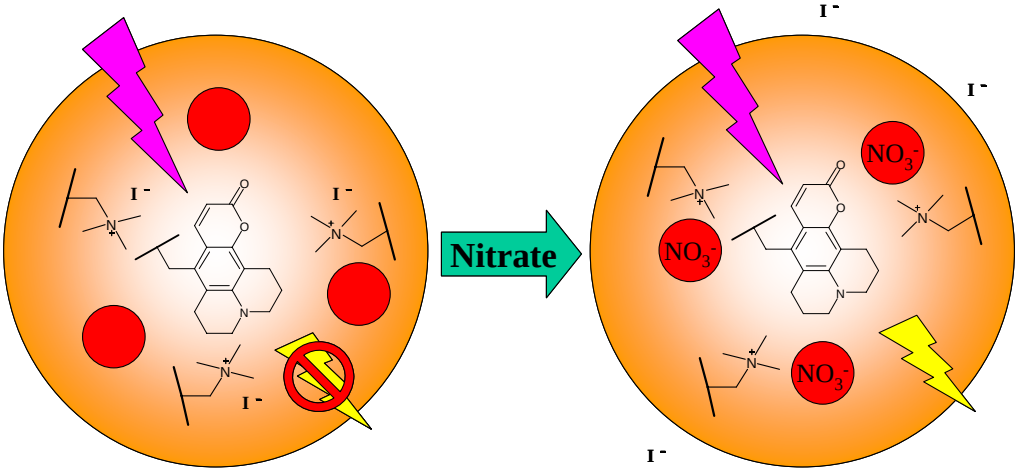

Figure 6. Mechanism of signaling action with quenching anions. The presence of iodide anions quenches the emission of the coumarin fluorophore (center), when nitrate binds to the receptors, the iodide will be expelled from the dendrimer restoring the emission.

Displacement of a quenching anion by nitrate as a sensing strategy. Although nitrate typically is incapable of quenching fluorescence, large anions (e.g. Br^-, I^-) quench fluorescence through enhancement of spin orbit coupling (SOC) [29] and electron deficient anions (e.g. 4-nitrobenzene-sulfonate) through SOC [30] or EET [17]. A simple proof-of-principle sensor for this strategy can be constructed with a dendrimer functionalized with ammonium iodide groups and fluorophores. Diffusion of nitrate into the interior will displace the quenching anions restoring fluorescence (Fig 6).

Principle Findings: Progress Report

General progress. Our work has focused on proof-of-concept research that is focused on two main areas: 1) synthesis of water soluble dendrimers; and 2) synthesis and characterization of sensors containing an anion receptor, a fluorophore and dendrimer mimetic substituents. We have enlisted an undergraduate researcher to continue work this summer. Future progress will be dependent on finding new personnel and additional mechanisms of financial support.

Dendrimers and Water Soluble Dendrimer synthesis. During our first year we synthesize poly(benzyl ether) dendrimers, and to date we have prepared G_1-G_3 dendrimers with

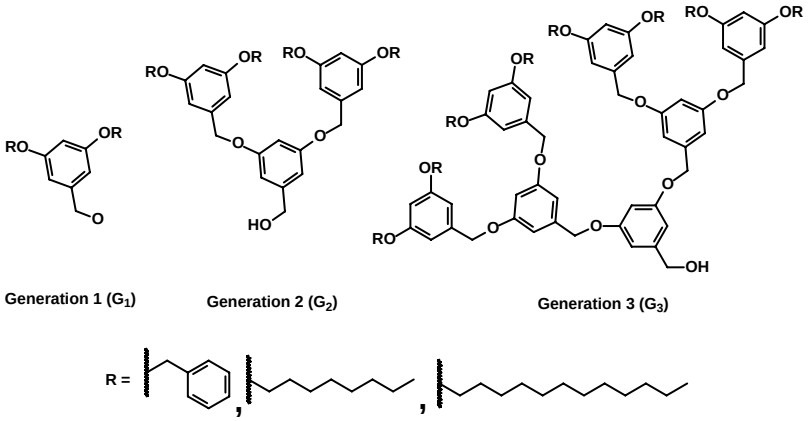

Figure 7. Dendrimers prepared to date

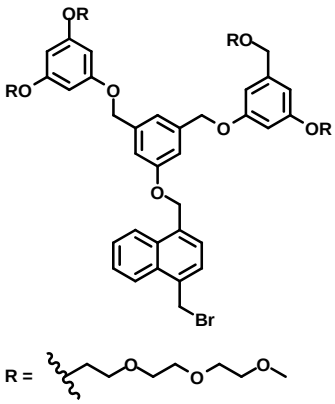

Figure 8. G2 water soluble dendrimer. G1 and G3 versions have also been prepared for incorporation into sensors.

benzyl groups as well as alkyl chains on the periphery (Fig. 7). These components of the model nitrate sensor are available for assembly of the dendritic nitrate sensors proposed originally. We have expanded our efforts in anticipation of constructing sensors for deployment in aqueous solution. Shown in Figure 8 is a water soluble benzyl ether dendron with a naphthalene group at the focal point. The poly(ethylene glycol) units will impart water solubility to future sensors and the naphthalene group is an emissive species that will provide the sensor readout.

Ntrate receptor synthesis. As we suggested in our original proposal, our 1st generation of nitrate sensors will utilize known nitrate receptors that we will modified to act as fluorescence switches or to contain synthetic handles for attachment to macromolecules. Based on literature precedence, we have modified the structure of a biphenyl-based nitrate receptor to contain a binaphthol scaffold (Fig. 9). We accessed the binaphthol ligand through a multi-step synthesis that is amenable to making a variety of related derivatives or making additional structural modifications. Unlike biphenyl, binaphthol is inherently fluorescent, but we have not observed any emission changes when it binds nitrate; however, this may still be a useful sensor component by incorporating components of the anion sensing mechanism described below.

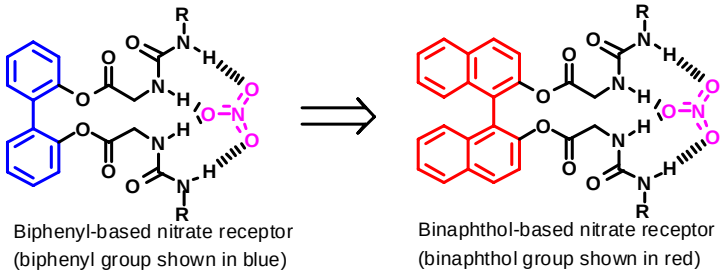

Figure 9. We have substituted a fluorescent binaphthol group (red) for biphenyl one (blue) in a nitrate (magenta) receptor. Additional structural changes are possible using our synthesis, and fluorescence studies are being initiated on the new receptor.

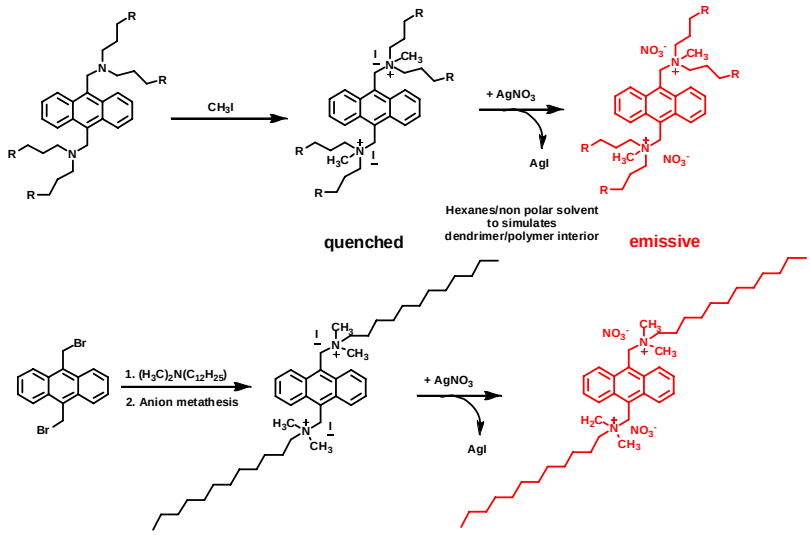

Figure 10. Proof-of-concept nitrate sensing with alkyl ammonium anthracene compounds. Displacement of iodide anions with nitrate anions removes a potent fluorescence quencher away from the sensor.

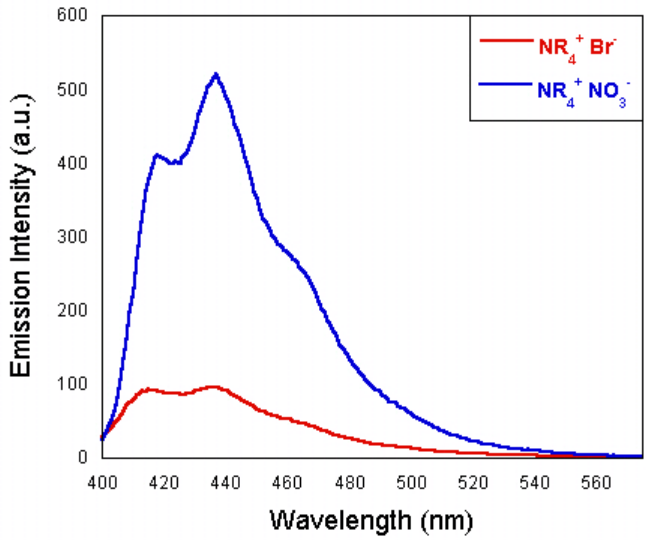

Figure 11. Fluorescence response of ammonium iodide species to silver nitrate. The nitrate species is approximately 5X more emissive than the iodide consistent with our predictions.

Investigation of sensing mechanisms. One of the key preliminary studies we have undertaken is an attempt to determine a mechanism that will allow changes in nitrate concentration to increase sensor emission. As described in the proposal, nitrate in a weakly coordinating anion, and therefore many common sensing mechanisms are not appropriate for our purposes. We successfully tested the hypothesis that nitrate can displace a quenching group that is in proximity to a fluorophore by preparing an anthracene derivatives containing two tetra-alkyl ammonium iodides (Fig. 10). The lipophilic alkyl groups to give the resulting sensor solubility in nonpolar solvents like hexanes or toluene. In these nonpolar solvents, the iodide anions form a close ion pair with the

ammonium cation, and the close proximity of the iodide quenches the emission of the anthracene by creating spin-orbit coupling quenching pathways. Subsequent displacement of the quenching iodides by with nitrate restores anthracene fluorescence (Figure 11). The formation of insoluble silver iodide drives the formation of the ammonium nitrate species. we will begin to integrate our sensing strategy with the receptor and dendrimer components. An sample target we are working toward is shown in Figure 12.

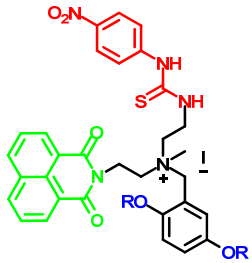

Figure 12. Modular representative of nitrate sensors under preparation. The sensor incorporates dendrimer (blue), anion receptor (red) and a fluorophore (green).

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CTIWR ADMIN NOTE: The PI was contacted and said that last year's status report was complete – that there was nothing new to report. So I have repeated it this year.

