

Discriminating Biotic and Abiotic Arsenic Release Processes under Highly Reduced Ground Water Conditions

2013 Annual Report

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Statement of Critical Regional or State Water Problem

Ground water is important to the residents of Ohio, with approximately 76% of the community water systems, over 99% of the non-community water systems, and nearly 1 million rural homes utilizing ground water. In total, approximately 4.5 million residents of Ohio, or roughly 40% of the population, depend upon ground water as their source for drinking water. In Ohio ground water, arsenic is the contaminant that most frequently exceeds a health-related drinking-water standard with approximately 17% of the public supply wells producing water that exceeds the Maximum Contaminant Level of 10 $\mu\text{g/L}$. Unfortunately, domestic wells are not routinely tested for arsenic, so most well owners do not know whether their water has elevated concentrations. As a general trend, ground water arsenic concentrations in Ohio are relatively insensitive to the amount of arsenic in the aquifer solids and are instead sensitive to the redox state of the aquifer.

Since ground water concentrations of arsenic in Ohio are tied to the predominant redox state of the system, understanding the conditions that foster certain redox states is critical in siting an appropriate location for a drinking water well. Identifying specific processes or mechanisms responsible for inducing shifts in ground water systems from redox conditions that hinder arsenic mobilization to those that enhance arsenic mobilization is a critical component of this procedure. Details of these processes are lacking, however, and their importance in driving arsenic release in the ground waters of Ohio and elsewhere are still poorly understood. This research will evaluate how redox state changes influence arsenic release behavior. Such knowledge could be used to identify sites with conditions likely to produce arsenic release and is important for maintaining the quality of ground water that approximately five million people in Ohio depend upon for their daily needs.

Research Objective

The proposed research is driven by two overarching objectives that speak to critical knowledge gaps regarding how arsenic release in aquifer systems depends upon redox conditions. They are:

- A. Characterize mechanisms and pathways responsible for arsenic release from aquifer solids under methanogenic conditions.**
- B. Relate redox conditions and changes in arsenic release/sequestration to dominant microbial community members.**

In order to address these objectives, we propose to link detailed macroscopic-level and atomic-level characterization of chemical processes with gene-based microbial community analyses in order to elucidate details of the chemical and biological processes that drive As release and sequestration under transient redox conditions. To do so requires testing the following hypotheses.

Hypothesis A: Arsenic release under methanogenic conditions results from microbially catalyzed dissolution of residual iron oxides in the absence of sulfur or sulfide.

Hypothesis B: Redox-state dependent arsenic release patterns (see Table 1) can be reproduced by changing the concentration of dissolved organic matter in the system.

Hypothesis C: Under similar redox conditions, microbial community profiles do not differ significantly between soil depths; however, archaea species are in greater abundance under methanogenic conditions with accumulated dissolved arsenic.

Methods and Procedures

The project combines bench-scale laboratory experimental work with atomic-level spectroscopy and molecular techniques to evaluate arsenic release and sequestration under transient redox conditions. The research plan was divided into two research tasks necessary to address the proposed objectives and hypothesis. Task 1 assesses arsenic release/sequestration from iron-reducing, sulfate-reducing and methanogenic systems under ambient conditions and as influenced by transients in DOM concentration. In Task 2, we will characterize arsenic speciation in the solids under select iron-reducing, sulfate-reducing and methanogenic conditions.

Study Materials

Aquifer materials for this work were provided courtesy of Dr. Mary Ann Thomas of the USGS. These samples were collected in 2004 - 2005 from southwestern Ohio as part of an effort headed by the USGS to characterize and relate the solid-phase properties of aquifer material and ground water quality to arsenic concentrations (Thomas et al. 2008). The sample site is located in an agricultural and rural residential area thought to be free of any anthropogenic inputs of contamination. The aquifer is comprised of glacial deposits that range in depth from 35 to 120 ft. The samples were collected during the drilling of two monitoring wells as described by Thomas et al. (Thomas et al. 2008). The samples consist of primarily silty, sandy till and some sand or gravel. Transitions between glacial episodes are evident in discontinuities in the physical and chemical properties of the solids. After collection, the samples were air dried, purged with nitrogen and sealed with tape for storage (Thomas et al. 2008). The physical and chemical properties of the samples are described by Thomas et al. (Thomas et al. 2008). For this work, we restricted our analyses to samples from depths in the aquifer that correspond to iron-reducing, sulfate-reducing or methanogenic conditions. Prior to analyses the samples were ground to a uniform particle size and autoclave sterilized.

