Importance of exposure history when using single well push-pull tests to quantify in situ ethanol biodegradation rates

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Importance of Exposure History When Using Single Well Push–Pull Tests to Quantify In Situ Ethanol Biodegradation Rates

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Abstract

Single well push-pull tests (PPTs) were used to characterize in situ biodegradation rates of ethanol in groundwater at a leaking underground fuel tank (LUFT) site at Site 60, Vandenberg Air Force Base (VAFB), CA. For the tests, local groundwater was spiked with bromide and ethanol and injected at different times into three different wells throughout the experimental area. The spiked water was allowed to remain in the aquifer for 1 to 15.9 hours prior to extraction. Biodegradation of ethanol was not observed within 15 hours of the aquifer’s first exposure to ethanol near any test well; the ethanol/Br ratio was nearly constant in the extraction samples. Biostimulation treatments (ethanol injections) over the course of 1 to 2 weeks resulted in a linear decrease in ln(ethanol/Br) with time in the extraction samples indicating that ethanol was biodegrading with a first order rate constant of about 0.3/h. After exposing an area to ethanol for 3 months, the biodegradation rate increased further by about a factor of 2. Ethanol degradation rates in the aquifer at this site were temporally variable based on the ethanol exposure history. Our results suggest that PPTs were an effective tool for examining such variability. PPT investigations should be valuable at other locations because ethanol degradation rates in groundwater should vary spatially and temporally depending on the type and timing of fuel releases as well as other factors that control the history of ethanol exposure to an aquifer.

Introduction

The Alternative Fuel Standard Act of 2007 calls for an increase in the use of ethanol as a biofuel. As a result, ethanol’s fate in groundwater has become a question of concern for the U.S. Environmental Protection Agency (EPA) and has stimulated research on the fate and transport of ethanol in the subsurface environment (NRC 2007). Much of this effort has focused on the impacts of ethanol on the fate and transport of petroleum compounds (Powers et al. 2001; Adam et al. 2002; Amro et al. 2004; Corseuil et al. 2004; Mackay et al. 2006, 2007).

Compared to other fuel components, ethanol can dissolve into groundwater at high concentrations and is not retarded during migration by sorption (Zhang et al. 2006). High groundwater concentrations of ethanol could lead to a co-solvent effect, potentially increasing the concentrations and enhancing the mobility of other contaminants, and/or a toxicity effect, potentially stopping microbial activity and biodegradation of itself and other contaminants. However, field and laboratory studies of ethanol and gasohol spills rarely note ethanol concentrations in groundwater high enough to lead to either significant co-solvent or toxicity effects (Wilson, USEPA, personal communication, 2009). Nevertheless, it is known that ethanol released into groundwater can have significant impacts on the fate of other contaminants. In particular, preferential biodegradation of ethanol can quickly lower or deplete concentrations of available electron acceptors and nutrients (Corseuil et al. 1998; Powers et al. 2001; Mackay et al. 2006). Thus, the biodegradation of other contaminants may be restricted to less optimal redox conditions down gradient of the spill site. For example, microcosms and field studies have shown that such effects lead to slower overall rates of biodegradation of BTEX species in ethanol-impacted groundwater (Corseuil et al. 1998; Mackay et al. 2006).

Wilson and Adair (2009, unpublished results) examined field and laboratory estimates of ethanol degradation rate in groundwater and microcosms meant to represent groundwater conditions and found the estimated zero-order degradation rates spanned over three orders of magnitude. The reason for the wide range of apparent degradation rates is unknown, but may be in part a result of variations in the microbial populations in various hydrogeologic settings and in part a function of the prior exposure of the native populations to ethanol or other labile substrates. If the rate of ethanol degradation truly varies so widely among sites, it would
be important to have site-specific estimates of the degradation rate to optimize monitoring and remediation strategies.

Laboratory studies are not well suited for determining site-specific biodegradation rates because it is difficult to capture the complexity of microbial communities and the multiple natural factors that control the population in the laboratory (Istok et al. 1997). For this reason, it is desirable to gather additional insights from biodegradation experiments conducted within natural systems. In the work reported here, we utilize a field method called the push-pull test (PPT) (Leap and Kaplan 1988; Istok et al. 1997; Snodgrass and Kitanidis 1998) to study in situ biodegradation of ethanol in a shallow aquifer in which sulfate is the dominant dissolved electron acceptor. Most fuel-contaminated sites in the United States overlie aquifers in which sulfate presents the highest capacity for fuel component degradation compared to other dissolved electron acceptors (Wiedemeier et al. 1999). PPTs are useful for establishing the potential for biodegradation of contaminants in aquifers and to compare substrate utilization rates with different electron acceptors.

