

Rates of As and Trace-Element Mobilization Caused by Fe Reduction in Mixed BTEX–Ethanol Experimental Plumes

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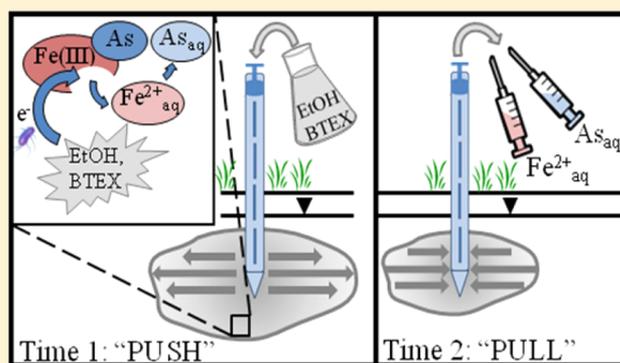
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Supporting Information

ABSTRACT: Biodegradation of organic matter, including petroleum-based fuels and biofuels, can create undesired secondary water-quality effects. Trace elements, especially arsenic (As), have strong adsorption affinities for Fe(III) (oxyhydr)-oxides and can be released to groundwater during Fe-reducing biodegradation. We investigated the mobilization of naturally occurring As, cobalt (Co), chromium (Cr), and nickel (Ni) from wetland sediments caused by the introduction of benzene, toluene, ethylbenzene, and xylenes (BTEX) and ethanol mixtures under iron- and nitrate-reducing conditions, using in situ push–pull tests. When BTEX alone was added, results showed simultaneous onset and similar rates of Fe reduction and As mobilization. In the presence of ethanol, the maximum rates of As release and Fe reduction were higher, the time to onset of reaction was decreased, and the rates occurred in multiple stages that reflected additional processes. The concentration of As increased from $<1 \mu\text{g/L}$ to a maximum of $99 \mu\text{g/L}$, exceeding the $10 \mu\text{g/L}$ limit for drinking water. Mobilization of Co, Cr, and Ni was observed in association with ethanol biodegradation but not with BTEX. These results demonstrate the potential for trace-element contamination of drinking water during biodegradation and highlight the importance of monitoring trace elements at natural and enhanced attenuation sites.



INTRODUCTION

Biodegradation of contaminated groundwater can be an effective means of removing organic contaminants and reducing the risk of human exposure to harmful chemicals. However, the natural environment in which these reactions occur is complex, and undesired reactions such as the mobilization of trace elements from contaminated sediments can occur and negatively impact water quality.^{1–3} Predicting the effects of biodegradation becomes more difficult when a mixture of contaminants such as petroleum hydrocarbons and biofuels is released to the environment, resulting in complex biodegradation rates and mechanisms.

Often, remediation at a contaminated site focuses on the primary contaminant (e.g., crude oil, landfill leachate, or human and animal waste). However, both natural attenuation and enhanced remediation strategies may amplify secondary water quality effects initiated during the remediation process. Furthermore, contaminated sites commonly contain mixtures of co-contaminants that make it more difficult to predict the overall effect that biodegradation has on water quality and human health. For example, the addition of ethanol to hydrocarbon plumes containing BTEX, either by addition to transport fuels or via accidental release, can impact BTEX biodegradation rates and mechanisms.^{4–8} The addition of labile organic matter can also cause the reduction of Fe(III) and the

release of trace elements associated with Fe(III) to groundwater.^{2,3}

The release of arsenic (As) to groundwater is of concern because As is known to adversely affect human health as both a toxin and a carcinogen.⁹ Although a $10 \mu\text{g/L}$ limit for drinking water has been set by the U.S. Environmental Protection Agency and World Health Organization,^{10,11} recent studies have found that adverse effects can occur at chronic exposures less than $10 \mu\text{g/L}$. A recent study observed lower intelligence quotients (IQs) in children chronically exposed to $5 \mu\text{g/L}$ As.¹² Another study showed that As exposure at $6 \mu\text{g/L}$ may be linked to the development of type 2 diabetes.¹³

Arsenic exposure can stem from a variety of natural and anthropogenic sources. It is a naturally occurring contaminant present in soils, sediment, and rocks, with an average concentration in U.S. soil of 5.2 mg/kg ,^{14,15} and correlates with Fe(III) (oxyhydr)-oxides in sediments due to strong adsorption affinities.^{16–18} This relationship is stable under oxic conditions in the absence of pH change or introduction of oxyanions, such as phosphate, that can compete with As for

Received: May 11, 2015

Revised: October 14, 2015

Accepted: October 20, 2015

Published: October 20, 2015

adsorption sites. However, reducing conditions stimulated by an influx of organic carbon can cause the microbial reduction of Fe(III) (oxyhydr)-oxides coupled with oxidation of carbon, releasing aqueous Fe^{2+} to groundwater and, along with it, adsorbed As.^{3,19,20} This reductive dissolution mechanism is thought to be the main contributor to As-contaminated groundwater in glacial and alluvial aquifers in the Midwest United States.²¹

Much of the work showing the reductive dissolution of As from Fe(III) (oxyhydr)-oxides stems from Southeast Asia, where the organic carbon source is naturally occurring in deltaic aquifers.^{21–25} However, recent studies have shown that As can also be mobilized from Fe(III) (oxyhydr)-oxides stimulated by an influx of anthropogenic carbon.^{2,3,26–28} Cozzarelli et al.³ recently observed concentrations of As in groundwater, reaching 230 $\mu\text{g/L}$ in the Fe-reducing zone of the petroleum-contaminated surficial sand-and-gravel aquifer at Bemidji. Concentrations in the plume are well above the USEPA 10 $\mu\text{g/L}$ limit for drinking water. Another study observed that leachate from a landfill created Fe-reducing conditions that released naturally occurring As to groundwater.² Although these studies document the occurrence of aqueous As from aquifer sediments due to Fe reduction coupled with the oxidation of organic carbon, little is known about the rates and timing at which these mechanisms occur. Furthermore, the effects of simultaneous release of competing electron donors (e.g., BTEX and ethanol) on reaction mechanisms and rates are poorly understood. Several studies have shown that a co-release of ethanol and petroleum hydrocarbons can cause different BTEX plume properties than releases without ethanol. Examples include ethanol stimulating a more rapid growth in selected microbial communities,^{29,30} faster consumption of electron acceptors,^{7,31,32} and plume elongation.^{4,32,33} These properties may also affect the kinetics and magnitude at which As and other associated trace elements are released from sediments.