We used ground water collected directly from the site as a nutrient and microorganism inoculum in our experiments. The samples were collected in August 2012 from depths that correspond with those for the specific aquifer solids examined. These samples were collected by the PI, one

graduate student, one undergraduate student and two USGS personnel using a submersible pump. Sample pH, dissolved oxygen, temperature, oxidation-reduction potential and total dissolved solids were measured immediately in the field using a YSI 556 multiprobe system calibrated with standard solutions prior to each use. Samples were stored in zero-head space sterilized containers, placed on ice and immediately transferred to the environmental engineering laboratories at Ohio State. Aliquots of the samples were analyzed as described in the following section and the remainder was stored at 4 °C until use.

Analytical Methods

Assessment of the dissolved concentration of inorganic elements (e.g., As, Ca, Cr, Fe, K, Pb, Mg, Mn, Na, S, U, Zn) was measured using inductively coupled plasma atomic emission spectrometry (ICP-AES) or graphite furnace atomic absorption (GFAA) spectrometry. All samples were filtered through a 0.22 µm PTFE membrane prior to analyses and acidified using trace-metal grade nitric acid. Calibration was conducted using ICP grade standards (Ricca Chemicals). Anion concentrations (acetate, bromide, chloride, nitrate, nitrite, phosphate, and sulfate) were analyzed using a Dionex ICS-2100 ion chromatography (IC) with an AS19 column and bicarbonate eluent. Dissolved and total organic and inorganic carbon (DOC/TOC and DIC/TIC) was conducted using a Shimadzu TOC 5000 carbon analyzer. Nitrogen species, sulfide and phosphorus were measured with a UV/vis spectrophotometer using the hypochlorite method (ammonium), persulfate digestion (total nitrogen), methylene blue (sulfide) and molybdovanadate method (total phosphorus). Organic nitrogen (TON) were calculated as the difference between the total and inorganic nitrogen species. Arsenic speciation was evaluated using As speciation cartridges purchased from MetalSoft Center and GFAA. Iron speciation was determined using the ferrozine method as described by Hansel et al. (Hansel et al. 2003).

Specific Research Tasks

Task 1A: Evaluate and characterize arsenic release/sequestration under ambient redox conditions. A series of three experiments were conducted to investigate As release and sequestration from the aquifer solids under ambient iron-reducing, sulfate-reducing and methanogenic conditions that approximate those *in situ*. Biotic microcosm experiments were conducted by combining groundwater containing indigenous microorganisms isolated from iron-reducing, sulfate-reducing or methanogenic depths of the site with autoclave-sterilized aquifer media from the same depth into sterile serum bottles (150 ml) at approximately a 2:1 ratio. The serum bottle liquid, media, and headspace were gassed with 80:20% N₂:CO₂, sealed with rubber septa, and crimped closed to maintain anaerobic conditions. Bottles were incubated at 30°C for several weeks in the dark. Biogeochemical changes in the systems were tracked by periodically extracting samples to evaluate solution chemistry and microbial community dynamics throughout the experiment. Abiotic experiments were conducted as described for biotic treatments, with the exception that in addition to the aquifer media being sterilized, the fluids were also be autoclave-sterilized of biological organisms.

Periods of rapid change in solution biogeochemistry in these experiments was used as a guide for selecting samples for profiling the microbial community. Nucleic acids for microbial community analysis were extracted using the PowerSoil DNA Isolation Kit (MoBio Labs, Carlsbad, CA). Dynamics were assessed using bacterial and archaeal primers targeting the 16S rRNA gene. Profiles were created using terminal restriction fragment length polymorphism (T-RFLP) by

labeling the forward primer with a fluorescein dye and digesting with restriction enzymes as described by (Mouser et al. 2010). Electropherogram analysis was conducted using Genemapper and T-REX software. For sequencing of the 16S rRNA gene, unlabeled PCR products were cloned into the TOPO TA vector and chemically competent *E. coli* cells (Invitrogen, Carlsbad, CA). Key inserts from 96 clones identified during fragment analysis and cloning were amplified with the M13F primer and purified for sequence analysis at OSU's Plant-Microbe Genomics Facility. Key sequences were compared to closest known relatives in the NCBI GenBank database, to elucidate species phylogeny and possible function in the systems.

