

**Water Resources Center
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Introduction

Research Program

Evaluating the Utility of Fluorescence In Situ Hybridizations as a Regular Process Monitoring Tool to Improve Reliable Wastewater Treatment

Basic Information

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PI: Daniel B. Oerther, Ph.D., Tenure-Track Assistant Professor, Department of Civil and Environmental Engineering, University of Cincinnati

I. Executive Summary.

The microorganisms in the aeration basin are the most important yet least monitored part of a municipal activated sludge sewage treatment plant. Primarily, this is due to the inability of traditional monitoring technology to identify and quantify individual groups of microorganisms in a timely fashion. Therefore, we propose to develop “base-line” data demonstrating the value of regular process monitoring using a modern genetic tool - fluorescence *in situ* hybridization (a.k.a. **FISH**) - to track activated sludge microorganisms in municipal sewage treatment plants operated to remove solids, organics, and excess nitrogen. We expect that the information collected using FISH will empower plant operators to diagnose, alleviate, and most importantly avoid treatment process upsets. With the knowledge gained in this project, the water quality community will be able to move toward adopting state-of-the-art microbial treatment process monitoring technology with confidence. Ultimately this will lead to improved treatment process performance and enhanced protection of environmental water quality, which is a critical component for protecting raw water supplies identified as a research priority by the U.S. Geological Survey.

II. Introduction.

The stable performance of biological wastewater treatment systems hinges critically upon maintaining a diverse community of appropriate microorganisms including heterotrophic floc formers and filaments as well as autotrophic ammonia and nitrite oxidizing bacteria (AOB and NOB, respectively). Despite the reliance of the Nation’s water quality professionals on microorganisms to treat more than 30 billion gallons of sewage each day, traditional methods for tracking “bugs”, such as growth on a Petri dish or looking at them in the microscope, successfully identify less than 1% of microorganisms in the environment (Amann et al., 1995). In the past ten years, researchers in the emerging interdisciplinary field of environmental biotechnology have adapted and improved state-of-the-art molecular biology-based genetic methods for identifying and quantifying environmental microorganisms (Amann et al., 1998; Keller et al., 2002; Oerther and de los Reyes, 2001; 2002; Rittmann, 1998; 2002a; 2002b; Wilderer, 2002). The most universally accepted of these genetic methods targets 16S ribosomal ribonucleic acid (16S rRNA) using fluorescence *in situ* hybridization (FISH) to identify, enumerate, and localize evolutionarily-related groups of microorganisms directly in environmental samples (16S rRNA-targeted FISH as described in Oerther et al., 2002). Although academic and applied research using the 16S rRNA-targeted FISH approach has provided a significant amount of useful information regarding specific microbial groups including floc formers, filaments, AOB, and NOB (Grady and Filipe, 2000; Yuan and Blackall,

2002), as yet no one has demonstrated the value of FISH as a regular process monitoring tool for full-scale biological wastewater treatment systems (Oerther and Love, 2003). Therefore, **this important tool is currently not used on a regular basis to monitor treatment process performance.**

Historically, sewage treatment process performance has been monitored on a regular basis by measuring a limited number of priority pollutants including solids as well as organic pollution determined as five-day biochemical oxygen demand (BOD₅). Relying on the robust nature of microbial metabolism, traditional activated sludge treatment systems have provided adequate protection of environmental water quality for nearly a century (in particular since the passage of the Clean Water Act and the best management practice requiring a minimum of a secondary sewage treatment process). Moving into the 21st century, water quality professionals have been challenged with an expanding mission. Because of increasingly concentrated population centers and limited water supplies, treated sewage is used more and more as a raw water supply for the (in)direct production of potable drinking water. Thus, to track quality trends in raw water supply, it is of the highest priority that the water quality community find cost efficient ways to use the Nation's existing sewage treatment infrastructure to more consistently remove traditional pollutants to new lower levels and to remove an increasing number of pollutants (i.e., nutrients as well as personal care products and endocrine disrupting compounds). To effectively optimize existing activated sludge treatment process performance to meet increasingly stringent environmental regulations, water quality professionals need better information provided by improved process monitoring tools. Thus, the **significant question** posed in this project is to determine if the long-term value of 16S rRNA-targeted FISH is primarily as a research tool, or if FISH can be used as a regular process monitoring tool to track the diverse community of microorganisms in a municipal sewage treatment plant.

Since joining the Department of Civil and Environmental Engineering at the University of Cincinnati three years ago in the autumn of 2000, the Principal Investigator (PI), tenure-track Assistant Professor Daniel B. Oerther, Ph.D., has developed an active program of research and outreach focused upon the development, demonstration, and successful transfer to practice of effective microbial monitoring technology that is cost efficient, reliable, and easy to use by environmental professionals with minimal retraining. Although a new faculty member, Dr. Oerther's growing program at the University of Cincinnati has gained recognition as a leader in the State of Ohio and in the Nation in the emerging interdisciplinary field of environmental biotechnology (please see academic resume attached at the end of this application). Therefore, it is expected that the research plan outlined in this proposal will be successfully completed under Dr. Oerther's guidance. Furthermore, it is expected that the successful results of this research project will be broadly disseminated to the water quality protection community throughout the State of Ohio as well as the Nation. It is expected that the information collected using 16S rRNA-targeted FISH on a regular basis will empower plant operators to improve the reliable performance of biological wastewater treatment systems and to routinely meet increasingly stringent regulatory requirements. This project is expected to help 16S rRNA-targeted FISH "cross the threshold of acceptance," and for FISH to be

considered as a regular process monitoring tool in biological wastewater treatment facilities. The interdisciplinary team assembled for this project is well prepared to successfully complete the proposed research because we have a proven track record of working together to transfer to practice 16S rRNA-targeted FISH.

III. Project Objective and Tasks.

The overall **OBJECTIVE** of this project is determining if 16S rRNA-targeted FISH is a useful, regular process-monitoring tool in municipal sewage treatment plants operated to remove solids, organics, and excess nitrogen.

To accomplish this objective, the following **THREE TASKS** will be addressed over a one-year period:

Task One: Assess the appropriateness of existing 16S rRNA-targeted oligonucleotide hybridization probes to track major phylogenetic groupings of heterotrophic floc-forming microorganisms.

Despite the acceptance of 16S rRNA-targeted FISH as a research tool, only a handful of systematic studies have been performed to demonstrate the value of regular process monitoring with FISH to track microorganisms in full-scale municipal sewage treatment plants (Biesterfeld and Figueroa, 2002; Oerther et al., 2001; de los Reyes and Raskin, 2002; Zilles et al., 2002a; 2002b). Documenting the success of a demonstration project is necessary to “cross the threshold of acceptance” and to show water quality professionals that 16S rRNA-targeted FISH is a useful regular process monitoring tool to track important groups of microorganisms. Because the phylogenetic diversity of heterotrophic floc-forming bacteria is enormous, it is experimentally infeasible to develop a suite of 16S rRNA-targeted oligonucleotide hybridization probes to just track “heterotrophic-floc forming microorganisms.” Therefore, to address Task One, we propose to follow the “top-down” phylogenetic approach employed repeatedly by the research group of Prof. Dr. Rudolf Amann, Max Planck Institute for Marine Microbiology, Bremen, Germany (where the PI was a visiting scholar in 1998 and 1999, see Amann et al, 1995 as a representative publication). In the top-down phylogenetic approach, major groups of phylogenetically-related bacteria are targeted by individual oligonucleotide hybridization probes (i.e., probe S-D-Bact-0338-a-A-18 targets all Bacteria; probe S-P-Grps-1200-a-A-13 targets all Gram positive bacteria; probe L-Sc-bProt-1207-a-A-17 targets all bacteria in the beta subclass of the Proteobacteria, etc.). Changes in the relative abundance of these major groups indicate shifts in the structure of the microbial community. Ultimately, these changes in the overall structure of the microbial community can be correlated with the results of traditional process monitoring tools (e.g., solids and BOD₅) to develop relationships linking 16S rRNA-targeted FISH as a process monitoring tool with traditional process monitoring tools. *The expected outcome of Task One is “base-line” data establishing whether FISH should be used as a regular process monitoring tool for heterotrophic floc-forming bacteria.*

Task Two: Assess the appropriateness of existing 16S rRNA-targeted oligonucleotide hybridization probes to identify and enumerate major populations of filamentous microorganisms.

The simplest procedures for identifying filamentous microorganisms in samples of activated sludge use direct microscopic examination of morphology coupled with staining procedures (Eikelboom, 2000; Jenkins et al., 1993). Although these methods have been used to study filamentous microorganisms for more than a decade, reliable identification of filaments still requires a high level of experience on the part of the examiner. In contrast, filament identification using 16S rRNA-targeted FISH does not rely upon the experience of the examiner to recognize morphologic differences. Instead, molecular biology-based tools such as FISH are used to identify unique molecular signatures that are unambiguously linked to specific microbial populations (Zuckerhandl and Pauling, 1965). Previously, Wagner and co-workers developed a suite of 16S rRNA-targeted oligonucleotide hybridization probes to identify Gram negative filamentous microorganisms using FISH (Wagner et al., 1994). Following a similar approach, the PI collaborated with Dr. Francis de los Reyes, currently in the Department of Civil Engineering at North Carolina State University, to develop probes to identify important groups of Gram positive filamentous microorganisms including Mycobacteria (de los Reyes et al., 1997; de los Reyes et al., 1998; Oerther et al., 1999) as well as Microthrix (de los Reyes et al., 2002). These existing 16S rRNA-targeted oligonucleotide hybridization probes will be used in FISH assays to identify and enumerate major populations of Gram positive and Gram negative filaments. Samples with filaments that are not targeted by these existing probes will be preserved for potential follow-up analysis in future projects with the intention of developing additional probes targeting previously uncharacterized filamentous bacteria as documented in publications by the PI (Oerther et al., 2002; Iyer and Oerther, 2003). *The expected outcome of Task Two is linking filament identity and abundance with operating decisions and environmental conditions to begin to develop rationale “rules-of-thumb” relating filament occurrence to process performance.*

Task Three: Assess the appropriateness of existing 16S rRNA-targeted oligonucleotide hybridization probes to track bacteria responsible for nitrification.

The process of nitrification, the step-wise biochemical oxidation of ammonia-N to nitrite-N and ultimately nitrate-N, was first described more than one hundred years ago by Jean Jacques Theophile Schloesing (Schloesing and Muntz, 1877; Warrington, 1878; and Winogradsky, 1891). Today, an average of more than one hundred papers appear annually in the peer-reviewed scientific literature describing various aspects of “nitrification” AND “wastewater” (based upon a keyword search of the Institute for Scientific Information [ISI] Citation Database for 1997 – 2001). Although the nitrification process has been studied for more than 100 years, it still escapes our complete understanding. Perhaps even more astounding is that revolutionary discoveries involving nitrification still remain to be made. For example, during the past decade molecular biology techniques targeting 16S rRNA challenged the long-held “text book”

notion that *Nitrosomonas europaea* (Warrington, 1878) and *Nitrobacter winogradskyi* (Winogradsky, 1891) are the predominant AOB and NOB, respectively, in activated sludge systems (Grady et al., 1999; Madigan et al., 2002; Metcalf and Eddy, 2003; Rittmann and McCarty, 2001). Instead, 16S rRNA-targeted FISH and other molecular biology techniques showed that although *Nitrosomonas* spp. may be a predominant AOB, they are not the only ammonia oxidizing microorganism in activated sludge; furthermore, *Nitrobacter* spp. are rarely found in activated sludge systems and *Nitrospira* spp. are perhaps the more dominant NOB (Daims et al., 2001; Dionisi et al., 2002; Gieseke et al., 2001; Juretschko et al., 1988; Mobarry et al., 1996; Okabe et al., 1999; Purkhold et al., 2000; Sakano and Kerkhof, 1998; Sakano et al., 2002; Schramm et al., 1999; 1998; 1996; Wagner et al., 1995). In this project, 16S rRNA-targeted oligonucleotide hybridization probes will be used to document the identity and abundance of nitrifying bacteria over the year-long operation of a municipal sewage treatment plant operated to eliminate excess nitrogen to reduce nutrient loading on the environment. *The expected outcome of Task Three is documenting the stability of the nitrifying bacteria community in a full-scale municipal activated sludge sewage treatment plant during one-year of operation.*

IV. Benefits of the Project.

Environmental biotechnologies used to track environmental microorganisms are an area of evolving technology. The successful adoption of 16S rRNA-targeted FISH to track microorganisms in treatment processes should help to improve reliable process performance leading to improved trends in raw water quality. One of the primary obstacles to the successful use of FISH as a regular process monitoring tool is the lack of a successful demonstration project that can be cited as a definitive example that FISH provides much needed, highly useful information about the microorganisms in a wastewater treatment plant.

