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Evaluation of sulfate reduction at experimentally induced mixing interfaces using small-scale push–pull tests in an aquifer–wetland system

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Abstract

This paper 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, SO_4^{2-}-rich anaerobic aquifer water (~ 100 mg/L SO_4^{2-}) was introduced into SO_4^{2-}-depleted wetland porewater via push–pull tests. Results showed SO_4^{2-} reduction was stimulated by the mixing of these waters and first-order rate coefficients were comparable to those measured in other push–pull studies. 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 SO_4^{2-} 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 the apparent rate is actually a function of time and that complexities in rate data be considered.

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) (Vroblesky and Chapelle, 1994; McGuire

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et al., 2000; van Breukelen and Griffioen, 2004; Báez-Cazull et al., 2007) indicating high levels of reactivity at sharp interfaces. At interface zones, biogeochemical activity is enhanced by the availability of limiting electron acceptors such as O_2, Fe(III), NO_3^-, and 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).

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) (Báez-Cazull et al., 2007) 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, is 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, (Wilson et al., 1983; D'Angelo and Reddy, 1999; Cozzarelli et al., 2000) analysis of geochemistry data (Lovley and Goodwin, 1988; Chapelle et al., 1996), 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 (Schroth et al., 1998; Luthy et al., 2000; Istok et al., 2001; McGuire et al., 2002; Harris et al., 2005). 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 3 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. (1998) and 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 (Luthy et al., 2000; Cunningham et al., 2001; Istok et al., 2001; Schroth et al., 2001; Kleikemper et al., 2002; McGuire, 2002; Ulrich et al., 2003; Harris et al., 2005). 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 community 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 (Luthy et al., 2000; Istok et al., 2001; Schroth et al., 2001; Addy et al., 2002; Kleikemper et al., 2002; McGuire et al., 2002). 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 within the complex aquifer–wetland system that exhibit steep geochemical gradients, representing several important mixing zones (Báez-Cazull et al., 2007). This study utilized push–pull tests designed to help 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.

2. 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 C (DOC), Cl^-, NH_3 and CH_4 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 (Fig. 1).

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 (Schlottmann et al., 1999; Cozzarelli et al., 2000; Eganhouse et al., 2001; Grossman et al., 2002), documentation of the microbiological processes (Beeman and Suflita, 1987; Harris et al., 1999; Ulrich et al., 2003), kinetic studies (Beeman and Suflita, 1990; Adrian et al., 1994; Senko et al., 2002), and quantification of groundwater–surface water fluctuations at the site (Christenson and Cozzarelli, 1999; Schlottmann et al., 1999; Scholl, 2000).

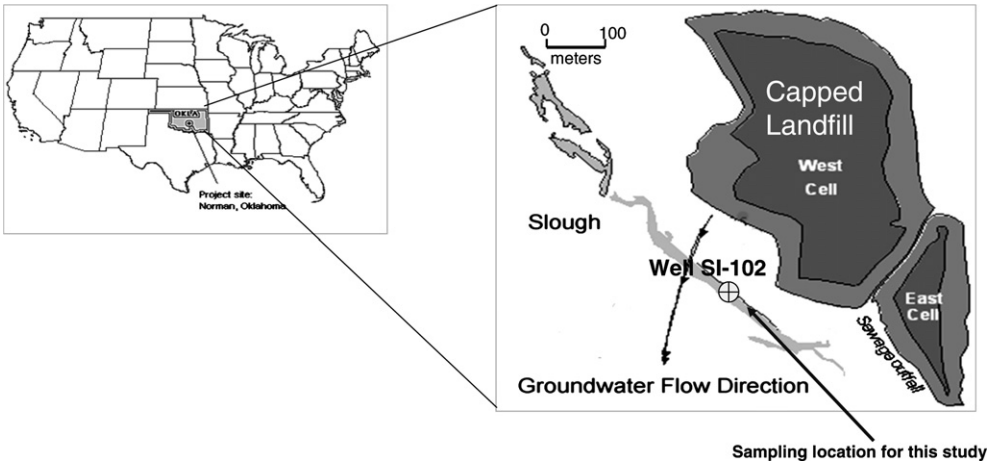

Fig. 1. Map showing study site location, Norman Landfill Research Site, Norman, OK, USA.

3. Methods

3.1. Push–pull well instrumentation

Small-scale push–pull wells were constructed from 3.34 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 plug 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 reducing coarse sand layer between two silty clay layers at 41.5–53 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 reducing), 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 (Fig. 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.

