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Laboratory Evaluation of Remediation Alternatives for Groundwater at the Lane Street Superfund Site

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LABORATORY EVALUATION OF REMEDIATION ALTERNATIVES FOR GROUNDWATER AT THE LANE STREET SUPERFUND SITE

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by
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Accepted by:

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ABSTRACT

At the Lane Street Groundwater Contamination Superfund site in Elkhart, Indiana, which includes industrial, commercial, and residential properties, a plume of contaminated groundwater was identified that requires remediation. The plume is chiefly composed of trichloroethylene (TCE) and has impacted residents' drinking water. A microcosm study was performed to evaluate the potential of various treatment methods to remediate the Lane Street Superfund Site groundwater. The objectives of this microcosm study are to evaluate the potential for biostimulation, bioaugmentation, and abiotic degradation for implementation at this Site and to evaluate toxicity factors that have precluded natural degradation of the site contaminants. The microbiological community within the microcosms was analyzed at the beginning and end of the experiment to evaluate the impacts of each treatment. Anaerobic microcosms were constructed with aquifer sediment and groundwater from the Site. The microcosms were amended with combinations of lactate, emulsified vegetable oil (EVO), microscale zero-valent iron (ZVI), sulfidated microscale ZVI, activated carbon, and a commercial bioaugmentation culture with *Dehalococcoides (Dhc)*.

Bioaugmented microcosms amended with *Dhc* revealed the highest dechlorination rates among the treatment options. Bioaugmentation lowered the methane production and demand for electron donor and completely reduced TCE to ethene. There were no signs of inhibition due to toxicity factors in the bioaugmented microcosms. Two of the three lactate amended microcosms revealed complete dechlorination activity of TCE despite initial concentrations of dechlorinating microbes, specifically *Dhc*, below detection.

Compared to the bioaugmented treatment, the lactate amended microcosms showed less consistent rates of dechlorination and vinyl chloride (VC) accumulation reaching stoichiometric levels. The EVO amended microcosms did not demonstrate the same dechlorinating activity as the lactate amended microcosms, which suggests that the fermenters required for long-chain fatty acids were absent.

Microcosms amended with microscale ZVI powder reduced the concentration of TCE at a first-order rate, in proportion to the dose of ZVI. However, by the end of the incubation period, the concentration of TCE persisted above the clean-up goal. Sulfidated-ZVI completely reduced TCE to ethene in a shorter time than the powder ZVI. VC accumulation was not observed with either of the ZVI products evaluated. Activated carbon rapidly adsorbed the TCE but showed no appreciable dechlorination activity.

The lactate amended microcosms revealed the presence of *Dhc*, despite initial levels of dechlorinating microbes being below detection. The lactate amended microcosms consisted primarily of tceA reductase (tceA) genes, compared to the bioaugmented microcosms which contained a larger percentage of vinyl chloride reductase (vcr) and BAV1 vinyl chloride reductase (bvc) genes. *Dhc* and *Dehalogenimonas (Dhgm)* were below detection in the unamended, EVO amended, and ZVI and EVO amended microcosms, which exhibited limited biological reductive dechlorination. These results reiterate the importance and value of microcosm studies when evaluating remediation methods to implement at a contaminated site.

The results of this microcosm study suggest that bioaugmentation has notable advantages as a treatment alternative over biostimulation or activated carbon. ZVI

powder and sulfidated-ZVI were also effective treatment methods and reduced TCE to ethene without accumulating VC. Additional considerations, especially regarding cost, will need to be considered before arriving at a final recommendation for remediation of the Lane Street Superfund Site.

