EXECUTIVE SUMMARY

Extending The Applicability of Compound-Specific Isotope Analysis To Low Concentrations Of 1,4-Dioxane Phase II

SERDP Project ER-2535

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ACRONYMS AND ABBREVIATIONS

ACB	Aerobic co-metabolic biodegradation
AFB	Air Force Base
AFP	Air Force Plant
C	Carbon, an element
^{12}C	Carbon-12, most abundant isotope of carbon with atomic mass of 12
^{13}C	Carbon-13, less abundant isotope of carbon with atomic mass of 13
^{14}C	Carbon-14 (radiocarbon), a radioactive isotope of carbon with atomic mass of 14
$^{13}C/^{12}C$	Stable carbon isotope ratio
CCAFS	Cape Cañaveral Air Force Station
CSIA	Compound-specific isotope analysis
$\delta^{13}C$	Stable carbon isotope ratio as per mill (‰) difference from internationally accepted standard
DoD	U.S. Department of Defense
EPA	U.S. Environmental Protection Agency
ERSON	Statement of Need, Environmental Restoration Program Area
ESTCP	Environmental Security Technology Certification Program
H	hydrogen, an element
^{1}H	protium, the most abundant isotope of hydrogen with atomic mass of 1
^{2}H	deuterium, less abundant isotope of hydrogen with atomic mass of 2
$^{2}H/^{1}H$	stable hydrogen isotope ratio
MNA	Monitored natural attenuation
NRC	National Research Council
SERDP	Strategic Environmental Research and Development Program
SLC-16	Space Launch Complex 16
THF	Tetrahydrofuran
µg/L	Micrograms per liter
VPDB	Vienna Pee Dee Belemnite (internationally accepted standard for referencing carbon isotope ratios)
VSMOW	Vienna Standard Mean Ocean Water (internationally accepted standard for referencing hydrogen isotope ratios)

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1.0 INTRODUCTION

At many DoD sites, even the most costly and aggressive groundwater cleanup methods will not be able to achieve the low concentration cleanup goals set by the U.S. Environmental Protection Agency (EPA) and/or state agencies for a variety of contaminants in a reasonable timeframe (NRC, 2013). A transition from active remediation to long-term management with monitored natural attenuation (MNA) is the most realistic outcome for many sites (NRC, 2013). ERSON-15-01, issued by the Strategic Environmental Research and Development Program (SERDP), calls for research that will lead to an improved understanding of long-term natural attenuation processes on contaminants in groundwater.

A critical process of natural attenuation is contaminant degradation. It is difficult to demonstrate degradation of 1,4-dioxane, which is often present at chlorinated solvent sites but is considered recalcitrant in the subsurface. This research project aimed to develop a method to provide direct evidence for the intrinsic degradation of 1,4-dioxane at low concentrations in groundwater. This limited-scope research project is important to the Department of Defense (DoD) because the expected outcome will allow for a cost-effective and readily implementable "field method" to assess the natural and enhanced biodegradation of 1,4-dioxane in groundwater. Specifically, this project sought to extend the applicability of compound-specific isotope analysis (CSIA) to low 1,4-dioxane concentrations in groundwater so that CSIA can be used to document *in situ* biodegradation of 1,4-dioxane at DoD sites.

CSIA is a powerful tool for providing evidence of degradation of many groundwater contaminants including fuel-related hydrocarbons, oxygenates, and chlorinated solvents (Hunkeler et al. 2009), where a change in the ratio of certain stable isotopes comprising the contaminant of interest (e.g., stable isotope ratios of carbon [$^{13}C/^{12}C$] and hydrogen [$^{2}H/^{1}H$]) typically occur with biodegradation. By convention, stable isotope ratios are reported in "delta" (δ) notation as the relationship between the isotopic ratio of a sample and an internationally defined standard, as defined in Equation 1. Here, the standard used for the $^{13}C/^{12}C$ ratio is the Vienna Pee Dee Belemnite (VPDB) and the standard for the $^{2}H/^{1}H$ ratio is Vienna Standard Mean Ocean Water (VSMOW). Each sample's stable isotopic ratio is expressed relative to that of the standard in parts per thousand, or per mill (‰), using the following expression:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000\ ‰ \qquad \textbf{Equation 1}$$

