An Evaluation of Compound-Specific Isotope Analyses for Assessing the Biodegradation of MTBE at Port Hueneme, CA

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The use of compound-specific isotope analysis (CSIA) as a diagnostic tool for MTBE biodegradation in aquifers was tested at the Port Hueneme, CA site. There, a 1500-m long dissolved MTBE plume and associated engineered aerobic flow-through biobarrier have been well-studied, leading to delineation of regions of known significant and limited bioattenuation. This allowed comparison of field-scale CSIA results with *a priori* knowledge of aerobic MTBE biodegradation, leading to conclusions concerning the utility of CSIA as a diagnostic tool for other aerobic biodegradation sites. Groundwater samples were collected and analyzed for both ^{13}C and ^{2}H (D) in MTBE through the bioactive treatment zone and within the larger MTBE plume. For reference, the ^{13}C enrichment factor for MTBE biodegradation in laboratory-scale microcosms using site groundwater and sediments was also quantified. Aerobic microcosms showed a ^{13}C enrichment of 5.5 to 6.4 $\pm$ 0.2‰ over a two-order of magnitude concentration decrease, with an average isotope enrichment factor (ϵ_c) of −1.4‰, in agreement with other aerobic microcosm studies. Less ^{13}C enrichment (about 25%) was observed for similar MTBE concentration reductions in groundwater samples collected within the aerobic biotreatment zone, and this enrichment was comparable to the scatter in $\delta^{13}C$ values within the source zone. Increasing enrichment with decreasing MTBE concentration seen in microcosm data was not evident in either the ^{13}C or D field data. The discrepancy between field and laboratory data may reflect small-scale (<1 m) spatial heterogeneity in MTBE biodegradation activity and the mixing of water from adjacent strata during groundwater sampling; for example, relatively nonattenuated MTBE-impacted water from one stratum could be mixed with highly attenuated/low-MTBE concentration from another, and this could produce a sample with both reduced MTBE concentration and low enrichment. Overall, the results suggest that ^{13}C data alone may produce inconclusive results at sites where MTBE undergoes aerobic biodegradation, and that even with two-dimensional CSIA (^{13}C and D), an increase in the confidence of data interpretation may only be possible with data sets larger than those typically collected in practice.

Introduction

MTBE, a fuel oxygenate, has been used in gasoline to diminish carbon monoxide (CO) emissions and enhance octane. As a result, it is a frequent groundwater contaminant at gasoline-release sites, and relative to other cocontaminants of regulatory concern (i.e., benzene, toluene, xylenes, etc.), it has a higher solubility, lower sorption coefficient, and lower Henry's Law constant (*1*). Its transport is relatively unretarded relative to groundwater flow, and concentration attenuation with distance results primarily from physical mixing processes (i.e., dispersion and dilution) and biodegradation. With respect to the latter, biodegradation has been observed over a range of aerobic and anaerobic conditions in the laboratory and field (*2–7*); however, the significance of MTBE bioattenuation with distance at field sites appears to be more widely variable than its cocontaminants, as reflected in dissolved MTBE plume lengths ranging from about 100 m to 1−2 kilometers. Furthermore, the site-specific factors affecting dissolved MTBE plume bioattenuation are not well understood; for example, some of the well-known sites with 1000-m long dissolved MTBE plumes also have rapidly bioattenuated benzene plumes.

It has become increasingly important to be able to assess if bioattenuation of dissolved MTBE plumes is occurring, as corrective action decisions are based to some extent on anticipated plume dynamics (i.e., growth, stability, or shrinking), and these are impacted by the significance of bioattenuation. This has led to interest in the use of compound-specific isotope analysis (CSIA) as a diagnostic tool for assessing MTBE biodegradation in aquifers (*8–15*). As hydrocarbon biodegradation occurs, the relative fraction of higher mass isotopes (i.e., ^{13}C and ^{2}H (D)) increases in the remaining parent compound with time (*16*). Thus, if a ^{13}C or D isotope enrichment trend is correlated with decreasing parent compound concentrations in the field, then the decline with time or distance is attributed in part to biodegradation. CSIA has been used to assess the biodegradation of aromatic and chlorinated hydrocarbons and more recently has been applied to MTBE in groundwater. For reference, the term "enrichment" in the discussion below refers to changes in $^{13}C/^{12}C$ and $D/^{1}H$ ratios, where these ratios are commonly reported as $\delta^{13}C$ and δD % defined, for example by $\delta^{13}C\ \% = (R_{sample}/R_{standard} - 1) \times 1000$, and R_{sample} and $R_{standard}$ are $^{13}C/^{12}C$ ratios of the sample and the international standard (Vienna Pee Dee Belemnite for $\delta^{13}C$ (*17*)). Enrichment factors are the slope of the line fit through data plotted as $1000\ \ln(R/R_o)$ vs $\ln F$, where R/R_o is the isotopic ratio at some MTBE fraction remaining (F) divided by the initial isotopic ratio at $F = 1$.