- Pat Bresnahan, June 1, 2010

The Geochemical Record of Cultural Eutrophication in Sediments of Beseck Lake and Lake Waramaug, Connecticut: Implications for Nutrient Cycling and Remediation Efforts

Basic Information

Title:	The Geochemical Record of Cultural Eutrophication in Sediments of Beseck Lake and Lake Waramaug, Connecticut: Implications for Nutrient Cycling and Remediation Efforts
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Principal Investigators:	Timothy Ku

Publication

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The Geochemical Record of Cultural Eutrophication in Sediments of Beseck Lake and Lake Waramaug, Connecticut: Implications for Nutrient Cycling and Remediation Efforts

Proposal IDs: Ku2007_104B_R, Ku20082009_104B_R
Principle Investigator: Timothy Ku, Wesleyan University

Problem and Research Objectives

Anthropogenic activities have dramatically altered the biogeochemical cycles of carbon, sulfur, nitrogen, and phosphorous in nearly every major aquatic ecosystem on Earth (Smith, 2003). Cultural eutrophication is the process whereby human activity increases the amount of nutrients, primarily nitrogen and phosphorous, entering an aquatic ecosystem causing excessive biological growth. The accelerated production of autochthonous organic matter results in anoxic conditions within the water column, thereby changing the community structure of aquatic ecosystems and degrading the recreational and retail value of the surrounding land. Eutrophication is a widespread environmental problem as it accounts for ~50% of impaired lake area and 60% of impaired river reaches in the U.S. (U.S. EPA, 1996). In Connecticut, prior to the mid-1800s the land was mostly forested, but increases in agriculture, urban, and residential land areas contributed to the eutrophication of several Connecticut lakes by the 1930s (Deevey, 1940; Bell, 1985). Progressive eutrophication associated with land-use changes continued in many Connecticut lakes as average Secchi disk depths decreased by 1.2m and total phosphorous concentrations doubled from the late 1930s to the early 1990s (Siver et al., 1996). During approximately the same time, mean estimated total phosphorous (eTP) and mean estimated total nitrogen (eTN) concentrations increased from 15 and 374 μg/L to 25 and 450 μg/L, respectively (Field et al., 1996). These results, together with biological-based paleolimnology studies, clearly demonstrate that anthropogenic activities have accelerated the eutrophication process in many Connecticut lakes (Siver et al., 1999).

To reverse or decelerate cultural eutrophication, many regulatory agencies have implemented stringent laws intended to lower the delivery rate of nutrients into impacted water bodies such as the Chesapeake Bay, Lake Erie, and Lake Baldeggersee (Switzerland) (Lotter, 1998; Boesch, 2002). In Connecticut, the Long Island Sound study aims to reduce nutrient inputs delivered to Long Island Sound, but restoration or preservation of inland lakes is usually the responsibility of local governing agencies working in conjunction with the Connecticut Department of Environmental Protection (NYSDEC and CTDEP, 2000). This study focuses on two eutrophic Connecticut lakes that have been the focus of major remediation efforts, Lake Waramaug and Beseck Lake. Lake Waramaug experienced significant eutrophication from the 1950s through the 1980s and two hypolimnetic withdrawal systems were installed in 1983 to contain the phosphorous in the bottom waters of the lake. Since 1983, additional remediation efforts have included the installation of two in-lake layer aeration systems, the passing of new zoning regulations to limit soil and water runoff, and the stocking and seeding of fish and zooplankton to improve water quality (http://www.lakewaramaug.org). The Lake Waramaug Task Force (LWTF) is a non-profit organization of volunteers and scientists and together with Ecosystem Consulting Services, Inc. has continuously monitored the lake since 1977. During this time, the lake water clarity has improved and epilimnion phosphorous concentrations have declined (ECS data, pers. comm.). Beseck Lake is a manmade impoundment created by a dam in

the mid 1800s and has experienced episodic eutrophic conditions from the 1970s to the 1990s, in part due to the addition of nutrients from failing septic systems (Canavan and Siver, 1995; Cinotti, 1997; Jacobs and O'Donnell, 2002). To decrease the flux of nutrients entering the lake, surrounding residences were converted from septic systems to a city sewer system. This transition was completed in 2002 and the Lake Beseck Association now helps maintain and monitor the lake water quality (R. Boyton, pers. comm.).

While these efforts to decelerate the eutrophication process have yielded positive results, future remediation policies must set realistic goals of water quality (chemical composition and biologic activity). By using historical data, time series data, or reference region data, regulatory agencies can determine pristine water quality conditions that are absent of the effects of human activity (Smith, 2003). Sediment cores collected from Beseck Lake and Lake Waramaug record the pre-anthropogenic lake conditions and the onset and remediation of cultural eutrophication. This project examines the history of these two lakes, which will help guide future remediation efforts in Beseck Lake and Lake Waramaug as well as in other worldwide lakes experiencing similar eutrophication problems.

Methodology

The three main objectives of this study are 1) determine the sedimentation rates of organic C, organic N, and detrital minerals, 2) determine the source of organic matter and detrital minerals, and 3) determine the paleoredox history of these lakes. Sediment push cores and freeze cores were collected using a pontoon boat. Linear sedimentation rates (LSR, cm/yr) and mass accumulation rates (MAR, g/cm^2/yr) were determined by ^{210}Pb, ^{137}Cs, and Hg and Pb methods (Appleby and Oldfield, 1992; Siver and Wozniak, 2001; Callender, 2004; Fitzgerald and Lambourg, 2004; Varekamp et al., 2005). Organic C and N concentrations were analyzed with an elemental analyzer. Major, minor, and trace element compositions were determined by digesting sediments in a HCl/ HNO_3/ HF/ $HClO_4$ solution followed by ICP and ICP-MS analyses. δ^{13}C and δ^{15}N organic matter measurements were performed at the Stable Isotope facility at Indiana University. Paleoredox indicators (DOP, $\delta^{34}S_{pyrite}$, and pyrite framboid size distributions) were analyzed using Fe-S methods and sediment phosphorus species (labile P, Al-P, organic P, and Fe-P) were determined by a sequential sediment extraction method (Canfield et al., 1986; Raiswell et al., 1994; Wilkin et al., 1996; 1997).

Principal Findings and Significance

Eight and seven sediment cores were collected from Lake Waramaug and Beseck Lake, respectively. Three sediment cores have been dated and represent 200 to 400 years of lake history. In Lake Waramaug, the Hg and Pb sediment concentrations increase at ~1900 A.D. due to fossil fuel use related to the Industrial Revolution and peak concentrations are found between 1950-1970 A.D. Compared to times before ~1900 A.D., Lake Waramuag experienced a period of higher C/N ratios shortly after 1900 A.D. that indicates a greater proportion of allochthonous organic matter being delivered from the surrounding watershed. This could be related to increased forest clearing, a major storm event, or a period of increased rainfall. The organic C, C/N, and δ^{15}N values (background 0.4‰) all indicate increasing cultural eutrophication throughout the 1900s and the highest organic matter δ^{15}N value of +3.1‰ occurring in the 1970s-1980s. The δ^{15}N values decrease from this peak time to values of +1.9‰ today and this is likely related to the remediation efforts of the Lake Waramaug Task Force, which significantly

decreased the external nutrient inputs and implemented in-lake restoration solutions since the 1970s-1980s. This finding is significant because this represents one of the few cases where remediation results are documented by lake sediments. Sediment iron-sulfur paleoredox indicators show high spatial and temporal variability demonstrating that Fe-S-P cycling has been greatly altered by human pollution and remediation activities. In general, our data show that the oxic/anoxic interface has fluctuated significantly over the last ~70 years, which has caused the fraction of Fe-bound phosphorus to decrease in recent times. Additionally, sulfuric acid from acid rain sources appears to have increased the ratio of pyrite Fe to reactive Fe, which would further decrease the amount of reactive iron available to bind phosphorus. Due to the high variability of Fe-S-P chemistries between sediment cores, we have had to 210Pb-date more cores than anticipated to firmly establish sediment chronologies. This data is currently being collected and we anticipate that all data will be collected during the summer of 2009 and the first manuscript focusing on Lake Waramaug will be submitted for publication in August 2009.

In Beseck Lake, the sediments document the time from prior to the mid-1800s, when the lake water was raised by damming the outflow, to the last few years. Higher C/N ratios mark the older swamp sediments and increased cultural eutrophication results in greater concentrations of organic C, lower C/N ratios, and higher $\delta^{15}N$ values. Unlike Lake Waramaug, a decrease in $\delta^{15}N$ values is not observed, however, that signal may be lost due to moderate bioturbation of the bottom sediments. The sediment Fe-S-P chemistries do not show significant changes over time, but rather show clear correlations with current water column heights and average overlying dissolved oxygen concentrations. In shallow, oxic portions of the lake, there is low total phosphorus and most of the phosphorus is found in non-Fe phases. However, in the deeper, anoxic portions of the lake, there are higher total phosphorus concentrations with iron-phases comprising the most important P-binding phase. These results highlight the importance of iron and sulfur cycling to the available phosphorus budget and demonstrate that the dominant P-bearing phase may shift due to human activities.

Our findings will help the Lake Waramaug Task Force and Beseck Lake Association with future remediation decisions and provide the scientific community with a rare opportunity to compare recent sediment geochemistry with long-term, remediation efforts. We expect that future researchers will use the techniques and results from this study to examine other eutrophic water bodies, thereby making Beseck Lake and Lake Waramaug the model examples for this type of work.

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Rapid and Sensitive Detection of Total Coliforms and E. coli Using SWNT Membrane

Basic Information

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Publications

1. L. Su, W.Z. Jia, C.B. Beacham, Yu Lei. Rapid and sensitive detection of E. coli using SWNT membrane. Applied Biochemistry and Biotechnology. 2010. In preparation.
2. L. Su, W.Z. Jia, , L.C. Zhang, H.V.S. de los Monteros, Yu Lei. Novel free-standing monolayer Pt nanoflowers-SWCNT composite membrane: preparation, characterization and glucose sensing application. 2010. Advanced Materials. In preparation.
3. L. Su, W.Z. Jia, C.J. Hou, Yu Lei. Recent progress in microbial biosensors. 2010. Biosensors and Bioelectronics. In preparation.
4. Y. Ding, Y. Wang, Yu Lei. Direct electrochemistry and electrocatalysis of novel single-walled carbon nanotubes-hemoglobin composite microbelts – Towards the development of sensitive and mediator-free biosensor. 2010. Biosensors and Bioelectronics. Submitted.
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9. L. Su, W.Z. Jia, A. Schempf, Y. Ding, Yu Lei, Free-standing Palladium/polyamide 6 Nanofibers for Electrooxidation of Alcohols in Alkaline Medium. 2009. Journal of Physical Chemistry C. 113 (36) pp. 16174-16180.
10. L. Su, W.Z. Jia, A. Schempf, Yu Lei, Palladium/Titanium Dioxide Nanofibers for Electrooxidation of Glycerol in Alkaline Medium. 2009. Electrochemistry Communications. 11 (11) pp. 2199-2202.