The goal of this study differed from earlier PPT studies; it was to evaluate the PPT field method as a tool for examining the spatial and temporal variability of ethanol biodegradation within the relatively small area typically impacted by fuel spills. This information is often desirable when developing remediation plans, yet can be difficult to obtain for specific sites. PPTs are relatively fast and inexpensive compared to alternative field approaches such as dual- and multiwell forced or natural gradient tests (Wiedemeier et al. 1999). Other advantages of PPTs are that they can be repeated at a number of wells within a site to observe spatial variations or they can be repeated at a single well to observe temporal variations.

In many PPT studies, it has been necessary to stimulate the subsurface microbial community to overcome the lag time leading up to the full biodegradation capacity (Kim et al. 2004). This implies that spatial variability of biodegradation capacity should exist in the vicinity of a fuel spill where exposure of aquifers to substrates should be heterogeneous for a number of reasons including spill characteristics (e.g., size, rate, composition, etc.), complex groundwater transport pathways, and degradation processes in the vadose zone and elsewhere in the subsurface. Our experiment was designed to investigate the effects of exposure history on the ethanol biodegradation rate.

Site Location

Our study was conducted at Site 60, VAFB, near Lompoc, California where a gasoline leak at an underground storage tank was noted in the mid-1990s. This leak created an MTBE contamination plume that extended for over 500 m down gradient from the original service station (Wilson et al. 2002). The site is extremely well characterized because of more than a decade of consulting and academic study that included a series of controlled field experiments. One of these studies examined the impact of ethanol on co-existing or pre-existing contaminants using the multiwell natural gradient tracer approach (Mackay et al. 2006, 2007).

At the time of this study, the research infrastructure (depicted in Figure 1) included 10 “background” wells up gradient of the original spill location, a full-scale aerobic biobarrier treating pre-existing contamination, 158 wells in the primary experimental area between the former source and the biobarrier, and 16 wells down gradient of the biobarrier (not all shown in Figure 1) to confirm its ability to prevent contaminants from migrating beyond the experimental area.

The subsurface at the site is composed of a sequence of thin aquifer and aquitard layers; the experimental aquifer, called the S3 sand (porosity 0.33 to 0.35), is confined and on the order of 1 m thick, making it an ideal location for our experiments. The field area has been instrumented with plume-normal rows of monitoring wells (Figure 1). The wells are 1.3 to 2.5 cm Schedule 40 PVC screen and casing, with screened intervals (0.51 mm slots) from approximately 3 to 4 m below ground surface (bgs), that is, through the full thickness of the S3 aquifer. The piezometric surface averaged 1.8 m bgs with fluctuations on the order of a third of a meter during the experimental period March 26, 2008 to November 3, 2008, but was always above the top of the S3 layer.

On the basis of nearly a decade of monitoring by UC Davis, the mean direction of groundwater flow is to the northeast, with up to 15° eastward then westward shifts during December to April depending on seasonal precipitation. During field research in 2004 to 2005, the groundwater velocity was estimated as approximately 0.6 m/day (Mackay et al. 2006); similarities in the piezometric surface
estimates of the steady state biodegradation rate for ethanol are 340 µg/L/min (assuming a zero-order reaction) or 0.26/h (assuming a first order reaction). It is important to note that this is a mixed degradation rate. Sufficient ethanol was injected during these experiments to deplete the available SO$_4^{2-}$ and initiate methanogenesis (Mackay et al. 2006, 2007). Nevertheless, the field biodegradation rate for ethanol at Site 60 under these mixed redox conditions was much higher than published microcosm rates with sediments from different sites, e.g. 0.0042/h (Corseuil et al. 1998) and the field value (0.01/h) determined by Zhang et al. (2006) at a different location.