Where R_{sample} is the $^{13}C/^{12}C$ or $^{2}H/^{1}H$ ratio measured in a 1,4-dioxane sample, and $R_{standard}$ is the $^{13}C/^{12}C$ or $^{2}H/^{1}H$ ratio of VPDB or VSMOW, respectively. In delta notation, the $^{13}C/^{12}C$ ratio is expressed as "$\delta^{13}C$" and the $^{2}H/^{1}H$ ratio is expressed as "$\delta^{2}H$." All isotope ratios reported herein use the delta notation, in units of per mill (‰).

Laboratory-controlled degradation reactions of 1,4-dioxane under various conditions were performed in connection with this project (ER-2535) and SERDP Project ER-2303. These studies found that the degree of enrichment in ^{13}C and ^{2}H varied among the reaction conditions, as shown by the slope of the dual isotope plot ($\Delta\delta^{2}H/\Delta\delta^{13}C$). These new findings demonstrate the unique potential of CSIA for assessing 1,4-dioxane degradation at field sites. First, the dual-isotope slope may provide evidence for *in situ* biodegradation of 1,4-dioxane and insights on the activities of monooxygenases degrading 1,4-dioxane in groundwater. Second, the dual isotope slope facilitates the selection of enrichment factors for the quantification of the extent of 1,4-dioxane degradation using the Rayleigh equation.

2.0 OBJECTIVES

The objectives of Project ER-2535 are aligned with the overall objective of the Statement of Need for the Environmental Restoration Program Area (ERSON-15-01): *to improve estimates of the long-term impact of natural attenuation processes on groundwater contaminants*.

Specific objectives of this research are to:

1. Develop a reliable method for performing CSIA on low levels of 1,4-dioxane in groundwater (e.g., one to 10 micrograms per liter (µg/L);
2. Assess the fractionation of stable carbon and hydrogen isotope ratios during the aerobic co-metabolic biodegradation (ACB) of 1,4-dioxane under laboratory and field conditions; and
3. Assess the use of stable carbon and hydrogen isotope ratios as tools to evaluate biodegradation of 1,4-dioxane at U.S. Department of Defense (DoD) sites with different groundwater conditions.

The project was implemented in two phases. Phase I completed the objectives listed above and the results are presented in the *Final Report: Extending the Applicability of Compound-Specific Isotope Analysis to Low Concentrations of 1,4-Dioxane, SERDP Project ER-2535* (Haley & Aldrich, 2018). Phase II of Project ER-2535 sought to expand the results for the third objective by:

1. Increasing the database of stable isotopic composition of neat 1,4-dioxane sources by 10 in order to improve interpretation of field data; and
2. Increasing the number of field sites where CSIA is applied to low concentrations of 1,4-dioxane in groundwater at DoD sites.

This report documents the Phase II results.

3.0 TECHNICAL APPROACH

The overall technical scope of work was designed to achieve the Phase II objectives and included the following three tasks:

1. Assessment of 1,4-Dioxane Source Isotope Composition;
2. CSIA of 1,4-Dioxane at Field Sites; and
3. Final Report Addendum.

Under Task 1, the stable carbon and hydrogen isotope ratios of 1,4-dioxane from ten different commercially available samples were analyzed to expand the isotopic database. In addition to analyzing different 1,4-dioxane suppliers, a simple evaporation experiment was performed to assess whether the isotopic composition of 1,4-dioxane changes when neat 1,4-dioxane undergoes evaporation. As part of Task 2, six case studies were completed at DoD sites while two were completed at non-DoD sites. An average of six samples were collected from each site for CSIA of 1,4-dioxane. When combined with the four Phase I case studies, a total of 12 sites have been sampled for this project; 68 groundwater samples have been analyzed for $\delta^{13}C$, and 54 have been analyzed for $\delta^{2}H$, not including duplicate and replicate analyses and samples with insufficient mass for isotopic characterization.