In addition, the successful results of the proposed research plan should provide a starting point for developing (semi-)automated techniques to make FISH into a routine process monitoring tool. This is important because the research in this project will lead to the use of FISH to monitor microorganisms in additional unit processes such as potable drinking water production as well as bacterial source tracking to identify pathogen pollution in the environment.

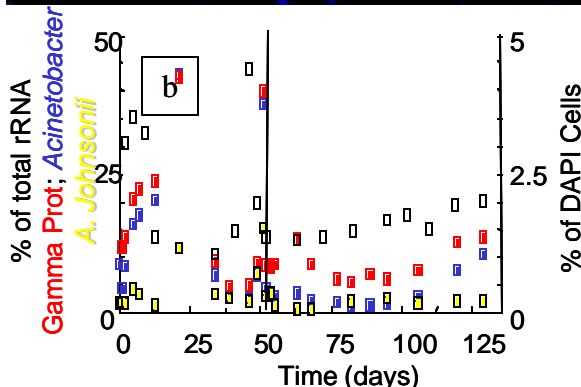
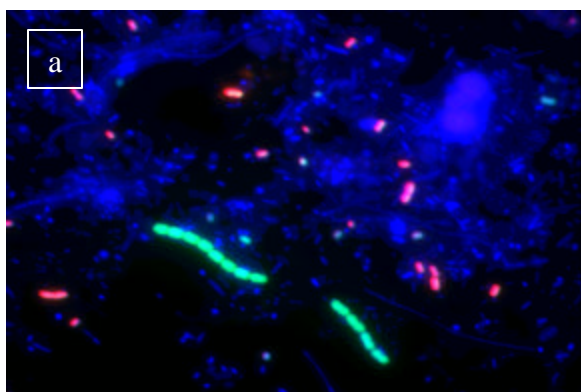
The proposed research plan will provide interdisciplinary training for one graduate student. Together with the PI and his existing research team of graduate and undergraduate students, the graduate student on this project will actively participate in regular monthly research group meetings as well as weekly one-on-one progress meetings with the PI. These meetings provide the student with an opportunity to present research results and defend the work in a public setting.

The results of the project will be broadly disseminated through active participation at local and international research conferences including the Water Environment Federation Technical Exposition and Conference and the general meetings of the American Chemical Society and the International Water Association. These meetings will provide

an excellent learning experience for the student researcher, and they will afford the PI with an opportunity to discuss the results of the project with other faculty and practitioners in the water quality community. The PI anticipates submitting the successful results of the research project to peer reviewed journals such as Applied and Environmental Microbiology, Biotechnology and Bioengineering, and Water Research.

V. Preliminary Results.

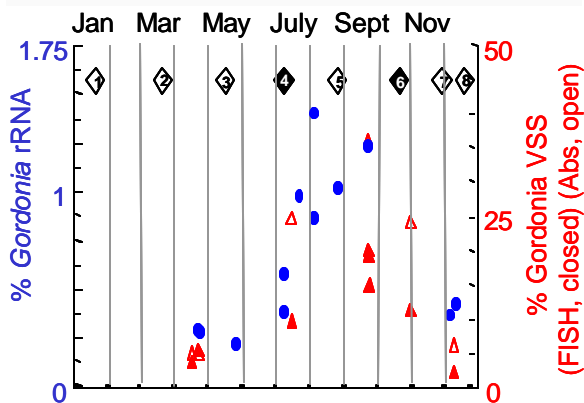
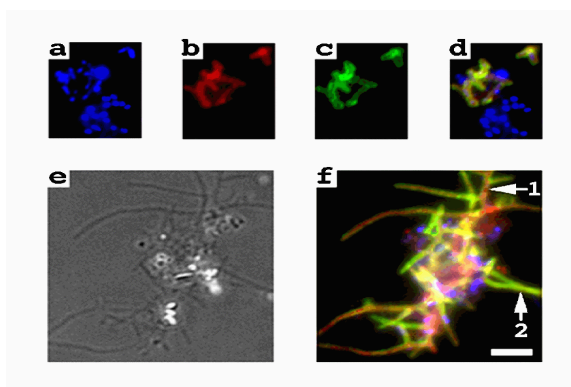
Preliminary results relevant to each of the Three Tasks described above in Section III Project Objective and Tasks are presented below.



Task One: To track floc-forming heterotrophic bacteria in a 5-liter lab-scale activated sludge sewage treatment system during one hundred and thirty days of operation, the PI used 16S rRNA-targeted oligonucleotide probe hybridizations with the FISH assay as well as with quantitative hybridizations of membrane immobilized total RNA extracts. The hybridization results followed a “top-down” phylogenetic approach with all microorganisms detected using a universal probe, S*-Univ-1390-a-A-18, while phylogenetically-nested groups of bacteria were detected using probe L-ScgProt-1207-a-A-17 (targeting the gamma-subclass of the Proteobacteria), probe S-G-Acin-0659-a-A-24 (targeting the genus *Acinetobacter*), and probe S-S-A.john-0451-a-A-22 (targeting *Acinetobacter johnsonii* type strain). Representative

results of FISH are provided in Figure 1 panel A; while summary results collected during the one hundred and thirty day operation of the lab-scale reactor are provided in Figure 1 panel B. Individual cells in each sample were visualized using DAPI to stain genomic DNA (Fig. 1a, blue cells). Hybridization results with a general probe targeting all of the genus *Acinetobacter* (Fig. 1a, green and red cells) versus hybridization results with a specific probe targeting just *A. johnsonii* type strain (Fig. 1a, red cells) showed that *Acinetobacter* represented less than 5% of DAPI stained cell counts (Fig. 1b, open square symbols reported on right y-axis), and *A. johnsonii* was not the only *Acinetobacter* present in this sample. The summary results of quantitative membrane hybridizations showed a similar trend with the abundance of *A. johnsonii* (Fig. 1b, filled yellow square symbols reported on left y-axis) less than or equal to the abundance of the genus *Acinetobacter* (Fig. 1b, filled blue square symbols reported on left y-axis) as well as the abundance of the gamma subclass of the Proteobacteria (Fig. 1b, filled red square symbols reported on left y-axis). Of note, the contribution of *Acinetobacter*-related

microorganisms to the total community 16S rRNA pool (Fig. 1b, values on left y-axis) was an order of magnitude higher as compared to the contribution of *Acinetobacter*-related biomass (Fig. 1b, values on right y-axis). These results strongly suggest that heterotrophic floc-forming bacteria such as *Acinetobacter*-related microorganisms can make a disproportionately large contribution to the overall microbial metabolic activity as compared to biomass levels suggesting that the net yield value of *Acinetobacter*-related microorganisms is lower as compared to the net yield value of other microorganisms in this particular lab-scale activated sludge system. *Collectively these results demonstrate that the PI has developed effective technology to identify and enumerate representative heterotrophic floc forming microorganisms using a top-down phylogenetic approach. These results strongly support the capabilities of the PI to complete the research proposed in Task One.*

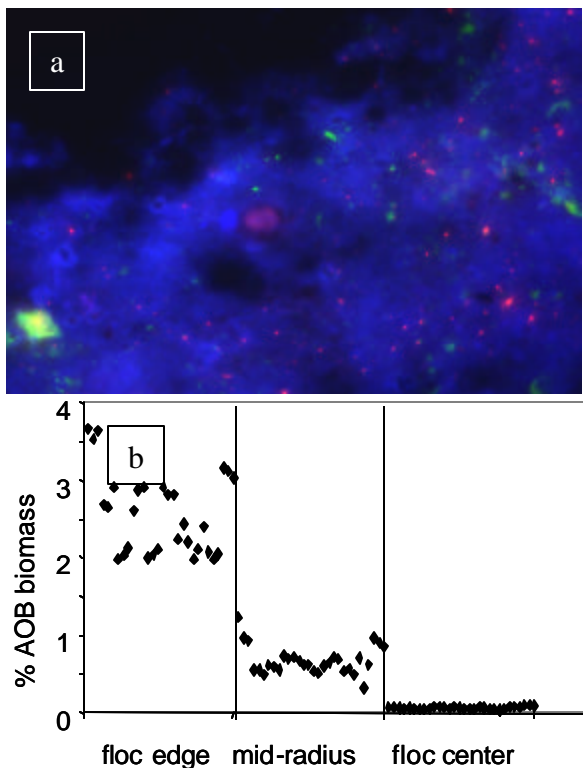


Task Two: To track filamentous bacteria in a full-scale municipal sewage treatment plant during one year of monitoring, the PI used 16S rRNA-targeted oligonucleotide probe hybridizations with the FISH assay as well as with

quantitative hybridizations of membrane immobilized total RNA extracts. In addition, to track individual cells immunostaining with antibodies was performed. The complementary molecular biology methods used in this year-long study (16S rRNA-targeted FISH; membrane hybridizations; and immunostaining) were selected to specifically target a phylogenetically-coherent population of filamentous Mycobacteria, namely the *Gordonia amarae*-like organisms (formerly known as the *Nocardia amarae*-like organisms or Nocardiaforms). Representative results of whole cell FISH combined with antibody

immunostaining are provided in Figure 2 panels A-F; while summary results collected during the year-long sampling campaign for the full-scale system are provided in Figure 2 panel G. Individual cells in each sample were visualized using DAPI to stain genomic DNA (Fig. 2a, 2d, and 2f blue cells). Hybridization results with a general probe targeting all of the genus *Gordonia* (Fig. 2b, 2f red cells) versus antibody staining (Fig. 2c, 2f green cells) were combined to provide two independent methods confirming the identity of *Gordonia* cells in mixtures of pure cultures (Fig. 2d, yellow cells) as well as in samples of activated sludge (Fig. 2f, yellow cells). A comparison of the year-long summary results with the membrane hybridizations (Fig. 2g, filled blue circle symbols reported on the left y-axis) with the results of FISH (Fig. 2g, filled red triangle symbols reported on the right y-axis) and whole cell antibody immunostaining (Fig. 2g, open red

triangle symbols reported on the right y-axis) show that the abundance and activity of the genus *Gordonia* increased during the warmer summer-time months (July through October). In contrast to the results discussed above for *Acinetobacter*-related microorganisms, the abundance of *Gordonia* (Fig. 2g, filled and open red triangle symbols reported on the right y-axis) was at least one order of magnitude greater than the relative activity of the *Gordonia* biomass (Fig. 2, filled blue circle symbols reported on the left y-axis). These results strongly suggest that some filamentous bacteria may have high levels of total biomass although they only contribute a minimal amount to the overall metabolic activity of the activated sludge microbial community. *Collectively these results demonstrate that the PI has developed effective technology to identify and enumerate heterotrophic filamentous microorganisms using a phylogenetically-targeted approach. These results strongly support the capabilities of the PI to complete the research proposed in Task Two.*



Task Three: To track autotrophic nitrifying bacteria inside individual activated sludge flocs removed from a full-scale municipal sewage treatment plant, the PI used 16S rRNA-targeted FISH and compared the results with measurements of chemical microenvironments determined with microelectrodes. The microelectrode measurements (not reported here) showed that dissolved oxygen levels decreased moving toward the center of individual flocs while ammonia levels dropped, nitrite levels rose, and nitrate levels rose, respectively. As expected, FISH results targeting ammonia and nitrite oxidizing bacteria showed a greater abundance of AOB and NOB at the edge of the flocs as compared to the interior. Representative results of FISH are provided in Figure 3 panel A; while summary results of FISH

for ten individual flocs are provided in Figure 3 panel B. Individual cells in each sample were visualized using DAPI to stain genomic DNA (Fig. 3a, blue cells). Hybridization results with probe S-*₁-Nso-1225-a-A-18 targeting ammonia oxidizing members of the beta subclass of the Proteobacteria (primary the genus *Nitrosomonas* and relatives) (Fig. 3a, red cells) corrected for measurements of autofluorescence (Fig. 3a, green speckles) showed that *Nitrosomonas*-related AOB represented less than 5% of DAPI stained cell counts. Summary results collected for hybridizations of thin cryo-sections of individual flocs showed a strong correlation between location in the floc and the abundance of AOB (Fig. 3b) suggesting that the abundance of AOB decreased as the levels of dissolved oxygen decreased (moving from the floc edge to the floc center). These results strongly suggest a spatial organization of individual microorganisms inside individual activated

sludge flocs according to microenvironments measured using microelectrodes. *Collectively these results demonstrate that the PI has developed effective technology to identify and enumerate autotrophic nitrifying microorganisms using 16S rRNA-targeted FISH. These results strongly support the capabilities of the PI to complete the research proposed in Task Three.*

Collectively, the results reported in this section, V. Preliminary Results, effectively demonstrate that the PI has the experience, expertise, and facilities necessary to undertake the proposed research project including sampling for floc forming and filamentous heterotrophic microorganisms as well as autotrophic nitrifying bacteria. Furthermore, the PI has demonstrated that he can successfully monitor both lab-scale and full-scale activated sludge sewage treatment systems during prolonged operation. Thus, it is expected that the PI will successfully complete the proposed research tasks in the proposed timeframe of one-year.