MTBE-specific proof-of-concept studies have generally coupled CSIA with laboratory microcosm experiments to identify the expected enrichment. For example, Hunkeler et al. (*8*) reported ^{13}C enrichments of 5.1 to 6.9‰, and carbon enrichment factors (ϵ_c) ranging from −1.5 to −2.0‰ during aerobic biodegradation. Gray et al. (*9*) observed similar ^{13}C enrichment (5.2 to 8.1‰ with ϵ_c from −2.0 to −2.4‰) and

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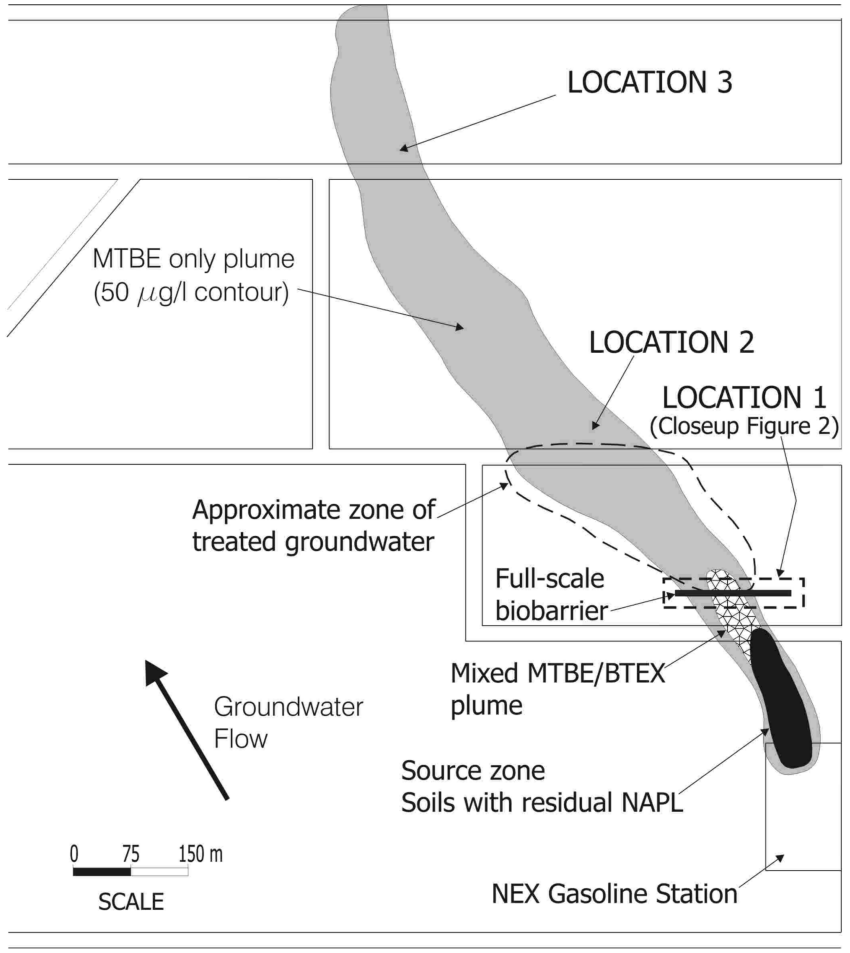

FIGURE 1. Dissolved MTBE plume at the Naval Base Ventura County, Port Hueneme, CA, showing the three general sampling areas in this study.

much larger D enrichment from 75 to 150‰ with hydrogen enrichment factors (ϵ_H) from −29 to −66‰, again in aerobic microcosms. Kuder et al. (*11*) report on results from anaerobic microcosms, and their data show much larger ^{13}C enrichment (ϵ_c about −13‰) and less D enrichment (ϵ_H about −16‰) than in the aerobic microcosms discussed above. The results from these and other studies suggest that enrichment factors may be site- and microbial consortium-specific (*9, 15*) and that the sensitivity of the CSIA tools may be different in aerobic and anaerobic settings (*10, 11*); for example, Kuder et al. (*11*) suggest that D enrichment data may yield a more easily interpreted result at sites where aerobic biodegradation is occurring and ^{13}C analysis may be easier to interpret at anaerobic biodegradation sites. Furthermore, the data from two-dimensional CSIA (^{13}C and D) might help identify the mechanism of bioattenuation (aerobic vs anaerobic).

This study is complementary to those MTBE-specific CSIA studies discussed above in that it seeks to better understand the utility of carbon and hydrogen CSIA as diagnostic tools for assessing MTBE biodegradation at field sites. This study is unique relative to the others for two reasons. First, this study was conducted at a field site with a well-studied engineered aerobic biobarrier; thus, use of the CSIA tools could be tested under field conditions in regions known to have significant and insignificant biodegradation. Previously published field studies applied the CSIA tools and inferred the presence or absence of biodegradation from CSIA results from microcosm studies but did not have independent assessment of biodegradation. Second, the number of CSIA analyses at this site is large compared to other studies (and sample numbers typical of practice), and so the data are useful for assessing how confidence in conclusions might be affected by different numbers of samples.