Rapid and sensitive detection of *E. coli* using SWNT membrane

1. Introduction

Escherichia coli, which belong to fecal coliform, is the most important indicator for fecal contamination in aqueous systems.[1] Therefore, the fast determination of *E. coli* is essential for monitoring the microbiological water quality and mitigating the risks associated with water contamination. *E. coli* have the ability to produce β-D-galactosidase and β-D-glucuronidase activity, both of which are inducible and can be employed in enzyme assays for the bacterial detection.[2]

In the past decades, a variety of technologies have been developed for determining the number of waterborne microorganisms in drinking water. Among all the techniques, amperometric method has demonstrated to be rapid, sensitive, cost-effective, and user-friendly.[3,4] Many efforts have been focused on the construction and modification of the working electrode with batch analysis mode or flow injection analysis mode.[5,6,7,8] A detection limit of 1×10^{6} cfu of *E. coli* mL^{-1} in 5 mL working solution was obtained using a tyrosinase composite biosensor without any pre-concentration or per-enrichment.[8] To the best of our knowledge, this is the best result for the electrochemical detection of *E. coli* reported to date. However, redundant operation, such as permeabilization step, was still included, therefore, inevitably increase the overall analysis time.

Carbon nanotube (CNT) has drawn considerable attentions due to its unique electrical and mechanical properties.[9] Recently, highly porous CNT membrane was prepared by chemical vapor deposition (CVD).[10] Enlightened by this study, we consider to fabricate free-standing CNT membrane electrode with a more straightforward method and apply it in the electrochemical detection of *E. coli*. Preferably, conductive free-standing membrane can make the bacterium of interest directly contact the electrode and keep them within a tiny space, therefore, greatly increase the efficiency of electron transfer and dramatically concentrate the bacteria without actually increase the number, respectively. At the end of this report, we also

demonstrate the electrodeposition of platinum (Pt) nanoparticles on the CNT membrane electrode, which makes it possible to further increase the sensitivity of this method due to the catalytic potential of Pt on the oxidation of the electroactive compounds applied in this work.

2. Experimental section

2.1 The fabrication of CNT membrane

0.4 g/L Single-walled carbon nanotubes (Unidym) and 10 g/L sodium dodecyl sulfate (SDS) were sonicated for 30 min by ultrasonic processor (Cole Parmer) in deionized (DI) water, and then diluted to different concentrations (5 mg/L or 10 mg/L) for further treatment. 5 mL CNT solution was filtrated through a Nuclepore polycarbonate (PC) membrane (Whatman, 47 mm diameter) with diverse pore sizes (0.4 μm or 0.05 μm). The CNT-PC was then thoroughly washed with DI water and dried at room temperature.

2.2 Bacteria cultivation and immobilization

Wild-type *E. coli* belong to the microorganism biosafety level Two (BSL-2) group, and all safety considerations concerning this group were accomplished through out the experiments.

Wild-type *E. coli* stock cultures, collected from Mirror Lake, Storrs, CT, were recovered overnight (10 hrs) in LB medium at 37 ˚C with shaking. 10 μL overnight culture solution was added in 10 mL fresh LB medium supplemented with 1 mM isopropyl β-D-galactropyranoside (IPTG) or methyl β-D-glucuronide sodium salt (MetGlu) for certain time (3 hrs or 5 hrs). Different numbers of *E. coli* were immobilized on pristine PC membrane (0.4 μm) and covered on the free-standing CNT-PC electrode to carry out the amperometric detection. In this way, the bacteria were confined between the two membranes. The immobilized *E. coli* was re-suspended in phosphate buffer solution (0.1 M, pH 7.4) for 10 min. 4-aminophenyl-β-D-galactopyranoside (4APGal, Sigma-Aldrich) or 8-hyroxyquinoline glucuronide (8-HQG, Sigma-Aldrich) was added in the system according to the purpose and the current response was recorded.

2.3 Electrodeposition of Pt

2 mL sulfuric acid (H_2SO_4, 0.1 M) with 1 mM hydrogen hexachloroplatinate (H_2PtCl_6) served as the electroplating solution and cyclic voltammetry (CV) was performed in the range of 0 to -1 V with a scan rate of 0.01 V/s for 10 cycles.

2.4 Equipments

The morphology of the pristine PC membrane, CNT-PC membrane, and Pt deposited CNT-PC membrane was examined by field emission scanning electron microscope (FESME, JEOL 6335F). All the electrochemical experiments were carried out using a home-made plate material evaluating cell (Figure 1) on CHI 601C electrochemical workstation (CH Instruments) at room temperature. A platinum electrode and a Ag/AgCl (0.21 V vs. SHE) electrode were used as the counter and reference electrode, respectively.

3. Results and discussions

3.1 Characterization of CNT-PC working electrode

Fig. 2 shows the SEM images of the as-prepared CNT-PC membrane with different CNT loadings (Fig. 2A and B) as well as the pristine 0.4 μm PC membrane (Fig. 2C) serving as the template. One can see that CNT networks were formed on the PC membrane with relative uniform distribution. The pores of PC membrane were totally covered by CNT. It is obvious that the higher CNT concentration gives more concentrated CNT networks, which is consistent with the fact that higher CNT concentration possesses a higher electrical conductivity. In addition, the as-prepared CNT-PC membrane also inherits the excellent mechanical strength of PC membrane, making it a promising free-standing electrode material in the sensor applications.

3.2 Electrochemical detection of 4-aminophenol and 8-hydroxyquinoline

4-aminophenol (4AP) and 8-hydroxyquinoline (8HQ) are the two electroactive compounds

which are the products of the enzymatic reaction in *E. coli*, and can be amperometrically determined by appropriate working electrode. The hydrodynamic voltammograms in Fig 3 shows that the optimal oxidation potential for 4AP and 8HQ are 0.3 V and 0.7 V, respectively. It is necessary to point out that the response with the CNT-PC membrane is also better than that with bare glassy carbon electrode (GCE) or modified GCE. Taking into account the free-standing capacity as well as the convenient operation, CNT-PC electrode is much superior to GCE.

Fig. 4 shows the effect of CNT concentration on the anodic current of 4AP and 8HQ. One can see that the CNT concentration does not affect too much on the amperometric response. In addition, both 4AP and 8HQ demonstrated good linear relationship with increasing the concentration of the analytes. Furthermore, the free-standing CNT-PC electrode is able to detect the analytes with the concentration as low as 500 nM or even lower. These traits ensure the good repeatability and high sensitivity of the constructed sensor in the application of *E. coli* detection.

3.3 Electrochemical detection of *E. coli*

β-D-galactosidase and β-D-glucuronidase can hydrolyze their corresponding substrates, 4APGal and 8-HQG, respectively, to produce the electroactive compounds (4AP or 8HQ), which can be oxidized on the working electrode and give current response.

Fig. 5 verifies that both β-D-galactosidase and β-D-glucuronidase activities in wild-type *E. coli* can be specifically induced by IPTG and MetGlu, respectively. The induced strain exhibited at least 100 times increase of the current response compared to the uninduced strain. This result is in accordance with the one in literature.[2] One can also see that IPTG can only induce the activity of β-D-galactosidase and vice versa for MetGlu. This provides more options with regard to the enzyme for *E. coli* detection. The decrease of each injection is consistent with Michaelis-Menten equation. However, due to the saturation of the active sites on the enzyme, the response from the first injection is more accurate to reflect to amount of *E. coli* than that from the later injections. Since the prices of the substrates applied in this system are normally high (0.1 g 4APGal for $ 52.4 and 0.05 g 8HQG for $ 52.8), low concentrations of the substrates are

desired in order to decrease the operational cost at utmost. Therefore, it is necessary to investigate the loading of the substrates. Fig. 6 examines the effect of concentration of 4APGal on the current response. In the investigated range, 10 μM 4APGal gives the best result. Even though further increasing the amount of substrate would produce bigger response, taking into account that the detection of low concentration of *E. coli* are always required, the concentration of 10 μM is high enough to reflect the total enzyme amount, which is equivalent to the total number of *E. coli* in the detection system.

Fig. 7 demonstrates the limit of detection of wild-type *E. coli* with the constructed sensor. The bacteria were induced by IPTG for 3 hrs and diluted to 5×10^6 cfu/mL with LB medium. 1 mL culture solution was filtrated and 5×10^6 cfu of *E. coli* were immobilized on the pristine PC membrane. This result is comparable to the most impressive result applying electrochemical method published so far.[8] The total number of *E. coli* in that paper was also 5×10^6 cfu. As shown in Fig. 7, the current response of this number of *E. coli* is about 150 nA/cm^2. Theoretically, 30 nA/cm^2 is also distinguishable in the figure. Therefore, further decrease of the detection limit is still possible. Moreover, there is no permeabilization step and enzymatic reaction step in this method, which can save at least 1 hour.

3.4 Electrodepostion of Pt nanoparticles on CNT-PC membrane

CNT-PC electrodes with different pore sizes of PC membrane (0.4 μm or 0.05 μm) served as the substrate of Pt electrodepostion. Fig. 8 shows the SEM images of the as-prepared Pt-CNT-PC membranes. PC membrane with the smaller pore size (Fig. 8A) can generate the Pt nanoparticles with higher density of more uniform distribution. The average diameter of the particles is around 150 nm. The formation procedure and the effect of different electrodepostion substrates are still under investigation. However, we believe that the sensitivity of our method for the determination of *E. coli* can further be improved with the Pt-CNT-PC free-standing electrode due to the outstanding catalytic capacity of platinum along with the huge specific area of the nanoparticles.

4. Conclusions and future work

In this report, we successfully fabricated carbon nanotube networks on polycarbonate membrane. This CNT-PC membrane demonstrated good electrical conductivity and excellent mechanical flexibility. The as-prepared CNT-PC membrane was applied as free-standing working electrode in the oxidation of 4-aminophenol and 8-hydroxyquinoline, and further in the electrochemical detection of wild-type *E. coli*. 5×10^6 cfu of *E. coli* was determined without any pre-concentration and pre-culture. No permeabilization and enzymatic step were included in the operation. We further modify the CNT-PC with electrodepostion of Pt on it. The resulting Pt-CNT-PC membrane is expected to be more sensitive in the application of *E. coli* detection in our detection system.

In the future, the electrochemical property of the Pt-CNT-PC membrane will be investigated and its applicability in *E. coli* detection will be verified. We will also examine the optimal pH value of the working solution in the system. With the Pt-CNT-PC free-standing electrode in the optimized system, we will plot the curve of current vs. culturing time and demonstrate the detection of extremely low initial concentration of *E. coli* in water.

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Figure Captions

Figure 1. The plate evaluating cell applied through out the experiments.

Figure 2. The SEM images of the as-prepared CNT-PC membrane (400 nm pore size) with 2 mg/L CNT loading (A), 5 mg/L CNT loading (B), and without CNT loading (C).

Figure 3. Hydrodynamic voltammograms of 8HQ (A) and 4AP (B) at the concentration of 5 μM with the free-standing CNT (10 mg/L)-PC (400 nm pore size) electrode.

Figure 4. Current vs. time curve of 500 nM, 1 μM, and 5 μM of 4AP at 0.3 V and of 8HQ at 0.7 V with the free-standing CNT-PC (400 nm pore size) membrane. The red and blue line correspond to 5 mg/L and 2 mg/L CNT loading, respectively.

Figure 5. The effects of inducing agents on the current response of *E. coli* detection. 5×10^7 cfu of *E. coli* were immobilized and 25 μM of 4APGal or 8HQG was injected with the free-standing CNT (5 mg/L)-PC (400 nm pore size) electrode.