Task 3 included the management and coordination of research activities as well as routine project reports and technology transfer. This report comprises the final Task 3 deliverable.

4.0 RESULTS AND DISCUSSION

4.1 ISOTOPIC COMPOSITION OF 1,4-DIOXANE OBTAINED FROM CHEMICAL SUPPLIERS

As shown in **Figure ES-1**, the range and median $\delta^{13}C$ values for undegraded "source" 1,4-dioxane are like those previously reported by Wang (2016) but higher than those reported by Pornwongthong (2014). The range in $\delta^{13}C$ values of 1,4-dioxane for each of the three studies is relatively small, approximately 5‰, and when the three studies are combined, the 7.2‰ range (from -36.0‰ to -28.8‰) is relatively small compared to the typical range in source values for common organic pollutants. Hunkeler et al. (2009) recommends a minimum enrichment in $\delta^{13}C$ of 2‰ beyond the undegraded "source" isotopic composition to demonstrate biodegradation, if such a conclusion were to be based solely on enrichment in $\delta^{13}C$. Based on this guidance and the maximum $\delta^{13}C$ values for source 1,4-dioxane shown in **Figure ES-1**, it can be concluded that $\delta^{13}C$ values greater than -26.8‰ may reflect degradation of 1,4-dioxane. This value may need to be adjusted if new source materials indicate higher $\delta^{13}C$ values for undegraded 1,4-dioxane.

Figure ES-2 shows that $\delta^{2}H$ values for source 1,4-dioxane reported in this study are substantively different from those reported by Wang (2016), where the median value reported by Wang is nearly 100‰ "lighter" than 1,4-dioxane sources analyzed in this study. The $\delta^{2}H$ values reported by Wang (2016) are substantially depleted compared to the Pornwongthong (2014) results, even for the same manufacturer; Pornwongthong (2014) $\delta^{2}H$ values for 1,4-dioxane are closer to those reported in this study (**Figure ES-2)**, but the median value is 30‰ lighter. The range in $\delta^{2}H$ values shown in **Figure ES-2** (-149‰ to -17‰) is large, but not beyond what has been reported for other groundwater contaminants. The relatively large range in $\delta^{2}H$ values of 1,4-dioxane can complicate biodegradation assessments based solely on the enrichment in $\delta^{2}H$.

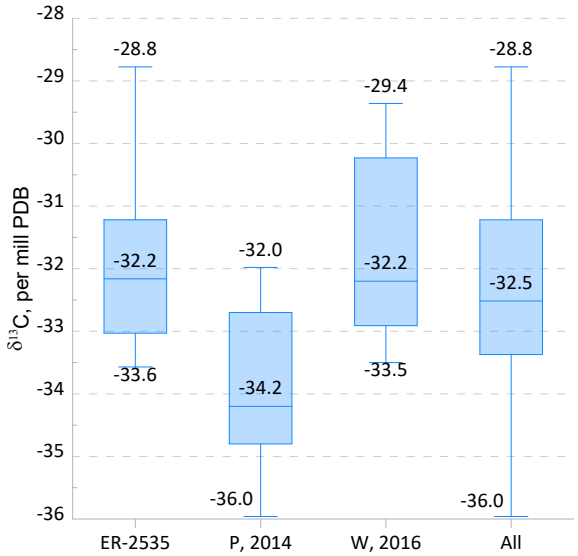

Figure ES-1. Carbon Isotopic Composition of Manufactured (Undegraded) 1,4-Dioxane Samples.