3.2. Push–pull tests

Four push–pull tests, referred to as PPT1 through PPT4, were performed during two separate

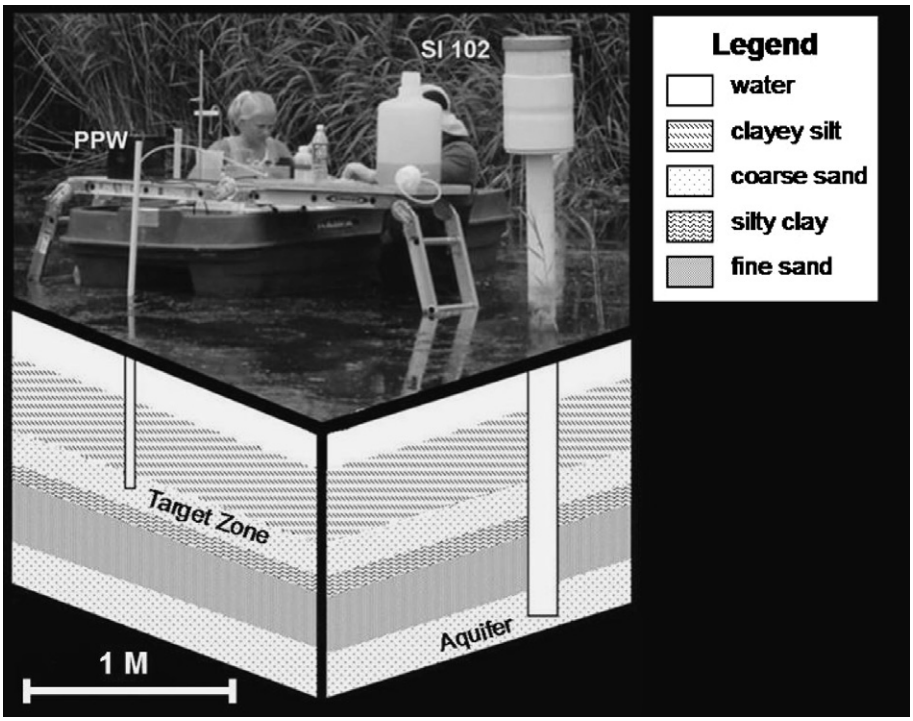

Fig. 2. Schematic of study site showing location of well SI 102 (source of injection water) and the push–pull well (PPW).

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 SO_4^{2-} 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, Figs. 1 and 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 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 (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 °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^-), NH_4^+, 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).

3.3. 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:

$$\ln\left(\frac{C_d(t^*)}{C_{tr}(t^*)}\right) = \ln\left[\frac{(1-e)^{kt_{inj}}}{kt_{inj}}\right] - kt^*, \quad (1)$$

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 Eq. (1) must be corrected using a mixing ratio following Eqs. 2 and 3, respectively (McGuire, 2002):

$$C_d = \left(\frac{d_m(t^*) - d_b}{X}\right), \quad (2)$$

$$C_t r = \left(\frac{tr_m(t^*) - tr_b}{X}\right), \quad (3)$$

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 $C_d = d_m$.

4. Results and discussion

4.1. 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 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-} (Fig. 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., 2001).

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 h. After a 22 h lag phase, SO_4^{2-} decreased coincident with an increase in H_2S indicating SO_4^{2-} reduction (Fig. 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 h. 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

Table 1
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

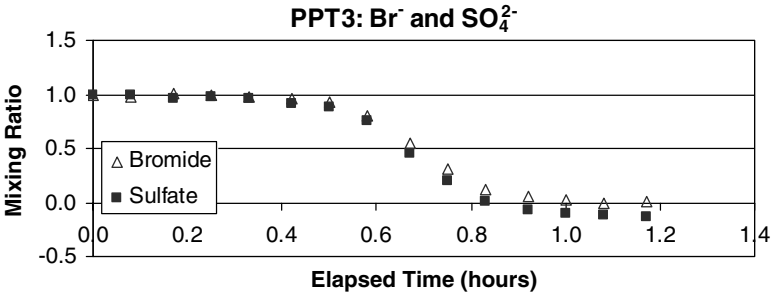

Fig. 3. Example comparison of breakthrough curves for conservative tracer, Br^-, and reactive solute, SO_4^{2-} (results from PPT3).

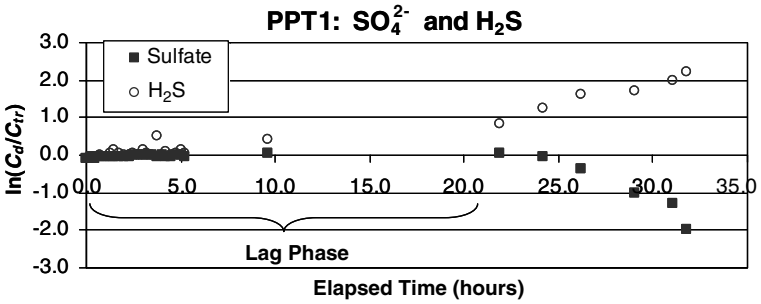

Fig. 4. Example rate data for H_2S and SO_4^{2-} (results from PPT1) showing an increase of H_2S coincident with a decrease in SO_4^{2-} indicating SO_4^{2-} reduction.

