EVALUATION OF KINETIC CONTROLS ON SULFATE REDUCTION IN A CONTAMINATED WETLAND-AQUIFER SYSTEM

A Dissertation

by

TARA ANN KNEESHAW

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2008

Major Subject: Geology

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Approved by:

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ABSTRACT

Evaluation of Kinetic Controls on Sulfate Reduction in a Contaminated Wetland-Aquifer System. (August 2008)

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Chair of Advisory Committee: Dr. Jennifer T. McGuire

Our ability to understand and predict the fate and transport of contaminants in natural systems is vital if we are to be successful in protecting our water resources. One important aspect of understanding chemical fate and transport in natural systems is identifying key kinetic controls on important redox reactions such as sulfate reduction. Anaerobic microbial activities like sulfate reduction are of particular interest because of the important role they play in the degradation of contaminants in the subsurface. However, current rate estimates for sulfate reduction have a wide range in the literature making it difficult to determine representative rates for a given system. These differences in rate data may be explained by varying kinetic controls on reactions.

Push-pull tests were used to evaluate sulfate reduction rates at the wetland-aquifer interface. Anaerobic aquifer water containing abundant sulfate was injected into sulfate-depleted wetland porewater. The injected water was subsequently withdrawn and analyzed for geochemical indicators of sulfate reduction. Complexities in rate data, such as presence of a lag phase, changing rate order and spatial variability, were observed and are hypothesized to be linked to activities of the native microbial population.

Subsequent experiments explored the response of native microorganisms to geochemical perturbations using a novel approach to measure directly the effects of a geochemical perturbation on an *in situ* microbial population and measure rates of resulting reactions. *In situ* experiments involved colonization of a substrate by microorganisms native to the wetland sediments followed by introductions of native water amended with sulfate and tracer. Experimental results showed that higher sulfate concentrations and warmer seasonal temperatures result in faster sulfate reduction rates and corresponding increases in sulfate reducing bacteria. Findings from this research provide quantitative evidence of how geochemical and microbiological processes are linked in a system not at equilibrium.

DEDICATION

I dedicate this dissertation to David Pearce whom I never would have met had if it had not been for this research. His patience and support have kept me going and smiling through all the ups and downs. I am particularly grateful for the strong support of my family, especially my mother, who has always been my biggest fan and to my brothers, Brent and Jacob, whose humor and support have meant so much. I also dedicate this dissertation to the loving memory of my grandmother who nearly saw me complete this journey. Her love and support meant the world to me and I know she is still cheering me on. And to the memory of Buster Dog whose recent passing was another reminder of the important role family and friends have had in helping me succeed. He will always be considered one of my most faithful friends.

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Finally I would like to thank the National Science Foundation for providing funding for this project through the Biocomplexity in the Environment Grant EAR 0418488 and the U.S. Geological Survey's Toxic Substances Hydrology Program.

NOMENCLATURE

TEAP	Terminal Electron Accepting Process
PPT	Push-Pull Test
RAMPP	Radial Array Mini Push-Pull
MDP	Mini Drive Point
NOGEE	Native Organism Experimentation Enclosure
BDL	Below Detection Limit

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CHAPTER I

INTRODUCTION

Protecting groundwater resources is a priority environmental problem in nearly all regions of the world. This issue is increasingly important as existing potable water supplies are depleted and water quality diminished in response to anthropogenic pressures such as overuse and contamination. In the United States alone approximately 46% of the population depends on groundwater for drinking water from either public sources or private wells (National Groundwater Association). Because of this dependence on groundwater we need to understand how to protect water quality so that we have safe water supplies for the future. Water quality is affected by a combination of human activities and natural processes which control the chemical, physical, and microbiological reactions that occur in the subsurface. The key to protecting water quality is understanding and predicting these reactions in groundwater systems.

Groundwater systems are complex natural environments where hydrologic processes along with geochemical and microbiological reactions ultimately dictate water quality. Chemical and biological processes, that transform available organic material, and physical processes, that transport it, control the fate and transport of organic matter and organic contaminants in these complex systems. Factors such as redox conditions and chemical make-up of the subsurface determine the resulting reaction rates and transformation pathways.

This dissertation follows the style of Applied Geochemistry.

Redox reactions in the subsurface are coupled reactions, requiring both an electron acceptor and an electron donor to proceed. Organic matter and organic contaminants can serve as electron donors and those oxidation reactions are coupled to the reduction of terminal electron acceptors (e.g. O_2, NO_3^-, Fe^{3+}, SO_4^{2-}). As a result, terminal electron-accepting processes (TEAPs) are an important control on the carbon flow and the fate of contaminants in subsurface systems via oxidation of organic material to CO_2 (Ball and Reinhard, 1996; Cozzarelli et al., 1999; Wilson et al., 2000; Wilson et al., 2004). The resulting reactions and reaction rates in groundwater systems thus partially depend on the availability of electron acceptors and donors. In addition to availability of electron acceptors and donors, rates of reactions are also affected by microbial metabolism, which mediates many redox reactions in the subsurface. Understanding rates of reactions is important in natural systems because ever-changing environmental conditions from events such as rainfall (recharge) or the introduction of contaminants often prevent systems from reaching equilibrium. The number and complexity of factors controlling reaction rates makes measuring rates in dynamic subsurface systems a challenge.

Measurement of redox reactions in natural systems can be done using a number of *in situ* techniques such as *in situ* microcosms (Bjerg et al., 1999; Gillham et al., 1990; Godsy et al., 1999), push-pull tests (Istok et al., 1997; Kleikemper et al., 2002; Kneeshaw et al., 2007; McGuire et al., 2002; Schroth et al., 1998; Schroth et al., 2001b), and tracer tests (Rugge et al., 1999; Sandrin et al., 2004; Smith et al., 1996) but as with any study it is important to understand what conditions the measured rates represent. If the complexities and linkages between geochemical, microbiological, and hydrological

controls on reaction rates can be understood, measured rate estimates can be used to predict the chemical fate and transport of contaminants in complex subsurface systems. However, much work still needs to be done *in situ* to evaluate factors controlling rates of individual redox reactions dominant in natural environments. Sulfate reduction, for example, is one important redox reaction that has been observed in many natural systems (Harris et al., 2005; Ingverson et al., 1981; Jakobsen and Postma, 1999; Scholl et al., 2006; Sinke et al., 1992) but *in situ* factors controlling the rates of sulfate reduction are not well understood. Evaluating the controls on *in situ* rates of a common redox reaction, like sulfate reduction, in unequilibrated natural systems will provide valuable insight into predicting carbon flow, including rates of natural attenuation or bioremediation of contaminated systems. Thus, the objective of this dissertation is to evaluate the factors controlling rates of sulfate reduction using *in situ* experiments in a landfill-leachate contaminated aquifer-wetland system.

Presentation of the *in situ* experiments in this dissertation follows the order in which research was conducted and is broken into four main chapters. Chapter II presents small-scale push-pull tests designed to evaluate the kinetic controls on SO_4^{2-} reduction *in situ* at mixing interfaces between a wetland and aquifer impacted by landfill leachate at the Norman Landfill research site, Norman, OK. Quantifying the rates of redox reactions initiated at interfaces is of great interest because interfaces have been shown to be zones of increased biogeochemical transformations and thus may play an important role in natural attenuation. To mimic the aquifer-wetland interface and evaluate reaction rates, sulfate-rich anaerobic aquifer water (~100 mg/L SO_4^{2-}) was introduced into sulfate-depleted wetland porewater via push-pull tests. Results showed sulfate reduction was

stimulated by the mixing of these waters and first-order rate coefficients were comparable to those measured in other push-pull studies (Harris et al., 2005; Istok et al., 2001; Kleikemper et al., 2002; Luthy et al., 2000; McGuire et al., 2002; Schroth et al., 2001b). However, rate data were complex involving either multiple first-order rate coefficients or a more complex rate order. In addition, a lag phase was observed prior to sulfate reduction that persisted until the mixing interface between test solution and native water was recovered irrespective of temporal and spatial constraints. The lag phase was not eliminated by the addition of electron donor (acetate) to the injected test solution. Subsequent push-pull tests designed to elucidate the nature of the lag phase support the importance of the mixing interface in controlling terminal electron accepting processes. These data suggest redox reactions may occur rapidly at the mixing interface between injected and native waters but not in the injected bulk water mass. Under these circumstances, push-pull test data should be evaluated to ensure that the apparent rate is actually a function of time and that complexities in rate data have been considered.

To further explore and describe the findings from both published and unpublished push-pull data, a series of follow-up studies were conducted. The results of these studies are presented in Chapter III. These experiments were specifically designed to target possible reactions or physical conditions that could be responsible for generating the features observed in push-pull data. Push-pull tests were repeated with different test solutions to address the possibility of 1) the presence of an inhibitor in the injected test solution and 2) a key component (e.g., electron donor) of sulfate reduction lacking in the injected test solution. In addition, the possibility of abiotic reactions between waters was evaluated by mixing end member waters at the surface, and the presence of a mixing

interface between end member waters was evaluated by sampling the mixing interface *in situ*.

Results from these studies indicate two probable reasons for the observed complexities in push-pull data. The first explanation is that push-pull rate data are likely a function of space rather than time due to the presence of a mixing interface and therefore rates are spatially variable during push-pull tests. The second explanation is that the microbial population also plays an important role in controlling the resulting reaction rates. Both explanations have implications for the interpretation of rate data. Traditional methods for rate determination from push-pull tests only work well for reactions that do not vary in space and have a single, unchanging kinetic control throughout the experiment. Data from push-pull tests show that this condition is often not satisfied. Results from the data presented in this chapter suggest that the additional features present in complex rate data should be described to improve our understanding of kinetic controls and enhance our ability to apply measured rates to other systems.

The role of microorganisms in controlling the kinetics of reactions in groundwater systems was evaluated by quantifying the response of a native microbial population to a geochemical perturbation such as would occur during a recharge event. Data from existing sampling techniques designed to measure changes in geochemistry and microbial community *in situ* (Bakermans and Madsen, 2002a; Bakermans and Madsen, 2002b; Jeon et al., 2003; Kleikemper et al., 2005; Pombo et al., 2002) often do not adequately demonstrate the linkages between geochemistry and microbial population because they don't allow for direct measurement. To address this issue a new technique, Native Organism Geochemical Experimentation Enclosures (NOGEEs), was developed to

further evaluate the role of microorganisms through direct measurement of the effect of geochemical perturbations on a native microbial population. Chapter IV presents the design and construction elements of this new technique. NOGEEs were designed to 1) trap a native microbial population *in situ*, 2) isolate the population, and 3) introduce and remove test solutions to measure resulting reactions rates. This novel technique allows for the direct measurement of both geochemical and microbiological parameters providing for the quantification of rate data more representative of complex natural systems not at equilibrium. Chapter IV also presents the results from a test designed to evaluate the effectiveness of this technique. The test consisted of repeated introductions of a sulfate test solution over time and findings showed increased rates of sulfate reduction corresponding to an increase in the number of sulfate reducing microorganisms. These results provided direct evidence of the linkage between microbial population and geochemistry, validating the effectiveness of this technique. Most importantly, this technique can be used to address a number of complex *in situ* questions.

This new technique was then used to further evaluate the kinetic controls on sulfate reduction *in situ*, by testing the importance of changes in sulfate concentration and temperature on sulfate reduction rates. Two comparative tests using NOGEEs were conducted to evaluate these parameters. The results of these experiments are presented in Chapter V. These NOGEE experiments were designed to evaluate differences in zeroth-order sulfate-reduction rates for three different sulfate concentrations (10, 25, and 100 mg/L SO_4^{2-}) during both warm and cold seasonal temperatures. Geochemical results indicated that higher concentrations of sulfate resulted in faster sulfate reduction rates. Variability in rates determined during the two different seasons indicated that warmer

temperatures also resulted in faster sulfate reduction rates. Microbial analyses supported geochemical data, in that faster rates corresponded with increases in the number of sulfate reducing bacteria. These data provide much needed information about the response of native microbial communities to changing geochemical conditions.

In this research novel techniques were used to evaluate *in situ* rates of microbial sulfate reduction. The use and evaluation of these techniques to simulate conditions of complex natural systems provided information about some of the factors important for determining representative reaction rates. In addition, the research encompassed in this dissertation highlights the importance of developing new techniques for obtaining samples from natural systems and for evaluating complexities in *in situ* studies.

CHAPTER II

EVALUATION OF SULFATE REDUCTION AT EXPERIMENTALLY INDUCED MIXING INTERFACES USING SMALL-SCALE PUSH-PULL TESTS IN AN AQUIFER-WETLAND SYSTEM[1]

INTRODUCTION

In subsurface aqueous systems, it is well recognized that interfaces between distinct water masses may be the most active zones of biogeochemical activity (Kappler et al., 2005); however, quantification of the complex suite of reactions initiated at these interfaces has been poorly documented. Steep geochemical gradients have been observed where waters with differing chemical/physical properties come in contact (e.g., the interface zone surrounding a contaminant plume or an aquifer-wetland interface) (Cazull et al., 2006; McGuire et al., 2000; van Breukelen and Griffioen, 2004; Vroblesky and Chapelle, 1994) indicating high levels of reactivity at sharp interfaces. At interface zones biogeochemical activity is enhanced by the availability of limiting electron acceptors such as oxygen (O_2), iron (Fe(III)), nitrate (NO_3^-), and sulfate (SO_4^{2-}) or electron donors such as acetate and lactate (Ball and Reinhard, 1996; Cozzarelli et al., 1999; Wilson et al., 2000; Wilson et al., 2004). Under these conditions, interfaces can become zones of rapid biogeochemical transformations (Harris et al., 2005).

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[1]Reprinted with permission from "Evaluation of Sulfate Reduction at Experimentally Induced Mixing Interfaces Using Small-Scale Push-Pull Tests in a Wetland-Aquifer System" by Tara A. Kneeshaw, Jennifer T. McGuire, Erik W. Smith, and Isabelle M. Cozzarelli, Applied Geochemistry, 22, 2618-2629 (2007). Copyright by Elsevier.

In natural systems, waters often exist as distinct masses that do not easily mix resulting in steep geochemical gradients at the interfaces between water masses. The physical characteristics of the system, such as temperature, grain size, and recharge events (Scholl et al., 2006) as well as chemical characteristics, such as reduction-oxidation (redox) potential and solute transport differences (McGuire et al., 2004) give rise to important distinguishing properties of water masses. Water masses occur coincident with changes in lithology (e.g., wetland-aquifer interface (Cazull et al., 2006) as well as within bulk lithologies (e.g., contaminant plume fringe; recharge water-contaminant plume) (McGuire et al., 2004; Scholl et al., 2006). Study of the dynamics of interface zones, including rates of transformation, has been limited by difficulties in obtaining representative measurements. Sampling mixing zones using conventional techniques (wells and drive points) is problematic due to the zones often small spatial scale (mm-cm), small volumes of fluid, and transient nature. However, knowledge of the scale at which interfaces persist, as well as detailed documentation of the biogeochemical processes occurring are important to understand and predict the fate and transport of nutrients and contaminants in aqueous-subsurface systems.

To quantitatively assess the role of interfaces on system-scale biogeochemical cycling, detailed measurements of the complex reactions occurring at interfaces and their rates need to be made. Though a wide variety of methods have been used to quantify subsurface activities of microorganisms, determining representative reaction rates has proven challenging. Methods including microcosm studies, (Cozzarelli et al., 2000; D'Angelo and Reddy, 1999; Wilson et al., 1983) analysis of geochemistry data (Chapelle et al., 1996b; Lovley and Goodwin, 1988), direct observations of changes in solid-phase

electron acceptors (Jakobsen and Postma, 1999), and molecular techniques (Bowman et al., 1993) provide a wide range of reaction rates making it difficult to apply these rates to natural systems.

In situ experiments, though more complex to interpret, provide more realistic conditions because complexities in mineralogy, microbiology, and geochemistry (including complex organic matter distribution) are maintained. The push-pull test has proven to be a useful technique for obtaining a wide range of *in situ* data while maintaining many of the natural system complexities necessary to consider when interpreting rate data (Harris et al., 2005; Istok et al., 2001; Luthy et al., 2000; McGuire et al., 2002; Schroth et al., 1998). Unlike well-mixed microcosm-type experiments, push-pull tests have the additional advantage of generating an interface between water masses allowing for the investigation of steep geochemical gradients as might be observed in nature.

Push-pull tests consist of a controlled rapid injection of a test solution into a single well followed by the slow recovery of the test solution from the same well.(Istok et al., 1997) Though push-pull tests vary based on their intent, all push-pull tests contain three phases: (1) extraction of groundwater from the push-pull well for preliminary geochemical characterization; (2) injection (push) of a test solution containing a conservative solute as a tracer to account for advection and dispersion and reactive solute(s); and (3) extraction (pull) of the test solution, sometimes after an incubation period, and measurement of solute concentrations over time.

Several studies have used push-pull tests to describe *in situ* microbial reaction kinetics. Studies by Haggerty et al (Haggerty et al., 1998) and Snodgrass and Kitanidis

(Snodgrass and Kitanidis, 1998) provide simplified methods of calculating first and zero-order *in situ* microbial reaction rate coefficients. These studies account for decreases in solute concentration as a result of dilution from diffusion and dispersion and require no knowledge of aquifer porosity, dispersivity, or hydraulic conductivity, nor the use of flow and transport models. Several studies have used these methods to interpret rate data from push-pull tests for various chemical species (Cunningham et al., 2001; Harris et al., 2005; Istok et al., 2001; Kleikemper et al., 2002; Luthy et al., 2000; McGuire, 2002; Schroth et al., 2001b; Ulrich et al., 2003). One complexity associated with push-pull test data is the often observed lag phase prior to reaction. Some studies interpret this lag phase as simply the time required by the microbial population to adjust to new conditions (Chapelle, 2001). Others have suggested the lag in microbial activity is due to lack of electron donor in the injection water, suggesting that the lag phase is controlled by the rate of desorption of organic matter and mixing with native water containing sufficient electron donor (Addy et al., 2002; Istok et al., 2001; Kleikemper et al., 2002; Luthy et al., 2000; McGuire et al., 2002; Schroth et al., 2001b). The nature and controls on this lag phase have not been adequately addressed but may represent an important control on reaction processes when distinct waters come in contact.

This paper presents small-scale push-pull tests designed to evaluate kinetic controls on SO_4^{2-} reduction at *in situ* mixing interfaces between a wetland and aquifer impacted by landfill leachate. Recent studies have identified multiple small (cm) scale mixing interfaces exhibiting steep geochemical gradients within the complex aquifer-wetland system representing several important mixing zones (Cazull et al., 2006). This study utilized push-pull tests designed to better understand the reaction kinetics

associated with these interface zones. This study demonstrates the importance of the mixing interface on initiating SO_4^{2-} reduction and demonstrates the utility of push-pull tests to explore complex reactions occurring at the mixing interface between water masses of differing redox potential.

STUDY SITE DESCRIPTION

The location of this study is the Norman Landfill research site in Norman, OK, a closed municipal landfill near the Canadian River. This unlined landfill received unrestricted waste from 1922 until 1985 when it was closed and covered with an earthen cap (Adrian et al., 1990; Christenson and Cozzarelli, 2003). A leachate plume containing elevated concentrations of dissolved organic carbon (DOC), chloride, ammonia, and methane developed, extending at least 225 m downgradient from the landfill and flowing under/through the wetland system (Christenson and Cozzarelli, 1999). The size and shape of the plume is controlled by the complex interactions between biogeochemical and hydrogeological processes including: biodegradation, sorption, dispersion, dilution, physical heterogeneities and changes in recharge conditions at the site. Plume dimensions also suggest the interface between the contaminated aquifer and overlying wetland porewater may be an important zone of biodegradation. The locations of the wells described in this study were within a slough adjacent to the capped landfill (Figure 2.1).

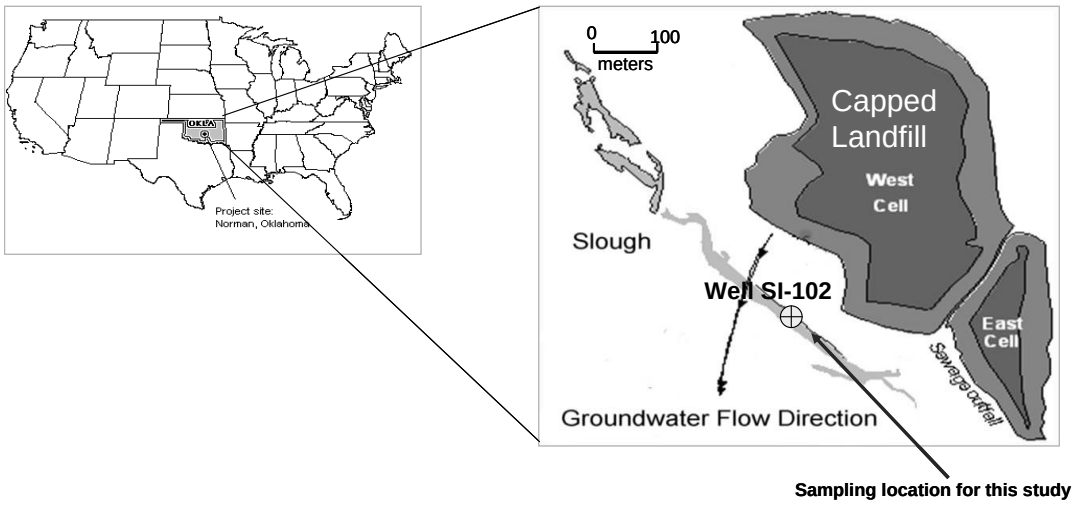

Figure 2.1. Map showing study site location. Norman Landfill research site, Norman, OK, USA. Figure modified from U.S. Geological Survey Fact Sheet 040-03 (Christenson and Cozzarelli, 2003).

The Norman Landfill is the site of an intensive investigation by USGS and university research groups. Knowledge of processes occurring in the aquifer includes characterization of the nature and magnitude of biotic and abiotic geochemical reactions (Cozzarelli et al., 2000; Eganhouse et al., 2001; Grossman et al., 2002; Schlottmann et al., 1999), documentation of the microbiological processes (Beeman and Suflita, 1987; Harris et al., 1999; Ulrich et al., 2003), kinetic studies (Adrian et al., 1994; Beeman and Suflita, 1990; Senko et al., 2002), and quantification of groundwater-surface water fluctuations at the site (Christenson and Cozzarelli, 1999; Schlottmann et al., 1999; Scholl, 2000).

METHODS

Push-Pull Well Instrumentation

Mini push-pull wells were constructed from 2.54 cm, (O.D.) schedule 40 PVC pipe with machined Delrin drive-points. The bottom 3 cm of the wells were screened with 0.5 mm slots and the screened interval was isolated from the remainder of the well casing interior with an o-ringed Delrin packer fitted with 0.635 cm (O.D.) polyethylene tubing. Water was delivered to and withdrawn from the screened interval through the tubing to eliminate the potential for errors due to unmixed space in the well casing. Sediment cores from within the slough were taken prior to installing push-pull wells to aid in determining the targeted zone for the tests. The cores show a reduced coarse sand layer between two silty clay layers at 41.5 to 53 cm depth. The upper silty-clay layer is 31.5 cm thick, bioturbated and mottled, light brown in color (less reduced), and has an erosional contact with the coarse sand layer. The lower silty-clay layer is uniform, black in color (more reduced), and has a sharp erosional contact with the coarse sand layer. The two silty-clay layers appear to confine the coarse sand layer but the lateral extent of the layers is unknown. The coarse sand layer is thought to have negligible flow, as the slough above is stagnant and very limited vertical flow has been measured. Thus, the coarse sand layer was determined to be the best location to conduct push-pull tests.

The injection water used in the test was collected from the aquifer underlying the targeted wetland sediments from a permanent landfill monitoring well, well SI 102-3 (Figure 2.2) with the goal being to simulate an *in situ* small-scale mixing interface between the anaerobic aquifer water and wetland porewater. A PVC drive-point well, hand-driven into the targeted sand lens approximately 50 cm below the sediment-water

interface, was utilized for the experiments during each field session. The well was placed in approximately the same location for each field session and was within 2 m of well SI 102. Wetland surface water overlying the wetland sediments at the well locations was less than 1 m deep.

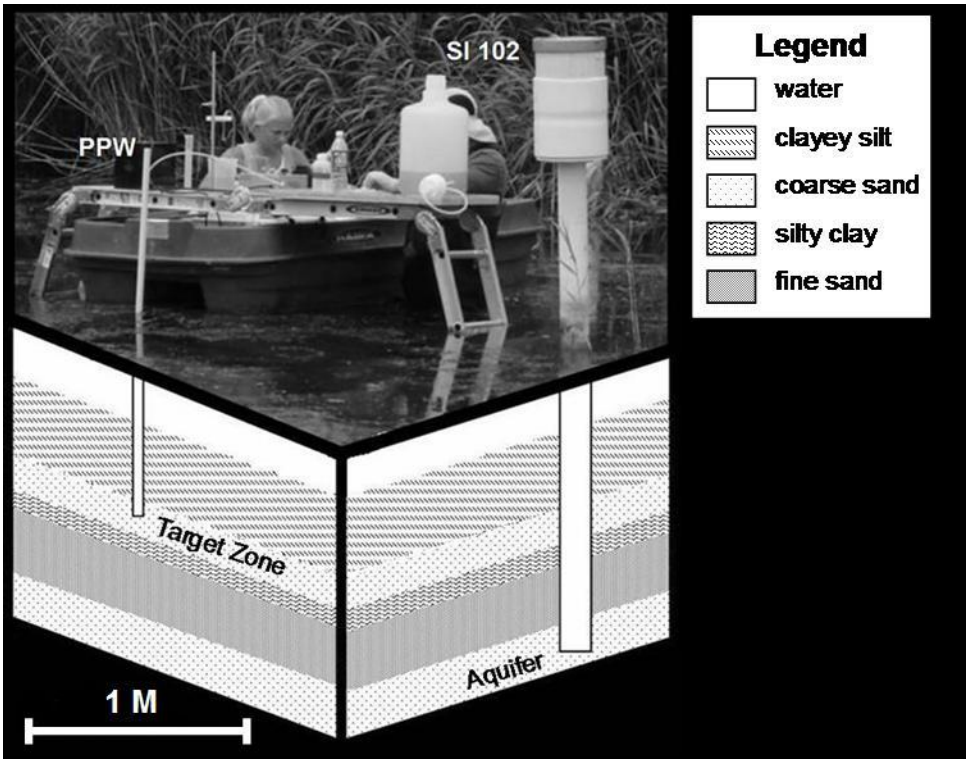

Figure 2.2. Schematic of study site. Well SI 102 is the source of injection water and PPW is the push-pull well.

Push-Pull Tests

Four push-pull tests, referred to as PPT1 through PPT4, were performed during two separate field sessions; PPT1 in May 2004 and PPT2, PPT3, and PPT4 in August 2004. The goal for each push-pull test was to create a mixing interface between

anaerobic aquifer water and more reducing wetland porewater and 1) observe the terminal electron accepting processes (TEAPs) stimulated by the mixing event and 2) quantify the rates of those reactions. These tests specifically targeted sulfate reduction by mixing SO_4^{2-} rich aquifer water and more reducing wetland porewater.

For each push-pull test wetland porewater was first withdrawn from the underlying aquifer (Well SI 102-3, Figures 2.1 and 2.2) using a peristaltic pump (GeoTech) and collected in a 20 L Nalgene carboy. Ten liters were collected for PPT1, PPT2, and PPT4 and 3 L were collected for PPT3. Test solutions were augmented with 100 mg/L sodium bromide ($NaBr^-$, prepared from NaBr, Acros Organics, New Jersey, USA) to serve as a conservative tracer to account for abiotic processes such as dilution from mixing, dispersion, and advection. Acetate (CH_3COOH, prepared from $NaCH_3COOH$, Acros Organics, New Jersey, USA) was added in stoichiometric proportion to SO_4^{2-} (~30 mg/L per test) as an electron donor in PPT4. Glove bags filled with N_2 gas were fitted to valves on the carboy caps to prevent the introduction of O_2 while preparing and injecting the test solutions. Aluminum foil was wrapped around the carboys to block sunlight and maintain aquifer water temperature (~18 $^{\circ}$C in May 2004 and ~23 °C in August 2004). For each push-pull test, the injection volume was pumped rapidly (~500 mL/min) into the push-pull well using the peristaltic pump; any residual solution was gravity drained by inverting the carboy.

Prior to each push-pull test, geochemical parameters were measured in the underlying aquifer water (Well SI 102-3), the push-pull well, and the carboys containing the injection (push) solution. Water samples were also collected at regular time intervals during the extraction (pull) phase of each test. These samples were analyzed for anions

(Cl^-, Br^-, SO_4^{2-}, NO_3^-), ammonium, organic acids (acetate), Fe^{2+} and H_2S. All samples were syringe filtered using Millex-HA 0.45 μm filters (Millipore, Bedford, MA). Anion samples were preserved with formaldehyde and organic acid and NH_4^+ samples were preserved by flash freezing; all were measured in the laboratory using a capillary electrophoresis system (Agilent Technologies, Wilmington, DE). Samples for Fe^{2+} and H_2S determination were preserved with trace metal grade HCl and zinc acetate, respectively; concentrations for both were determined photometrically in the field using a Spectronic20D+ spectrophotometer (Thermo Spectronic, Rochester, NY). Cation samples collected for initial end member water concentrations were preserved with HCl and analyzed by capillary electrophoresis (Agilent Technologies, Wilmington, DE).

Determination of First-Order Rate Coefficients

First-order rate coefficients were determined from reactant and tracer breakthrough curves following the methods of Haggerty et al. (1998). Assuming the tracer and reactant have similar retardation factors, this approach accounts for non-reactive (conservative) processes such as the degree of mixing between native and injected waters. Using this method, rate coefficients were determined according to:

$$\ln\left(\frac{C_d(t^*)}{C_{tr}(t^*)}\right) = \ln\left[\frac{(1-e)-kt_{inj}}{kt_{inj}}\right] - kt^* \qquad \text{Equation 2.1.}$$

where, C_d is the concentration of the reactant, C_{tr} is the concentration of the tracer, t^* is time elapsed since the end of the injection of the test (push) solution, and t_{inj} is the duration of the test solution injection. A plot of $\ln(C_d(t^*)/C_{tr}(t^*))$ versus t^* generates a straight line with a slope $-k$, the first-order rate coefficient. A linear regression was applied to the experimental data to obtain estimates of SO_4^{2-} reduction first-order rate coefficients. Because the determination of k is based on the ratio of C_d/C_{tr}, complete mass recovery is not necessary to obtain accurate estimates of k. Similarly, a portion of the breakthrough curve may be used to estimate k. This is particularly useful in instances where a lag phase is observed. To account for low levels of tracer and/or reactive species in background water, C_d and C_{tr} in equation 2.1 must be corrected using a mixing ratio following equations 2.2 and 2.3 respectively (McGuire, 2002):

$$C_d = \left(\frac{d_m(t^*) - d_b}{X} \right) \qquad \text{Equation 2.2.}$$

$$C_{tr} = \left(\frac{tr_m(t^*) - tr_b}{X} \right) \qquad \text{Equation 2.3.}$$

where $d_m(t^*)$ is the measured reactant concentration at time t, d_b is the measured background concentration of reactant and $tr_m(t^*)$ is the measured tracer (Br^-) concentration at time t, tr_b is the measured background Br^- concentration. X is the slope of the line generated from a plot of the percent input solution (0-100%) versus concentration. This line represents the mixing curve between the injected solution and the background water. If the background concentration is zero then the slope (X) equals one and $C_d = d_m$.

RESULTS AND DISCUSSION

Push-Pull Tests (Geochemical Analyses)

Initial geochemical characterization confirmed that both injected and native waters were depleted with respect to O_2 and NO_3^- (Table 2.1) and contained abundant DOC (~10-50 mg/L in aquifer water and ~30-120 mg/L in wetland porewater); these concentrations remained constant throughout the tests. Aquifer water from well SI 102 contained abundant SO_4^{2-} (~90-114 mg/L SO_4^{2-}) while water from the push-pull wells contained low levels of SO_4^{2-} (~2-14 mg/L). Despite varying test parameters, results for each test were similar and support experimental assumptions. Regardless of test duration or injected volume, breakthrough curves for Br^- and SO_4^{2-} were similar throughout the initial extraction phase of the test differing only upon microbial reduction of SO_4^{2-} (Figure 2.3). This indicates that retardation of Br^- and SO_4^{2-} was negligible, and confirms the assumption made in rate determination that tracer and reactant results were similar (Haggerty et al., 1998; Schroth et al., 2001b).

Table 2.1. Geochemical parameters. Summary of initial geochemical parameters measured in the injection water (underlying aquifer) and the push-pull well water (wetland porewater).

Initial Parameter	PPT1		PPT2		PPT3		PPT4	
	Wetland	Aquifer	Wetland	Aquifer	Wetland	Aquifer	Wetland	Aquifer
pH	6.7	6.6	7.1	6.8	6.9	7.1	6.9	6.8
Temperature (C)	18.4	18.6	24.7	23.9	23.7	22.8	23.8	23.1
ORP (mV)	-143.3	-131.2	-136.3	-104.3	-133.0	-92.5	-132.7	-99.6
SO_4^{2-} (mg/L)	13.5	113.6	5.3	92.0	12.3	93.9	2.3	94.2
H_2S (mg/L)	1.6	0.7	0.07	0.05	0.07	5.8	0.1	0.05
Fe^{2+} (mg/L)	4.2	15.4	3.2	7.5	5.8	13.7	7.7	13.6
O_2 (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
NO_3^- (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
NH_4^+ (mg/)L)	< 0.5	6.9	1.1	0.8	1.2	1.7	5.0	< 0.5

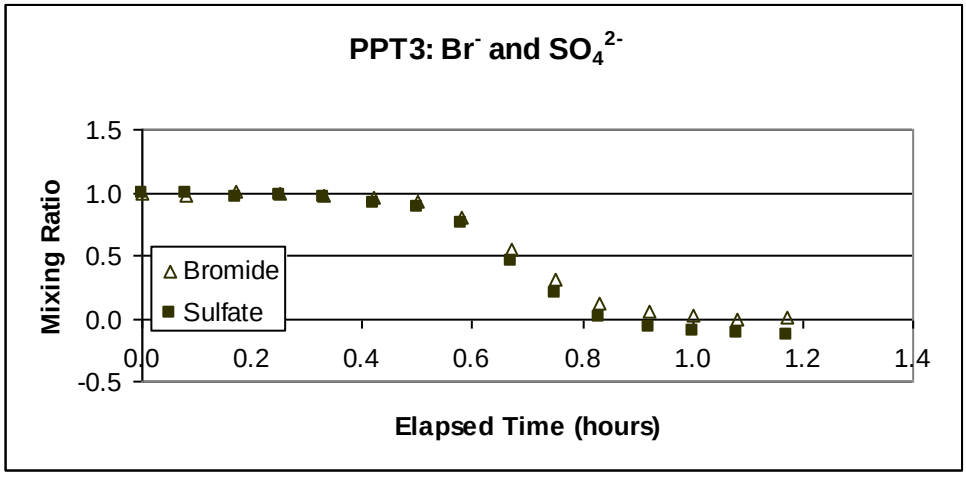

Figure 2.3. Example comparison of breakthrough curves. Plot showing conservative tracer, Br^-, and reactive solute, SO_4^{2-} (results from PPT3).

PPT 1, conducted in May 2004, was performed to evaluate the length of time needed to observe SO_4^{2-} reduction and lasted a total of 32 hours. After a 22 hour lag phase, SO_4^{2-} decreased coincident with an increase in H_2S indicating SO_4^{2-} reduction (Figure 2.4). Interestingly, SO_4^{2-} reduction began at approximately the volume where the mixing interface between injected solution and native water was extracted (~10 L). One possible explanation for the observed lag phase is that the native microorganisms required an incubation time of ~22 hours. Alternatively, as subsequent tests support, SO_4^{2-} reduction did not occur in the bulk injected water but rather only occurred at the mixing interface between injected and native water due to either a lack of critical reactant such as electron donor or the presence of an inhibitory substance. Subsequent push-pull tests (PPTs 2-4, August 2004) were conducted to further explore the nature and cause of the lag phase to better understand how to interpret our results. It should be noted that this study design cannot distinguish any "background" sulfate reduction that may be occurring in the native wetland porewater from sulfate reduction stimulated by the push-pull tests particularly given the heterogeneous nature of wetland sediments.

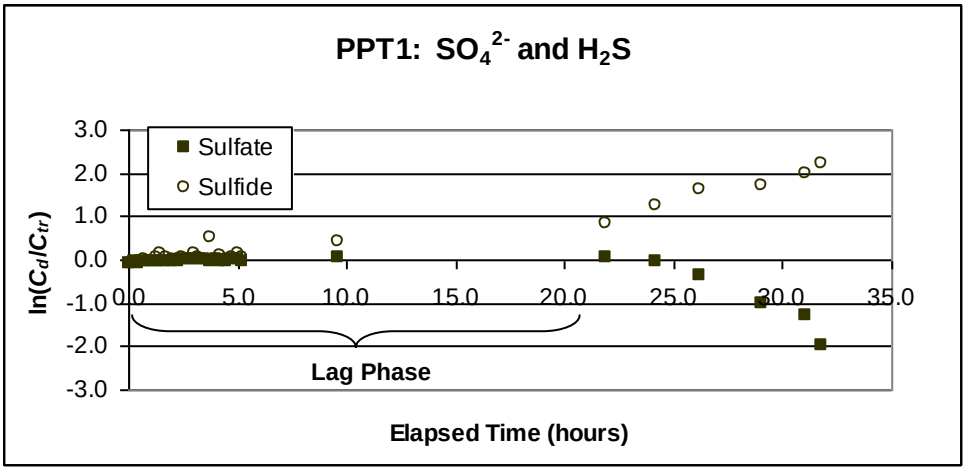

Figure 2.4. Example rate data for H_2S and SO_4^{2-}. Results from PPT1, showing an increase of H_2S coincident with a decrease in SO_4^{2-} indicating SO_4^{2-} reduction.

Nature and Cause of Lag Phase

PPT2 duplicated the test conditions of PPT1 but was conducted over a shorter period of time (3.5 hours versus 32 hours). A lag phase was again observed but in this case it was only ~2.4 hours long, compared to the ~22 hour lag phase observed in PPT1 (Figure 2.5) suggesting that a standard incubation period is not required. Interestingly, the lag phase again coincided with the extraction of the majority of the injected test solution (Figure 2.6) supporting the idea that the mixing interface is the zone of greatest activity. To further explore the spatial relationship of the mixing interface with the reaction front, PPT3 was performed using a smaller injection volume (3 L injected versus 10 L as in other tests). Sulfate reduction was again observed at approximately the same time that the bulk of the injection water was removed (Figure 2.6). These findings suggest that the lag phase was not the result of a simple incubation period but rather was related to the nature of the mixing interface.

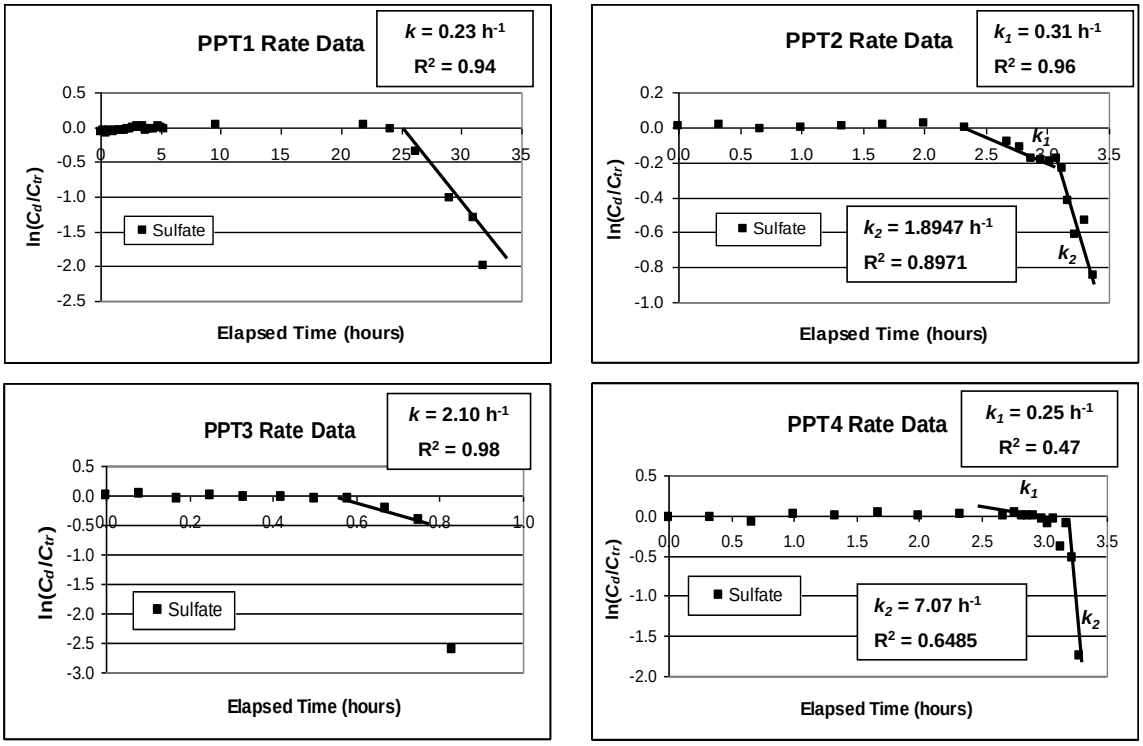

Figure 2.5. First-order rate data verses time. Rate coefficients were determined on portions of the dataset by linear regression. Solid lines show data points used to determine rates.

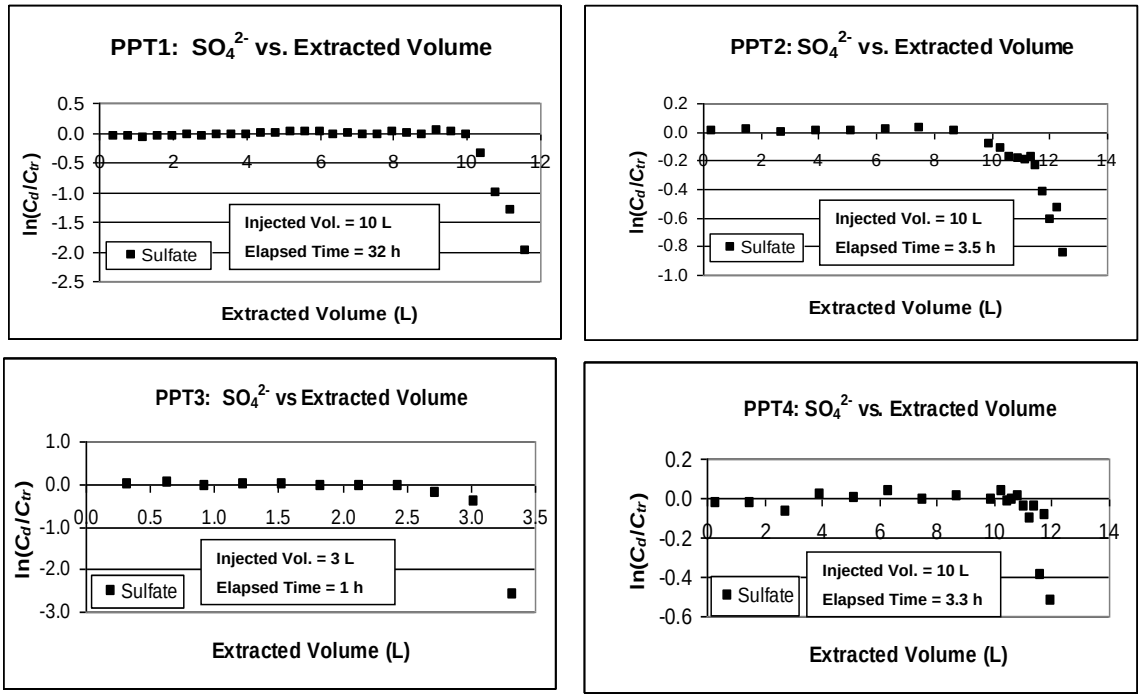

Figure 2.6. First-order rate data verses volume. Plots for SO_4^{2-} versus volume of test solution extracted (L) demonstrating that SO_4^{2-} reduction occurs irrespective of injected volume or total elapsed time.