In this study, we report field-derived rates of release of naturally occurring As, as well as Co, Cr, and Ni, to groundwater caused by microbial Fe reduction coupled with the biodegradation of BTEX and ethanol mixtures. Our field study used push–pull tests to estimate the kinetic influences of competing electron donors such as BTEX and ethanol and electron acceptors, Fe(III) and NO_3^- , on the mobilization of As, Co, Cr, and Ni from wetland sediments at a wetland-aquifer interface. This study reports: (1) the initial onset of mobilization; (2) mobilization rate estimations during various stages of release; (3) the overall magnitude of element release (i.e., the maximum concentration of mobilized elements); and (4) the duration of mobilization. Currently, trace elements in groundwater are not commonly monitored at sites where organic carbon is attenuated by biodegradation. Results from this study show that the biodegradation of organic contaminants can release trace elements at rates fast enough to elevate groundwater concentrations above drinking-water standards over short time frames (days), highlighting the importance of monitoring trace elements at these sites.

EXPERIMENTAL SECTION

Site Description. In 1979, a crude oil pipeline ruptured near Bemidji, Minnesota, releasing 10 700 barrels of crude oil onto a glacial outwash deposit.³⁴ Oil flowed over land and settled in topographic depressions, where it percolated through the unsaturated zone and settled on the water table, forming

subsurface oil pools and, later, dissolved hydrocarbon plumes. The wetland used as the study site for this research was contaminated by the southernmost overland flow of oil.³⁵ Detectable levels of BTEX remain in the wetland subsurface, and groundwater chemistry indicates that Fe reduction and methanogenesis are the dominant redox processes in the wetland (Table 1). Over 30 years of interdisciplinary research

Table 1. Initial Geochemical Conditions in Wetland Drive-Point Wells

| parameter | PPB | PPBE | PPBN | PPBEN |
|---------------------------|-------|-------|-------|-------|
| pH | 6.5 | 6.7 | 6.0 | 5.9 |
| NO_3^- (mg/L) | <0.03 | <0.03 | <0.03 | <0.02 |
| Mn^{2+} (mg/L) | <0.5 | <0.5 | <0.5 | <0.5 |
| Fe (mg/L) | 3.6 | 4.1 | 9.1 | 6.1 |
| SO_4^{2-} (mg/L) | <0.06 | <0.06 | <0.05 | <0.07 |
| CH_4 (mg/L) | 17.63 | 17.53 | 13.18 | 10.2 |
| As ($\mu\text{g/L}$) | <1 | <1 | 57 | 1.2 |
| Cr ($\mu\text{g/L}$) | <10 | <10 | 116 | <10 |
| Co ($\mu\text{g/L}$) | 1.1 | 1.3 | 4.3 | 1.7 |
| Ni ($\mu\text{g/L}$) | 34.1 | 22.8 | 55.6 | 30.8 |
| NVDOC (mg/L C) | 22.0 | 21.6 | 54.9 | 50.7 |

has been conducted at the site, making the Bemidji site the most thoroughly studied hydrocarbon-spill site in the world. Research at the site has resulted in over 200 publications (see <http://toxics.usgs.gov/bib/bib-bemidji.html>).

Push–Pull Tests. A total of four single-well push–pull tests were conducted in individual drive-point wells in the sandy subsurface of a wetland screened 65–90 cm below land surface. Each well was equipped with a 122 cm long, 0.16 cm inner diameter polyethylene tube for pumping into and out of the wetland. The volume for each pumping tube totaled 19.3 cm^3 . Drive-point wells were spaced approximately 2 m apart to ensure there was no mixing of test solutions between wells. On the basis of an estimated porosity of 0.3, we determined that the maximum radial influence of the injected volume for a single test is ~ 0.16 m from the well. Push–pull tests for this study consisted of three phases.^{36,37} In phase one, unique test solutions were prepared for each of the four push–pull tests. For each test, 5 L of groundwater from the Fe-reducing zone of the contaminated aquifer were pumped into a clean N_2 -flushed anaerobic Kynar bag using a submersible Keck pump. Aquifer water was selected as the test solution solvent to simulate an aquifer–wetland interface upon injection because several studies have shown that the bulk of biogeochemical reactions occur at mixing interfaces of geochemically distinct waters.^{38–41} Test solutions were amended with a conservative tracer (Cl^- or Br^-) and reactive solutes. The four test solutions (Table 2) included BTEX compounds that were added for test PPB to evaluate the impact of BTEX alone. BTEX and ethanol were added in PPBE to test the effect of two competing electron donors with different labilities on biodegradation and consequent trace-metal release rates. Nitrate was added to two tests (PPBN with BTEX and PPBEN with BTEX and ethanol). Nitrate, which is thermodynamically preferred over Fe(III), was added to evaluate if a shift in redox conditions to NO_3^- reduction would promote the stability of Fe(III) and inhibit the release of adsorbed As to groundwater. In phase two, the well-mixed test solutions were rapidly injected, or “pushed,” into the wetland drive-point wells at ~ 150 mL/min using a peristaltic pump. In phase three, 120 mL aliquots of sample

Table 2. Test Solutions for Push–Pull Tests

| analyte | PPB | PPBE | PPBN | PPBEN |
|---|-------|-------|------|-------|
| electron donor | | | | |
| benzene ($\mu\text{g/L}$) | 753 | 738 | 820 | 756 |
| toluene ($\mu\text{g/L}$) | 403 | 307 | 463 | 415 |
| ethylbenzene ($\mu\text{g/L}$) | 271 | 165 | 332 | 276 |
| <i>m/p</i> -Xylenes ($\mu\text{g/L}$) | 470 | 271 | 547 | 471 |
| <i>o</i> -Xylene ($\mu\text{g/L}$) | 282 | 179 | 325 | 298 |
| ethanol (mg/L) | – | 154 | – | 148 |
| electron acceptor | | | | |
| NO_3^- (mg/L) | <0.03 | <0.03 | 101 | 102 |
| SO_4^{2-} (mg/L) | <0.06 | 0.31 | 0.33 | <0.07 |
| tracer | | | | |
| Br^- or Cl^- (mg/L) | 111 | 80.3 | 103 | 76.7 |

were manually extracted, or “pulled,” from each well using syringes. The first 60 mL were purged to ensure that stagnant water sitting in the 19.3 cm³ tube was discarded, and water used for analysis was from the wetland. The second 60 mL portions were used for analyses. Samples were collected over a two month period, and the volume extracted during each test totaled approximately 2.0 L.