Approximately 3 mg of SO$_4^{2-}$ are needed to completely biodegrade (mineralize) 1 mg of ethanol. Therefore, each liter of Site 60 groundwater, which had a SO$_4^{2-}$ concentration of 100 to 200 mg/L at the time of this research, had the capacity to mineralize 33 to 66 mg of ethanol.

**Methods**

For each PPT, a volume of groundwater was extracted from a location >10 m distant from the test well, spiked with ethanol and bromide tracer, and injected (pushed) into the aquifer at the test well. After a pre-planned “drift time,” groundwater samples were extracted (pulled) from the aquifer using the same well. Initial PPTs were conducted with only bromide in the injected water to evaluate efficiencies of capture of the tracer for various experimental conditions and thus to select optimal experimental procedures for PPTs at the site (Kline 2009).

Table 1 lists the details for each ethanol-bromide PPT. To limit the introduction of oxygen or loss of ethanol during all tests, a plastic 20-L collapsible water bag was used as

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**Table 1**

<table>
<thead>
<tr>
<th>PPT #</th>
<th>Date (2008)</th>
<th>Well</th>
<th>Drift Time (h)</th>
<th>Vol Inj. (L)</th>
<th>Vol Ext. (L)</th>
<th>Inj. Etoh (mg/L)</th>
<th>% Br Recovery</th>
<th>% Etoh Recovery</th>
<th>First Order Rate Constant k (1/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No biostimulation prior to PPT1-4</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>March 26</td>
<td>EJ19</td>
<td>2.4</td>
<td>16.4</td>
<td>63.2</td>
<td>90</td>
<td>92</td>
<td>90</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>March 27</td>
<td>EJ19</td>
<td>2.1</td>
<td>15.9</td>
<td>51.0</td>
<td>87</td>
<td>87</td>
<td>85</td>
<td>—</td>
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<tr>
<td>3</td>
<td>May 8–9</td>
<td>EJ19</td>
<td>9.4</td>
<td>16.2</td>
<td>54.9</td>
<td>103</td>
<td>66</td>
<td>68</td>
<td>—</td>
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<tr>
<td>4</td>
<td>July 15–16</td>
<td>EJ9</td>
<td>15.6</td>
<td>16.1</td>
<td>64.9</td>
<td>112</td>
<td>29</td>
<td>42</td>
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<tr>
<td>Biostimulation of four spikes over 10 days prior to PPT5</td>
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<tr>
<td>5</td>
<td>June 28–29</td>
<td>EJ17</td>
<td>9.5</td>
<td>14.2</td>
<td>47.6</td>
<td>108</td>
<td>68</td>
<td>27</td>
<td>0.31 ± 0.02</td>
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<td>No additional biostimulation prior to PPT6</td>
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<tr>
<td>6</td>
<td>July 24–25</td>
<td>EJ17</td>
<td>15.4</td>
<td>14.0</td>
<td>44.9</td>
<td>110</td>
<td>49</td>
<td>30</td>
<td>0.13 ± 0.01</td>
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<td>Biostimulation of three spikes over 1 week prior to PPT7</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>July 28–29</td>
<td>EJ9</td>
<td>15.5</td>
<td>14.7</td>
<td>45.3</td>
<td>109</td>
<td>28</td>
<td>4</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>Biostimulation of an additional three spikes over 1 week prior to PPT8 and after PPT7</td>
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<tr>
<td>8</td>
<td>August 4–5</td>
<td>EJ9</td>
<td>15.9</td>
<td>14.7</td>
<td>45.1</td>
<td>109</td>
<td>32</td>
<td>&lt;1</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Biostimulation of six spikes over 2 weeks prior to PPT9 following a period of quiescence and earlier biostimulation$^1$</td>
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</tr>
<tr>
<td>9</td>
<td>November 3</td>
<td>EJ9</td>
<td>1.2</td>
<td>15.3</td>
<td>45.1</td>
<td>99</td>
<td>85</td>
<td>40</td>
<td>0.56 ± 0.16</td>
</tr>
</tbody>
</table>

$^1$Prior to PPT 9, biostimulation in EJ9 was conducted during two distinct periods: July 21 to September 22 (9 weeks) and October 17 to October 31 (2 weeks).
the container for the injectate. The injectate was prepared by dissolving sufficient NaBr into 15 to 20 L of groundwater to produce an approximately 350 mg/L bromide solution, two orders of magnitude higher than the native groundwater concentration, which ranged between 2 and 5 mg/L at the PPT locations during this study. After sparging with either nitrogen or helium for 15 min to remove any introduced oxygen, ethanol was added to produce a solution of approximately 100 mg/L (Table 1). The bag was sealed (with a head space volume of less than 250 mL) and shaken for 5 min to ensure mixing. Samples of the injectate were collected from the tubing leading into the well two to five times during the push phases and confirmed approximately constant concentrations of bromide and ethanol and that no oxygen was introduced. These data were averaged for later calculations.