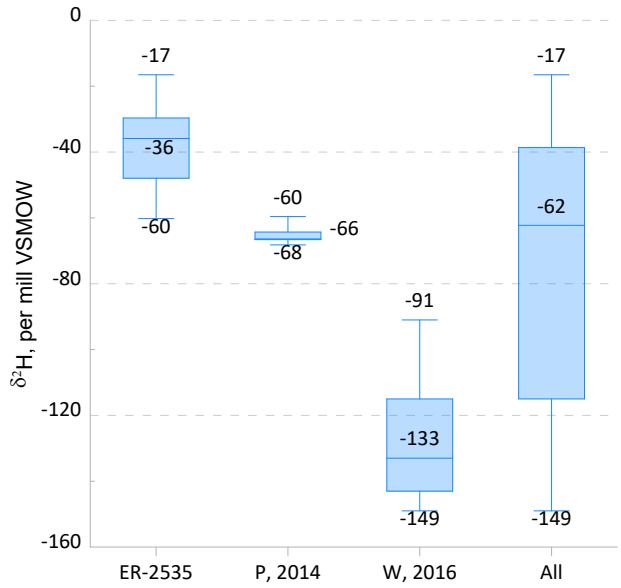

Figure ES-2. Hydrogen Isotopic Composition of Manufactured (Undegraded) 1,4-Dioxane Samples.

Note: Samples of 1,4-dioxane are from this study (ER-2535; n=10) and compared to previous studies: Pornwongthong (2014) (P, 2014; n=5), Wang (2016) (W, 2016; n=7) and all three datasets combined (All; n=22). Range and median values for each are posted on the whisker plots.

Ten manufacturers of 1,4-dioxane were present in the mid-1980s across the globe and, by 2000, only five were operating (Mohr, 2010). This indicates that sources of 1,4-dioxane procured today cannot represent the full range of manufacturers for historical releases which may have occurred several decades ago. Therefore, it is possible that the full range in isotopic composition of 1,4-dioxane is not entirely represented in **Figure ES-1** and **Figure ES-2**. Industrial and municipal wastewater effluent can also be a source for 1,4-dioxane in groundwater. In many cases, the 1,4-dioxane is not directly manufactured, but is instead a byproduct of manufacturing. The isotopic composition of byproduct 1,4-dioxane is not represented by the samples analyzed in this study.

No enrichment trend with evaporation of neat 1,4-dioxane was observed in passive evaporation experiments. The $\delta^{13}C$ and $\delta^{2}H$ values at 80% evaporation are within 0.2‰ and 3‰ of the initial values prior to evaporation (the analytical precision reported by the laboratory for $\delta^{13}C$ and $\delta^{2}H$ is ±0.5‰ and ±5‰, respectively). These results suggest that the isotopic composition of manufactured 1,4-dioxane may not be substantively affected by passive evaporative losses.

4.2 ISOTOPIC COMPOSITION OF 1,4-DIOXANE IN GROUNDWATER SAMPLES

The CSIA method developed in Phase I of this project was applied to 78 groundwater samples from 12 different field sites at concentrations ranging from non-detect to 17,200 µg/L with a median concentration of 18 µg/L. Of these samples, 68 measurements of $\delta^{13}C$ and 54 measurements of $\delta^{2}H$ were reported. The $\delta^{13}C$ and $\delta^{2}H$ values for 1,4-dioxane measured at the 12 field sites are summarized in **Figure ES-3** and **Figure ES-4**, respectively.

As shown in **Figure ES-3**, most $\delta^{13}C$ values for 1,4-dioxane in groundwater samples fall between -34‰ and -28‰, within the range of $\delta^{13}C$ values for undegraded source 1,4-dioxane. The lowest $\delta^{13}C$ values between -38‰ and -36‰ indicate that "lighter" $\delta^{13}C$ values for sources of 1,4-dioxane exist compared to those shown in **Figure ES-2**. Some of the "heavier" $\delta^{13}C$ values between -26‰ and -22‰ reflect biodegradation due to biostimulation; others may be related to natural biodegradation and/or source variability and/or analytical uncertainty (for low concentrations).