VI. Methods, Procedures, and Facilities.

The procedures for 16S rRNA-targeted fluorescence in situ hybridizations (16S rRNA-targeted FISH) are employed daily in the regular operation of the PI's laboratory at the University of Cincinnati. A brief description of the procedure (adapted from Oerther et al., 2002) follows:

For most applications of FISH with metabolically active samples (i.e., grab samples of activated sludge mixed liquor collected fresh and processed immediately), often one milliliter of a cell suspension provides adequate biomass for all subsequent analyses. Samples are typically fixed using 4% (wt/vol) paraformaldehyde prepared in a solution of 1x phosphate buffered saline for either a short duration (one minute, typically for Gram positive microorganisms) or long duration (12 hours, typically for Gram negative microorganisms) or fixation with paraformaldehyde is eliminated from the procedure (typically for Gram positive microorganisms). After fixation, sample preservation is carried out by storing the samples in an equal volume of ethanol and 1xPBS with storage at -20°C for up to one year before analysis. Fixed, stored samples are typically immobilized on a Heavy Teflon Coated microscope slide (Catalog Number 10-619, Cel-Line/Erie Scientific Co. [800]258-0834) for processing. Alternatively, samples could be filtered using 0.2 micron nominal pore size Sudan Black prestained nylon filters and each filter can be subsequently hybridized, washed, and analyzed. Immobilized cells are air dried, and dehydrated by passing through a 50%, 80%, and 100% ethanol bath for 1 min each. Unlike traditional membrane hybridizations where the hybridization step is performed at low stringency and the subsequent wash step is performed at high stringency; for FISH both the hybridization and wash steps must be performed at high stringency to reduce non-specific background fluorescent signal. Traditionally, the level of formamide in the hybridization buffer is used to control stringency for the hybridization step while the level of sodium chloride in the wash buffer is used to control stringency for the wash step. An appropriate hybridization solution (containing between 0% of 70% formamide) is placed on top of each sample of fixed bacteria. At least 50 ng / 10 microliters of 16S rRNA-targeted fluorescently-

labeled oligonucleotide hybridization probe is added to the hybridization cocktail. The microscope slide is placed in a hybridization chamber and incubated for 2 hrs at 46°C. The washing step is carried out using high stringency with the substitution of sodium chloride for formamide in the wash buffer. Washing is performed at 48°C for at least 30 min. After washing, each slide is rinsed with distilled water and air dried in the dark to avoid unnecessary fluorescence quenching due to exposure to bright light.

Hybridized slides (or filters) are subsequently stored at -20°C until they are analyzed using epifluorescence or confocal laser scanning microscopy. The PI's laboratory is well equipped to visualize samples. We have exclusive use of three epifluorescence microscopes including an upright Nikon model E-600, an upright Nikon model E-400 and an inverted Nikon model TE-2000. A slow scan cooled charged coupled device (CCD) camera is available to capture digital images and Metamorph software is routinely used to perform semi-automated digital image analysis. In addition to epifluorescence capability, the PI's laboratory has recently been equipped with a state-of-the-art Nikon model C-1 Confocal Laser Scanning Microscope equipped with lasers for green, red, and infrared excitation. This microscope was provided through a grant from the Ohio Board of Regents to the PI, and is available for the exclusive use of the PI's research team. The typical cost for hourly-use of the shared CLSM available in the College of Medicine at the University of Cincinnati is \$150/hr. We expect to use the CLSM for approximately 5 hrs/wk during this project. Therefore, because we have exclusive use of our own CLSM system, we are saving \$39,000 of user-fees that would have otherwise been associated with this project.

Samples of mixed liquor will be collected weekly throughout one year of operation from a municipal sewage treatment plant in Greater Cincinnati. The PI has been working with the Greater Cincinnati Metropolitan Sewer District for the past three years. In particular, the PI has used 16S rRNA-targeted FISH to examine heterotrophic floc forming and filamentous microorganisms as well as autotrophic nitrifying bacteria present in samples collected from the Mill Creek and the Muddy Creek Wastewater Treatment Plants. The Mill Creek plant operates two parallel conventional activated sludge treatment basins to process a daily average flow of 120 million gallons of sewage and a peak capacity of approximately 400 million gallons per day during wet weather events. The influent characteristics include 150 mg-chemical oxygen demand per liter of flow and 25 mg-N per liter of flow. The Muddy Creek plant operates a single pass activated sludge system with a suspended growth nitrification system to process a daily average flow of 14 million gallons of sewage and a peak capacity of approximately 60 million gallons per day during wet weather events. The influent characteristics include 60 mg-chemical oxygen demand per liter of flow and 10 mg-N per liter of flow. These two plants are easily accessible by public transportation from the campus of the University of Cincinnati. The Greater Cincinnati MSD shares all process performance data as well as log books of operator observations with the PI as part of ongoing research. Therefore, the PI has adequate access to the necessary full-scale municipal sewage treatment plants as demonstration sites for determining the value of regular process monitoring using 16S rRNA-targeted fluorescence in situ hybridization.

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Rapid Characterization of PCB Contaminated Sediments toward Effective Enhanced Remediation

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Principal Investigators:	Cynthia Gruden, Cyndee Gruden

Publication

Completion Report

Project Title: Rapid Characterization of PCB Contaminated Sediments toward Effective Enhanced Remediation

Project Team: Cyndee L. Gruden, Olya Mileyeva-Biebesheimer (MS student), and Qi Wang (PhD Student)

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Summary. Point and non-point source discharges from industrial facilities, municipalities, and dumpsites combined with urban and agricultural runoff to the Great Lakes have resulted in major contamination issues. Many of the toxic substances (e.g., polychlorinated biphenyls (PCBs), heavy metals) identified accumulate in sediments. The Maumee River area of concern (AOC) located in Northwest Ohio is defined as the area from River Mile 22.8 in the city of Bowling Green downstream to the Maumee Bay and Lake Erie. Sediment cores collected from the Ottawa River located in the Maumee AOC contain PCBs concentrations up to 1,000 ppm. These levels are harmful for humans, aquatic species and wild life. PCBs are known to be carcinogenic to animals and might be carcinogenic to humans. PCBs cause damage to the immune, endocrine and reproductive systems.

It has been observed that PCBs can be degraded by indigenous microorganisms resulting in lower chlorinated and thus less toxic compounds. However, this is typically an extremely slow process that requires up to one year for the removal of one chlorine. Microorganisms need carbon as a nutrient source and electron donors as a source of energy. Sediment microbial communities compete for available resources. In an environment that has a limited source of electron donors, these microorganisms may out compete indigenous dechlorinating bacteria, in turn, adversely affecting PCB degradation. The addition of excess electron donors can dramatically increase the number and activity of dechlorinating bacteria, resulting in an increased PCB dechlorination rate. Significant interest exists in the development of techniques to accelerate the dechlorination process in the field to provide a viable sediment remediation alternative to dredging.

The purpose of this research was to determine the potential for accelerated PCB dechlorination in freshwater sediments by providing excess electron donors, delivered in the form of Hydrogen Release Compound (HRCTM, Regeneration, San Clemente, CA). This experiment involved the incubation of sediment samples collected along the Ottawa River from four different locations (at depths ranging from 2" to 24" below the surface). Triplicate samples with HRC added in proportion 1% V/V were incubated along with unamended control samples under anaerobic conditions for up to 6 months. Periodically (1, 3, and 6 months), microbial numbers, percent activity, biogas production, and PCB levels were recorded. During the first month of incubation, a significant increase in the percent of active bacteria was observed in all HRC-amended samples (14-32%) as compared to the unamended control samples (5-14%). Gas production was observed in amended samples within one week of incubation, suggesting anaerobic biological activity. Biogas produced in amended samples (90mL/month) was significantly higher

than that observed in unamended samples (10mL/month) during the first 3 months of incubation. Results following 6 months of incubation, suggest that hydrogen donor amendment increases anaerobic microbial activity which may lead to increasing dechlorination rates in contaminated sediments.

INTRODUCTION. Point and non-point source discharges from industrial facilities, municipalities, and dump sites combined with urban and agricultural runoff to the Great Lakes have resulted in major contamination issues. Many of the toxic substances (e.g., polychlorinated biphenyls (PCBs), heavy metals accumulate in sediments. The Maumee River Area of Concern (AOC) located in Northwest Ohio is defined as the area from River Mile 22.8 in the city of Bowling Green downstream to the Maumee Bay and Lake Erie. PCBs have been identified as a problematic toxic substance in the Maumee AOC, particularly in the Ottawa River which has been impacted by industrial activities and leaky landfills (Figure 1).

Sediment management strategies pose some of the most challenging issues facing the Great Lakes region. Chlorinated contaminants (e.g., PCBs) are strongly sorbing and bioaccumulative, thus impacting ecosystem degradation, public health, and economic development. Volumes of contaminated sediments tend to be large and contaminant concentrations low such that application of expensive conventional

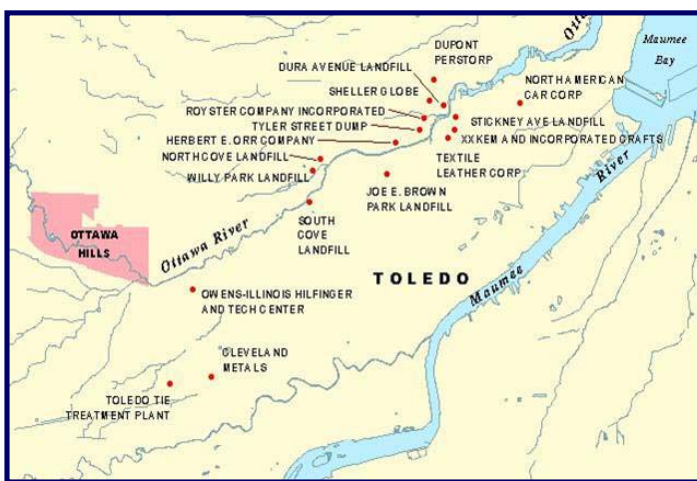


FIGURE 1: Map of Ottawa River in the Maumee AOC

control technologies such as incineration or disposal in a secure landfill may be cost-prohibitive. Under such conditions, enhanced recovery processes that involve manipulation strategies designed to stimulate natural fate pathways may be investigated.

The use of organic electron donors such as aliphatic and aromatic acids is a common technique to supply reducing equivalents for enhanced microbial activity and to stimulate dechlorination of aliphatic and aromatic compounds (Bedard and Quensen, 1995; Adriaens et al., 1999). From a thermodynamic perspective, hydrogen has the potential to serve as an electron donor for dehalogenation of aromatic compounds such as PCBs. Generally, freshwater sediments are characterized by a sharp redox-cline from the sediment bed surface downward, resulting in a gradient from aerobic processes near the surface, to denitrifying, iron-reducing and methanogenic conditions within short distances in the vertical direction. Among these processes, the hydrogen concentrations in sediment pore water are controlled by competitive metabolic conditions (Hoehler et al., 1998). Growing evidence indicates that hydrogen is a key electron donor used in the dehalogenation of lesser chlorinated organics (e.g. cis-DCE and VC to ethene), and organic electron donors appear to serve mainly as primary precursors to supply the needed hydrogen via fermentation (DiStefano et al., 1992; Fennell et al., 1997).

METHODOLOGY.

The overarching goal of the proposed work was to assess the efficacy of a hydrogen-based microbial enhancement technology on PCB contaminated sediments from the Maumee AOC. The following specific objectives were addressed:

Objective 1: Collected representative sediment samples from the Ottawa River.

Four samples of Ottawa River sediments were collected from previously characterized locations known to have a range of PCB contamination (up to 1000 ppm). Grab samples (4L) of surface sediments were be decanted, sealed and delivered to the laboratory on ice for homogenization, and split into aliquots for analysis of total organic carbon, physical parameters (percent solids, total/volatile solids), microbial indicators (total number, live/dead/injured cells, and respiratory competence), and PCB screening.

Objective 2: Characterized sediment samples collected in Objective 1. Each sample was subjected to (i) physical characterization (total organic carbon; total/volatile solids); (ii) microbial assessment (total/active number of microorganisms present, amenability to microbial enhancement); and (iii) determination of historical PCB contamination levels.

Task 1: Physical characterization of sediments. Organic content (total/volatile solids) was measured for each sample collected (Standard Methods for the Examination of Water and Wastewater, Method 209G). Metals were extracted from sediment samples using USEPA's Method 3050 for acid-digestion of sediments (Edgell 1988). After filtration of digested samples, the supernatants were analyzed for metals concentrations using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Perkin-Elmer® Plasma II).