The injectate was left within the aquifer for a 1 to 16 h drift period during which it migrated and mixed with groundwater flow. After the drift, groundwater was extracted at a rate of 9 to 10 L/h. The first extraction sample was taken after 1 h and had been produced and the well thoroughly purged; additional samples were taken after every 2 L until the total volume extracted equaled or exceeded the total injected, then every 5 L thereafter. Approximately three times the injected volume was extracted during each PPT. The mass of ethanol and bromide recovered was calculated by integration of the plot of concentration vs. extracted volume (Table 1).

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The microbial community was expected to change in the vicinity of the test well after the injection of ethanol, because ethanol would serve as a new, presumably easily degraded, substrate to this part of the aquifer, which had not seen significant concentrations of ethanol or other fuel compounds for a number of years (Mackay, unpublished data). In many PPT studies, biostimulation has been shown to be necessary to overcome the lag time leading up to full biodegradation capacity (Kim et al. 2004). Therefore, we conducted two biostimulation experiments.

During biostimulation periods, groundwater spiked with ethanol and Br was injected into the test well for a period of time prior to the PPTs. The idea was to provide a time significantly longer than the PPT for the native microbial populations to acclimate to the presence of ethanol without setting up a continuous release. Therefore a series of ethanol-rich slugs were injected (see Table 1 for a summary of the timing and number of spikes prior to PPTs).

For biostimulation, 10 to 12 L of a solution similar to a typical PPT injectate was prepared and injected into the test well following the PPT procedure. A minimum of 2.5 days was allowed between the last spike of each series and the subsequent PPT. Furthermore, immediately before the start of the subsequent PPT, 15 L of native groundwater was injected into the test well. This “buffer” volume was intended to clear the aquifer in the vicinity of the test well of most, if not all, of the bromide and ethanol remaining from the prior biostimulation events. Samples taken from the test well before PPTs verified that bromide and ethanol were at background levels both before and after the buffer injection (see Table 1 and Figure 1 for test wells and their locations). Ethanol was not detected in any samples taken at wells downgradient of the PPT well (along the EJP well transect).

For this work, we were unable to conduct ethanol and bromide analyses in the field. Instead, we collected samples for later analysis in off-site labs, generally long after each PPT and, unfortunately, not soon enough to help plan the next PPT or set of PPTs. Samples for bromide analysis were filtered with 0.22-µm membrane filters into glass bottles and analyzed on a DX-120 Dionex Ion Chromatograph (IC) containing an IonPac AS22 anion exchange column (4 x 250 mm) with an AG22 guard column (4 x 50 mm). The detection limit was less than 1 ppm and the analytical uncertainty based on replicates was ±5%. Sulfate peaks were also identified on the IC. However, because these samples were neither preserved with biocides nor stored continuously at a low temperature, some sulfate loss may have occurred if biodegradation of substrates in the samples (e.g., ethanol) occurred. Thus, we do not trust, report, or use results of sulfate analyses in this study.

Samples for ethanol analysis were collected according to the methods of Mackay et al. (2006). About 0.5 g of sodium tripolyphosphate was added as a preservative at the time of collection. Sample bottles were inverted and stored at 4 °C until shipped to UC Davis (in a cooler under ice) for analysis on a gas chromatograph. The detection limit for this method is 1 mg/L, 100 times lower than the target injectate concentration. All samples were analyzed in duplicate and mean values interpreted. The analytical agreement of the duplicates was typically better than ±10%.

Temperature and pH were measured with a field meter and DO in the injectate was measured using CHEMetrics DO Self-Filling Ampoules (0 to 1 ppm, detection limit 0.025 ppm). As there were no significant changes in pH and all DO concentrations were less than 0.025 ppm (Kline 2009), we do not discuss the results in this paper.