Water Analyses. Water samples collected for measurement of CH₄ concentrations were collected in Glaspak syringes that were connected directly to the syringe.⁴² The water samples for CH₄ analyses were transferred from the syringe into 25 mL serum bottles containing mercuric chloride (resulting in a concentration of 0.2 mM Hg) or TSP (trisodium phosphate dodecahydrate). Dissolved CH₄ concentrations were measured by headspace analysis using a 5890 Series II HP gas chromatograph split and splitless inlet FID (flame ionization detector) with a fused silica capillary column. Water samples collected for anions (Cl^- , Br^- , SO_4^{2-} , NO_3^- , NO_2^- , and PO_4^{3-}) were syringe-filtered (Millex-HA 0.45 μm ; Millipore, Bedford, MA) and analyzed by using a Dionex Model DX-600 Ion Chromatograph equipped with an AS15 analytical column. Cation (Fe, Mn, As, Co, Ni, and Cr) samples were syringe-filtered (Millex-HA 0.45 μm ; Millipore, Bedford, MA), preserved with trace-metal-grade nitric acid to pH < 2, and analyzed by inductively coupled plasma optical-emission spectrometry (ICP-OES) for Fe and ICP mass spectrometry (ICP-MS) for Mn, As, Co, Ni, and Cr. Samples for acetate analyses were collected without filtration in prebaked glass vials with Teflon-lined caps. Sample vials were filled 2/3 full and frozen as soon as possible in a freezer or on dry ice. Samples for acetate were analyzed on a Thermo Scientific ICS 2100 ion chromatograph with a gradient pump and eluent generator using an AS11 HC column.

As and Fe are reported as total concentrations. Due to the limited sample volume, we were unable to determine valences for Fe and As. Previous work at this site has shown that the total dissolved Fe reflects the dissolved Fe²⁺, and Fe³⁺ contributes minimally to Fe in solution at the circumneutral pH in the aquifer (unpublished data). Thus, we will use total Fe as a proxy for dissolved Fe²⁺. Arsenic species could not be identified, although recent work at the Bemidji site has shown that As(III) accounts for 80–100% of As species released to groundwater during biodegradation.³

Determination of First-Order Rate Coefficients. For our experiments, reaction rates were estimated with first-order kinetics using the reactant and tracer breakthrough curve method described in Haggerty et al.⁴³ and Snodgrass and

Kitanidis.⁴⁴ Although the Monod model is considered the most robust description of biodegradation kinetics, Schreiber and Bahr⁴⁵ showed that first-order kinetics accurately describe BTEX biodegradation rates, especially at low concentrations. Bekins et al.⁴⁶ discusses the conditions when it is appropriate to use first-order kinetics in lieu of the Monod model and shows that first-order kinetics are appropriate and accurately approximate “true” Monod values under conditions when the substrate concentration is much less than the half-saturation constant (K_s) for the substrate. This is likely the case in our experiments, making the first-order analytical solution an appropriate kinetic model to describe reaction rates. This method provides an analytical solution for interpreting first-order rates from push–pull test data as opposed to requiring an approximate numerical solution using a radial-flow model approach. This method has been shown to provide robust solutions to similar biogeochemical reactions in situ and is appropriate here, having produced excellent best-fit lines for the processes described.

This method uses a conservative tracer to account for the dilution from physical processes such as advection, diffusion, and dispersion.^{37,43} Rates were determined by assuming that the injected solutes and mobilized species from the sediment were transformed following the first-order rate equation, $\delta C_d/\delta t = -kC_d$, so that

$$C_d(t) = C_d^0 e^{-kt} \quad (1)$$

where $C_d(t)$ is the reactive solute concentration at time t , C_d^0 is the initial reactive solute concentration, and k is the first-order rate coefficient. Thus, the breakthrough curve for a reactive solute $C_d(t^*)$ can be solved by

$$C_d(t^*) = \frac{C_{tr}(t^*)}{kt_{inj}} [e^{-kt} - e^{-k(t_{inj}-t^*)}] \quad (2)$$

where t^* is the time elapsed after injection, $C_{tr}(t^*)$ is the breakthrough curve for the injected conservative tracer (Cl^- or Br^-), and t_{inj} is the duration of the injection of the test solution. Eq 2 can be rewritten as

$$\ln \left[\frac{C_d(t^*)}{C_{tr}(t^*)} \right] = \ln \left[\frac{(1 - e)^{kt_{inj}}}{kt_{inj}} \right] - kt^* \quad (3)$$

where C_d is the concentration of the reactive solute (e.g., As, Fe²⁺, and acetate) at time t normalized to the concentration at time $t = 0$, and C_{tr} is the concentration of the tracer at time t normalized to the tracer concentration at time $t = 0$. Plotting $\ln[(C_d(t^*)/C_{tr}(t^*))]$ versus t^* yields a straight line with the slope of the first-order rate coefficient, k . Applying a linear regression to the straight-line experimental data provides a first-order rate coefficient for reactions (e.g., Fe reduction and trace-element release). Only a portion of the breakthrough curve is needed to estimate k because k is based on the ratio of C_d/C_{tr} .³⁸ This allows us to account for a lag phase, which is often observed in push–pull tests. When background concentrations of a reactive solute were present, C_d from eq 3 was corrected using a mixing ratio (eq 4),

$$C_d = \frac{[d_m - d_p](t^*) - [d_m - d_p]_{\min}}{[d_m - d_p]_{\max} - [d_m - d_p]_{\min}} \quad (4)$$

where d_m is the measured concentration of the reactive solute, d_p is the predicted concentration of the reactive solute based on

a linear regression of the percent test solution of injected tracer (0–100%) versus d_m , representing the mixing curve between the injected solution and background water. Concentrations of Cl^- and Br^- were low in background waters, so mixing corrections were not needed for conservative tracers in this study.

RESULTS AND DISCUSSION

Initial characterization of the background water chemistry in the wetland shows low concentrations of BTEX ranging from <1 to 73 $\mu\text{g/L}$, mostly as benzene. These residual concentrations remain from the initial contamination in 1979, highlighting that BTEX biodegradation, especially benzene biodegradation, is slow. However, the microbial community in the wetland is likely primed for BTEX biodegradation. Redox geochemistry (Table 1) shows concentrations of total dissolved Fe and CH_4 in the wetland, suggesting that redox conditions are strongly reducing with Fe reduction and methanogenesis occurring in the wetland. Although these redox reactions are thought to occur sequentially, several studies have documented overlapping redox zones with co-occurring redox reactions.^{3,36,47} Other electron acceptors, NO_3^- and SO_4^{2-} , and electron acceptor byproducts (Mn^{2+}) were below detection in the wetland, suggesting that denitrification, manganese reduction, and sulfate reduction were not important redox reactions in the wetland, and Fe reduction and methanogenesis were coupled with the biodegradation of organic matter. Background data also show detectable As in native groundwater for PPBN and PPBE. As is <1 $\mu\text{g/L}$ in PPB and PPBE. This indicates As mobilization from reductive dissolution of Fe(III) (oxyhydr)-oxides may occur heterogeneously in the undisturbed wetland by coupling with oxidation of organic carbon already existing in the wetland. We suspect wetland heterogeneity is responsible for the elevated Fe and As in PPBN and PPBE. These wells have higher nonvolatile dissolved organic carbon (NVDOC) than PPB and PPBE, which may serve as a natural driver to more reducing conditions in these wells. Other studies have shown that wetlands are especially heterogeneous with respect to geochemistry, observing concentrations of aqueous species ranging orders of magnitude over cm-m scales.^{40,48,49} When a chemical of interest was detected in native groundwater, we applied eq 4 to account for background concentrations.