Figure 6. The effects of concentration of the substrate on the current response of *E. coli* detection. 1×10^7 cfu of *E. coli* were immobilized and different concentrations of 4APGal (10, 5, and 2 μM) was injected with the free-standing CNT (5 mg/L)-PC (400 nm pore size) electrode.

Figure 7. The current response of four successive injection of 4APGal (10 μM) for 5×10^7 cfu of *E. coli* with the free-standing CNT (5 mg/L)-PC (400 nm pore size) electrode. The bacteria were

induced by 1 mM IPTG for 3 hrs.

Figure 8. The SEM images of electrodeposition of Pt on CNT-PC membrane. The pore size of PC membrane was 50 nm (A) and 400 nm (B) with 5 mg/L of CNT loading.

Fig. 1

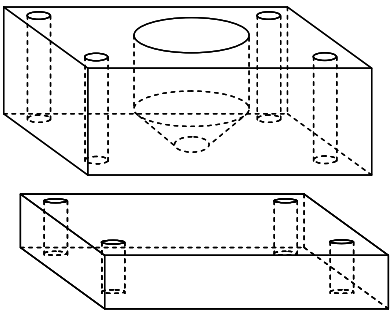
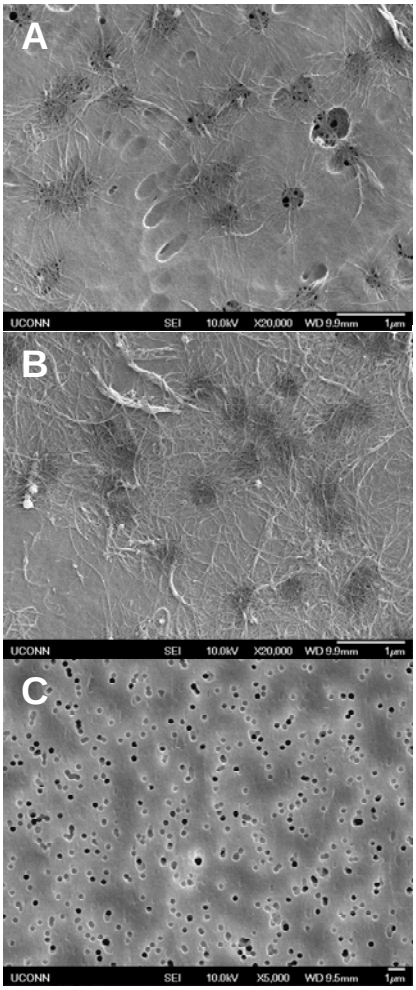


Fig. 2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B
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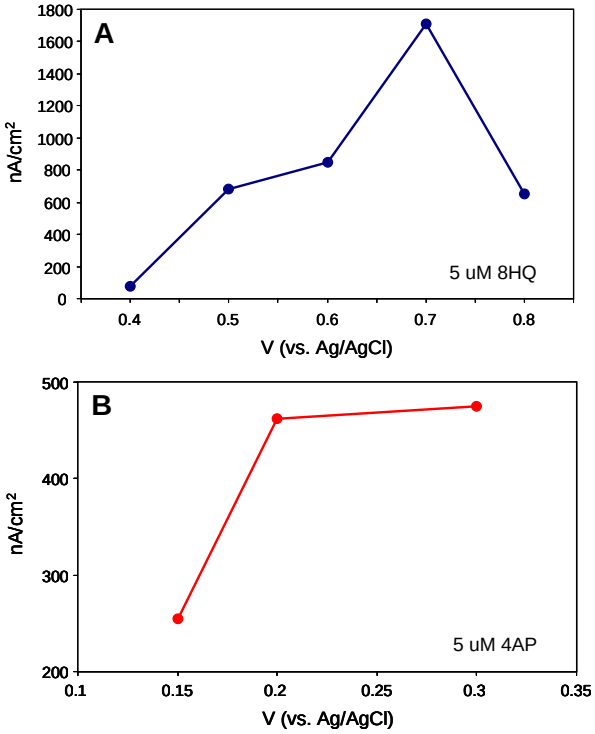
C
UCONN SEI 10.0kV X5,000 WD 9.5mm 1μm

Fig. 3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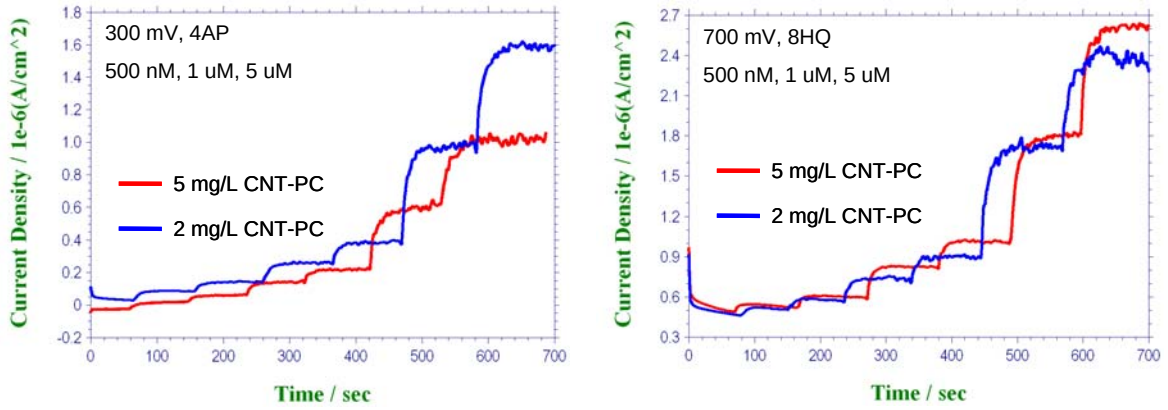
nA/cm2
1800
1600
1400
1200
1000
800
600
400
200
0
0.4
0.5
0.6
0.7
0.8
V (vs. Ag/AgCl)
5 uM 8HQ
B
nA/cm2
500
400
300
200
0.1
0.15
0.2
0.25
0.3
0.35
V (vs. Ag/AgCl)
5 uM 4AP

Fig. 4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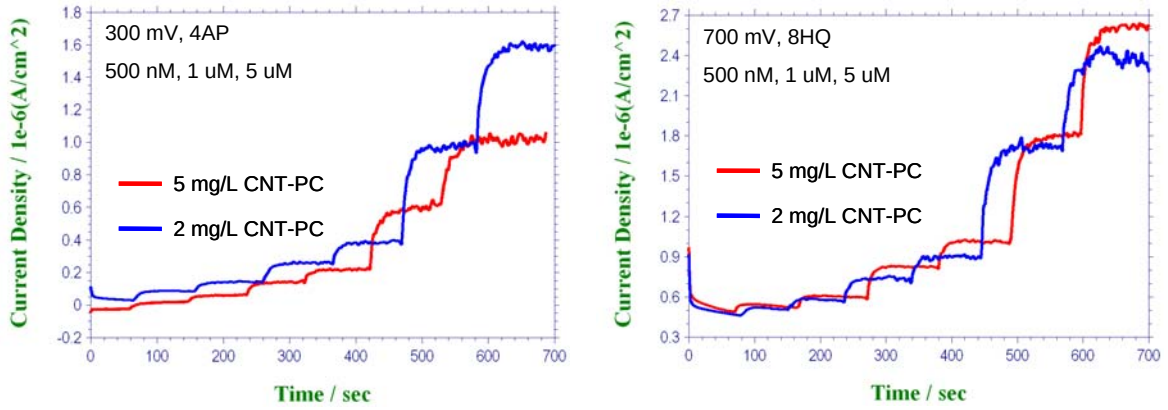
500 nM, 1 uM, 5 uM
5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec
700 mV, 8HQ
500 nM, 1 uM, 5 uM
5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec

Fig. 5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5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec
700 mV, 8HQ
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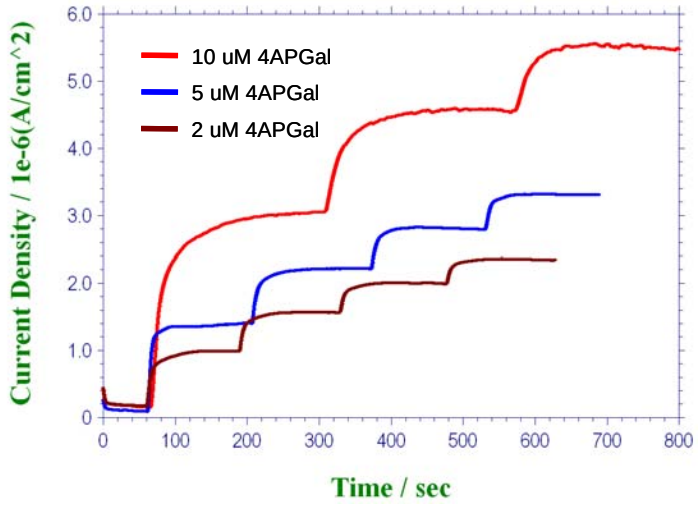
5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec

Fig. 6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5 uM 4APGal
2 uM 4APGal
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Time / sec
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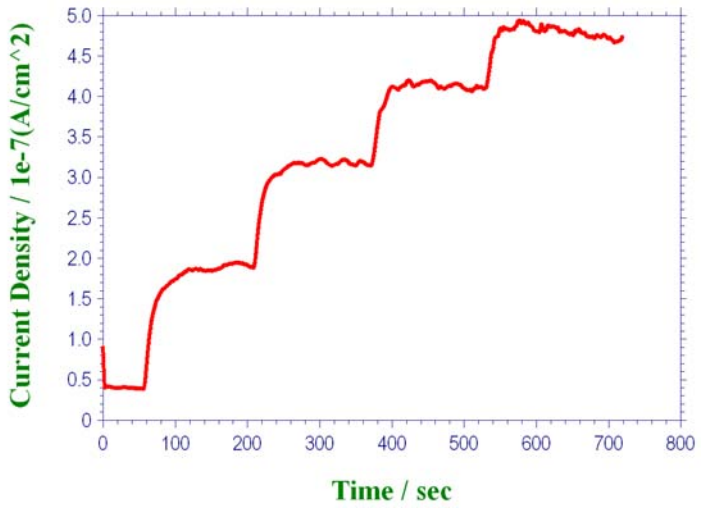
3.0
4.0
5.0
6.0
0
100
200
300
400
500
600
700
800

Fig. 7


Current Density / 1e-7(A/cm^2)
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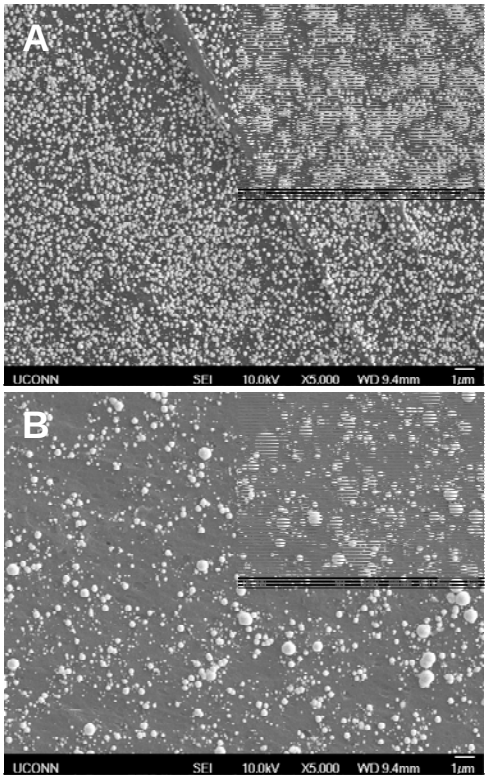
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
100
200
300
400
500
600
700
800
Time / sec