Results and Discussion

Ethanol Biodegradation without Biostimulation

During PPT1 and PPT2, the injection concentrations of ethanol and drift time of the tests were approximately...
100 mg/L and 2 h, respectively. These PPTs were performed on two consecutive days at Well EJ19 (Figure 1). The results for the two days were very similar (Figure 2), showing no evidence for biodegradation occurring in the vicinity of EF19. While the ln(ethanol/Br) ratio of the extraction samples showed some variability, there was no trend.

Two additional PPTs were conducted at wells prior to biostimulation. During PPT3, which was also conducted in EJ19 more than a month after PPT2, the drift time was increased to 9 h to give the microbial community longer to consume the ethanol. The shape of the ln(ethanol/Br) curve during the extraction (data plots not shown) was similar to those of the earlier PPTs, with no decrease as would be expected if biodegradation had occurred. This was also true for PPT4 (data plots not shown) at a second well, EJ9 (about 7 m cross gradient from EJ19), using a 15.5 h drift. However, unlike the earlier PPTs, the mass recovery during PPT4 was low, less than 50% with slightly more ethanol recovered than Br during this test. Presumably, we were approaching the methodological limit of the PPT at this site.

PPTs at wells with no known prior exposure to ethanol using a drift time from 2 to 15.5 h were unable to detect ethanol biodegradation. These results differed from the minimum mixed-rate estimate (0.26/h) for the continuous release study that was conducted in a different portion of the same aquifer (Mackay et al. 2006), but are consistent with results of the field slug experiment of Zhang et al. (2006) that yielded a very low ethanol degradation rate (0.01/h).

Among many possible reasons for the difference between the Site 60 experimental results, two appeared to us as most likely. First, the two areas may have had different initial microbial communities; the continuous injection wells were within the area impacted directly by the original fuel spill. Second, the microbial communities had different time of exposure to ethanol. The rapid degradation of ethanol during Mackay et al. (2006)'s experiment was observed after a few days/weeks of injection. This is a significantly longer exposure history than the PPTs, where the communities were exposed for hours.

**Biostimulation Treatments**

To test for a biostimulation effect, PPT5 was conducted in a well (EJ17) not yet used for PPTs but after a 10-day biostimulation period consisting of four ethanol spikes (using approach described above) introduced 2 to 3 days apart. A drift period of 9.5 h was used. The recovery curves of ethanol and bromide were similar in shape (Figure 3) but the recovery of ethanol was much less than that of bromide (27% vs 68%; Table 1). The results from PPT5 show a decrease in recovery of ethanol relative to bromide from the beginning of the extraction and closely fit the theoretical line of Snodgrass and Kitanidis (1998) for first order degradation (Figure 3). Therefore, the data could be used to calculate the first order reaction rate, which was 0.31 ± 0.02/h (Table 1). It appears that the biostimulation period was sufficient to reduce the lag time to less than the drift time, that is, less than 9.5 h. After the microbial community was exposed to ethanol for 10 days, the biodegradation rate increased substantially above rates from PPTs without stimulation. Thus a short series of exposures to ethanol was adequate to stimulate the microbial populations to biodegrade ethanol at a rate on the order of that observed over the multimonth continuous release study (0.26/h) reported by Mackay et al. (2006).

EJ17 was then left for 4 weeks with no biostimulation, after which PPT6 was performed with a slightly longer drift of 15 h. The recovery plots for PPT6 (Figure 4) had a shape similar to those of PPT5, but yielded a significantly lower first order rate estimate of 0.13 ± 0.01/h. This suggests that, during the 4 weeks between PPT5 and PPT6, much of the biostimulation effect was lost, perhaps because of a decrease in the ability of the native microbial population to metabolize ethanol or to changes in the makeup of the microbial population.

A second set of tests to evaluate the impact of biostimulation was conducted using well EJ9, approximately 7 m cross gradient from the well used for the PPT5 and PPT6 biostimulation test (Figure 1). Recall that PPT4 had been
injected into the aquifer before the extraction began; only 4% of the injected ethanol was recovered, significantly less than the 28% recovery rate for Br (Table 1). The observed rate is very similar to that observed after 10-days of biostimulation at EJ17 during PPT5.