Push–Pull Tests. Concentrations of trace elements released as a secondary product from biodegradation can raise water quality and human health concerns. An example of As, Fe, and Cl^- (tracer) concentrations observed during PPBE, the test in which BTEX and ethanol were added, shows that As is released to groundwater during the experiment (Figure 1). After the test solution was injected, As concentrations were low (<10 $\mu\text{g/L}$) up to 27 h after injection. After 47 h, there were rapid increases in As, reaching a maximum concentration of 99 $\mu\text{g/L}$ at 116 h. This is nearly an order of magnitude greater than the 10 $\mu\text{g/L}$ limit for drinking water set by the USEPA and WHO. As the push–pull test progressed, concentrations of reactive solutes and tracer were diluted as the test slug mixes with native background water.

We can calculate the concentration for each solute at time t relative to the initial concentration at the start of each experiment (Figure 2). When the relative concentration for a reactive solute deviates from the relative concentration for the tracer, it is indicative of a biogeochemical process that is either producing or consuming the reactive solute. A decreasing

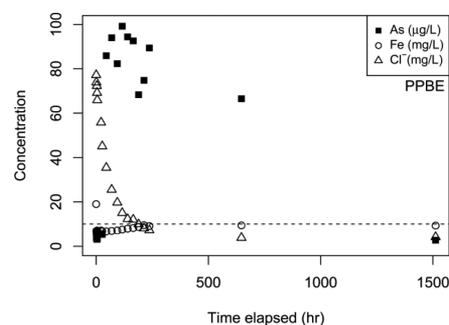


Figure 1. Concentrations of Fe (○), As (■), and Cl^- (△) tracer for the duration of the push–pull test in PPBE. The dashed line represents the 10 $\mu\text{g/L}$ limit for arsenic in drinking water set by the USEPA and the WHO. Arsenic concentrations plotted above this exceed the limit for drinking water. In background conditions and at the beginning of the experiment, As is less than 10 $\mu\text{g/L}$. A total of 23 h after injection, As concentrations rapidly increase and are elevated nearly an order of magnitude above 10 $\mu\text{g/L}$, while Fe concentrations gradually increase for the duration of the experiment.

deviation in the relative concentration of the reactive solute from the tracer indicates solute consumption, and increasing deviation in the reactive concentration of a reactive solute from the tracer indicates solute being produced in solution. For example, Fe is being released in PPB and PPBE (Figure 2A,B), indicating Fe reduction. In contrast, relative concentrations of nitrate are decreasing faster than the tracer in PPBN and PPBE (Figure 2C,D), suggesting denitrification.

First-Order Rates. Although relative concentrations can help identify biogeochemical processes and trace-element release for public health considerations, understanding rates of release is also important for water-quality assessment. The push–pull test data were analyzed to obtain first-order rate coefficients for reactions occurring in each test (Figures 3 and 4). We interpret the changing slopes in the first-order rate plots to suggest changes in processes controlling trace-element release in the tests. Therefore, linear regressions were conducted on straight-line portions of the rate data to obtain first-order rate coefficients for each apparent process. Where data were sparsely collected, a straight line was drawn between two data points to show changes in slope that reflect changes in processes. However, we do not quantitatively report rate estimates when a line was drawn between data points. Rates of increased dissolved Fe were used as a proxy for overall Fe reduction. It should be noted that rate measurements based on the production of dissolved Fe are likely an underestimate (potentially a substantial underestimate) of the actual rate of Fe reduction because studies have shown that most Fe that is reduced is retained in the sediment.^{1,34,47} However, we interpret the observed increases in the rate of Fe release to solution as a reflection of increases in overall Fe reduction while acknowledging these limitations.

More complex Fe and As cycling occurred in PPBE than PPB (Figure 3). Acetate, a common intermediate generated from the biodegradation of ethanol⁷ and BTEX,^{50,51} was used as a proxy for microbial activity. In both tests, Fe and As were released to solution after a lag phase. The production of Fe reflects the reduction of solid-phase Fe(III) from the sediment to aqueous Fe in response to the sudden influx of electron donors (BTEX or ethanol). Due to strong adsorption affinities between As and Fe(III) (oxyhydr)-oxides, As production in the push–pull tests, hereafter referred to as “As release”, is interpreted as the

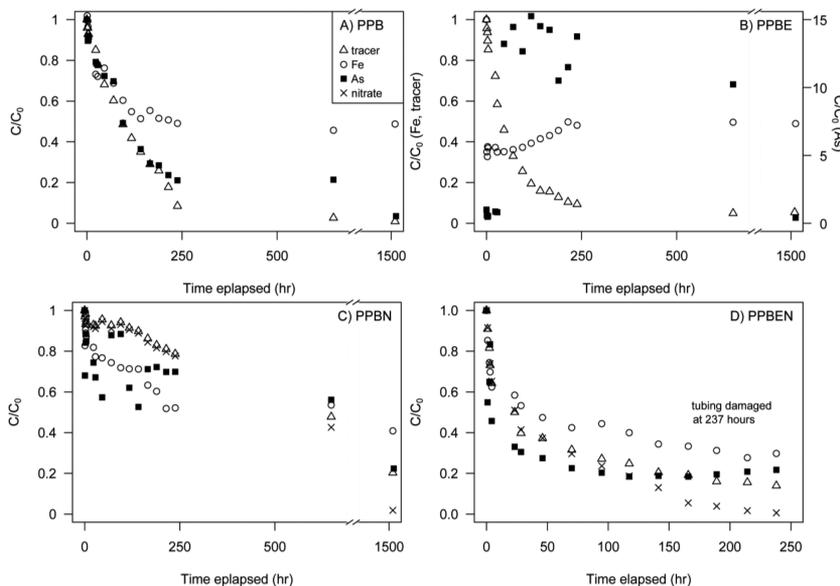


Figure 2. Relative concentrations for tracers and reactive species in (A) PPB, (B) PPBE, (C) PPBN, and (D) PPBE. A positive deviation (increase) of relative concentrations for a reactive solute from the tracer indicates production of the reactive solute in groundwater. A negative deviation (decrease) from the tracer indicates that the reactive solute is consumed in groundwater.