Task 1B: Evaluate and characterize arsenic release/sequestration under transient conditions.

Experiments evaluating system behavior in response to changes in DOM concentration were conducted as described in Task 1A for the ambient systems. The DOM amendments were comprised of (a) DOM concentrated from approximately 5 L of groundwater after first removing suspended solids and microbial cells (0.22 glass fiber filter) and (b) 10 mM sodium acetate. The DOM was concentrated on a reversed-phase silica SPE Bond Elut C18 column (Agilent Technologies). Concentrations tested varied across a similar range to those observed in these aquifer systems. Each of these experiments was conducted in triplicate and the solution phase chemistry and community structure analyses followed methods previously described.

Task 2: Investigate solid-state As speciation under specific redox conditions. Samples were collected from select experiments in Task 1 for analyses using x-ray absorption spectroscopy (XAS) at Argonne National Laboratory in Argonne, IL in order to identify the predominant As oxidation state and structure. Identification of oxidation state will depend upon analyses of the x-ray absorption near edge structure (XANES), whereas structural analyses will rely on computational fits to the extended x-ray adsorption fine structure (EXAFS). Both of these require analyses using proper standards. The XAS sample preparation, data collection and model fits will follow methods described in (Stuckman et al. 2012).

Progress Summary

Work over the past year comprised much of that listed under Task 1, with samples needed for Task 2 analyses also collected and currently being stored for future analyses.

Principal Findings

A series of batch microcosm experiments were conducted with the solids and ground water from the three redox zones: iron-reducing, sulfate-reducing, and methanogenic. At the same time a parallel set of experiments was conducted using DOM-amended microcosms. In both cases, very little activity was observed, indicating the levels of carbon were not sufficient to spur microbial growth (data not shown). The microcosms were subsequently amended with 10 mM sodium acetate and allowed to equilibrate for several weeks. The results of these experiments are still being analyzed and interpreted and thus will those results collected for the iron reducing systems (Site A) will be described here.