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 the results. It should be noted that this study design cannot distinguish any "background" SO_4^{2-} reduction that may be occurring in the native wetland porewater from SO_4^{2-} reduction stimulated by the push–pull tests, particularly given the heterogeneous nature of wetland sediments.

4.2. Nature and cause of lag phase

PPT2 duplicated the test conditions of PPT1 but was conducted over a shorter period of time (3.5 h versus 32 h). A lag phase was again observed but in this case it was only ~2.4 h long, compared to the ~22 h lag phase observed in PPT1 (Fig. 5) suggesting that a standard incubation period is not required. Interestingly, the lag phase again coincided with the extraction of the majority of the

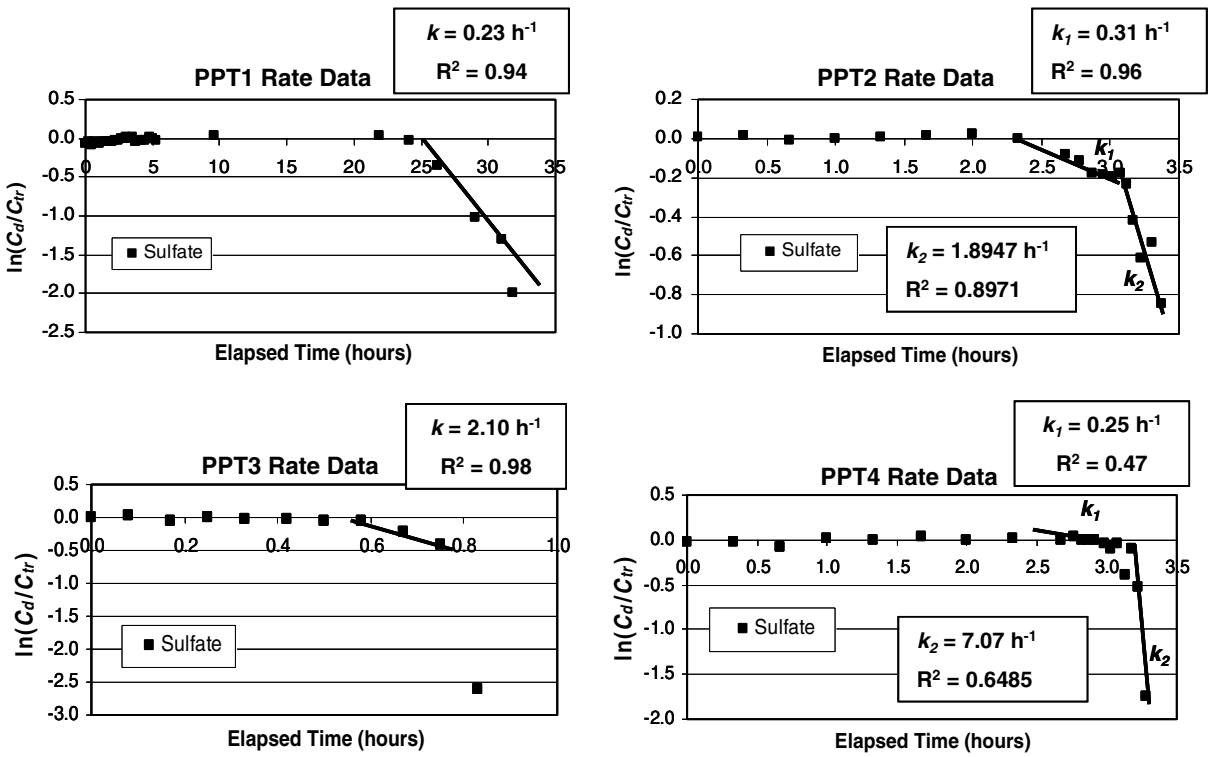

Fig. 5. First-order rate data. Rate coefficients were determined on portions of the dataset by linear regression. Solid lines show data points used to determine rates.