Field Site. The dissolved MTBE plume at the Naval Base Ventura County, Port Hueneme, CA is attributed to a gasoline release that occurred in the mid-1980s at the Base service station. At the time of this study, the MTBE plume was about 1500-m long and about 150-m wide as shown in Figure 1. Dissolved MTBE was present across the 3-m vertical extent of the surficial aquifer, which is found roughly 3-m below ground surface (bgs). The aquifer is bounded below by a clay aquitard, and the saturated zone extends about 0.5 m into an upper silty fill layer. The majority of aquifer sediments between 3 to 6 m bgs are fine to medium sands, characterized by increasing coarseness with depth. Groundwater flows southwest, with hydraulic gradients ranging from 0.001 to 0.003 m/m, and the groundwater seepage velocity has been estimated from MTBE plume history and hydraulic property data to be about 0.3 m/d along the faster flow paths. The groundwater table fluctuates approximately 0.5 m throughout the year. The dissolved MTBE plume is anoxic, and while the MTBE plume is only weakly attenuated with distance, the dissolved benzene, toluene, ethylbenzene, and xylene co-contaminants are naturally attenuated within about 50 m of the down-gradient edge of gasoline-containing source zone aquifer sediments. More site and MTBE plume details can be found in refs *18* and *19*.

In August 2000 a full-scale aerobic flow-through biobarrier was installed immediately down-gradient of the source zone. The 150-m long biobarrier was oriented perpendicular to groundwater flow so that dissolved contaminants (MTBE, TBA, benzene, toluene, etc.) in groundwater would be

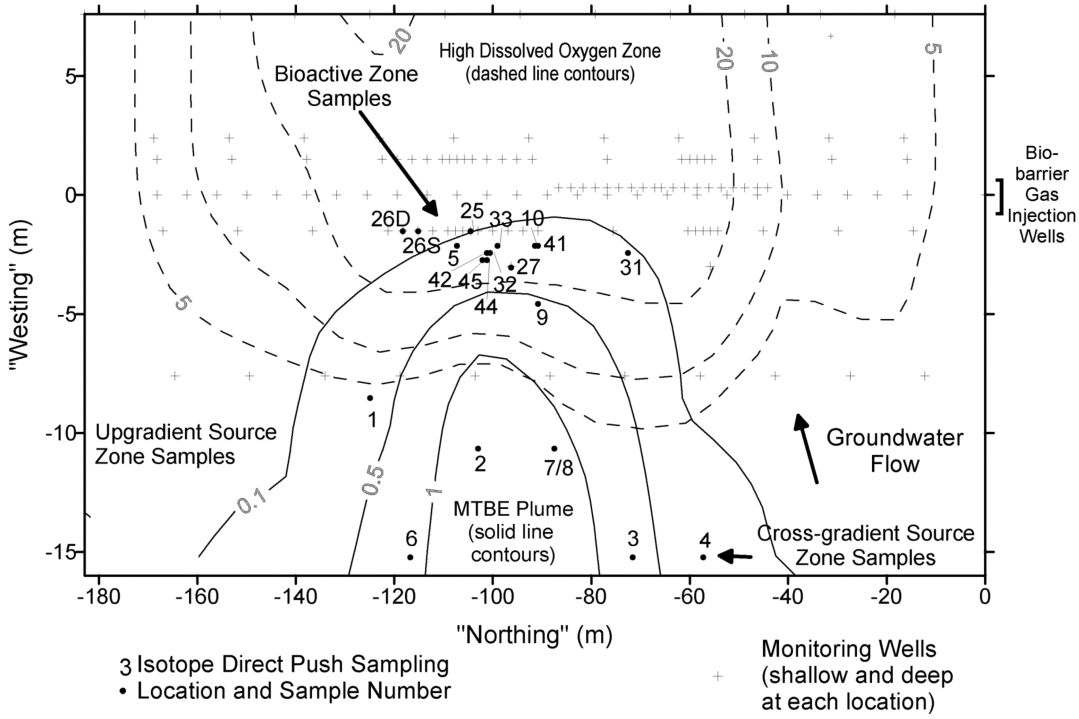

FIGURE 2. Dissolved MTBE plume [mg/L] and dissolved oxygen (DO) [mg/L] concentration contours near the aerobic biobarrier. Modified from Johnson et al. (*18*).

aerobically biodegraded as they flowed through a well-oxygenated treatment zone. Oxygen was delivered in a pulsed mode by 144 injection wells and air by 108 injection wells, and the central section of the biobarrier was seeded with MTBE-degrading mixed and single cultures. Performance was assessed by sampling from about 400 monitoring wells. Within about one year, dissolved oxygen levels (DO) in groundwater increased from <1 mg/L to >5 mg/L in air injection zones and >20 mg/L in oxygen injection zones; influent MTBE concentrations ranging from 10 to 10 000 μg/L across the width of the biobarrier were reduced to nondetect levels (<10 μg/L) as shown in Figure 2. The biobarrier and associated monitoring network were designed to ensure insignificant volatilization losses and to prevent groundwater flow from bypassing the treatment zone. Thus, the MTBE attenuation along flow paths entering the biobarrier can confidently be attributed to aerobic biodegradation.