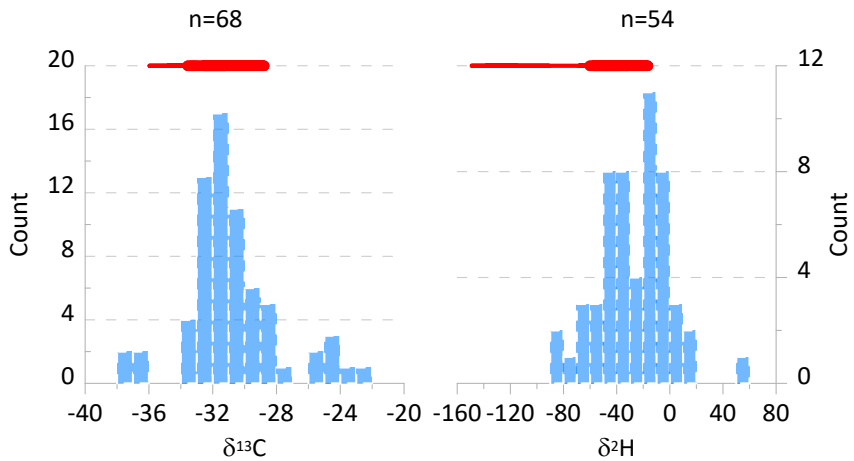

Figure ES-3. Isotopic Composition of 1,4-Dioxane in Groundwater Samples from Field Sites.

***See* figure at left.**

Figure ES-4. Isotopic Composition of 1,4-Dioxane in Groundwater Samples from Field Sites.

***See* figure at right.**

Figure ES-3 compares the frequency distribution for $\delta^{13}C$ values of 1,4-dioxane in 68 groundwater samples from Phase I and Phase II field sites with the range in source isotopic composition shown in **Figure ES-1**. The thick red bar is the range in isotopic composition of the 10 samples analyzed in this study; the thin red bar extends the range to include other published values shown in **Figure ES-1**. **Figure ES-4** compares the frequency distribution for $\delta^{2}H$ values of 1,4-dioxane in 54 groundwater samples from Phase I and Phase II field sites with the range in isotopic composition of the 10 samples analyzed in this study (thick red bar) and other published values shown in **Figure ES-2** (thin red bar).

As shown in **Figure ES-4**, the majority of $\delta^{2}H$ values for 1,4-dioxane measured in groundwater samples fall between -50‰ and 0‰, overlapping the full range of $\delta^{2}H$ values for undegraded source 1,4-dioxane, but with a proportion of samples being enriched by 10 to 20‰ relative to the maximum $\delta^{2}H$ value for undegraded source 1,4-dioxane of -17‰. Based on the expected error for determination of $\delta^{2}H$ values by CSIA, the $\delta^{2}H$ values in **Figure ES-4** between -17‰ and 0‰ cannot be attributed to biodegradation alone without considering other lines of evidence (e.g., concurrent enrichment in $\delta^{13}C$ along a dual isotope enrichment trend). The values greater than 0‰ include samples from sites where other lines of evidence do not support biodegradation, as discussed in **Section 4.4.2** of the Final Report.

The δ^2H values lower than -60‰ were reported in groundwater samples from three sites, indicating that some "lighter" δ^2H values for sources of 1,4-dioxane exist compared to the sources of 1,4-dioxane analyzed in this study. Based on the large range in δ^2H values for source 1,4-dioxane and given the larger uncertainty in analytical results, the use of δ^2H values alone as an indicator for biodegradation is not recommended. Instead, comparison of $\delta^{13}C$ and δ^2H values along flowpaths can be useful, particularly when interpreted using a "dual isotope" approach on a case by case basis; this dual-isotope approach was applied to the case studies where paired $\delta^{13}C$ and δ^2H values were available.