Task 2: Microbial assessment of sediments. Microorganisms were eluted from sediments following an established elution protocol of surfactant addition, sonication, centrifugation, and resuspension in river water (Barkovskii and Adriaens, 1996; Gruden et al., 2003). Sediment-eluted microorganisms were collected, amended with CTC, a redox dye which is reduced to a fluorescent red intracellular precipitate by metabolically active (respiring) bacteria, and counter-stained with the DNA intercalator picogreen (PG, Molecular Probes, Oregon). Epifluorescent microscopy was used for enumeration of total and active bacteria.

Task 3: Determination of historical PCB contamination. Sediment PCB levels were determined using EPA SW-846 Method #4020. This antibody-based assay is commonly used by EPA in the field as a screening method applicable to PCB contaminated sediments (0.5 to 500 ug/kg) (Strategic Diagnostics Inc., Newark, DE). This proven method provides a rapid, qualitative assessment of PCB concentration.

Objective 3: Incubation studies targeted at assessing the efficacy of enhanced PCB dechlorination in sediments collected and characterized in Objectives 1 and 2.

The four sediment samples with distinct characteristics (PCB concentration) were selected for the sediment incubation studies. The sediments were collected, divided into six aliquots of 100 g solids each (100 mL serum vials). Ottawa River water was added to a total volume of 90 mL. Half of vials were amended with a hydrogen donor (1% V/V)

(HRC™ Regenesis, San Clemente, CA). Dechlorination activity was monitored for one, three and six months, and the samples were sacrificed for microbiological assessment and chemical (PCBs) characterization. Samples were originally characterized via the colorimetric assay, however, for the incubation study, congener-specific analysis (GC-MS) will be carried out.

RESULTS AND DISCUSSION.

Following collection, physical, chemical, and microbiological characterization of the samples was completed (Table 1). All of the sediment samples, which were collected in the same river mile along the Ottawa River, had similar physical and chemical characteristics with the exception of sample #53 which had an elevated PCB concentration (~1000 ppm) and volatile solids (VS) (22%). The higher VS will result in more significant partitioning of hydrophobic contaminants (e.g., PCBs) to sediments and is usually associated with higher numbers of bacteria. TOC values, which were between 150 and 250 mg/L, were measured in an effort to determine if ample organic matter was present to support microbial activity and to delineate any differences between samples. It should be noted that metals analysis (Al, As, Cd, Fe, Cu, Ni, Pb, and Zn) indicated that none were above recommended levels. Microbial assessment of samples was also completed as outlined in Objective 2. The percent of active bacteria in initial samples ranged from 22 to 36%, while the total (~1x10⁷/mL) and active numbers (~5x10⁶/mL) were similar for all four samples.

For the incubation study, it was necessary to select an appropriate hydrogen release compound. For short term impact, a technology that has been used in the field and is relatively easy to implement was needed. For this work, we chose Hydrogen Release

TABLE 1: Selected Characteristics of Sediment Samples

Sample	TOC (mg/L)	Volatile Solids	Total PCBs (mg/L)	% Active	Active Number (#/mL)	Total Number (#/mL)
#32	164	10%	<1	36	7.08x10 ⁶	1.97x10 ⁷
#37	151	6%	10-100	28	1.32x10 ⁷	4.53x10 ⁷
#441	255	9%	10-100	32	6.24x10 ⁶	1.97x10 ⁷
#53	154	22%	100-1000	22	5.89x10 ⁶	2.66x10 ⁷

Compound or HRC (Regenesis; San Clemente, CA). HRC is a viscous fluid at room temperature that breaks down to lactic acid, which can be biologically degraded to produce low concentrations of hydrogen in sediments. This technology has previously been applied to contaminated groundwater sites with success.

Following initial characterization, triplicate samples from each of the four locations were incubated both with and without HRC amendment. HRC addition resulted in a short term (up to 7 days) increase in total and active numbers of sediment microorganisms in all samples (Figures 2A and 2B). Although the total numbers were

the same, the active number of bacteria in all four HRC amended samples was statistically higher than in unamended control samples after 1 month of incubation. After 3 months of incubation the increase in active numbers had diminished in most samples (except #53) (data not shown). HRC resulted in an order of magnitude increase in TOC in amended samples. However, there was no statistically significant difference in TOC concentrations of amended samples as compared to unamended samples after 3 months of incubation.

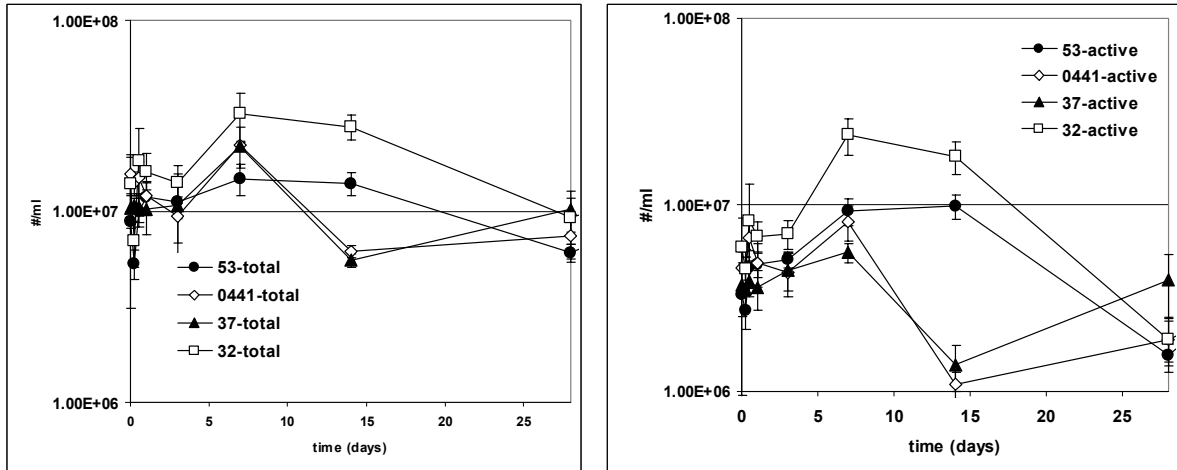


FIGURE 2: Total (A) and active (B) number of bacteria in sediment samples amended with HRC as a function of incubation time.

During incubation, significant gas production was noted in the amended samples within the first week (Figure 3). Gas production was significantly higher in sample #53 following 8-10 weeks of incubation, sample #37 following 12-15 weeks of incubation, and sample # 32 following 13-15 weeks of incubation.

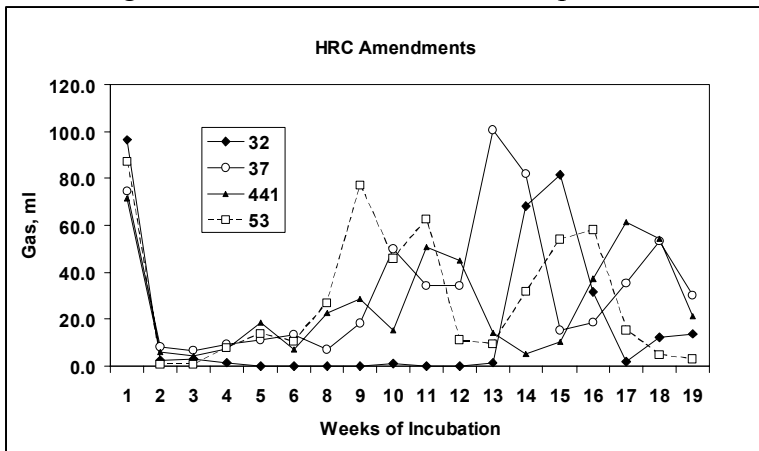


FIGURE 3: Gas production samples with HRC amendment measured weekly during 5 months of incubation.

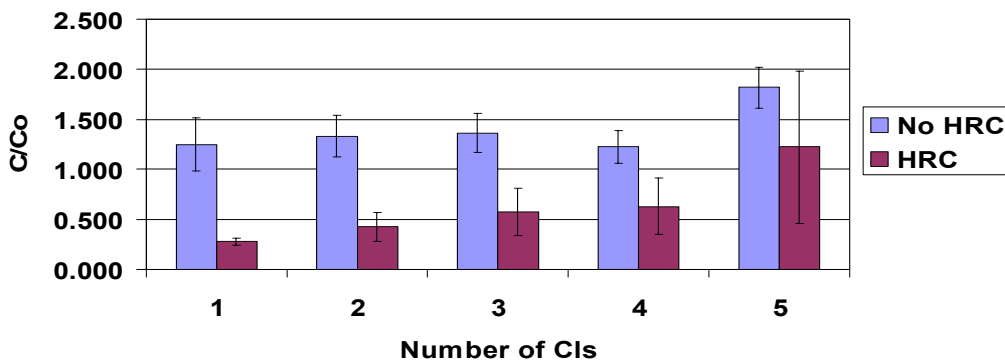
Weekly production ranged from negligible to 100mLs of biogas. During the first 3 months of incubation, biogas production in samples with amendment were significantly higher (approximately 100mL/month) than in unamended samples (<10mL/month). This result is likely attributed to the original TOC of amended samples, which was an order of magnitude higher (~1000 mg/L TOC) than unamended samples. After 3 months of incubation the significant increase in gas production was no longer observed, which concurs with microbiological data which demonstrated no difference between active numbers of bacteria in amended and unamended samples following 3 months of incubation.

and sample # 32 following 13-15 weeks of incubation. Weekly production ranged from negligible to 100mLs of biogas. During the first 3 months of incubation, biogas production in samples with amendment were significantly higher (approximately 100mL/month) than in unamended samples (<10mL/month). This result is likely attributed to the original TOC of amended

samples, which was an order of magnitude higher (~1000 mg/L TOC) than unamended samples. After 3 months of incubation the significant increase in gas production was no longer observed, which concurs with microbiological data which demonstrated no difference between active numbers of bacteria in amended and unamended samples following 3 months of incubation.

Since the results of the colorimetric assay for PCB determination were primarily qualitative. Congener specific analyses are currently under way to analyze samples from the incubation study. The results after 1 month of incubation of triplicates of Sample #53 suggest (Figure 4) that only lower chlorinated congeners (up to 5 Cls) are present in the original sample and dechlorination (25-50%) occurs within the first month. After six months of incubation, there is no statistically significant increase in dechlorination of this sample, suggesting that dechlorination activity peaked within the first month of the study.

After 1 Month of Incubation



CONCLUSIONS. Results following 6 months of incubation, suggest that hydrogen donor amendment increases anaerobic microbial activity as evidenced by increased biogas production and active numbers of bacteria in all sediment samples amended with HRC and, subsequently, a reduction in the concentration of lower chlorinated congeners in a highly contaminated (>1000 ppm PCB) sample (#53) amended with HRC. For sample #53, it appeared that a positive response in terms of both gas production and enhanced activity suggested the potential for PCB dechlorination. However, this result must be verified with subsequent analysis. Congener-specific PCB analysis of the remaining sediment samples is ongoing as part of a related research project.

This project investigates an enhanced remediation alternative for PCB-contaminated sediments. Results from this study will provide the basis for establishing causal relationships between sediment characterization and efficacy of hydrogen donor amendment strategies toward PCB dechlorination in sediments. Although enhanced natural recovery strategies represent some of the most promising and cost-effective medium-term sediment management approaches, causal relationships have yet to be established. In addition, this proposed technology may provide a viable alternative for contaminated areas where sediment removal technologies are cost prohibitive yet remediation is warranted.

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The Scour and Deposition around River and Estuarine Bridges

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Publication

The Scour and Deposition around River and Estuarine Bridges

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Rationale:

This investigation is motivated by the amount of river, estuarine, and coastal infrastructure which is susceptible to extreme wave and flooding events. The high velocities and resulting shear stresses are capable of scouring or depositing large quantities of sediment from around hydraulic structures. Preventing the failure of these structures and sedimentation in inlets alone costs federal and state agencies billions of dollars annually. In addition to being costly, the manual monitoring of bridge scour as mandated by the Federal Highway Administration can be inefficient in states such as Ohio where the flood events which initiate the scour process occur sporadically.

According to the National Scour Evaluation Database, there are 23326 bridges over waterways in the state of Ohio. 5273 of these bridges are considered scour susceptible and of those 191 of the bridges are considered 'scour critical'. Previous methods for observing bridge scour have relied on the manual sampling of local water depths and have been generally limited to sampling periods of low water flow. As the dynamic scour and deposition of sediments around structures is strongly correlated to periods of high flow, traditional sampling methods have limited our ability to evaluate scour models.