Study Design

Concept. Groundwater samples were collected across the down-gradient width of the source zone and in the well-oxygenated biobarrier treatment zone, identified as Location 1 in Figure 1. Sampling points were selected in order to assess (a) the isotopic enrichment vs MTBE concentration along flow paths through the aerobic biobarrier treatment zone, and (b) isotopic enrichment vs MTBE concentration across the MTBE plume width immediately up-gradient of, and unaffected by, the biobarrier. For comparison, aquifer sediment samples were also collected in Location 1 to quantify the isotope enrichment factor for MTBE biodegradation using microcosm experiments. Dissolved oxygen concentrations (DO) >2 mg/L were used to delineate aerobically active zones. Groundwater samples were also collected for isotopic analysis in Locations 2 and 3 (in Figure 1) far down-gradient of the source zone and unaffected at that time by the biobarrier operation to determine if biodegradation was occurring in this marginally attenuated MTBE plume.

The study occurred in a phased approach and included two field sampling events. Phase 1 focused on the use of ^{13}C CSIA and included groundwater and aquifer sediment sampling and microcosm experiments. Phase 2 included only groundwater sampling, but with ^{13}C and D CSIA. A total of 50 groundwater samples were collected from Locations 1, 2, and 3 in the first phase and another 28 samples were collected from Locations 1 and 2 in the second phase of this study. Phase 2 samples were collected as close as possible to phase 1 sampling locations and are designated with an asterisk on the plots. For reference, these are large sample numbers relative to the sampling and analyses at other CSIA field sites (*10*, *11*, *13*).

Sample Collection. Aquifer sediment and groundwater samples were collected using Geoprobe direct-push tools. Groundwater samples were collected at discrete shallow (2.5–3 m bgs) and deep (5.5–6 m bgs) intervals using a peristaltic pump; slow flow conditions were used and samples were collected in 40-mL VOA vials after purging and stabilization of the DO measured in a flow-through small-volume DO measurement cell. Aquifer sediment samples were collected in clear plastic liners that were capped in the field and then later subsampled for the microcosm studies. Two sets of triplicate groundwater samples were collected in the first phase of this study and one triplicate set of samples was collected in the second phase.

Laboratory Microcosms. Microcosms were performed in 1 L clear-glass narrow-neck bottles containing 320 g of aquifer sediment. Site groundwater (650 mL) was added after filtering and sparging with pure oxygen for about 10 min. The remaining 200 mL of headspace was also purged with oxygen gas and the bottles were sealed with butyl rubber screw-cap septa and tape to prevent volatilization. Respiration-inhibited controls were prepared by adding 8 g of solid sodium azide. MTBE was added to achieve initial concentrations ranging from 1 to 10 mg/L, and the bottles were placed on a shaker device to ensure continuous mixing.

The dissolved MTBE concentrations were analyzed by headspace analyses as described below. After headspace sampling, 0.5 mL of oxygen was injected into the microcosm to replace the extracted headspace. As MTBE concentrations declined to predetermined target levels, water samples were

collected for CSIA in 40 mL VOA vials. These were preserved by adding 0.2 mL of saturated mercuric chloride solution and then shipped to the University of Waterloo for ^{13}C analysis.

Analytical Methods. Dissolved MTBE concentrations were analyzed by headspace gas analysis using an SRI Model 8610C GC with flame ionization (FID) and photoionization (PID) detectors, and fitted with a 0.53-mm i.d. × 60-m MXT-Vol capillary column. Headspace samples (0.5 mL) were analyzed under isothermal oven conditions (70 °C) unless other fuel compounds were detected, and then an oven temperature program was used (70 °C for 4 min, heating at 15 °C/min to 180 °C, hold at 180 °C for 10 min). Four-point calibrations were conducted each day with known standards (10, 1, 0.1, and 0.01 mg/L), and after every 10 samples, one of the standards was run to assess detector consistency. MTBE analyses were conducted using this method in the field during sampling and in the laboratory during the microcosm tests.

Phase 1 groundwater samples collected for ^{13}C analysis were stored in 40 mL VOA vials, preserved with 0.2 mL of saturated mercuric chloride solution, stored on ice, and sent for analyses to the Environmental Isotope Laboratory at the University of Waterloo. The CSIA method employed has been described by Hunkeler et al. (*8*). Phase 2 samples collected for ^{13}C and D analyses were stored in 40 mL VOA vials, preserved with 0.5 mL of a saturated trisodium phosphate (TSP) solution, stored on ice, and sent for analyses to the School of Geology and Geophysics at The University of Oklahoma. The CSIA method used for those samples has been described by Kolhatkar et al. (*10*). The isotope data is generally reported in δ per mil unit (‰) and the analytical precision is ±0.2‰ for ^{13}C and ±3‰ for D. As discussed below, CSIA differences (possibly an off-set) between the two external laboratories were noted when comparing results from similar areas of the site. These observed difference, however, are immaterial to the analyses and conclusions presented below. Phase 1 results and conclusions are based only on phase 1 data and phase 2 results and conclusions are based only on phase 2 data. No mixing of the data sets occurred in the analysis and data presentation.