Fig. 8


A
UCONN SEI 10.0kV X5,000 WD 9.4mm 1μm
B
UCONN SEI 10.0kV X5,000 WD 9.4mm 1μm

Evaluation of Turbidity Acidification During Sampling and Analytical Preparation as the Cause of Observed Manganese Anomalies in Drinking Water Wells

Basic Information

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Focus Category:	Groundwater, Water Quality, Geochemical Processes
Descriptors:	None
Principal Investigators:	Gary A. a Robbins

Publications

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Evaluation of Turbidity Acidification During Sampling and Analytical Preparation as the Cause of Observed Manganese Anomalies in Drinking Water Wells

by

Lisandro Suarez
and
Gary A. Robbins

Department of Natural Resources and the Environment
University of Connecticut
1376 Storrs Road
W.B. Young Bldg, Room 313
Storrs, CT 06269-4087

Submitted to

Connecticut Institute of Water Resources
W.B. Young Building, Rm. 224
1376 Storrs Rd, UNIT 4018
Storrs, CT 06269-4018

June 1, 2010

Abstract

According to the Connecticut Department of Environmental Protection (DEP) files more than 80 locations (involving one or more bedrock water wells) have been identified where concentrations of manganese have exceeded the State action level of 0.5 mg/L. In Connecticut, manganese concentrations have been observed to range up to 100 mg/L. A number of potential anthropomorphic causes of the anomalous well concentrations have been proposed. By law, if wells are contaminated the DEP is charged with providing an alternative water supply. In cases of rural private wells, the solution applied is an expensive filter system that is usually maintained by the DEP or local health officials. Recent analyses of the DEP data files suggest that the high manganese concentrations observed are strongly correlated with turbidity. There are two possible explanations for this observation. The high manganese levels may be an artifact of leaching metals from suspended rock flour or fragments of rusted well casing when the samples are collected and then preserved by acidification, which is the standard approach for the collection of samples of water for metals analyses. In cases in which samples are not preserved in the field, standard protocol for metal analysis by inductively coupled plasma (ICP) (e.g. EPA Method 6010) calls for the pretreatment with acid if the water sample exhibits turbidity greater than 1 NTU. ICP has become the most common method of analysis for metals. In this study we have tested the acidification hypothesis by conducting systematic laboratory leaching studies and field tests that involve collecting filtered and unfiltered samples. The results of the study indicate that acidification of turbidity, caused from suspended rock flour or weathered well casing material, can cause manganese to be leached into solution and result in a false positive indication of dissolved manganese contamination.

INTRODUCTION

Background

According to the Connecticut Department of Environmental Protection (DEP) files more than 80 wells have been identified where concentrations of manganese have exceeded the State action level of 0.5 mg/L (Real-Munroe, 2008). The action level was set by the Department of Public Health to ensure protection against manganese toxicity and neurological hazards if the water is used for drinking purposes. Manganese concentration levels above 0.05 mg/L are reported to produce noticeable changes in color, odor, or taste in water. In Connecticut, concentrations have been observed to range up to 100 mg/L. Statistical studies indicate the manganese levels are anomalous with respect to background levels in Connecticut and throughout New England, which average about 0.1 mg/L (Robbins, 2007a, Robbins and Goldman, 2007). A number of potential anthropomorphic causes of the anomalous well concentrations have been proposed (Robbins, 2007b). These relate to conditions that can either lower pH or lower redox (Eh). These geochemical changes result in converting immobile Mn^{+4} to soluble Mn^{+2}. It should be noted that manganese is a major constituent in Connecticut rocks. It also occurs in the relatively insoluble oxide form ($Mn^{+4}O_2$) coating mineral grains in soil and on fracture surfaces. Manganese is a major constituent in the steel used in well casing here in Connecticut. In fact, manganese is present in almost all steel alloys. Manganese stabilizes austenite and, in combination with sulfur, prevents the formation of iron sulfide (FeS) on the surface of the steel during the process of lamination thus, increasing the steel's capacity to harden (Corporación Venezolana de Guayana, 2009 personal comm.). Oxidation of the steel well casing commonly occurs creating a coating of manganese dioxide along with ferric oxide and hydroxides.

The redox of the ground water may be lowered by releasing biodegradable organic substances into the subsurface, such as septic or sewer effluent, fuel oil, blasting agents, stumps and vegetation buried at construction sites, and fertilizer. The pH of the ground water may be lowered by exposing rocks at the surface to rainwater at construction sites. Rainwater seeping into the fractures at the surface has a pH on the order of 4.5, sufficient to increase the solubility of manganese in the ground water by several orders of magnitude (Hem, 1970). Another mechanism to lower the pH would be exposing rocks rich in pyrite to the surface. Oxidation of the pyrite by rainwater produces sulfuric acid, which would cause manganese to leach from the rock.

Connecticut General Statues Section 22a-471, charges the DEP to provide an alternative water supply to contaminated wells. In cases of manganese contamination to domestic wells, the long-term solution applied is an expensive (in excess of $5000 dollars) reverse osmosis filter system that is usually maintained by the DEP or local health officials. Our initial analyses of DEP data files suggest that the high manganese concentration observed is strongly correlated with

turbidity. This is exemplified by Figure 1 below for a home monitored periodically over a three-year period.

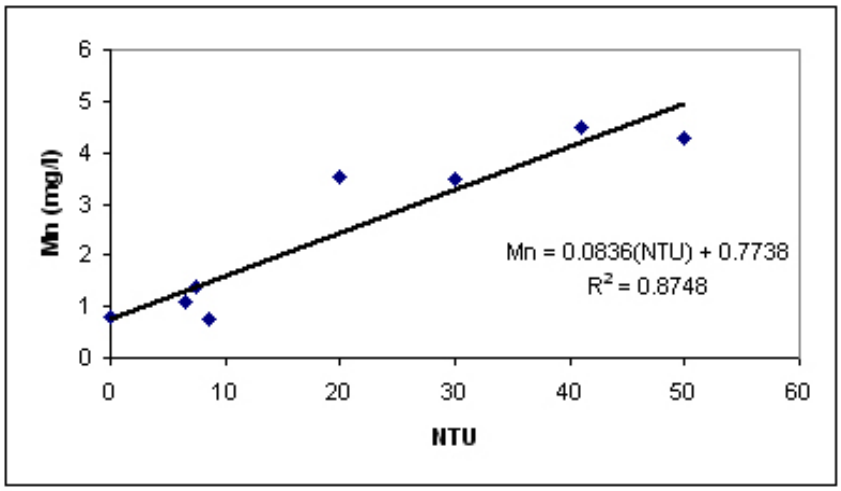

Figure 1: Example correlation of manganese and turbidity in water samples from a home monitored periodically over a three-year period.

There are two possible explanations for this observation. The high manganese levels may be an artifact of leaching metals from suspended rock flour or fragments of oxidized well casing when the samples are collected and then preserved by acidification. Acid preservation is a standard approach used by the Department of Public Health (DPH) when groundwater samples are collected for metal analyses. Even if samples are not preserved in the field, the standard protocol for metal analysis by inductively coupled plasma (ICP), (e.g. EPA Method 6010/ICP), is pretreatment of samples with acid if they exhibit turbidity greater than 1 NTU. The ICP method has become the most common method for the analysis of metals used by the DPH laboratory and private certified laboratories in the State, laboratories also check the pH of the samples when they arrive at the lab, if the pH is more than 2, samples may be acidified by the laboratory and then stored until analyzed.

Problem Statement

Currently the DEP is faced with having to find alternative water supplies and installing expensive filter systems when rural wells are found to have manganese levels that exceed the State action level. Such systems have to be perpetually maintained. If indeed the problem were an artifact of turbidity leaching, the solution would simply require particulate filtering at greatly reduced expense. The high levels of manganese observed may impact obtaining certificates of occupancy by homeowners, require local health officials to use resources to address the problem, and create anxiety for homeowners worried about the health of their families and the value of their property.

Objective

The objective of this study is to test the hypothesis that acidification in preserving water well samples and/or pretreatment for ICP analysis could be the cause of the anomalous manganese levels. To test this hypothesis we conducted leaching studies using rock flour obtained from wells drilled in different locations around the state and oxidized well casing.

METHODOLOGY

Laboratory Experiments

Rock Flour Testing

Leaching studies were conducted on samples of rock flour obtained from standard air percussion drilling of domestic wells in different rock types in Connecticut. Metamorphic and igneous rocks such as schists, gneisses and granite underly 75% of the State of Connecticut. The remaining 25% of the state is underlain by clastic sedimentary rocks (i.e. brownstone) and intrusions of basalt (Burton et al, 2003). Samples from the following rock formations were tested: Hebron Gneiss, Straits Schist, Trap Falls Schist, New London Gneiss, and Portland Arkose. The Hebron Gneiss is composed of andesine, quartz, biotite, and K-feldspar. The Straits Schist is composed of layers of amphibolite, marble, calc-silicate rock, and quartzite. The Trap Falls Schist is generally composed of quartz, sodic plagioclase, biotite, muscovite, sillimanite, kyanite, and garnet. The mineralogy of the New London Gneiss consists of oligoclase, biotite, quartz, plagioclase, K-feldspar, and magnetite. Portland Arkose is a feldspar-rich sandstone which is composed predominantly of quartz and at least, 25% feldspar (YNHTI, 2009).

The first test performed was an EPA Method 3010, Synthetic Precipitation Leaching Procedure (SPLP) test to evaluate the extent of which the rock flour will leach manganese under pH conditions (4-5) that simulate rainwater.

The second test was designed to evaluate the effects of acidification on leaching of manganese (Mn) from rock flour when mixed with deionized water. Testing of the deionized water showed no detections of manganese. Its turbidity was 0 NTUs and its pH was 6.95. Rock flour samples were oven dried for 24 hours at 100-110°C. The dried rock flour samples were mixed with deionized water in a 1:1 weight ratio (about 300 grams each) in one-liter polyethylene bottles. Half of the mixed samples were treated with nitric acid while the other half were not. A solution of nitric acid was prepared by diluting 1N nitric acid with deionized water to obtain a volume ratio of 1:7, HNO_3:H_2O which resulted in a pH of 1.365. The acid solution was added to the rock flour deionized mixture in three polyethylene bottles in three different amounts (15 µL, 180 µL, and 345µL). The polyethylene bottles were stored at room temperature. Half the bottles were

analyzed after one week and the other half after three months. It should be noted that EPA protocol allows storage of preserved samples for six months after analysis. The analyses consisted of the following: measurement of pH, Oxidation-Reduction Potential (ORP), turbidity, electrical conductivity (EC), temperature (T), and dissolved oxygen (DO); measurement of dissolved manganese on three aliquots (10 ml each) using a Hach test kit; measurement of dissolved iron on three aliquots (25ml each) using a Chemets kit; laboratory measurement of dissolved iron and manganese on an unfiltered aliquot (250 ml); and laboratory measurement of dissolved iron and manganese on an aliquot (250 ml) filtered with a 0.45 micron syringe filter. The Hach test kit uses the Periodate Oxidation Method and a colorimeter for quantification. To prepare the aliquots for manganese analysis using the Hach kit, a 0.45 µm syringe filter was used to filter out turbidity which could influence the Hach kit colorimetric analysis. The use of the 0.45 µm filter typically produced turbidity levels below 1 NTU. Turbidity was measured using a Orbeco-Hellige, model 966 portable turbidity meter. Quantification of dissolved iron with the Chemets kit entailed a color comparison. Laboratory analysis for iron and manganese was also conducted at the University of Connecticut, Center for Environmental Sciences and Engineering Laboratory (CESE lab) using EPA method 6010 (ICP). It should be noted that the laboratories used in this study typically check the pH of the incoming water samples. If the pH is more than 2 then the water sample is preserved to a pH of 2. This is usually done by adding concentrated trace metal grade nitric acid. In addition, if a request for total metals analyses is made to the laboratory, the laboratory acidifies the sample first and then the sample is filtered. If a request for dissolved metal analyses is made to the laboratory then the sample is filtered first and then acidified.