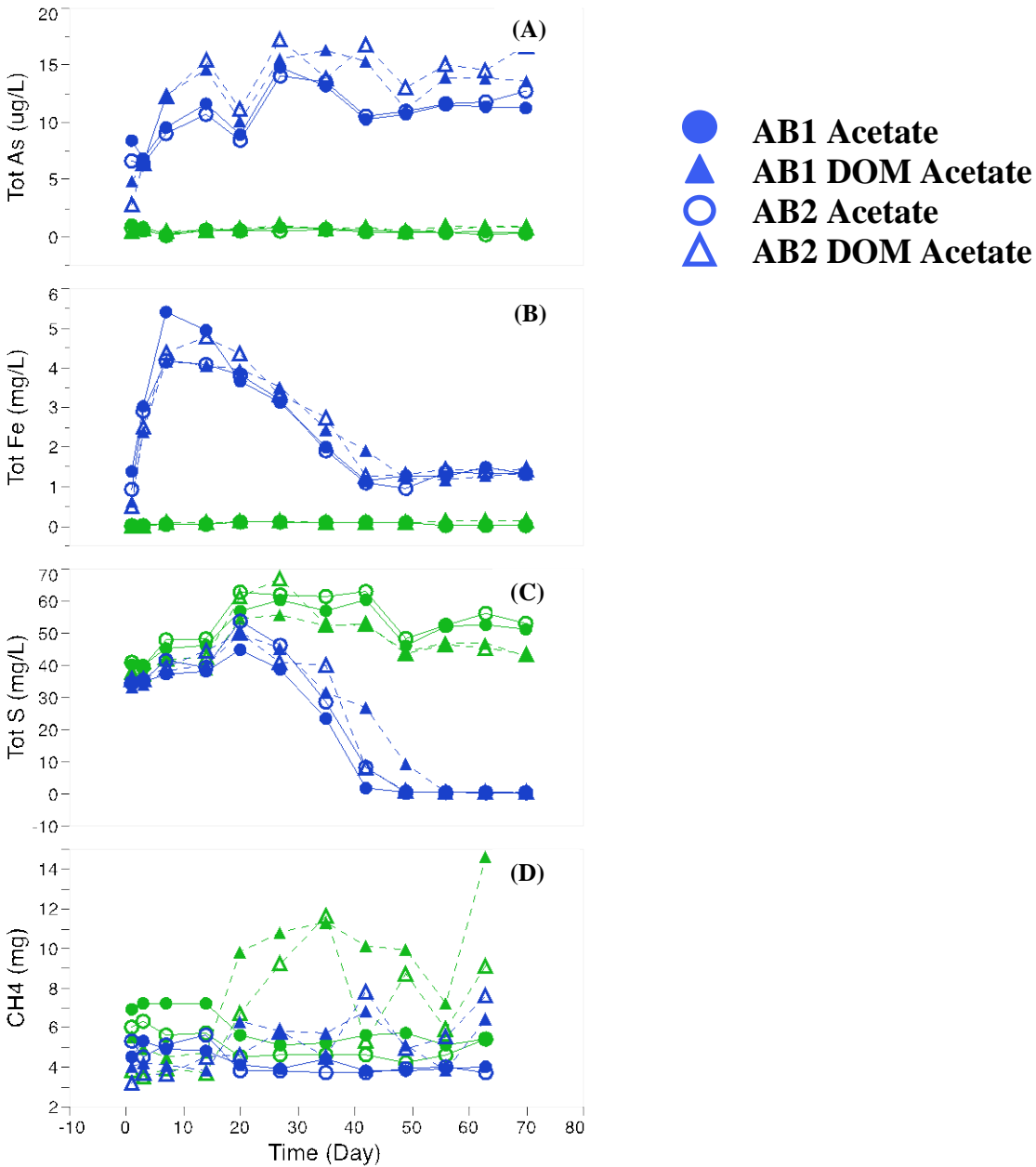


Figure 1 – Concentrations of (A) total arsenic, (B), total iron, (C), total sulfur, and (D) methane released during the incubation of ground water and sediments from iron reducing aquifer. Results are presented for duplicate samples amended with 10 mM sodium acetate (closed symbols) or 10 mM sodium acetate plus additional native DOM (open symbols). The green symbols were for abiotic controls.

Over the course of the study, concentrations of total arsenic were observed to roughly double (Figure 1A) from an initial concentration of approximately 5 ppb to 10 ppb. Most of this increase occurred over the first forty days and coincided with the rapid increase and subsequent

decline in iron (Figure 2B) as well as a decrease in total sulfur (Figure 2C). Over this time methane produced was observed to be small and near that measured for the control samples (Figure 1D). Overall these trends are consistent with release of arsenic concurrent with the reductive dissolution of iron. Similar results collected for samples from the sulfate-reducing and methanogenic aquifer layers produced results that paralleled these results indicating iron- and sulfate-reducing conditions were achieved. Additional work is being conducted to investigate release trends between iron and arsenic for the two systems to better identify potential mechanisms and processes.

Analyses of microbiological community structure (data not shown) in the systems noted that initial communities for the iron-reducing and sulfate-reducing systems were very similar. Upon spurring growth with the addition of acetate, however, both systems converged to have very similar community structures.

Future work consists of (1) correlating release of arsenic and other elements to specific pathways denoted by the presence of particular microorganisms and (2) evaluating sulfur, iron and arsenic speciation in the sediments pre- and post-incubation.

Publications

Stuckman, M.Y., Lenhart, J.J., Mouser, P.J. and Z. Zheng, 2013, "Biotic and Abiotic Release Processes from Groundwater Aquifers under Methanogenic and Transient Redox Conditions", Presented at the 245th ACS National Meeting, New Orleans, LA.

Students Supported

Mengling Stuckman (Ph.D. student in the Environmental Science Graduate Program at The Ohio State University)

Awards or Achievements

None at this time.

References cited

- Hansel, C. M., et al. (2003). "Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow." Geochimica et Cosmochimica Acta **67**(16): 2977-2992.
- Mouser, P. J., et al. (2010). "Enhanced detection of groundwater contamination from a leaking waste disposal site by microbial community profiles." Water Resources Research **46**.
- Stuckman, M. Y., et al. (2012). "Evaluating the Release of Arsenic from Drinking Water Absorbents under Regulatory and Landfill Leaching Conditions Using X-ray Adsorption Fine Structure (XAFS) Spectroscopy." Environmental Science & Technology **In Review**.
- Thomas, M. A., et al. (2008). Relation Between Solid-Phase and Dissolved Arsenic in the Ground-Water System Underlying Northern Preble County, Ohio, United States Geological Society.