Results and Discussion

Microcosm Experiments. Microcosms were conducted with initial MTBE concentrations at 6.0, 3.2, and 0.7 mg/L, and for all, the initial $\delta^{13}C$ value was approximately −31‰. The final $\delta^{13}C$ values ranged from −24.6 to −25.5 ± 0.2‰ after one- to two-orders of magnitude concentration reduction, or a ^{13}C enrichment of 5.5 to 6.4 ± 0.2‰ as shown in Figure 3. The carbon isotope enrichment factor (ϵ_c) was on average −1.4‰. Control microcosms did not show any biodegradation activity and no change in δ ^{13}C for the duration of the experiment. These results are similar to those previously published for aerobic microcosm experiments; for example, Hunkeler et al. (*8*) reported ^{13}C enrichments of 5.1 to 6.9‰, and ϵ_c from −1.5 to −2.0‰, and Gray et al. (*9*) reported ^{13}C enrichment of 5.2 to 8.1‰ and ϵ_c from −2.0 to −2.4‰.

Phase 1 Field Results. Figures 4a to 4c represent $\delta^{13}C$ [‰] vs MTBE concentration [μg/L] results from Locations 1 to 3 shown in Figure 1, respectively. The data values are also summarized in Table S1 found in the Supporting Information. Of the 50 samples collected and analyzed, only 39 samples with measurable MTBE concentrations resulted in measurable ^{13}C values. All plots present, for reference, results from samples collected across the width of the low-DO downgradient edge of the source zone. Those samples ranged in MTBE concentration from 4900 to 70 μg/L and from −30.6 to −29.1‰ in $\delta^{13}C$ values (mean = −29.8 ± 0.5‰). There also is no clear trend in the source zone $\delta^{13}C$ vs MTBE concentration. Variations in source zone $\delta^{13}C$ values are not unexpected and may reflect contributions from different fuel

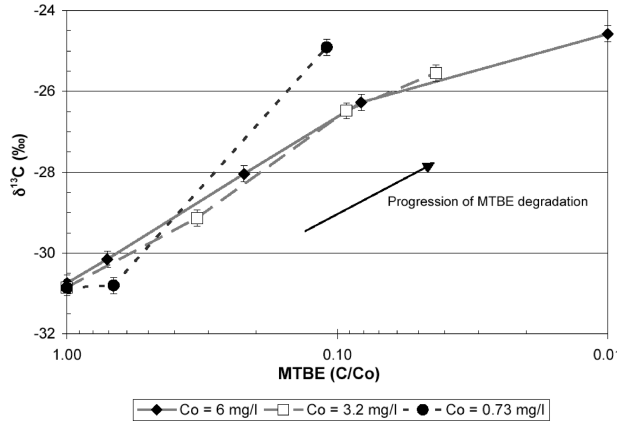

FIGURE 3. $\delta^{13}C$ results from aerobic biodegradation microcosms. Error bars correspond to the laboratory analytical uncertainty. The isotope enrichment factor (ϵ_c) is on average −1.37‰.

releases over time (*11*). At this study site the near-source $\delta^{13}C$ variation is about twice the analytical error and comparable to the variability for triplicate samples.

As shown in Figure 4a, samples collected from the well-oxygenated biotreatment zone range in MTBE concentration from 30 to 720 μg/L, and in $\delta^{13}C$ value from −29.5 to −26.4‰. The mean $\delta^{13}C$ value of all treatment zone samples is −28.2 ± 0.9‰, and about 75% of the results fall in the range −29 to −27‰. Overall, treatment zone $\delta^{13}C$ values are slightly enriched relative to near-source values (a 1.6‰ difference between the mean values, with overlapping data ranges).

The observed enrichment of roughly 1 to 2‰ for most field samples is significantly less than that observed in microcosm studies for similar MTBE concentration reductions ($\delta^{13}C$ change of about 5‰ for a 1–2 order of magnitude change in MTBE concentration). Bennett et al. (*20*) also report a very limited aerobic isotopic enrichment (~1‰) at another field site characterized by a large change in MTBE mass. It is worth noting that no significant enrichment is observed along some possible flow paths; for example, concentrations are reduced from 610 to 50 μg/L from 45S to 25S with no discernible $\delta^{13}C$ change (−28.2 to −28.4‰). In addition, there are significant differences in $\delta^{13}C$ values for some closely spaced points; for example, samples 10S and 41S have similar MTBE concentrations of about 100 μg/L but $\delta^{13}C$ values of −28.2 and −26.4‰, respectively. For reference, the average analytical error for laboratory duplicates is about 0.2‰ and the standard deviation for triplicate field samples was about 0.4‰ on ^{13}C analysis.