4.3 SUMMARY OF CASE STUDIES APPLYING CSIA TO GROUNDWATER SITES

Dual isotope trends consistent with biodegradation of 1,4-dioxane were found at four of the 12 sites evaluated under Phase I and Phase II of Project ER-2535:

1. McClellan Air Force Base (*see* **Section 4.4.1.1** of the Final Report), only within the area where 1,4-dioxane was being treated *in situ* through biostimulation with propane and oxygen addition. The dual-isotope enrichment trend aligned with the trend observed in laboratory reactions of *Mycobacterium* 1A stimulated with gaseous alkanes (**Figure ES-1**).
2. Cape Cañaveral Air Force Station (CCAFS) Space Launch Complex 16 (SLC-16) (*see* **Section 4.4.1.3** of the Final Report), where one of the groundwater samples was strongly enriched relative to the other samples from this site, with $\delta^{13}C$ and δ^2H of -23.4‰ and -10‰, respectively, and plotting on a dual isotope trend consistent with that observed for laboratory degradation reactions with *R. rhodochrous ATCC 21198* grown on propane. A second sample had a smaller isotopic enrichment plotting on a dual-isotope enrichment trend similar to that determined for *P. dioxanivorans CB1190* (1,4-dioxane substrate, orange line; **Figure ES-6**).
3. Confidential DoD Site #2 (*see* **Section 4.4.2.5** of the Final Report), where the dual isotope enrichment aligned with trends established for *Mycobacterium* 1A and *P. tetrahydrofuranoxidans K1* downgradient of the source zone (**Figure ES-7**).
4. Confidential Non-DoD Site #1 (*see* **Section 4.4.2.7** of the Final Report), where the dual isotope enrichment aligned with the trend established for *P. tetrahydrofuranoxidans K1* in an area where a biostimulation pilot test (propane and oxygen addition) was underway, and farther downgradient, at the distal end of the plume where other lines of evidence suggest natural attenuation processes were occurring (**Figure ES-8**). Tetrahydrofuran (THF) is a co-contaminant with 1,4-dioxane in groundwater at this site, and THF had previously been implicated in the natural attenuation of 1,4-dioxane in earlier studies on this site using a multiple lines of evidence approach (Gedalanga et al. 2016).

CSIA was inconclusive with respect to biodegradation at eight of the 12 sites, either because the enrichment trends associated with biodegradation of 1,4-dioxane were not observed, or because concentrations were too low for analysis.

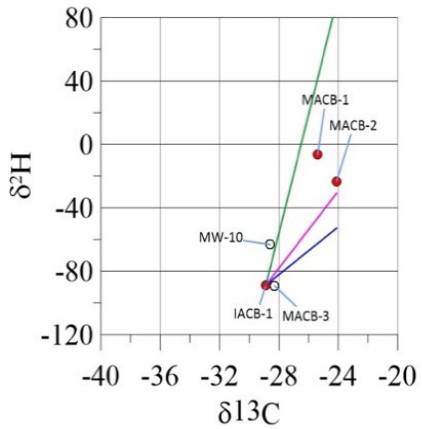

Figure ES-5. Dual Isotope Plot for McClellan AFB Samples.

This figure shows paired $\delta^{13}C$ and $\delta^{2}H$ values (‰) for groundwater samples collected in June 2016 from biostimulation pilot test (red dots). MACB-1 and MACB-2 are in the biostimulation zone; IACB-1 is undegraded 1,4-dioxane. Laboratory-determined dual-isotope enrichment trends are shown for R. rhodochrous ATCC 21198 (propane substrate, blue line), Mycobacterium sp. 1A (propane substrate, magenta line) and P. tetrahydrofuranoxidans K1 (THF substrate, green line).

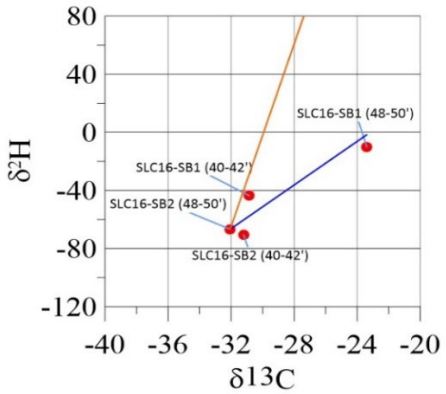

Figure ES-6. Dual Isotope Plot for CCAFS SLC-16.