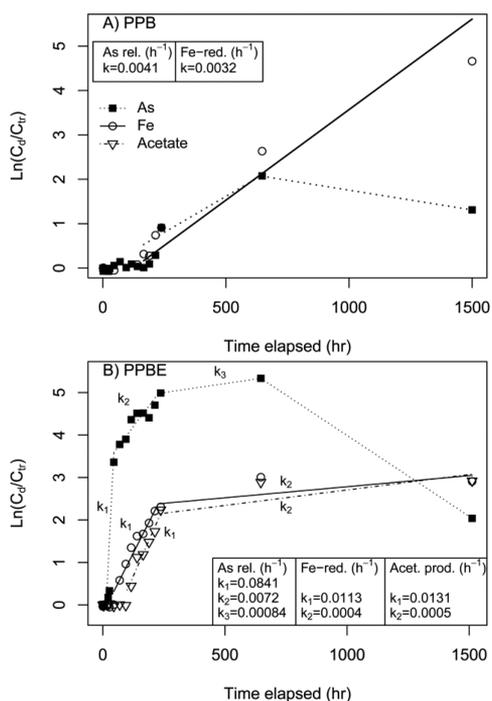


Figure 3. (A) First-order rate plots for Fe (O) and As (■) in PPB. After an initial lag phase, Fe reduction occurs for the duration of the push–pull test, whereas As release occurs until 647 h, after which it begins to be removed from groundwater. Significant acetate production was not observed in PPB. (B) First-order rate plots for Fe, As, and acetate (▽) in PPBE. Arsenic release occurs in three stages with three different rates. k_1 occurs before Fe is released to groundwater. k_2 and k_3 for As release corresponds with Fe reduction and acetate production through 647 h. After 647 h, Fe reduction and acetate production continue at the same rate, whereas the rate of As release decreases.

reductive dissolution of As adsorbed to Fe(III) (oxyhydr)-oxides that are microbially reduced due to the biodegradation of BTEX or ethanol.^{2,3} An exception occurs in PPBE, where As

is initially released prior to observed Fe reduction. For simplicity, we will also refer to this process as “As release.”

Effect of BTEX (PPB). In PPB, the onset of Fe reduction began after an initial 166 h lag phase, coincident with an increase in dissolved As (Figure 3A). This suggests that the influx of BTEX stimulated the microbial population, causing Fe reduction and the release of As from wetland sediments to groundwater. Linear regressions through the straight-line portions of data show similar first-order rate coefficient estimates for Fe reduction and As release: 0.0032 h^{-1} ($R^2 = 0.966$) and 0.0041 h^{-1} ($R^2 = 0.911$), respectively. The rate coefficient for Fe reduction is consistent with literature results.⁵² Acetate was not observed in PPB. This may be caused by biogeochemical processes occurring at slow rates that do not cause appreciable acetate concentrations at our sampling intervals. Alternatively, complex biogeochemical processes may occur such that acetate is microbially produced and consumed so that it was not observed at our sampling points.

Although the initial stages of Fe reduction coincide with the onset of As release, the two reactions have different durations. After an initial lag phase, Fe reduction occurs at a constant rate for the duration of the experiment, whereas the rate of As release changes after 647 h as shown by the new slope from 647 to 1500 h. This suggests that although Fe reduction appears to continue uninhibited for the duration of the experiment, the process controlling the distribution of As changes after 647 h, and As concentrations are no longer a function of the reductive dissolution mechanism. In fact, the negative slope from 647 to 1500 h indicates that As is being removed from groundwater faster than it is being released. If the wetland sediments are stripped of all or most of the labile adsorbed As within 647 h, a change in rate, and perhaps a reversal of slope for As release, would be expected thereafter, even though Fe reduction continues at the same rate.

Effect of Ethanol (PPBE). Adding ethanol to evaluate the effects of electron-donor competition demonstrated complex cycling of Fe and As in PPBE (Figure 3B). The lag phase was reduced, and there was earlier onset of Fe reduction and As

release. For simplicity of discussion, PPBE has been divided into four time stages after the initial lag phase to highlight different processes.

Stage 1 (23–47 h). After the initial 23 h lag phase, As release was observed with a rate coefficient, $k_1 = 0.0841 \text{ h}^{-1}$ ($R^2 = 0.9732$), and lasted up to 47 h after injection (Figure 3B). During this stage, As concentrations rapidly increase from <10 to 84 $\mu\text{g/L}$, well above the 10 $\mu\text{g/L}$ limit for drinking water. This initial release occurred without the production of Fe^{2+} , suggesting the initial stage of As release occurred independent of Fe reduction and the reductive dissolution mechanism. Kocar and Fendorf⁵³ discuss the microbial use of As(V) as an electron acceptor and the consequences for As mobilization from sediments via a reductive desorption mechanism. Tufano and Fendorf⁵⁴ report the desorption of As after the reduction of As(V) to As(III) at a circumneutral pH due to increased solubility. Additionally, As(III) desorbs more rapidly and extensively than does As(V) from Fe(III), so microbial respiration of As(V) can cause elevated As in groundwater without observed Fe reduction via a reductive desorption mechanism. This process may be responsible for the first stage of As release in PPBE prior to the onset of Fe-reducing conditions. After the As(V) has been consumed, the dominant electron-accepting process can shift to Fe reduction, which can further mobilize As via the reductive dissolution of Fe(III) (oxyhydr)-oxides.⁵⁵

Interestingly, the release of As prior to Fe reduction was observed in PPBE but not PPB. This may be explained by microbial constraints of the electron donor. Several studies have shown that As(V)-reducing bacteria commonly biodegrade simple organic compounds including ethanol and its common byproduct, acetate.⁵⁶ It is rare that As(V)-reducing bacteria biodegrade aromatic compounds, although one bacterium, *Desulfosporosinus* strain Y5, isolated from Onondaga Lake, Syracuse, New York, was shown to biodegrade toluene.⁵⁷ Thus, ethanol biodegradation is likely compatible with the As(V)-reducing microbial community, whereas the As(V)-reducing microbes likely cannot biodegrade BTEX, resulting in As release prior to Fe reduction in PPBE but not PPB.

The apparent independence of As release from Fe reduction may also be explained by the retention of reduced Fe(II) in the wetland sediments. Modeling results from nearby contaminated aquifer sediments presented in Ng et al.¹ report that >99.9% of Fe(II) that is produced from Fe(III) reduction is retained on the solid phase. However, the precise mechanism retaining Fe(II) (i.e., solid-phase reduction versus the release of Fe^{2+} followed by rapid resorption to sediments) is difficult to distinguish in field experiments, and implications for mobilization of associated As mobilization are poorly understood. However, if Fe(II) was retained due to solid-phase Fe reduction, As released during that process would appear independent of Fe reduction because reduced Fe^{2+} would not be detected in water samples. Field data alone are not enough to identify the reason behind the apparent independence of As release from Fe reduction for 23–47 h. However, it is clear that As and Fe cycling are complex and dynamic in PPBE.