After an additional week of biostimulation in EJ9 (stimulation sequence of three additional spikes over the next week-PPT7-3 spikes over a week), the biodegradation rate was too fast for the chosen drift time 15.9 h used in PPT8 (data plots not shown). In this test, the first extraction sample was only slightly above the detection limit for ethanol and all subsequent points were at or below the detection limit. For this PPT, ethanol concentrations could only be quantified at two times: the injection condition and the first extraction point. Consequently the apparent rate was estimated from a two-point fit using the first order kinetic rate equation and is considered uncertain. During the rest of the treatments and showed no evidence for ethanol biodegradation. The effects of biostimulation were then evaluated in a series of six more PPTs over the course of 3.5 months interspersed with regular ethanol spikes for biostimulation. Three of these PPTs are shown in Table 1 and discussed hereafter (PPTs 7 to 9).

After 1 week of biostimulation with three spikes (i.e., three injections of ethanol-spiked groundwater), PPT7 was conducted (data plots not shown) with a drift time of 15.5 h. The ethanol concentration of the first extraction sample, 6 mg/L, was much lower than during earlier PPTs. Subsequent extraction samples had even lower ethanol concentrations, quickly dropping below the detection limit of 1 mg/L. Using the results when ethanol concentrations were still quantifiable (data plots not shown), a first order rate constant of 0.34 ± 0.08/h was calculated. The biodegradation rate was sufficient to degrade almost all of the ethanol

![Figure 3. Results of PPT5 (EJ17, 9.5 hour drift, 108 mg/L injectate ethanol concentration) following 10 days of biostimulation (four spikes).](image)

![Figure 4. Results of PPT6 (EJ17, 15.4 h drift, 110 mg/L injectate ethanol concentration) following 25 days of quiescence after PPT5, which was conducted after 10 days of biostimulation (see Figure 3).](image)
PPTs conducted in this sequence (these PPTs not numbered herein) until the drift time was adjusted for PPT9, the biodegradation rate was too fast relative to the ca. 15 h drift time and the PPTs were uninterpretable because no ethanol was detected during the extraction phase; these tests are discussed in more detail by Kline (2009).

After 9 weeks of biostimulation interspersed with PPTs, Well EJ9 was left under natural conditions, with no exposure to ethanol, for 1 month. A second set of biostimulation injections, which consisted of six spikes over the course of 2 weeks, were conducted prior to a final test, PPT9. During this test a shorter drift time of 1.2 h was used. In the recovery curves, bromide started at about 90% of the injection concentration and ethanol at about 70% (Figure 5). Ethanol was above the limit of detection in all samples collected during the first 3 hours of the extraction. Similar to all

PPTs conducted after biostimulation, biodegradation was observed based on the ln(ethanol/bromide) plot at a rate of $0.56 \pm 0.16$/h. The rate observed in this PPT was 40 to 70 times greater than that observed by Zhang et al. (2006) during their slug experiment in a different aquifer and previously reported microcosm rates (Powers et al. 2001). This rate is also 1.6 to 2.1 times greater than the minimum reaction rate estimated at this site by Mackay et al. (2006) during that continuous release study.

Conclusions

The PPTs performed at VAFB show that biostimulation over a period of a week can induce the in situ microbial population to acclimate to ethanol biodegradation causing the biodegradation rate to increase by an order of magnitude over the rate determined without stimulation. With further biostimulation, the rate increased by a factor of two. The PPT method was useful in this work for evaluating operational factors affecting rates of ethanol biodegradation. However, more research is needed to determine a practical approach for optimizing PPTs, though it is clear that on site analytical capabilities are desirable to allow field decisions not possible in this work. Our approach of adjusting the drift time had mixed results. The history of exposure of groundwater to ethanol and other fuel constituents may vary widely depending on rate, size and type of fuel spilled as well as processes controlling transport and fate of ethanol and other species in the vadose zone. As PPTs can be easily performed at many wells throughout a site, they could be used to characterize spatial variability in biodegradation rates that is expected because the exposure history should be variable at a spill site. Our work clearly shows that exposure history is critically important to the ethanol biodegradation rate. Because ethanol affects the microbial and chemical environment of a plume, understanding variations in existing or potential biodegradation rates may be necessary to evaluate the risk, both short- and long-term, of gasohol spills.

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