This figure shows paired $\delta^{13}C$ and $\delta^{2}H$ values (‰). For reference, dual-isotope enrichment trends are shown for laboratory-controlled degradation reactions of P. dioxanivorans CB1190 (1,4-dioxane substrate, orange line) and R. rhodochrous ATCC 21198 (propane substrate, blue line).

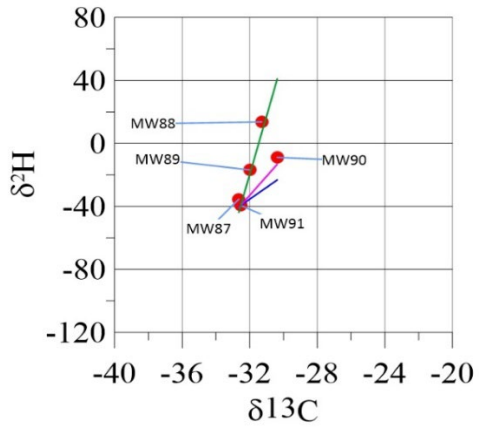

Figure ES-7. Dual Isotope Plot for Confidential DoD Site #2.

This figure shows paired $\delta^{13}C$ and $\delta^{2}H$ values (‰, red dots) for groundwater samples collected from locations in the 1,4-dioxane source area (MW87 and MW91) and further downgradient (MW88, MW89, MW90). Laboratory-determined dual-isotope enrichment trends are shown for R. rhodochrous ATCC 21198 (propane substrate, blue line), Mycobacterium sp. 1A (propane substrate, magenta line) and P. tetrahydrofuranoxidans K1 (THF substrate, green line).

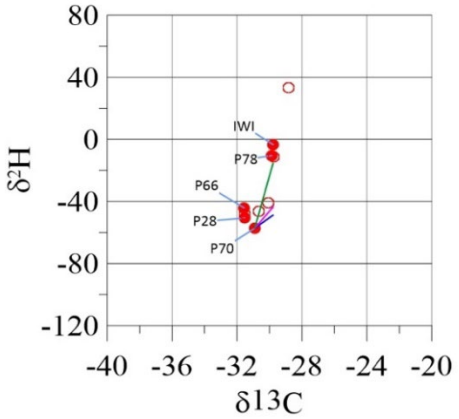

Figure ES-8. Dual Isotope Plot for Confidential Non-DoD Site #1.

This figure shows paired $\delta^{13}C$ and $\delta^{2}H$ values (‰, red dots) for groundwater samples collected along a mixed 1,4-dioxane/THF plume; duplicate samples are shown in red circles. IW1 was sampled after several months of biostimulation of 1,4-dioxane biodegradation as part of a pilot test. P78 is near the distal end of the plume. Laboratory-determined dual-isotope enrichment trends are shown for R. rhodochrous ATCC 21198 (propane substrate, blue line), Mycobacterium sp. 1A (propane substrate, magenta line) and P. tetrahydrofuranoxidans K1 (THF substrate, green line).

5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

The method developed under Project ER-2535 allows for applying CSIA to low concentrations of 1,4-dioxane in groundwater to demonstrate the natural or enhanced biodegradation of 1,4-dioxane at DoD sites. Through the use of dual isotope plots, CSIA results can be compared to published enrichment trends to provide insights into the degradation conditions by comparison with published dual-isotope trends for laboratory-controlled degradation reactions. Future research in laboratory and field-based degradation studies may strengthen the link between dual-isotope 1,4-dioxane degradation trends and *in situ* enzyme activity. The dual isotope plot can also be useful in selecting the appropriate enrichment factor for Rayleigh-type degradation calculations at groundwater sites.

6.0 LITERATURE CITED

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