The smaller enrichment in ^{13}C and the lack of a trend in $\delta^{13}C$ [‰] vs MTBE concentration in the field data suggest that interpretation of stable carbon isotope data from some sites will be more complex than suggested from laboratory studies, and occasionally the results will be inconclusive. Recall that this is a field site where aerobic MTBE biodegradation is known confidently because it has been engineered and verified by other independent measures; however, the $\delta^{13}C$ results provide a relatively weak biodegradation signal compared to what would be expected based on the laboratory microcosm studies. Also, the data point to challenges associated with small $\delta^{13}C$ data sets. For example, if the only data available were those results from 8S, 9S, 10D and 12D, 13S, and 36D, one might conclude that MTBE biodegradation was not occurring in the biotreatment zone.

For this site, we hypothesize that the reduced isotopic enrichment results from a combination of spatial heterogeneity in the aerobic biodegradation activity and the mixing of water from different vertical strata during sampling (even for the relatively discrete-depth samples collected in this

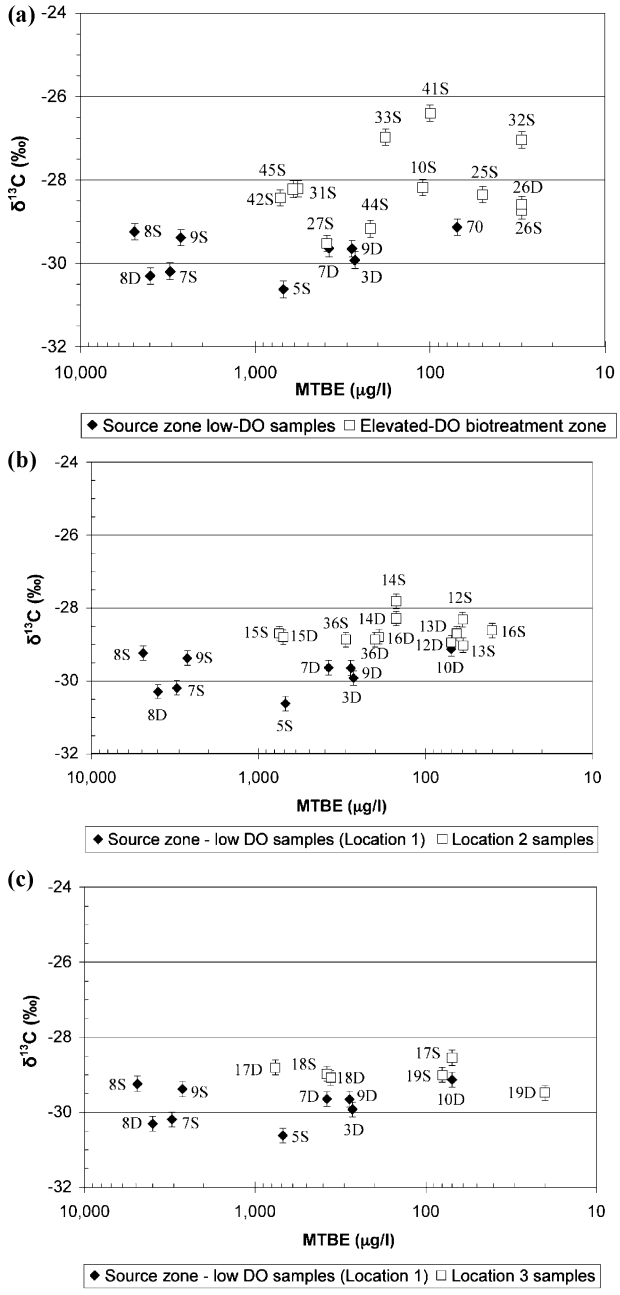

FIGURE 4. (a) Phase 1 δ^{13}C results from Location 1 samples (Figure 2). Error bars correspond to the laboratory analytical uncertainty. Method error from field triplicate samples is 0.4‰ for δ^{13}C. (b) Midplume δ^{13}C results (Location 2) from phase 1 sampling, compared with source zone sample results (Location 1). Error bars correspond to the laboratory analytical uncertainty. Method error from field triplicate samples is 0.4‰ for δ^{13}C. (c) Leading-edge MTBE-plume δ^{13}C results (Location 3) from phase 1 sampling, compared with source zone sample results (Location 1). Error bars correspond to the laboratory analytical uncertainty. Method error from field triplicate samples is 0.4‰ for δ^{13}C.

work). For example, relatively nonattenuated MTBE-impacted water from one stratum could be mixed with highly attenuated/low-MTBE concentration from another, and this could produce a sample with both reduced MTBE concentration and low ^{13}C enrichment. This mixing effect during sampling has been proposed previously to explain low isotope enrichment observed at other field sites (*11*). At the Port Hueneme site, spatial heterogeneity in the aerobic biodegradation activity over short distances was evident during the