Though the test solution contained abundant DOC, it did not contain common electron donors such as acetate. Thus in PPT4 acetate was added to test the possibility that the lag phase was caused by donor limitation. This test revealed a decrease in acetate coincident with a decrease in SO_4^{2-} (Figure 2.7). This is consistent with the observations of previous research (Chapelle, 2001; Kleikemper et al., 2002; Pombo et al., 2002) that demonstrated acetate is a preferred electron donor for SO_4^{2-} reducing bacteria. Unexpectedly, the addition of acetate did not eliminate the observed lag phase before SO_4^{2-} reduction indicating the lag phase is not related to desorption or mixing with waters of higher acetate concentration. However, this does not rule out electron donor limitation

as a possible explanation for the observed lag phase. Mixing of test solution with water containing a more favorable electron donor, such as dissolved hydrogen is a possible scenario. For example, Brown et al. (2005) discussed a slight competitive inhibition between hydrogen and acetate utilization, as well as the possibility of simultaneous utilization of the two electron donors.

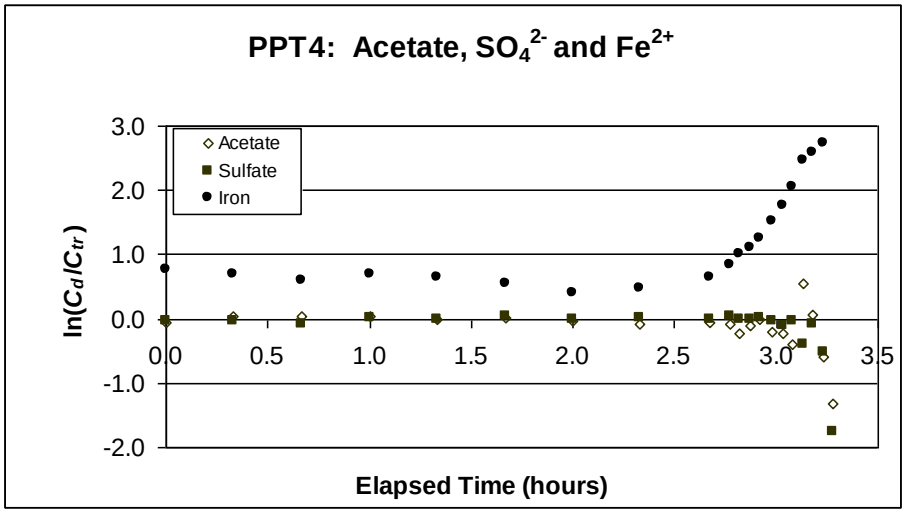

Figure 2.7. Rate data. Plot shows a lag phase followed by the production of Fe^{2+} (indicating Fe(III) reduction) and the simultaneous consumption of acetate and sulfate (indicating SO_4^{2-} reduction).

At approximately the same time SO_4^{2-} was reduced, Fe^{2+} increased suggesting a similar lag phase was also present for Fe(III) reduction TEAPs (Figure 2.7). Though Fe(III) was not directly measured in these tests the increase in Fe^{2+} was interpreted to be an indicator of iron reduction. The mechanism by which Fe(III) reduction occurred during these tests cannot be definitively concluded but two possible scenarios are

suggested: 1) Fe(III) was microbially reduced by iron-reducing microorganisms simultaneously with SO_4^{2-} (Chapelle, 2001) or 2) Fe(III) was reduced via an abiotic chemical reaction, such as reductive dissolution of Fe(III) oxyhydroxide minerals by a reductant (ex.H_2S) (Kostka et al., 2002; Stumm and Morgan, 1996). In the case of direct microbial reduction, the lag phase can be explained as simultaneous Fe(III) and SO_4^{2-} reduction in the mixing fringe water (outer edge of the injected test solution). Simultaneous Fe(III) and SO_4^{2-} reduction has been observed at mixing interfaces within the wetland-aquifer system (Cazull et al., 2006). If Fe(III) was reduced abiotically, then the lag phase would likely be due to lack of sufficient concentration of reductant (H_2S) to initiate dissolution.

Unlike SO_4^{2-} and Fe^{2+}, a steady increase in NH_4^+ concentration was observed from the onset of the extraction phase of each test with no apparent lag phase. Reactive NH_4^+ processes in the subsurface are typically controlled by sorption as a result of cation exchange reactions and biological degradation (Buss et al., 2004). In natural waters NH_4^+ must compete for exchange sites with other more electrostatically favorable cations (Domenic and Schwartz, 1998). Sorption and retardation data are not known for the geologic material present in the test area; however, cation data from the end member waters (data not shown) suggest cation concentrations in both waters had similar concentrations. Therefore the increase in NH_4^+ concentration is likely due to cation exchange reactions occurring upon injection of the test solution resulting in a physical flushing of the *in situ* sediments and subsequent exchange of NH_4^+ into solution. Although unlikely at the flow rates used in this study, another possible explanation for the observed lag phase is a similar physical flushing of the microorganisms within the test

zone. This could potentially result in the physical displacement of the native microbial population explaining the lack of reaction prior to extraction of the mixing interface.

Estimation of Sulfate Reduction Rates

For each push-pull test, first-order reaction rate coefficients were calculated using the Haggerty et al. (1998) method discussed above (equation 2.1). Plots of $\ln(C_d(t^*)/C_{tr}(t^*))$ versus t^* showed a lag time (values near 0) followed by a period of reaction characterized by straight line(s) with a slope $-k$, the first-order rate coefficient (Figure 2.5). Linear regressions were performed on the straight portion(s) of the curves to obtain estimates of SO_4^{2-} reduction first-order rate coefficients. For each push pull test this analysis yielded rate coefficients for SO_4^{2-} reduction that were comparable to those found in previous studies (Harris et al., 2005; Istok et al., 2001; Kleikemper et al., 2002; Luthy et al., 2000; McGuire et al., 2002; Schroth et al., 2001b). For PPT1 the determined rate coefficient for SO_4^{2-} reduction was approximately 0.23 h^{-1} ($R^2 = 0.9398$) (Figure 2.5). Two rate coefficients for PPT2 were estimated. The first SO_4^{2-} rate coefficient was slower, 0.31 h^{-1} ($R^2 = 0.9593$), followed by a second faster rate coefficient of 1.89 h^{-1} ($R^2 = 0.8971$). The rate coefficient for SO_4^{2-}consumption during PPT3 was determined to be approximately 2.10 h^{-1} ($R^2 = 0.9835$). Lastly, two rate coefficients for PPT4 revealed SO_4^{2-} was consumed first at a slower rate of 0.25 h^{-1} ($R^2 = 0.4748$) and then at a faster rate of 7.07 h^{-1} ($R^2 = 0.6485$). Though these rates are consistent with rates found in previous push-pull studies, it should be noted that other studies did not necessarily observe a similar change in slope. Closed-form analytical solutions may not be able to describe the complexities in experimental data observed here, including the lag phase and

potentially complex rate order, and alternative rate determination methods based on numerical approaches (Navaneethakrishnan et al., in review; Phanikumar and McGuire, in review) may be required.

CONCLUSIONS

Small-scale push-pull tests were successfully used to create mixing interfaces in an aquifer-wetland system and explore the *in situ* kinetic controls on TEAPs at cm-scale interfaces. First-order rate coefficients for SO_4^{2-} reduction measured in these tests were similar to those found in previous studies. However, complexities in experimental data, including the presence of a lag phase and potential complex reaction order, demonstrate that a simple first-order rate description does not provide enough information to understand the kinetic controls on sulfate reduction at mixing interfaces.

In all push-pull tests, a lag phase was observed prior to the TEAPs sulfate and iron reduction. The lag phase persisted irrespective of temporal or spatial considerations as evidenced by the reproducibility of the lag phase during tests of differing total length and injection volume. In all cases, the onset of reaction coincided with the removal of water representing a mixture of injected test solution and native waters (the mixing interface). This suggests that the lag phase was not related to a standard incubation period in which the organisms adjust to new conditions but rather was related to the reactions initiated at the mixing interface. Two possible scenarios may explain this phenomenon. Either there was something lacking in the injection water limiting sulfate reduction or there was something present inhibiting reactions. The addition of acetate to the complex natural aquifer water used as the injection solution did not eliminate the lag

phase as expected given that acetate has been shown to be a favorable electron donor for sulfate reduction. Geochemical analyses revealed that not all changes induced during the tests exhibited a lag phase. Ammonium concentrations increased immediately likely due to cation exchange with low conductivity sediments adjacent to the targeted sand layer where push-pull tests were performed. It is unclear the extent to which similar exchange processes might affect microbial populations.

These findings demonstrate that push-pull tests are an important tool to investigate the linked hydro-bio-geochemical processes occurring at complex mixing interfaces. However, interpretation of data retrieved from push-pull tests should be carefully evaluated to ensure the apparent rate is actually a function of time and not another parameter such as degree of mixing.

CHAPTER III

FOLLOW-UP STUDIES OF KINETIC CONTROLS ON SULFATE REDUCTION

INTRODUCTION

A series of small-scale push-pull tests designed to evaluate the kinetic controls on SO_4^{2-} reduction *in situ* at mixing interfaces between a wetland and aquifer impacted by landfill leachate were conducted at the Norman Landfill research site, Norman, OK (see Chapter II). Resulting rate data were complex, involving either multiple first-order rate coefficients or more complex rate orders. In addition, a lag phase was observed prior to sulfate reduction that persisted until the mixing interface between test solution and native water was recovered irrespective of temporal and spatial constraints. The lag phase was not eliminated by the addition of electron donor (acetate) to the injected test solution. A number of questions arose as to the cause of the observed complexities and what they may mean for the interpretation and use of rate data from push-pull tests.

These complexities in rate data combined with the fact that field and laboratory rate estimates for a given reaction can range several orders of magnitude make it difficult to discern a representative rate for a system of interest. To confidently apply a rate, it is critical to understand the controls on the reaction rate being evaluated and how rates may vary over time. For example, it is important to understand the controls on the presence/absence of a lag phase and the conditions under which a rate will change from a simple first-order reaction to a fractional order. Push-pull tests have been commonly used to determine *in situ* rates for subsurface processes such as microbial respiration, contaminant degradation, and aquifer properties. Published and unpublished push-pull

data, including data presented in Chapter II of this dissertation, often contain complexities, which if further evaluated, could provide important information on additional kinetic controls for the processes being investigated. This chapter examines probable explanations of the observed complexities in push-pull rate data through a series of complementary field investigations.

The spatial and temporal variability of factors controlling reaction rates complicate the quantification of reaction rates natural systems. Spatial heterogeneities in the distribution of mineral phases, and gradients in geochemical solutes, temperature, pH, and microbial populations, dictate that reaction rates will vary over small (cm) spatial scales. Superimposed on these spatial heterogeneities are temporal variations in microbial growth/decay, microbial population structure, temperature, and hydrologic flow conditions (i.e., delivery of reactants and removal of products). In addition, the effects of linked reactions and non-linear feedback complicate our theoretical understanding of kinetic controls. These processes can result in complex rate data that may include features observed in push-pull test data. Evaluation of these features thus becomes critical to understanding and predicting the rates of key reactions in natural systems.

Many methods exist and much work has been done to tease out the kinetic controls, though the current state of knowledge regarding kinetic controls is imperfect for even simple reactions in the natural environment. Push-pull tests have proven to be useful for obtaining a wide range of *in situ* data, while maintaining many of the natural system complexities (Hageman et al., 2001; Haggerty et al., 1998; Harris et al., 2005; Luthy et al., 2000). Processes investigated include microbial transformations of

hydrocarbons (Azizian et al., 2005; Hageman et al., 2001; Istok, 1997; Kleikemper et al., 2002; Pombo et al., 2002; Reinhard et al., 1997; Reusser et al., 2002), radionuclides (Senko et al., 2002), electron acceptors (Haggerty et al., 1998; Harris et al., 2005; Kneeshaw et al., 2007) and nutrients (Luthy et al., 2000), groundwater flow velocities (Leap and Kaplan, 1988), solute retardation (Schroth et al., 2001a), sorption (Cassiani et al., 2005; Davis et al., 2002; Hageman et al., 2001), cation exchange (Drever and McKee, 1980) and other aquifer properties (Hall et al., 1991; Hellerich et al., 2003). Though the design of push-pull tests vary based on their intent, protocol consists of a controlled rapid injection of a test solution into a single well followed by the slow recovery of that test solution, sometimes after an incubation period, from the same well (Istok et al., 1997).

To date, push-pull test data have been primarily interpreted using analytical solutions (Gelhar and Collins 1971; Hsieh, 1986), which work well to describe rates over spatial and temporal scales where rate limiting factors are constant. Studies by Haggerty et al. 1998 and Snodgrass and Kitanidis, 1998 provide simplified methods of calculating first and zero-order *in situ* microbial reaction rate coefficients in the absence of sorption and negligible background concentrations, assuming complete and instantaneous mixing of the injected test solution in the portion of the aquifer investigated by the test (i.e., the system can be described as a well-mixed reactor). These methods account for decreases in solute concentration as a result of dilution from diffusion and dispersion and require no knowledge of aquifer porosity, dispersivity, or hydraulic conductivity, nor the use of flow and transport models.

However, the conditions for applying the analytical solution are not always satisfied. If the injection of test solution creates an aqueous interface (Kneeshaw et al.,

2007; Schroth and Istok, 2006) between the native and injected waters (i.e., the assumption of a well-mixed system is violated), rates may be spatially variable. For example, a greater reaction rate may be observed at the mixing interface than in either end-member waters. Spatially variable rates cannot be described using this simplified analytical method and require a numerical simulation (Schroth and Istok, 2006). In addition, rate data obtained from push-pull tests do not always follow the idealized example described in Haggerty et al. (1998) (Haggerty et al., 1998), creating difficulties with interpretation. Complexities often observed in rate data are similar to those presented in Chapter II of this dissertation and include the presence of a lag phase, complex reaction order, and the presence of multiple rate constants. A lag phase prior to reaction has been observed for a wide range of microbial transformations (Addy et al., 2002; Kleikemper et al., 2002; McGuire et al., 2002; Navaneethakrishnan et al., in review; Schroth et al., 2001b). Though the cause of the lag phase varies and is a topic of ongoing studies, a straight analytical solution cannot describe the presence of a lag phase leading to an incomplete description of kinetic controls. This becomes particularly important when applying rate estimates to other systems. Another commonly observed complexity is the apparent "scatter" within first-order rate coefficient data. Though a best-fit line is often constructed, this is less than ideal and valuable information on the processes controlling kinetics can be obscured. Closer examination of data published in the literature shows poor linear fits due to trends in data points that are curved or appear to have linear changes in slope suggesting multiple/fractional order rate constants or multiple first-order rate constants respectively (e.g., McGuire et al, 2002-figure 3, Schroth et al., 1998-figure 6b, Haggerty et al., 1998-figures 7 c and d, and Schroth et al.,

2001a-figure 4). These changes in slope may represent important shifts in physical, chemical, or microbial processes that control rates of reaction. Thus, careful description of complexities such as lag phase, complex rate order, and changing rate order in the analysis of rate data is a critical step to understanding underlying kinetic controls. Creation of an alternative method for analyzing push-pull tests using a new numerical model, PPTEST, is the focus of ongoing studies not included in this dissertation.

This chapter presents a series of experiments conducted to address some of these complexities so that understanding of kinetic controls in complex *in situ* conditions can be improved. These experiments were specifically designed to target possible reactions or physical conditions that could be responsible for generating the features observed in push-pull data including: an inhibitor in the injected test solution, a key constituent of sulfate reduction lacking in the injection solution, abiotic reactions between end member waters, and the presence of a mixing interface between end member waters.

The findings from these complementary studies suggested two probable causes for the observed complexities in push-pull data. The first is that push-pull rate data are a function of space rather than time due to the presence of a mixing interface between end-member waters and thus spatial variability in rates during push-pull tests. The second cause is that the microbial populations also play an important role in controlling the resulting reaction rates. Both factors have implications for how push-pull rate data should be interpreted. Traditional methods for rate determination from push-pull tests only work well for reactions that do not vary in space and have a single, unchanging kinetic control throughout the experiment. My data show that this condition often remains unsatisfied. Results from the data presented in this chapter suggest that the

additional features present in complex rate data should be described and that further work evaluating the role of microorganisms in mediating reaction rates will be necessary to improve our understanding of kinetic controls and enhance our ability to apply measured rates to other systems.

STUDY SITE DESCRIPTION

Experiments were conducted at the Norman Landfill research site in Norman, OK, a closed municipal landfill near the Canadian River. The Norman Landfill received unrestricted waste from 1922 until 1985 when it was closed and covered with an earthen cap (Adrian et al., 1990; Christenson and Cozzarelli, 2003). A leachate plume developed beneath the landfill moundd that extends downgradient from the landfill (Christenson and Cozzarelli, 1999). Overlying the leachate plume is a wetland system and slough where a number of studies have been conducted by the USGS and other research groups. This research has resulted in detailed knowledge of the processes at this site including, detailed analyses of the biogeochemical and hydrological processes (Cozzarelli et al., 2000; Eganhouse et al., 2001; Grossman et al., 2002; Schlottmann et al., 1999) that control contaminant transport and remediation at the Norman Landfill research site, as well as . Wells for this study were located within the slough adjacent to the capped landfill (see Figure 2.1).

METHODS

Geochemical Samples

Samples for experiments discussed in this chapter were analyzed for anions (Cl^-, Br^- SO_4^{2-}, NO_3^-), ammonium, organic acids (acetate), Fe^{2+} and H_2S. All samples were syringe filtered using Millex-HA 0.45 µm filters (Millipore, Bedford, MA). Anion samples were preserved with formaldehyde and organic acid and NH_4^+ samples were preserved by flash freezing; all were measured in the laboratory using a capillary electrophoresis system (Agilent Technologies, Wilmington, DE). Precision for capillary electrophoresis analyses is better than 0.1 mg/L. Samples for Fe^{2+} and H_2S determination were preserved with trace metal grade HCl and zinc acetate, respectively; concentrations for both were determined photometrically in the field using a Spectronic#20D+ spectrophotometer (Thermo Spectronic, Rochester, NY). Precision for Fe^{2+} analyses was better than 0.1 mg/L and better than 0.001 mg/L for H_2S. Cation samples collected for initial end member water concentrations were preserved with HCl and analyzed by capillary electrophoresis (Agilent Technologies, Wilmington, DE). All method details are described in the appendix.

Surface Reaction Vessel Tests

Summary

Surface reaction vessel (SRV) tests were designed to simulate the experimental conditions of push-pull tests conducted at the Norman Landfill research site in Norman, OK (see Chapter II) except that the end member waters (landfill-leachate contaminated aquifer water and wetland porewater) were mixed completely and were not in contact

with native sediments. These tests were conducted to evaluate the resulting reactions when complete and uniform mixing occurs, i.e., when a mixing interface is not present. SRV experiments were conducted in anaerobic, climate controlled carboys at the surface. Tests were repeated three times during three separate field trips. Each SRV test consisted of pumping 1 L of landfill leachate contaminated aquifer water and 1 L of wetland porewater pumped from a drive point well placed in the same shallow sand layer as previous push-pull tests (see Figure 2.2). Both waters were pumped into a 3 L carboy attached to a glove bag filled with N_2 gas to maintain anaerobic conditions and shaken to ensure complete mixing. During each of the three field trips both a "live" and a "killed" SRV test was conducted (Figure 3.1). Both of the SRV experiments were set up in the same manner but one SRV carboy was amended with mercuric chloride to stop all microbial activity. This served as the "killed" control to account for any abiotic reactions that may be occurring while the SRV carboy that received no amendments served as the "live" control. All carboys were kept in water baths to maintain constant temperature (groundwater temperature) with the temperature closely monitored. Aluminum foil was wrapped around the carboys to block sunlight. Prior to each test geochemical parameters were measured for the landfill-leachate contaminated aquifer water (Well SI 102-3) and wetland porewater. Samples were then collected at regular intervals for the duration of a typical push-pull test (~3 hours).

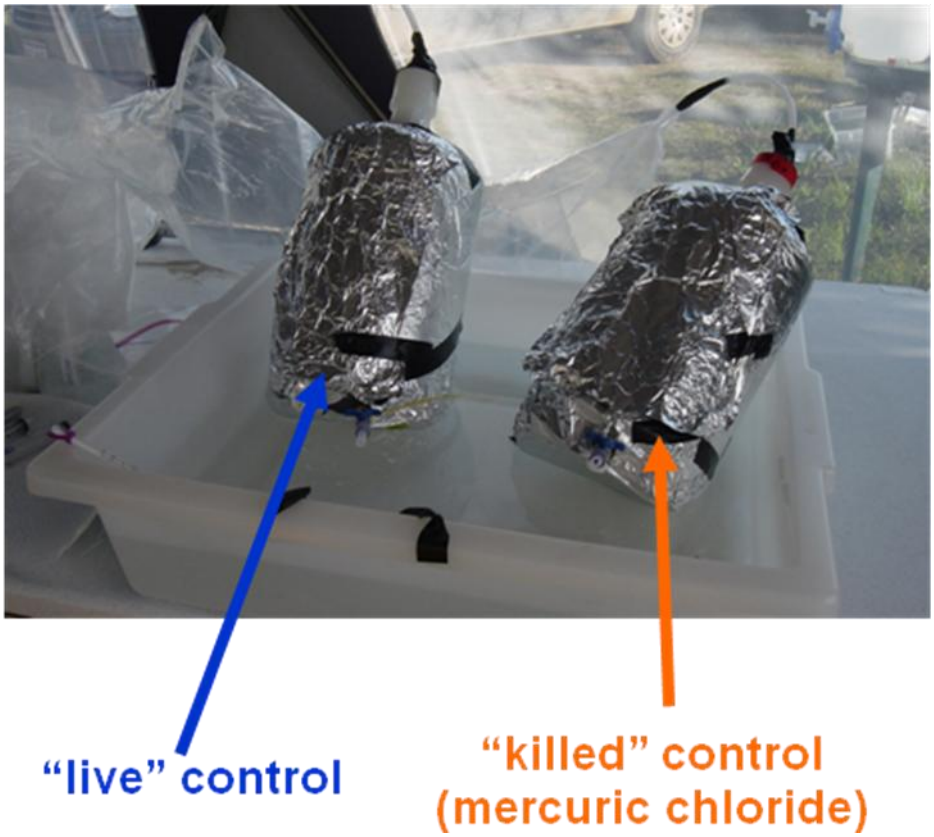

Figure 3.1. Surface reaction vessels. Picture depicts surface reaction vessels (SRVs) in which landfill-leachate contaminated aquifer water and wetland porewater were uniformly mixed and sampled over time. Live control contained no amendments; killed control was amended with mercuric chloride.

Results and Discussion

A total of six replicate SRV experiments were performed, three "live" and three "killed" experiments. In each case the two end member waters (landfill-leachate contaminated aquifer water and wetland porewater) were allowed to react for the length of a typical push-pull test (3-4 hours). Results from all of the "live" and "killed" SRV experiments revealed little or no change in sulfate concentration other than that due to

dilution from mixing of landfill-leachate aquifer water (abundant sulfate) and wetland porewater (depleted with respect to sulfate). One "killed" SRV test was thrown out because it was contaminated with oxygen. There was no significant difference between the results from the "live" and "killed" SRV tests.

Homogeneous mixing of the two end member waters resulted in no change in important geochemical reaction indicators, including sulfate, for both the "live" and "killed" SRV tests. These results indicate that reactions observed during push-pull tests do not occur as a result of abiotic reactions occurring when the two waters come in contact. In addition, results indicate that any redox reactions observed during push-pull tests do not occur in the reduced wetland porewater without influence of the sediments and their associated microbial communities. This could mean that highly oxidized compounds like sulfate may persist in reduced groundwater. It cannot be said for certain from SRV results whether sulfate reduction did not occur due to the absence of solid phase material or its associated microbial population. However, these results are valuable in that they identify two possible controls (sediments and microorganisms) on sulfate reduction rates. Additionally, these results demonstrate the necessity of understanding solid phase reactions and native microbial populations in order to understand how reactions like sulfate reduction will proceed in complex natural systems.

Forced Gradient Test

Summary

A forced gradient test was designed to evaluate the effects of transport processes on mixing interface data under similar experimental conditions as push-pull tests conducted at the Norman Landfill research site in Norman, OK (see Chapter II). The forced gradient test consisted of injecting the same test solution used in push-pull tests (landfill-leachate contaminated aquifer water with abundant sulfate) into reduced shallow wetland sediments (see Figure 2.2) and then pulling it out across a 1 m gradient in the direction of groundwater flow through those sediments. The goal of this experiment was to capture the mixing interface between the two end member waters and evaluated how transport processes affect the resulting reaction rates.

Forced gradient wells were constructed from 2.54 cm, (O.D.) schedule 40 PVC pipe with machined Delrin drive-points. The bottom 3 cm of the wells were screened with 0.5 mm slots and the screened interval was isolated from the remainder of the well casing interior with an o-ringed Delrin packer fitted with 0.635 cm (O.D.) polyethylene tubing. Water was delivered to FGT Well A and withdrawn from FGT Well B (Figure 3.2) across the screened interval and through the tubing to eliminate the potential for errors due to unmixed space in the well casing. FGT Well A and FGT Well B were hand-driven into the targeted sand lens approximately 50 cm below the sediment-water interface and placed 1 m apart.

Test solution was made by withdrawing 30 L of landfill-leachate contaminated aquifer water from the underlying aquifer (Well SI 102, Figures 3.2) using a peristaltic pump (GeoTech) into 50 L Nalgene carboy. The test solution was augmented with 100

mg/L sodium bromide ($NaBr^-$, prepared from NaBr, Acros Organics, New Jersey, USA) to serve as a conservative tracer to account for abiotic processes such as dilution from mixing, dispersion, and advection. A glove bag filled with N_2 gas was fitted to a valve on the carboy cap to prevent the introduction of O_2 while preparing and injecting the test solution. Aluminum foil was wrapped around the carboy to block sunlight and maintain aquifer water temperature. The injection volume was then pumped rapidly (~500 mL/min) into the FGT Well A using a peristaltic pump; any residual solution was gravity drained by inverting the carboy. Prior to the forced gradient test, geochemical parameters were measured for the underlying aquifer water (Well SI 102), wells FGT A and B, and the carboy containing the amended injection solution. After injection of test solution into FGT Well A, water was pumped from FGT Well B at 250 ml/min for approximately 6 hours. Water samples were collected at regular time intervals throughout the duration of the test from FGT Well.

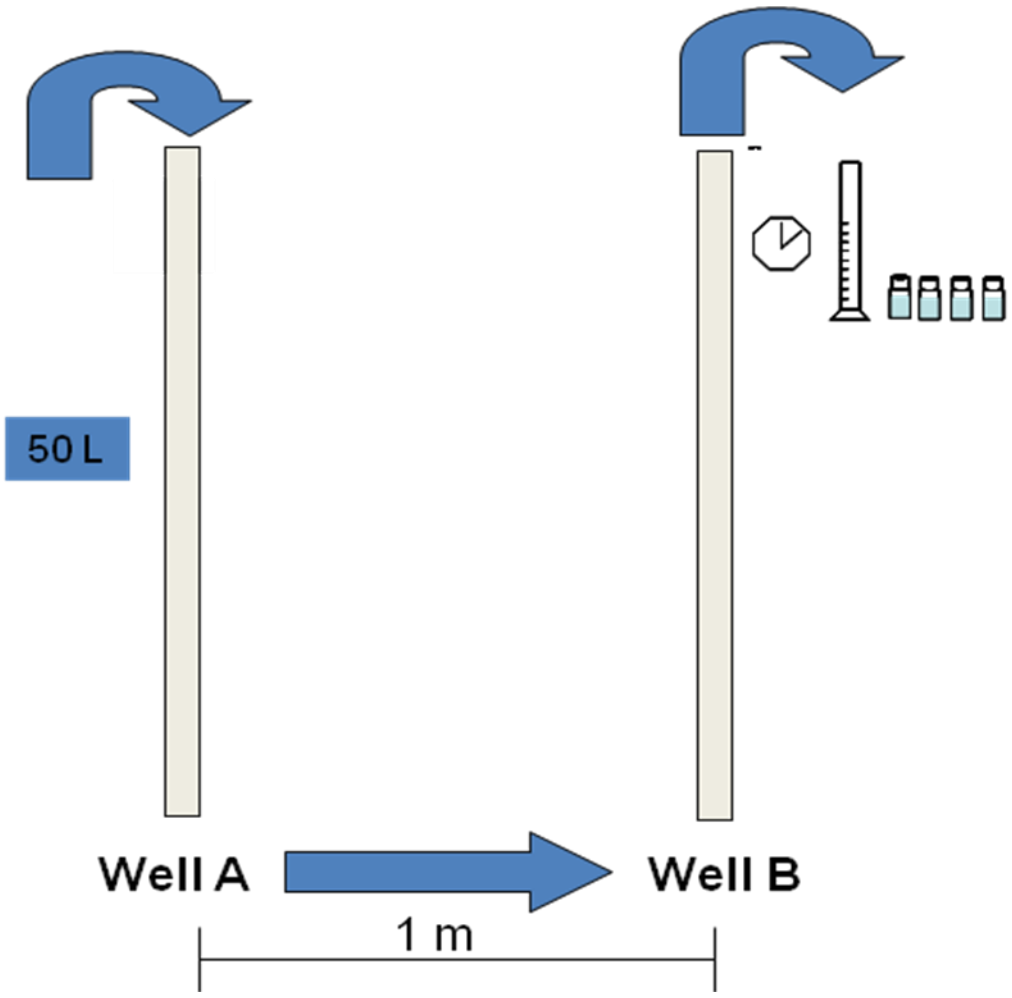

Figure 3.2. Schematic showing forced gradient test (FGT) set-up. Both wells were placed in the reduced shallow sand layer.

Results and Discussion

A forced gradient test was conducted at the Norman Landfill research site, Norman, OK in which landfill-leachate contaminated aquifer water containing abundant sulfate (~100 mg/L) and amended with a conservative tracer (Br^-) was injected into wetland sediments reduced with respect to sulfate. Samples were collected

approximately every 20 minutes for 6 hours. Evaluation of measured geochemical parameters indicated that the injected test solution was never recovered. Bromide was not detected in any of the samples despite low hydraulic conductivity in the wetland sediments. Results from the forced gradient test are thus inconclusive. Spatial heterogeneity of the shallow sand layer in which the wells were placed is unknown and likely played a role in the lack of test solution recovery and could be explained by differences in porosity and hydraulic conductivity between the two wells. It is also possible that well depth, pump speeds and pumping volume were not adequate to pull the injected water across the defined gradient. This test should be repeated in the future under well-defined conditions.

Pull-Push-Pull Test

Summary

A lag phase was consistently observed in push-pull tests conducted at the Norman Landfill research site, Norman, OK. The lag phase was hypothesized to be the result of spatial variability in rate data due to the creation of a mixing interface between end member waters after injection of test solution (see Chapter II). To provide further evidence for this scenario a new test, referred to as a pull-push-pull test, was designed to test other hypotheses for the lag phase. These hypotheses include the presence of an inhibitor in the injected test solution and/or that the test solution lacks a key component necessary for sulfate reduction.

Push-pull tests conducted at the Norman Landfill research site, Norman, OK simulated a mixing interface between waters from two different zones by injecting

landfill-leachate contaminated aquifer water with abundant SO_4^{2-} into native wetland porewater reduced with respect to SO_4^{2-}. Pull-push-pull test did not use landfill-leachate contaminated aquifer water as the injected test solution. Instead, water from the same zone (the reduced wetland porewater) was augmented with SO_4^{2-} thereby eliminating any inhibitors and ensuring components necessary for sulfate reduction were available.

The pull-push-pull well was constructed in the same manner as previous push-pull wells. The well was made from 2.54 cm, (O.D.) schedule 40 PVC pipe with machined Delrin drive-points. The bottom 3 cm of the well were screened with 0.5 mm slots and the screened interval was isolated from the remainder of the well casing interior with an o-ringed Delrin packer fitted with 0.635 cm (O.D.) polyethylene tubing. Water was delivered to and withdrawn from the well across the screened interval and through the tubing to eliminate the potential for errors due to unmixed space in the well casing. The drive-point well was hand-driven into the targeted sand lens approximately 50 cm below the sediment-water interface.

Test solution was made by withdrawing 10 L of wetland porewater from the pull-push-pull well using a peristaltic pump (GeoTech) into 20 L Nalgene carboy. The test solution was augmented with 100 mg/L sodium bromide ($NaBr^-$, prepared from NaBr, Acros Organics, New Jersey, USA) to serve as a conservative tracer to account for abiotic processes such as dilution from mixing, dispersion, and advection and with 100 mg/L sulfate (prepared from Na_2SO_4, Acros Organics, New Jersey, USA). A glove bag filled with N_2 gas was fitted to a valve on the carboy cap to prevent the introduction of O_2 while preparing and injecting the test solution. Aluminum foil was wrapped around the carboy to block sunlight and maintain aquifer water temperature. The injection volume

was then pumped rapidly (~500 mL/min) back into the pull-push-pull well using a peristaltic pump; any residual solution was gravity drained by inverting the carboy. Prior to the pull-push-pull test, geochemical parameters were measured for the wetland porewater and the carboy containing the amended injection solution. After injection of test solution into the pull-push-pull well, water samples were collected at regular time intervals for approximately 4 hours, just as in a typical push-pull test. First-order rate coefficients were determined from reactant and tracer breakthrough curves following the methods of Haggerty et al. (1998) and are described in detail in Chapter II (pages 17, Equations 2.1-2.3).

Results and Discussion

Despite the use of a different test solution, sulfate reduction was stimulated and results for the pull-push-pull test were similar to results from push-pull tests conducted at the Norman Landfill research site and support experimental assumptions. Breakthrough curves for Br^- and SO_4^{2-} were similar throughout the initial extraction phase of the test differing only upon microbial reduction of SO_4^{2-} (Figure 3.3). This indicates that retardation of Br^- and SO_4^{2-} was negligible, and confirms the assumption made in rate determinations that tracer and reactant results were similar (Haggerty et al., 1998; Schroth et al., 2001b). Just as in push-pull tests, sulfate reduction began at approximately the volume where the mixing interface between injected solution and native water was extracted (~10 L) (Figure 3.4). This suggests that sulfate reduction did not occur in the bulk injected water but rather only occurred at the mixing interface between injected and background water. Because this test used native wetland porewater from the same zone

in which the test was conducted, the lag phase can no longer be attributed to either a lack of critical reactant necessary for sulfate reduction or the presence of an inhibitory substance.

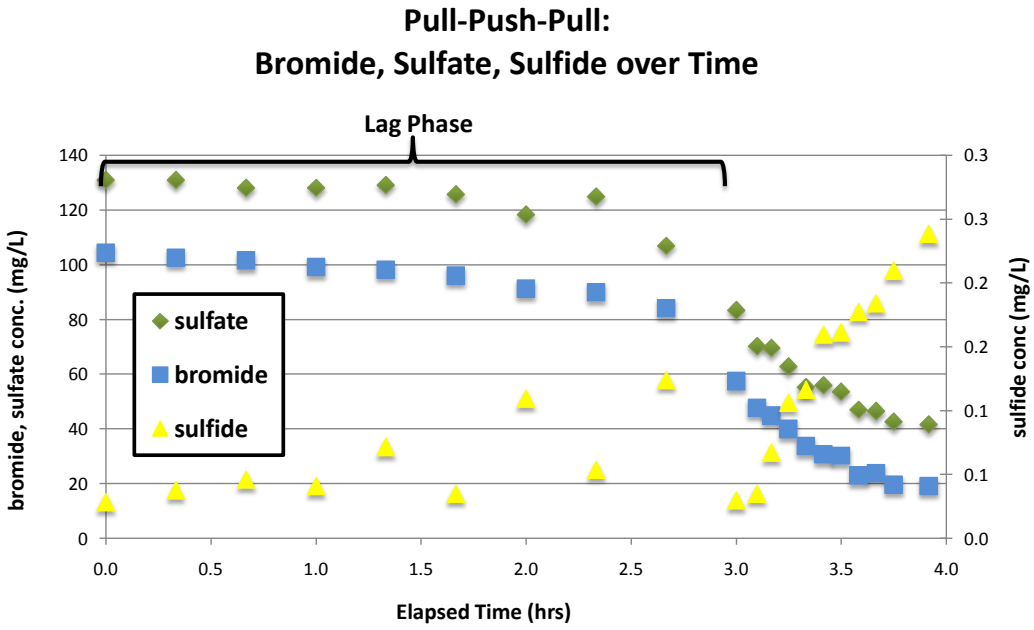

Figure 3.3. Breakthrough curves from pull-push-pull tests. Wetland porewater was extracted, amended with SO_4^{2-} and tracer and pumped back into wetland sediments. Presence of lag phase prior to sulfate reduction is the the same as the lag phases found in push-pull tests (see Chapter II).

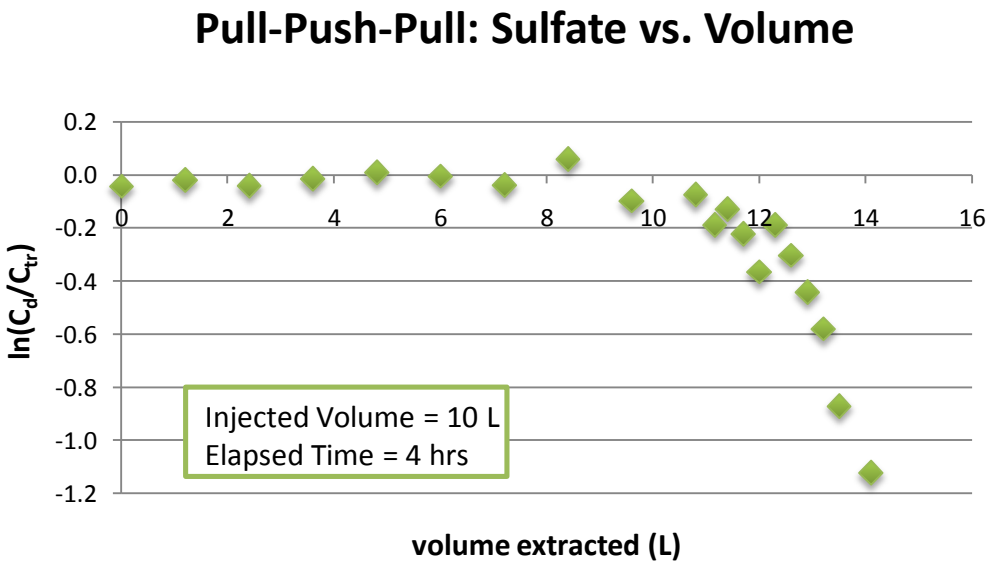

Figure 3.4. Rate data verses volume. Plots show first-order rate data for SO_4^{2-} versus volume of test solution extracted (L) demonstrating that SO_4^{2-} reduction occurs irrespective of injected volume or total elapsed time.

First-order reaction rate coefficients were calculated using the Haggerty et al. (1998) method discussed above (equation 3.1). A plot of $\ln(C_d(t^*)/C_{tr}(t^*))$ versus t^* showed a lag time (values near 0) followed by a period of reaction characterized by straight line(s) with a slope $-k$, the first-order rate coefficient (Figure 3.5). Linear regressions were performed on the straight portion(s) of the curves to obtain estimates of SO_4^{2-} reduction first-order rate coefficients. This analysis yielded rate coefficients for SO_4^{2-} reduction that were comparable to those found in previous studies (Harris et al., 2005; Istok et al., 2001; Kleikemper et al., 2002; Luthy et al., 2000; McGuire et al., 2002; Schroth et al., 2001b), including those conducted at the Norman Landfill research site

(see Chapter II). Two rate coefficients were estimated. The first SO_4^{2-} rate coefficient was slower, 0.32 h^{-1} (R^2 = 0.7 53), followed by a second faster rate coefficient of 1.95 h^{-1} (R^2 = 0.982). This complexity in rate data was also observed in push-pull tests.

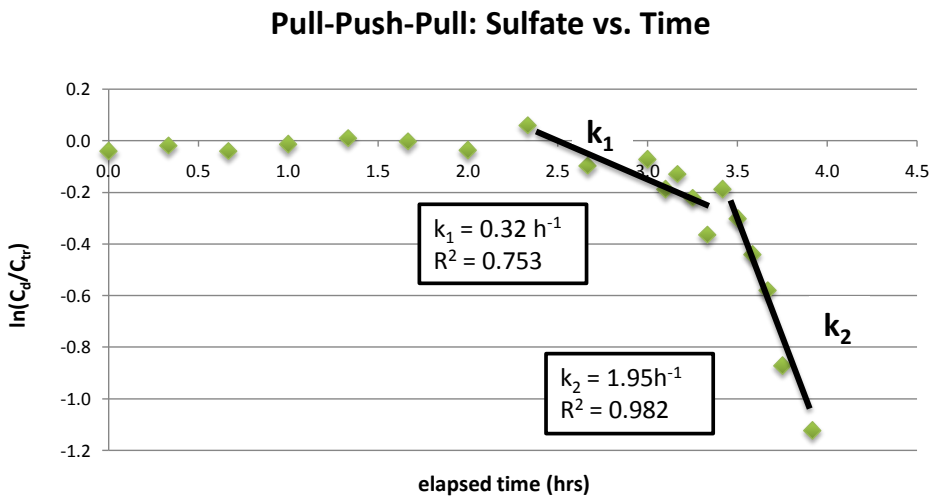

Figure 3.5. Rate data verses time. Rate coefficients were determined on portions of the dataset by linear regression. Solid lines show data points used to determine rates.

In summary, a pull-push-pull test was successfully used to test possible explanation for the lag phase observed in push-pull tests. First-order rate coefficients for SO_4^{2-} reduction measured in this test were similar to those found in previous studies. However, complexities in experimental data, including the presence of a lag phase and potential complex reaction order, were not eliminated by using a test solution made from wetland porewater instead of landfill-leachate contaminated aquifer water. The consistency of the data obtained from pull-push-pull tests to data from push-pull tests

conducted at the Norman Landfill research site provide further evidence that the lag phase is indeed related to the nature of the mixing interface.

Radial Array Mini Push-Pull Tests

Summary

Push-pull tests along with follow-up studies have indicated that the creation of a mixing interface between end-member waters during push-pull tests produces spatial variability in rate data. In push-pull tests and a pull-push-pull test conducted at the Norman Landfill research site, Norman, OK, the onset of sulfate reduction coincided with the removal of water representing a mixture of injected test solution and native waters (the mixing interface). Spatial variability in rate data affects the interpretation and use of rate estimates and as such the interpretation of data retrieved from push-pull tests should be carefully evaluated to ensure the apparent rate is actually a function of time and not another parameter such as degree of mixing. Because of this further research to try to characterize the occurrence of spatial variability in rate data is important and prompted the development of a new test designed to target this question *in situ*.