Stage 2 (47–237 h). In stage two, the onset of Fe reduction was observed at 47 h with a rate coefficient of $k_1 = 0.0113 \text{ h}^{-1}$ ($R^2 = 0.9483$). The rate of As release also changed after 47 h ($k_2 = 0.0072 \text{ h}^{-1}$, $R^2 = 0.8825$) and ultimately reached a maximum concentration of 99 $\mu\text{g/L}$ at 116 h. The rate of As release mimics Fe reduction at constant rates up to 237 h. Additionally, the production of acetate begins during stage two.

Acetate production is interpreted as a response from microbial activity because acetate is a common metabolite of BTEX and ethanol. The rate of acetate production ($k_1 = 0.0131 \text{ h}^{-1}$, $R^2 = 0.9493$) is similar to that of Fe reduction during stage two of PPBE, suggesting that acetate and Fe are byproducts of one oxidation–reduction reaction (e.g., ethanol biodegradation coupled with Fe reduction). The co-occurrence of As release, Fe reduction, and acetate production, coupled with similar rate coefficients for the three processes, suggests that reductive dissolution of Fe(III) (oxyhydr)-oxides is the mechanism responsible for As release.

Stage 3 (237–647 h). A third stage beginning at 237 h shows slower but sustained rates of As release, Fe reduction, and acetate production. Despite the slowed rates, As concentrations were measured at 66.5 $\mu\text{g/L}$ at 647 h, well above the nondetectable As concentrations (<1 $\mu\text{g/L}$) in background waters. Interestingly, although the rates for As release, Fe reduction, and acetate production changed, they continued to occur at similar rates of $k_3 = 0.00084 \text{ h}^{-1}$, $k_2 = 0.0004 \text{ h}^{-1}$, and $k_2 = 0.0005 \text{ h}^{-1}$, respectively, in stage three. This suggests that the microbial reduction of Fe is still responsible for As release. The decrease in the rates for the three processes from the 47–237 h interval may be due to a change in electron donor in redox reactions. Ethanol was likely consumed after 237 h, and the microbial community shifted to a more recalcitrant electron donor, causing a slower rate of acetate production from the biodegradation coupled with Fe reduction. The slower rate of As release, coinciding with the slower Fe reduction and acetate production, provides further support that As release is controlled by Fe reduction.

Stage 4 (647–1500 h). After 647 h, Fe reduction and acetate production continue for the duration of the PPBE at the same rates as the 237–647 h interval, but the trend for As release changes. Arsenic release no longer appears to be a function of Fe reduction. The negative slope for As release for this stage suggests that As is being removed from groundwater faster than it is being released, similar to what was observed at 647–1500 h in PPB (Figure 3A). This may be explained by the precipitation of As-bearing minerals. Other studies have observed that dissolved arsenic in the presence of biogenic Fe(II) can precipitate As from solution as Fe–As compounds such as symplectite ($\text{Fe}_3(\text{AsO}_4)_2 \cdot 8\text{H}_2\text{O}$).^{58,59} The rate of Fe reduction in PPBE is an order of magnitude slower than PPB for the 647–1500 h interval. Several studies have demonstrated that ethanol is more labile than BTEX and is preferentially consumed during biodegradation.^{5,7} Consequently, we expect an earlier depletion of easily reducible Fe(III) in wetland sediments when ethanol is the electron donor, as well as earlier decreases in longer-term Fe-reduction rates. The strongly coupled rates of acetate production and Fe release suggest that the apparent change in Fe reduction is microbially controlled. Because ethanol is biodegraded preferentially over BTEX, a co-release of BTEX and ethanol suggests a faster growth of microbial populations than a release of BTEX alone, resulting in the more rapid consumption of electron acceptors, driving the redox conditions to more reducing and less thermodynamically favorable redox reactions.^{7,31,32}

Differences in Lag Phases. Lag phases are commonly observed in microbially mediated reactions and have been attributed to an equilibration period in which the native microbial community responds to perturbations such as the addition of new electron donors or electron acceptors.^{36,38,60} In PPB, the lag phase prior to Fe reduction and As release was 166

h. When ethanol was added in PPBE, the lag phase was reduced to 47 h. This is likely due to ethanol being more easily biodegraded than BTEX.^{5,7} BTEX molecules have a more complex chemical structure than ethanol, making them more recalcitrant to biodegradation. Additionally, BTEX is less soluble in water than ethanol, which can cause BTEX to adsorb to sediment upon the injection of a test solution. Because microorganisms generally cannot biodegrade BTEX unless it is dissolved in the bulk solution,⁶¹ the lower solubility of BTEX may also contribute to a delayed microbial response and longer lag phase. Combined, these properties allow ethanol to spur the more rapid onset of microbial activity and redox processes than BTEX. In the absence of more thermodynamically favorable electron acceptors (e.g., O₂ and NO₃⁻), a release of ethanol with BTEX, compared to a release of BTEX alone, causes (1) an earlier onset of As mobilization from reductive dissolution of Fe(III) (oxyhydr)-oxides (lag time with ethanol = 47 h versus lag time without ethanol = 166 h) and (2) a faster initial rate of As release ($k_1 = 0.0841 \text{ h}^{-1}$ (independent of Fe reduction) and $k_2 = 0.0072 \text{ h}^{-1}$ (dependent on Fe reduction) with ethanol versus $k = 0.0041 \text{ h}^{-1}$ without ethanol).

Effect of Nitrate (PPBN and PPBE). Nitrate and BTEX were added with (PPBE) and without (PPBN) ethanol in two separate push-pull tests to investigate the role that NO₃⁻ might have on BTEX degradation and associated As mobilization. NO₃⁻ is a more thermodynamically preferred electron acceptor than Fe(III) and thus should cause the biodegradation of organic matter to couple with NO₃⁻ reduction, which would not affect the stability of As sorbed to Fe(III).

Neither Fe reduction nor As release were observed in either of the push-pull tests containing NO₃⁻ (Figure 2C,D). NO₃⁻ is thermodynamically favored over Fe(III) as an electron acceptor, so biodegradation of BTEX or ethanol was coupled with denitrification rather than Fe reduction. Therefore, As remained associated with solid Fe(III) in wetland sediments and was not released to groundwater. It should be noted that PPBE was shorter than the other tests. The drive-point well for PPBE was damaged after 237 h, so water samples were not collected at 647 and 1500 h. However, NO₃⁻ was almost entirely removed from the system after 237 h in PPBE (0.6 mg/L), suggesting denitrification would soon thereafter cease, and a less thermodynamically favorable reaction, such as Fe reduction, would begin.