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ABBREVIATIONS AND ACRONYMS

ASL	Agricultural Service Laboratory
BGS	below ground surface
BVC	BAV1 vinyl chloride reductase
DCA	dichloroethane
DCE	dichloroethene
DCM	dichloromethane
DDI	distilled deionized
DHC	*Dehalococcoides*
DHGM	*Dehalogenimonas*
EPA	Environmental Protection Agency
EVO	emulsified vegetable oil
FID	flame ionization detector
GC	gas chromatograph
HPLC	high performance liquid chromatograph
IC	ion chromatograph
IDEM	Indiana Department of Environmental Management
MCL	Maximum contaminant level
NPL	National Priorities List
PAU	peak area unit
PCE	perchloroethylene
qPCR	quantitative polymerase chain reaction

RPD	relative percent difference
TCA	trichloroethane
TCE	trichloroethylene
TCEA	tceA reductase
VC	vinyl chloride
VCR	vinyl chloride reductase
VOC	volatile organic compound
ZVI	zero valent iron

1.0 INTRODUCTION

Chlorinated solvents, including trichloroethylene (TCE), have been used extensively in many industrial and commercial applications because of their low cost, easy availability, and chemical stability. The extensive use is often followed by poor management, leading to widespread soil and groundwater contamination.[1] TCE is an unsaturated aliphatic chlorinated hydrocarbon and is one of the most common volatile organic compounds (VOCs) detected in groundwater in the United States.[2,3] Due to the widespread contamination of TCE in groundwater, extensive research has been conducted on methods to reduce and degrade TCE at contaminated sites, especially in groundwater.[4] Many contaminated subsurface environments are anaerobic or anoxic.[5,6] Treatment methods that may be implemented to remediate contaminated groundwater include anaerobic biostimulation with the addition of an electron donor[6], anaerobic bioaugmentation with the addition of dechlorinating microbes[5], abiotic degradation with ZVI[7], abiotic sequestration with activated carbon[8], aerobic cometabolism[9], and monitored natural attenuation.[10]

The degradation mechanisms that have played the most significant role in this study are anaerobic biological reductive dechlorination, abiotic hydrogenolysis, and abiotic β-elimination. Microbiological dechlorination of TCE utilizes dechlorinating bacteria, specifically *Dehalococcoides (Dhc)* and *Dehalogenimonas (Dhgm)*, to entirely reduce TCE to ethene through organohalide respiration.[11] Reductive dechlorination consists of transformation steps where chlorine atoms are replaced with hydrogen atoms. This process transforms perchloroethylene (PCE) and TCE into daughter products cis-

1,2-dichloroethene (DCE), vinyl chloride (VC) and, ultimately, ethene. Ethene is a non-toxic, environmentally suitable end product, while cDCE and VC are toxic products, not fit for release into the environment.[4,12] Biological reductive dechlorination is a favorable remediation alternative because it is environmentally friendly, cost-effective, and reliable.[6,11] Dechlorinating microbes use chlorinated solvents as an electron acceptor and hydrogen as an electron donor.[4,13] In the environment, dechlorinating microbes compete with other hydrogen-consuming microorganisms, including sulfate and nitrate-reducing bacteria.[14,15] To ensure enough hydrogen is present for reductive dechlorination, fermentable electron donors, such as lactate and emulsified vegetable oil (EVO), are often added to provide additional hydrogen for this reaction.[16]

Abiotic degradation of TCE with zero-valent iron (ZVI) involves dechlorination reactions between the contaminant and the ZVI. The two major pathways are hydrogenolysis and reductive β-elimination.[17] In the β-elimination pathway, two chlorine atoms are removed and an additional bond between the carbon atoms forms.[18] In this pathway, the reaction bypasses the toxic degradation products cDCE and VC and can transform TCE to acetylenes, and ultimately, ethene.[19,20] Abiotic degradation with activated carbon is another effective treatment often used to remediate TCE in soil and groundwater. Activated carbon uses both adsorption and degradation to remove contaminants. Contaminants are quickly adsorbed to the activated carbon and then destroyed through biotic or abiotic degradation.[21,22]

At the Lane Street Groundwater Contamination Superfund site in Elkhart, Indiana, a plume of contaminated groundwater was identified that requires remediation.