To examine spatial variability in rates radial array, mini push-pull (RAMPP) tests were designed to physically capture the mixing interface created during a typical push-pull test. The RAMPP test works by providing a method for *in situ* sampling of an injected push-pull test solution so that zeroth order rates can be quantified and evaluated spatially. RAMPP tests were designed to collect samples in the same way as the previous push-pull tests except that 15 mini drive point (MDP) wells were added in a radial pattern over the potential zone of influence generated upon injecting the push-pull test solution.

Test solutions for the RAMPP tests were the same as those for previous push-pull tests at the Norman Landfill research site (see Chapter II).

RAMPP well construction consisted of a center push-pull well was made from 2.54 cm, (O.D.) schedule 40 PVC pipe with machined Delrin drive-points. The bottom 3 cm of the well were screened with 0.5 mm slots and the screened interval was isolated from the remainder of the well casing interior with an o-ringed Delrin packer fitted with 0.635 cm (O.D.) polyethylene tubing. Water was delivered to and withdrawn from the well across the screened interval and through the tubing to eliminate the potential for errors due to unmixed space in the well casing.

The MDP wells were spaced to ideally capture the mixing interface between the injected test solution and the native porewater. Three "arms" spaced 120^{o} apart, each containing five evenly spaced MDPs, extend in a radial pattern from the center mini push-pull well (Figure 3.6). Given a porosity of 30%, which was roughly determined for the targeted sand layer and a 20 L injection, the calculated "sphere" of influence would have a 50.4 cm diameter. Thus, the MDP wells extended laterally to just outside this zone (~60 cm), targeting the mixing interface between the injected test solution and the native porewater. The MDP wells were made of 0.3 cm (I.D.) stainless steel tubes with ceramic cups adhered to the tip. A frame was built to house the push-pull well and MDP wells (Figure 3.7). Gas impermeable tubing was attached to the end of each MDP well from which samples were withdrawn using luer-lock syringes. The RAMPP well was set within a few meters of the previous push-pull tests and targeted the same reduced sand layer (see Figure 2.2).

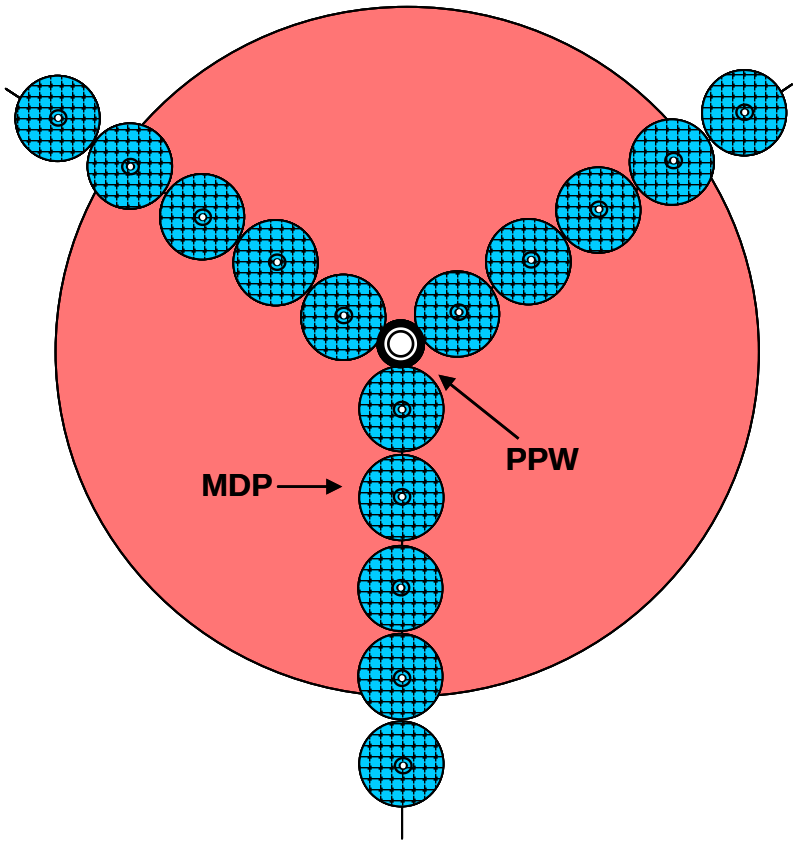

Figure 3.6. Schematic of RAMPP set-up (plan view). PPW is the center push-pull well from which test solution will be injected and extracted as in a normal push-pull test. MDP refers to the mini drive point wells from which samples will be extracted at various time points to try and capture the mixing interface. The red circle is the expected zone of influence upon injection of test solution (20 L).

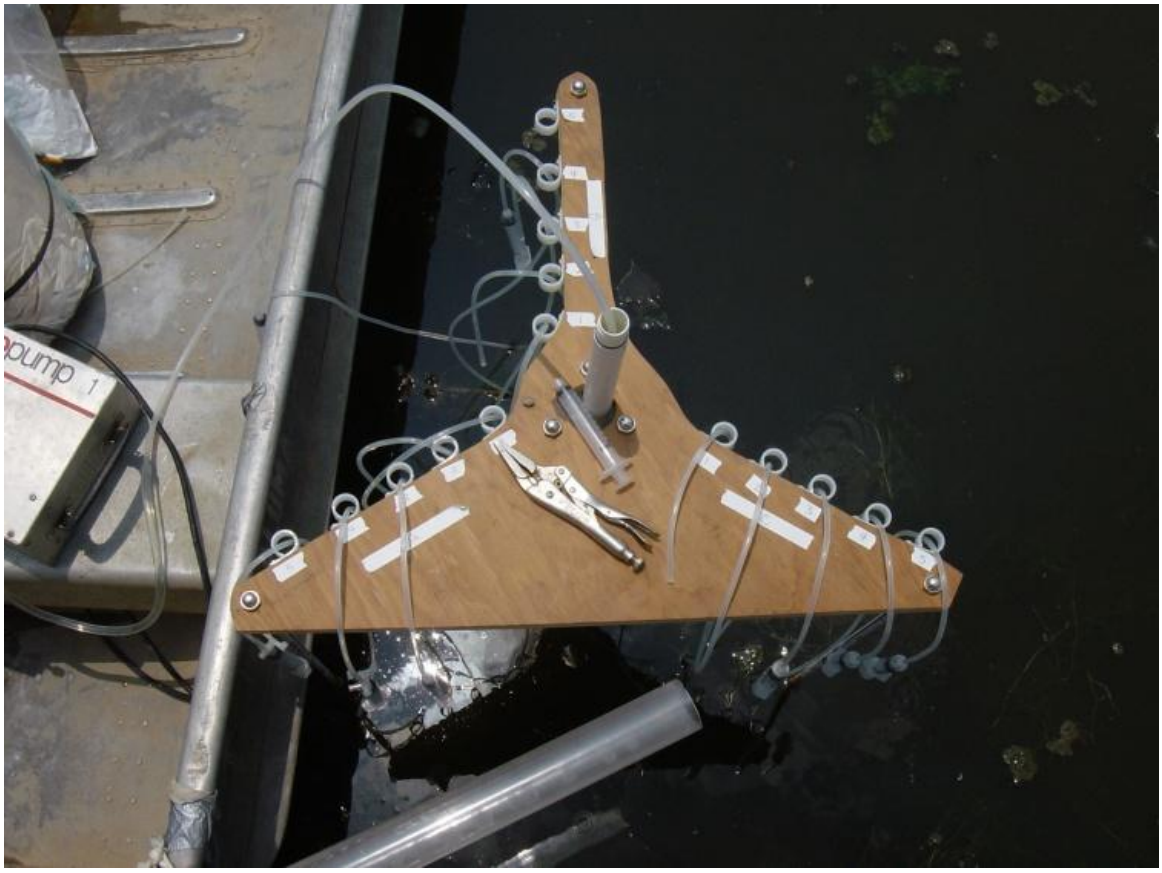

Figure 3.7. Picture depicting actual RAMPP construction.

Test solutions for RAMPP tests was made by withdrawing 20 L of landfill-leachate contaminated aquifer water from the underlying aquifer (Well SI 102, see Figure 2.2) using a peristaltic pump (GeoTech) into 50 L Nalgene carboy. The test solution was augmented with 100 mg/L sodium bromide ($NaBr^-$, prepared from NaBr, Acros Organics, New Jersey, USA) to serve as a conservative tracer to account for abiotic processes such as dilution from mixing, dispersion, and advection. Acetate (CH_3COOH, prepared from $NaCH_3COOH$, Acros Organics, New Jersey, USA) was added in stoichiometric

proportion to SO_4^{2-} (~30 mg/L per test) as an additional (energetically favorable) electron donor in one RAMPP test. In addition, lactate was added as another possible electron donor in the same manner to a subsequent RAMPP test. A glove bag filled with N_2 gas was fitted to a valve on the carboy cap to prevent the introduction of O_2 while preparing and injecting the test solution. Aluminum foil was wrapped around the carboy to block sunlight and maintain aquifer water temperature. The injection volume was then pumped rapidly (~500 mL/min) into the FGT Well A using a peristaltic pump; any residual solution was gravity drained by inverting the carboy.

Prior to starting the experiment, geochemical parameters were measured for the wetland porewater and the carboy containing the amended injection solution. After injection of test solution into the center well water samples were collected at regular time intervals (every 10 to 15 minutes) during the extraction (pull) phase of each test from both the push-pull well and the MDP wells. Each arm (5 MDP wells) of the RAMPP set-up was to be sampled simultaneously at designated time points but due to technical difficulties in the field the three outermost MDP wells on each arm were sampled at three different time points followed by the 2 innermost MDP wells on each arm being sampled at three different time points.

Results and Discussion

Sampling each arm of the RAMPP apparatus simultaneously proved difficult and the method ultimately used to collect samples was not ideal as large assumptions were made as to the location of the mixing interface so that it could be captured. Additional difficulties existed as well since each sampling event removed some fluid and created a slight vacuum, likely having an overall effect on mixing and the zone of influence. As such, the resulting dataset was complex to interpret and the design of this test will need to be reevaluated. Nonetheless, zeroth order sulfate reduction rates were calculated for each sample collected from the MDP wells. Calculated rates from MDP well samples showed a great deal of variability in zeroth order sulfate reduction rates (Figure 3.8). Interestingly, in scrutinizing the data, faster rates bracketed by slower rates are hypothesized to be in the zone of mixing between the two end member waters. If this is indeed the mixing interface then it appears true that there is increased biogeochemical activity within the zone of mixing. Despite complexities with the RAMPP experiments and interpreting the resulting datasets RAMPP tests did provide more evidence of spatial variability in rate data.

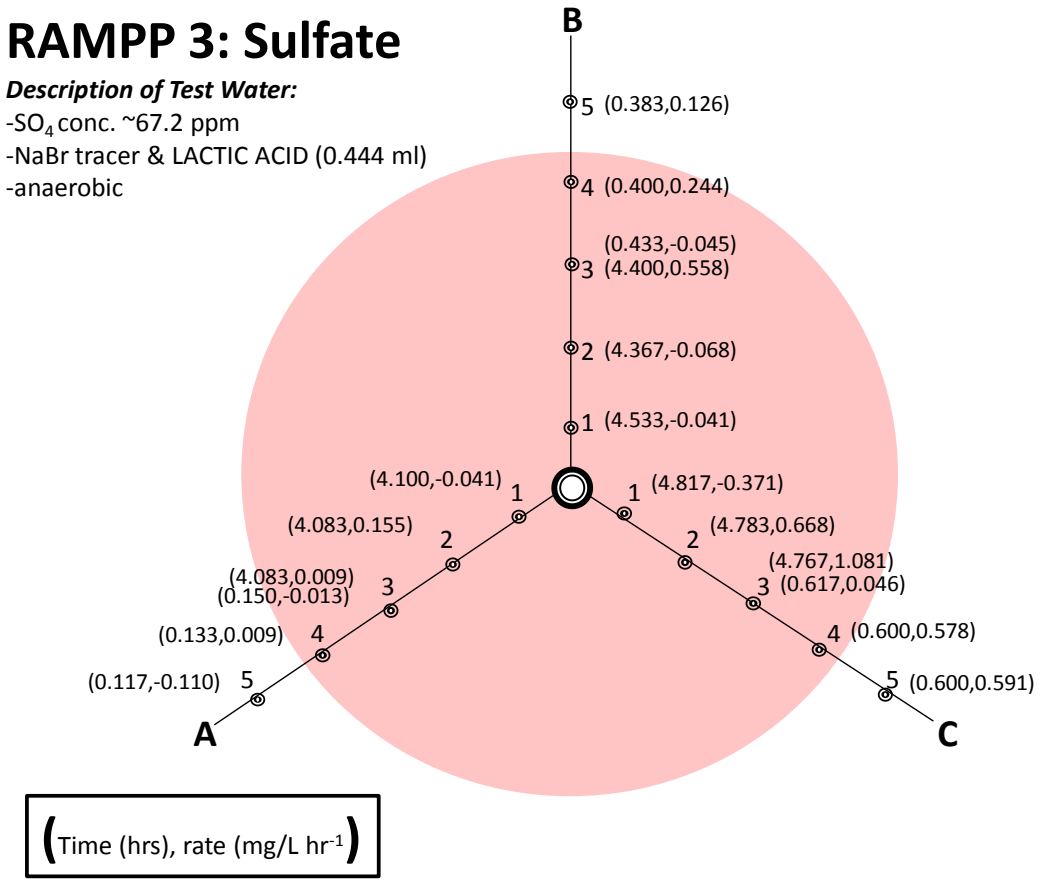

Figure 3.8. Example dataset from a RAMPP test. Figure shows the elapsed time and zeroth-order sulfate reduction rate for each MDP well (shown in plan view). In some cases two samples were collected at different time points.

CONCLUSIONS

The experimental results discussed in this chapter provide strong evidence of spatial variability in rate data due to the creation of a mixing interface during push-pull tests. This is not however considered a problem as this provides a new opportunity for evaluating dynamic natural systems where mixing interfaces are likely present due to constantly changing environmental conditions. Push-pull tests can be used to simulate these natural mixing zones and resulting rate estimates can be incorporated into system level evaluations. The studies presented here also indicate that complexities involving changing reaction rates and rate order can be described and are likely due to the response of the native microbial population. These results strongly suggest that further research evaluating the factors controlling the kinetics redox reactions such as microbial sulfate reduction in complex natural systems is necessary if we are to make accurate estimates of reactions rates. As such, new *in situ* techniques need to be developed which allow for direct measurement of geochemical and microbiological activities in the subsurface so that linkages between them can be quantified.

CHAPTER IV

A NEW APPROACH FOR DETERMINING *IN SITU* MICROBIAL RESPONSE TO GEOCHEMICAL PERTURBATIONS

INTRODUCTION

The metabolic activity of microorganisms in subsurface systems is often dictated by geochemical conditions (Lovley et al., 1994; Smith, 1997). Availability of electron acceptors and donors thus controls what redox processes will dominate in a system. In subsurface aqueous systems, the chemical and physical properties of the subsurface, including hydraulic conductivity and amount of reactive materials, primarily control the abundance of electron acceptors and donors (Chapelle and McMahon, 1991; McMahon and Chapelle, 1991; McMahon et al., 1991; Ulrich et al., 1998). Introduction of new electron acceptors and donors to the subsurface can occur as a result of natural events such as changing hydrologic conditions (wetting/drying events) and as a result of human events such as the introduction of contaminants (Chapelle et al., 1996a; Cozzarelli et al., 1999; McGuire et al., 2004; McGuire et al., 2002; Scholl et al., 2006). Native microbial communities adapt and respond to these perturbations and in turn control the resulting geochemical concentrations. Understanding the changes in microbial activity that occur as a result of such perturbations is critical for predicting chemical fate and transport in natural systems, as well as for providing insight into nutrient and carbon cycling, and development of *in situ* biotechnological applications (Ulrich et al., 1998).

Numerous challenges exist in studying natural systems due to heterogeneities, disequilibrium conditions, and issues with sampling scale (Adrian et al., 1994; Barlaz and

Borden, 1999; Beeman and Suflita, 1990; Cazull et al., 2006; Christensen et al., 2000; Cozzarelli et al., 2000). To date, *in situ* studies have relied largely on geochemical analyses of dissolved solutes to 1) infer indirectly the activity of native microbial communities (Cazull et al., 2006; Christensen et al., 2000; Christensen et al., 2001; Cozzarelli et al., 1999; Istok et al., 1997) and 2) assess native microbial communities through analyses of native sediments and water (Beeman and Suflita, 1987; Bekins et al., 1999; Bjerg et al., 1999; Bowman et al., 1993; Martino et al., 1998). Field experiments that try to capture or grow native microorganisms *in situ* have also been tried (ex., glass slides, Biosep®-immobilized cells, mineral substrates) (Bengtsson, 1989; Biggerstaff et al., 2007; Ekendahl and Pedersen, 1994; Poindexter et al., 2000; Rogers et al., 1998). Field investigations of combined geochemical and microbiological parameters have proven valuable and necessary for the development of better predictive techniques. Several studies have demonstrated comparable trends in microbial abundance and concentrations of dissolved constituents. For example, the abundance of methanogens has been shown to be consistent with dissolved methane concentrations (Bakermans and Madsen, 2002b; Bekins et al., 1999; Ludvigsen et al., 1999). Other studies, have however shown examples of discrepancies in interpretations of geochemical data and expected microbial results (Bekins et al., 2001). These studies used innovative methods to evaluate microbial communities and functions, but difficulties still exist in evaluating the complex linkages between geochemistry and microbiology. Laboratory results provide evidence of microbial response and allow for the quantification of microbial reaction rates (Chapelle et al., 1996a; Cozzarelli et al., 2000; D'Angelo and Reddy, 1999; Kneeshaw et al., 2007; Lovley and Goodwin, 1988; Wilson et al., 1983), but replication

of natural conditions is difficult so that laboratory and field rate measurements often vary by many orders of magnitude. In addition to variability in rate data, current sampling methods do not facilitate direct measurement of microorganisms and geochemical concentrations, making it difficult to assign representative reaction rates for a given system. Development of a new *in situ* method that directly assesses *in situ* response of native microbial communities to changing geochemical conditions would provide rate data representative of a complex natural environment.

Combined *in situ* analyses of geochemistry and microbiology are preferred for evaluating the behavior of complex natural systems, but problems exist in how these samples are collected. One challenge is to obtain geochemical and microbiological samples from the same spatial and temporal scales. In response to this need, we have developed a new *in situ* sampling technique referred to as NOGEEs (Native Organism Geochemical Experimentation Enclosures). NOGEEs allow the isolation of a native microbial population and subsequent introduction and removal of test solutions *in situ* providing direct measurement of geochemical parameters and native microbial population response to a perturbation. This technique has been applied at the Norman Landfill research site in Norman, OK to simulate the introduction of landfill leachate to wetland sediments as would be expected to occur during changing hydrologic conditions (ex. recharge). Results from experiments demonstrate the feasibility of this technique for *in situ* quantification of microbial reaction rates coincident with change in microbial population structure in response to a geochemical perturbation.

STUDY SITE DESCRIPTION

NOGEE experiments were conducted at the Norman Landfill research site in Norman, OK. This unlined landfill is located near the Canadian River in an alluvial aquifer system and received unrestricted, solid waste for sixty three years (1922 through 1985) at which time it was closed and covered with an earthen cap (Adrian et al., 1990; Christenson and Cozzarelli, 2003). A leachate plume containing elevated concentrations of dissolved organic carbon (DOC), chloride, ammonia, and methane developed in the alluvial aquifer beneath the landfill resulting in a layered system with a series of interfaces between different water masses (Cozzarelli et al., 2000). Areas of ponding (referred to here as a wetland/slough) have resulted from beaver dams in a shallow stream adjacent to the landfill mound (see Figure 2.1). Groundwater flow in this region is from the landfill toward the slough and the Canadian River (Scholl and Christenson, 1998). The locations of the NOGEEs described in this study were within the slough adjacent to the landfill (see Figure 2.1).

Intensive investigations of the Norman Landfill site have been conducted by the U.S. Geological Survey as part of the Toxic Substances Hydrology Program as well university research groups for over ten years. Results from these studies have provided detailed knowledge of processes occurring within the aquifer system. A number of studies have evaluated the nature and magnitude of biotic and abiotic geochemical reactions (Cozzarelli et al., 2000; Eganhouse et al., 2001; Grossman et al., 2002; Schlottmann et al., 1999; Scholl et al., 2006) and microbiological processes (Beeman and Suflita, 1987; Harris et al., 2005; Harris et al., 1999; Ulrich et al., 2003). Other studies have evaluated the kinetics of reactions that occur within the aquifer system (Adrian et

al., 1994; Beeman and Suflita, 1990; Senko et al., 2002), and quantified groundwater-surface water fluctuations at the site (Christenson and Cozzarelli, 1999; Schlottmann et al., 1999; Scholl, 2000). The wealth of data that exists for this site makes it an ideal location to conduct *in situ* rate experiments.

MATERIALS AND METHODS

NOGEE Design

An *in situ* sampling apparatus, referred to as NOGEE, was designed and constructed for the specific goal of directly obtaining geochemical and microbiological evidence of a native microbial population's response to a geochemical perturbation. NOGEEs were designed to accomplish 4 main tasks: 1) trap a native microbial population, 2) isolate the population for the surrounding environment *in situ*, 3) introduce a geochemical solution, and 4) measure the resulting effect on the microbial population and geochemical concentrations. The physical construction for a single NOGEE consisted of a well-like apparatus made of schedule 40 PVC pipe. The lower screened interval was packed and housed a main chamber (60 mL) that was connected to the surface by tubing (Figure 4.1). The chamber area was screened and covered with a 5.0 μm polycarbonate membrane filter (Sterlitech Corporation) to prevent sediment from entering while allowing the passage of native water and microorganisms into the chamber. Additionally, the chamber enclosed a chemically inert polycarbonate sponge (Honeywell, Des Plaines, IL) housed in a perforated PVC tube, which served as a substrate for microbial colonization (Figure 4.1).

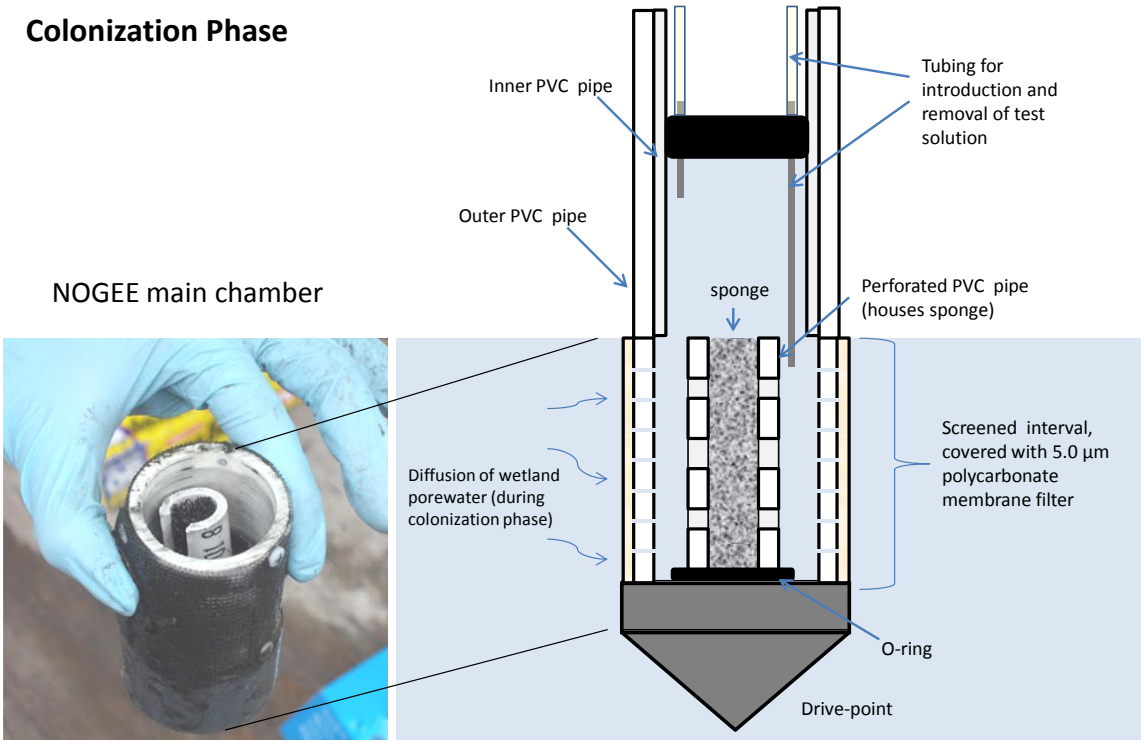

Figure 4.1. Photograph of NOGEE main chamber (left). Schematic cross-section through NOGEE main chamber (right) during the colonization phase. During the colonization phase the internal tube is raised to allow microbial colonization of a sponge or sponges housed inside the membrane enclosed chamber.

There were two phases for the NOGEE experiments, phase 1- colonization, and phase 2- experimentation. NOGEEs were designed so that during phase 1, the incubation phase, an internal PVC pipe was raised above the sponge chamber allowing passive diffusion of native porewater and microorganisms into the chamber. During phase 2, the experimental phase, this pipe is lowered over the sponge where it passes over an o-ring creating a seal, effectively isolating the chamber area from the surrounding environment (Figure 4.2). Isolating the sponge chamber *in situ* allowed test solution to be introduced and samples to be collected through two tubing ports set at the bottom and top of the sponge chamber (Figure 4.2). Additionally, several non-experimental NOGEE's were constructed in the same manner but without the ability to conduct the isolation step in the experimental phase. These NOGEEs were placed as close as possible to the experimental NOGEEs for the purpose of providing the initial sponge samples for molecular analyses of the microbial population established during the colonization phase and assessing heterogeneity between NOGEEs.

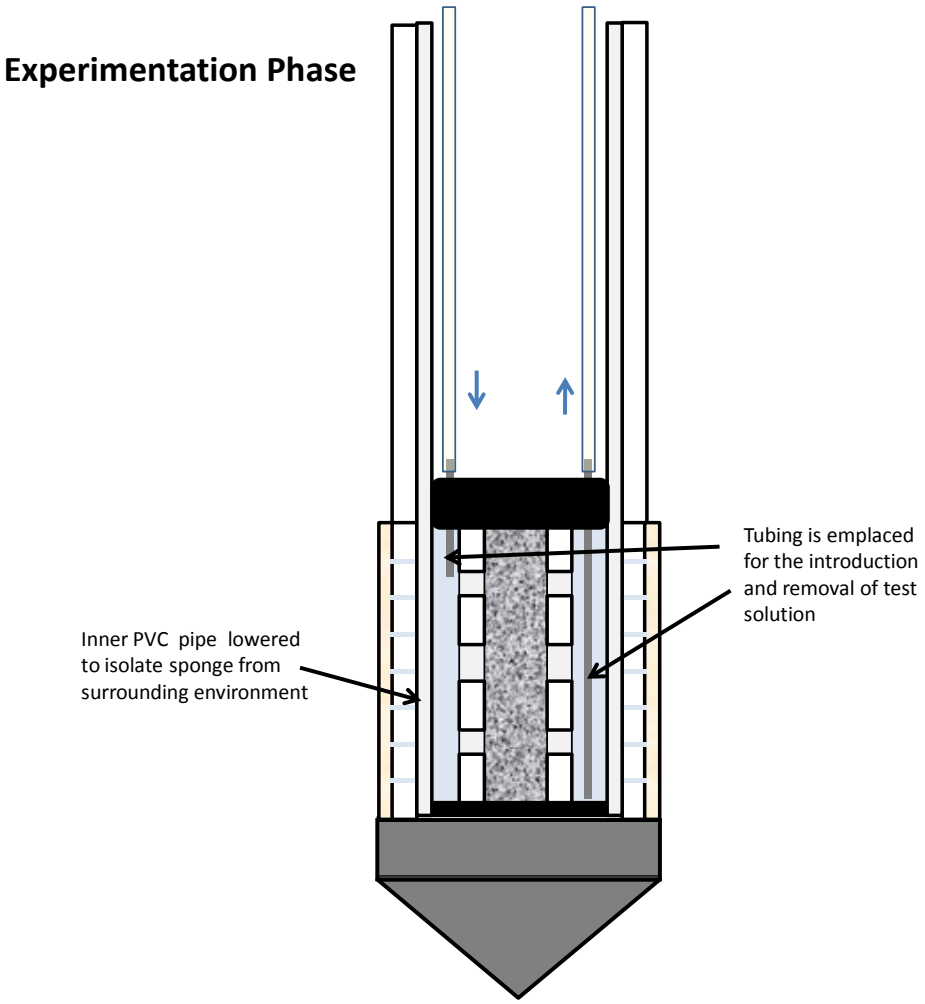

Figure 4.2. Schematic cross-section through NOGEE main chamber. During the experimentation phase the internal tube is lowered over the sponge, isolating it from the surrounding environment. Tubing allows introduction of test solutions and sample collection.

NOGEE Experiment

Experiments were conducted in the landfill-leachate contaminated wetland at the Norman Landfill Research Site, Norman, OK (see Figure 2.1). In May 2007, four NOGEEs (two reactive, S1 and S2 and two controls, C1 and C2) and four non-experimental NOGEEs (D1-D4) were installed in the wetland sediments by hand so that the screened interval was in a shallow, reduced silty-clay layer (Figure 4.3) within the wetland sediments. After installation NOGEE chambers were filled with deoxygenated Nanopure water and all tubing was sealed. NOGEEs were left to colonize for approximately 6 weeks. After the colonization period, initial water samples were collected from the sponge chamber of all eight NOGEEs immediately prior to beginning the experimentation phase. The internal tube of experimental NOGEEs was then lowered to isolate the sponge chambers. The non-experimental NOGEEs were removed from the wetland sediments and sponges were collected for initial molecular microbiology analyses. Native water from the landfill-leachate contaminated aquifer beneath the wetland sediments where the NOGEEs were deployed was used to make test solutions. This was done to simulate a natural event resulting in hydrologic fluctuations (due to a recharge event) at this site that would result in mixing of these two waters. Test solutions consisted of landfill-leachate contaminated aquifer water amended with sulfate (~100 mg/L SO_4, prepared from Na_2SO_4, Acros Organics, New Jersey, USA) to serve as electron acceptor, lactate and acetate (~30 mg/L, prepared from $C_3H_6O_3$ and $NaCH_3CO_2$, respectfully, Acros Organics, New Jersey, USA) to serve as electron donor, and bromide (~100 mg/L Br^-, prepared from NaBr, Acros Organics, New Jersey, USA) as a conservative tracer. All test solutions were made in a climate-controlled, argon

atmosphere to maintain aquifer conditions. Test solution was introduced to the sponge chamber of the experimental NOGEEs using a peristaltic pump at a pumping rate of 100 ml/min. A volume of test solution greater than three times the volume of the chamber (~180 ml) was flushed through the sponge chamber to ensure maximum displacement of the liquid already in the chamber. Outlet tubing was purged with argon gas to eliminate mixing and dilution with residual water during subsequent sampling events. Tubing was then sealed and the test solution was left in the sponge chamber until sample collection (71 hours for the first test and 45-53 hours for the following four tests). At designated sampling times (Table 4.3, page 73) water was pumped using a peristaltic pump from the sponge chamber into an attached syringe. The exposed tubing end was connected to a Tedlar bag filled with argon gas so as not to introduce oxygen during sample collection. Once samples for geochemical samples were collected, fresh test solution was again introduced to the chamber. This process was repeated a total of 5 times over the course of approximately 11 days (Table 4.3, page 73). After the last sampling event NOGEEs were removed from wetland sediments and sponges were collected for final molecular analyses.

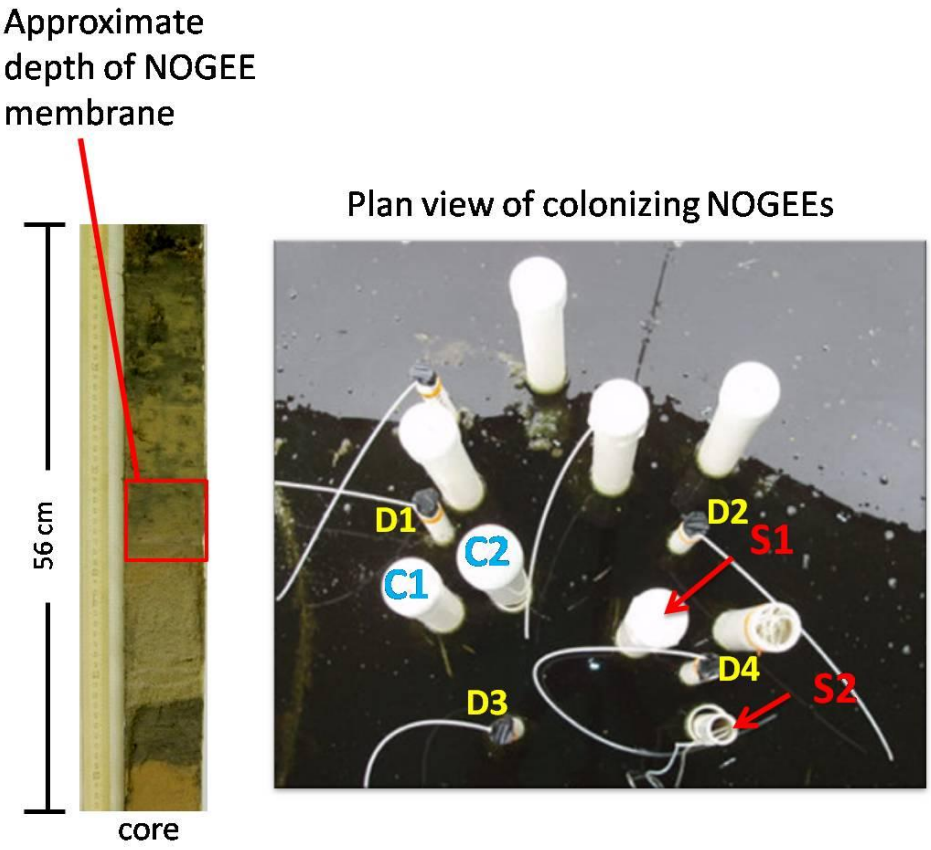

Figure 4.3. Core showing placement of NOGEEs in wetland sediments (left). Photo shows plan view of spacing for the two reactive, S1 and S2, two controls, C1 and C2, and four non-experimental NOGEEs (D1-D4).

Analytical Methods

Geochemistry samples were collected for initial water samples (collected from the sponge chamber of all eight NOGEEs immediately prior to beginning the experimentation phase), all test solutions and for each sampling event (see appendix for details of analytical techniques). Samples were collected and analyzed for anions (Cl^-, Br^-, SO_4^{2-}, NO_3^-), ammonium, organic acids (acetate and lactate), Fe^{2+}, H_2S, dissolved

organic carbon (DOC), alkalinity, and CH_4. All samples were syringe filtered using Millex-HA 0.45 μm filters (Millipore, Bedford, MA). Anion samples were preserved with formaldehyde and organic acid and NH_4^+ samples were preserved by flash freezing; all were measured in the laboratory using a capillary electrophoresis system (Agilent Technologies, Wilmington, DE). Precision for capillary electrophoresis analyses is better than 0.1 mg/L. Samples for Fe^{2+} and H_2S determination were preserved with trace metal grade HCl and zinc acetate, respectively; concentrations were determined photometrically in the field using a Spectronic#20D+ spectrophotometer (Thermo Spectronic, Rochester, NY). Precision for Fe^{2+} analyses was better than 0.1 mg/L and better than 0.001 mg/L for H_2S. Cation samples were preserved with HCl and analyzed by capillary electrophoresis (Agilent Technologies, Wilmington, DE). Alkalinity samples were measured upon collection by acid titration and Gran plots for graphical determination (Stumm and Morgan, 1996). Samples for DOC were filtered thru a 0.20 μm syringe tip filter into a baked glass bottle, preserved with hydrochloric acid to a pH of <2 and analyzed using a Shimadzu TOC Vcsn analyzer (Shimadzu Corporation). Methane samples were collected following the method of (Baedecker and Cozzarelli, 1992) and analyzed using a 5890 Series II HP Gas Chromatograph split/splitless inlet FID (flame ionization detector) with a fused silica capillary column.

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Rate Determination

Initial reactant (sulfate) and tracer (bromide) concentrations were compared to final reactant and tracer concentrations. Any loss of tracer was noted and used to correct for actual loss of reactant. The change in concentration of the initial reactant (c_{ri}) measured in the test solution minus the concentration of the final reactant (c_{rf}) collected at the end of each sampling event was determined. This change in concentration over time exposed to test solution (t) was then used to determine sulfate reduction rates. Rates were assumed to be independent of the concentration of sulfate, so zeroth-order sulfate reduction rates (k) were calculated for each sampling event.

$$k = \frac{c_{ri} - c_{rf}}{t} \qquad \text{Equation 4.1}$$

Microbial Methods

Prior to NOGEE experiments, colonization of sponge material was tested in the laboratory. Sponges were put inside NOGEE chambers and placed in beakers of wetland sediment collected from the site. Beakers were kept under anaerobic conditions for a colonization period of ~5 weeks at which point sponges were removed and frozen for later molecular analyses.

After the final sampling event for the NOGEE experiments the lower portion of the sponge chamber was extracted by removing screws and carefully pulling the chamber apart from the rest of the NOGEE apparatus. Sponges were then removed using sterilized tweezers, placed in sterile bags, and immediately frozen on dry ice and stored at -80^{o}C until DNA extraction and analysis via real-time quantitative PCR (qPCR). In the

laboratory DNA was extracted from approximately 0.5-1.5 gm of sponge material according to manufacturer's instructions using the Gentra Puregene kit (Gentra, Inc., Minneapolis, MN). DNA was quantified in a Stratagene MX3000P using the Quant-it picogreen dsDNA assay kit (Molecular Probes, Eugene, OR). Real-time quantitative PCR reactions were then performed in a Stratagene MX3000P to assess the abundance of the following organisms of interest using the primer sets indicated in Table 4.1: *Geobacter*, sulfate-reducing bacteria, and methanogens. Reactions were performed using the Quantitect SYBR green PCR kit (Qiagen, Valencia, CA) and 0.6 μM final primer concentration, with melting curves performed at the end of each reaction to ensure product integrity. Cycling conditions were 40 cycles at the Quantitect manufacturer recommended cycling temperatures and times with one modification. The fluorescence reading was taken after extension followed by a post-extension heating step at the temperature indicated in Table 4.1. Plasmids containing the gene of interest were used as quantitation standards, and were prepared by cloning PCR products into the pcR2.1 plasmid using the Topo TA cloning kit (Invitrogen, Carlsbad, CA). Reported numbers were normalized to ng of DNA.

Table 4.1. Summary of molecular methods. Summary of parameters used to target specific functional groups in DNA extracted from colonized sponges.

Functional group	Target gene	Primers	Anneal Temp (°C)	Read	Primer Reference
***Geobacter* (as proxy for Fe-reducers)**	***16s rRNA***	**Geo494f, Geo825R**	**53**	**83**	**(Anderson et al., 1998) (Holmes et al., 2002)**
Sulfate-reducing bacteria	***dsrB***	**dsrp2060f, dsr4r**	**55**	**82**	**(Geets et al., 2006) (Wagner et al., 1998)**
Methanogens	***mcrA***	***mcrA*f, *mcrA*R**	**56**	**82**	**(Luton et al., 2002)**

RESULTS

Geochemical Response

To ensure adequate time for reaction to occur, the first introduction of amended landfill-leachate (sampling event 1) was left in the sponge chamber for 71 hours (Tables 4.2-4.4 show elapsed times and geochemical concentrations for test solutions and sampling events). Geochemical samples collected at the end of the first sampling event provided evidence of sulfate reduction, thus the time interval between sampling events was shortened (45-53 hours). All sampling events for S1 and S2 (Table 4.3) revealed lower concentrations of sulfate than in the initial test solution compared to small changes in concentrations of tracer (bromide), which could be accounted for by mixing or dilution (any loss of tracer was not more than 15% and could be accounted for), which indicated

microbial sulfate reduction rather than dilution. Concentrations of sulfide increased in the final samples compared to concentrations in the initial test solution, and provided another indicator of microbial sulfate reduction. Concentrations of iron (Fe^{2+}) showed a decrease in final samples from initial test solution concentrations. This decrease is likely due to the increased sulfide, which sequesters Fe^{2+} out of solution. Little change was observed in other measured geochemical parameters (Table 4.4).

Initial geochemical samples from all NOGEEs (non-experimental, experimental, and control) indicated that chemical heterogeneity exists even in the small (<0.25 m) spatial scale between the NOGEEs (Figure 4.2). Initial sulfate concentrations, for example, ranged from 36.6 to 331.7 mg/L (Table 4.2). Geochemical analyses from control NOGEES, C1 and C2, which received landfill leachate with no addition of electron acceptor or donor revealed, as expected, little change in concentrations of measured geochemical parameters throughout the experiment.

Table 4.2. Summary of geochemical parameters. Geochemical concentrations of measured species for initial samples (prior to experimentation phase). BDL = below detection limit.

NOGEE ID Initial Samples	Cl^- mg/L	Br- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	Acetate mg/L	Lactate mg/L	NH_4^+ mg/L	H_2S mg/L	Fe^{2+} mg/L	CH_4 mg/L	DOC mg/L of C
D1	516.4	bdl	279.5	bdl	bdl	bdl	13.1			0.2	54.0
D2	558.2	bdl	331.7	bdl	bdl	bdl	0.5			0.1	59.5
D3	502.3	8.9	36.3	5.1	bdl	bdl	4.9	1.1	bdl	0.3	67.8
D4	524.4	5.7	266.5	1.8	bdl	bdl	0.5	0.3	bdl	0.2	54.2
D5	509.0	6.3	90.8	bdl	bdl	bdl	2.5	0.1	bdl	0.3	56.6
D6	489.5	5.6	45.1	bdl	bdl	bdl	bdl	1.1	bdl	0.2	55.3
S1 initial	491.5	5.4	142.8	bdl	bdl	bdl	bdl	1.4	bdl	0.6	94.3
S2 initial	471.5	bdl	47.3	bdl	bdl	bdl	1.0	2.3	bdl	0.7	99.3
C1 initial	483.1	bdl	145.4	bdl	bdl	bdl	19.6	1.3	bdl	1.5	78.8
C2 initial	444.1	bdl	68.2	bdl	bdl	bdl	12.5	bdl	bdl		70.2

Table 4.3. Sulfate NOGEEs. Geochemical concentrations for sulfate test solutions and experimental NOGEEs (S1 and S2) from each sampling event. BDL = below detection limit.