In contrast to problems generated by sediment scour is the problem of sediment deposition in navigational channels. On the Maumee river alone, the Army Corp of Engineers spends millions of dollars annually to dredge an average of 850,000 cubic yards of sediment. With the elimination of open lake disposal of dredged sediments, an inter-agency collaboration of government and private citizens has been formed to identify possible methods for reducing the amount of deposition by reducing the soil erosion along river banks. Clearly, an increase in our understanding of how sediment is scoured or deposited around structures will improve our ability to utilize available resources in the most efficient manner.

Anticipated Benefits:

Beyond the scientific merit of better understanding the scour process, we anticipate that these results may be used by both scientists and planners interested in improved parameterization of the sediment transport around structures under river and combined wave-current flow. The results may also be used to identify locations for future sampling sites. Specifically, we will identify locations and structures which should be more closely monitored and possibly altered. Combined these model-data results will highlight potential areas of concern.

Objectives:

The objective of this research is to increase our ability to predict how variations in flow conditions will affect the scour and/or deposition of sediment around estuarine and river bridges. Two specific goals for this project are listed below.

1. Evaluate an existing three-dimensional flow and sediment transport model with observations of river morphology and flow velocity at structures within the Lake Erie tributary.
2. Examine the effect variations in river stage will have on bridge scour. Particular attention will be paid to locations where complex three-dimensional velocities resulting from mean and oscillatory motions exist.

Timeline:

In the spring and summer of 2004, we will identify possible bridges susceptible to scour or deposition. In collaboration with ODOT (contact Bill Krouse), we will consider the five USGS-ODOT previously established bridge-scour measurement sites and also several bridges along the Maumee river. The site will be determined through a collaboration with the Ohio Department of Transportation (ODOT) and by a preliminary sediment and bathymetry sampling. In the fall of 2004, static bed model simulations of flooding conditions of the selected bridge will be performed. Through the fall of 2004 and beyond scour observations will be sampled on an as-needed rapid response basis during periods of high flow. At least one deployment of the in-situ instruments will be performed during this period. In spring and summer of 2005 full morphologic simulations of the near field bathymetry will be performed for both the available observations and for a variety of statistically extreme storm events.

Methodology:

Modeling of the scour and depositional process will be performed with the computational fluid dynamics model, FLOW-3D (Flow Science, Santa Fe, NM). FLOW-3D is a three-dimensional non-hydrostatic computational fluid dynamics model which employs the FAVOR method to resolve the flow around obstacles without mesh regeneration. The primary strength of FLOW-3D is its ability to accurately resolve three-dimensional flows in great detail, while tracking complex flow behavior at fluid-structure and fluid-sediment interfaces. The flow-sediment-structure modules allow for coupled flow-sediment equations to be incorporated. The model will be used to first, simulate the riverine flow conditions for a static bed and second, simulate the morphologic evolution of the river bed. The static bed simulations will provide the initial flow conditions and allow for model-data comparisons between the water column velocity profiles and, when available, the surface velocity observations described below. For the static bed simulations performed in Year 1, regions of potential scour will be identified when the local bed stress exceeds the critical bed stress. Regions of potential deposition will be assumed when the Rouse number is of order 10.

In this investigation, the model geometry will be initialized with observed river bathymetry obtained from a WaveRunner survey system consisting of a differential GPS receiver and base station, dual-frequency sonic altimeter, and custom navigation software, Figure 1. The system is in-hand and has been tested in coastal marine and fresh water environments where waves and currents are present and sometimes energetic. The system has accuracies of about $\pm 5\text{-}10\text{ cm}$ in both the horizontal and vertical coordinates of the measured bathymetry.

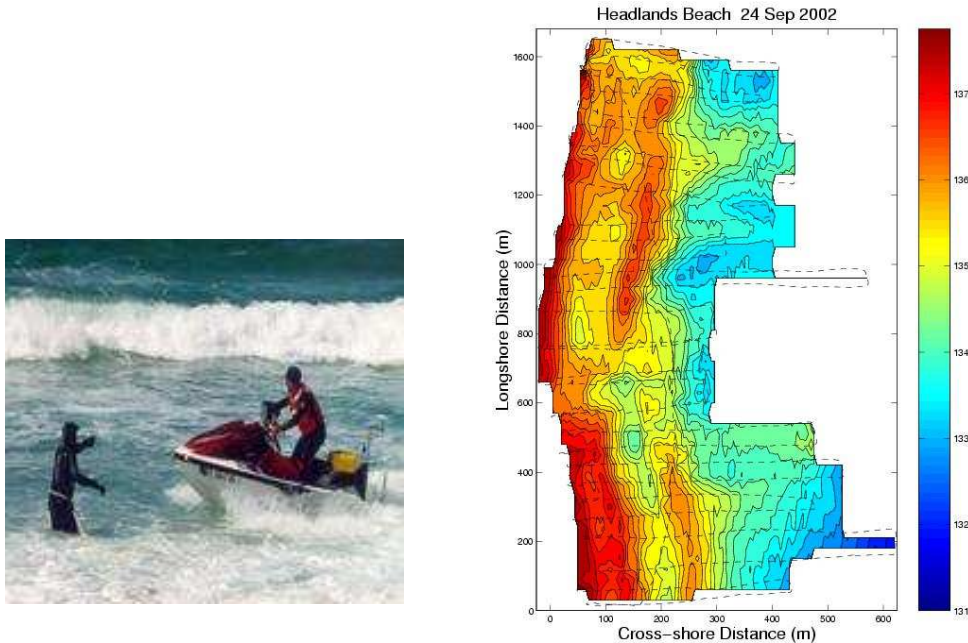


Figure 1. The wave runner survey system is capable of measuring water depths from approximately 0.5 m to 15 m. The left panel shows the survey system just following deployment and the right panel shows a sample bathymetry from Headlands state park.

Field observations of water column velocity, surface flow, and local scour will be obtained during at least one field deployment, Figure 2. Vertical profiles of velocity will be measured at a single upstream location with a Pulse-Coherent Acoustic Doppler Profiler (PC-ADP). The sensor can remotely sample three-components of velocity at 5 cm range bins at a 2 Hz sampling rate. The observations will be used to specify the upstream boundary condition and evaluate the surface flow observations described below. A single point measurements of three-components of velocity will be measured immediately downstream of the structure with a new Acoustic Doppler Velocimeter (ADV). The observations will be used to quantify the incipient motion velocity and to evaluate the model simulations. Measurements of the surface flow in and around the pier piling will be obtained from analysis of video data that utilizes particle image velocimetry (PIV) techniques. The recently developed PIV system uses visible particles on the water surface (such as from sediment patches, bubble clouds, or other passively floating detritus) to identify displacements between individual frames of the video imagery. Correlation and filtering techniques have been developed that allow mean and oscillatory flow to be measured with high accuracy, on the order of 10% of the measured velocity field (results presented at the Fall AGU meetings in 2000, 2001, and 2002 by PI

Lippmann and colleagues). For this project, cameras will be deployed on both the upstream and downstream sides of the bridge, Figure 2. The labor intensive nature of the PIV system will limit surface flow analyses to the initial model evaluation and to several high-flow events. If needed, additional funds may be sought from other agencies to perform additional surface velocity PIV analyses. The near-field scour and sediment suspension will be measured with a rotating two-axis IMAGENEX profiling sonar. The sonar will resolved the two-dimensional centimeter-scale bathymetric variations over a 5 m radius.

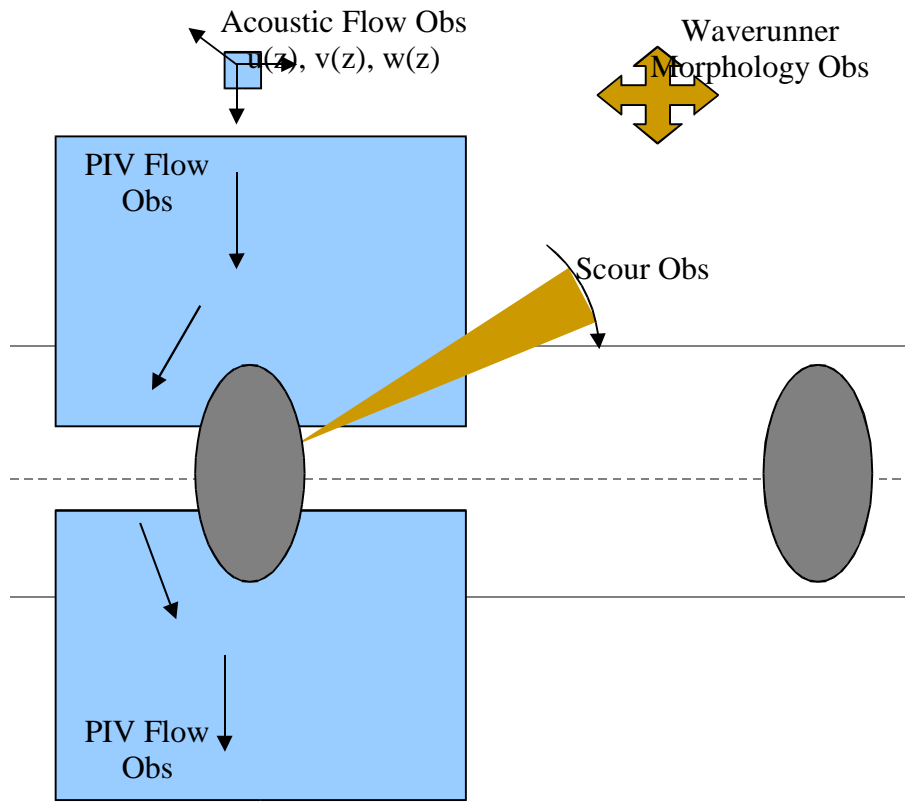


Figure 2. Sketch of proposed observation. Blue highlighted area represents area of potential particle-image-velocimetry observations. Tan highlighted wedge represents a single slice of the IMAGENEX local scour observations. Far-field bathymetric observations will be obtained with the WaveRunner survey system.

The in-situ instruments will be deployed during one low flow event (summer 2004) and at least one high flow event (2005). Observations will be used to evaluate model performance as well as identify any flow patterns related to structure scour and deposition in mean and oscillatory flow environments. Model simulations may also be compared with observations from the National Bridge Scour Database (USGS). Ongoing model-data comparisons will improve model strengths and set limitations on present capabilities. Following successful model-data comparisons, the model can be used to predict the scour and deposition around bridge piers for extreme storm events with a variety of return periods that may include, for example, 20, 50, or 100 year events.

Facilities:

All existing facilities required to complete both the observational and modelling objectives currently exist. Two site license for FLOW-3d are available to fulfill the flow and bathymetric modelling objectives. PI Lippmann has the WaveRunner and PIV systems. PI Foster has the IMAGENEX sonar and PC-ADP velocimetry profiler systems. With matching funds provided by a Board of Regents Grant, a two-axis near bed Acoustic Doppler Velocimetry (ADV) system will be purchased for the sampling of the near bed velocity observations.

Related Research:

Through an Office of Naval Research project, we have evaluated FLOW-3D for both the scour and deposition around a two-dimensional pipeline subjected to steady flow (Smith and Foster, 2002a; and Smith and Foster, 2002b) and around a three-dimensional cylindrical submarine mine subjected to both steady and wave flow, Figures 3 and 4. We have also examined the free surface module of FLOW-3D with wave transformation observations of velocity and wave breaking (Chopakarla and Lippmann, 2003). The model-data comparisons of pipeline flow and scour are generally excellent, Figure 3. An ongoing field experiment at the Martha's Vineyard Coastal Observatory (MVCO) is providing observations for the evaluation of mine scour and burial. The IMAGENEX sonar is currently deployed in 15 m of water at the MVCO and is observing a 5 m radius of sediment suspension and mine scour and deposition.

The wave runner survey system has been evaluated for both lakes (Alum Creek Reservoir and Lake Erie) and oceans (Duck, NC and La Jolla, CA), Figure 1. In a recent Office of Naval Research funded three-month long field experiment, PI Lippmann examined wave breaking processes with a 7 camera array covering a 10 km stretch of the Southern California coast. The PIV system resolved the temporally varying two-dimensional surface flow. Coincident bathymetric surveys were obtained with the wave runner system. The Pulse Coherent Acoustic Doppler Profiler (PC-ADP) has been deployed in two locations on Lake Erie to examine the effects of lake level variations on the suspension of sediment (funded by Sea Grant).

Training Potential:

This project will provide for funds for one dedicated graduate student, Ms. Kimberley Hatton. Ms. Hatton has a 3.8 GPA and is currently completing her senior honors thesis on critical scour regimes around cylindrical objects. She has experience with both FLOW-3D and with the PIV PC-ADP, and wave runner instrumentation. This project will provide her with an opportunity to fully develop skills acquired this past year while examining riverine hydraulics. We will also attempt to mentor an undergraduate honors thesis student in the area of bridge hydraulics. This project will also provide for the collaboration between university researchers, students, and ODOT engineers.