Well Casing Testing

To evaluate whether dissolved manganese could be generated by the leaching of well casing, the following testing was performed. Well casing typically used in domestic wells in Connecticut was analyzed using X-ray fluorescence spectroscopy (XRF) to measure the elemental composition of manganese. The composition was measured at two different places on the casing.

The leaching test consisted of the following: a cutting (1.375’ long, 0.54’ outer diameter, 0.34’ inner diameter cylinder) from well casing was placed inside a polyethylene container and submerged partially in 1.5 gallons of deionized water. The water level was allowed to fluctuate on the weekly basis to simulate the weathering of the casing under conditions of water table fluctuations. Weekly water level adjustment was performed and recorded immediately after extracting water samples to be used for analysis. Approximately 2000 ml of deionized water was added between weekly measurements. Concentrations of dissolved manganese and iron, in both

filtered and unfiltered samples, were monitored using the Hach and the Chemets kit. The pH was also monitored. The test ran for approximately two months.

Immediately following the deionized weathering test the casing was exposed to acidic conditions. Nitric acid was added until the pH was approximately 2. On average, approximately 90 mL of 1N nitric acid was added each time the water level adjustment was performed. The acid leaching test was continued for approximately one month. Because the concentrations of dissolved iron and manganese exceeded the maximum detectable concentrations of the portable test kits, the samples were submitted to the CESE laboratory for analysis.

Other Laboratory Testing

The deionized water used in the experiments was tested for manganese, pH and turbidity by the CESE laboratory to establish background levels.

Private Well Water Quality Testing

To evaluate whether filtering and acidification affects the manganese and iron concentrations, four homes were sampled on four different locations in the state. The procedure used to collect water samples from private wells consisted of the following steps: 1) Water samples were taken at locations prior to any filter, water treatment system, and the water storage tank. 2) The cold tap water was allowed to run for approximately five minutes prior to taking a sample if there was not much water use (flushing toilet, shower, etc.) for a few hours prior to sampling, otherwise two minutes was considered appropriate. 3) One-liter plastic bottles were used to collect water samples for the metal analyses. The samples were not acidified in the field. Samples were tested in the field for dissolved manganese, dissolved iron, turbidity, DO, pH, EC, T and ORP. 4) At each home, two samples were collected, one was filtered and the other was not. Given the low turbidity, the samples were filtered using a 0.45 micron syringe filter. 5) Once water samples were taken, they were placed in a cooler and maintained at 4 ± 2 degrees centigrade. 6) Water samples were submitted to either the CESE laboratory or the DPH laboratory for analysis via ICP.

RESULTS AND DISCUSSION

Rock Flour Testing

Table 1 provides a summary of pH, oxidizing conditions, and conductivity of the test samples. The July sampling represents one week of leaching whereas the October sampling represents three months of leaching. The July samples had near neutral pH, were oxidizing and had moderate levels of turbidity and low conductivity. The samples in October showed an increase in pH and moderate level of alkalinity.

Table 1 – Physical parameters of samples in the deionized water leaching tests

TEST DATE	JULY 2009					OCTOBER 2009	
Rock Formation (Town)	**pH**	**ORP**	**DO**	**Turbidity**	**Conductivity**	**pH**	**Alkalinity**
		mV	mg/L	NTUs	µS/cm		mg/L
Hebron Gneiss (Deep River)	7.4	191	9.03	27.3	225	7.9	82
Straits Schists (Chesire#1*)	7.7	144	11.12	88.7	160	8	65
Trap Falls (Chesire#2*)	7.8	183	7.34	64.5	303	8.3	154
New London Gneiss (Deep River)	7.4	119	9.41	24.1	383	NA	NA
Portland Arkose (Guilford) (350’)	7.6	118	15.06	38.8	200	8.1	118
Portland Arkose (Guilford) (250’)	7.8	212	12.85	133.6	43	8.1	163
Portland Arkose (Durham)	7.7	214	7.65	125.2	50	7.2	31
Straits Schists (Burlington)	7.3	220	8.28	12.8	142	NA	NA

Notes:
NA = Not Analyzed
(250') = sample taken at a depth of 250 feet below ground surface

Table 2 shows the results of the SPLP tests. Under the SPLP test conditions, both manganese and iron exhibited low leachability. Only the Portland Arkose had a leachate concentration that exceeded the drinking water standard for manganese.

Table 2 – SPLP test results.

Rock Formation	**[Fe]**	**[Mn]**	**[Fe]**	**[Mn]**
(Town)	CESE Lab	CESE Lab	Calculated	
	(mg/L)	(mg/L)	μg/g	μg/g
Hebron Gneiss (Deep River)	0.579	0.009	28.8	0.4
Straits Schists (Chesire#1*)	1.371	0.028	68.4	1.4
Trap Falls (Chesire#2*)	0.391	0.007	19.7	0.4
New London Gneiss (Deep River)	0.081	0.02	4.0	1.0
Portland Arkose (Guilford) (350')	0.078	0.071	3.9	3.5
Portland Arkose (Guilford) (250')	0.087	0.005	4.4	0.3
Portland Arkose (Durham)	0.266	0.013	13.1	0.6
Straits Schists (Burlington)	0.162	0.002	8.0	0.1

Table 3 summarizes the deionized water leaching tests. The initial analysis were based on the Hach and the Chemets kits. It was found that little manganese and iron leached out initially. The October samples were acidified prior to sending them to the laboratory. The October filtered values of iron and manganese represent dissolved concentrations and were low although two of the samples exceeded the drinking water standards. The unfiltered but acidified samples exhibited much higher values of manganese and iron. All the unfiltered samples tested for manganese were above the drinking water standard and three of the samples were above the Department of Health action level (0.5 mg/L). Also, the unfiltered concentrations of iron and manganese, with one exception, appeared highly correlated ($R^2 > 0.9$) as shown on Figure 2.

Table 3 – Manganese and Iron leaching from rock flour when mixed with deionized water but not acidified.

TEST DATE>>	JULY 2009		OCTOBER 2009			
Rock Formation	**[Mn]**	**[Fe]**	**[Mn]**	**[Fe]**	**[Mn] Filtered**	**[Fe] Filtered**
(Town)	Hach	Chemets	CESE Lab	CESE Lab	CESE Lab	CESE Lab
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Hebron Gneiss (Deep River)	0.6	<1	0.443	26.89	0.014	ND
Straits Schists (Cheshire#1*)	0.1	<1	0.256	16.61	0.006	ND
Trap Falls (Cheshire#2*)	0.2	<1	1.228	81.37	0.076	ND
New London Gneiss (Deep River)	0.3	<1	NA	NA	NA	NA
Portland Arkose (Guilford)(350')	0.1	<1	0.622	17.26	0.088	ND
Portland Arkose (Guilford)(250')	0.4	<1	1.627	44.62	0.020	ND
Portland Arkose (Durham)	0.2	<1	0.097	1.716	0.049	ND
Straits Schists (Burlington)	0.2	<1	0.384	43.85	0.013	ND

Notes:
Note 1: Blank samples were Non-Detect.
Note 2: "Cheshire #1" is at a different location than "Cheshire#2" but within same town.
Note 3: The Hach test kit has a precision error of ±0.2 mg/L
Note 4: NA = Not Analyzed
Note 5: ND means Non-detect
Note 6: Chemets test kit was used to measure dissolved iron
Note 7: All samples unfiltered unless noted

Figure 2: Correlation of manganese and iron.

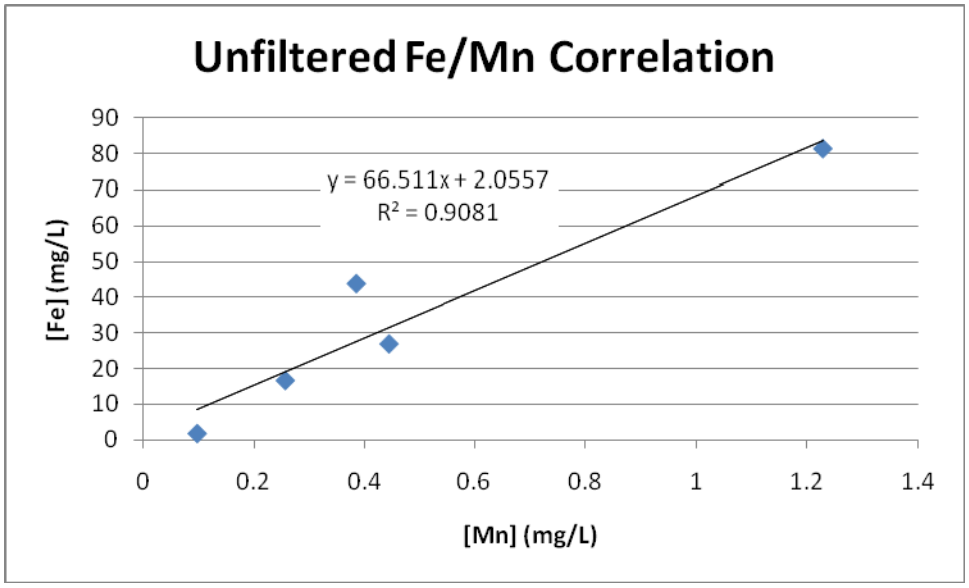

Table 4 shows the results from the rock flour acidification test after one week of exposure to the acidic conditions. Overall, the filtered samples had very low concentrations of manganese and iron in comparison to the unfiltered samples. In certain cases, unfiltered samples were orders of magnitude higher than the filtered samples. In general, concentrations of iron and manganese in unfiltered samples were higher at lower pH. The Hach kit manganese measurements for the filtered samples were near the detection limit for the method. The Hach kit manganese results for the unfiltered samples differed significantly from the results of the laboratory. Although the results are different, they showed similar trends when comparing the unfiltered versus the filtered samples. The difference between the Hach kit results and the laboratory results may be due to differences in degree of turbidity in the aliquots that were used for measurements.