Experimental NOGEEs	Time Exposed to Test Solution (hrs)	Cl^- mg/L	Br- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	Acetate mg/L	NH_4^+ mg/L	H_2S mg/L	Fe^{2+} mg/L	Alkalinty mmol/L	CH_4 mg/L	DOC mg/L of C
Test Solution 1		638.7	108.6	104.5	bdl	90.2	22.5	bdl	13.2		1.4	180.5
S1-1	71.2	602.8	96.1	39.8	bdl	110.6	8.5	3.0	1.5	47.6	2.0	215.1
S2-1	71.0	597.7	88.7	18.1	bdl	88.6	0.7	2.1	1.8	47.2	1.0	175.8
Test Solution 2		624.3	106.7	78.8	bdl	77.7	0.7	bdl	20.0	48.1	4.5	165.5
S1-2	45.6	619.1	102.1	9.2	bdl	111.0	57.8	1.8	1.3	56.4	0.7	210.8
S2-2	45.5	615.0	92.6	14.4	bdl	87.4	2.1	1.4	0.2	51.4	1.5	162.6
Test Solution 3		611.2	102.8	97.3	bdl	121.8	2.0	bdl	11.7	50.0	3.2	180.3
S1-3	45.2	606.1	95.9	10.0	bdl	91.9	117.5	2.3	0.4	52.8	0.2	206.0
S2-3	45.3	593.5	95.3	15.5	bdl	31.7	3.5	2.0	1.0	53.1	1.6	163.3
Test Solution 4		612.9	103.9	91.9	bdl	84.7	2.0	bdl	11.7	52.1	2.9	184.0
S1-4	52.5	596.3	90.4	0.0	bdl	8.6	198.3	1.8	0.4	56.6	0.8	213.5
S2-4	52.5	594.9	91.3	18.0	bdl	81.7	150.6	1.1	0.8	73.1	1.7	154.9
Test Solution 5		608.8	96.1	97.0	bdl	77.4	154.6	bdl	12.0	67.0	2.0	190.2
S1-5	48.0	612.7	94.3	5.4	bdl	84.2	126.9	2.2	0.0	63.8	0.9	206.7
S2-5	47.7	592.9	83.1	9.6	bdl	82.0	165.2	0.1	0.3	63.8	1.6	154.0

Table 4.4. Control NOGEEs. Geochemical concentrations for control test solutions and control NOGEEs (S1 and S2) from each sampling event. BDL = below detection limit.

Control NOGEEs	Time Exposed to Test Solution (hrs)	Cl^- mg/L	Br- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	Acetate mg/L	Lactate mg/L	NH_4^+ mg/L	H_2S mg/L	Fe^{2+} mg/L	Alkalinty mmol/L	CH_4 mg/L	DOC mg/L of C
Test Solution 1		594.5	92.9	17.0	bdl	bdl	bdl	314.3	bdl	12.7		4.9	84.7
C2-1	70.5	573.6	70.5	46.0	bdl	bdl	bdl	bdl	0.3	2.0	37.6	1.2	102.4
C2-1	70.3	556.7	86.0	19.1	bdl	bdl	bdl	83.9	bdl	5.0	47.0	0.8	113.9
Test Solution 2		600.6	88.9	bdl	bdl	bdl	bdl		bdl	12.1	27.2	8.5	113.9
C1-2	47.4	566.8	78.4	bdl	bdl	bdl	bdl	390.7	bdl	2.2	54.7	2.1	149.1
C2-2	47.8	553.3	89.3	bdl	bdl	bdl	bdl	bdl	bdl	7.8	53.5	1.5	142.0
Test Solution 3		597.7	104.0	bdl	bdl	bdl	bdl	10.5	bdl	13.4	54.8	6.5	122.7
C1-3	48.1	571.0	94.4	bdl	bdl	bdl	bdl	21.8	0.2	4.1	44.5	2.7	182.6
C2-3	48.1	575.7	89.3	bdl	bdl	bdl	bdl	31.8	bdl	8.7	49.2	2.1	157.1
Test Solution 4		582.7	105.0	bdl	bdl	bdl	bdl	15.3	bdl	10.9	52.0	4.2	123.0
C1-4	47.2	571.7	96.2	bdl	bdl	bdl	bdl	15.3	0.6	3.9	58.3	3.1	181.7
C2-4	47.5	581.1	87.4	bdl	bdl	bdl	bdl	20.8	bdl	8.1	66.1	1.6	157.3
Test Solution 5		573.2	92.3	bdl	bdl	bdl	bdl	21.5	bdl	12.7	71.7	4.2	122.4
C1-5	48.6	567.0	92.9	bdl	bdl	bdl	bdl	9.8	bdl	4.3	75.3	2.5	186.5
C2-5	48.4	568.3	bdl	bdl	bdl	bdl	bdl	25.0	bdl	8.8	71.3	2.0	152.7

Sulfate Reduction Rates

For both S1 and S2, measurable sulfate reduction occurred. Sulfate reduction rates were calculated using equation 4.1. Comparison of S1 and S2 sulfate reduction rates for each of the 5 sampling events revealed a strong similarity in rates and trends (Table 4.5). Sulfate reduction rates increased over the first three sampling events. The first sampling event resulted in the slowest sulfate reduction rate; likely representing a transitional period in which the microbial population is adjusting to the introduction of new electron acceptors and donors. Rates in S1 and S2 increased with the same magnitude for the second and third sampling events. During the fourth sampling event there was a slight decrease in the rates of both S1 and S2, suggesting a threshold of sulfate reducing activity had been reached. In the final sampling event the rate continued to decrease slightly in S1 while there was an increase in the rate measured for S2 back to a rate similar to that measured during sampling event three.

Table 4.5. Results. Time sponges were exposed to test solution and rates of sulfate reduction determined from each sampling event.

	NOGEE S1		NOGEE S2	
Sampling Event	Time Exposed to Test Solution (hrs)	Sulfate Reduction Rate (mg/L hr^{-1})	Time Exposed to Test Solution (hrs)	Sulfate Reduction Rate (mg/L hr^{-1})
1	71	0.974	71	0.559
2	46	1.038	45	1.127
3	45	1.933	45	1.929
4	53	1.664	53	1.751
5	48	1.255	48	1.920

Microbial Response

Molecular analyses of sediment and sponge samples tested in the laboratory prior to NOGEE experiments revealed successful colonization of the sponges inside the NOGEE chambers and that results were comparable to molecular analyses of sediment microbiology. Abundance of sulfate reducing bacteria (sponge average = 3.33E+03 copies *dsrB*/ng DNA, sediment = 9.79E+03 copies *dsrB*/ng DNA), iron reducing bacteria (sponge average = 1.25E+04 copies *Geobact 16S rDNA*/ng DNA, sediment = 1.20E+04 copies *Geobact 16S rDNA*/ng DNA), and methanogens (sponge average = 5.23E+02 copies *mcrA*/ng DNA, sediment = 3.29E+02 copies *mcrA*/ng DNA) for the sediment and sponge samples from the laboratory experiment were comparable within an order of magnitude. Molecular analyses of sponges collected from the field experiments showed abundant DNA, further indicating that the polycarbonate sponge provided a suitable substrate for colonization of a native microbial population.

Results from qPCR analyses (Figures 4.4-4.6) indicate abundant sulfate reducers (average for initial sponge samples ~1.70E+03 copies *dsrB* /ng DNA) and iron reducers (average for initial sponge samples 4.00E+03 copies *Geobact 16S rDNA* /ng DNA) and few or no methanogens (average for initial sponge samples 1.48+E00 copies *mcrA*/ng DNA). Comparison of molecular results from initial sponge samples (D1-D4) reveals some spatial heterogeneity in the microbial populations colonized (Figures 4.4-4.6).

In both S1 and S2, the average abundance of sulfate reducers (Figure 4.4) increased from initial to final sampling intervals by upwards of an order of magnitude, while little change was observed in the concentration of iron reducers (Figure 4.5). These results correspond well with the change observed in sulfate reduction rates. Interestingly,

there was an increase in methanogens (Figure 4.6) from below detection limit in most initial sponge samples to approximately 1.99E+00 copies *mcrA*/ng DNA in S1 and 4.16E+00 copies *mcrA*/ng DNA in S2 final sponge samples. This may be because test solution contained acetate, which in addition to acting as an electron donor for sulfate reduction, is also a growth substrate for methanogens (Watson et al., 2003). Little change was observe in the concentration of sulfate reducers in final sponge samples from control NOGEEs but an increase in iron reducers was observed (Figures 4.4-4.6).

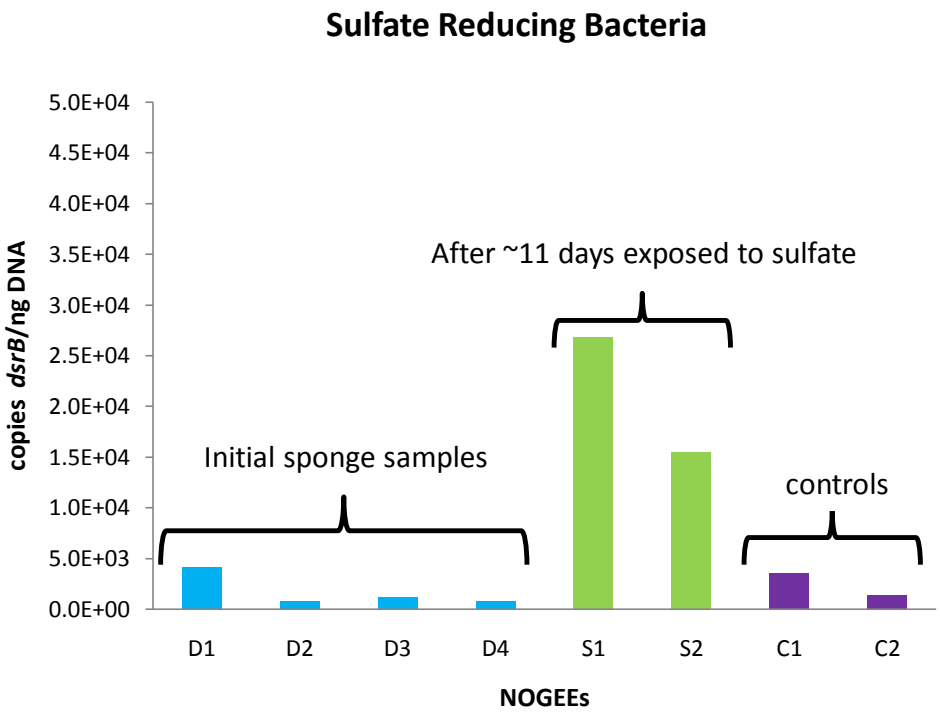

Figure 4.4. Abundance of sulfate reducing bacteria (copies *dsrB*/ng DNA). Initial sponge samples (D1-D4, blue) received no test solution. Final sponge samples (S1 and S2) are shown in green, received 100 mg/L sulfate test solution 5 times over 11 days. The control sponges received the same test solution without the addition of sulfate.

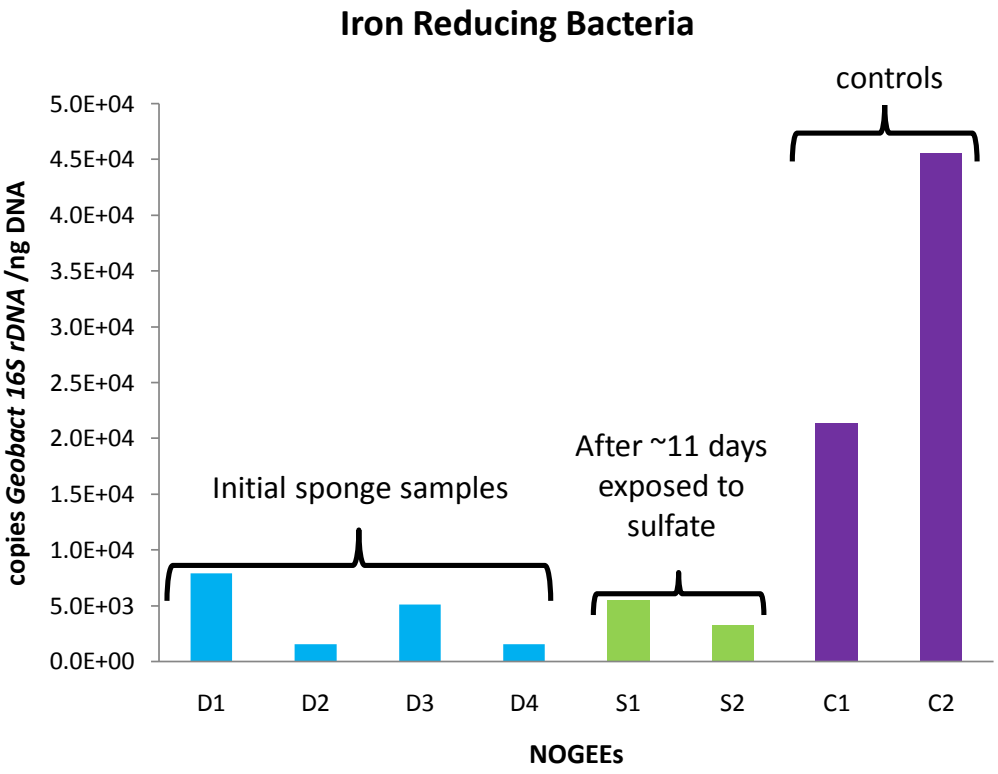

Figure 4.5. Abundance of iron reducing bacteria (copies *Geobact 16S rDNA* /ng DNA). Initial sponge samples (D1-D4, blue) received no test solution. Final sponge samples (S1 and S2) are shown in green, received 100 mg/L sulfate test solution 5 times over 11 days. The control sponges received the same test solution without the addition of sulfate.

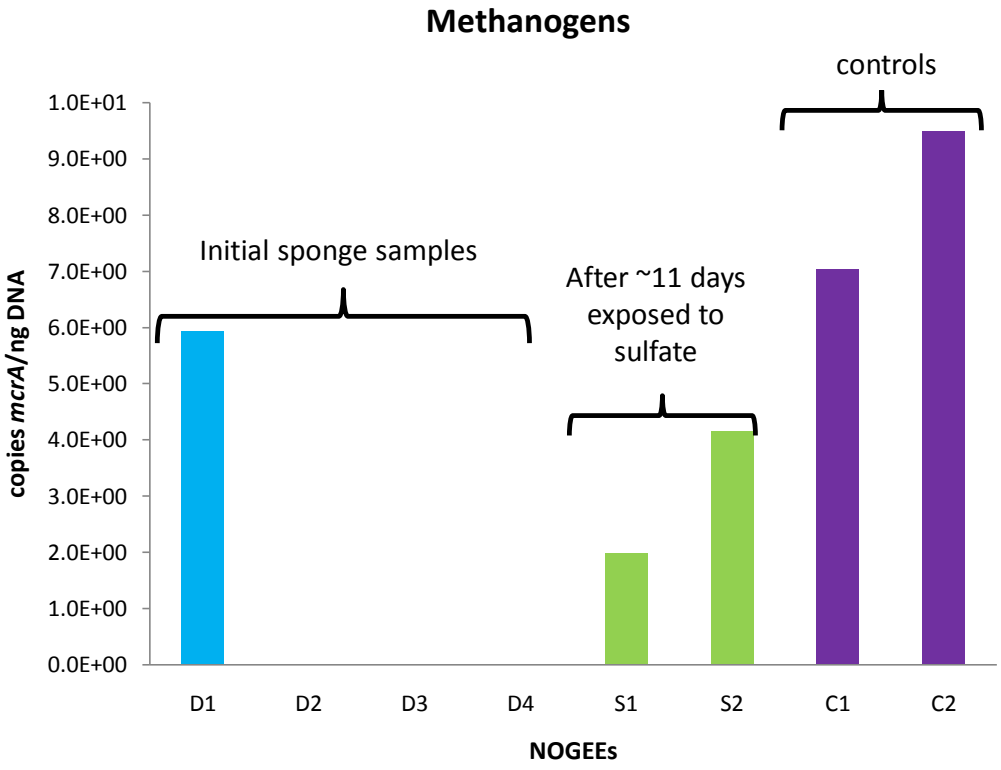

Figure 4.6. Abundance of methanogens (copies *mcrA*/ng DNA). Note different scale than Figures 4.4 and 4.5. Initial sponge samples (D1-D4, blue) received no test solution. Final sponge samples (S1 and S2) are shown in green, received 100 mg/L sulfate test solution 5 times over 11 days. The control sponges received the same test solution without the addition of sulfate.

CONCLUSIONS

Native organism geochemical experimentation enclosures (NOGEEs) enabled successful isolation of a native microbial population *in situ* and measurement of the population's response to a geochemical perturbation. This method is unique, in that the colonized sponges were isolated *in situ*, which allowed for the introduction and removal of test solution with little disturbance to the established microbial population. In this manner NOGEEs were successful in eliminating problems with disturbance and contamination that often occur when samples for laboratory experiments and analyses are collected (Roling and van Verseveld, 2002). This method also provided a sampling mechanism in which the aqueous samples collected were known to be in direct contact with the microbial population analyzed. Results could then be used to evaluate direct linkages between geochemical concentrations and microbial abundances. This method improves upon other integrative studies, which cannot confidently make these linkages because of temporal and spatial variability in the collection of geochemical and microbial samples (Bekins et al., 2001; Cozzarelli et al., 2001; Martino et al., 1998).

Introduction of a native solution (landfill-leachate contaminated aquifer water) amended with sulfate and electron donor resulted in measureable sulfate reduction rates and substantial growth of sulfate reducing bacteria when compared to results from control experiments. The resulting sulfate reduction rates were comparable within an order of magnitude to rates found in previous studies (Istok et al., 2001; Kneeshaw et al., 2007; McGuire et al., 2002; Schroth et al., 2001b). Further validation of the results obtained from this new technique is evident in the similarities in the rates and trends of the two experimental NOGEEs. The resulting rate data combined with the change in specific

microbial populations provided information on how quickly a native population can respond to a change in *in situ* conditions. These results provide important information for the evaluation of dynamic natural systems where environmental conditions are often changing and equilibrium states are rarely achieved.

Combined geochemical and microbiological analyses from NOGEE experiments suggest that the introduction of new electron acceptors and donors results in a relatively fast change in native microbial populations. These data provide direct evidence on how events, such as changing hydrologic conditions and the introduction of contaminants, which introduce new electron acceptors and donors to the subsurface, can affect reaction rates in natural systems. Implications for these data may include improving the application of natural attenuation and bioremediation in contaminated natural systems. NOGEEs provide a powerful new method for *in situ* quantification of reaction rates in complex natural systems. The applications for NOGEEs are broad and experiments could be designed to evaluate other more specific controls on redox reactions; including effects such as electron acceptor concentration, temperature, and organic matter quality.

CHAPTER V

IN SITU EVALUATION OF THE EFFECTS OF CONCENTRATION, TEMPERATURE AND MICROBIAL POPULATION RESPONSE ON SULFATE REDUCTION RATES IN A WETLAND-AQUIFER SYSTEM

INTRODUCTION

The activities carried out by microbial communities in the subsurface directly impact carbon and nutrient cycles as well as contaminant degradation (Ajwa et al., 1998; Christenson and Cozzarelli, 1999; Cozzarelli et al., 2001; Cozzarelli et al., 2000; Gaudinski et al., 2000; Konopka, Turco, 1991). Microorganisms play important functional roles in the subsurface because their metabolic activities mediate redox reactions that ultimately dictate the fate of both naturally occurring and xenobiotic chemicals (Lehman et al., 2001). As such, there is an increasing need to understand the controls on microbial activities in both pristine (Kieft et al., 1995; Krumholz, 2000; Lehman et al., 2001; Shi et al., 1999; Zhou et al., 2002) and contaminated (Cho and Kim, 2000; Fang and Barcelona, 1998; Rooney-Varga et al., 1999) subsurface systems.

The structure and function of subsurface microbial communities is dependent upon the interaction of a myriad of physical, chemical and biological parameters. In anaerobic subsurface systems microorganisms mediate redox reactions through respiration of organic material coupled to the transfer of alternate electron acceptors such as NO_3^-, Fe(III), or SO_4^{2-} (Cozzarelli et al., 2001; McGuire et al., 2000; Schreiber et al., 2004; Vroblesky and Chapelle, 1994). These reactions are dependent upon the availability of electron acceptors and donors which vary in response to events such as

rainfall (recharge), abiotic reactions and/or anthropogenic inputs. When parameters such as hydrologic and geochemical conditions change, the dominant terminal electron accepting processes (TEAPs) shift, resulting in different rates of redox reactions (Chapelle et al., 1995; Cozzarelli et al., 2000). Many natural systems are in a continual state of disequilibrium due to changing environmental conditions making it a challenge to estimate reaction rates for a given system. Yet it is necessary to determine rates of important reactions like sulfate reduction in complex systems so that estimates of carbon turnover can be made. This is especially important in contaminated subsurface systems where a reliable assessment of the *in situ* degradation of a contaminant in an aquifer is essential for the successful application of natural attenuation and bioremediation techniques (National Research Council, 2000).

The key to determining reaction rates in dynamic natural systems is understanding the important kinetic controls on reactions like sulfate reduction. A number of methods exist for evaluating microbially mediated reactions in the subsurface including; laboratory batch and column experiments (Beeman and Suflita, 1990; Bengtsson, 1989; Shi et al., 1999), field-based *in situ* microcosms (Bjerg et al., 1999; Watson et al., 2003), and tracer and push-pull tests (Addy et al., 2002; Azizian et al., 2005; Haggerty et al., 1998; Harris et al., 2005; Istok et al., 1997; Kneeshaw et al., 2007; McGuire et al., 2004; Reinhard et al., 1997). These methods have been valuable for characterizing chemical reactions (biotic verses abiotic), determining rates of reactions, evaluating toxicity effects, determining electron acceptor and donor availability, and measuring biodegradation rates. However, applying rates obtained from these methods to systems not at equilibrium presents problems because 1) most studies are performed under

controlled equilibrium conditions which may not be appropriate, and 2) samples for geochemistry and microbiology are generally not spatially and temporally representative making it difficult to measure linked geochemical and microbiological kinetic controls.

These limitations can best be overcome using *in situ* experimental investigations to assess geochemical reactions and microbial population metabolism directly within a natural subsurface environment. This study examined the response of an *in situ* native microbial population to different electron acceptor concentrations and seasonal temperature change using NOGEEs (Native Organism Geochemical Experimentation Enclosures) which facilitate the direct evaluation of geochemical and microbial parameters in response to geochemical perturbations (Chapter IV). The effect of sulfate concentration was evaluated through introductions of sulfate test solutions covering a range of sulfate concentrations (10, 25, 100 mg/L SO_4^{2-}) to native microbial communities on colonized substrate within NOGEEs. Replicate tests were conducted during a warm and a cold season to examine the effect of seasonal variations in temperature. Results show changes in microbial population structure corresponding with changes in geochemical parameters and reduction rates in response to both sulfate concentration and seasonal temperature change.

STUDY SITE DESCRIPTION

Experiments presented in this chapter were conducted in a wetland/slough system (see Figure 2.1) at the Norman Landfill research site in Norman, OK. The Norman Landfill is an unlined landfill which received unrestricted waste from 1922 through 1985 when it was covered and vegetated (Adrian et al., 1990; Christenson and Cozzarelli, 2003). The landfill is situated in alluvium near the Canadian River where the depth to water is shallow, ranging from land surface to ~4 m (Christenson and Cozzarelli, 2003). In the alluvial aquifer beneath the landfill, a leachate plume has developed that extends approximately 225 m downgradient from the landfill mound. The plume has elevated concentrations of dissolved organic carbon (DOC), chloride, ammonia, and methane and flows under/through the adjacent wetland system (Christenson and Cozzarelli, 1999). The biotic and abiotic reactions that occur in the aquifer have been well documented through intensive investigations by the USGS as well as university research groups (Cozzarelli et al., 2000; Eganhouse et al., 2001; Grossman et al., 2002; Schlottmann et al., 1999). In addition, a number of kinetic studies (Adrian et al., 1994; Beeman and Suflita, 1990; Senko et al., 2002) have also been conducted at the site along with documentation of microbial processes (Beeman and Suflita, 1987; Harris et al., 1999; Ulrich et al., 2003) and quantification of groundwater-surface water fluctuations (Christenson and Cozzarelli, 1999; Schlottmann et al., 1999; Scholl, 2000).

The geographic location of the Norman Landfill research site results in seasonal variability in temperatures and rainfall which change the hydrologic and biogeochemical processes at the site and result in the interaction of wetland porewater with landfill-leachate contaminated aquifer water. Seasonal temperature changes are thought to effect

rates of reactions and changes in hydrologic conditions which result in mixing of waters with very different redox conditions (Cazull et al., 2006). As a result, this is a dynamic system that is likely in a constant state of disequilibrium. Experiments described in this study were designed to simulate the complexities likely to be associated with this site.

MATERIALS AND METHODS

NOGEE Description

NOGEEs are a new sampling device (see Chapter IV) designed to accomplish 4 main tasks: 1) trap a native microbial population, 2) isolate the population from the surrounding environment *in situ*, 3) introduce a geochemical solution, and 4) measure the resulting effect on the microbial population structure through geochemical and microbial analyses. A single NOGEE is essentially a drive-point well which houses an inert polycarbonate sponge (Honeywell, Des Plaines, IL) suitable for microbial colonization (see Figure 4.1). After a colonization period of 4-6 weeks an inner pipe can be lowered over the colonized sponge, isolating it from the surrounding environment. Lowering this inner pipe also emplaces Teflon tubes that are connected to the surface to facilitate the introduction and removal of test solutions (see Figure 4.2). Detailed construction of NOGEEs is outlined in Chapter IV.

Eight NOGEEs (S1-S6, and C1, C2 [controls]) were installed in the reduced wetland sediments at the Norman Landfill research site, Norman, OK in October 2007 and February 2008. All NOGEEs were hand driven into the wetland sediments so that the sponge chamber was in a shallow (~30 cm) reducing muddy clay layer. After installation, each NOGEE was filled with deoxygenated Nanopure water and tubing ends were sealed. NOGEEs were left undisturbed in the wetland sediments for 4-6 weeks to allow colonization of the polycarbonate sponge. Additionally, several non-experimental NOGEEs (four in October 2007 and six in February 2008 [small wells in Figure 5.1]) were constructed in the same manner but without the ability to conduct the isolation step in the experimental phase. These NOGEEs were placed as close as possible to the experimental NOGEEs for the purpose of providing the initial sponge samples for molecular analyses of the microbial population established during the colonization phase.

NOGEE Experiments

NOGEE experiments were designed to evaluate the effect of sulfate concentration and seasonal temperature change on sulfate reduction rates and microbial population structure. Experiments were conducted during two different seasons, October 2007 when the surface water temperature for the slough was warm (average surface water temperature for October 2007 ~76.2^{o}F) and in February 2008 when the surface water

Figure 5.1. NOGEE placement for February 2008 experiments. October 2007 NOGEEs were conducted in the same vicinity and with similar spacing. Non-experimental NOGEEs (small wells) provided initial sponge samples for molecular analyses of the microbial population.

temperature for the slough was cold, (average surface water temperature for February 2008 ~39.2^{o}F) to examine the effect of natural changes in temperature. During both experiments the effect of sulfate concentration was evaluated using test solutions prepared with native water (landfill leachate) collected from a multi-level well (well MLS 36, see Figure 2.1) at the research site. Native water was used for test solutions to represent a mixing event likely to occur during recharge at the site. Three sulfate test

solutions of approximately 10, 25, and 100 mg/L sulfate were made by amending landfill leachate in an argon atmosphere with sulfate (prepared from Na_2SO_4, Acros Organics, New Jersey, USA) in the three different concentrations to serve as electron acceptor. Each test solution also received 30 mg/L of lactate and acetate (prepared from $C_3H_6O_3$ and $NaCH_3CO2$, respectfully, Acros Organics, New Jersey, USA) to serve as electron donor and bromide (~100 mg/L Br^-, prepared from NaBr, Acros Organics, New Jersey, USA) to serve as a conservative tracer to account for loss, mixing or dilution. All test solutions were made in an argon atmosphere to maintain anaerobic conditions. NOGEEs S1 and S2 received the 10 mg/L sulfate test solution. NOGEEs S3 and S4 received the 25 mg/L test solution and NOGEEs S5 and S6 received the 100 mg/L test solution (Figure 5.1). Two control NOGEEs received test solution consisting of the landfill leachate without any addition of sulfate or electron donor. Test solutions were made in the same manner for October 2007 and February 2008 experiments.

After the colonization period in October 2007 (warm) and February 2008 (cold), initial water samples were collected from the sponge chamber of all non-experimental and experimental NOGEEs immediately prior to beginning the experimentation phase. The internal tube of experimental NOGEEs was then lowered to isolate the sponge chambers and non-experimental NOGEEs were removed from the wetland sediments and sponges were collected for initial molecular microbiology analyses.

Following isolation of the sponge chamber and collection of initial samples test solution was introduced to NOGEE sponge chambers using a peristaltic pump at a pumping rate of 100 ml/min. A volume of test solution greater than three times the volume of the chamber (~180 ml) was flushed through the sponge chamber to ensure

maximum displacement of the solution already in the chamber. To ensure anaerobic conditions were maintained, tubing was purged with argon gas to eliminate mixing and dilution with residual water during subsequent sampling events. Tubing was then sealed and the test solution was left in the sponge chamber until sample collection (19-23 hours). At designated sampling times water was pumped using a peristaltic pump from the sponge chamber into an attached syringe. The exposed tubing end was connected to argon gas so as not to introduce oxygen during sample collection. Once samples for geochemical analyses were collected fresh test solution was again introduced to the chamber. This process was repeated 3 times. After the last sampling event NOGEEs were removed from wetland sediments and sponges were extracted using sterile tweezers, placed in sterile bags and immediately frozen on dry ice for final molecular microbiology analyses.

Analyses

Geochemistry samples were collected for initial water samples (collected from NOGEEs prior to beginning the experimentation phase), all test solutions and for each sampling event. Sample collection methods and geochemical parameters and analyses were the same as those in Chapter IV (page 67). Sulfate reduction rates were determined by comparing initial reactant (sulfate) and tracer (bromide) concentrations to final reactant and tracer concentrations. All rates were calculated following the method presented in Chapter IV (page 69, Equation 5.1).

All collected sponges were immediately frozen on dry ice and stored at -80^{o}C until DNA extraction and analysis via real-time quantitative PCR (qPCR). DNA was

extracted from sponges and quantified as described in Chapter IV (page 69). Reported numbers were normalized to ng of DNA.

RESULTS AND DISCUSSION

Geochemical Indicators

All warm NOGEEs (October 2007) showed depletion of sulfate at each of the three sampling points (Table 5.1). Zeroth-order sulfate reduction rates were calculated for each time point (Table 5.1). Rates for each pair of NOGEEs receiving the identical test solutions were similar (positive correlation, mean r = 0.84) but the rates varied by initial concentrations. Sulfate reduction rates were fastest in NOGEEs that received the highest concentrations of sulfate (80 – 115 mg/L SO_4^{2-}) and slowest in NOGEEs that received the lowest concentrations of sulfate concentrations (9 – 12 mg/L SO_4^{2-}) . It should be noted that there was some variability in initial test solution concentrations of sulfate that resulted in overlap of the different experiments. For example, the first introduction of "10 mg/L" sulfate test solution to NOGEEs S1 and S2 (Table 5.1) was actually 24.7 mg/L sulfate, creating overlap with test solution concentrations of sulfate for NOGEEs S3 and S4. All rates were determined based on actual measured concentrations from test solutions; rates were not quantified using intended concentrations (i.e., 10, 25, and 100 mg/L).

All cold NOGEEs (February 2008) showed some depletion of sulfate at each of the three sampling points but sulfate reduction was not as fast as during the warm NOGEE experiments. Zeroth-order sulfate reduction rates were calculated for each time point (Table 5.2) and rates were found to be an order or magnitude slower in most

NOGEEs than were observed in the warmer season. Rates for NOGEE pairs S1 and S2 and S5 and S6 were similar (positively correlated with r = 0.990 and r = 0.956, respectively) while rates for S1 and S2 showed no correlation (r = -0.133). Additionally, sulfate concentration appeared to have very little effect on the resulting rates. A comparison of rates for October 2007 (warm) and February 2008 (cold) is shown in Table 5.3. In addition, a plot of the sulfate reduction rates for each NOGEE verses initial test solution sulfate concentrations for the warm and cold NOGEE experiments is shown in Figure 5.2. This figure illustrates two results; first, sulfate reduction rates were on average much faster in October 2007 than in February 2008 and second, the effect of sulfate concentration on rate was more apparent in October 2007 than in February 2008. A comparison of the slopes (m= 0.008 in October 2007 and m= 0.000 in February 2008) from the linear regression equations for the two experiments shows an increase in sulfate reduction rates corresponding to higher initial sulfate concentrations during the warm NOGEEs while no relationship is evident for the cold NOGEEs .

Table 5.1. October 2007 results. Summary of test conditions and sulfate reduction rates for October 2007 NOGEEs. Red number indicates sulfate concentration higher than intended and that results should be group with results from NOGEEs receiving similar sulfate concentrations (bdl = below detection limit).

Sampling Event	Time Exposed to Test Solution (hrs)	Initial Test Solution Conc. (mg/L)	Final Sulfate Conc. (mg/L)	Rate (mg/L hr^{-1})	Final Sulfate Conc. (mg/L)	Rate (mg/L hr^{-1})
Intended concentration: 10 mg/L Sulfate						
NOGEE			**S1**	**S1**	**S2**	**S2**
1	22	24.7	bdl	1.138	bdl	1.136
2	21.5	8.6	bdl	0.399	5.9	0.125
3	21	10.9	bdl	0.524	bdl	0.530
Intended concentration: 25 mg/L Sulfate						
NOGEE			**S3**	**S3**	**S4**	**S4**
1	22	34.7	20.7	0.642	15.9	0.854
2	21	20.9	10.2	0.499	6.2	0.684
3	21	28.02	14.3	0.660	bdl	1.367
Intended concentration: 100 mg/L Sulfate						
NOGEE			**S5**	**S5**	**S6**	**S6**
1	22	114.9	93.6	0.968	84.8	1.363
2	21	91.2	54.2	1.739	52.2	1.834
3	20	97.1	79.3	0.910	65.4	1.636

Table 5.2. February 2008 results. Summary of test conditions and sulfate reduction rates for February 2008 NOGEEs. Red number indicates sulfate concentration higher than intended and that results should be group with results from NOGEEs receiving similar sulfate concentrations (bdl = below detection limit).

Sampling Event	Time Exposed to Test Solution (hrs)	Initial Test Solution Conc. (mg/L)	Final Sulfate Conc. (mg/L)	Rate (mg/L hr^{-1})	Final Sulfate Conc. (mg/L)	Rate (mg/L hr^{-1})
Intended concentration: 10 mg/L Sulfate						
NOGEE			**S1**	**S1**	**S2**	**S2**
1	20	12.0	11.8	0.010	12.0	none
2	21	11.6	10.5	0.052	11.4	0.012
3	21	20.0	18.8	0.057	19.8	0.009
Intended concentration: 25 mg/L Sulfate						
NOGEE			**S3**	**S3**	**S4**	**S4**
1	20	23.5	22.6	0.046	4.4	0.948
2	21	22.5	18.4	0.194	16.5	0.269
3	21	21.0	20.4	0.026	20.6	0.017
Intended concentration: 100 mg/L Sulfate						
NOGEE			**S5**	**S5**	**S6**	**S6**
1	18	80.0	72.5	0.415	72.4	0.415
2	23	87.5	88.1	none	87.5	0.003
3	21	81.5	80.8	0.036	78.2	0.160

Table 5.3. Comparison of sulfate reduction rates. October 2007 (warm) and February 2008 (cold). Concentrations in red and blue boxes are intended concentrations.

	Warm		Cold	
Sampling Event	Rate (mg/l hr^{-1})	Rate (mg/l hr^{-1})	Rate (mg/l hr^{-1})	Rate (mg/l hr^{-1})
	S1 10 ppm	S2	S1 10 ppm	S2
1	1.138	1.136	0.010	none
2	0.399	0.125	0.052	0.012
3	0.524	0.530	0.057	0.009
	S3 25 ppm	S4	S3 25 ppm	S4
1	0.642	0.854	0.046	0.948
2	0.499	0.684	0.194	0.269
3	0.660	1.367	0.026	0.017
	S5 100 ppm	S6	S5 100 ppm	S6
1	0.968	1.363	0.415	0.415
2	1.739	1.834	none	0.003
3	0.910	1.636	0.036	0.160

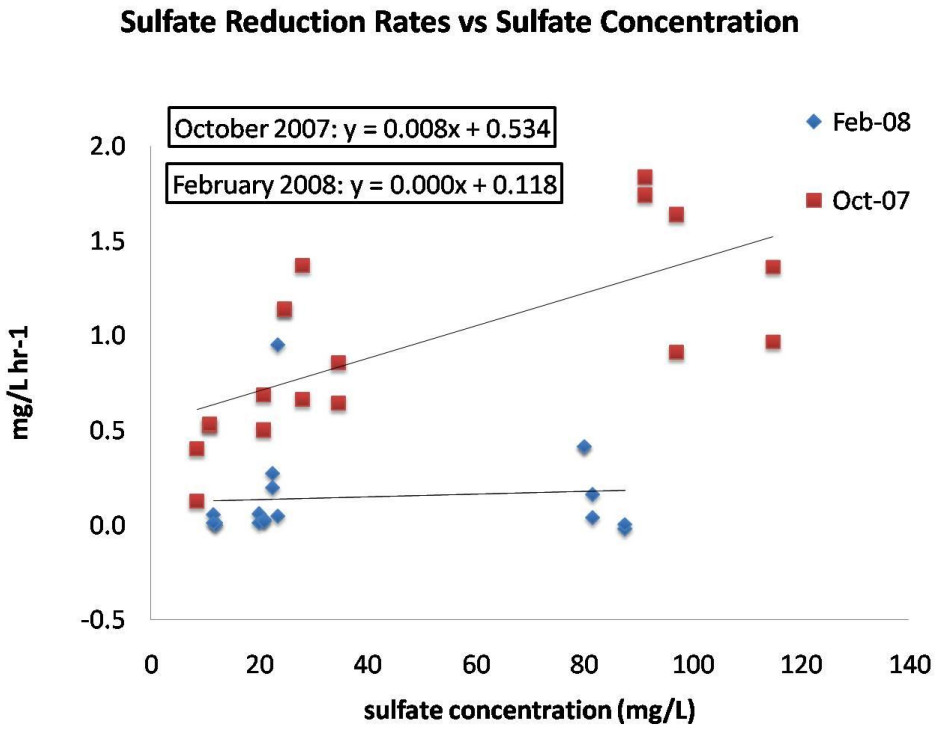

Figure 5.2. Rate results. Comparison of sulfate reduction rates verses test solution sulfate concentrations for October 2007 and February 2008 NOGEEs. Sulfate reduction rates were faster and exhibited more of a concentration effect in October 2007 (average surface water T = 76.2°F) than in February 2008 (average surface water T = 39.2°F).

Sulfide (H_2S) was not detected in any NOGEE samples from October 2007 or February 2008 which is likely due to interactions with iron. Iron (Fe^{2+}) was high in test solutions (~10.5 mg/L) compared to initial porewater samples (~4 mg/L). Iron (Fe^{2+}) decreased from initial test solution concentrations in October 2007 and February 2008 samples (Table 5.4) with the exception of a few instances in which increases in iron were observed. The lack of measureable sulfide and decrease in iron is attributed to the

formation of iron sulfide minerals during the experiment. Concentrations of electron donors (lactate and acetate) both decreased from initial test solution concentrations but were never completely consumed (concentrations >1 mg/L).

Table 5.4. Iron. Comparison of initial and final iron (Fe^{2+}) concentrations for October 2007 (warm) and February 2008 (cold) NOGEEs.

	warm			cold		
Sampling Event	**Iron (Fe^{2+}) Initial Test Solution Conc. (mg/L)**	**Iron Final Conc. (mg/L)**	**Iron Final Conc. (mg/L)**	**Iron (Fe^{2+}) Initial Test Solution Conc. (mg/L)**	**Iron Final Conc. (mg/L)**	**Iron Final Conc. (mg/L)**
Intended concentration: 10 mg/L Sulfate						
NOGEE		**S1**	**S2**		**S1**	**S2**
1	10.8	2.8	3.1	11.1	5.1	4.9
2	11.8	5.4	5.1	9.2	6.0	5.2
3	10.9	6.0	4.7	10.5	11.0	10.2
Intended concentration: 25 mg/L Sulfate						
NOGEE		**S3**	**S4**		**S3**	**S4**
1	12.0	5.2	13.8	12.2	0.7	3.8
2	11.1	4.6	4.2	10 .8	6.0	6.8
3	9.6	4.3	2.8	10.4	10.4	9.4
Intended concentration: 100 mg/L Sulfate						
NOGEE		**S5**	**S6**		**S5**	**S6**
1	8.8	3.9	2.0	13.0	7.6	4.9
2	11.9	11.7	14.7	10.5	8.3	6.0
3	18.2	5.0	2.8	10.3	10.1	9.3

DOC in landfill leachate prior to amendments was nearly identical (~112 mg/L C) in both the warm and cold sampling events. After amendments of lactate and acetate DOC increased by ~60 mg/L C (test solution DOC was ~170 mg/L, Table 5.6). As such the DOC in the introduced test solution was a combination of carbon from landfill leachate and from acetate and lactate additions. Test solution DOC concentrations were roughly twice as high as background concentrations in initial porewater samples from the wetland sediments (~70 mg/L of C in October and ~80 mg/L of C in February). DOC decreased during roughly half the sampling events in both warm and cold NOGEEs. Decreases in DOC occurred more frequently in NOGEEs which received test solution with higher sulfate concentrations. Decreases in DOC for the warm NOGEEs ranged from 2-8 mg/L of C while decreases in DOC for the cold NOGEEs ranged from 5-64 mg/L of C (Table 5.6). In some cases larger decreases in DOC correspond with faster sulfate reduction rates. For example, the fastest sulfate reduction rate in February 2008 (NOGEE S4 sampling event 1, Table 5.3) was 0.948 mg/L hr^{-1} (after 20 hrs exposure to test solution), which corresponded to the largest decrease in DOC in February 2008 (63 mg/L of C, rate = 3.15 mg/L of C hr^{-1}, NOGEE S4 sampling event 1 in Table 5.6).

Increases in DOC ranged from 0.2-10 mg/L of C in the warm NOGEEs and 4-16 mg/L in the cold NOGEEs (Table 5.6). Increases in DOC concentrations were surprising but may be due to the remineralization of particulate organic carbon (POC) through extracellular hydrolysis which forms DOC (Hee et al., 2001) or to other mechanisms such as the introduction of cellular carbon as a result of cell lysis.

Table 5.6. DOC. Comparison of initial and final DOC concentrations for October 2007 (warm) and February 2008 (cold) NOGEEs. Gray values indicate decreases in DOC from initial test solution concentrations.

	warm			cold		
Sampling Event	**DOC Initial Test Solution Conc. (mg/L C)**	**DOC Final Conc. (mg/L C)**	**DOC Final Conc. (mg/L C)**	**DOC Initial Test Solution Conc. (mg/L C)**	**DOC Final Conc. (mg/L C)**	**DOC Final Conc. (mg/L C)**
Intended concentration: 10 mg/L Sulfate						
NOGEE		**S1**	**S2**		**S1**	**S2**
1	175.0	181.3	185.1	165.1	155.5	164.8
2	175.2	172.0	175.2	170.6	165.3	181.4
3	153.4	155.2	158.0	173.9	153.3	170.3
Intended concentration: 25 mg/L Sulfate						
NOGEE		**S3**	**S4**		**S3**	**S4**
1	183.8	184.0	183.4	175.2	171.4	111.8
2	162.2	165.8	156.8	162.3	182.9	173.0
3	158.5	155.7	150.0	173.9	182.2	187.5
Intended concentration: 100 mg/L Sulfate						
NOGEE		**S5**	**S6**		**S5**	**S6**
1	156.9	161.4	160.0	171.2	174.9	164.8
2	170.0	165.1	163.2	178.3	194.5	188.2
3	170.5	164.8	163.4	178.5	185.4	170.7

Methane values (Table 5.7) were slightly higher in initial porewater samples during the cold experiments (~3 mg/L CH_4) than warmer experiments (~2 mg/L CH_4). Similarly, aquifer leachate used to make test solutions also contained slightly higher methane concentrations during the colder sampling event (mean of ~2 mg/L CH_4 in October 2007 and mean of ~2.5 mg/L CH_4 in February 2008). During the warm NOGEE

experiments, methane decreased in the majority of time points. These decreases in methane were generally between 0.1-1.5 mg/L CH_4. During our cold NOGEE experiments, methane was produced (average increase of ~1.5 mg/L CH_4) during several sampling events. The decreases in methane observed during colder NOGEE experiments were comparatively greater than the decreases observed during warmer experiments (~0.2 in Oct. ~2.5 mg/L in Feb). Both production and loss of methane can be explained by the activites of microorganisms. For example, methane production is likely due to methanogenesis and methane loss may be due to anaerobic methane oxidation coupled to sulfate reduction, a microbial reaction that has been documented at this site (Grossman et al., 2002).