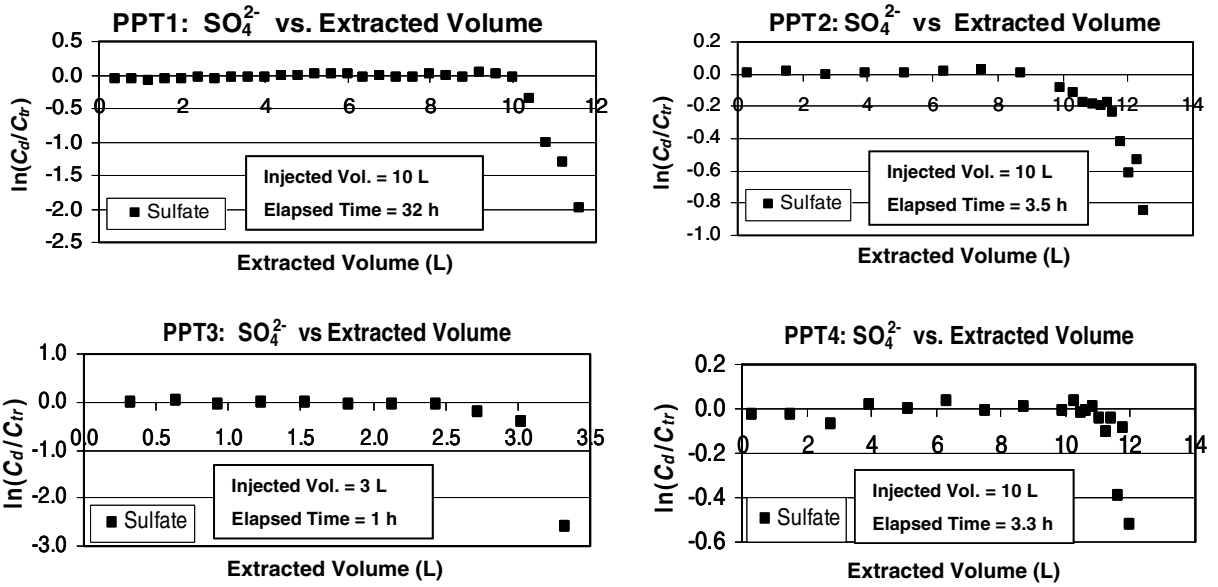

Fig. 6. Plots of first-order rate data for SO_4^{2-} versus volume of test solution extracted (L) demonstrating that SO_4^{2-} reduction occurs irrespective of injected volume or total elapsed time.

injected test solution, (Fig. 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 (Fig. 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.

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-} (Fig. 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

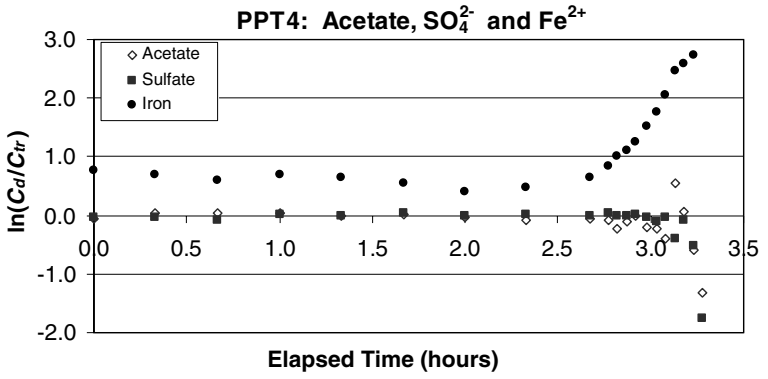

Fig. 7. Rate data showing a lag phase followed by the production of Fe^{2+} (indicating Fe(III) reduction) and the simultaneous consumption of acetate and sulfate (indicating SO_4^{2-} reduction).

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.

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 (Fig. 7). Though Fe(III) was not directly measured in these tests the increase in Fe^{2+} was interpreted to be an indicator of Fe 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 Fe-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 (e.g. H_2S) (Stumm and Morgan, 1996; Kostka et al., 2002). 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 (Báez-Cazull et al., 2007). 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 (Domenico 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 cations 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 release 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 community explaining the lack of reaction prior to extraction of the mixing interface.

4.3. 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 Eq. (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 (Fig. 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 (Luthy et al., 2000; Istok et al., 2001; Schroth et al., 2001; Kleikemper et al., 2002; McGuire et al., 2002; Harris et al., 2005). For PPT1 the determined rate coefficient for SO_4^{2-} reduction was approximately $0.23\ h^{-1}$ ($R^2 = 0.9398$) (Fig. 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 (Phanikumar and McGuire, in review) may be required.

5. 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 SO_4^{2-} reduction at mixing interfaces.

In all push–pull tests, a lag phase was observed prior to the TEAPs SO_4^{2-} and Fe 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 SO_4^{2-} 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 SO_4^{2-} 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.

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