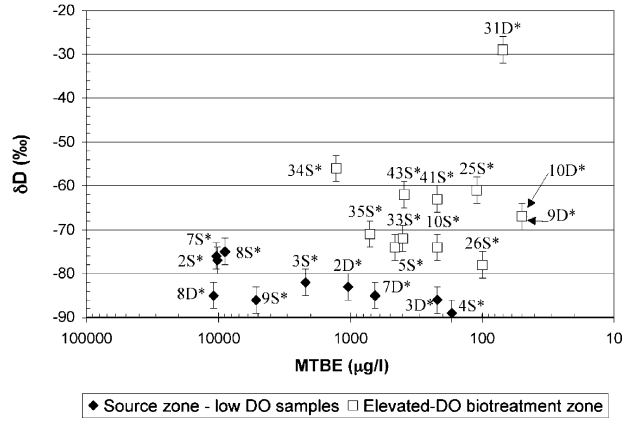

FIGURE 5. δD results from phase 2 near-biobarrier sampling (locations shown in Figure 2). Error bars correspond to the laboratory analytical uncertainty. Method error (not shown) from field triplicate samples was 6‰ for δD. Samples denoted with an asterisk reflect their collection as close as possible to phase 1 locations.

field sampling; in particular it was a challenge to obtain samples with MTBE concentrations in the low 100s of μg/L, as the concentrations would be attenuated to nondetect levels over very short distances and it was not unusual to have more than an order-of-magnitude difference in MTBE concentration between discrete samples collected at different vertical intervals at the same location.

For midplume Location 2, the δ^{13}C values presented in Figure 4b range from −29.0 to −27.8‰ for MTBE concentrations between 750 and 40 μg/L. The mean δ^{13}C value (−28.6 ± 0.3‰) is slightly enriched relative to source samples (mean = −29.8 ± 0.5‰) and is similar to the mean of values from the aerobic biotreatment zone (−28.2 ± 0.9‰), suggesting attenuation by biodegradation. For leading-edge MTBE plume Location 3, the δ^{13}C values ranged from −29.5 to −28.6 ± 0.2‰ for MTBE concentrations between 770 and 20 μg/L as shown in Figure 5c. The Location 3 mean δ^{13}C value (−29.0 ± 0.3‰) falls midway between source samples and aerobic biotreatment zone samples, only weakly suggesting attenuation by biodegradation. Neither the data in Figure 4b nor the data in Figure 4c show trends in δ^{13}C [‰] vs MTBE concentration.

Phase 2 Field ^{13}C and D-MTBE Results. In phase 2, 11 source zone, six biotreatment zone, and six leading-edge plume (Location 3) samples were collected and analyzed for δ^{13}C and δD. The δ^{13}C showed similar trends to those in phase 1, with the exception that δ^{13}C values were about 1‰ greater in phase 2 than in phase 1. For example, the mean source zone δ^{13}C value was −28.2 ± 0.3‰ in phase 2 and −29.8 ± 0.5‰ in phase 1, the well-oxygenated biotreatment zone mean δ^{13}C value was −27.5 ± 0.5‰ in phase 2 and −28.2 ± 0.9‰ in phase 1, and the leading-edge plume Location 3 mean δ^{13}C value was −27.8 ± 0.1‰ in phase 2 and −29.0 ± 0.3‰ in phase 1. These differences are attributed to the use of different laboratories for CSIA in phase 1 and phase 2, and as noted above, these differences are immaterial to the analyses and conclusions presented below. Phase 1 results and conclusions are based only on phase 1 data and phase 2 results, and conclusions are based only on phase 2 data.

As in phase 1, biotreatment zone δ^{13}C values are slightly enriched relative to source zone values but smaller than microcosm enrichments). Location 3 δ^{13}C values are less enriched, and the data do not show any clear δ^{13}C vs MTBE concentration trends.

Figure 5 presents δD vs MTBE concentration results from source zone and biotreatment zone samples (Location 1 in

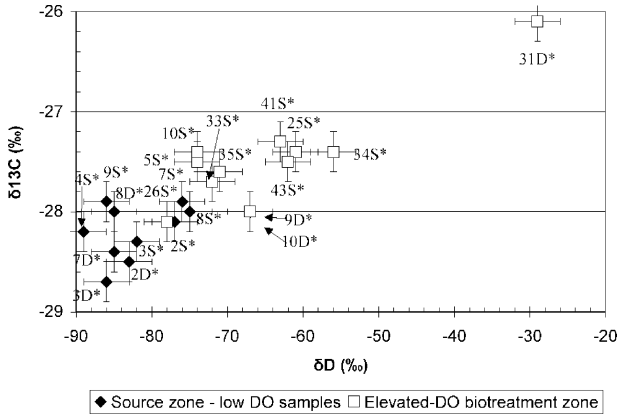

FIGURE 6. $\delta^{13}C$ vs δD for source zone and biobarrier samples during phase 2 sampling. The error bars indicate the laboratory analytical error. Method error (not shown) from field triplicate samples are 0.03‰ for $\delta^{13}C$ and 6‰ for δD. Samples denoted with an asterisk reflect their collection as close as possible to phase 1 direct-push locations shown in Figure 2.