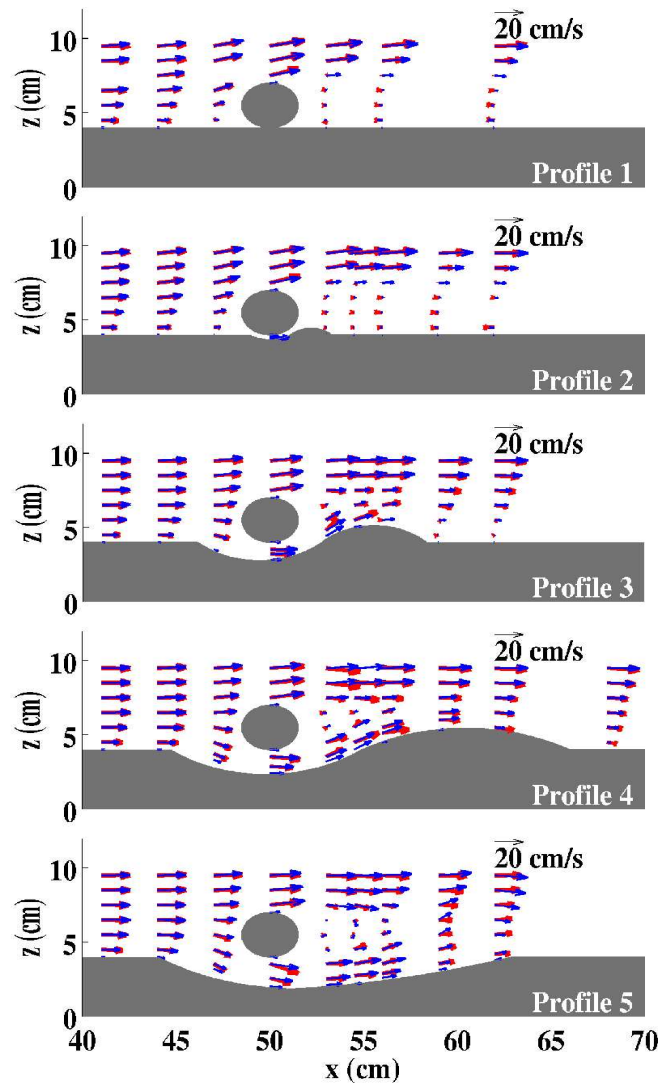


Figure 3. Observed (blue) and simulated (red) velocity profiles around a two-dimensional pipeline over 5 bed static-bed profiles. The model resolves the recirculating region present on planar and near-planar beds (profiles 1 and 2) and vortex shedding present on scoured beds (profiles 4 and 5).

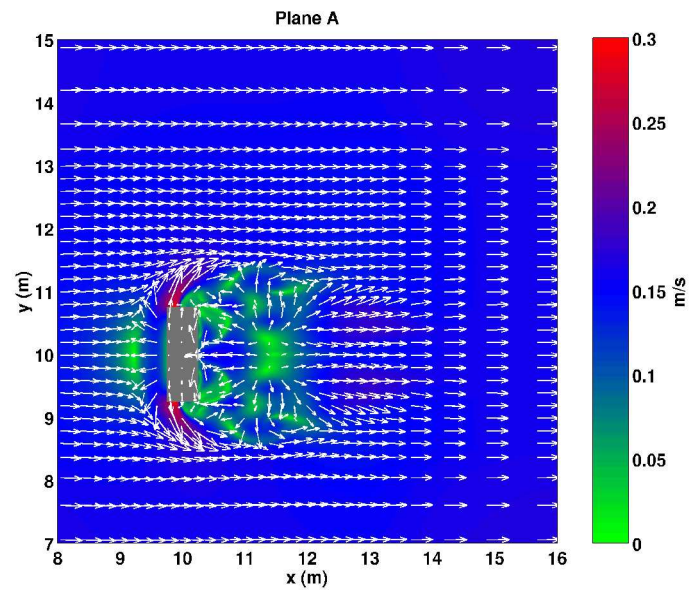


Figure 4. A plan view of near bed simulated velocities resulting from a 25 cm/s transverse current applied to a three-dimensional cylindrical object. Image color depicts velocity magnitude with red intensities highlighting regions of scour.

The Effect of Humic and Fulvic Acids on Arsenic Solubility in Drinking Water Supplies

Basic Information

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The Effect of Humic and Fulvic Acids on Arsenic Solubility in Drinking Water Supplies

Final Report

Statement of Critical Regional or State Water Problem

Arsenic in ground water results primarily from natural geochemical interactions that occur between water and As-containing rocks and minerals (Fleischer 1983). Due to its known toxic effects on humans, arsenic in drinking water is a threat to public health and is regulated in the United States by the Safe Drinking Water Act. On January 22, 2001 the United States Environmental Protection Agency (USEPA) published a Final Rule in the Federal Register (40 CFR 141.62(b)(16)) establishing a new maximum contaminant level (MCL) for arsenic of 10 µg/L, down from 50 µg/L. This revision reflects an improved understanding of the toxic effects of arsenic on humans, and is expected to substantially decrease annual deaths from cancer (USEPA 2002). On February 22, 2002 the new arsenic drinking water limit became effective. Enforcement of the Rule begins on January 23, 2006 and it is expected to increase annual treatment costs by approximately \$181 million (USEPA 2002).

Ground water is crucial to meeting the industrial and domestic needs of the residents of Ohio, with 79% of the community water systems, over 99% of the non-community water systems, and nearly 1 million rural homes utilizing ground water (OhioEPA 2000). Arsenic concentrations in ground water above the MCL of 10 µg/L occur throughout Ohio, particularly in areas with sand and gravel aquifers (OhioEPA 2000). In many instances treating As-containing ground water to meet regulatory needs and protect public health will require costly modifications to optimize existing treatment processes or the addition of point-of-use treatment techniques. The United States Environmental Protection Agency has issued guidance in selecting treatment methods for removing arsenic, and emerging technologies utilizing membranes (e.g., reverse osmosis), adsorptive processes (e.g., activated alumina), and precipitative processes (e.g., enhanced coagulation/filtration) show particular promise (Hering et al. 1997; Brandhuber and Amy 2001). Common inorganic and organic ground water constituents reduce removal efficiency, and natural organic matter (NOM) decreases removal by 20 - 50 % (Hering et al. 1997; Brandhuber and Amy 2001). Recent evidence suggests that NOM can complex arsenic to form stable solution complexes (Redman et al. 2002), and the increase in arsenic solubility resulting from the presence of such stable As-NOM complexes could be responsible for reduced removal efficiency. Details of these interactions are limited, however, and their importance is currently unknown. Knowledge of the fundamental processes that control As solubility, transport, and treatment, including interactions between arsenic and NOM, is crucial to maintaining the quality of ground water that approximately five million people in Ohio depend upon for their daily needs.

Nature and Scope of the Research

Arsenic (As) is a naturally occurring trace element in the earth's crust and is a common constituent in many igneous and sedimentary rocks. Arsenic is readily mobilized into solution through the combined effects of geochemical interactions and biological activity, and is normally present in surface and ground water at low concentrations (≤ 1 µg/L) (Welch et al. 2000;

Smedley and Kinniburgh 2002). Although high As concentrations are associated with anthropogenic sources, the majority of environmental As problems are the consequence of natural processes (Welch et al. 2000; Smedley and Kinniburgh 2002). Arsenic toxicity to humans is well documented (Council 1999), and its presence at elevated concentrations in the public water supply is of great contemporary concern (Nickson et al. 1998; Council 1999; Welch et al. 2000; Berg et al. 2001; Smedley and Kinniburgh 2002).

In natural water systems, arsenic predominately exists in the inorganic form as oxyanions of trivalent arsenite, As(III), or pentavalent arsenate, As(V). Oxidizing conditions favor the formation of arsenate species (H_3AsO_4 , H_2AsO_4^- and HAsO_4^{2-}), whereas reducing conditions favor arsenite species (H_3AsO_3 and H_2AsO_3^-). The species H_2AsO_4^- , HAsO_4^{2-} and H_3AsO_3 prevail under environmental conditions, where the pH spans 4 to 9 (Baes and Mesmer 1976). Although the redox state of a system is important, arsenic solubility and transport is dominated by adsorption reactions that occur at the surface of reactive iron and aluminum oxide minerals. Adsorption of arsenic oxyanions by mineral surfaces is favored at low pH, and adsorption decreases in magnitude with increasing pH in a manner consistent with other anions (Sigg and Stumm 1981). In general, arsenate is adsorbed to a greater extent than arsenite, except at elevated pH (≥ 9) where the opposite occurs (Xu et al. 1988; Wilkie and Hering 1996; Raven et al. 1998). Consequently, in most environmental systems arsenite is more mobile and bioavailable, hence more toxic than arsenate (Council 1999; Smedley and Kinniburgh 2002).

Co-occurring anionic solutes alter the adsorption and thus the solubility of arsenic. Sulfate and phosphate directly compete with arsenic for surface sites on reactive metal oxides, particularly at low pH, and increase arsenic solubility (Xu et al. 1988; Manning and Goldberg 1996; Wilkie and Hering 1996); molybdate, however, has little net effect on As adsorption or mobility (Manning and Goldberg 1996). Surface complexation models suggest that dissolved carbonate should interfere with arsenic adsorption on mineral surfaces at carbonate concentrations typically measured in ground and soil waters (Appelo et al. 2002). Experimental evidence in support of these calculations is still lacking because carbonate adsorption reactions are difficult to study (Wilkie and Hering 1996).

The formation of solution complexes between arsenic oxyanions and other elements is limited (Cullen and Reimer 1989), however, even such limited interactions still influence arsenic speciation (Lowenthal et al. 1977; Wilkie and Hering 1996; Redman et al. 2002). For example, in artificial seawater arsenate forms ion pairs with magnesium and calcium (Lowenthal et al. 1977). These ion pairs result from charge screening that is induced by the high solution ionic strength and their presence increases the concentration of arsenic (Lowenthal et al. 1977). A similar process decreases arsenic solubility by enhancing As (V) adsorption at elevated pH (Wilkie and Hering 1996) where the adsorption of calcium reduces unfavorable coulombic interactions that otherwise would limit the adsorption of arsenate oxyanions.

The adsorption of arsenate and arsenite to mineral surfaces is reduced in the presence of natural organic matter (NOM) (Xu et al. 1988; Xu et al. 1991; Bowell 1994; Grafe et al. 2001; Grafe et al. 2002; Redman et al. 2002). NOM is ubiquitous in aquatic systems and consists of a heterogeneous mixture of polyfunctional molecules of varying size and reactivity. The ability of NOM to bind contaminants and mineral surfaces can markedly alter contaminant mobility and

has resulted in extensive research (e.g., (Davis 1984; Pignatello and Xing 1996; McCarthy et al. 1998; Lenhart and Honeyman 1999). The effects of NOM on As adsorption differ depending upon the NOM source, as well as the charging characteristics and surface area of the adsorbent mineral (Xu et al. 1988; Xu et al. 1991; Howell 1994; Grafe et al. 2001; Grafe et al. 2002). Like sulfate and phosphate, the reduction in arsenic adsorption is presumed to result from competition between As and NOM for surface sites (Xu et al. 1988; Xu et al. 1991; Howell 1994; Grafe et al. 2001; Grafe et al. 2002). Redman et al. (Redman et al. 2002), however, present evidence that supports the formation of stable As-NOM solution complexes, which could be the reason for the reduced As adsorption. The complexation of As by NOM depended upon the NOM source and increased with NOM-bound cationic metals, particularly Fe (Redman et al. 2002).

A comprehensive framework for understanding the extent and importance of arsenic complexation by NOM in natural waters awaits development. Scant evidence, other than that presented by Redman et al. (Redman et al. 2002), exists examining the formation of solution complexes between NOM and As (Tanizaki et al. 1985; Thanabalasingam and Pickering 1986). Thanabalasingam and Pickering (Thanabalasingam and Pickering 1986) find that the association of As(V) and As(III) with two commercial humic acids followed a Langmuir relationship, and that NOM binds arsenate more strongly than arsenite. Tanizaki et al. (Tanizaki et al. 1985) sampled river water in Japan and report that approx. 60% of the As was associated with colloidal matter that consisted primarily of organic carbon. These results provide little additional insight into the complexation of As by NOM, and many questions remain, including the role of coexisting cationic solutes, the impact of solution pH, and the dependence of As complexation on the physicochemical properties of NOM.

Research Objective

The objective of this research is to investigate the association of inorganic arsenic with different sources of NOM in the presence of metal cations (e.g., Ca^{2+}). Arsenic is highly toxic and readily mobilized in significant concentrations by natural processes that occur in ground water. The EPA considers arsenic to be a priority pollutant and recently lowered the MCL to 10 $\mu\text{g/L}$ from 50 $\mu\text{g/L}$. Results focused on examining interactions between As, metal cations, and NOM using potentiometric titration, dialysis and for speciation, capillary electrophoresis.