This Site occupies 65 acres and consists of active and inactive industrial, commercial, and residential properties. The contamination plume is primarily composed of TCE with lesser amounts of PCE, cDCE, and 1,1-dichloroethane (DCA) observed. There is limited evidence of anaerobic biodegradation of TCE due to the lesser amount of cDCE observed than TCE. The contamination at this Site has impacted the quality of residents' drinking water, with the primary concern being TCE. This Site was listed on the National Priorities List (NPL) in 2009, and the investigation process under the Environmental Protection Agency (EPA) Superfund program began.[23] Under the Safe Drinking Water Act, the EPA has set maximum contaminant levels (MCL) for the highest concentrations of contaminants allowed in drinking water.[24] TCE, cDCE, and VC concentrations at this Site must be lowered to meet their respective MCLs of 5, 70, and 2 μg/L.[25]

Site characterization is essential for developing a remediation strategy. This includes quantification of contaminants, geochemical parameters, and microbiological composition. Such information can be used to interpret ongoing activity such as the extent of reductive dechlorination. In some cases, this level of information is considered sufficient to proceed with a site remedy. However, laboratory studies may be advisable to better assess the potential for various strategies. For example, if *Dhc* or *Dhgm* are not detected, that might guide the decision to evaluate bioaugmentation. A microcosm study can reveal if site conditions are unfavorable to bioaugmentation, e.g., due to the presence of inhibitory co-contaminants such as heavy metals. Laboratory microcosm studies have proven to be effective for this purpose.[14] Simulating the natural environment in a microcosm study provides an opportunity to observe the impacts of electron donors,

microbiological populations, other contaminants, and various limiting factors on the effectiveness of remediation treatments.[26,5] In this study, microcosms were constructed with aquifer material and groundwater from the Lane Street site.

The overall objective of this microcosm study was to evaluate the effectiveness of various TCE remediation methods for potential implementation at the Lane Street Superfund Site. The specific objectives were: 1) to evaluate the potential for biostimulation and bioaugmentation to achieve the clean-up goals for TCE, as well as other VOCs that persist at the Site; 2) to evaluate toxicity factors that may have precluded natural degradation of the site contaminants; 3) to evaluate the potential for other measures that will most effectively address groundwater contamination at the Site such as the use of ZVI powder and activated carbon to promote abiotic degradation, in combination with biological dechlorination; and 4) to evaluate the impacts of the treatments on the microbiological community at the contaminated site.

2.0 MATERIALS AND METHODS

2.1 Site Description and Sample Collection

The Lane Street Superfund Site is located in Elkhart, Indiana. The Site includes active and inactive industrial, commercial, and residential areas and consists of two TCE plumes. One plume is located in the northeast industrial area and is composed primarily of PCE and TCE. The second plume is larger, located in the central industrial area, and extends into the residential areas of the Site (**Figure 2.1**).[27] The larger plume is primarily composed of TCE. A small drainage ditch is located in the industrial portion of the Site (**Appendix A.1)**[23]. Groundwater has potentially been intersecting the drainage ditch during the spring and summer seasons.

Figure 2.1. Location and site boundary of the Lane Street Superfund Site. The approximate site boundary and contaminant plumes are indicated. The smaller plume is primarily composed of PCE while the larger plume is primarily TCE. The larger plume extends from the industrial area into the residential area.

Grab groundwater and soil samples from the Site were collected and analyzed for multiple VOCs prior to this study. These samples indicated the potential that

contamination has stemmed from multiple releases from different sources. The depth to the water table at this Site is approximately 4 to 10 feet below ground surface (bgs). The aquifer consists of unconsolidated sand and gravel materials.[23]

S.S. Papadopulos & Associates collected a sample of aquifer material (~2 kg) from within the saturated zone (21-27 ft bgs) using techniques that minimized the risk for exogenous contamination. Sediment samples were collected from monitoring well SB-MW-12i in the area of highest groundwater contamination. Core samples of water-saturated aquifer material were shipped to Clemson University (**Appendix A.2**). The sediment is primarily fine to coarse sand.