It should be noted that the rock flour from different formations reacted differently to the addition of nitric acid. When a fixed amount of nitric acid was added to the rock flour solution, some of the samples were found to resist a drop of pH (i.e. Trap Falls C2, $\Delta pH=1$; Portland Arkose 250', $\Delta pH= 0.988$) while others exhibited a considerable drop in pH (i.e. New London Gneiss $\Delta pH= 4.54$; Portland Arkose Du, $\Delta pH= 4.876$). The differences likely refer the buffering capacity of the different rock formations. The resistance to pH changes is most noticeable in unfiltered samples. Since the rocks contain little carbonate, if any, the buffering may be due to the hydrolosis which involves the feldspars releasing bicarbonate.

Table 4 – Manganese leachability from rock flour solution when subjected to incremental acidic conditions.

	ROCK FORMATION							
sample ID	Hebron Gneiss (D)							
	Unfiltered (mg/L)				Filtered (mg/L)			
Acidification	pH	Mn (H)	Mn (C)	Fe (C)	pH	Mn (H)	Mn (C)	Fe (C)
High	4.867	4.9	7.419	472.9	2.674	0.4	0.018	1.085
Medium	6.700	1.3	NA	NA	5.417	0.3	NA	NA
Low	6.961	1.3	7.167	472.2	6.918	0.6	0.009	0.459
	Straits Schists (C1)							
High	3.300	2.4	2.216	158.6	2.690	0.1	0.007	0.551
Medium	6.433	1.3	NA	NA	4.541	0.1	NA	NA
Low	6.883	2.1	1.495	110.5	6.512	0.2	0.001	0.118
	Trap Falls (C2)							
High	6.800	4.6	45.440	1823.0	2.913	0.3	0.012	0.148
Medium	7.071	1.9	NA	NA	6.702	0.7	NA	NA
Low	7.105	1.1	14.070	894.0	7.074	0.5	0.013	0.114
	New London Gneiss (D)							
High	2.860	0.7	1.362	47.0	2.722	0	0.060	0.409
Medium	6.652	0.3	NA	NA	6.665	0.2	NA	NA
Low	6.718	0.7	0.913	26.6	5.840	0.1	0.042	0.340
	Portland Arkose 350’ (G)							
High	5.227	4.0	3.808	173.8	2.732	0.1	0.031	0.052
Medium	6.795	0.9	NA	NA	6.940	0.3	NA	NA
Low	6.988	1.8	1.554	50.9	6.167	0.3	0.033	0.036
	Portland Arkose 250’ (G)							
High	6.812	1.6	4.058	115.5	2.824	0.2	0.014	0.134
Medium	7.074	1.1	NA	NA	7.378	0.1	NA	NA
Low	7.218	0.9	3.728	135.6	6.484	0.3	0.011	0.075
	Portland Arkose (Du)							
High	2.824	0.8	0.601	26.7	2.690	0.2	0.017	0.275
Medium	4.264	1.9	NA	NA	3.353	0	NA	NA
Low	6.644	3.0	0.442	21.5	6.637	0.3	0.014	0.275
	Straits Schists (B)							
High	3.605	3.4	1.304	200.4	2.773	0.2	0.002	0.031
Medium	6.585	1.0	NA	NA	4.862	0	NA	NA
Low	7.066	15.8*	0.200	21.7	6.904	0.1	0.002	0.010

Notes for Table 4 above:

1. (H) means test performed with Hach kit; (C) means analytical test performed by CESE Metals Lab using ICP technique; (D) means sample taken from Deep River, CT; (C1) means sample taken from location #1 in Cheshire, CT; (C2) means sample taken from location #2 in Cheshire, CT; (G) means sample taken from Guilford, CT, (Du) means sample taken from Durham, CT; (B) means sample taken from Burlington, CT.
2. (*) Visual observation of unfiltered sample Straits Schists (B) showed significant turbidity.
3. ("letter") after rock formation refers to the first letter of the town's name where the rock flour sample was taken from.
4. High, Medium and Low refer to the different acidification levels. (See rock flour testing methodology section).

Weathered Casing Exposed to Deionized Water and Acidic Conditions

Table 5 shows results from the XRF test on the casing. It was found that both fresh and weathered casings have an average of 22% manganese by weight. Table 6 shows test results of the leachability of manganese and iron from the casing when immersed in deionized water. It can be noted that oxidation of iron was visually noticeable within 24 hours. As can be seen the amount of manganese leachate increased as a function of the duration of testing. A possible explanation for these observations is that the casing continued to oxidize with time and generated an increase in turbidity in the water. Upon acidification in the laboratory (starting after the October 2 sampling), an increase in manganese and iron concentrations was occurred.

Table 5 – XRF test results on the fresh and weathered casings.

XRF Test	Mn	Fe
	mg/Kg	mg/Kg
Casing		
Fresh	214,426	5,988,352
Weathered	228,455	6,707,609

Table 6 – Mn and Fe leaching of a weathered casing in deionized water.

Test Date	pH	Mn	Fe
		mg/L	mg/L
8/7/2009	6.7	NT	NT
9/11/2009	6.43	0.6	NT
9/18/2009	5.65	0.3	NT
9/25/2009	5.46	1.9	NT
10/2/2009	5.59	5.4	NT
10/10/2009	4.68	10.59	412.5
10/26/2009	4.32	32.01	818.3

Note: NT = Not Tested

Private Well Water Quality Testing – Home Case Studies

Table 7 shows the results from groundwater testing of homes in Connecticut. Two out of the four homes show manganese levels exceeding the National Secondary Drinking Water Standard. One of the homes (Bethany) exhibited a manganese concentration above the state action level of 0.5 mg/L. A home in Colchester had iron concentration well above the National Secondary Drinking Water Standard. Both of the homes that showed NSDWRS exceedances in either manganese or iron had acidic pH levels in their groundwater. Alkalinity and turbidity levels appeared to be normal when compared to the NSDWRS.

Table 7 – Groundwater testing from homes.

CASE STUDIES						
		[Mn]	**[Fe]**	**Ph**	**Alkalinity**	**Turbidity**
		(mg/L)	(mg/L)		(mg/L)	NTU
Criteria>>	**MCL/NSDWRS>>**	0.05	0.3	6.5-8.5	300	5
Test Date	**Home Location**					
7/31/2009	Bethany	1.326	0.169	6.2	27	<0.2
10/29/2009	Colchester #1	0.195	12.91	6	NA	3
10/29/2009	Colchester #2	ND	ND	6.9	27	0.8
6/18/2009	Broad Brook	<0.04	0.09	7.4	79	2

Notes: NA = Not Analyzed; ND = Not Detected
MCL = Maximum Contaminant Level (MCL)
NSDWRS = National Secondary Drinking Water Regulations

CONCLUSIONS

Based on laboratory and field testing, manganese concentrations being reported from wells in the in Connecticut may be an artifact of sample preservation and analytical preparation. The results of this study demonstrate that the leaching of rock derived turbidity and weathered well casing can result in false positive exceedance of regulatory standards when samples are acidified.

RECOMMENDATIONS

In order to avoid the false positive problem, the following is recommended:

1. In conducting well testing for manganese and other metals, a test should be performed to determine whether or not the metals are dissolved or an artifact of turbidity leaching. The test should consist of dividing water samples into two aliquots: One aliquot should be filtered using an inline filter and the other should be left unfiltered. The samples should then be preserved with acid. If the unfiltered sample concentration is higher than the filtered, the filtered sample concentration should be relied upon for characterizing the dissolved metal concentration.

2. Caution must be taken when obtaining an unfiltered and unpreserved groundwater sample, in consideration of the procedure followed by water quality laboratories. Typically, laboratories acidify a non-acidic sample (i.e., when the pH > 2) first and then the sample gets filtered (if the turbidity is > 1) if a total metal analysis by ICP is requested.

ACKNOWLEDGEMENTS

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Stream chemical interactions within the urban environment: assessing the fate of nitrogen and mercury in a stream impacted by combined sewer overflows

Basic Information

Title:	Stream chemical interactions within the urban environment: assessing the fate of nitrogen and mercury in a stream impacted by combined sewer overflows
Project Number:	2009CT207B
Start Date:	3/1/2009
End Date:	1/7/2011
Funding Source:	104B
Congressional District:	2
Research Category:	Water Quality
Focus Category:	Nutrients, Surface Water, Wastewater
Descriptors:	None
Principal Investigators:	Joseph T Bushey

Publications

There are no publications.

CONNECTICUT INSTITUTE OF WATER RESOURCES

Project Title: Dynamics of nitrogen loading and speciation in urban combined sewer catchments: An assessment of the effects of flow conditions

QUARTERLY REPORT

Period: April 2009 – May 2010
Submitted: May 28, 2010

PRINCIPAL INVESTIGATOR

Joseph T. Bushey
Department of Civil & Environmental Engineering, University of Connecticut

ADDITIONAL PROJECT PARTICIPANTS

Graduate Student, Mykel Mendes, MS

OVERVIEW:

Efforts during the first year of the project (April 2009 – May 2010) have focused on establishing field sampling sites, purchasing equipment hiring and training a graduate student and collecting samples. Because of budgetary delays, the project support was not finalized until April 2009, in line with the proposed start date. As such, a graduate student was not able to be hired until August 2009 rather than April 2009 as proposed. Dr. Bushey and Ms. Mendes have toured the sampling sites in April 2009 and October 2010. Following field site selection, equipment was purchased and arrived in August 2009. Summer 2009 also involved the development of a conceptual design for the pore water samplers. Site access to the proposed sites was obtained in October 2009. Stream water collection began in November 2009 with installation of pore water samples in early December 2009. Pore water samplers were purged in April 2010 and sediment samples were collected in May 2010.

<u>Accomplishments to date:</u>

- Finalized sampling site selection and instrumentation plan
- Obtained site access
- Designed a pore water sampler and ordered necessary equipment to conduct sampling/analyses
- Hired and trained a graduate student to execute sampling and chemical analysis
- Conducted literature review of relevant chemical analyses (DOC characterization and fractionation; sulfide). Ordered supplies for analytical methods of DOC and S^{2-}.
- Began collection of monthly stream water samples (November 2009)
- Installed porewater samplers (December 2009)
- Purged porewater samplers (April 2010)
- Collected sediment samples (May 2010)

RESEARCH AND EDUCATIONAL ACTIVITIES:

Field Deployment*:*

Dr. Bushey and Mr. Perkins from the University of Connecticut Center for Environmental Sciences and Engineering made an initial evaluation of perspective field sites in April 2009 in association with a related project for The Metropolitan District Commission. This was followed by two additional site visits by Dr. Bushey during Summer 2009 and by Dr. Bushey and Ms. Mendes in November 2009. Based on these initial discussions regarding The MDC study, a stream reach was selected to bracket the influence of combined sewer overflows (CSOs) and untreated wastewater on water quality in the North Park River (NPR), in Northwestern Hartford (CT). Concerns remained regarding the suitability of the field sampling sites relative to (1) the CT IWR study objectives, (2) the physical potential to install and maintain porewater samplers, and (3) site access approval. Based on preliminary site visits, four sites were selected within the initial stream reach for stream water sampling: UConn Law School, Albany Avenue, University of Hartford and Portage Road moving upstream, respectively (Figure 1). These four sites were approximately equally-distributed along the reach with two each above and below the uppermost CSO. Site access for the respective sites was obtained in Fall 2009 during discussions with homeowners (Portage Rd), the University of Hardford, the University of Connecticut Law School and Hartford and MDC personnel (Albany Ave).