Materials and Methods

Materials. Stock solutions of arsenate (As(V)) and arsenite (As(III)) were prepared using sodium hydrogenarsenate heptahydrate, $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, and sodium metaarsenite, Na_3AsO_3 , respectively (both purchased from Aldrich). Water for all experiments was supplied from a Milli-Q water system ($>18 \text{ M}\Omega \times \text{cm}$ resistance, Millipore). Three samples of NOM were purchased from the International Humic Substances Society, IHSS (Table 1); Suwannee River NOM (SRNOM), Nordic Lake NOM (NLNOM) and Pahokee Peat. The Suwannee River and Nordic Lake NOM were used as supplied. DOM from Pahokee peat was isolated following IHSS standard methods for soil, followed by dialysis using a 1000MW cutoff membrane. The Pahokee Peat NOM extracted in this manner was then stored in the dark at 4°C after being freeze dried. The elemental compositions of the NOM samples were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Varian) and graphite furnace atomic

absorption spectrometry (GFAA; Varian) and confirmed the arsenic content in each sample was negligible (e.g., results for SRNOM in Table 2).

Table 1 - IHSS NOM Elemental Compositions.

NOM Type	H ₂ O	Ash	C	H	O	N	S	P
Suwannee River	8.15	7.0	52.47	4.19	42.69	1.10	0.65	0.02
Nordic Lake	nd	41.4	53.17	5.67	nd	1.10	nd	nd
Pahokee Peat*	6.2	12.7	46.90	3.90	30.3	3.42	0.58	Nd

*Typical results, not necessarily representative of those for the substance examined in this study.

Table 2 - Elemental Analysis of SRNOM (mg/g).

Al	As	Ba	Ca	Cu	Fe	K	Mg	Pb	S	Se	Si	Sr	Zn
0.85	nd*	<0.01	0.35	0.01	2.19	0.28	0.09	nd	2.90	nd	4.20	<0.01	1.44

* Limit of detection by GFAA for As is 0.9 µg/L

Potentiometric Titrations. Titrations were performed on three solutions: (1) 20 mg/L As(V) in 0.1 M NaCl, (2) 170 mg/L PPNOM in 0.1 M NaCl, and (3) 20 mg/L As(V) and 170 mg/L PPNOM using a computer controlled automatic titrator (Mettler-Toledo DL77). Experiments were performed at a constant temperature of 25 °C in a nitrogen atmosphere. Prior to titration, appropriate aliquots of PPNOM, As(V), and NOM + As were prepared in CO₂-free Milli-Q water and allowed to equilibrate overnight. The ionic strength was adjusted to 0.1 M using NaCl. Titrations were run from the initial starting pH to a pH of 10 – 10.5 using standardized 0.1 or 0.01 N NaOH. Equilibrium between additions was defined to be when the pH drift was less than 0.01 pH/min.

Dialysis Experiments. Experiments were performed to examine interactions between As(V) and PPNOM using a batch dialysis experimental approach. Solutions of variable As and 10 mg/L PPNOM were equilibrated in the dark at pH 6.0 in solutions comprised of 0.01 M NaCl with and without 0.0005 mM CaCl₂. 100 mL aliquots of these solutions were transferred to dialysis tubing with a nominal molecular weight cutoff of 1 kDa. The dialysis tubing was placed within 2 L beakers and the As-PPNOM solutions were dialyzed against 2000 mL of the electrolyte solution. Solutions within the beaker were stirred continuously using a Teflon coated magnetic stir bar and samples were periodically extracted to determine As and TOC concentrations. To complete the mass balance, at the conclusion of the experiment the final solution concentrations of As and TOC within the tubing and the dialysis solutions were determined.

Arsenic Speciation using Capillary Electrophoresis. An HP^{3D} capillary electrophoresis (CE) system (Agilent Technologies, Inc.) with direct UV detection and normal electroosmotic flow (EOF) was used as the CE device to separate arsenate and arsenite. A fused-silica capillary of 50 μm i.d. \times 48.5 cm was used in all experiments. The effective length of the capillary to the detector is 40 cm and the capillary temperature was maintained at 20°C. The separation voltage was set to +15 kV, although a range in values between +10 kV and + 25 kV was investigated. Hydrodynamic injection at a pressure of 50 mbar was used for sample introduction and an on-capillary UV diode-array was used for detection at a wavelength of 192 nm. (The minimum wavelength is 191 nm for the Agilent, diode-array with a deuterium lamp). The total injection time was 5 s or 10 s. To ensure uniform capillary surface conditions, the capillary was washed with a 0.1 M NaOH solution for 10 min at the beginning of each workday. Prior to each injection, the fused-silica capillary was flushed with 1 M NaOH for 1 min, Milli-Q water for 1 min and electrolyte buffer for 2 min.

Computational Platform. All model calculations and parameter optimizations presented in this report were performed using the nonlinear least-squares optimization program FITEQL, Version 3.2 (Herbelin and Westall 1996). Specific details of this program are discussed by Westall *et al.* (Westall and Hohl 1980; Westall *et al.* 1995; Herbelin and Westall 1996). The determination of the model's goodness of fit is provided using a weighted sum of squares term (WSOS/DF), which is the sum of the squares of residuals divided by the degrees of freedom. The WSOS/DF term is a function of the absolute (s_{abs}) and relative errors (s_{rel}) of the data. In general, lower values of WSOS/DF indicate a better fit of the model to the data. Appropriate model fits are indicated when $0.1 < \text{WSOS/DF} < 20$.

Results

Potentiometric Titration Studies: Potentiometric titration results for PPNOM, As(V) and PPNOM + As(V) in 0.1 M NaCl are shown in Figures 1A, 1B, and 1C, respectively. The titration curves for PPNOM and PPNOM+As(V) demonstrate the characteristic broad, featureless, "smeared" titration curve associated with natural organic substances (Bartschat *et al.* 1992). Titrations of arsenate solutions are characterized by buffering associated with the second dissociation of arsenic acid at pH 6.8. The first and third dissociation reactions are obscured by the hydrolysis of water at low and high pH, respectively.

Model fits to the PPNOM were accomplished using the discrete-ligand approach described by Westall *et al.* (Westall *et al.* 1995). This approach allows for the estimation of NOM acid-base chemistry by depicting NOM as a suite of monoprotic acidic ligands, HL_i , with defined pK_{a} values (e.g., $\text{pK}_{\text{a},1} = 2$; $\text{pK}_{\text{a},2} = 4$, etc.). No direct physical relationship of the acid groups to the NOM 'molecule' is implied; instead the acids are treated as a suite of discrete ligands that act independently of each other. Total concentrations of each acid site ($T_{\text{HL}(i)}$) result from simulating the potentiometric titration data. To limit the number of model variables, no explicit corrections are employed to account for activity or electrostatics.

The data are simulated within FITEQL using a set of protolysis reactions and mass-action expressions for each acid site, HL_i :



where i refers to the specific monoprotic ligand. Model results using a 4-ligand model with pKa 4, 6, 8 and 10 provide excellent fit to the data (solid line in Figure 1A). We next applied this model, amended with the constants for arsenate protolysis, to predict the As(V)+NOM data. As shown in Figure 1C, the model fit predicts the behavior very closely indicating that for the conditions studied that interactions between PPNOM molecules and arsenate if present are very weak and do not perturb the protonation state of either reactant.

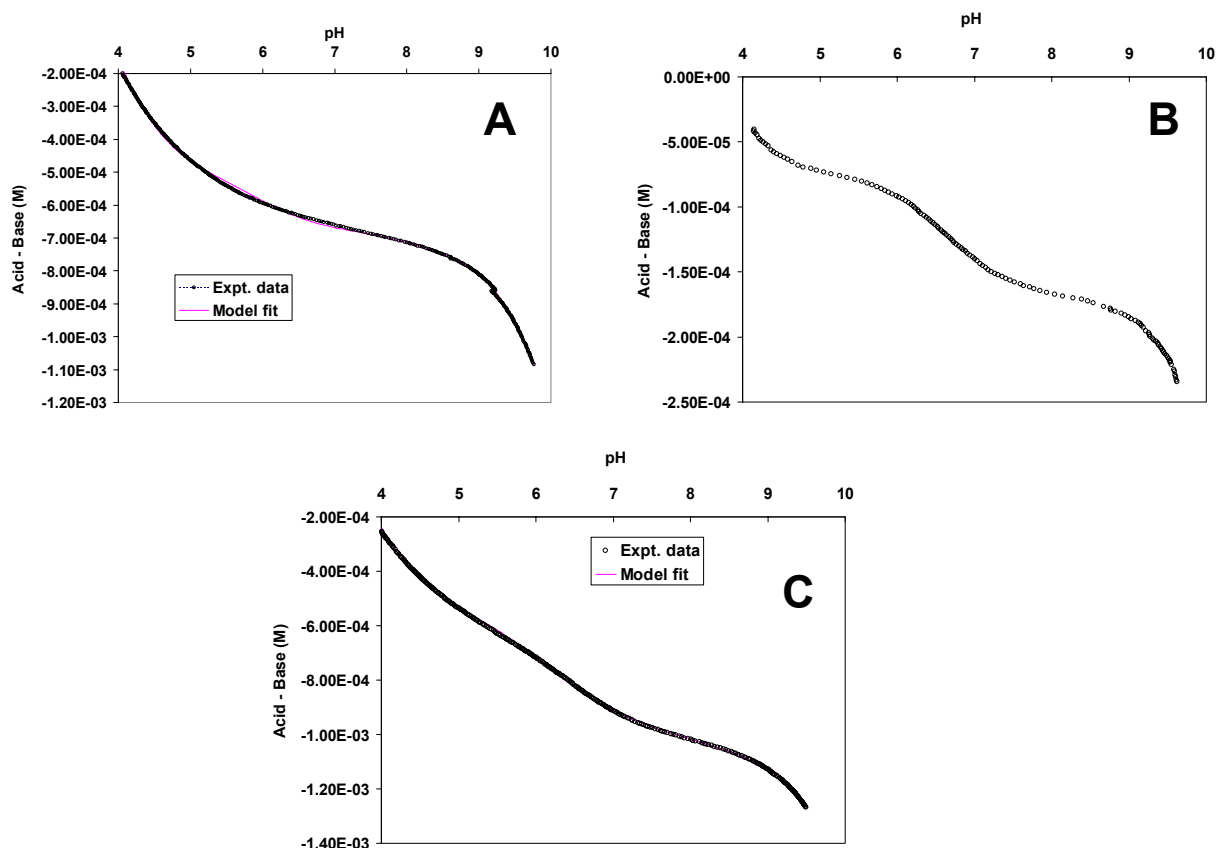


Figure 1 - Acid-base titration of (a) 170 mg/l PPNOM, (b) 20 mg/L As(V) and (c) 170 mg/L PPNOM and 20 mg/L As(V). Solid lines represent model fits.

Dialysis of As(V)-PPNOM Solutions: Dialysis experiments also did not provide evidence for sodium or calcium mediated interactions between PPNOM and arsenate as the concentration of As(V), corrected for dilution, approached the starting values of 20 mg/L, 100 mg/L and 200 mg/L (e.g., see Figure 2 for results for systems with calcium). Experiments were conducted for over 100 hrs, although steady-state conditions appear to have been reached in each system at approximately 24 hours. Concentrations of carbon in the solution outside the dialysis bags remained at background levels and at the conclusion of the experiment the carbon content was essentially the same as at the beginning of the experiment (data not shown). These results confirm that all of the PPNOM was retained within the dialysis tubing and that the arsenate distributed uniformly throughout the hydraulically connected solutions inside and outside the dialysis tubing to the same concentration.

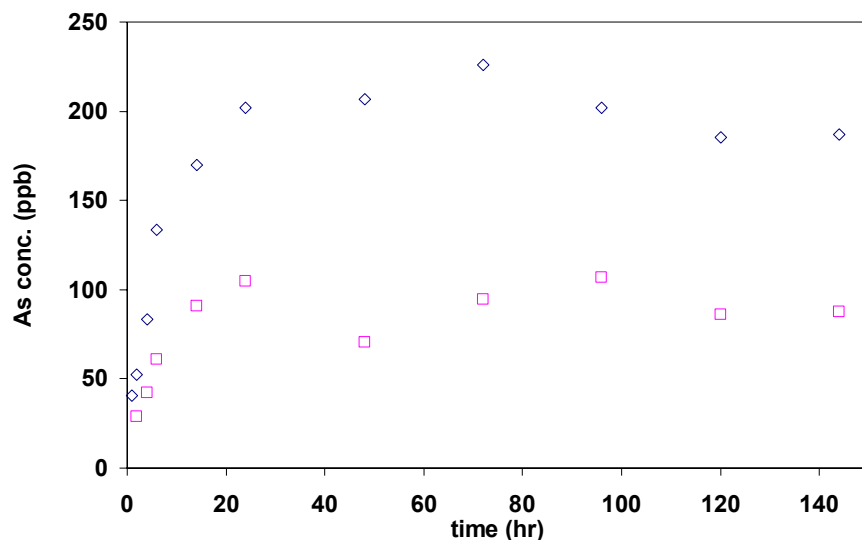


Figure 2 – Measured As(V) concentrations outside dialysis tubing, corrected for dilution, as a function of time in the presence of 0.0005 M Ca^{2+} .