No significant changes were observed in other measured parameters, including concentrations of tracer (bromide). Other than DOC, changes in measured parameters for control NOGEEs were not significant (see appendix for data).

Table 5.7. Methane. Comparison of initial and final methane (CH_4) concentrations for October 2007 (warm) and February 2008 (cold) NOGEEs. Gray values indicate decreases in DOC from initial test solution concentrations.

	warm			cold		
Sampling Event	**CH_4 Initial Test Solution Conc. (mg/L)**	**CH_4 Final Conc. (mg/L)**	**CH_4 Final Conc. (mg/L)**	**CH_4 Initial Test Solution Conc. (mg/L)**	**CH_4 Final Conc. (mg/L)**	**CH_4 Final Conc. (mg/L)**
Intended concentration: 10 mg/L Sulfate						
NOGEE		**S1**	**S2**		**S1**	**S2**
1	1.7	1.5	1.8	3.4	no sample	2.5
2	1.1	1.9	1.4	2.7	1.3	1.2
3	2.4	1.8	no sample	0.5	0.1	0.4
Intended concentration: 25 mg/L Sulfate						
NOGEE						
1	2.5	1.6	2.3	2.8	no sample	2.9
2	2.4	1.7	1.9	3.8	1.5	1.4
3	2.1	1.5	1.9	0.5	0.6	1.8
Intended concentration: 100 mg/L Sulfate						
NOGEE						
1	1.8	1.6	0.6	3.6	2.2	4.8
2	1.3	1.9	2.0	3.8	1.1	no sample
3	1.7	1.4	0.8	1.2	2.9	2.4

Microbial Response

Molecular analyses of initial sponge material from warm NOGEE experiments revealed high quantities of DNA (425 +/- 563 ng DNA, mean DNA yield from sponges). Of the three targeted groups (Geobacter, sulfate-reducing bacteria, and methanogens), initial sponge analyses showed Geobacter and sulfate-reducing bacteria to be the most abundant (Geobacter mean abundance ~9.5E+03 copies *Geobact 16S rDNA*/ng DNA, sulfate reducing bacteria mean abundance ~3.05E+03 copies *dsrB*/ng DNA [Figures 5.3 and 5.4]), while methanogens were significantly less abundant (mean abundance ~1.56E+02 copies *mcrA*/ng DNA [Figure 5.5]).

Molecular analyses of initial sponge material from cold NOGEE experiments revealed lower overall quantities of DNA (105 +/-78 ng DNA, mean DNA yield from sponges). Of the three targeted groups initial sponge analyses revealed a greater abundance of Geobacter (mean abundance ~2.13E+04 copies *Geobact 16S rDNA*/ng DNA [Figure 5.3]) than sulfate-reducing bacteria (mean abundance ~2.4E+03 copies *dsrB*/ng DNA [Figure 5.2]). Methanogens were significantly less abundant (mean abundance ~9.6E+00 copies *mcrA*/ng DNA [Figure 5.4]). Geobacter and methanogens showed larger changes in abundance from warm to cold. Geobacter increased by nearly an order of magnitude from October 2007 to February 2008 while methanogens decreased by nearly two orders of magnitude. These shifts in abundance are likely explained by different redox conditions due to variable hydrologic conditions during the two different seasons.

During the warm NOGEE experiments, there was an increase in sulfate reducing bacteria from initial to final sponge samples for all NOGEEs receiving sulfate test solutions and higher sulfate concentrations resulted in larger increases in sulfate reducing bacteria (Figure 5.3). It should be noted however, that in several cases the final microbial abundances were lower than the initial sponge averages. For example, the abundance of sulfate-reducing bacteria for the final sponges from NOGEEs S2 and S3 was lower than the mean abundance from initial sponges samples (Figure 5.3). This is likely due to heterogeneity between placement and colonization of experimental and non-experimental NOGEEs rather than loss of microorganisms and it represents a challenge in interpreting the molecular data. Nonetheless, several trends are obvious, for example, there was also an increase in the iron reducing bacteria in all NOGEEs as well as in the control NOGEEs (possibly from test solution). Methanogens on average showed only a significant increase in two NOGEEs, one 25 mg/L sulfate NOGEE and one control NOGEE. All results are illustrated in Figures 5.3, 5.4, and 5.5.

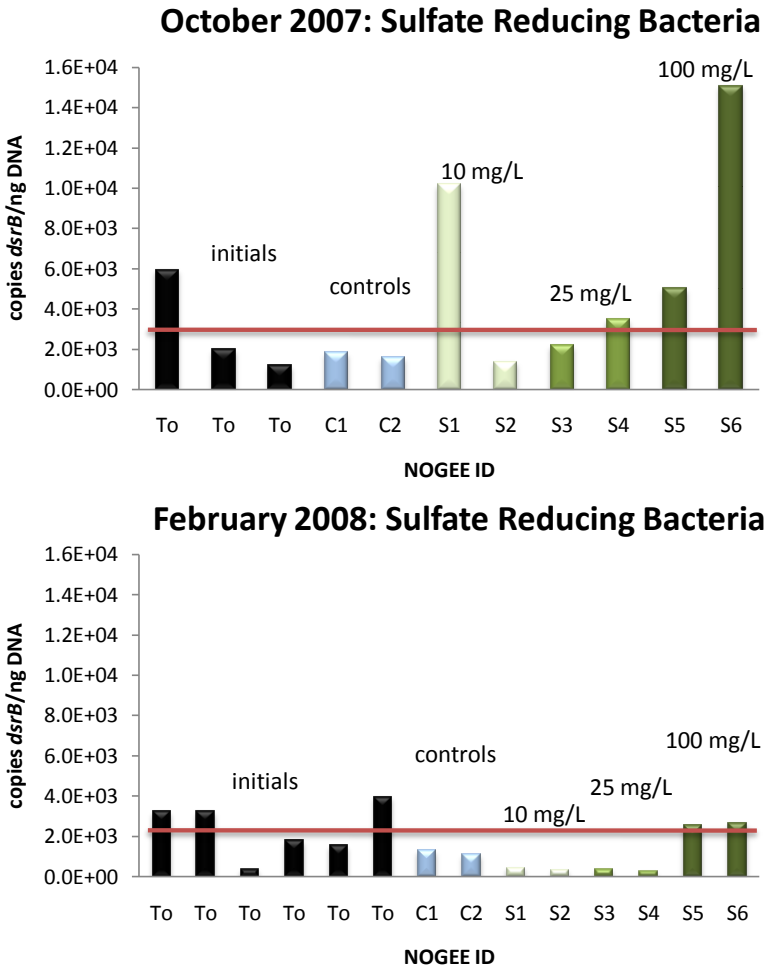

Figure 5.3. Sulfate reducing bacteria. Comparison of sulfate reducing bacteria (copies *dsrB*/ng DNA) for October 2007 and February 2008 NOGEEs. T_0 are initial sponge samples collected from non-experimental NOGEEs. C1 and C2 are controls (received landfill leachate with no sulfate or electron donor amendments) and S1-S6 are NOGEE pairs which received landfill leachate amended with different sulfate concentrations. Red line is the mean amount of copies *dsrB*/ng of DNA for the initial sponge samples collected from non-experimental NOGEEs.

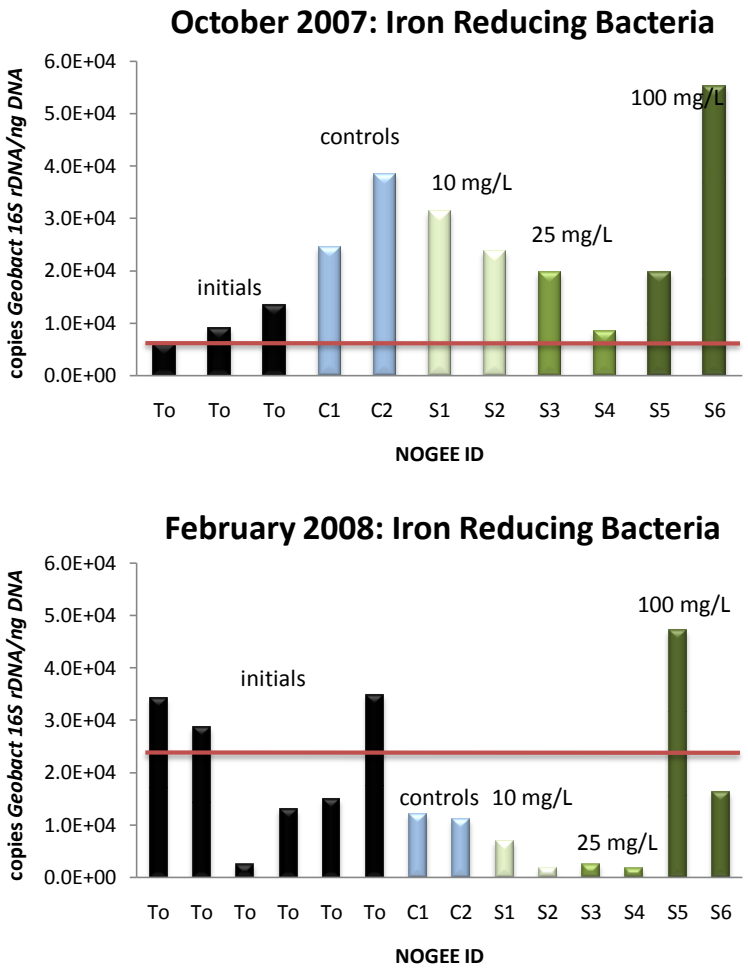

Figure 5.4. Iron reducing bacteria. Comparison of iron reducing bacteria (*Geobacter*) (copies *Geobact 16S rDNA* /ng DNA) for October 2007 and February 2008 NOGEEs. T_0 are initial sponge samples collected (prior to introduction of test solution). C1 and C2 are controls (received landfill leachate with no sulfate or electron donor amendments) and S1-S6 are NOGEE pairs which received landfill leachate amended with different sulfate concentrations. Red line is the mean amount of copies *Geobact 16S rDNA*/ng of DNA for the initial sponge samples.

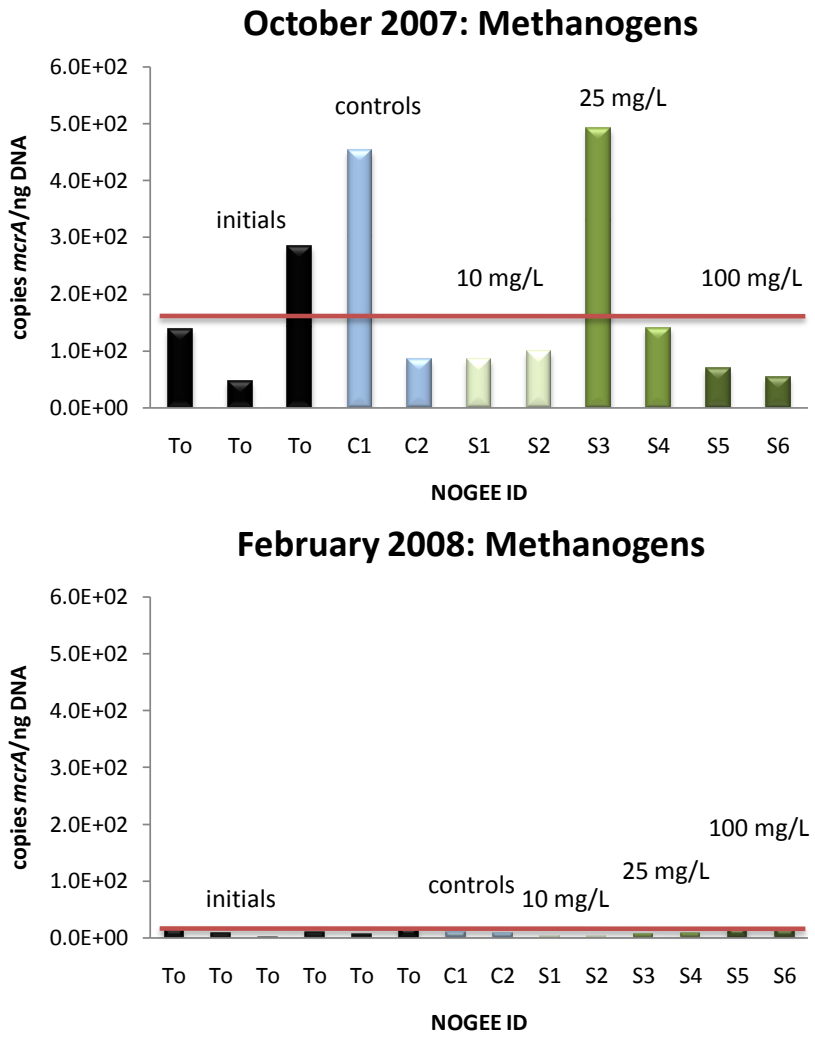

Figure 5.5. Methanogens. Comparison of methanogens (copies *mcrA*/ng DNA) for October 2007 and February 2008 NOGEEs. T_0 are initial sponge samples collected from non-experimental NOGEEs. C1 and C2 are controls (received landfill leachate with no sulfate or electron donor amendments) and S1-S6 are NOGEE pairs which received landfill leachate amended with different sulfate concentrations. Red line is the average amount of copies *mcrA*/ng of DNA for the initial sponge samples collected from non-experimental NOGEEs.

Microbial analyses of sponges from cold NOGEE experiments revealed a less apparent change in microbial population (Figures 5.3, 5.4, and 5.5). In February 2008 there was, on average, a slight decrease in sulfate reducing bacteria when compared to initial sponge values. However, the averages may not reveal the whole story given the heterogeneity of the system, particularly given the lower biomass measured during these colder experiments. When comparing only the results for NOGEEs that received sulfate test solutions (i.e., neglecting non-experimental NOGEEs), the number of sulfate reducing bacteria present in NOGEEs receiving 100 mg/L sulfate test solution (S5, S6) is much higher than those in the NOGEEs receiving smaller concentrations of test solution. This would suggest that the test solution concentration did have an effect on the microbial population but it cannot be decisively determined because the heterogeneity of the initial sponge values is greater than the changes due to the addition of sulfate, which complicates interpretation of the resulting data. One piece of data that supports the hypothesis that microbial activity was in fact stimulated by input of test solutions and the decrease is simply the result of initial heterogeneity in biomass within the system is that iron reducing bacteria also increase in all NOGEEs including the control NOGEEs in February 2008. Data for methanogens from the two experiments would however suggest the opposite conclusion and indicate a decrease in microorganisms.

CONCLUSIONS

Sulfate reduction rates were faster during October 2007 when average surface water temperature was about 37 degrees warmer than the average surface temperature in February 2008. Sulfate reduction rates were also faster at higher sulfate concentrations

and the observed effect was greater during warmer temperatures. This suggests that the effect of temperature may be two-fold in that, cooler temperatures result in slower sulfate reduction rates, which in turn lessens the effect of sulfate concentration (i.e., rates are more constant regardless of sulfate concentration under colder conditions). This suggests that temperature plays an important role in determining the appropriate rate order for quantification of sulfate reduction rates. For example, a first-order rate may be more appropriate for determination of sulfate reduction rates for warmer temperatures and a zero-order rate may be more appropriate for rate determination in colder temperatures. Molecular analyses suggest the presence of iron reducers, sulfate reducers, and methanogens during both warm and cold experiments though the overall DNA yields were lower during the colder season. The changes in microbial population initiated by the influx of sulfate-rich water were different during warm and cold seasons (a greater increase in sulfate-reducing bacteria at higher sulfate test solution concentrations was observed during the warm experiments than cold experiments). In addition, it was observed that these changes were linked to changes in measured rates of sulfate reduction.

This dataset is unique in that it reports direct measurements of linked changes in geochemistry and *in situ* microbial population structure and the resulting effects on reaction kinetics. It also demonstrates the role of temperature on both the microbiological and geochemical kinetic controls on sulfate reduction. To our knowledge, these measurements represent the first direct observations of linked geochemistry and microbiology in a complex natural system not at equilibrium.

CHAPTER VI

CONCLUSIONS

There are a number of controls on rates of terminal electron accepting processes (TEAPs) in subsurface systems, including concentrations of electron acceptors and donors, the presence of mixing interfaces, and microbial activities. The research presented in this dissertation suggests that these controls must be understood to provide representative rates for dynamic natural systems. Understanding these controls means that microbial processes in subsurface systems must be considered in conjunction with geochemical and hydrological processes. The complex linkages between these processes cannot be ignored when making rate estimates.

In the past we have needed many different approaches to study complex systems such as the wetland-aquifer system at the Norman Landfill research site in Norman, OK. These approaches have included geochemical analysis of field parameters such as electron acceptors and donors, introduction of perturbations, and lab experiments. Through these different studies it has become apparent that a multi-disciplinary approach is necessary to understand factors controlling important reactions in the subsurface. Increasing needs for prediction of fate and transport of contaminants in the environment has led to a number of new methods for predicting rates of reactions in contaminated and pristine subsurface systems. This dissertation evaluated the utility of one commonly used technique, push-pull tests, and in an effort to overcome problems with current samples methods provided a new technique for *in situ* investigation of kinetic controls on important subsurface reactions.

Field investigations conducted using push-pull tests have proven these tests to be a powerful tool for evaluating the rates of various hydro-bio-geochemical processes *in situ* and resulting rate data have been widely used. Data from push-pull tests and follow-up studies presented in this dissertation suggest that rate data obtained from push-pull tests works well under conditions in which rates are not spatially variable, and when a single unchanging kinetic control persists during the experiment. This means that in order to confidently apply push-pull rate data to complex natural systems natural heterogeneities, experimental conditions, and other rate controlling factors must be understood. Additionally, in many instances more information than a single rate estimate can be obtained from the data collected in the field. In fact many push-pull datasets have been shown to exhibit complexities including the presence of a lag phase and complex/changing rate order. These complexities should be described so that important information about kinetic controls can be obtained.

Two important conclusions were made based on results from push-pull tests and follow-up studies. The first is that the experimental results from push-pull tests and follow-up studies provided strong evidence of spatial variability in rate data due to the creation of a mixing interface and the second is that complexities involving changing reaction rates and rate order can be described and are likely due to the response of the native microbial population. Both of these observations provide new research avenues for evaluating dynamic natural systems. Push-pull tests can be used to simulate natural mixing zones and resulting rate estimates can be incorporated into system level evaluations. Further research evaluating the factors controlling the kinetics of microbial sulfate reduction is necessary to describe the complexities observed in rate data.

These findings led to the development of native organism geochemical experimentation enclosures (NOGEEs) which were successfully used to isolate native microbial communities *in situ* and subsequently measure the population's response to different geochemical perturbations. Measured geochemical parameters provided rate data and molecular analyses provided the coincident change in the microbial population. Several different experiments were conducted to evaluate the kinetic controls on sulfate reduction under conditions that would be expected in nature. The combined results from these experiments help demonstrate the important linkages between geochemistry and microbiology in complex natural systems where environmental conditions are often changing and equilibrium states are rarely achieved. This type of data provides direct evidence about controls on reaction rates in natural systems and has implications for improving upon application of natural attenuation and bioremediation in contaminated natural systems. NOGEEs provide a powerful new method for *in situ* quantification of reaction rates in complex natural systems and experiments can be designed to evaluate other more specific controls on redox reactions; including effects such as electron acceptor concentration, temperature, and organic matter quality.

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APPENDIX I

GEOCHEMICAL AND MICROBIAL DATA

The following pages provide detailed results from geochemical and microbial analyses of samples collected from experiments conducted at the Norman Landfill Research Site, Norman, OK in support of this dissertation.

Push-Pull Test Data

Push-Pull Test R2 (MPPT1) 8-May-04			uncorrected for mixing						corrected for mixing															
	hrs	L																			sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer	ammonium vs. tracer
Sample	Elapsed Time	Vol Removed	Br	SO_4^{2-}	H_2S	Fe^{2+}	Cl^-	NH_4^+	C/Co	Br^- Mixing Ratio	C/Co	SO_4^{2-} Mixing Ratio	C/Co	H_2S Mixing Ratio	C/Co	Fe^{2+} Mixing Ratio	C/Co	NH_4^+ Mixing Ratio	C/Co	Cl^- Mixing Ratio	$\ln(SO_4^{2-}$ mixing ratio/Br^- mixing ratio)	$\ln(H_2S$ mixing ratio/ Br^- mixing ratio)	$\ln(Fe^{2+}$ mixing ratio/ Br^- mixing ratio)	$\ln(NH_4^+$ mixing ratio/Br^- mixing ratio)
102-Initial Well			**5.35**	**13.48**	**1.57**	**4.15**	**454.30**																	
R2 Jug			**102.46**	**113.62**	**0.68**	**15.43**	**586.73**	**6.92**																
R2-1	0.00	0.4	112.38	116.43	0.69	15.75	628.35		1.10	1.10	1.02	1.03	1.02	0.99	1.02	1.03	0.00	0.00	1.07	1.31	-0.07	-0.11	-0.07	
R2-2	0.25	0.8	111.15	116.91	0.71	17.33	625.44	7.48	1.08	1.09	1.03	1.03	1.04	0.97	1.12	1.17	1.08	1.08	1.07	1.29	-0.05	-0.12	0.07	-0.01
R2-3	0.50	1.2	111.22	113.71	0.66	14.99	624.68	6.85	1.09	1.09	1.00	1.00	0.98	1.02	0.97	0.96	0.99	0.99	1.06	1.29	-0.09	-0.07	-0.13	-0.10
R2-4	0.75	1.6	109.78	115.55	0.62	16.38	624.97	9.58	1.07	1.08	1.02	1.02	0.92	1.07	1.06	1.08	1.38	1.38	1.07	1.29	-0.05	-0.01	0.01	0.25
R2-5	1.00	2	111.41	116.00	0.64	14.32	627.29	5.99	1.09	1.09	1.02	1.02	0.94	1.05	0.93	0.90	0.87	0.87	1.07	1.31	-0.06	-0.04	-0.19	-0.23
R2-6	1.25	2.4	106.46	113.14	0.61	14.74	622.15	11.06	1.04	1.04	1.00	1.00	0.90	1.08	0.96	0.94	1.60	1.60	1.06	1.27	-0.05	0.04	-0.10	0.43
R2-7	1.50	2.8	105.21	110.97	0.54	13.82	618.59	3.83	1.03	1.03	0.98	0.97	0.79	1.16	0.90	0.86	0.55	0.55	1.05	1.24	-0.05	0.12	-0.18	-0.62
R2-8	1.75	3.2	104.93	111.89	0.59	13.90	620.75		1.02	1.03	0.98	0.98	0.88	1.10	0.90	0.86	0.00	0.00	1.06	1.26	-0.04	0.07	-0.17	
R2-9	2.00	3.6	106.29	112.75	0.66	14.99	620.04		1.04	1.04	0.99	0.99	0.98	1.02	0.97	0.96	0.00	0.00	1.06	1.25	-0.05	-0.02	-0.08	
R2-10	2.25	4	104.93	112.76	0.66	13.48	617.90		1.02	1.03	0.99	0.99	0.98	1.02	0.87	0.83	0.00	0.00	1.05	1.24	-0.03	-0.01	-0.22	
R2-11	2.50	4.4	105.55	114.85	0.61	13.14	628.36	8.20	1.03	1.03	1.01	1.01	0.90	1.08	0.85	0.80	1.18	1.18	1.07	1.31	-0.02	0.05	-0.26	0.14
R2-12	2.75	4.8	106.04	115.69	0.65	13.14	620.68	33.21	1.04	1.04	1.02	1.02	0.96	1.03	0.85	0.80	4.80	4.80	1.06	1.26	-0.02	0.00	-0.26	1.53
R2-13	3.00	5.2	98.81	110.37	0.61	12.64	606.27	18.86	0.96	0.96	0.97	0.97	0.90	1.08	0.82	0.75	2.73	2.73	1.03	1.15	0.01	0.12	-0.25	1.04
R2-14	3.25	5.6	102.98	114.36	0.65	13.14	617.10	23.18	1.01	1.01	1.01	1.01	0.96	1.03	0.85	0.80	3.35	3.35	1.05	1.23	0.00	0.03	-0.23	1.20
R2-15	3.50	6	103.04	114.57	0.66	13.23	628.22	27.94	1.01	1.01	1.01	1.01	0.98	1.02	0.86	0.80	4.04	4.04	1.07	1.31	0.00	0.01	-0.22	1.39
R2-16	3.75	6.4	106.34	113.35		12.22	638.92	29.42	1.04	1.04	1.00	1.00	0.00	1.76	0.79	0.72	4.25	4.25	1.09	1.39	-0.04	0.53	-0.37	1.41
R2-17	4.00	6.8	101.24	110.50	0.69	12.39	620.86	36.82	0.99	0.99	0.97	0.97	1.02	0.99	0.80	0.73	5.32	5.32	1.06	1.26	-0.02	0.00	-0.30	1.68
R2-18	4.25	7.2	103.07	111.39	0.58	11.84	621.56	46.75	1.01	1.01	0.98	0.98	0.85	1.11	0.77	0.68	6.76	6.76	1.06	1.26	-0.03	0.10	-0.39	1.90
R2-19	4.50	7.6	101.74	110.37	0.71	11.67	619.09	42.54	0.99	0.99	0.97	0.97	1.04	0.97	0.76	0.67	6.15	6.15	1.06	1.24	-0.03	-0.02	-0.40	1.82
R2-20	4.75	8	92.10	103.55	0.72	11.13	592.15	46.80	0.90	0.89	0.91	0.90	1.06	0.95	0.72	0.62	6.76	6.76	1.01	1.04	0.01	0.07	-0.37	2.02
R2-21	5.00	8.4	90.67	100.38	0.69	11.00	600.81	43.21	0.89	0.88	0.88	0.87	1.02	0.99	0.71	0.61	6.24	6.24	1.02	1.11	-0.01	0.12	-0.37	1.96
R2-22	5.25	8.8	80.44	88.90	0.86	10.75	582.22	60.34	0.79	0.77	0.78	0.75	1.27	0.80	0.70	0.58	8.72	8.72	0.99	0.97	-0.03	0.03	-0.28	2.42
R2-23	9.58	9.2	66.31	79.03	0.71	10.62	565.62	48.06	0.65	0.63	0.70	0.65	1.04	0.97	0.69	0.57	6.94	6.95	0.96	0.84	0.04	0.44	-0.09	2.40
R2-24	21.92	9.6	49.41	60.03	0.64	10.33	549.50	55.01	0.48	0.45	0.53	0.46	0.94	1.05	0.67	0.55	7.95	7.95	0.94	0.72	0.02	0.84	0.19	2.86
R2-25	24.17	10	34.48	42.57	0.65	8.86	517.33	42.85	0.34	0.30	0.37	0.29	0.96	1.03	0.57	0.42	6.19	6.19	0.88	0.48	-0.03	1.24	0.33	3.03
R2-26	26.18	10.4	22.88	26.27	0.78	8.27	498.57	29.22	0.22	0.18	0.23	0.13	1.15	0.89	0.54	0.37	4.22	4.22	0.85	0.33	-0.35	1.60	0.70	3.15
R2-27	29.05	10.8	21.05	19.34	0.76	10.58	472.49	46.67	0.21	0.16	0.17	0.06	1.13	0.91	0.69	0.57	6.74	6.74	0.81	0.14	-1.02	1.72	1.26	3.73
R2-28	31.05	11.2	17.83	16.98	0.72	7.98	493.74	41.84	0.17	0.13	0.15	0.03	1.06	0.95	0.52	0.34	6.05	6.05	0.84	0.30	-1.30	2.01	0.97	3.85
R2-29	31.83	11.6	13.19	14.58	0.92	7.22	461.71	53.74	0.13	0.08	0.13	0.01	1.36	0.73	0.47	0.27	7.77	7.77	0.79	0.06	-1.99	2.20	1.21	4.57

Push-Pull Test R3 uncorrected for mixing corrected for mixing

8-May-04

																				sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer	acetate vs tracer
	hrs	L																					
Sample	**Elapsed Time**	**Vol Removed**	**Br**	**SO_4^{2-}**	**H_2S**	**Fe^{2+}**	**Cl^-**	**C/Co**	**Br^- Mixing Ratio**	**C/Co**	**SO_4^{2-} Mixing Ratio**	**C/Co**	**H_2S Mixing Ratio**	**C/Co**	**Fe^{2+} Mixing Ratio**	**C/Co**	**Cl^- Mixing Ratio**	**C/Co**	**Acetate Mixing Ratio**	**ln(SO_4^{2-} mixing ratio/Br^- mixing ratio)**	**ln(H_2S mixing ratio/ Br^- mixing ratio)**	**ln(Fe^{2+} mixing ratio/ Br^- mixing ratio)**	**ln(acetate dilution ratio/Br dilution ratio)**
pp102B-Initial Well	**0.00**	**0.40**	**10.05**	**19.11**	**1.77**	**-0.24**	**0.00**																
R3-Jug	**0.25**	**0.80**	**107.02**	**117.98**	**0.66**	**16.28**	**18.05**																
R3-1	0.50	1.20	100.06	111.02	0.64	15.64	18.65	0.93	0.93	0.94	0.93	0.96	1.03	0.96	0.96	1.07	1.31	0.96	1.03	0.00	0.10	0.04	0.11
R3-2	0.75	1.60	104.18	107.43	0.74	14.57	19.31	0.97	0.97	0.91	0.89	1.11	0.94	0.90	0.90	1.07	1.29	0.90	1.07	-0.08	-0.03	-0.08	0.10
R3-3	1.00	2.00	100.73	107.55	0.74	14.49	17.88	0.94	0.94	0.91	0.89	1.11	0.94	0.89	0.89	1.06	1.29	0.89	0.99	-0.04	0.01	-0.05	0.06
R3-4	1.25	2.40	106.40	115.96	0.66	14.62	17.06	0.99	0.99	0.98	0.98	1.00	1.00	0.90	0.90	1.07	1.29	0.90	0.95	-0.01	0.01	-0.10	-0.05
R3-5	1.50	2.80	107.10	112.63	0.69	13.90	20.49	1.00	1.00	0.95	0.95	1.04	0.98	0.85	0.86	1.07	1.31	0.85	1.14	-0.06	-0.02	-0.16	0.13
R3-6	1.75	3.20	100.33	108.23	0.72	13.65	17.87	0.94	0.93	0.92	0.90	1.09	0.95	0.84	0.84	1.06	1.27	0.84	0.99	-0.03	0.02	-0.10	0.06
R3-7	2.00	3.60	104.93	112.52	0.68	11.63	21.31	0.98	0.98	0.95	0.94	1.02	0.99	0.71	0.72	1.05	1.24	0.71	1.18	-0.03	0.01	-0.31	0.19
R3-8	2.25	4.00	101.57	109.66	0.74	12.26	18.43	0.95	0.94	0.93	0.92	1.11	0.94	0.75	0.76	1.06	1.26	0.75	1.02	-0.03	0.00	-0.22	0.08
R3-9	2.50	4.40	106.99	109.98	0.69	12.14	16.07	1.00	1.00	0.93	0.92	1.04	0.98	0.75	0.75	1.06	1.25	0.75	0.89	-0.08	-0.02	-0.29	-0.12
R3-10	2.75	4.80	109.37	115.87	0.82	11.59	20.60	1.02	1.02	0.98	0.98	1.24	0.86	0.71	0.72	1.05	1.24	0.71	1.14	-0.05	-0.17	-0.36	0.11
R3-11	3.00	5.20	104.06	118.73	0.82	11.17	18.62	0.97	0.97	1.01	1.01	1.24	0.86	0.69	0.69	1.07	1.31	0.69	1.03	0.04	-0.12	-0.34	0.06
R3-12	3.25	5.60	107.36	116.99	0.81	11.30	24.71	1.00	1.00	0.99	0.99	1.21	0.88	0.69	0.70	1.06	1.26	0.69	1.37	-0.01	-0.14	-0.36	0.31
R3-13	3.50	6.00	109.95	115.59	0.88	11.17	18.29	1.03	1.03	0.98	0.98	1.32	0.81	0.69	0.69	1.03	1.15	0.69	1.01	-0.05	-0.24	-0.40	-0.02
R3-14	3.75	6.40	104.14	118.81	0.91	10.54	16.97	0.97	0.97	1.01	1.01	1.36	0.79	0.65	0.65	1.05	1.23	0.65	0.94	0.04	-0.21	-0.40	-0.03
R3-15	4.00	6.80	106.83	116.86	1.02	10.83	18.09	1.00	1.00	0.99	0.99	1.53	0.68	0.67	0.67	1.07	1.31	0.67	1.00	-0.01	-0.38	-0.40	0.00
R3-16	4.25	7.20	102.77	115.12	1.12	9.57	20.82	0.96	0.96	0.98	0.97	1.68	0.59	0.59	0.59	1.09	1.39	0.59	1.15	0.02	-0.48	-0.48	0.19
R3-17	4.50	7.60	105.49	114.40	1.05	9.70	19.85	0.99	0.98	0.97	0.96	1.58	0.66	0.60	0.60	1.06	1.26	0.60	1.10	-0.02	-0.40	-0.49	0.11
R3-18	4.75	8.00	102.70	115.08	1.13	8.31	17.77	0.96	0.96	0.98	0.97	1.70	0.58	0.51	0.52	1.06	1.26	0.51	0.98	0.02	-0.50	-0.61	0.03
R3-19	5.00	8.40	99.12	106.16	1.19	8.61	18.93	0.93	0.92	0.90	0.88	1.79	0.53	0.53	0.54	1.06	1.24	0.53	1.05	-0.04	-0.55	-0.54	0.13
R3-20	5.25	8.80	86.66	101.97	1.22	7.39	15.82	0.81	0.79	0.86	0.84	1.83	0.50	0.45	0.46	1.01	1.04	0.45	0.88	0.06	-0.45	-0.54	0.10
R3-21	5.50	9.20	77.06	90.91	1.20	6.88	9.54	0.72	0.69	0.77	0.73	1.81	0.52	0.42	0.43	1.02	1.11	0.42	0.53	0.05	-0.29	-0.47	-0.27
R3-22	5.75	9.60	65.46	75.82	1.25	6.42	11.20	0.61	0.57	0.64	0.57	1.88	0.48	0.39	0.40	0.99	0.97	0.39	0.62	0.00	-0.18	-0.35	0.08
R3-23	6.00	10.00	52.95	68.81	1.44	6.17	9.35	0.49	0.44	0.58	0.50	2.17	0.30	0.38	0.39	0.96	0.84	0.38	0.52	0.13	-0.40	-0.13	0.16
R3-24	6.25	10.40	39.63	53.61	1.39	5.35	5.72	0.37	0.31	0.45	0.35	2.09	0.35	0.33	0.34	0.94	0.72	0.33	0.32	0.13	0.13	0.10	0.04
R3-25	6.50	10.80	32.09	43.90	1.57	9.49	2.37	0.30	0.23	0.37	0.25	2.37	0.18	0.58	0.59	0.88	0.48	0.58	0.13	0.10	-0.23	0.95	-0.55
R3-26	6.75	11.20	25.11	33.59	1.40	4.89	0.00	0.23	0.16	0.28	0.15	2.11	0.33	0.30	0.31	0.85	0.33	0.30	0.00	-0.06	0.77	0.69	
R3-27	7.00	11.60	18.14	28.14	1.57	4.26	0.00	0.17	0.08	0.24	0.09	2.37	0.18	0.26	0.27	0.81	0.14	0.26	0.00	0.09	0.77	1.18	
R3-28	7.25	12.00	10.70	20.28	0.92	4.47	0.00	0.10	0.01	0.17	0.01	1.38	0.77	0.27	0.29	0.84	0.30	0.27	0.00	0.57	4.75	3.75	
R3-29			7.63	17.77	1.27	3.94	0.00	0.07	-0.02	0.15	-0.01	1.92	0.45	0.24	0.25	0.79	0.06	0.24	0.00	-0.61			
R3-30			9.07	16.01	1.17	3.40	0.00	0.08	-0.01	0.14	-0.03	1.77	0.54	0.21	0.22			0.21	0.00	1.13			

Push-Pull Test R4 (MPPT2) uncorrected for mixing corrected for mixing

16-Aug-04

																					sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer	NH4+ vs. tracer
	hrs	L																						
Sample	**Elapsed Time**	**Vol Removed**	**Br**	**SO_4^{2-}**	**H_2S**	**Fe^{2+}**	**Cl^-**	**NH_4^+**	**C/Co**	**Br^- Mixing Ratio**	**C/Co**	**SO_4^{2-} Mixing Ratio**	**C/Co**	**H_2S Mixing Ratio**	**C/Co**	**Fe^{2+} Mixing Ratio**	**C/Co**	**NH_4^+ Mixing Ratio**	**C/Co**	**Cl^- Mixing Ratio**	**$\ln(SO_4^{2-}$ mixing ratio/Br^- mixing ratio)**	**$\ln(H_2S$ mixing ratio/ Br^- mixing ratio)**	**$\ln(Fe^{2+}$ mixing ratio/ Br^- mixing ratio)**	**$\ln(NH_4$ mixing ratio/Br^- mixing ratio)**
R4-initial			**5.50**	**5.30**	**0.09**	**3.20**	**488.08**	**1.07**																
R4-Jug			**104.53**	**91.96**	**0.05**	**7.47**	**587.55**	**0.75**																
R4-1	0.00	0.3	104.03	92.34	0.03	10.54	590.58	1.65	1.00	0.99	1.00	1.00	0.63	1.78	1.41	2.55	2.22	-1.75	1.01	1.03	0.01	0.58	0.94	
R4-2	0.33	1.5	103.39	92.02	0.04	11.38	594.24	0.22	0.99	0.99	1.00	1.00	0.69	1.66	1.52	1.64	0.29	2.60	1.01	1.07	0.01	0.52	0.51	0.97
R4-3	0.67	2.7	102.69	89.75	0.03	11.41	588.53	4.45	0.98	0.98	0.98	0.97	0.59	1.84	1.53	1.45	5.98	-10.24	1.00	1.01	-0.01	0.63	0.39	
R4-4	1.00	3.9	101.68	89.65	0.03	14.09	588.91		0.97	0.97	0.97	0.97	0.63	1.78	1.89	1.84	0.00	3.25	1.00	1.01	0.00	0.61	0.64	1.21
R4-5	1.33	5.1	100.82	89.25	0.04	10.21	585.18	1.11	0.96	0.96	0.97	0.97	0.76	1.54	1.37	1.74	1.49	-0.11	1.00	0.98	0.01	0.47	0.59	
R4-6	1.66	6.3	98.63	88.02	0.03	9.37	583.02	3.11	0.94	0.94	0.96	0.95	0.66	1.72	1.26	1.17	4.18	-6.18	0.99	0.95	0.02	0.61	0.22	
R4-7	2.00	7.5	98.19	88.21	0.04	11.08	586.77	8.74	0.94	0.94	0.96	0.96	0.76	1.54	1.48	1.18	11.74	-23.24	1.00	0.99	0.02	0.50	0.23	
R4-8	2.33	8.7	89.47	78.97	0.05	10.64	567.17	8.53	0.86	0.85	0.86	0.85	0.94	1.25	1.43	1.27	11.45	-22.59	0.97	0.80	0.00	0.38	0.41	
R4-9	2.67	9.9	70.88	58.08	0.06	8.20	595.62	7.02	0.68	0.66	0.63	0.61	1.24	0.71	1.10	1.50	9.42	-18.02	1.01	1.08	-0.08	0.07	0.82	
R4-10	2.78	10.3	44.68	35.92	0.06	8.24	519.17	2.37	0.43	0.40	0.39	0.35	1.18	0.83	1.10	1.28	3.18	-3.93	0.88	0.31	-0.11	0.74	1.17	
R4-11	2.87	10.6	43.60	33.21	0.06	8.64	517.31	2.82	0.42	0.38	0.36	0.32	1.21	0.77	1.16	1.18	3.79	-5.30	0.88	0.29	-0.18	0.69	1.12	
R4-12	2.95	10.9	35.70	27.26	0.07	9.61	532.02	3.51	0.34	0.30	0.30	0.25	1.31	0.59	1.29		4.71	-7.39	0.91	0.44	-0.19	0.66		
R4-13	3.02	11.2	31.00	23.71	0.06	8.67	508.30	0.10	0.30	0.26	0.26	0.21	1.24	0.71	1.16	1.13	0.14	2.95	0.87	0.20	-0.19	1.01	1.48	2.44
R4-14	3.07	11.4	25.98	20.30	0.66	8.24	504.46	4.64	0.25	0.21	0.22	0.17	12.63		1.10	0.99	6.22	-10.79	0.86	0.16	-0.18		1.57	
R4-15	3.12	11.5	22.57	17.10	0.07		508.32	0.41	0.22	0.17	0.19	0.14	1.28	0.65	0.00		0.55	2.01	0.87	0.20	-0.24	1.33		2.46
R4-16	3.17	11.8	20.72	14.08	0.06	8.04	497.43	2.03	0.20	0.15	0.15	0.10	1.21	0.77	1.08	1.19	2.72	-2.90	0.85	0.09	-0.42	1.61	2.05	
R4-17	3.23	12.0	21.07	12.70	0.07	7.43	518.71	3.27	0.20	0.16	0.14	0.09	1.35	0.53	1.00	0.97	4.39	-6.65	0.88	0.31	-0.61	1.22	1.82	
R4-18	3.30	12.3	16.09	10.74	0.08	6.90	524.23	3.89	0.15	0.11	0.12	0.06	1.49	0.29	0.92	-0.75	5.22	-8.53	0.89	0.36	-0.53	1.00		
R4-19	3.37	12.5	14.57	8.69	0.08	8.30	524.92		0.14	0.09	0.09	0.04	1.49	0.29	1.11	-0.75	0.00	3.25	0.89	0.37	-0.85	1.16		3.57
R4-20	3.57	13.2	11.50	5.24	0.09	7.33	492.40	6.21	0.11	0.06	0.06	0.00	1.66	-0.01	0.98	-0.75	8.34	-15.56	0.84	0.04				