At 1500 h, measured concentrations of nitrate in PPBE, 2 mg/L, were elevated enough to inhibit Fe reduction and As release. This faster depletion of NO₃⁻ for PPBE is likely due to ethanol acting as a more labile electron donor for the microbial community. Therefore, ethanol would deplete a system of NO₃⁻ sooner than if BTEX acted as the lone electron-donor source, creating an earlier onset of the redox conditions necessary for Fe reduction and associated As mobilization. However, if the wetland received a large enough influx of NO₃⁻ so that the biodegradation of hydrocarbons (e.g., ethanol or BTEX) was continually coupled to denitrification, As would remain immobile and adsorbed to thermodynamically stable Fe(III) (oxyhydr)-oxides. However, an influx of NO₃⁻ may contribute to other undesired water-quality effects.

Mobilization of Co, Ni, and Cr (PPBE). This study also documents the mobilization of other redox-sensitive trace elements stimulated by the microbial reduction of Fe(III) (oxyhydr)-oxides in the presence of ethanol (PPBE).

Significant release of other trace elements was not observed in other tests. The release of Co, Ni, and Cr was observed in PPBE, resulting in elevated concentrations in groundwater. Ni was elevated from 22.8 μg/L in background waters to a maximum of 137 μg/L 94 h after injection, above the 100 μg/L chronic exposure health advisory set by the USEPA.¹⁰ Cr and Co in groundwater were also elevated from <10 to 70.5 μg/L and from 1.3 to 18.9 μg/L, respectively. Cr did not exceed the 100 μg/L limit for drinking water, and no regulatory limit exists for Co.

The release of these elements was quantified using eqs 1 and 2 to estimate first-order rate coefficients (Figure 4); Cr was released at a faster rate ($k = 0.0166 \text{ h}^{-1}$, $R^2 = 0.8115$) than Co (0.0079 h^{-1} , $R^2 = 0.9423$) and Ni (0.0055 h^{-1} , $R^2 = 0.7615$). All three reactions were preceded by a lag phase, although the lag phase for Cr (23 h; prior to Fe reduction) was shorter than for Co and Ni (47 h; coincident with Fe reduction), suggesting, perhaps, that a different mechanism was responsible for the initial release of Cr than for Co and Ni.

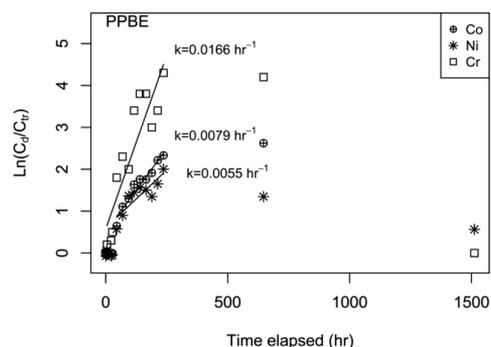
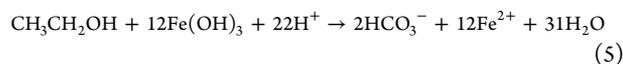


Figure 4. First-order rate plots for the release of Co (⊕), Ni (*), and Cr (□) for PPBE. Cr release began 23 h after injection, whereas Co and Ni release began after 47 h.

Several studies have documented the association of Co,^{62–64} Ni,^{62,65–67} and Cr^{62,66,68} with Fe(III) (oxyhydr)-oxides. Some have observed that they can coprecipitate with Fe and are incorporated into the mineral structure of Fe(III) minerals,^{62,63,65,68} while others have found adsorption to be a primary attachment mechanism.^{64,66,67} Although timing and mobilization rates from this study provide support for the Cr, Co, and Ni release to groundwater associated with Fe reduction, kinetics alone are not enough to distinguish the release mechanisms, and a more detailed investigation is necessary to definitively identify the processes controlling the mobilization of these trace elements.

Electron-Acceptor Demand. Ethanol and BTEX require different amounts of electron acceptors to undergo biodegradation and thus would have different demands for Fe(III) in these experiments. Biodegradation of ethanol, the simplest organic molecule evaluated in this study, coupled with Fe reduction is described by eq 5.



Using the known concentration of injected ethanol and the injection volume, we can calculate how much Fe(III) was required to completely biodegrade ethanol. For example, in PPBE, we used a 5 L test solution with an ethanol concentration of 154 mg/L. Using eq 5, we calculate that

11.2 g of Fe(III) from the sediment were required for complete ethanol biodegradation.

$$\begin{aligned} & 5 \text{ L of injected solution} \times \frac{154 \text{ mg of EtOH}}{\text{L}} \times \frac{1 \text{ g of EtOH}}{1000 \text{ mg of EtOH}} \\ & \times \frac{1 \text{ mol of EtOH}}{46.068 \text{ g of EtOH}} \times \frac{12 \text{ mol of Fe(OH)}_3}{1 \text{ mol of EtOH}} \times \frac{1 \text{ mol of Fe}^{3+}}{1 \text{ mol of Fe(OH)}_3} \\ & \times \frac{55.845 \text{ g of Fe}^{3+}}{1 \text{ mol of Fe}^{3+}} = 11.2 \text{ g of Fe}^{3+} = 11\,200 \text{ mg of Fe}^{3+} \end{aligned}$$

Using the volume of sediment in the radial injection sphere (0.01169 m³, calculation shown in the [Supporting Information](#)), a porosity of 30%, and a particle density of 2.65 g/cm³, we calculate that an Fe(III) concentration of 361 mg/kg in the aquifer sediments was required to fully biodegrade ethanol.

Similarly, we can calculate the Fe(III) demand for BTEX biodegradation. Fe reduction reactions for biodegradation of benzene, toluene, and combined ethylbenzene plus xylenes are described in eqs 6–8, respectively.



Using initial concentrations for PPBE of 738 μg/L benzene, 307 μg/L toluene, and a summed 615 μg/L for ethylbenzene and xylenes (Table 2), we calculate that 5.7 mg/kg Fe(III) was required to biodegrade the injected BTEX. Calculations are detailed in the [Supporting Information](#).

It is clear that more Fe(III) was required to biodegrade ethanol than BTEX in these experiments. Independent of kinetics, the greater Fe(III) demand by ethanol would result in more Fe reduction overall. Because the extent of As release was related to the amount of Fe reduction, the high Fe(III) demand resulted in an overall greater release of As mass from the sediment during biodegradation, and thus PPBE saw a greater magnitude of As release compared to that in PPB.