Equipment

Following site selection, the samplers and flow monitoring devices were ordered. Equipment was ordered and porewater samplers were designed based on an installation approach. The installation approach was selected over composite sampling using a temporary pumping probe due to the consistent potential for the permanent devices. A sediment sampler, flow-meter and two water chemistry probes were ordered and prepared for use. Sufficient 1-ft and 2-ft plastic sediment core sleeves were ordered to collect the proposed number of sediment cores from the sites.

For porewater sampling, 30 mL Teflon vials were ordered with one transfer port on the tip. Holes were drilled into the sides of the vials to allow for uniform water flow into the vials during sampling. The smallest possible drill bit was used to minimize the entrainment of solids

during sampling. Additionally, the sediment corer was retrofitted to enable easy installation of the porewater samples at the appropriate depth (Figure 2). A prototype temporary porewater sampling device also was explored and constructed due to potneitla issues with the permanent installations and site channel sediment composition as discussed below.

Student Training

In addition to the set up of instrumentation, a graduate student, Mykel Mendes, has been hired and trained. Due to the timing of the grant, the student did not begin until August 2009. However, in this short time frame, the student has been trained by Dr. Bushey and CESE personnel on laboratory protocol as well as the analysis of dissolved organic carbon (DOC), anions and mercury (Hg). Ms. Mendes has also investigated DOC characterization techniques and is setting up the capabilities to sample for and fractionate DOC in the UConn laboratory. Additionally, Ms. Mendes (MS) has become familiar with sampling and field work as well as obtained knowledge of the relevant research in the field.

Sampling and piezometers installation

Stream water sampling was initiated in November 2009 following the granting of site access. Samples were collected at each of the four sampling sites for Hg speciation, DOC characterization, anions and metals. Monthly stream samples have been collected through May 2010 with subsequent trips planned through January 2011.

Bed type and suitability were examined for porewater samplers during the initial stream water sampling at the four sites in November 2009 (Figure 1). The Portage Road site was eliminated for porewater samplers due to the bedrock stream bed characteristics. At the UConn Law School site, the stream channel is composed of a thick clay layer. However, a sandy layer exists near rip-rap installed to protect a recently modified MDC sewer pipe crossing beneath the channel. Three samplers were installed in this sandy layer as these relatively exchangeable sites are Denitrification and methylation hotspots. The transect of three porewater samplers at the Albany Avenue site was installed perpendicular to the channel (Figure 3) at an approximate depth of 4 in to the top of the sampler. This depth was deeper than initially intended but necessary to prevent the sampler from washing downstream during high flow. The two transects at the University of Hartford site included two samplers in the stream channel at 4 in depth with

four samplers installed in sand/gravel bar on a bend in the river (Figure 4). Two samplers were installed at different depths of the bar, one shallow (8 in below surface) and one deep (22 in below surface). The transects at the UConn Law School stream channel site were installed similarly to those at Albany Ave.

At the UConn Law School, a small side channel which floods during elevated discharge conditions was instrumented with porewater samplers as a thick littoral layer existed over the clay layer. However, two of the lysimeter tubes were vandalized in the month following installation while the others filled with clay making pumping difficult. The stream bank samplers at the University of Hartford location were also vandalized. Finally, a decision was made not to install porewater samplers in the small pond on the University of Hartford campus in lieu of sediment core collection. Due to vandalism we are re-exploring the temporary porewater collection device as described by the USGS. Permanent samplers are difficult and costly to install particularly given the lack of protection from vandalism afforded at the urbanized sites.

Initial discharge readings were to be calculated from readings across the channel, with channel morphology noted. However, this has proved difficult and inconsistent. A set of water level loggers has been ordered and will be installed in June 2010 at the Portage Rd and the UConn Law School sites. These will be calibrated during multiple events to discharge using ISCO® discharge recorders.

Dissolved Organic Carbon Characterization

A column set up is being set up to separate the humic acid, fulvic acid, hydrophobic acid and hydrophilic acid fractions. Additionally, DOC will be characterized for aromaticity via SUVA254. Organic characteristics have been documented to reflect source contributions to the watershed and also to influence contaminant, particularly trace metal, mobilization.

Future Work

- Collect stream water samples monthly through January 2011
    - Analyze for Hg speciation, N speciation, metals, DOC characterization and ancillary parameters
- Collect porewater samples quarterly
    - Analyze for Hg speciation, N speciation, metals and ancillary parameters

- Analyze sediment samples for Hg speciation, metals, POC characterization, organic content and ancillary parameters
- Collect stream water samples across precipitation events, during which CSOs are likely to occur. Assess pore water chemical changes with following precipitation events.
- Begin manuscript preparation regarding Hg-DOC relationships, shifts in N speciation during events and with CSO inputs

Figure Captions

Figure 1 Location of stream water sampling sites (✦) and porewater sampling sites (■) in the North Park River.

Figure 2 Porewater sampler.

Figure 3 Sampler layout at the side channel near the UConn Law School.

Figure 4 Sampler installation at the University of Hartford site.

The Metropolitan District
Sanitary and Storm Sewers
North Branch of the Park River - Hartford

Figure 1

Figure 2

Figure 3

Figure 4

Information Transfer Program Introduction

The Connecticut Institute of Water Resources information transfer program has several components: 1. CT IWR web site; 2. Publications; 3. Seminar Series; 4. Conferences and Workshops; 5. Service and Liaison Work. This work is supported through a separate 104B information transfer project, described below.

Water Resources Technology Transfer Program

Basic Information

Title:	Water Resources Technology Transfer Program
Project Number:	2006CT128B
Start Date:	3/1/2007
End Date:	2/28/2011
Funding Source:	104B
Congressional District:	2nd
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Glenn Warner, Patricia Bresnahan

Publications

1. Bresnahan, P., G.S. Warner, R.A. Jacobson and J.M. Stella. 2007. Modeling the effects of reservoir release practices on downstream flows. Connecticut Conference on Natural Resources. March 9, 2007. Storrs, CT
2. Warner, G.S., P. Bresnahan, R.A. Jacobson, J.M. Stella. 2007. Modeling the Effect of Reservoir Release Practices on Available Water Supply Using STELLA. Massachusetts Water Resources Conference. April 9, 2007. Amherst, MA.
3. Warner, G.S., P. Bresnahan, and R.A. Jacobson, 2007. Modeling Flows Downstream of Water Supply Reservoirs. Paper # 072092. Annual International Conference, ASABE. Minneapolis, MN; June 17-20, 2007.
4. Warner, G.S. and P.A. Bresnahan. 2007. Final Report for Project entitled: "Modeling Flows Downstream of Reservoirs" submitted to Connecticut Department of Environmental Protection, April 6, 2006.

The Connecticut Institute of Water Resources information transfer program has several components: 1. CT IWR web site; 2. Publications; 3. Seminar Series; 4. Conferences and Workshops; 5. Service and Liaison Work.

Web Site: Our office maintains the CT IWR web site, which is updated on a quarterly basis (or as needed). It includes information about the WRI program, our institute and its board, a listing of the current year's seminars, a list of sponsored projects and publications, and access to electronic copies of our "Special Reports" series. We also use the web to announce special events and our RFP. We continue to cooperate with the University of Connecticut's digital archives department, which maintains our electronic reports as a part of its "Digital Commons @ University of Connecticut" project.

- **Publications:** We have been asked to maintain a copy of two statewide water conference proceedings available through our Institute's Digital Archives project. These should be added some time this year. Publication of the Water Law conference proceedings was made possible in part by a contribution from our Institute. We also cosponsored the event and served on the steering committee for the event.

*** Connecticut Instream Flow Conference. March 23, 2001: Water Conflicts in Connecticut: Balancing Needs, and May4 2001: Working Toward a Connecticut Instream Flow Standard. Yale Center for Coastal and Watershed Systems.**

***The Connecticut Water Law Conference. Fish and Faucet: Ensuring a Healthy Future. December 2, 2005. Berlin, Connecticut.**

Seminar Series. The CTIWR has begun co-sponsoring the seminar series offered by the Department of Natural Resources Management and engineering, the administrative home for our Institute, instead of holding its own, separate series. Pat Bresnahan serves on the steering committee and actively seeks out speakers with a water interest. Each semester the CTIWR provides financial support to bring in one outside speaker as the "Kennard Water Resources Lecturer." Dr. William Kennard was the first Director of our Institute, and we honoring his contribution to our program in this way. Copies of the series are attached.

Conferences. The Institute co-sponsored and served on the steering committee for the annual. Connecticut Conference on Natural Resources. Steering Committee: Warner, co-chair, Bresnahan, Member. CTIWR also Contributed $500.

Service and Liaison Work. Both the Director and Associate Director actively serve on a number of water related panels.

- Scientific and Technical Standards Workgroup of the CT Stream Flow Advisory Group. Glenn Warner, invited member.

- Willimantic River Aquatic Study, UCONN Wellfield Impacts on Streamflow: Glenn Warner is a member of the technical advisory group.

- The Nature Conservancy / Green Valley Institute's Conservation Action Planning for the Natchaug Basin. Pat Bresnahan and Glenn Warner participated on the panel.

- CT Governor's Steering Committee on Climate Change. Adaptation Subcommittee Workgroups. Glenn Warner serves on the Agriculture workgroup, and Pat Bresnahan serves on the infrastructure workgroup.

- Monitoring the Impact of Invasive Shrub Removal in a Riparian Corridor in Schoolhouse Brook Park, Mansfield, CT. Pat Bresnahan is working as a volunteer on this project, serving mainly as the field data coordinator, and is also contributing a few hours per month of CTIWR time to maintain the project's web site as a page off of the CTIWR site.

- Governor's Climate Change Adaptation Subcommittee Workgroups. Glenn Warner is serving on the Agriculture workgroup and Pat Bresnahan is serving on the Infrastructure workgroup.

- Managed Flows Planning Workshop, sponsored by the Nature Conservancy and Aquarion Water Company. Glenn Warner and Pat Bresnahan were asked to participate in this event which related to the development of instream flow standards for a reservoir system.

USGS Summer Intern Program

None.

Notable Awards and Achievements

Yu Lei, 2008CT170B:Up to date, 8-hydroxyquinoline glucuronide and p-nitrophenyl- -D-galactopyranoside (and/or p-aminophenyl- -D-galactopyranoside) are specifically selected as the substrate of E. coli and total coliform, respectively, from a pool of available glucuronide and galactopyranoside derivatives. Hydrodynamic voltammograms of the substrates and their corresponding hydrolysis product, 8-hydroxyquinoline and p-nitrophenol (and/or p-aminophenol) were performed to determine the optimal detection potential of the products. After that, a sensitive method for rapid detection of E. coli by flow injection analysis was developed.

In addition, we successfully fabricated carbon nanotube networks on polycarbonate membrane. This CNT-PC membrane demonstrated good electrical conductivity and excellent mechanical flexibility. The as-prepared CNT-PC membrane was applied as free-standing working electrode in the oxidation of 4-aminophenol and 8-hydroxyquinoline, and further in the electrochemical detection of wild-type E. coli. 5×106 cfu of E. coli was determined without any pre-concentration and pre-culture. No permeabilization and enzymatic step were included in the operation. We further modify the CNT-PC with electrodepostion of Pt on it. The resulting Pt-CNT-PC membrane is expected to be more sensitive in the application of E. coli detection in our detection system.