Push-Pull Test R5 uncorrected for mixing corrected for mixing
17-Aug-04

																					sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer	ammonium vs. tracer
	hrs	L																						
Sample	**Elapsed Time**	**Vol Removed**	**Br**	**SO_4^{2-}**	**H_2S**	**Fe^{2+}**	**Cl^-**	**NH_4^+**	**C/Co**	**Br^- Mixing Ratio**	**C/Co**	**SO_4^{2-} Mixing Ratio**	**C/Co**	**H_2S Mixing Ratio**	**C/Co**	**Fe^{2+} Mixing Ratio**	**C/Co**	**NH_4^+ Mixing Ratio**	**C/Co**	**Cl^- Mixing Ratio**	**$\ln(SO_4^{2-}$ mixing ratio/Br^- mixing ratio)**	**$\ln(H_2S$ mixing ratio/ Br^- mixing ratio)**	**$\ln(Fe^{2+}$ mixing ratio/ Br^- mixing ratio)**	**$\ln(NH_4^+$ mixing ratio/Br^- mixing ratio)**
R5-initial			**7.11**	**0.17**	**0.19**	**4.74**	**670.42**	**0.81**																
R5-Jug			**110.25**	**85.63**	**0.06**	**11.79**	**563.84**	**1.13**																
R5-1	0.00	0.3	110.90	91.50	0.04	12.84	680.59	3.62	1.01	1.01	1.07	1.07	0.20	1.14	1.09	1.15	3.19	8.77	1.21	1.10	0.06	0.12	0.13	2.16
R5-2	0.33	1.5	109.97	92.60	0.04	13.13	682.00	4.23	1.00	1.00	1.08	1.08	0.20	1.14	1.11	1.19	3.73	10.68	1.21	1.11	0.08	0.13	0.18	2.37
R5-3	0.67	2.7	110.21	89.04	0.03	11.17	670.35	1.21	1.00	1.00	1.04	1.04	0.17	1.18	0.95	0.91	1.07	1.24	1.19	1.00	0.04	0.17	-0.09	0.21
R5-4	1.00	3.9	109.78	91.80	0.04	11.37	683.31	2.57	1.00	1.00	1.07	1.07	0.20	1.14	0.96	0.94	2.27	5.51	1.21	1.12	0.07	0.13	-0.06	1.71
R5-5	1.33	5.1	109.47	91.21	0.04	10.78	674.04	2.12	0.99	0.99	1.07	1.07	0.20	1.14	0.91	0.86	1.87	4.10	1.20	1.03	0.07	0.14	-0.15	1.42
R5-6	1.67	6.3	107.51	88.27	0.05	10.46	674.48	2.03	0.98	0.97	1.03	1.03	0.27	1.04	0.89	0.81	1.79	3.79	1.20	1.04	0.06	0.07	-0.18	1.36
R5-7	2.00	7.5	102.15	83.78	0.05	10.04	668.82	6.69	0.93	0.92	0.98	0.98	0.27	1.04	0.85	0.75	5.90	18.37	1.19	0.98	0.06	0.12	-0.20	2.99
R5-8	2.33	8.7	98.60	80.10	0.06	8.69	664.35	4.14	0.89	0.89	0.94	0.94	0.34	0.95	0.74	0.56	3.65	10.40	1.18	0.94	0.05	0.07	-0.46	2.46
R5-9	2.67	9.9	73.92	58.92	0.07	8.21	641.40	0.35	0.67	0.65	0.69	0.69	0.39	0.88	0.70	0.49	0.31	-1.43	1.14	0.73	0.06	0.30	-0.27	
R5-10	2.78	10.3	53.47	40.40	0.08	9.19	613.23	0.20	0.49	0.45	0.47	0.47	0.43	0.81	0.78	0.63	0.18	-1.89	1.09	0.46	0.05	0.59	0.34	
R5-11	2.83	10.6	44.75	31.78	0.10	9.93	599.63	9.62	0.41	0.36	0.37	0.37	0.52	0.69	0.84	0.74	8.49	27.51	1.06	0.34	0.01	0.63	0.70	4.32
R5-12	2.88	10.7	34.96	24.92	0.10	9.78	587.70	0.15	0.32	0.27	0.29	0.29	0.52	0.69	0.83	0.72	0.13	-2.07	1.04	0.22	0.07	0.93	0.97	
R5-13	3.26	10.9	27.67	18.31	0.04	6.26	560.15		0.25	0.20	0.21	0.21	0.23	1.10	0.53	0.22	0.00	-2.54	0.99	-0.03	0.06	1.71	0.08	
R5-14	3.33	11.2	26.00	15.02	0.05	7.56	582.32	2.81	0.24	0.18	0.18	0.17	0.28	1.03	0.64	0.40	2.48	6.25	1.03	0.17	-0.05	1.73	0.78	3.53
R5-15	3.38	11.4	23.45	15.85	0.07	7.30	569.56	10.92	0.21	0.16	0.19	0.18	0.40	0.86	0.62	0.36	9.64	31.58	1.01	0.05	0.15	1.70	0.83	5.29
R5-16	3.75	11.5	23.72	11.10	0.04	7.21	570.79	6.84	0.22	0.16	0.13	0.13	0.21	1.13	0.61	0.35	6.04	18.84	1.01	0.07	-0.23	1.94	0.78	4.76
R5-17	3.80	11.6	21.39	11.03	0.06	8.92	581.71	11.68	0.19	0.14	0.13	0.13	0.30	1.00	0.76	0.59	10.31	33.98	1.03	0.17	-0.09	1.98	1.46	5.50
R5-18	3.85	11.8	16.63	11.23	0.08	8.04	571.94	11.62	0.15	0.09	0.13	0.13	0.41	0.85	0.68	0.47	10.26	33.77	1.01	0.08	0.34	2.22	1.62	5.90
R5-19	4.23	11.9	14.20	8.05	0.05	8.24	580.49	6.12	0.13	0.07	0.09	0.09	0.29	1.02	0.70	0.50	5.40	16.59	1.03	0.16	0.29	2.69	1.98	5.49
R5-20	4.28	12.0	13.42	5.62	0.10	8.24	571.96	4.65	0.12	0.06	0.07	0.06	0.51	0.70	0.70	0.50	4.10	11.99	1.01	0.08	0.04	2.44	2.10	5.28
R5-21	4.33	12.2	13.17	4.83	0.10	7.50	568.72	0.09	0.12	0.06	0.06	0.05	0.56	0.63	0.64		0.08	-2.25	1.01	0.05	-0.07	2.37		

Push-Pull Test R6 (MPPT3) uncorrected for mixing corrected for mixing

17-Aug-04

																					sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer	NH4+vs. tracer
	hrs	L																						
Sample	**Elapsed Time**	**Vol Removed**	**Br**	**SO_4^{2-}**	**H_2S**	**Fe^{2+}**	**Cl^-**	**NH_4^+**	**C/Co**	**Br^- Mixing Ratio**	**C/Co**	**SO_4^{2-} Mixing Ratio**	**C/Co**	**H_2S Mixing Ratio**	**C/Co**	**Fe^{2+} Mixing Ratio**	**C/Co**	**NH_4^+ Mixing Ratio**	**C/Co**	**Cl^- Mixing Ratio**	**ln(SO_4^{2-} mixing ratio/Br^- mixing ratio)**	**ln(H_2S mixing ratio/ Br^- mixing ratio)**	**ln(Fe^{2+} mixing ratio/ Br^- mixing ratio)**	**ln(NH_4^+ mixing ratio/Br^- mixing ratio)**
R6 Initial PP Well 102- C			**8.77**	**12.25**	**0.07**	**5.82**	**500.46**	**1.19**																
R6 Jug			**99.23**	**93.88**	**0.04**	**13.72**	**600.50**	**1.74**																
R6-1	0.00	0.33	97.87	92.88	0.03	6.91	595.57	0.80	0.99	0.98	0.99	0.99	0.44	1.38	0.50	0.14	0.46	-0.70	0.99	0.95	0.00	0.34	-1.96	
R6-2	0.08	0.64	96.97	93.74	0.03	9.22	594.90		0.98	0.98	1.00	1.00	0.44	1.38	0.67	0.43	0.00	-2.17	0.99	0.94	0.02	0.35	-0.82	
R6-3	0.17	0.93	99.40	90.20	0.03	9.31	591.40	9.20	1.00	1.00	0.96	0.95	0.37	1.56	0.68	0.44	5.29	14.56	0.98	0.91	-0.05	0.44	-0.82	2.68
R6-4	0.25	1.23	98.69	92.43	0.03	8.95	596.10		0.99	0.99	0.98	0.98	0.44	1.38	0.65	0.40	0.00	-2.17	0.99	0.96	-0.01	0.33	-0.92	
R6-5	0.33	1.53	97.16	90.31	0.03	7.59	597.52	0.26	0.98	0.98	0.96	0.96	0.44	1.38	0.55	0.22	0.15	-1.69	1.00	0.97	-0.02	0.35	-1.47	
R6-6	0.42	1.83	95.11	87.21	0.03	5.36	592.35	4.62	0.96	0.95	0.93	0.92	0.44	1.38	0.39	-0.06	2.66	6.24	0.99	0.92	-0.04	0.37		1.88
R6-7	0.50	2.13	92.16	83.54	0.04	7.92	588.60	4.86	0.93	0.92	0.89	0.87	0.58	1.02	0.58	0.27	2.79	6.67	0.98	0.88	-0.05	0.11	-1.24	1.98
R6-8	0.58	2.43	81.56	74.17	0.03	7.33	576.34	5.52	0.82	0.80	0.79	0.76	0.46	1.32	0.53	0.19	3.18	7.87	0.96	0.76	-0.06	0.50	-1.44	2.28
R6-9	0.67	2.73	58.58	48.72	0.03	8.54	553.95	7.23	0.59	0.55	0.52	0.45	0.46	1.32	0.62	0.34	4.16	10.98	0.92	0.53	-0.21	0.88	-0.47	2.99
R6-10	0.75	3.03	36.65	28.81	0.05	7.77	530.62	17.20	0.37	0.31	0.31	0.20	0.61	0.96	0.57	0.25	9.90	29.11	0.88	0.30	-0.42	1.14	-0.22	4.55
R6-11	0.83	3.33	19.41	12.96	0.04	7.86	505.85	7.62	0.20	0.12	0.14	0.01	0.58	1.02	0.57	0.26	4.38	11.68	0.84	0.05	-2.61	2.16	0.79	4.60
R6-12	0.92	3.63	13.73	6.83	0.06	6.79	510.41	2.96	0.14	0.05	0.07	-0.07	0.83	0.43	0.50	0.12	1.70	3.22	0.85	0.10		2.05	0.81	4.07
R6-13	1.00	3.93	10.32	3.57	0.06	7.30	503.42	22.07	0.10	0.02	0.04	-0.11	0.87	0.31	0.53	0.19	12.70	37.96	0.84	0.03		2.89	2.39	7.70
R6-14	1.08	4.23	8.34	2.59	0.05	6.44	498.38	29.05	0.08	0.00	0.03	-0.12	0.71	0.73	0.47	0.08	16.71	50.65	0.83	-0.02	3.22			
R6-15	1.17	4.53	8.99	1.60	0.07	6.41	505.23	14.11	0.09	0.00	0.02	-0.13	0.92	0.19	0.47	0.07	8.12	23.49	0.84	0.05		4.35	3.41	9.16

Push-Pull Test R7 (MPPT4) uncorrected for mixing corrected for mixing

17-Aug-04

	hrs	L																			sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer	NH4+vs. tracer
Sample	**Elapsed Time**	**Vol Removed**	**Br**	**SO_4^{2-}**	**H_2S**	**Fe^{2+}**	**Cl^-**	**NH_4^+**	**C/Co**	**Br^- Mixing Ratio**	**C/Co**	**SO_4^{2-} Mixing Ratio**	**C/Co**	**H_2S Mixing Ratio**	**C/Co**	**Fe^{2+} Mixing Ratio**	**C/Co**	**NH_4^+ Mixing Ratio**	**C/Co**	**Cl^- Mixing Ratio**	**$\ln(SO_4^{2-}$ mixing ratio/Br^- mixing ratio)**	**$\ln(H_2S$ mixing ratio/ Br^- mixing ratio)**	**$\ln(Fe^{2+}$ mixing ratio/ Br^- mixing ratio)**	**$\ln(NH_4^+$ mixing ratio/Br^- mixing ratio)**
R7-initial			**7.80**	**2.30**	**0.13**	**7.70**	**508.99**	**4.98**																
R7-Jug			**111.74**	**94.20**	**0.05**	**13.57**	**606.39**	**bdl**																
R7-1	0.00	0.33	111.18	91.11	0.03	13.42	594.52	3.12	1.00	0.99	0.97	0.97	0.22	-0.26	0.99	2.15	0.05	0.37	0.98	0.88	-0.03		0.77	-0.98
R7-2	0.33	1.53	105.56	86.64	0.03	11.93	588.80	2.23	0.94	0.94	0.92	0.92	0.24	-0.22	0.88	1.90	0.04	0.55	0.97	0.82	-0.02		0.70	-0.53
R7-3	0.67	2.73	109.25	85.64	0.05	11.17	603.54	2.82	0.98	0.98	0.91	0.91	0.38	0.01	0.82	1.77	0.05	0.43	1.00	0.97	-0.07	-5.22	0.59	-0.81
R7-4	1.00	3.93	108.19	92.90	0.04	12.14	602.77	2.83	0.97	0.97	0.99	0.99	0.31	-0.11	0.89	1.93	0.05	0.43	0.99	0.96	0.02		0.69	-0.81
R7-5	1.33	5.13	108.44	91.25	0.04	11.74	603.23	1.31	0.97	0.97	0.97	0.97	0.31	-0.11	0.87	1.87	0.02	0.74	0.99	0.97	0.00		0.66	-0.27
R7-6	1.67	6.33	106.38	92.24	0.06	10.42	606.39	3.27	0.95	0.95	0.98	0.98	0.43	0.09	0.77	1.64	0.05	0.34	1.00	1.00	0.03	-2.30	0.55	-1.02
R7-7	2.00	7.53	105.43	87.64	0.07	9.03	599.38	2.42	0.94	0.94	0.93	0.93	0.50	0.21	0.66	1.40	0.04	0.51	0.99	0.93	-0.01	-1.52	0.40	-0.60
R7-8	2.33	8.73	101.10	85.82	0.07	9.24	594.13	4.63	0.90	0.90	0.91	0.91	0.54	0.27	0.68	1.44	0.08	0.07	0.98	0.87	0.01	-1.19	0.47	-2.57
R7-9	2.67	9.93	78.39	64.17	0.10	8.36	570.41	6.16	0.70	0.68	0.68	0.67	0.73	0.59	0.62	1.29	0.10	-0.24	0.94	0.63	-0.01	-0.15	0.64	
R7-10	2.77	10.29	64.13	53.66	0.09	8.21	551.06	4.96	0.57	0.54	0.57	0.56	0.65	0.45	0.60	1.27	0.08	0.00	0.91	0.43	0.03	-0.18	0.85	-4.85
R7-11	2.82	10.47	59.57	47.47	0.08	8.78	572.82	2.91	0.53	0.50	0.50	0.49	0.62	0.41	0.65	1.36	0.05	0.42	0.94	0.66	-0.01	-0.20	1.01	-0.18
R7-12	2.87	10.65	50.44	39.75	0.09	8.03	541.85	2.87	0.45	0.41	0.42	0.41	0.70	0.54	0.59	1.23	0.05	0.42	0.89	0.34	-0.01	0.28	1.10	0.03
R7-13	2.92	10.83	43.65	34.18	0.10	7.82	543.76	5.10	0.39	0.34	0.36	0.35	0.74	0.61	0.58	1.20	0.08	-0.03	0.90	0.36	0.01	0.57	1.25	
R7-14	2.98	11.07	35.10	25.47	0.11	7.76	546.83	5.55	0.31	0.26	0.27	0.25	0.80	0.70	0.57	1.19	0.09	-0.12	0.90	0.39	-0.04	0.98	1.51	
R7-15	3.03	11.25	29.97	19.88	0.10	8.12	537.56	1.30	0.27	0.21	0.21	0.19	0.72	0.56	0.60	1.25	0.02	0.74	0.89	0.29	-0.11	0.97	1.77	1.24
R7-16	3.08	11.43	24.45	16.38	0.10	8.06	532.23	6.06	0.22	0.16	0.17	0.15	0.72	0.56	0.59	1.24	0.10	-0.22	0.88	0.24	-0.04	1.26	2.05	
R7-17	3.13	11.61	18.76	8.83	0.10	8.12	510.34	4.32	0.17	0.11	0.09	0.07	0.76	0.63	0.60	1.25	0.07	0.13	0.84	0.01	-0.39	1.79	2.47	0.22
R7-18	3.18	11.79	17.27	9.94	0.09	7.91	513.41	6.22	0.15	0.09	0.11	0.08	0.66	0.47	0.58	1.21	0.10	-0.25	0.85	0.05	-0.09	1.65	2.59	
R7-19	3.23	11.97	17.90	7.60	0.10	9.66	521.17	3.42	0.16	0.10	0.08	0.06	0.74	0.61	0.71	1.51	0.06	0.31	0.86	0.13	-0.52	1.83	2.74	1.17
R7-20	3.28	12.15	32.05	6.03	0.09	6.73	520.68	6.92	0.29	0.23	0.06	0.04	0.69	0.52	0.50	1.01	0.11	-0.39	0.86	0.12	-1.75	0.80	1.47	

Surface Reaction Vessel Data

SRV 23-Jun-05						
	hrs	concentration (ppm)				
Sample	Elapsed Time	Bromide	Sulfate	Sulfide	Iron	Chloride
SRV (SI 102-3) init		**bdl**	**80.982**	**0.04**	**10.12**	**615.321**
SRV (pp102) init		**7.440**	**3.899**	**0.09**	**3.13**	**465.050**
SRV-A JUG		bdl	46.18	0.06	**6.10**	655.36
SRV A-1	0.000	bdl	50.28	0.05	7.31	621.41
SRV A-2	0.333	bdl	29.38	0.04	6.44	397.48
SRV A-3	0.666	bdl	49.82	0.04	6.06	630.66
SRV A-4	1.000	bdl	50.36	0.04	7.20	632.90
SRV A-5	1.333	bdl	46.77	0.04	7.11	641.99
SRV A-6	1.667	bdl	55.85	0.04	7.17	797.28
SRV A-7	2.000	bdl	55.09	0.03	6.66	784.12
SRV A-8	2.333	bdl	29.80	0.03	7.06	403.42
SRV A-9	2.667	bdl	35.68	0.03	6.59	507.58
SRV A-10	3.000	bdl	31.09	0.03	6.16	407.10
SRV-B JUG		bdl	57.87	0.03	7.46	647.93
SRV B-1	0.000	bdl	38.20	0.02	3.48	495.97
SRV B-2	0.333	bdl	45.43	0.02	2.48	586.84
SRV B-3	0.666	bdl	9.62	0.01	1.67	147.97
SRV B-4	1.000	bdl	26.38	0.02	0.92	355.15
SRV B-5	1.333	bdl	31.39	0.01	0.91	410.55
SRV B-6	1.667	bdl	27.36	0.01	0.72	356.51
SRV B-7	2.000	bdl	14.68	0.01	-0.49	198.47
SRV B-8	2.333	bdl	33.00	0.02	-0.37	462.60
SRV B-9	2.667	bdl	41.48	0.02	-0.24	526.42
SRV B-10	3.000	bdl	43.39	0.02	-0.57	563.88

Radial Array Mini Push-Pull (RAMPP) Data

RAMPP 1			uncorrected for mixing					corrected for mixing										sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer
	hrs	L	conc (ppm)						Bromide		Sulfate		Sulfide		Iron		Chloride			
Sample	Elapsed Time	Vol Removed	Br	SO_4^{2-}	H_2S	Fe^{2+}	Cl^-	C/Co	Br^- Mixing Ratio	C/Co	SO_4^{2-} Mixing Ratio	C/Co	H_2S Mixing Ratio	C/Co	Fe^{2+} Mixing Ratio	C/Co	Cl^- Mixing Ratio	$\ln(SO_4^{2-}$ mixing ratio/Br^- mixing ratio)	$\ln(H_2S$ mixing ratio/ Br^- mixing ratio)	$\ln(Fe^{2+}$ mixing ratio/ Br^- mixing ratio)
RAMPP 1 INIT			**7.44**	**3.90**	**0.17**	**1.27**	**465.05**													
RAMPP 1 JUG			**113.26**	**80.98**	**0.04**	**13.58**	**615.32**													
RAMPP 1-1	0.00	0.00	103.70	80.31	0.04	8.20	609.47	0.92	0.91	0.99	0.99	1.10	0.94	0.54	0.56	0.99	0.96	0.09	0.04	-0.48
RAMPP 1-2	0.27	0.96	108.58	79.92	0.03	12.41	609.24	0.96	0.96	0.99	0.99	0.85	1.01	0.82	0.91	0.99	0.96	0.03	0.06	-0.05
RAMPP 1-3	0.58	2.10	109.67	80.01	0.03	12.17	616.86	0.97	0.97	0.99	0.99	0.70	1.05	0.81	0.89	1.00	1.01	0.02	0.08	-0.09
RAMPP 1-4	0.93	3.36	98.76	78.92	0.03	11.72	591.27	0.87	0.86	0.97	0.97	0.83	1.02	0.78	0.85	0.96	0.84	0.12	0.16	-0.02
RAMPP 1-5	1.23	4.44	105.18	78.61	0.03	11.51	605.84	0.93	0.92	0.97	0.97	0.88	1.00	0.76	0.83	0.98	0.94	0.05	0.08	-0.10
RAMPP 1-6	1.58	5.70	113.72	76.94	0.03	11.70	602.39	1.00	1.00	0.95	0.95	0.86	1.01	0.78	0.85	0.98	0.91	-0.06	0.00	-0.17
RAMPP 1-7	1.85	6.66	102.10	83.10	0.04	11.57	601.81	0.90	0.89	1.03	1.03	1.10	0.94	0.77	0.84	0.98	0.91	0.14	0.05	-0.07
RAMPP 1-8	2.10	7.56	100.81	77.06	0.05	11.17	588.27	0.89	0.88	0.95	0.95	1.41	0.86	0.74	0.80	0.96	0.82	0.07	-0.02	-0.09
RAMPP 1-9	2.43	8.76	99.63	75.89	0.05	10.77	593.69	0.88	0.87	0.94	0.93	1.48	0.85	0.71	0.77	0.96	0.86	0.07	-0.03	-0.12
RAMPP 1-10	2.60	9.36	100.41	74.60	0.05	10.41	578.90	0.89	0.88	0.92	0.92	1.46	0.85	0.69	0.74	0.94	0.76	0.04	-0.03	-0.17
RAMPP 1-11	2.85	10.26	103.89	71.65	0.06	10.43	574.30	0.92	0.91	0.88	0.88	1.56	0.82	0.69	0.74	0.93	0.73	-0.04	-0.10	-0.20
RAMPP 1-12	3.10	11.16	104.29	68.12	0.05	9.73	576.18	0.92	0.92	0.84	0.83	1.46	0.85	0.65	0.69	0.94	0.74	-0.09	-0.07	-0.29
RAMPP 1-13	3.35	12.06	93.69	71.07	0.06	8.94	586.60	0.83	0.82	0.88	0.87	1.49	0.84	0.59	0.62	0.95	0.81	0.07	0.03	-0.27
RAMPP 1-14	3.68	13.26	93.43	66.33	0.07	9.29	573.73	0.82	0.81	0.82	0.81	1.85	0.75	0.62	0.65	0.93	0.72	0.00	-0.08	-0.22
RAMPP 1-15	3.95	14.22	91.92	62.90	0.07	9.01	575.46	0.81	0.80	0.78	0.77	1.94	0.72	0.60	0.63	0.94	0.73	-0.04	-0.10	-0.24
RAMPP 1-16	4.18	15.06	84.90	60.70	0.07	8.85	551.98	0.75	0.73	0.75	0.74	1.97	0.71	0.59	0.62	0.90	0.58	0.01	-0.03	-0.17
RAMPP 1-17	4.43	15.96	78.11	53.47	0.07	8.41	536.00	0.69	0.67	0.66	0.64	1.88	0.74	0.56	0.58	0.87	0.47	-0.04	0.10	-0.14
RAMPP 1-18	4.68	16.86	69.24	50.70	0.08	8.30	532.40	0.61	0.58	0.63	0.61	2.10	0.68	0.55	0.57	0.87	0.45	0.04	0.15	-0.02
RAMPP 1-19	4.83	17.40	66.48	45.85	0.09	7.99	527.90	0.59	0.56	0.57	0.54	2.43	0.59	0.53	0.55	0.86	0.42	-0.02	0.06	-0.02
RAMPP 1-20	4.97	17.88	60.01	42.02	0.09	7.95	518.58	0.53	0.50	0.52	0.49	2.31	0.62	0.53	0.54	0.84	0.36	0.00	0.23	0.09
RAMPP 1-21	5.10	18.36	49.05	38.70	0.09	8.11	516.75	0.43	0.39	0.48	0.45	2.51	0.57	0.54	0.56	0.84	0.34	0.14	0.38	0.35
RAMPP 1-22	5.18	18.66	52.00	34.67	0.11	7.08	576.30	0.46	0.42	0.43	0.40	2.84	0.48	0.47	0.47	0.94	0.74	-0.05	0.14	0.11
RAMPP 1-23	5.22	18.78	53.14	33.16	0.10	7.41	571.85	0.47	0.43	0.41	0.38	2.76	0.51	0.49	0.50	0.93	0.71	-0.13	0.16	0.14
RAMPP 1-24	5.25	18.90	48.72	31.14	0.11	7.41	562.02	0.43	0.39	0.38	0.35	2.88	0.48	0.49	0.50	0.91	0.65	-0.10	0.20	0.25
RAMPP 1-25	5.28	19.02	48.15	29.22	0.11	6.87	553.51	0.43	0.38	0.36	0.33	2.93	0.46	0.46	0.46	0.90	0.59	-0.16	0.18	0.17
RAMPP 1-26	5.32	19.14	45.26	30.57	0.10	7.50	548.23	0.40	0.36	0.38	0.35	2.59	0.55	0.50	0.51	0.89	0.55	-0.03	0.43	0.35
RAMPP 1-27	5.35	19.26	42.92	27.49	0.10	7.61	523.25	0.38	0.34	0.34	0.31	2.70	0.52	0.51	0.52	0.85	0.39	-0.09	0.44	0.43
RAMPP 1-A5	0.03		43.97	25.52	0.03	1.01	507.40	0.39	0.35	0.32	0.28	0.93	0.99	0.07	-0.02	0.82	0.28	-0.21	1.05	
RAMPP 1-A4	0.07		111.60	75.18	0.02	-0.31	583.53	0.99	0.98	0.93	0.92	0.59	1.08	-0.02	-0.13	0.95	0.79	-0.06	0.09	
RAMPP 1-A3	0.38		91.54	60.46	0.03	6.28	570.09	0.81	0.79	0.75	0.73	0.88	1.00	0.42	0.41	0.93	0.70	-0.08	0.23	-0.67
RAMPP 1-B5	0.63		15.00	6.01	0.06	1.57	472.87	0.13	0.07	0.07	0.03	1.52	0.83	0.10	0.02	0.77	0.05	-0.96	2.46	-1.09
RAMPP 1-B4	0.67		20.89	6.79	0.05	2.17	489.05	0.18	0.13	0.08	0.04	1.28	0.90	0.14	0.07	0.79	0.16	-1.22	1.95	-0.56
RAMPP 1-B3	0.72		7.52	3.65		2.25	496.46	0.07	0.00	0.05	0.00			0.15	0.08	0.81	0.21			4.69
RAMPP 1-C5	1.00		15.99	6.49	0.05	0.99	459.73	0.14	0.08	0.08	0.03	1.46	0.85	0.07	-0.02	0.75	-0.04	-0.88	2.35	
RAMPP 1-C4	1.02		17.38	7.88	0.05	-0.98	463.23	0.15	0.09	0.10	0.05	1.27	0.90	-0.07	-0.18	0.75	-0.01	-0.60	2.26	
RAMPP 1-C3	1.03		30.86	19.93	0.06	0.69	488.42	0.27	0.22	0.25	0.21	1.62	0.81	0.05	-0.05	0.79	0.16	-0.06	1.29	
RAMPP 1-A3-2	3.82		106.17	74.01	0.05	1.50	589.12	0.94	0.93	0.91	0.91	1.23	0.91	0.10	0.02	0.96	0.83	-0.03	-0.03	-3.95
RAMPP 1-A2	3.87		92.78	63.55	0.06	4.62	577.74	0.82	0.81	0.78	0.77	1.49	0.84	0.31	0.27	0.94	0.75	-0.04	0.04	-1.09
RAMPP 1-A1	3.88		91.40	65.66	0.05	7.37	572.09	0.81	0.79	0.81	0.80	1.35	0.88	0.49	0.50	0.93	0.71	0.01	0.10	-0.47
RAMPP 1-B3-2	4.15		76.77	55.08	0.09	2.57	547.76	0.68	0.66	0.68	0.66	2.31	0.62	0.17	0.10	0.89	0.55	0.01	-0.05	-1.83
RAMPP 1-B2	4.17		79.37	42.61	0.07	3.42	548.39	0.70	0.68	0.53	0.50	1.99	0.71	0.23	0.17	0.89	0.55	-0.30	0.04	-1.36
RAMPP 1-B1	4.18		74.90	45.25	0.07	1.41	545.72	0.66	0.64	0.56	0.54	1.86	0.74	0.09	0.01	0.89	0.54	-0.17	0.15	-4.09
RAMPP 1-C3-2	4.50		84.46	55.58	0.06	-0.60	539.63	0.75	0.73	0.69	0.67	1.67	0.79	-0.04	-0.15	0.88	0.50	-0.08	0.09	
RAMPP 1-C2	4.55		20.13	11.12	0.07	1.07	473.89	0.18	0.12	0.14	0.09	1.78	0.76	0.07	-0.02	0.77	0.06	-0.25	1.85	
RAMPP 1-C1	4.82		33.29	19.74	0.07	0.59	494.04	0.29	0.24	0.24	0.21	1.80	0.76	0.04	-0.06	0.80	0.19	-0.17	1.14	

RAMPP 2			uncorrected for mixing						corrected for mixing												sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer	acetate vs. tracer
	hrs	L	conc (ppm)																					
Sample	Elapsed Time	Vol Removed	Br	SO_4^{2-}	H_2S	Fe^{2+}	Cl^-	Acetate	C/Co	Br^- Mixing Ratio	C/Co	SO_4^{2-} Mixing Ratio	C/Co	H_2S Mixing Ratio	C/Co	Fe^{2+} Mixing Ratio	C/Co	Cl^- Mixing Ratio	C/Co	Acetate Mixing Ratio	$\ln(SO_4^{2-}$ mixing ratio/Br^- mixing ratio)	$\ln(H_2S$ mixing ratio/ Br^- mixing ratio)	$\ln(Fe^{2+}$ mixing ratio/ Br^- mixing ratio)	ln(Acetate mixing ratio/Br^- mixing ratio)
RAMPP 2 INIT			**16.50**	**8.90**	**0.19**	**6.02**	**525.69**	**0.00**																
RAMPP 2 JUG			**103.47**	**71.40**	**0.03**	**13.91**	**597.86**	**29.59**																
RAMPP 2-1	0.00	0.00	111.01	70.85	0.01	8.83	598.60	34.52	1.07	1.09	0.99	0.99	0.20	1.18	0.63	0.36	1.00	1.01	1.17	1.17	-0.09	0.08	-1.12	0.07
RAMPP 2-2	0.23	0.84	108.76	69.49	0.01	11.18	603.36	30.85	1.05	1.06	0.97	0.97	0.25	1.17	0.80	0.65	1.01	1.08	1.04	1.04	-0.09	0.10	-0.48	-0.02
RAMPP 2-3	0.50	1.80	105.52	68.77	0.02	13.21	596.27	33.84	1.02	1.02	0.96	0.96	0.48	1.12	0.95	0.91	1.00	0.98	1.14	1.14	-0.07	0.09	-0.12	0.11
RAMPP 2-4	0.78	2.82	103.38	69.14	0.03	13.36	585.33	34.73	1.00	1.00	0.97	0.96	0.94	1.03	0.96	0.93	0.98	0.83	1.17	1.17	-0.04	0.03	-0.07	0.16
RAMPP 2-5	0.98	3.54	103.66	68.32	0.03	13.10	590.90	32.52	1.00	1.00	0.96	0.95	0.92	1.03	0.94	0.90	0.99	0.90	1.10	1.10	-0.05	0.03	-0.11	0.09
RAMPP 2-6	1.23	4.44	101.12	64.87	0.03	13.03	571.76	25.09	0.98	0.97	0.91	0.90	0.83	1.05	0.94	0.89	0.96	0.64	0.85	0.85	-0.08	0.08	-0.09	-0.14
RAMPP 2-7	1.48	5.34	101.94	68.50	0.03	13.54	595.86	33.59	0.99	0.98	0.96	0.95	0.98	1.02	0.97	0.95	1.00	0.97	1.14	1.14	-0.03	0.04	-0.03	0.14
RAMPP 2-8	1.73	6.24	100.84	65.81	0.03	12.66	577.48	30.53	0.97	0.97	0.92	0.91	0.96	1.03	0.91	0.84	0.97	0.72	1.03	1.03	-0.06	0.06	-0.14	0.06
RAMPP 2-9	1.98	7.14	100.48	63.41	0.03	12.27	575.39	27.46	0.97	0.97	0.89	0.87	0.96	1.03	0.88	0.79	0.96	0.69	0.93	0.93	-0.10	0.06	-0.20	-0.04
RAMPP 2-10	2.23	8.04	101.72	66.00	0.04	11.86	563.94	30.78	0.98	0.98	0.92	0.91	1.17	0.99	0.85	0.74	0.94	0.53	1.04	1.04	-0.07	0.01	-0.28	0.06
RAMPP 2-11	2.48	8.94	104.58	66.01	0.04	12.14	557.69	27.26	1.01	1.01	0.92	0.91	1.26	0.97	0.87	0.78	0.93	0.44	0.92	0.92	-0.10	-0.05	-0.27	-0.09
RAMPP 2-12	2.73	9.84	102.85	63.15	0.05	11.86	556.22	26.09	0.99	0.99	0.88	0.87	1.41	0.94	0.85	0.74	0.93	0.42	0.88	0.88	-0.13	-0.06	-0.29	-0.12
RAMPP 2-13	2.98	10.74	102.64	72.31	0.08	11.83	570.70		0.99	0.99	1.01	1.01	2.56	0.71	0.85	0.74	0.95	0.62	4.83	4.83	0.02	-0.34	-0.30	1.59
RAMPP 2-14	3.23	11.64	96.20	62.75	0.08	11.39	550.86	26.30	0.93	0.92	0.88	0.86	2.47	0.72	0.82	0.68	0.92	0.35	0.89	0.89	-0.06	-0.23	-0.30	-0.03
RAMPP 2-15	3.57	12.84	99.48	64.19	0.10	10.59	560.70	22.87	0.96	0.95	0.90	0.88	3.01	0.62	0.76	0.58	0.94	0.49	0.77	0.77	-0.08	-0.44	-0.50	-0.21
RAMPP 2-16	3.85	13.86	97.81	60.42	0.08	9.61	550.19	25.92	0.95	0.93	0.85	0.82	2.64	0.69	0.69	0.45	0.92	0.34	0.88	0.88	-0.13	-0.30	-0.72	-0.07
RAMPP 2-17	4.17	15.00	95.24	57.98	0.09	8.92	545.22	25.71	0.92	0.91	0.81	0.79	2.88	0.64	0.64	0.37	0.91	0.27	0.87	0.87	-0.14	-0.34	-0.90	-0.04
RAMPP 2-18	4.50	16.20	84.96	51.73	0.09	8.86	544.82	39.63	0.82	0.79	0.72	0.69	2.73	0.67	0.64	0.36	0.91	0.26	1.34	1.34	-0.14	-0.16	-0.78	0.53
RAMPP 2-19	4.68	16.86	81.95	48.44	0.10	8.98	536.61	16.96	0.79	0.75	0.68	0.63	2.97	0.62	0.65	0.38	0.90	0.15	0.57	0.57	-0.17	-0.19	-0.70	-0.27
RAMPP 2-20	4.85	17.46	72.04	42.63	0.12	8.69	512.12	15.94	0.70	0.64	0.60	0.54	3.83	0.45	0.63	0.34	0.86	-0.19	0.54	0.54	-0.17	-0.34	-0.63	-0.17
RAMPP 2-21	5.02	18.06	60.17	37.07	0.13	8.06	501.51	14.00	0.58	0.50	0.52	0.45	4.18	0.38	0.58	0.26	0.84	-0.34	0.47	0.47	-0.11	-0.27	-0.66	-0.06
RAMPP 2-22	5.15	18.54	65.53	33.34	0.14	7.35	505.03	13.77	0.63	0.56	0.47	0.39	4.46	0.33	0.53	0.17	0.85	-0.29	0.47	0.47	-0.37	-0.55	-1.21	-0.19
RAMPP 2-23	5.20	18.72	55.69	33.80	0.14	7.80	576.45	29.30	0.54	0.45	0.47	0.40	4.46	0.33	0.56	0.22	0.96	0.70	0.99	0.99	-0.12	-0.32	-0.70	0.79
RAMPP 2-24	5.25	18.90	55.61	32.83	0.14	8.15	602.22	0.00	0.54	0.45	0.46	0.38	4.29	0.36	0.59	0.27	1.01	1.06	0.00	0.00	-0.16	-0.22	-0.51	
RAMPP 2-25	5.30	19.08	46.55	29.34	0.13	8.04	579.58	6.61	0.45	0.35	0.41	0.33	4.18	0.38	0.58	0.26	0.97	0.75	0.22	0.22	-0.05	0.10	-0.30	-0.44
RAMPP 2-26	5.35	19.26	49.44	27.46	0.13	10.69	576.93	13.88	0.48	0.38	0.38	0.30	4.15	0.39	0.77	0.59	0.97	0.71	0.47	0.47	-0.24	0.03	0.45	0.21
RAMPP 2-27			45.19	27.91	0.14	0.64	571.91	6.35	0.44	0.33	0.39	0.30	4.33	0.35	0.05	-0.68	0.96	0.64	0.21	0.21	-0.08	0.07		-0.43
RAMPP 2-A5	0.17		59.29	34.89	0.02	5.58	578.07	67.54	0.57	0.49	0.49	0.42	0.48	1.12	0.40	-0.06	0.97	0.73	2.28	2.28	-0.17	0.83		1.53
RAMPP 2-A4	0.18		109.13	67.58	0.01	12.89	616.55	32.21	1.05	1.07	0.95	0.94	0.38	1.14	0.93	0.87	1.03	1.26	1.09	1.09	-0.13	0.07	-0.20	0.02
RAMPP 2-A3	0.20		111.28	70.67	0.01	5.06	627.67	36.73	1.08	1.09	0.99	0.99	0.46	1.13	0.36	-0.12	1.05	1.41	1.24	1.24	-0.10	0.03		0.13
RAMPP 2-B5	0.55		27.67	14.77	0.11	5.40	542.73	0.00	0.27	0.13	0.21	0.09	3.34	0.55	0.39	-0.08	0.91	0.24	0.00	0.00	-0.31	1.45		
RAMPP 2-B4	0.57		36.76	19.42	0.08	5.30	545.59	0.00	0.36	0.23	0.27	0.17	2.64	0.69	0.38	-0.09	0.91	0.28	0.00	0.00	-0.33	1.09		
RAMPP 2-B3	0.65		89.14	54.16	0.06	4.21	572.75	5.72	0.86	0.84	0.76	0.72	1.78	0.86	0.30	-0.23	0.96	0.65	0.19	0.19	-0.14	0.03		-1.46
RAMPP 2-C5	0.83		21.36	11.72	0.07	3.89	521.52	0.00	0.21	0.06	0.16	0.05	2.08	0.80	0.28	-0.27	0.87	-0.06	0.00	0.00	-0.21	2.67		
RAMPP 2-C4	0.85		28.19	15.61	0.08	4.96	518.52	0.00	0.27	0.13	0.22	0.11	2.58	0.70	0.36	-0.13	0.87	-0.10	0.00	0.00	-0.22	1.65		
RAMPP 2-C3	0.85		93.01	53.70	0.06	4.83	583.66	23.82	0.90	0.88	0.75	0.72	1.87	0.84	0.35	-0.15	0.98	0.80	0.81	0.81	-0.20	-0.04		-0.09
RAMPP 2-A3-2	3.68		96.45	57.93	0.07	4.98	599.56	25.12	0.93	0.92	0.81	0.78	2.23	0.77	0.36	-0.13	1.00	1.02	0.85	0.85	-0.16	-0.17		-0.08
RAMPP 2-A2	3.70		90.29	57.96	0.04	8.80	623.86	31.34	0.87	0.85	0.81	0.79	1.20	0.98	0.63	0.35	1.04	1.36	1.06	1.06	-0.08	0.14	-0.88	0.22
RAMPP 2-A1	3.72		93.32	62.28	0.06	2.77	624.23	54.69	0.90	0.88	0.87	0.85	1.76	0.87	0.20	-0.41	1.04	1.37	1.85	1.85	-0.03	-0.02		0.74
RAMPP 2-B3-2	4.02		22.95	12.13	0.09	5.51	555.13	0.00	0.22	0.07	0.17	0.05	2.92	0.64	0.40	-0.06	0.93	0.41	0.00	0.00	-0.36	2.15		
RAMPP 2-B2	4.02		84.19	49.74	0.09	10.31	595.66	18.95	0.81	0.78	0.70	0.65	2.79	0.66	0.74	0.54	1.00	0.97	0.64	0.64	-0.17	-0.16	-0.36	-0.20
RAMPP 2-B1	4.02		87.52	49.78	0.16	2.03	599.80	0.00	0.85	0.82	0.70	0.65	4.89	0.24	0.15	-0.51	1.00	1.03	0.00	0.00	-0.22	-1.22		
RAMPP 2-C3-2	4.38		28.48	9.77	0.13	2.50	515.11	0.00	0.28	0.14	0.14	0.01	4.16	0.39	0.18	-0.45	0.86	-0.15	0.00	0.00	-2.29	1.03		
RAMPP 2-C2	4.43		24.64	13.97	0.08	1.45	521.48	0.00	0.24	0.09	0.20	0.08	2.60	0.70	0.10	-0.58	0.87	-0.06	0.00	0.00	-0.14	2.01		
RAMPP 2-C1	4.47		60.87	27.64	0.05		568.65	0.00	0.59	0.51		0.30	1.54	0.91			0.95	0.60	0.00	0.00	-0.53	0.58		