Groundwater samples were collected from the closest monitoring wells: R-MW-14i, R-MW-12s, R-MW-12i, MW-04d, MW-02s, MW-02i, MW-01i, and MW-01d (**Appendix A.1**). Analysis of the samples indicated an average pH of 7.2, temperature of 19.0 °C, nondetectable dissolved oxygen, nitrate at <1 mg/L, and sulfate at 15 mg/L. The average oxidation/reduction potential (ORP) was 56 mV, ranging from 27 to 121 mV in individual samples (**Appendices A.3 and A.4**).[23]

2.2 Experimental Design

To evaluate the effectiveness of different TCE remediation methods at the Lane Street Superfund Site, microcosms were constructed in 160 mL glass serum bottles containing 70 mL groundwater and 50 g aquifer sediment. These microcosms were prepared in the anoxic glovebox and sealed with Teflon-faced butyl rubber septa and aluminum crimp caps. The following treatments were prepared in triplicate: unamended, lactate amended, lactate amended and bioaugmented, EVO amended, EVO amended and

bioaugmented, microscale ZVI powder, microscale ZVI powder and EVO amended, autoclaved controls, and water controls. A second set of microcosms was prepared in triplicate based on the following amendments: activated carbon, microscale ZVI powder, and sulfidated microscale ZVI. Resazurin was added to the groundwater at a concentration of 1 mg/L to serve as a redox indicator.[4] TCE saturated water was added to bring the initial concentration of TCE to ~1 mg/L.

In the bioaugmented microcosms, KB-1 (SiREM labs) was added to raise the initial concentration of *Dhc* to ~10^6 copies/mL.[28] EVO and lactate served as additional electron donors and were added at a dose to provide 10x the electron donor necessary for the TCE to be reduced to ethene. Additional doses of electron donor were added, as needed. Ferox Flow ZVI powder (Hepure) was used in the designated microcosms at doses of 2 and 5 g/L as ZVI. SMicro-ZVI (SmZVI), an injectable sulfidated-ZVI (Regenesis) was used at doses of 2 and 5 g/L. PlumeStop, a form of liquid activated carbon (Regenesis), was added at a dose of 0.4 mL/L. Autoclaved microcosms served as controls to separate the chemical reactions from the biological reactions happening within the microcosms.

At the start of the experiment, three extra unamended bottles were prepared, and groundwater samples were used to determine time zero conditions. The remaining microcosms were then incubated for eight months or less time if the VOCs decreased below their MCLs. At the end of incubation, the microcosms were moved into the glovebox, the solids were allowed to settle overnight, the septa were removed, and 10 mL of groundwater was withdrawn. These samples were filtered (0.2 µm) and used to

quantify nitrate, sulfate, and organic acids. One microcosm from each triplicate was then sent to Microbial Insights for quantitative polymerase chain reaction (qPCR) analysis (QuantArray-Chlor (DNA)). Results were compared to the time zero samples.

2.3 Chemicals

TCE (99.5%) was obtained from Avantor. A water-saturated solution of TCE (~1.1 mg/mL) was made with distilled deionized (DDI) water. Other chemicals used in this experiment are described in **Appendix A.5.**

2.4 Sample Preparation and Characterization

Groundwater from monitoring wells R-MW-14i, R-MW-12s, and R-MW-12i contained detectable concentrations of TCE and were used to prepare ~2.5 L of composited groundwater for this experiment, as described in **Appendix A.3**. Resazurin was added to the composited groundwater at a concentration of 1 mg/L to serve as a redox indicator.[4] The composited groundwater was placed in the anoxic chamber to prepare for microcosm construction.

Sediment samples were collected from the saturated zone (21-27 ft bgs) from monitoring well SB-MW-12i and were shipped as cores. Upon arrival, these cores were moved into the anoxic chamber, and the sediment was transferred to a sterile Tupperware container. The solids were homogenized using a sterile spoon in the anoxic chamber and covered tightly to prevent evaporation. Using analytical techniques described in **Appendix A.6**, the sediment was characterized as 87% dry and 0.28% organic carbon. Groundwater and sediment samples used in this study were taken from the area of highest TCE contaminant concentration, as indicated in **Figure 2.2.**