Figure 1). These data are summarized in Table S2 found in the Supporting Information. All plots present, for reference, results from samples collected in the low-DO source zone region. Those samples, collected across the width of the dissolved plume, ranged in MTBE concentration from 10400 to 50 μg/L and from −89 to −75‰ in δD values (mean = −82 ± 5‰). There is no clear trend in the source zone δD vs MTBE concentration, and the near-source δD variation is not large compared to the analytical error or the observed variability for triplicate samples (±3 and ± 5‰, respectively).

As shown in Figure 5, samples collected from the well-oxygenated biotreatment zone range in MTBE concentration from 1280 to 50 μg/L, and in δD value from −78 to −29‰. The mean δD value of all treatment zone samples is −65 ± 12‰, and about 75% of the results fall in the range −74 to −58‰. Overall, treatment zone δD values are enriched relative to source zone values, but the upper-end source zone δD values overlap with the lower-end biotreatment zone δD values. As in phase 1, phase 2 $\delta^{13}C$ results do not exhibit a trend in δD vs MTBE concentration, and this may reflect a combination of spatial heterogeneity in the aerobic biodegradation activity and the mixing of water from different vertical strata during sampling.

Location 3 (leading-edge plume samples) results are presented in Figure S1 in the Supporting Information. The δD values range from −71 to −56‰, with a mean value of −63 ± 5‰ for MTBE concentrations ranging from 1400 to 90 μg/L. Overall, Location 3 δD values are enriched relative to source zone values, but the upper-end source zone δD values overlap with the lower-end biotreatment zone δD values.

Kuder et al. and Zwank et al. (*11, 14*) suggest that two-dimensional ($\delta^{13}C$ and δD) CSIA is needed to have confidence in the interpretation of CSIA results for MTBE applications, and that $\delta^{13}C$ vs δD plots might be used to distinguish between aerobic and anaerobic biodegradation. To examine this, Figure 6 presents the $\delta^{13}C$ vs δD plot for Location 1, which includes both source zone and aerobic-biotreatment zone samples. The general trend in Figure 6 is consistent with that expected by Kuder et al. for aerobic biodegradation and is significantly different from the trend in their field data for sites that they assert are undergoing anaerobic MTBE biodegradation.

The Use of CSIA Tools for Determining Aerobic MTBE Biodegradation. Like others (i.e., 10, 11, 13−15, 20−22), the goal of this study was to better understand the utility of carbon and hydrogen CSIA as diagnostic tools for assessing MTBE biodegradation in groundwater at field sites. The unique feature of this work is that it was conducted at a site with a dissolved MTBE plume having known regions of significant and insignificant aerobic MTBE biodegradation, and the data density was much greater than other studies.

To place observations given below in context, it should be noted that CSIA is increasingly being used in practice as a line-of-evidence for determining if MTBE biodegradation is occurring, and a typical application involves the analysis of only about a half-dozen samples (i.e., 11), in part because the analyses are relatively expensive and only performed by a few laboratories. Thus, it is important for practitioners to understand the benefits vs costs of employing CSIA tools, and issues that might arise when interpreting CSIA data. The following are relevant for those considering or designing CSIA sampling plans.

The observed enrichment in the known aerobic biodegradation region for this field site is only about 25% of that observed in microcosm studies for similar MTBE concentration reductions, and the field data exhibit no trend in enrichment vs concentration reduction. Furthermore, the data scatter is significant compared to the mean enrichment and the standard laboratory error for the analyses. Thus, CSIA field efforts with typical sample numbers might conclude that less than an order-of-magnitude (or even no) reduction in MTBE concentration is attributable to aerobic biodegradation, when it is actually responsible for 1000× concentration reduction as at this site. The observation that enrichment observed in the field is less than that observed in laboratory microcosms has been reported by others who suggest that it could be the result of flow path mixing as well as spatial heterogeneity in microbial populations (*11, 15, 21, 22*).

Larger data sets than those typically collected are likely needed to ensure reasonable confidence in data interpretation at aerobic biodegradation sites. The use of two-dimensional analyses ($\delta^{13}C$ and δD), as noted by others (*11, 15, 21, 22*), can help increase confidence. Even with this, however, down-gradient field data need to span a $\delta^{13}C$ or δD range that is large compared to the spatial variability in $\delta^{13}C$ or δD in the source zone (approximately 1‰ at this study site), or the results will be inconclusive. Observing the needed enrichment may also be difficult because the operating range of groundwater concentrations may be less than two orders-of-magnitude given the minimum concentrations needed for CSIA analyses.

The value of laboratory microcosm CSIA data or its routine use in interpreting the contribution of biodegradation from field data is not clear. For example, microcosm plots of CSIA results vs MTBE concentration data are sometimes used to draw quantitative conclusions from field CSIA results; however, the change in field $\delta^{13}C$ values at the Port Hueneme study site are significantly less than that in the microcosms and the field $\delta^{13}C$ and δD data do not exhibit any dependence on MTBE concentration.

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Supporting Information Available

A table of all data values presented in the figures as well as a plot showing MTBE concentration vs δD in sampling Location 3. This information is available free of charge via the Internet at http://pubs.acs.org.

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