RAMPP 3			uncorrected for mixing					corrected for mixing											sulfate vs. tracer	sulfide vs. tracer	iron vs. tracer
	hrs	L	conc (ppm)																		
Sample	Elapsed Time	Vol Removed	Br	SO_4^{2-}	H_2S	Fe^{2+}	Cl^-	C/Co	Br^- Mixing Ratio	C/Co	SO_4^{2-} Mixing Ratio	C/Co	H_2S Mixing Ratio	C/Co	Fe^{2+} Mixing Ratio	C/Co	Cl^- Mixing Ratio		$\ln(SO_4^{2-}$ mixing ratio/Br^- mixing ratio)	$\ln(H_2S$ mixing ratio/ Br^- mixing ratio)	$\ln(Fe^{2+}$ mixing ratio/ Br^- mixing ratio)
RAMPP 3 INIT			**15.43**	**0.00**	**0.13**	**6.12**	**532.74**														
RAMPP 3 JUG			**111.49**	**67.27**	**0.03**	**14.81**	**617.93**														
RAMPP 3-1	0.00	0.00	112.83	67.99	0.03	12.17	622.60	1.01	1.01	1.01	1.01	0.87	1.08	0.82	0.69	1.01	1.05		0.00	0.06	-0.38
RAMPP 3-2	0.27	0.96	113.94	67.23	0.02	12.78	623.26	1.02	1.03	1.00	1.00	0.71	1.13	0.86	0.77	1.01	1.06		-0.03	0.10	-0.29
RAMPP 3-3	0.55	1.98	110.91	65.22	0.02	12.83	609.13	0.99	0.99	0.97	0.97	0.55	1.18	0.87	0.77	0.99	0.90		-0.03	0.17	-0.25
RAMPP 3-4	0.80	2.88	113.68	66.07	0.02	13.07	627.34	1.02	1.02	0.98	0.98	0.63	1.15	0.88	0.80	1.02	1.11		-0.04	0.12	-0.25
RAMPP 3-5	1.05	3.78	110.84	66.91	0.03	13.13	628.32	0.99	0.99	0.99	0.99	0.91	1.07	0.89	0.81	1.02	1.12		0.00	0.07	-0.21
RAMPP 3-6	1.30	4.68			0.04	13.02						1.32	0.94	0.88	0.79						
RAMPP 3-7	1.55	5.58	115.94	68.11	0.05	12.94	633.55	1.04	1.05	1.01	1.01	1.70	0.83	0.87	0.78	1.03	1.18		-0.03	-0.23	-0.29
RAMPP 3-8	1.80	6.48	112.96	67.86	0.04	12.41	623.49	1.01	1.02	1.01	1.01	1.48	0.90	0.84	0.72	1.01	1.07		-0.01	-0.13	-0.34
RAMPP 3-9	2.05	7.38	107.10	64.63	0.05	12.41	615.06	0.96	0.95	0.96	0.96	1.50	0.89	0.84	0.72	1.00	0.97		0.01	-0.07	-0.28
RAMPP 3-10	2.30	8.28	110.76	66.06	0.05	12.19	619.03	0.99	0.99	0.98	0.98	1.78	0.81	0.82	0.70	1.00	1.01		-0.01	-0.21	-0.35
RAMPP 3-11	2.55	9.18	111.55	65.49	0.04	12.17	619.87	1.00	1.00	0.97	0.97	1.46	0.90	0.82	0.69	1.00	1.02		-0.03	-0.10	-0.36
RAMPP 3-12	2.80	10.08	112.75	64.16	0.04	11.98	621.55	1.01	1.01	0.95	0.95	1.48	0.90	0.81	0.67	1.01	1.04		-0.06	-0.12	-0.41
RAMPP 3-13	3.05	10.98	109.72	65.32	0.04	11.60	613.33	0.98	0.98	0.97	0.97	1.46	0.90	0.78	0.63	0.99	0.95		-0.01	-0.09	-0.44
RAMPP 3-14	3.30	11.88	108.97	63.81	0.05	11.50	618.86	0.98	0.97	0.95	0.95	1.80	0.80	0.78	0.62	1.00	1.01		-0.03	-0.20	-0.45
RAMPP 3-15	3.63	13.08	109.76	62.07	0.06	11.11	618.63	0.98	0.98	0.92	0.92	2.08	0.72	0.75	0.57	1.00	1.01		-0.06	-0.32	-0.54
RAMPP 3-16	3.97	14.28	108.49	63.61	0.07	10.46	622.24	0.97	0.97	0.95	0.95	2.20	0.68	0.71	0.50	1.01	1.05		-0.02	-0.35	-0.66
RAMPP 3-17	4.32	15.54	97.36	57.47	0.07	10.50	599.75	0.87	0.85	0.85	0.85	2.34	0.64	0.71	0.50	0.97	0.79		0.00	-0.29	-0.53
RAMPP 3-18	4.62	16.62	94.34	55.27	0.09	9.98	575.96	0.85	0.82	0.82	0.82	2.99	0.44	0.67	0.44	0.93	0.51		0.00	-0.62	-0.62
RAMPP 3-19	4.95	17.82	84.15	48.56	0.08	9.40	582.74	0.75	0.72	0.72	0.72	2.73	0.52	0.63	0.38	0.94	0.59		0.01	-0.32	-0.64
RAMPP 3-20	5.20	18.72	72.04	39.57	0.12	9.35	583.10	0.65	0.59	0.59	0.59	4.12	0.10	0.63	0.37	0.94	0.59		0.00	-1.75	-0.46
RAMPP 3-21	5.37	19.32	59.22	32.96	0.17	8.56	575.85	0.53	0.46	0.49	0.49	5.63	-0.35	0.58	0.28	0.93	0.51		0.07		-0.49
RAMPP 3-22	5.42	19.50	52.96	27.38	0.11	5.78	579.64	0.48	0.39	0.41	0.41	3.65	0.25	0.39	-0.04	0.94	0.55		0.04	-0.46	
RAMPP 3-23	5.47	19.68	43.95	24.58	0.15	8.29	536.90	0.39	0.30	0.37	0.37	5.10	-0.19	0.56	0.25	0.87	0.05		0.21		-0.17
RAMPP 3-24	5.50	19.80	43.06	21.67	0.14	8.15	535.08	0.39	0.29	0.32	0.32	4.80	-0.10	0.55	0.23	0.87	0.03		0.11		-0.21
RAMPP 3-25	5.55	19.98	42.10	20.94	0.15	8.27	525.11	0.38	0.28	0.31	0.31	4.88	-0.12	0.56	0.25	0.85	-0.09		0.11		-0.12
RAMPP 3-26	5.60	20.16	41.77	21.93	0.14	9.22	543.77	0.37	0.27	0.33	0.33	4.58	-0.03	0.62	0.36	0.88	0.13		0.17		0.26
RAMPP 3-27	5.77	20.76	25.19	14.08	0.19	7.53	544.23	0.23	0.10	0.21	0.21	6.47	-0.60	0.51	0.16	0.88	0.13		0.72		0.47
RAMPP 3-A5	0.12		81.94	41.73	0.03	1.94	543.33	0.73	0.69	0.62	0.62	1.08	1.01	0.13	-0.48	0.88	0.12		-0.11	0.38	
RAMPP 3-A4	0.13		104.26	62.76	0.02	5.91	588.00	0.94	0.92	0.93	0.93	0.79	1.10	0.40	-0.02	0.95	0.65		0.01	0.18	
RAMPP 3-A3	0.15		101.35	59.38	0.03	10.13	562.98	0.91	0.89	0.88	0.88	0.93	1.06	0.68	0.46	0.91	0.36		-0.01	0.17	-0.66
RAMPP 3-B5	0.38		33.83	14.61	0.07	2.15	506.27	0.30	0.19	0.22	0.22	2.42	0.62	0.15	-0.46	0.82	-0.31		0.13	1.17	
RAMPP 3-B4	0.40		41.12	22.95	0.07	1.31	522.91	0.37	0.27	0.34	0.34	2.46	0.60	0.09	-0.55	0.85	-0.12		0.24	0.81	
RAMPP 3-B3	0.43		86.73	47.75	0.07	3.48	553.83	0.78	0.74	0.71	0.71	2.44	0.61	0.23	-0.30	0.90	0.25		-0.04	-0.20	
RAMPP 3-C5	0.60		24.83	11.88	0.07	1.82	470.65	0.22	0.10	0.18	0.18	2.36	0.63	0.12	-0.49	0.76	-0.73		0.59	1.87	
RAMPP 3-C4	0.60		35.22	24.71	0.06	0.78	444.95	0.32	0.21	0.37	0.37	1.90	0.77	0.05	-0.61	0.72	-1.03		0.58	1.32	
RAMPP 3-C3	0.62		86.71	52.26	0.06	2.26	551.75	0.78	0.74	0.78	0.78	1.90	0.77	0.15	-0.44	0.89	0.22		0.05	0.04	
RAMPP 3-A3-2	4.08		91.92	54.08	0.07	5.64	571.70	0.82	0.80	0.80	0.80	2.38	0.63	0.38	-0.06	0.93	0.46		0.01	-0.24	
RAMPP 3-A2	4.08		88.83	60.02	0.06	6.93	570.85	0.80	0.76	0.89	0.89	1.90	0.77	0.47	0.09	0.92	0.45		0.16	0.01	-2.10
RAMPP 3-A1	4.10		92.82	52.04	0.05	8.10	566.74	0.83	0.81	0.77	0.77	1.66	0.84	0.55	0.23	0.92	0.40		-0.04	0.04	-1.26
RAMPP 3-B3-2	4.40		25.78	12.65	0.12	1.76	516.32	0.23	0.11	0.19	0.19	4.02	0.13	0.12	-0.50	0.84	-0.19		0.56	0.21	
RAMPP 3-B2	4.37		86.23	46.31	0.08	4.56	562.53	0.77	0.74	0.69	0.69	2.73	0.52	0.31	-0.18	0.91	0.35		-0.07	-0.35	
RAMPP 3-B1	4.53		77.74	41.89	0.19	6.50	544.70	0.70	0.65	0.62	0.62	6.45	-0.59	0.44	0.04	0.88	0.14		-0.04		-2.68
RAMPP 3-C3-2	4.77		19.77	8.96	0.14	2.48	466.21	0.18	0.05	0.13	0.13	4.64	-0.05	0.17	-0.42	0.75	-0.78		1.08		
RAMPP 3-C2	4.78		23.72	11.32	0.09	2.12	467.25	0.21	0.09	0.17	0.17	3.13	0.40	0.14	-0.46	0.76	-0.77		0.67	1.54	
RAMPP 3-C1	4.82		58.16	20.66	0.11	3.21	522.19	0.52	0.44	0.31	0.31	3.53	0.28	0.22	-0.33	0.85	-0.12		-0.37	-0.46	

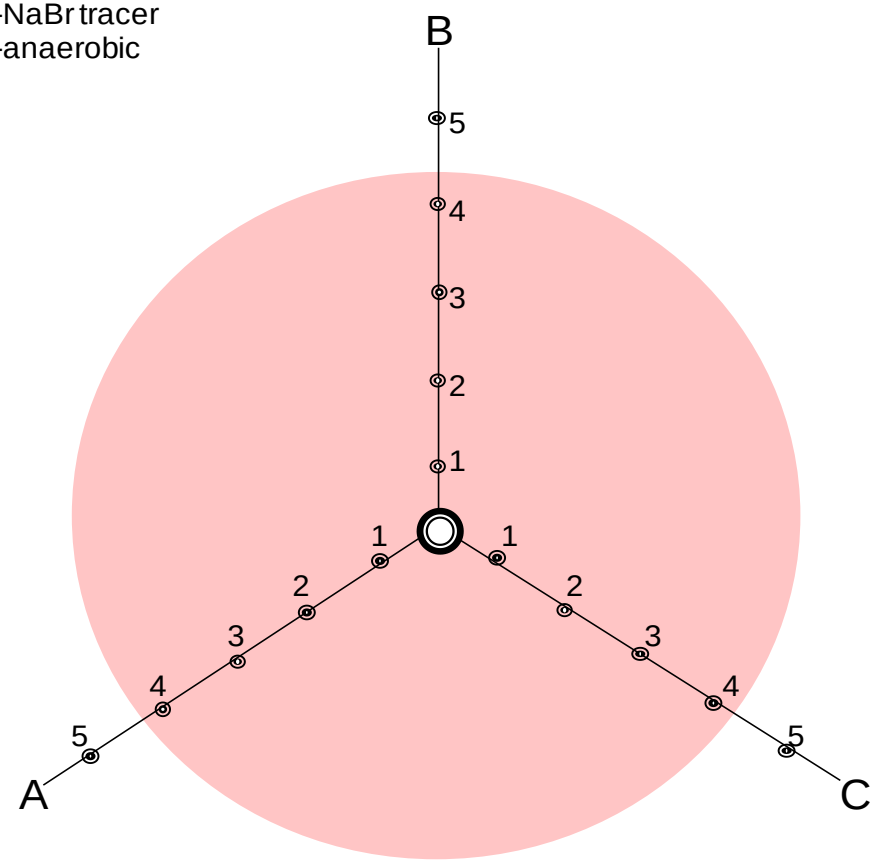
RAMPP 1: Sulfate

Description of Test Water:

-SO_4 conc. ~80.98 ppm

-NaBr tracer

-anaerobic



Rates appear to be faster at interface

	Sample Location	Time (hrs)	Rate (mg/L h-1)
1	RAMPP 1-A5	0.03	49.526
	RAMPP 1-A4	0.07	67.065
	RAMPP 1-A3	0.38	9.601
2	RAMPP 1-B5	0.63	0.418
	RAMPP 1-B4	0.67	0.912
	RAMPP 1-B3	0.72	0.020
3	RAMPP 1-C5	1.00	0.306
	RAMPP 1-C4	1.02	0.328
	RAMPP 1-C3	1.03	0.255
4	RAMPP 1-A3-2	3.77	0.460
	RAMPP 1-A2	3.82	0.541
	RAMPP 1-A1	3.83	-0.135
5	RAMPP 1-B3-2	4.10	-0.118
	RAMPP 1-B2	4.12	1.837
	RAMPP 1-B1	4.13	1.106
6	RAMPP 1-C3-2	4.45	0.716
	RAMPP 1-C2	4.50	0.065
	RAMPP 1-C1	4.77	0.160

= estimated interface location

= fastest rate per sampling event

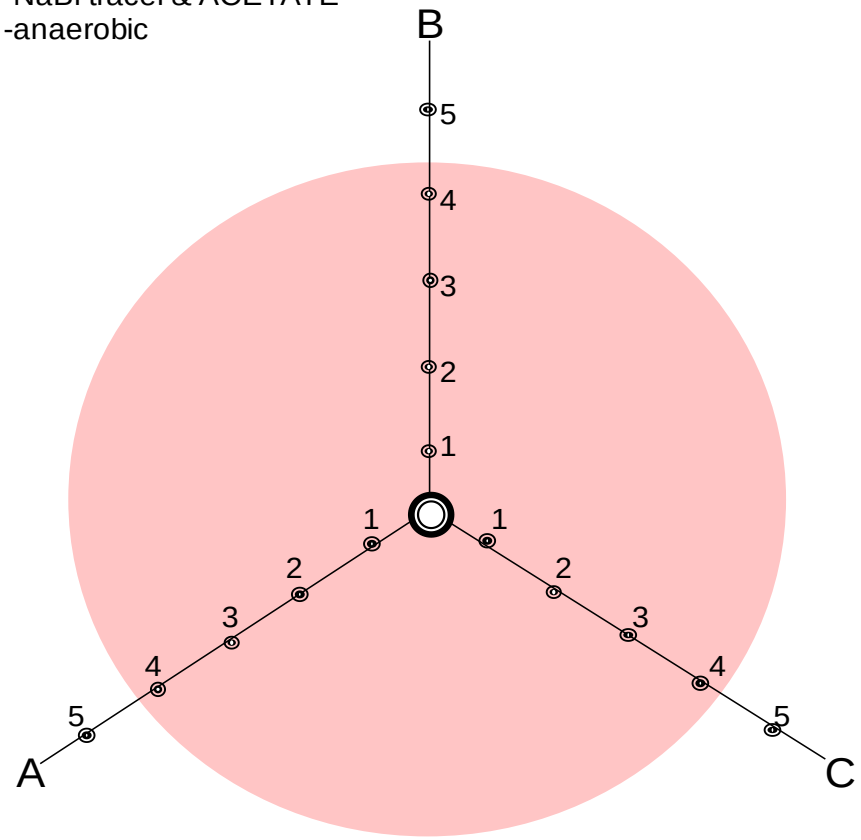
RAMPP 2: Sulfate

Description of Test Water:

-SO_4 conc. ~71.4 ppm

-NaBr tracer & ACETATE

-anaerobic



Again rates appear faster at interface but likely do not have good sense of where the interface is

	Sample Location	Time (hrs)	Rate (mg/L h-1)
1	RAMPP 2-A5	0.17	15.927
	RAMPP 2-A4	0.18	46.490
	RAMPP 2-A3	0.20	35.829
2	RAMPP 2-B5	0.55	0.927
	RAMPP 2-B4	0.57	2.217
	RAMPP 2-B3	0.65	9.256
3	RAMPP 2-C5	0.83	0.151
	RAMPP 2-C4	0.85	0.495
	RAMPP 2-C3	0.85	10.295
4	RAMPP 2-A3-2	3.85	2.027
	RAMPP 2-A2	3.87	0.950
	RAMPP 2-A1	3.88	0.467
5	RAMPP 2-B3-2	4.18	0.065
	RAMPP 2-B2	4.18	1.486
	RAMPP 2-B1	4.18	1.934
6	RAMPP 2-C3-2	4.55	0.266
	RAMPP 2-C2	4.60	0.038
	RAMPP 2-C1	4.63	1.254

= estimated interface location

= fastest rate per sampling event

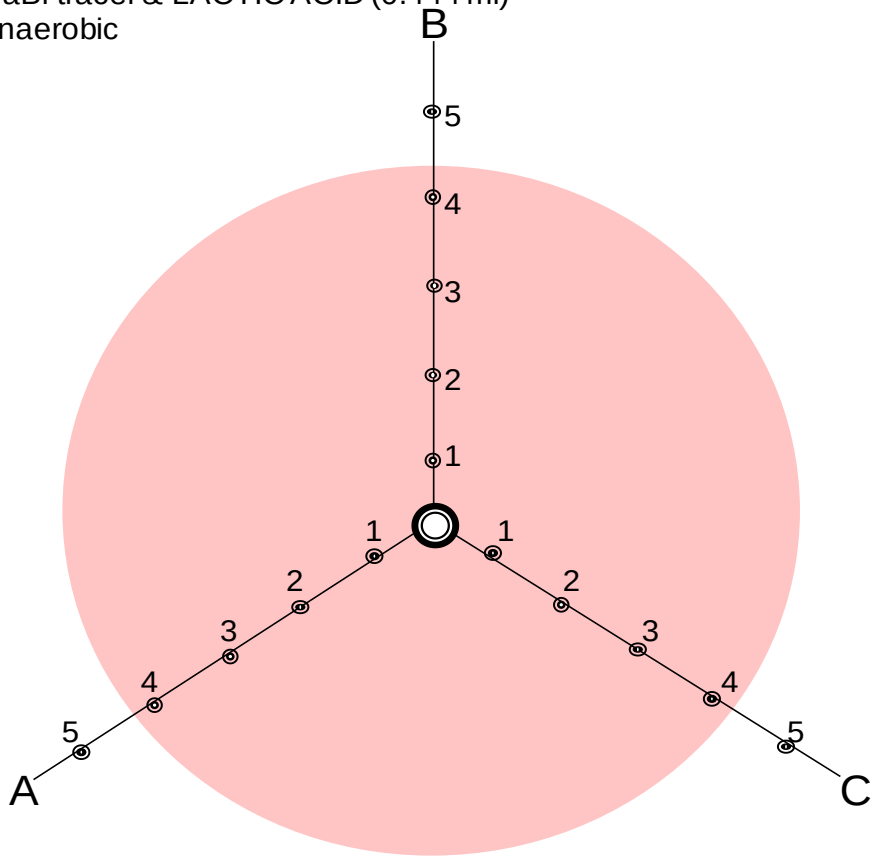
RAMPP 3: Sulfate

Description of Test Water:

-SO_4 conc. ~67.2 ppm

-NaBr tracer & LACTIC ACID (0.444 ml)

-anaerobic



Negative rates = sulfate production

	Sample Location	Time (hrs)	Rate (mg/L h-1)
1	RAMPP 3-A5	0.12	25.806
	RAMPP 3-A4	0.13	-3.871
	RAMPP 3-A3	0.15	4.651
2	RAMPP 3-B5	0.38	-0.980
	RAMPP 3-B4	0.40	-4.237
	RAMPP 3-B3	0.43	3.568
3	RAMPP 3-C5	0.77	-1.221
	RAMPP 3-C4	0.77	-5.200
	RAMPP 3-C3	0.78	-2.321
4	RAMPP 3-A3-2	4.08	-0.101
	RAMPP 3-A2	4.08	-1.883
	RAMPP 3-A1	4.10	0.406
5	RAMPP 3-B3-2	4.37	-0.233
	RAMPP 3-B2	4.40	0.513
	RAMPP 3-B1	4.53	0.240
6	RAMPP 3-C3-2	4.77	-0.165
	RAMPP 3-C2	4.78	-0.194
	RAMPP 3-C1	4.82	0.591

= estimated interface location

= fastest rate per sampling event

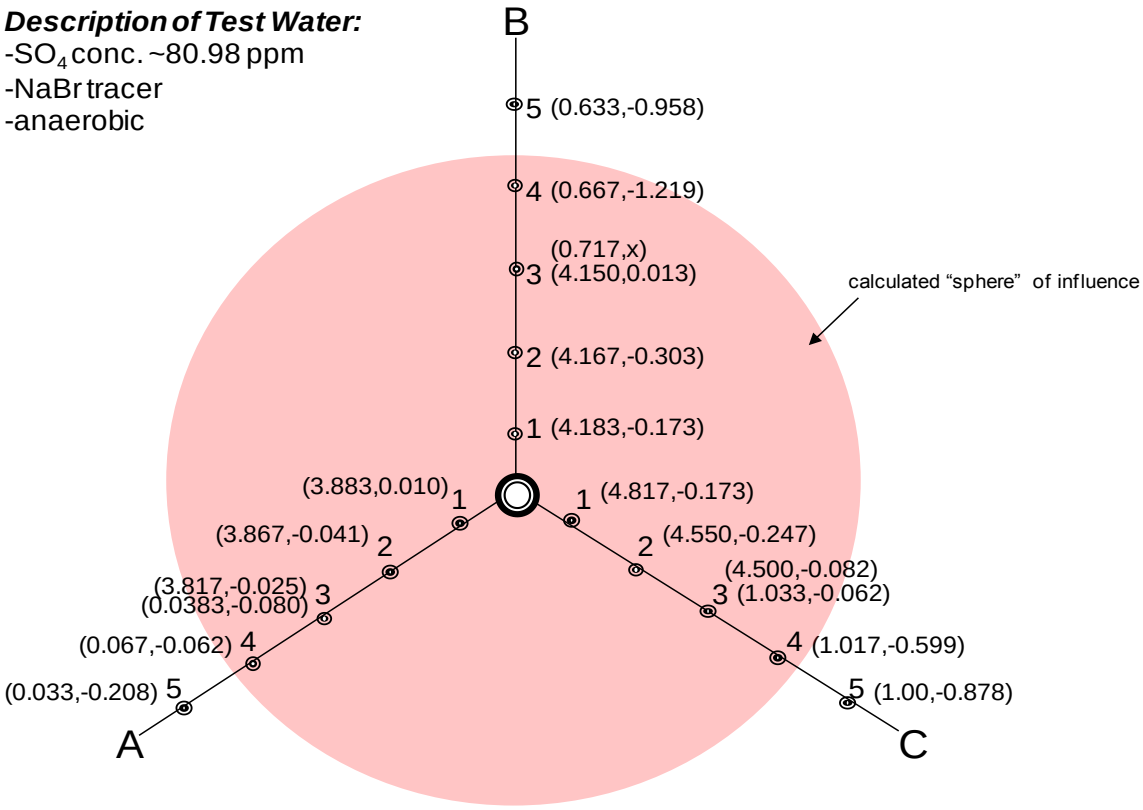
RAMPP 1: Sulfate

Description of Test Water:

-SO_4 conc. ~80.98 ppm

-NaBr tracer

-anaerobic



(Time (hrs), $\ln(r_m/tm)$)

Center Well

Time (hrs)	$\ln(r_m/t_m)$
0.000	0.086
0.267	0.031
0.583	0.022
0.933	0.120
1.233	0.048
1.583	-0.058
1.850	0.139
2.100	0.073
2.433	0.070
2.600	0.043
2.850	-0.036
3.100	-0.094
3.350	0.067
3.683	-0.003
3.950	-0.042
4.183	0.007
4.433	-0.038
4.683	0.039
4.833	-0.025
4.967	-0.004
5.100	0.138
5.183	-0.054
5.217	-0.129
5.250	-0.099
5.283	-0.158
5.317	-0.032
5.350	-0.091

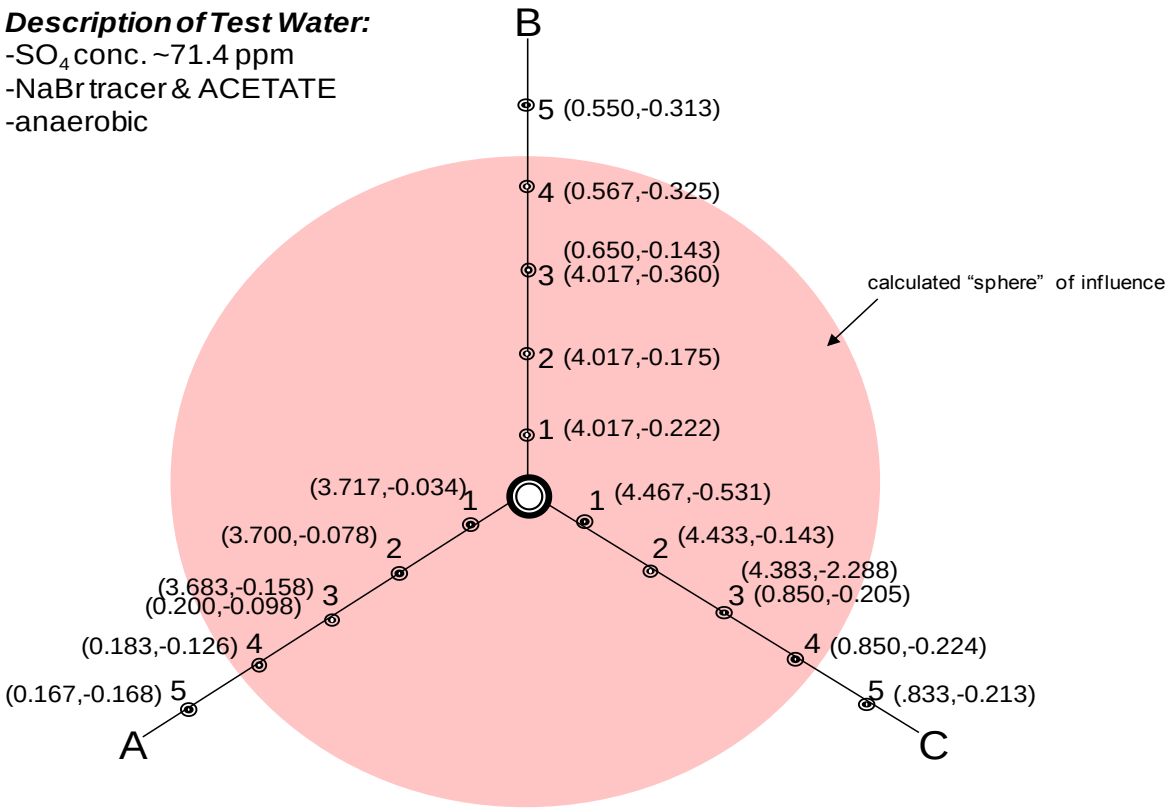
RAMPP 2: Sulfate

Description of Test Water:

-SO_4 conc. ~71.4 ppm

-NaBr tracer & ACETATE

-anaerobic



(Time (hrs), $\ln(r_m/tm)$)

Center Well

Time (hrs)	$\ln(r_m/t_m)$
0.000	-0.092
0.233	-0.090
0.500	-0.066
0.783	-0.036
0.983	-0.053
1.233	-0.083
1.483	-0.030
1.733	-0.063
1.983	-0.102
2.233	-0.070
2.483	-0.103
2.733	-0.134
2.983	0.024
3.233	-0.062
3.567	-0.076
3.850	-0.126
4.167	-0.142
4.500	-0.138
4.683	-0.174
4.850	-0.168
5.017	-0.108
5.150	-0.366
5.200	-0.123
5.250	-0.161
5.300	-0.055
5.350	-0.243

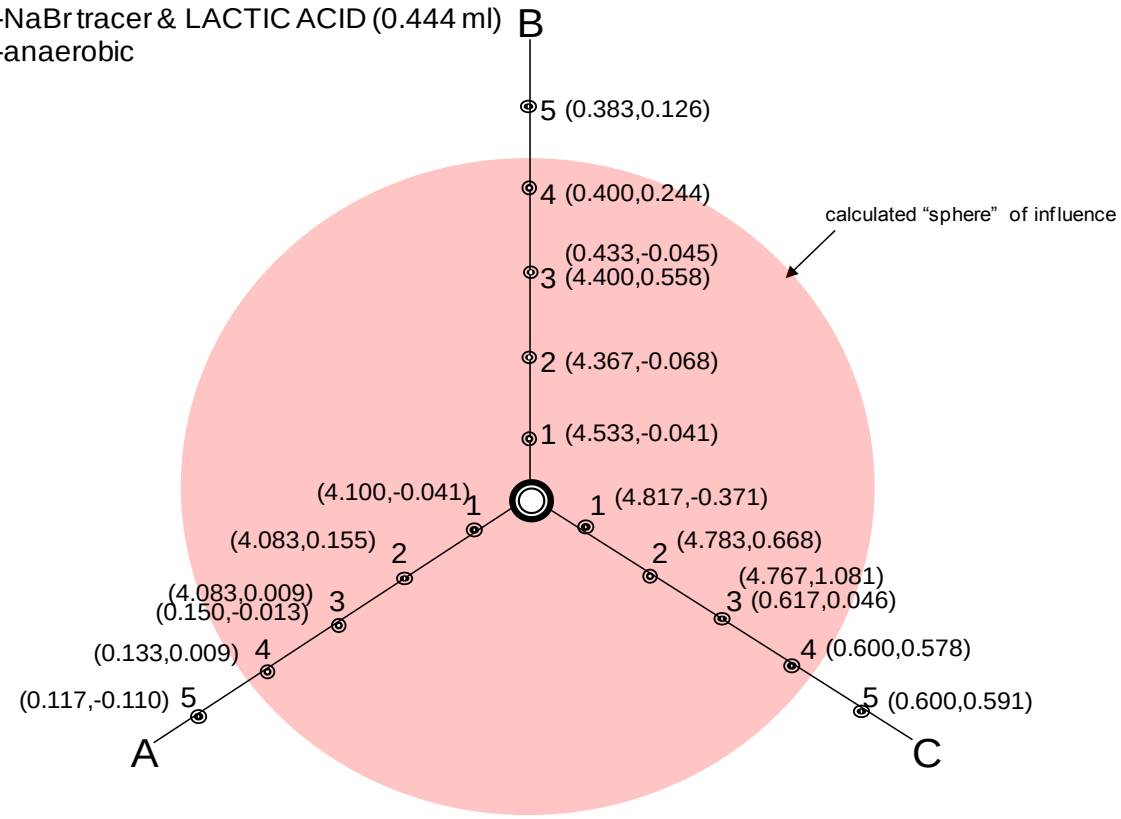
RAMPP 3: Sulfate

Description of Test Water:

-SO_4 conc. ~67.2 ppm

-NaBr tracer & LACTIC ACID (0.444 ml)

-anaerobic



(Time (hrs), $\ln(r_m/tm)$)

Center Well

Time (hrs)	$\ln(r_m/t_m)$
0.000	-0.003
0.267	-0.026
0.550	-0.025
0.800	-0.041
1.050	0.001
1.300	
1.550	-0.033
1.800	-0.006
2.050	0.007
2.300	-0.011
2.550	-0.028
2.800	-0.060
3.050	-0.011
3.300	-0.026
3.633	-0.062
3.967	-0.024
4.317	0.002
4.617	0.000
4.950	0.009
5.200	-0.002
5.367	0.072
5.417	0.041
5.467	0.208
5.500	0.113
5.550	0.114
5.600	0.173
5.767	0.723

Native Organism Geochemical Experimentation Enclosure (NOGEE) Data

Summer 2007 - Sulfate Test

NOGEE Experiments June/July2007		geochemistry										molecular							
	Time Exposed to Test Solution (hrs)	**Cl^-**	**Br^-**	**SO_4^{2-}**	**NO_3^{2-}**	**NH_4^+**	**H_2S**	**Fe^{2+}**	**Alkalinty**	**Methane**	**DOC**	**wet wt. extracted**	**DNA yield (ng)**	**dsr/gm ext**	**geo/gm ext.**	**mcr/gm ext.**	**dsr/ng DNA**	**geo/ng DNA**	**mcr/ng DNA**
Sulfate NOGEEs		**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mmol/L**	mg/L	**mg/L of C**								
D5 (T_o)		509.0	6.3	90.8	bdl	2.54	0.10	bdl	0.00	0.35	56.6	0.60	59.4	7.50E+04	1.49E+05	bdl	7.58E+02	1.51E+03	bdl
D6 (T_o)		489.5	5.6	45.1	bdl	bdl	1.11	bdl	0.00	0.22	55.3	0.60	268.5	5.12E+05	2.28E+06	bdl	1.14E+03	5.09E+03	bdl
S1 initial		491.5	5.4	142.8	bdl	bdl	1.36	bdl	0.00	0.63	94.3								
S3 initial		471.5	bdl	47.3	bdl	1.04	2.28	bdl	0.00	0.66	99.3								
ITS S1		638.7	108.6	104.5	bdl	22.49	bdl	13.17	0.00	1.44	180.5								
ITS S2		624.3	106.7	78.8	bdl	0.69	bdl	20.03	48.06	4.53	165.5								
ITS S3		611.2	102.8	97.3	bdl	2.01	bdl	11.75	49.97	3.22	180.3								
ITS S4		612.9	103.9	91.9	bdl	2.02	bdl	11.68	52.12	2.89	184.0								
ITS S5		608.8	96.1	97.0	bdl	154.56	bdl	12.05	66.97	2.04	190.2								
S1-1	71.2	602.8	96.1	39.8	bdl	8.51	3.01	1.48	47.56	1.99	215.1								
S1-2	45.6	619.1	102.1	9.2	bdl	57.82	1.83	1.27	56.39	0.66	210.8								
S1-3	45.2	606.1	95.9	10.0	bdl	117.52	2.27	0.39	52.77	0.23	206.0								
S1-4	52.5	596.3	90.4	0.0	bdl	198.26	1.76	0.38	56.61	0.82	213.5								
S1-5	48.0	612.7	94.3	5.4	bdl	126.87	2.23	0.04	63.81	0.93	206.7								
Total Time	**262.32**																		
S1f												0.24	598.5	6.69E+07	1.36E+07	4.97E+03	2.68E+04	5.45E+03	1.99E+00
S3-1	71.0	597.7	88.7	18.1	bdl	0.68	2.14	1.76	47.17	1.00	175.8								
S3-2	45.5	615.0	92.6	14.4	bdl	2.14	1.37	0.18	51.39	1.49	162.6								
S3-3	45.3	593.5	95.3	15.5	bdl	3.55	2.00	0.95	53.09	1.61	163.3								
S3-4	52.5	594.9	91.3	18.0	bdl	150.64	1.09	0.79	73.11	1.71	154.9								
S3-5	47.7	592.9	83.1	9.6	bdl	165.24	0.08	0.33	63.81	1.65	154.0								
Total Time	**261.97**																		
S3f												1.24	1055.2	1.31E+07	2.79E+06	3.54E+03	1.54E+04	3.28E+03	4.16E+00

Summer 2007 - Iron Test (ferrihydrite)

NOGEE Experiments June/July2007		geochemistry									molecular							
	Time Exposed to Test Solution (hrs)	**Cl^-**	**Br^-**	**SO_4^{2-}**	**NO_3^{2-}**	**NH_4^+**	**H_2S**	**Fe^{2+}**	**Alkalinty**	**Methane**	**DOC**	**wet wt. extracted**	**DNA yield (ng)**	**dsr/gm ext**	**geo/gm ext.**	**mcr/gm ext.**	**dsr/ng DNA**	**geo/ng DNA**	**mcr/ng DNA**
Iron NOGEEs																			
D1 (T_o)		516.4	bdl	279.5	bdl	13.07			0.00	0.17	54.0	2.07	203.4	2.98E+05	6.95E+05	bdl	3.03E+03	7.08E+03	bdl
D2 (T_o)		558.2	bdl	331.7	bdl	0.53			0.00	0.12	59.5	1.99	106.2	1.01E+05	2.74E+05	bdl	1.90E+03	5.14E+03	bdl
I-1* (T_o)												0.39	71.6	2.06E+05	5.17E+05	bdl	1.12E+03	2.82E+03	bdl
I-3* (T_o)												0.35	63.9	3.20E+05	1.13E+06	bdl	1.75E+03	6.20E+03	bdl
I2 initial		363.9		191.4	bdl	47.97	0.10	bdl	0.00	0.96	95.7								
I3 initial		391.0	13.5	121.6	bdl	18.78	1.22	bdl	0.00	1.96	111.1								
ITS I1		683.2	105.3	bdl	bdl	63.22	bdl	9.85	0.00	2.29	187.5								
ITS I2		706.4	106.7	bdl	bdl	no sample	bdl	9.46	52.07	3.33	146.5								
ITS I3		736.9	126.4	bdl	bdl	143.40	bdl	9.46	54.26	1.77	178.7								
ITS I4		854.9	123.5	bdl	bdl	20.60	bdl	7.88	49.70	0.93	184.7								
ITS I5		909.7	130.9	bdl	bdl	missing	bdl	8.46	65.19	0.41	173.9								
I2-1	71.82	661.9	108.0	bdl	bdl	143.08	bdl	2.09	48.57										
I2-2	47.52	737.0	118.9	bdl	bdl	no sample	bdl	7.32	56.39	no sample	196.4								
I2-3	47.70	638.4	97.7	bdl	bdl	no sample	bdl	15.54	69.60	no sample	227.8								
I2-4	47.45	861.4	118.0	bdl	bdl	no sample	bdl	14.98	96.00	no sample	242.8								
I2-5	47.60	928.9	132.5	bdl	bdl	no sample	bdl	11.94	96.00	no sample	254.1								
Total Time	**262.08**																		
I2f												0.33	102.6	4.92E+05	3.86E+06	bdl	1.58E+03	1.24E+04	bdl
I3-1	71.93	714.2	108.0	bdl	bdl	179.85	bdl	5.28	59.16	1.81	182.6								
I3-2	47.57	708.8	115.1	bdl	bdl	160.51	bdl	8.22	54.56	1.60	155.0								
I3-3	47.85	668.7	101.5	bdl	bdl	182.20	bdl	14.51	48.67	1.76	187.8								
I3-4	47.47	917.2	130.5	bdl	bdl	30.43	bdl	18.80	70.21	3.04	178.6								
I3-5	47.35	944.1	130.8	bdl	bdl	160.65	bdl	19.47	71.06	2.26	183.2								
Total Time	**262.17**																		
I3f												0.33	108.3	9.92E+05	4.32E+06	bdl	3.02E+03	1.32E+04	bdl

Summer 2007 - Control NOGEEs

NOGEE Experiments June/July2007		geochemistry										molecular							
	Time Exposed to Test Solution (hrs)	**Cl^-**	**Br^-**	**SO_4^{2-}**	**NO_3^{2-}**	**NH_4^+**	**H_2S**	**Fe^{2+}**	**Alkalinty**	**Methane**	**DOC**	**wet wt. extracted**	**DNA yield (ng)**	**dsr/gm ext**	**geo/gm ext.**	**mcr/gm ext.**	**dsr/ng DNA**	**geo/ng DNA**	**mcr/ng DNA**
Control NOGEEs																			
D3 (T_o)		502.3	8.9	36.3	5.1	4.90	1.13	bdl	0.00	0.28	67.8	2.10	478.3	9.45E+05	1.80E+06	1.35E+03	4.15E+03	7.89E+03	5.93E+00
D4 (T_o)		524.4	5.7	266.5	1.8	0.48	0.31	bdl	0.00	0.20	54.2	0.60	59.4	7.50E+04	1.49E+05	BLD	7.58E+02	1.51E+03	BLD
C2 initial		483.1	bdl	145.4	bdl	19.63	1.30	bdl	0.00	1.52	78.8								
C3 initial		444.1	bdl	68.2	bdl	12.53	bdl	bdl	0.00	no sample	70.2								
ITS C1		594.5	92.9	17.0	bdl	314.28	bdl	12.73	0.00	4.87	84.7								
ITS C2		600.6	88.9	bdl	bdl	no sample	bdl	12.15	27.23	8.46	113.9								
ITS C3		597.7	104.0	bdl	bdl	10.50	bdl	13.36	54.76	6.52	122.7								
ITS C4		582.7	105.0	bdl	bdl	15.34	bdl	10.90	52.00	4.21	123.0								
ITS C5		573.2	92.3	bdl	bdl	21.55	bdl	12.66	71.66	4.19	122.4								
C2-1	70.52	573.6	70.5	46.0	bdl	bdl	0.28	2.04	37.62	1.22	102.4								
C2-2	47.42	566.8	78.4	bdl	bdl	390.68	bdl	2.19	54.66	2.14	149.1								
C2-3	48.12	571.0	94.4	bdl	bdl	21.84	0.17	4.13	44.53	2.67	182.6								
C2-4	47.20	571.7	96.2	bdl	bdl	15.34	0.57	3.95	58.28	3.07	181.7								
C2-5	48.62	567.0	92.9	bdl	bdl	9.80	bdl	4.34	75.29	2.52	186.5								
Total Time	**261.87**																		
C2f												0.44	240.8	1.95E+06	1.17E+07	3.85E+03	3.56E+03	2.13E+04	7.04E+00
C3-1	70.27	556.7	86.0	19.1	bdl	83.94	bdl	4.98	46.95	0.81	113.9								
C3-2	47.77	553.3	89.3	bdl	bdl	bdl	bdl	7.81	53.54	1.53	141.96								
C3-3	48.12	575.7	89.3	bdl	bdl	31.75	bdl	8.71	49.16	2.05	157.1								
C3-4	47.47	581.1	87.4	bdl	bdl	20.78	bdl	8.07	66.08	1.57	157.3								
C3-5	48.38	568.3	bdl	bdl	bdl	25.04	bdl	8.82	71.29	2.04	152.7								
Total Time	**262.00**																		
C3f												0.33	152.98	6.20E+05	2.11E+07	4.41E+03	1.34E+03	4.56E+04	9.51E+00