The greater demand for Fe(III) by ethanol in these experiments is only due to the difference in initial concentrations between ethanol and BTEX. In eq 5–8, we see that the BTEX molecules have a much greater stoichiometric demand for Fe(III) than ethanol. For example, 12 mol of Fe(III) are required to biodegrade 1 mol of ethanol, whereas 30 mol of Fe(III) are required to biodegrade one mol of benzene. Toluene, ethylbenzene, and xylenes similarly have a higher demand for Fe(III). Thus, if BTEX and ethanol were present in similar concentrations, BTEX biodegradation would contribute more than ethanol biodegradation to the overall Fe reduction and associated As release. However, despite greater Fe(III) demand and a greater magnitude of As release, the added complexity of slow BTEX biodegradation rates brings into question whether or not As would be released from sediment fast enough with solely BTEX biodegradation for As to become elevated above the 10 μg/L limit for drinking water. In PPB, sufficiently low concentrations of BTEX likely allowed Fe reduction and the associated As release to occur at rates slow enough that mobilized As was sequestered either via resorption or diluted by physical processes so that aqueous As never exceeded 10 μg/L. Conversely, data from PPBE showed that ethanol biodegraded rapidly due to its labile nature and caused a pulselike release of As to concentrations well above the limit for drinking water. Under more spill-like conditions where BTEX concentrations approach saturation in groundwater, the same rate of biodegradation and As release might

cause dissolved As to exceed the drinking water standard simply due to the greater BTEX mass available to be degraded. Thus, a summary of this work highlights the importance of considering the following properties when assessing secondary mobilization of trace elements under Fe-reducing conditions: (1) the lability of organic matter that is biodegrading and its associated biodegradation rate, (2) the Fe(III) demand of the biodegrading organic matter, and (3) the initial mass (aqueous concentration) of organic matter undergoing biodegradation.

Implications for Trace-Element Transport. This study quantifies the rates, timing, and duration of trace element mobilization during the biodegradation of organic matter under nitrate- and iron-reducing conditions (Table 3). The effects of

Table 3. First-Order Rate Coefficient Estimates for Reactions in Push–Pull Tests

| | rate (hr ⁻¹) | lag time (hr) | duration (hr) |
|--------------|--------------------------|---------------|-------------------|
| PPB | | | |
| Fe reduction | 0.0032 | 189 | 1311 ^a |
| As release | 0.0041 | 189 | 458 |
| PPBE | | | |
| Fe reduction | 0.0113 | 47 | 190 |
| | 0.0004 | n.a. | 1263 ^a |
| As release | 0.0841 ^b | 23 | 24 |
| | 0.0072 | n.a. | 190 |
| | 0.00084 | n.a. | 410 |
| Co release | 0.0079 | 47 | 190 |
| Ni release | 0.0055 | 47 | 190 |
| Cr release | 0.0166 ^b | 23 | 214 |

^aReaction continued until end of experiment. ^bReaction occurred before Fe reduction was observed.

competing electron donors with in situ organic matter were evaluated by adding combinations of BTEX and ethanol during in situ rate experiments. The addition of ethanol resulted in more rapid and complex release of As and other trace elements. Although this study investigated trace element mobilization during the biodegradation of organic matter in the form of petroleum and biofuels, these results support the potential mobilization of trace elements during the biodegradation of other contaminant sources, as the redox reactions initiated are not unique to fuels. However, it should be noted that these results also show that the nature of the organic matter, including the specific competing electron donors present, will impact the initiated processes and rates. The overall fate of mobilized elements is likely dependent on the prevailing geochemistry downgradient from the contaminated site. If conditions are sufficiently reducing, there are possible implications for the large-scale transport of trace elements mobilized from the sediment. However, oxic conditions downgradient would likely immobilize trace elements.³ Another complexity to consider is the overall effect that sulfide may have on fate and transport of trace elements. Although this system was limited with respect to sulfur (S), sulfide generation caused by microbial sulfate reduction is a common redox process in most wetlands. Some studies have shown that systems of rapid Fe and S cycling can produce stable aqueous iron sulfide clusters, FeS_{aq}, that readily complex trace elements and transport them environmentally significant distances (m and km) from their source.^{69,70} A recent study showed increased As solubility under sulfate-reducing conditions due to the formation of thioarsenic species.⁷¹ Conversely, several studies

have shown that microbial sulfide generation can sequester As via coprecipitation mechanisms with sulfide or iron sulfide.^{72,73} This study emphasizes the need to have a broad understanding of the geochemistry of a contaminated site because naturally occurring contaminants can have significant impacts on water quality given the appropriate geochemical conditions.

The effects of competing electron donors, ethanol and BTEX, demonstrate that ethanol causes an earlier response in the microbial community, both in the presence and the absence of NO_3^- . Furthermore, a comparison of As release rates between PPB and PPBE show that As release occurs almost twice as fast when ethanol is present during the initial Fe-reducing stage ($k = 0.0072 \text{ h}^{-1}$ with ethanol versus $k = 0.0041 \text{ h}^{-1}$ with only BTEX). NO_3^- was shown to inhibit As mobilization by coupling biodegradation to denitrification rather than Fe reduction. However, ethanol caused a more rapid denitrification rate, suggesting that a corelease of BTEX and ethanol would result in faster depletion of NO_3^- and earlier onset of Fe reduction and As mobilization than a release of BTEX alone.

On the basis of these findings, we conclude that secondary water quality effects, such as the increased aqueous concentrations of trace elements, should be considered in systems where the biodegradation of organic matter is occurring under iron-reducing conditions. Thus, it is important to consider rates of trace-element mobilization at natural and enhanced attenuation sites impacted by organic matter. In addition, the nature of the organic matter (e.g., the presence of competing electron donors) may impact the processes initiated as well as the rates of those processes and requires further investigation for protective design and evaluation of drinking water remediation strategies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b02341.

Additional details regarding the calculation of the radius of injected solution in drive-point wells, the volume of sediment in the total injection sphere volume, and the Fe(III) demand by BTEX in PPBE (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We extend special thanks to Erik Smith, Ben Ruhme, Ryan Streitz, and Jeanne Jaeschke for their help with experimental design and collecting data. We thank Madeline Schreiber for useful discussions. This project was supported by the USGS Toxic Substances Hydrology Program and the National Research Program. Partial funding for this project was provided by the University of St. Thomas Grants and Research Office and the National Crude Oil Spill Fate and Natural Attenuation Research Site, a collaborative venture of the USGS, Enbridge

Energy Limited Partnership, the Minnesota Pollution Control Agency, and Beltrami County, MN. Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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