CE studies with Arsenite and Arsenate: The absorption of ultraviolet light by inorganic arsenic species increases as the wavelength is decreased below 250 nm and appears strongest near 190 nm. Sun et al. 2002 applied CE at a wavelength of 192 nm to measure arsenate and arsenite solutions down to approximately 1 to 6 mg/L. Under similar experimental conditions we measured arsenite at 5.2 min., but seemed unable to detect arsenate (Figure 3). Investigating further using a UV-VIS spectrophotometer (Shimadzu Co., Kyoto, Japan), we scanned arsenite and arsenate samples from a wavelength of 190 to 300 nm. For a 20 mg/L arsenite sample at pH 6.58 the maximum absorbance at a wavelength of 192.8 nm was 0.73; however, at the same wavelength the maximum absorbance of a 200 mg/L arsenate sample at a similar pH of 6.84 was only 0.12. This suggests that the low absorptivity of arsenate ($0.6 \text{ L cm}^{-1} \text{ g}^{-1}$) compared with arsenite ($36.5 \text{ L cm}^{-1} \text{ g}^{-1}$) might be responsible for our inability to detect arsenate and thus in the remainder of this report we focus on results obtained with arsenite.

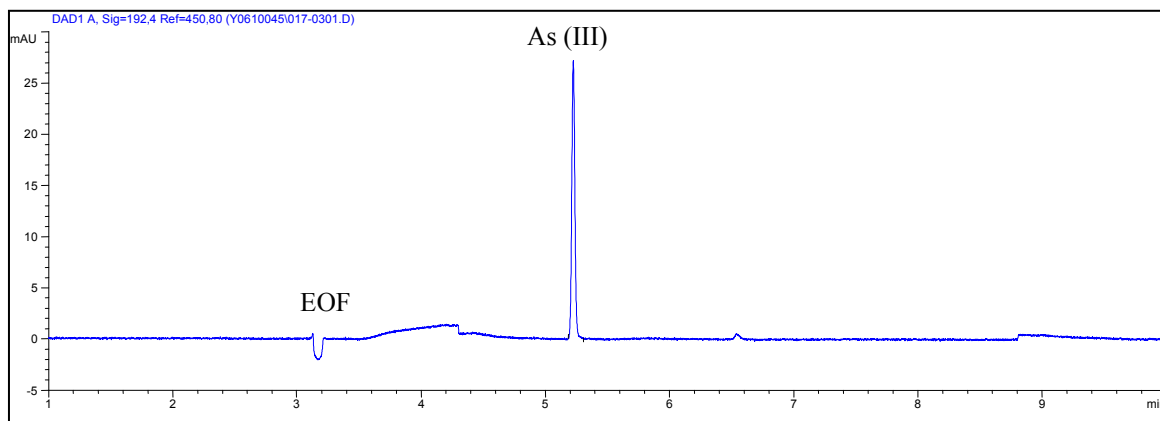


Figure 3. Electropherogram of arsenite (44.11 mg/L) in the borate buffer (20 mM, pH 10).

The ability of CE to separate analyte species is dependent upon a proper buffer selection. We tested four common buffer solutions; carbonate (20 mM, pH 10), borate (20 mM, pH 10), phosphate (20 mM, pH 5, 7, 9, 10, and 11), and acetate (20 mM, pH 7.3). Optimum separation of arsenite occurs at elevated pH where both borate and carbonate are the most effective buffers, but due to its lower UV background borate is a better choice than carbonate.

Studies examining the pH effect on arsenic and NOM complexation were performed in a phosphate buffer at pH values of 5, 7, 9, 10, and 11 to investigate the influence of buffer pH on arsenite detection. At pH values of 5 and 7, the arsenite peak was very close to the EOF peak, making it difficult to accurately analyze peak areas. The pH of the electrolyte has a significant influence on the migration time of analytes and potentially the separation efficiency as well. Furthermore, it is possible that the electrophoretic mobility of arsenic is a weighted average of individual arsenic species.

Evaluation of two-component interactions. Results examining interactions between arsenite and SRNOM were collected by mixing 5-mL aliquots of arsenite and SRNOM solutions in polyethylene tubes. The initial arsenite and SRNOM concentrations were 44 mg/L and 57 mg/L, respectively. The pH value was not recorded. Tubes were rotated in the dark for 24 hours at room temperature (25 °C). Blanks run in parallel ensured that adsorption of arsenite to the surface of the polyethylene tubes was negligible. After equilibration, the mixture of arsenite and SRNOM solution was analyzed using CE. The detection wavelength was fixed at 192 nm although the maximum absorbance of SRNOM was measured at 195.5 nm.

The peak in the electropherogram associated with As(III) exhibits a slight decrease (~ 3%) in the mixed arsenite-SRNOM sample (Figure 4a) compared with the peak in an NOM-free electropherogram at the same concentration (Figure 3). This we attribute to a decrease in the concentration of free arsenite anions due to the formation of a small amount of SRNOM-As(III) complexes. The arsenite peak in Figure 4a occurs simultaneously with an “NOM hump” in the arsenite-free SRNOM sample (Figure 5). Changes in the peaks associated with NOM also suggest the formation of As(III)-NOM complexes (Figure 4b). For example, the height of the NOM peak at 9 minutes, NOM C, decreases and splits in the presence of arsenite (Figure 4b) which occurs, according to Nordén and Dabek-Zlotorzynska (1996), when complexes are formed with NOM. Note that the sharp peaks in the SRNOM electropherogram (NOM A, NOM B, and NOM C) and the broad NOM hump occur as a result of the heterogeneity and polydispersity of NOM. NOM macromolecules span a range in size and functional group content, and thus exhibit nonuniform charge-to-mass ratios and electrophoretic mobilities. In general, during normal EOF molecules having more negative charge-to-mass ratios also have greater electrophoretic mobilities and longer migration times.

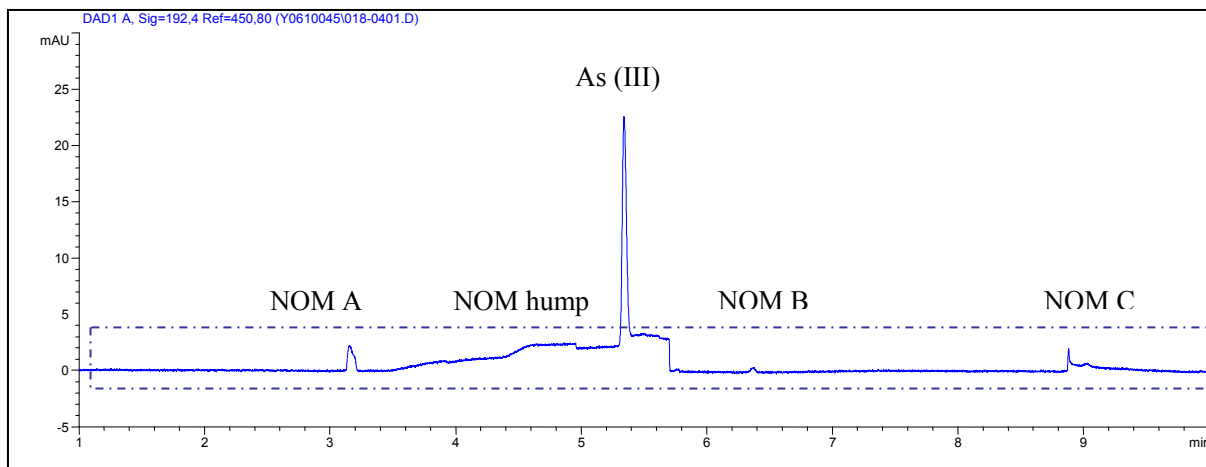


Figure 4a. Electropherogram of arsenite and SRNOM (20 mM borate, pH 10).

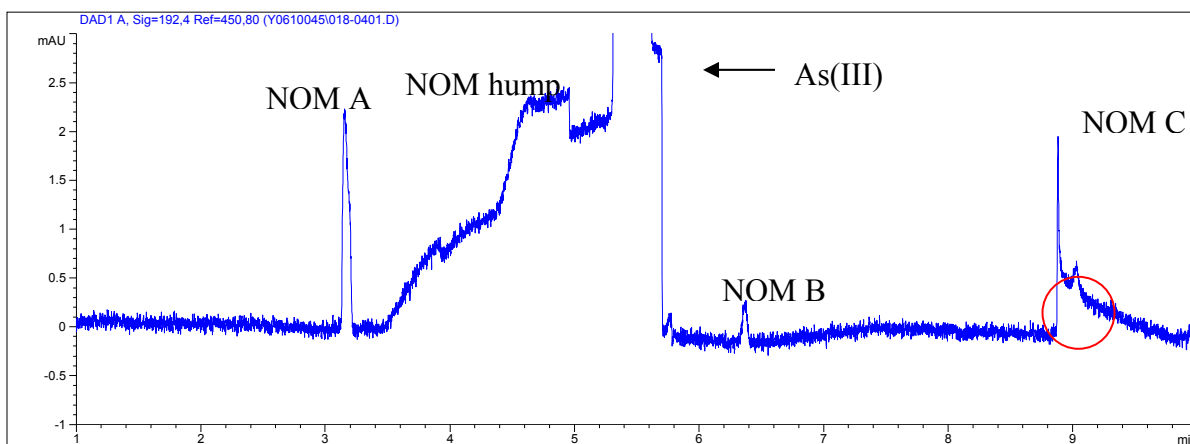


Figure 4b. Exaggeration of lower absorbance region in the electropherogram of arsenite and SR-NOM mixture in the borate buffer (Figure 2a).

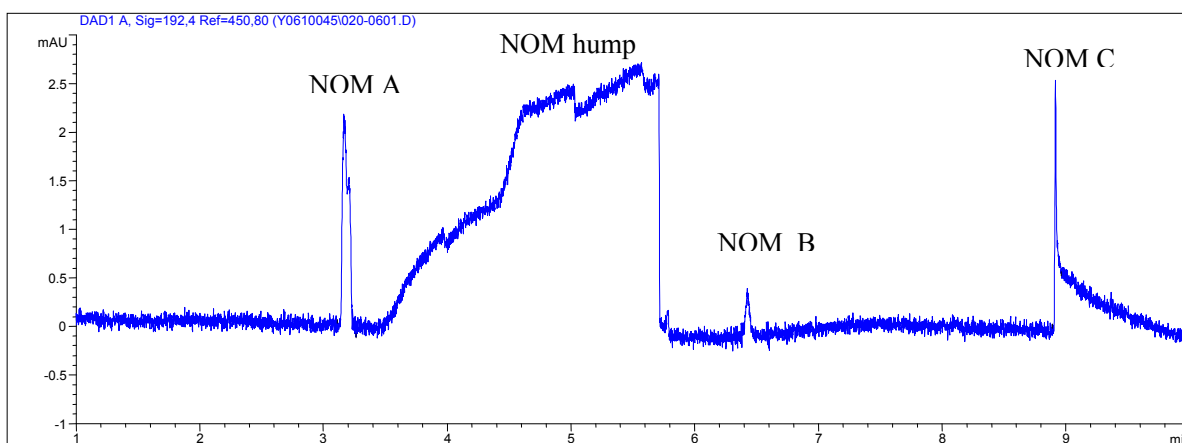


Figure 5. Electropherogram of SR-NOM (57.27 mg/L) in the borate buffer (20 mM, pH 10).

Conclusions

Results collected using potentiometric titration and dialysis procedures indicate that under the conditions studied that arsenate does not form complexes with PPNOM, even in the presence of a weakly binding ion (Ca^{2+}). Our results examining As(III) interactions with SRNOM, however, suggest attractive interactions are a possibility. The content of iron and other cations within SRNOM could be the root cause for this observation and thus the system merits further study. Experimental work further characterizing interactions between NOM and As(III), which could change oxidation state to As(V), are contingent on method development to simultaneously analyze As(III) and As(V). For example, by coupling capillary electrophoresis with a technique that better detects arsenate (e.g., ICP-AES or ICP-MS).

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Post-Doc.	0	0	0	0	0
Total	6	0	0	0	6

Notable Awards and Achievements

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