October 2007 NOGEEs (warm NOGEEs) – Initial and Control Samples

October 2007 NOGEEs			geochemistry									
									mmol/L		mg/L of C	ppm
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane
date collected	Sample ID	time exposed to test solution (hrs)										
DUMMY NOGEEs												
10/18/2007	**D1 (T_o)**		341.11	6.31	45.21	7.23	0.00	3.45	14.57	7.42	62.36	3.47
10/18/2007	**D2 (T_o)**		440.57	5.70	0.00	0.00	0.00	10.12	12.4	7.48	56.57	2.82
10/18/2007	**D3 (T_o)**		482.60	5.11	0.00	0.00	0.00	7.85	16.12	7.39	68.75	1.30
10/18/2007	**D4 (T_o)**		505.29	5.88	4.84	4.08	0.00	0.62	14.725	7.63	58.15	0.44
Initial Samples	**Sample ID**	**time exposed to test solution (hrs)**										
10/18/2007	C1 initial		265.28	3.81	0.00	0.00	0.18	-0.06	8.68	7.11	71.78	2.16
10/18/2007	C2 initial		283.37	0.00	19.50	0.00	0.00	1.35	8.37	7.14	89.80	1.53
10/18/2007	S1 initial		298.83	0.00	19.46	0.00	0.07	2.08	8.525	7.02	71.79	1.86
10/18/2007	S2 initial		313.87	0.00	0.00	0.00	0.00	5.23	7.13	6.92	95.39	2.94
10/18/2007	S3 initial		337.73	6.82	0.00	0.00	0.00	4.93	6.665	7.02	64.31	2.98
10/18/2007	S4 initial		339.04	0.00	0.00	0.00	0.01	3.27	7.75	7.01	61.68	2.78
10/18/2007	S5 initial		332.14	0.00	26.59	0.00	0.00	0.93	8.525	7.2	68.62	1.30
10/18/2007	S6 initial		342.33	0.00	0.00	0.00	0.78	2.44	9.3	7.21	no sample	1.75
Control NOGEEs	**Sample ID**	**time exposed to test solution (hrs)**										
10/18/2007	**ITS C1**		**774.77**	**143.09**	**0.00**	**0.00**	**0.00**	**10.01**	**18.755**	**7.38**	**112.40**	**1.67**
10/19/2007	C1-1	21.75	639.17	105.73	0.00	0.00	0.00	3.70	18.6	7.54	118.54	1.21
10/19/2007	C2-1	21.87	629.01	108.29	0.00	0.00	0.00	3.08	17.67	7.54	121.18	1.56
10/19/2007	**ITS C2**		**626.41**	**103.64**	**0.00**	**0.00**	**0.00**	**11.62**	**18.6**	**7.54**	**112.14**	**1.72**
10/20/2007	C1-2	21.58	637.61	99.60	0.00	0.00	0.00	5.23	17.205	7.58	120.46	1.21
10/20/2007	C2-2	21.55	624.97	99.30	0.00	0.00	0.00	3.54	18.29	7.53	119.72	0.64
10/20/2007	**ITS C3**		**544.68**	**100.93**	**0.00**	**0.00**	**0.00**	**11.87**	**18.6**	**7.25**	**110.87**	**3.49**
10/21/2007	C1-3	21.78	522.97	91.84	0.00	0.00	0.00	8.77	17.515	7.51	114.20	2.33
10/21/2007	C2-3	21.57	545.80	94.59	0.00	0.00	0.00	8.04	15.5	7.44	119.40	2.12

October 2007 NOGEEs (warm NOGEEs) – 10 mg/L sulfate NOGEEs

October 2007 NOGEEs			geochemistry										
									mmol/L		mg/L of C	ppm	
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane	SO4 red. rate
10 ppm NOGEEs	Sample ID	time exposed to test solution (hrs)											
10/18/2007	**ITS S1a**		**675.91**	**119.66**	**24.66**	**0.00**	**0.00**	**10.84**	**19.84**	**7.48**	**175.02**	**1.69**	
10/19/2007	S1-1	21.67	643.16	116.29	0.00	0.00	0.00	2.82	17.825	7.53	181.29	1.46	**1.138**
10/19/2007	S2-1	21.70	658.39	117.52	0.00	0.00	0.00	3.05	18.6	7.45	185.12	1.75	**1.136**
10/19/2007	**ITS S2a**		**631.24**	**96.70**	**8.58**	**0.00**	**0.00**	**11.80**	**19.84**	**7.19**	**175.17**	**1.12**	
10/20/2007	S1-2	21.50	416.17	71.25	0.00	0.00	0.00	5.39	18.755	7.27	171.94	1.85	**0.399**
10/20/2007	S2-2	21.58	406.90	72.47	5.89	0.00	0.00	5.06	15.5	7.5	175.17	1.44	**0.125**
10/20/2007	**ITS S3a**		**586.37**	**93.90**	**10.85**	**0.00**	**0.00**	**10.86**	**21.39**	**7.39**	**153.37**	**2.39**	
10/21/2007	S1-3	20.71	636.22	99.52	0.00	0.00	0.00	5.97	18.6	7.55	155.21	1.87	**0.524**
10/21/2007	S2-3	20.47	637.92	101.11	0.00	0.00	0.00	4.67	18.6	7.57	158.00	no sample	**0.530**

October 2007 NOGEEs (warm NOGEEs) – 25 mg/L sulfate NOGEEs

October 2007 NOGEEs			geochemistry										
									mmol/L		mg/L of C	ppm	
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane	SO4 red. rate
25 ppm NOGEEs	**Sample ID**	**time exposed to test solution (hrs)**											
10/18/2007	**ITS S1b**		**691.07**	**122.35**	**34.70**	**0.00**	**0.00**	**11.99**	**20.15**	**7.2**	**183.75**	**2.51**	
10/19/2007	S3-1	21.80	663.66	114.87	20.70	0.00	0.00	5.15	18.6	7.36	184.88	1.59	**0.642**
10/19/2007	S4-1	22.02	646.41	112.12	15.90	0.00	0.00	13.82	18.29	7.26	183.36	2.28	**0.854**
10/19/2007	**ITS S2b**		**639.96**	**96.40**	**20.90**	**0.00**	**0.00**	**11.10**	**18.6**	**7.31**	**162.24**	**2.42**	
10/20/2007	S3-2	21.38	384.16	57.65	10.23	0.00	0.00	4.58	17.67	7.54	165.77	1.69	**0.499**
10/20/2007	S4-2	21.45	401.93	63.95	6.23	0.00	0.00	4.15	18.755	7.47	156.75	1.88	**0.684**
10/20/2007	**ITS S3b**		**659.84**	**109.33**	**28.02**	**0.00**	**0.00**	**9.62**	**18.6**	**7.29**	**158.48**	**2.11**	
10/21/2007	S3-3	20.73	634.74	98.82	14.34	0.00	0.00	4.31	15.19	7.65	155.65	1.54	**0.660**
10/21/2007	S4-3	20.50	617.41	97.76	0.00	0.00	0.00	2.81	18.6	7.32	149.93	1.91	**1.367**

October 2007 NOGEEs (warm NOGEEs) – 100 mg/L sulfate NOGEEs

October 2007 NOGEEs			geochemistry										
									mmol/L		mg/L of C	ppm	
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane	SO4 red. rate
100 ppm NOGEEs	Sample ID	time exposed to test solution (hrs)											
10/18/2007	**ITS S1C**		**679.43**	**121.06**	**114.90**	**0.00**	**0.00**	**8.77**	**15.035**	**7.5**	**156.86**	**1.77**	
10/19/2007	S5-1	21.95	627.18	108.32	93.66	0.00	0.00	3.89	19.22	7.31	161.43	1.64	**0.968**
10/19/2007	S6-1	22.03	638.29	109.62	84.88	0.00	0.00	1.99	18.6	7.58	159.82	0.56	**1.363**
10/19/2007	**ITS S2c**		**625.19**	**103.58**	**91.23**	**0.00**	**0.00**	**11.92**	**21.7**	**7.15**	**170.02**	**1.28**	
10/20/2007	S5-2	21.28	427.40	74.96	54.22	0.00	0.00	11.66	15.655	7.62	165.07	1.91	**1.739**
10/20/2007	S6-2	21.30	423.42	76.73	52.16	0.00	0.00	14.69	18.6	7.41	163.18	1.97	**1.834**
10/20/2007	**ITS S3c**		**555.83**	**101.42**	**97.11**	**0.00**	**0.00**	**18.16**	**21.7**	**7.14**	**170.45**	**1.69**	
10/21/2007	S5-3	19.55	601.07	104.06	79.33	0.00	0.00	4.99	16.585	7.75	164.79	1.41	**0.910**
10/21/2007	S6-3	19.33	640.48	95.79	65.47	0.00	0.00	2.83	19.22	7.5	163.42	0.80	**1.636**

October 2007 NOGEEs (warm NOGEEs) – Microbiology Data (from polycarbonate sponges)

October 2007 NOGEEs				microbiology			
sample	DNA yield (ng)	dsr/gm ext	geo/gm ext.	mcr/gm ext.	dsr/ng DNA	geo/ng DNA	mcr/ng DNA
D1	141	2.77E+06	2.77E+06	6.45E+04	5.94E+03	5.96E+03	1.39E+02
D2	1075	1.03E+07	4.61E+07	2.39E+05	2.01E+03	8.97E+03	4.65E+01
D3	60.5	1.22E+05	1.38E+06	2.90E+04	1.20E+03	1.35E+04	2.84E+02
C1	134	6.19E+05	8.17E+06	1.51E+05	1.86E+03	2.46E+04	4.53E+02
C2	98.5	1.06E+06	2.53E+07	5.59E+04	1.61E+03	3.85E+04	8.51E+01
S1	222	4.76E+06	1.46E+07	3.96E+04	1.02E+04	3.14E+04	8.48E+01
S2	258.5	7.43E+05	1.27E+07	5.28E+04	1.39E+03	2.38E+04	9.87E+01
S3	252	8.55E+05	7.57E+06	1.88E+05	2.23E+03	1.98E+04	4.91E+02
S4	227	2.76E+06	6.61E+06	1.10E+05	3.52E+03	8.42E+03	1.41E+02
S5	77.5	2.56E+06	1.01E+07	3.53E+04	5.02E+03	1.97E+04	6.92E+01
S6	249	1.01E+07	3.69E+07	3.62E+04	1.51E+04	5.52E+04	5.42E+01

February 2008 NOGEEs (cold NOGEEs) – Initial and Control Samples

Feburary/March 2008 NOGEEs

									mmol/L		mg/L of C	ppm
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane
date collected	**Sample ID**	**time exposed to test solution (hrs)**										
DUMMY NOGEEs												
2/28/2008	D1		408.86	bdl	63.78	bdl	bdl	9.49	16.43	7.37	71.63	1.08
2/28/2008	D2		409.07	10.03	5.00	8.31	bdl	8.09	15.81	7.39	69.61	4.35
2/28/2008	D3		406.43	9.29	4.58	7.54	bdl	0.78	18.91	7.44	66.83	0.32
2/28/2008	D4		333.41	bdl	77.65	6.85	bdl	bdl	17.36	7.5	63.71	0.55
2/28/2008	D5		315.03	bdl	97.81	5.46	bdl	bdl	13.95	7.56	62.07	NS
2/28/2008	D6		442.11	4.98	14.03	bdl	bdl	6.37	12.71	7.26	64.06	4.80
Initial Samples	**Sample ID**	**time exposed to test solution (hrs)**										
2/28/2008	C1 initial		390.08	3.61	39.90	bdl	bdl	7.72	12.4	7.2	95.72	3.67
2/28/2008	C2 initial		288.83	4.89	36.90	bdl	bdl	9.89	7.75	5.59	78.60	4.80
2/28/2008	C3 initial (S4 initial)		213.95	3.62	37.46	bdl	bdl	2.55	6.51	6.93	132.01	2.68
2/28/2008	S1 initial		292.68	4.53	16.95	bdl	bdl	4.93	9.3	7.12	82.05	3.17
2/28/2008	S2 initial		240.51	bdl	43.65	bdl	bdl	1.65	6.51	7.08	88.43	1.74
2/28/2008	S3 initial		211.18	bdl	37.73	bdl	bdl	1.31	5.89	7.04	76.51	3.61
2/28/2008	S4 initial											
2/28/2008	S5 initial		204.75	bdl	35.86	bdl	bdl	3.87	6.2	6.94	107.10	NR
2/28/2008	S6 initial		222.19	bdl	24.16	bdl	bdl	2.22	4.96	6.96	42.29	NS
Control NOGEEs	**Sample ID**	**time exposed to test solution (hrs)**										
2/28/2008	ITS C1		592.05	110.78	bdl	bdl	bdl	8.03	17.67	7.22	113.14	1.55
3/1/2008	C1-1	21.52	599.34	109.95	bdl	bdl	bdl	8.94	19.84	7.33	109.01	2.57
3/1/2008	C2-1	21.07	614.43	108.78	bdl	bdl	bdl	5.66	20.46	7.28	110.54	3.02
3/1/2008	ITS C2		524.74	71.29	bdl	bdl	bdl	9.49	20.77	7.55	107.16	3.42
3/2/2008	C1-2	23.38	525.24	74.63	bdl	bdl	bdl	8.56			106.01	2.03
3/2/2008	C2-2	23.55	516.05	120.27	bdl	bdl	bdl	6.31			113.47	2.34
3/2/2008	ITS C3		407.29	70.44	bdl	bdl	bdl	9.71			113.98	3.16
3/3/2008	C1-3	23.05	405.52	72.73	bdl	bdl	bdl	10.31			112.46	1.27
3/3/2008	C2-3	23.18	412.90	70.91	bdl	bdl	bdl	8.45			115.65	0.86

February 2008 NOGEEs (cold NOGEEs) – 10 mg/L sulfate NOGEEs

Feburary/March 2008 NOGEEs

									mmol/L		mg/L of C	ppm	
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane	SO4 red. rate
10 ppm NOGEEs	**Sample ID**	**time exposed to test solution (hrs)**											
2/28/2008	ITS S1a		618.86	114.01	11.97	bdl	bdl	11.11	21.7	7.28	165.13	3.42	
3/1/2008	S1-1	20.12	622.07	112.81	11.77	bdl	bdl	5.08			155.52	NR	**0.0171**
3/1/2008	S2-1	20.30	620.49	115.55	12.03	bdl	bdl	4.85			164.76	2.48	**-0.0048**
3/1/2008	ITS S2a		534.91	119.99	11.60	bdl	bdl	9.15			170.61	2.74	
3/2/2008	S3-2	21.41	535.68	118.31	10.48	bdl	bdl	6.00			165.28	1.29	**0.0963**
3/2/2008	S4-2	21.55	546.62	116.97	11.35	bdl	bdl	5.23			181.42	1.23	**0.0216**
3/2/2008	ITS S3a		513.29	77.54	20.02	bdl	bdl	10.50			173.92	0.51	
3/3/2008	S1-3	21.57	512.24	78.48	18.78	bdl	bdl	10.99			153.25	0.06	**0.0619**
3/3/2008	S2-3	21.68	513.40	78.92	19.83	bdl	bdl	10.18			170.33	0.35	**0.0095**

February 2008 NOGEEs (cold NOGEEs) – 25 mg/L sulfate NOGEEs

Feburary/March 2008 NOGEEs													
									mmol/L		mg/L of C	ppm	
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane	SO4 red. rate
25 ppm NOGEEs	**Sample ID**	**time exposed to test solution (hrs)**											
2/28/2008	ITS S1b		642.57	116.63	23.54	bdl	bdl	12.16	21.7	7.25	175.21	2.76	
3/1/2008	S3-1	19.78	618.38	113.55	22.62	bdl	bdl	0.72			171.40	NR	**0.0388**
3/1/2008	S4-1	20.13	596.08	101.09	4.44	bdl	bdl	3.77			111.84	2.92	**0.8112**
3/1/2008	ITS S2b		541.63	121.20	22.51	bdl	bdl	10.82			162.33	3.75	
3/2/2008	S3-2	21.30	540.65	119.48	18.38	bdl	bdl	6.01			182.85	1.46	**0.1835**
3/2/2008	S4-2	22.43	485.00	118.75	16.47	bdl	bdl	6.84			172.98	1.35	**0.2684**
3/2/2008	ITS S3b		516.97	61.00	20.94	bdl	bdl	10.43			173.92	0.51	
3/3/2008	S3-3	20.98	396.24	62.06	20.40	bdl	bdl	10.42			182.16	0.57	**0.0256**
3/3/2008	S4-3	21.13	391.97	59.01	20.57	bdl	bdl	9.35			187.49	1.84	**0.0173**

February 2008 NOGEEs (cold NOGEEs) – 100 mg/L sulfate NOGEEs

Feburary/March 2008 NOGEEs

									mmol/L		mg/L of C	ppm	
			Cl^-	Br^-	SO_4^{2-}	NO_3^{2-}	H_2S	Fe^{2+}	Alkalinty	pH	DOC	Methane	SO4 red. rate
100 ppm NOGEEs	**Sample ID**	**time exposed to test solution (hrs)**											
2/28/2008	ITS S1C		526.90	55.09	80.02	bdl	bdl	12.97	22.01	7.38	171.20	3.56	
3/1/2008	S5-1	18.23	510.51	59.00	72.46	bdl	bdl	7.60			174.91	2.21	**0.0945**
3/1/2008	S6-1	18.38	504.31	54.26	72.40	bdl	bdl	4.92			164.77	4.76	**0.0953**
3/1/2008	ITS S2c		570.85	120.72	87.57	bdl	bdl	10.52			178.25	3.79	
3/2/2008	S5-2	22.73	554.98	119.66	88.05	bdl	bdl	8.33			194.49	1.12	**-0.0055**
3/2/2008	S6-2	22.87	545.41	101.20	87.49	bdl	bdl	5.95			188.19	NR	**0.0009**
3/2/2008	ITS S3c		389.26	64.41	81.55	bdl	bdl	10.26			178.50	1.16	
3/3/2008	S5-3	20.52	378.04	64.80	80.81	bdl	bdl	10.11			185.36	2.93	**0.0091**
3/3/2008	S6-3	20.63	362.48	70.19	78.24	bdl	bdl	9.28			170.66	2.38	**0.0406**

February 2008 NOGEEs (cold NOGEEs) – Microbiology Data

Feburary/March 2008 NOGEEs				microbiology				
Sample	**sponge wet wt. (g)**	**DNA yield (ng)**	**dsr/gm ext**	**geo/gm ext.**	**mcr/gm ext.**	**dsr/ng DNA**	**geo/ng DNA**	**mcr/ng DNA**
D1	1.77	11.5	2.10E+04	2.22E+05	9.60E+01	3.24E+03	3.42E+04	1.48E+01
D2	1.39	158.5	3.72E+05	3.27E+06	9.33E+02	3.26E+03	2.87E+04	8.18E+00
D3	1.7	28	6.00E+03	3.97E+04	2.41E+01	3.64E+02	2.41E+03	1.46E+00
D4	2.47	132.5	9.66E+04	6.93E+05	5.09E+02	1.80E+03	1.29E+04	9.50E+00
D5	1.82	212.5	1.79E+05	1.74E+06	6.88E+02	1.53E+03	1.49E+04	5.90E+00
D6	0.74	86	4.61E+05	4.05E+06	2.03E+03	3.96E+03	3.48E+04	1.74E+01
C1	0.65	19	3.87E+04	3.53E+05	3.02E+02	1.32E+03	1.21E+04	1.03E+01
C2	0.52	103	2.19E+05	2.20E+06	1.54E+03	1.11E+03	1.11E+04	7.78E+00
S1	0.73	150.5	8.91E+04	1.42E+06	5.17E+02	4.32E+02	6.87E+03	2.51E+00
S2	0.35	109.5	1.06E+05	5.63E+05	6.85E+02	3.37E+02	1.80E+03	2.19E+00
S3	0.54	47	3.22E+04	2.11E+05	5.75E+02	3.70E+02	2.43E+03	6.61E+00
S4	0.65	40	1.68E+04	1.10E+05	4.29E+02	2.74E+02	1.78E+03	6.98E+00
S5	0.53	31	1.48E+05	2.76E+06	1.00E+03	2.53E+03	4.73E+04	1.71E+01
S6	0.53	39	1.97E+05	1.20E+06	1.09E+03	2.68E+03	1.63E+04	1.48E+01

APPENDIX II

METHODS

A summary of the sample volumes, preservation methods, and analytical techniques used to analyze samples collected for this dissertation are presented on Table A1. Detailed method descriptions for each analysis follow and are presented in the order listed in the Table A1.

Table A1. Summary of Geochemical Sampling Methods

Method	Sample Vol.	Filter	Preservation	Analytical Technique	Field/Lab Analysis
Fe(II)	2 ml	0.45 µm	100 µl HCl Optima	Spectrophotometer	Field
H_2S	3 ml	0.45 µm	0.5 ml zinc acetate	Spectrophotometer	Field
Alkalinity	1 ml	0.45 µm	chilled	Titration/Gran plot	Field
Inorganic Anions	1 ml	0.45 µm	10 µl Formaldehyde (stored at 4°c)	Capillary Electrophoresis	Lab
Inorganic Cations	1 ml	0.45 µm	10 µl HCl Optima (stored at 4°c)	Capillary Electrophoresis	Lab
NH_4^+	1 ml	0.45 µm	Flash freeze (dry ice)	Capillary Electrophoresis	Lab
Organic Acids	1 ml	0.45 µm	Flash freeze (dry ice)	Capillary Electrophoresis	Lab

*All sample were collected by filling sterile, acid washed plastic syringes and then syringe filtered into sterile, acid washed plastic vials containing the appropriate preservatives.

Iron (Fe(II)) Method

Fe^{2+} Determination: Phenanthroline Method

Reagents:

1. Certified Fe Standard 1000 ppm (i.e. Fisher, Baker) - to provide convert Fe^{0} to Fe^{2+} in standards
2. Hydroxylamine Hydrochloride. Solution:
   Add approximately 100 ml of DDW to a 250 ml volumetric flask. Then add 25.0 g of hydroxylamine hydrochloride and 12.5 ml of trace metal grade HCl and dilute to 250 ml with DDW.
3. Phenanthroline Reagent:
   Add approximately 100 ml of DDW to a 250 ml volumetric flask. Then add 0.250 g of phenanthroline and 100 µl of trace metal grade HCl and dilute to 250 ml with DDW. This may take a while to dissolve.
4. Ammonium Acetate Buffer Solution:
   Combine 62.5 g ammonium acetate, 37.5 ml DDW, and 175 ml glacial acetic acid in a 500 ml beaker.

Standard Preparation:

Blank (0 ppm Fe): Add approximately 50 ml of DDW to a 100 ml volumetric flask. Add 2 ml of trace metal grade HCl, 2 ml hydroxylamine hydrochloride solution, and dilute to 100 ml with DDW.

1.0 ppm Fe: Add approximately 50 ml of DDW to a 100 ml volumetric flask. Add 2 ml of trace metal grade HCl, 2 ml hydroxylamine hydrochloride solution, 100 µl certified Fe standard, and dilute to 100 ml with DDW. Allow at least 30 minutes for the sample to stabilize before analysis.

5

~~10.0~~ ppm Fe: Add approximately 50 ml of DDW to a 100 ml volumetric flask. Add 2 ml of trace metal grade HCl, 2 ml hydroxylamine hydrochloride solution, ~~1 ml~~ certified Fe standard, and dilute to 100 ml with DDW. Allow at least 30 minutes for the sample to stabilize before analysis.

500µL

10.0 ppm Fe: Add approximately 50 ml of DDW to a 100 ml volumetric flask. Add 2 ml of trace metal grade HCl, 2 ml hydroxylamine hydrochloride solution, 1 ml certified Fe standard, and dilute to 100 ml with DDW. Allow at least 30 minutes for the sample to stabilize before analysis.

15.0 ppm Fe: Add approximately 50 ml of DDW to a 100 ml volumetric flask. Add 2 ml of trace metal grade HCl, 2 ml hydroxylamine hydrochloride solution, 1.5 ml certified Fe standard, and dilute to 100 ml with

DDW. Allow at least 30 minutes for the sample to stabilize before analysis.

20.0 ppm Fe: Add approximately 50 ml of DDW to a 100 ml volumetric flask. Add 2 ml of trace metal grade HCl, 2 ml hydroxylamine hydrochloride solution, 2 ml certified Fe standard, and dilute to 100 ml with DDW. Allow at least 30 minutes for the sample to stabilize before analysis.

Method of Analysis (as conducted for the Cannelton project 1997-98):

1. Pipet 100 µl of trace metal grade HCl into sample container.
2. Add 3-5 ml of filtered sample to sample container.
3. Pipet 2.5 ml of acidified sample to a couvet.
4. Add 1.0 mL phenanthroline solution.
5. Add 0.5 mL ammonium acetate buffer solution.
6. Add 1.0 ml DDW to the sample.
7. Wait 2-5 minutes before analyzing sample on the spectrophotometer.

or

1. Pipet 100 µl of trace metal grade HCl into couvet.
2. Pipet 2.5 ml of filtered sample to a couvet.
3. Add 1.0 mL phenanthroline solution.
4. Add 0.5 mL ammonium acetate buffer solution.
5. Add 1.0 ml DDW to the sample.
6. Wait 2-5 minutes before analyzing sample on the spectrophotometer.

References:

AWWA. (1971) Standard Methods for the Examination of Water and Wastewater. 13th edition, 189-192.

Suzuki, Y., K. Kuma, I. Kudo, K. Hasebe, and K. Matsunaga. (1992) Existence of stable Fe(II) complex in oxic river water and its determination. Water Research 26(11), 1421-1424.

Iron Field Sheet

Norman Landfill Initials_____
Trip Dates:

Field Notes

Iron Test

Date:_____

Sample Preparation

1. 100 µL of HCl Optima®
2. 2 mL sample
3. 1 mL phenanthroline
4. 0.5 mL acetate buffer
5. 2 mL nanopure water
6. Wait 3 minutes

Blank Preparation

1. 100 µL of HCl Optima®
2. 2 mL blank
3. 1 mL phenanthroline
4. 0.5 mL acetate buffer
5. 2 mL nanopure water
6. Wait 3 minutes

Spec 20 D

Filter Position: 340-599 nm
Wavelength: 510 nm

Sample ID	Wavelength (nm)	Absorbance	Notes

Sulfide (H_2S) Method

Solute HS after Aller
Method: After Cline 1969

Reagents

A. .05 M Zinc acetate -2.75 g zinc acetate/250 ml DW. Stable.
B. Diamine reagent: 4.0 g anhydrous ferric chloride and 1.6 g N,N-dimethyl-p-phenyleme diamine sulfate/100ml (6N HCl, solvent). Stable for about 3 months refridgerated

Standards
Add a weighed amount of Na2S.9HoH corresponding to about 0.12 g to exactly 100 ml of deareated distilled water under N2. Immediately add 6 ml of this solution ot 50 ml deaerated distilled water. Immediately use 0.1,0.25,0.5, 0.75 and 1.0 ml of this solution ot prepare standards corresponding to about 60-600uM S as described below

Sample size 0.02-1.0 ml, Standards = 1ml total volume

Procedure

1. Pipet sample (standard) into a scintillation vial containing .5 ml of reagent A. Dilute to 1.5 ml total volume with distilled water (= DW + sample+ reagent A) and the sample is stable when refrigerated for about one month
2. Add 0.05 ml reagent B
3. wait 20 min and add 8 ml dw
4. after 15 min read sample absorbance at 670 nm in a 1 cm cell

Sulfide Field Sheet

Norman Landfill Initials_____
Trip Dates:

Field Notes

Sulfide Test

Date:_____

Sample Preparation

1. 0.5 mL Zinc Acetate
2. 3 mL sample
3. 0.5 mL Amine-Sulfuric/Ferric Chloride Reagent
4. 1 mL nanopure water
5. wait 20 minutes

Blank Preparation

1. 0.5 mL Zinc Acetate
2. 3 mL nanopure water
4. 0.5 mL Amine-Sulfuric/Ferric Chloride Reagent
4. 1 mL nanopure water
5. wait 20 minutes

Spec 20 D

Filter Position: 600-950 nm
Wavelength: 670 nm

Sample ID	Wavelength (nm)	Absorbance	Notes

Alkalinity Field Sheet

Norman Landfill Initials_____
Trip Dates:

Field Notes

Alkalinity

Date: _____
Sample #:_____
Time:_____
Temperature:_____
2 Points Calibration: _____ _____
Slope Calibration: _____
Volume used:_____

Number	Vol. of H_2SO_4	pH	Temperature
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			

NOTES:

Methods used to analyze samples with Agilent Technologies Capillary Electrophoresis System

Notes: For all capillary electrophoresis (CE) analyses the following vial designations were used.

Vial 3- inlet home vial (buffer, charge is applied to this vial)

Vial 4-outlet home vial (buffer, charge is applied to this vial)

Vial 5- buffer (for flushing)

Vial 6- waste

Vial 7- water (Nanopure, for flushing)

Vial 47- water (dunk, Nanopure, for rinsing capillary tips)

For CE analyses in which the replenishment system cannot be used due to buffer properties (ex. if buffer is a surfactant) additional methods are created with different home vials. In most cases buffer must be replaced and replenished after six analyses as it becomes degraded with the charge applied during each analysis.

For all analyses standards were made using trace metal grade stock solutions or salts and Nanopure water.

CE method details follow.

Method for Inorganic Anion and Organic Acid determination (Agilent Technologies Capillary Electrophoresis System)

The buffer used for this method is a chromate buffer made by Agilent Technologies. The capillary electrophoresis replenishment system cannot be used with this buffer so several duplicate methods were required with different home vials. This method is the same for analysis of both inorganic anions and organic acids with the exception of the sample stop time which is increased to 30 minutes for organic acid determination.

```
Method: C:\HPCHEM\1\METHODS\TKCHR.M of 10/2/2007 1:10:00 PM



                          Method Information

anion chromate buffer


                          Run Time Checklist

       Pre-Run Cmd/Macro:  off

        Data Acquisition:  on

  Standard Data Analysis:  on

Customized Data Analysis:  off

           Save GLP Data:  off

      Post-Run Cmd/Macro:  off

   Save Method with Data:  skipped - no ACQ running

                     CAPILLARY ELECTROPHORESIS

CE mode:                   CE

Home values:

   Lift Offset               4
   Cassette Temperature     25.00 °C
   Inlet Home Vial          3: Inlet Home
   Outlet Home Vial         4: Outlet Home



Replenishment and Preconditioning:
   serial processing


Replenishment Entries:
   No Replenishment used


Preconditioning Entries:

   Function     Parameter

   1  INLET     47: nanowater
   2  FLUSH     5.00 min, I:5: Buffer, O:6: waste
```

Method: C:\HPCHEM\1\METHODS\TKCHR.M of 10/2/2007 1:10:00 PM

Postcondition Entries:

	Function	Parameter
1	INLET	47: nanowater
2	FLUSH	5.00 min, I:7: blank (nanowater, O:6: waste

Electric:

Electric	On
Polarity	Negative
Voltage	15.00 kV
Current	14.00 µA
Power	System Limit
Low Current Limit	0.00 µA

Injection Table Entries:

	Function	Parameter
1	PRESSURE	35.0 mbar, 2.0 sec, I:5: Buffer, O:6: waste
2	PRESSURE	35.0 mbar, 10.0 sec, I:InjectVial, O:6: waste
3	PRESSURE	35.0 mbar, 2.0 sec, I:5: Buffer, O:6: waste

Store Data:

Collect voltage	Yes
Collect current	Yes
Collect power	Yes
Collect pressure	Yes
Collect temperature	Yes

Time entries:

Stoptime	22.00 min
Posttime	Off

DIODE ARRAY DETECTOR

Settings:

Stop Time	as CE: 22.00 min
Post Time	Off
Response Time	2.6 sec
Peakwidth	>0.2 min
Prerun Autobalance	On
Postrun Autobalance	Off

Spectrum:

Method: C:\HPCHEM\1\METHODS\TKCHR.M of 10/2/2007 1:10:00 PM

Store	None
From	200 nm
To	350 nm
Threshold	40.00 mAu

Signals:

	Store	Signal,Bw	Reference,Bw	[nm]
A:	Yes	315 5	375 30	
B:	Yes	510 10	375 30	
C:	Yes	325 10	280 40	
D:	Yes	325 10	375 40	
E:	Yes	315 20	375 40	

Contacts:

Contact 1	Off
Contact 2	Off

Method: C:\HPCHEM\1\METHODS\TKCHR.M of 10/2/2007 1:10:00 PM

Weight : Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Migration Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1: DAD1 B, Sig=230,20 Ref=400,60

MigTime [min]	Sig	Lvl	Amount [mg/L]	Area	Amt/Area	Ref	Grp	Name
6.894	1	1	5.00000e-1	1.89340	2.64076e-1			Cl
		2	1.00000	3.00392	3.32898e-1			
		3	10.00000	26.43315	3.78313e-1			
		4	50.00000	122.46768	4.08271e-1			
7.054	1	1	5.00000e-1	5.55984e-1	8.99306e-1			Br
		2	1.00000	1.25199	7.98728e-1			
		3	10.00000	11.75681	8.50571e-1			
		4	50.00000	56.31440	8.87872e-1			
7.161	1	1	5.00000e-1	1.30739	3.82441e-1			NO2
		2	1.00000	2.25812	4.42846e-1			
		3	10.00000	20.99429	4.76320e-1			
		4	50.00000	96.79285	5.16567e-1			
7.386	1	1	5.00000e-1	1.48307	3.37139e-1			SO4
		2	1.00000	2.38914	4.18560e-1			
		3	10.00000	21.32265	4.68985e-1			
		4	50.00000	99.92426	5.00379e-1			
7.676	1	1	5.00000e-1	1.91689	2.60839e-1			NO3
		2	1.00000	2.70149	3.70166e-1			
		3	10.00000	21.14075	4.73020e-1			
		4	50.00000	98.25453	5.08882e-1			
8.408	1	1	5.00000e-1	4.23595	1.18037e-1			F
		2	1.00000	8.63875	1.15757e-1			
		3	10.00000	88.18819	1.13394e-1			
		4	50.00000	405.50391	1.23303e-1			
8.851	1	1	1.00000	6.02757	1.65904e-1			PO4
		2	2.00000	13.38817	1.49386e-1			
		3	20.00000	131.45103	1.52148e-1			
		4	100.00000	633.72565	1.57797e-1			

Methods for Inorganic Cation Method and NH_4^+ (Agilent Technologies Capillary Electrophoresis System)

Two methods are used to run these analyses. The methods are the same except that one method is used to replenish buffer vials and empty the waste vial. The buffer used in for these methods is dimethyldiphenylphosphonium hydroxide (DDP) made by Agilent Technologies. This method is the same for analysis of both inorganic cation and NH_4^+ with the exception of the sample stop time which is increased to 30 minutes for organic cation determination.

```
Method: C:\HPCHEM\1\METHODS\TAKDDP.M of 7/28/2007 5:43:54 PM


                          Method Information

NH4 method using cation DDP buffer


                          Run Time Checklist

       Pre-Run Cmd/Macro:  off

        Data Acquisition:  on

  Standard Data Analysis:  off

Customized Data Analysis:  off

           Save GLP Data:  off

      Post-Run Cmd/Macro:  off


   Save Method with Data:  off

                     CAPILLARY ELECTROPHORESIS

CE mode:                   CE

Home values:

   Lift Offset               4
   Cassette Temperature     25.00 °C
   Inlet Home Vial          3: Inlet Home
   Outlet Home Vial         4: Outlet Home



Replenishment and Preconditioning:
   serial processing



Replenishment Entries:
   No Replenishment used



Preconditioning Entries:

   Function     Parameter

   1  FLUSH     5.00 min, I:5: Buffer, O:6: waste

Postcondition Entries:

   Function     Parameter
```

Method: C:\HPCHEM\1\METHODS\TAKDDP.M of 7/28/2007 5:43:54 PM

1	INLET	47:
2	FLUSH	5.00 min, I:7: blank (nanowater, O:6: waste

Electric:

Electric	On
Polarity	Positive
Voltage	20.00 kV
Current	300.0 µA
Power	System Limit
Low Current Limit	0.00 µA

Injection Table Entries:

	Function	Parameter
1	PRESSURE	50.0 mbar, 3.0 sec, I:InjectVial, O:6: waste
2	PRESSURE	50.0 mbar, 2.0 sec, I:InHomeVial, O:6: waste

Store Data:

Collect voltage	Yes
Collect current	Yes
Collect power	Yes
Collect pressure	Yes
Collect temperature	Yes

Time entries:

Stoptime	7.00 min
Posttime	Off

DIODE ARRAY DETECTOR

Settings:

Stop Time	no Limit
Post Time	Off
Response Time	2.6 sec
Peakwidth	>0.2 min
Prerun Autobalance	On
Postrun Autobalance	Off

Spectrum:

Store	None
From	190 nm
To	600 nm
Threshold	100.00 mAu

Method: C:\HPCHEM\1\METHODS\TAKDDP.M of 7/28/2007 5:43:54 PM

Signals:

	Store	Signal,Bw	Reference,Bw	[nm]
A:	Yes	310 60	200 20	
B:	Yes	228 20	216 20	
C:	Yes	250 20	200 20	
D:	Yes	210 40	228 20	
E:	Yes	450 80	230 20	

Contacts:

Contact 1	Off
Contact 2	Off

Method: C:\HPCHEM\1\METHODS\TAKDDP.M of 7/28/2007 5:43:54 PM

Vialtable Information for Method:

Vial#	Used in	Name	Comment
3	METHOD	Inlet Home	
4	METHOD	Outlet Home	
5	METHOD	Buffer	
6	METHOD	waste	
7	METHOD	blank (nanowater	
47	METHOD		

Fraction Collection: OFF

Method: C:\HPCHEM\1\METHODS\TAKDDP.M of 7/28/2007 5:43:54 PM

Specify Report

Destination:	Screen
Quantitative Results sorted by:	Signal
Report Style:	Short
Sample info on each page:	No
Add Electropherogram Output:	Yes
Electropherogram Output:	Portrait
Size in Time direction:	100 % of Page
Size in Response direction:	40 % of Page

Signal Options

Include: Axes, Migration Times, Baselines, Tick Marks
Font: Arial, Size: 8

Ranges: Full
Multi Electropherograms: Separated, Each in full Scale

Calibration Table

Calib. Data Modified :

Calculate	:	Area Percent
Area Calculation Mode	:	Measured Area
Rel. Reference Window	:	5.000 %
Abs. Reference Window	:	0.000 min
Rel. Non-ref. Window	:	5.000 %
Abs. Non-ref. Window	:	0.000 min

Use Multiplier & Dilution Factor with ISTDs

Uncalibrated Peaks	:	not reported
Partial Calibration	:	Yes, identified peaks are recalibrated
Correct All Mig. Times:		No, only for identified peaks
Curve Type	:	Linear
Origin	:	Included
Weight	:	Equal

Recalibration Settings:

Average Response	:	Average all calibrations
Average Migration Time:		Floating Average New 75%

Calibration Report Options :
- Printout of recalibrations within a sequence:
  - Calibration Table after Recalibration
  - Normal Report after Recalibration
- If the sequence is done with bracketing:
  - Results of first cycle (ending previous bracket)

DDP Replenishment Method

Method: C:\HPCHEM\1\METHODS\TAKDDPRP.M of 7/28/2007 5:45:43 PM

Method Information

NH4 method using cation DDP buffer

Run Time Checklist

Pre-Run Cmd/Macro:	off
Data Acquisition:	on
Standard Data Analysis:	off
Customized Data Analysis:	off
Save GLP Data:	off
Post-Run Cmd/Macro:	off
Save Method with Data:	off

CAPILLARY ELECTROPHORESIS

CE mode: CE

Home values:

Lift Offset	4
Cassette Temperature	25.00 °C
Inlet Home Vial	3: Inlet Home
Outlet Home Vial	4: Outlet Home

Replenishment and Preconditioning:
serial processing

Replenishment Entries:

	Function	Parameter
1	REPLENISH	1.4 cm, InHomeVial
2	REPLENISH	1.4 cm, 5: Buffer
3	REPLENISH	1.4 cm, OutHomeVial
4	EMPTY	6: waste

Preconditioning Entries:

	Function	Parameter
1	FLUSH	5.00 min, I:5: Buffer, O:6: waste

Method: C:\HPCHEM\1\METHODS\TAKDDPRP.M of 7/28/2007 5:45:43 PM

Postcondition Entries:

	Function	Parameter
1	INLET	47:
2	FLUSH	5.00 min, I:7: blank (nanowater, O:6: waste

Electric:

Electric	On
Polarity	Positive
Voltage	20.00 kV
Current	300.0 µA
Power	System Limit
Low Current Limit	0.00 µA

Injection Table Entries:

	Function	Parameter
1	PRESSURE	50.0 mbar, 3.0 sec, I:InjectVial, O:6: waste
2	PRESSURE	50.0 mbar, 2.0 sec, I:InHomeVial, O:6: waste

Store Data:

Collect voltage	Yes
Collect current	Yes
Collect power	Yes
Collect pressure	Yes
Collect temperature	Yes

Time entries:

Stoptime	7.00 min
Posttime	Off

DIODE ARRAY DETECTOR

Settings:

Stop Time	no Limit
Post Time	Off
Response Time	2.6 sec
Peakwidth	>0.2 min
Prerun Autobalance	On
Postrun Autobalance	Off

Spectrum:

Store	None
From	190 nm
To	600 nm
Threshold	100.00 mAu

Signals:

	Store	Signal,Bw		Reference,Bw		[nm]
A:	Yes	310	60	200	20	
B:	Yes	228	20	216	20	
C:	Yes	250	20	200	20	
D:	Yes	210	40	228	20	
E:	Yes	450	80	230	20	

Contacts:

Contact 1	Off
Contact 2	Off

Method: C:\HPCHEM\1\METHODS\TAKDDPRP.M of 7/28/2007 5:45:43 PM

Vialtable Information for Method:

Vial#	Used in	Name	Comment
3	METHOD	Inlet Home	
4	METHOD	Outlet Home	
5	METHOD	Buffer	
6	METHOD	waste	
7	METHOD	blank (nanowater	
47	METHOD		

Fraction Collection: OFF

Specify Report

Destination:	Screen
Quantitative Results sorted by:	Signal
Report Style:	Short
Sample info on each page:	No
Add Electropherogram Output:	Yes
Electropherogram Output:	Portrait
Size in Time direction:	100 % of Page
Size in Response direction:	40 % of Page

Signal Options

Include: Axes, Migration Times, Baselines, Tick Marks
Font: Arial, Size: 8

Ranges: Full
Multi Electropherograms: Separated, Each in full Scale

Calibration Table

Calib. Data Modified :

Calculate	:	Area Percent
Area Calculation Mode	:	Measured Area
Rel. Reference Window	:	5.000 %
Abs. Reference Window	:	0.000 min
Rel. Non-ref. Window	:	5.000 %
Abs. Non-ref. Window	:	0.000 min
Use Multiplier & Dilution Factor with ISTDs		
Uncalibrated Peaks	:	not reported
Partial Calibration	:	Yes, identified peaks are recalibrated
Correct All Mig. Times:		No, only for identified peaks
Curve Type	:	Linear
Origin	:	Included
Weight	:	Equal

Recalibration Settings:

Average Response	:	Average all calibrations
Average Migration Time:		Floating Average New 75%

APPENDIX III

NOGEE SAMPLING PROCEDURE

1) Clear the bottom tube before sampling
    a. Connect bottom tube to pump tubing
    b. Connect top tube to tedlar bag fill with argon gas; open valve
    c. Turn pump on and fill syringe to 8 ml (volume in tubing)
    d. Turn pump off
    e. Get clean syringe and sample vials
    f. Turn pump back on, waste a few drops and collect samples
2) Pumping test solution in
    a. Connect tedlar bag containing test solution to pump tubing
    b. Disconnect bottom tube from pump
    c. Clear test solution tubing by switching pump direction and pumping to waste for about 2 minutes
    d. Close tedlar gas bag and disconnect top tube
    e. Reconnect bottom tube to pump tubing and continue pumping until approximately ~100 ml (or 3 times the chamber volume) has flushed through
    f. *pumping rate should never exceed 100 ml/min
3) Clear top tube with 6 ml of argon gas
    a. Why? To help eliminate mixing with test solution “in-line” during sampling
    b. Fill a syringe with argon gas from tedlar bag, close tedlar bag and waste syringe to atmosphere (repeat a couple of times)
    c. After final fill of syringe with argon, close tedlar bag and disconnect syringe while gently forcing argon out of the syringe
    d. While continually forcing gas from syringe carefully attach top tube and stop push gas
    e. Disconnect bottom tube from pump
    f. Force 8 ml (volume of tubing) of gas into top tube; this should force solution out of the bottom tube.
    g. Seal tubing ends (be sure to not let any oxygen in)

VITA

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Publications

Kneeshaw, T.A., McGuire, J.T., Smith, E.W., Cozzarelli, I.M., 2007. Evaluation of sulfate reduction at experimentally induced mixing interfaces using small-scale push-pull tests in an aquifer-wetland system. Applied Geochemistry, 22, 2618-2629.