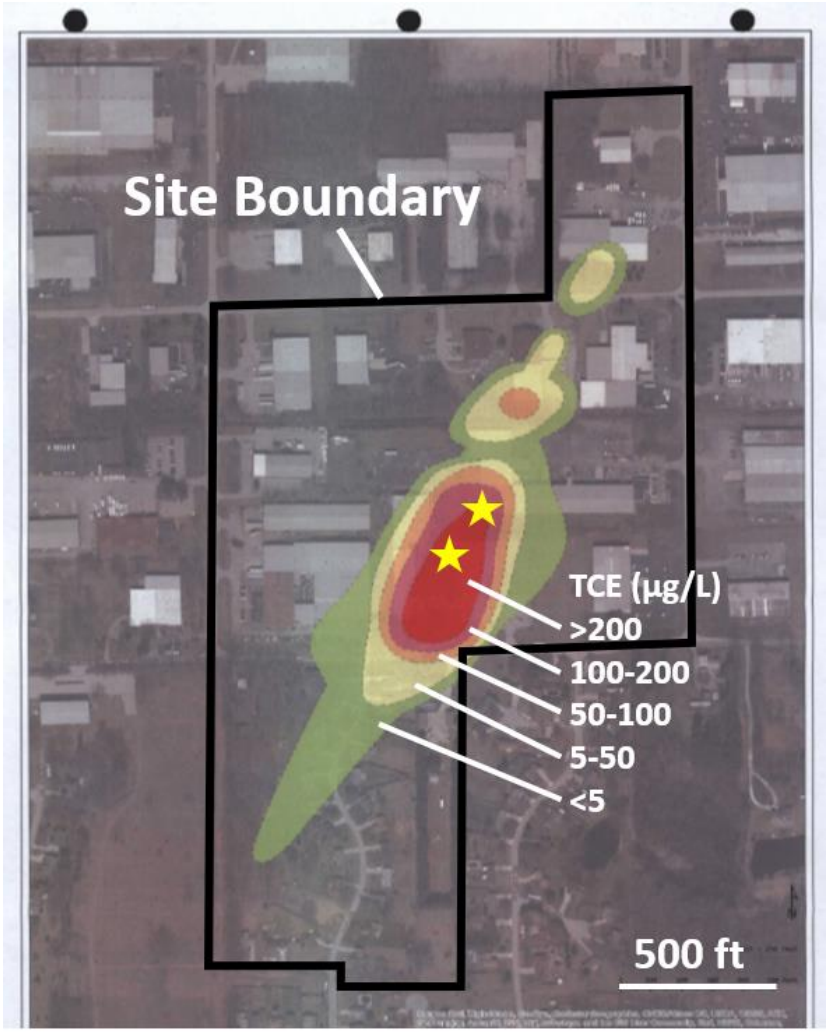

Figure 2.2. The approximate site boundary of the Lane Street Superfund Site with the shallow TCE concentration gradient at the Site. The concentrations of TCE at the site are indicated. The maximum TCE concentration measured was 320 µg/L. The yellow stars indicate monitoring wells R-MW-12 and R-MW-14 where groundwater was sampled for use in this study.

2.5 Additions to Microcosms

For the treatments amended with lactate and EVO, the amount added was calculated based on reduction of nitrate to nitrogen gas, sulfate to sulfide, and TCE to ethene. A safety factor of 10 was included in this calculation:

$$e^{-}demand\ (meq) = \{(mmol\ NO_3^- \times 5) + (mmol\ SO_4^{2-} \times 8) + (mmol\ TCE \times 6)\} \times 10 \quad (2.1)$$

In this calculation, 5 meq/mmol are required for nitrate reduction, 8 meq/mmol are required for sulfate reduction, and 6 meq/mmol are required for complete dechlorination of TCE to ethene. It was assumed that lactate provides 4 meq/mmol (via 2 mmol of H_2/mmol) and EVO provides 102 meq/mmol (via 51 mmol of H_2/mmol). This assumed that acetate formed during fermentation is not usable as an electron donor.

Stock solutions were prepared so that the required amount of electron donor was delivered in a 0.5 mL aliquot: ~7.77 g of 60% sodium lactate syrup (Sigma-Aldrich) and 2.65 g of Newman 55 EVO (>55% soybean oil, 4% sodium lactate, RNAS Remediation Products) per 100 mL DDI water. These amendments are further described in **Appendix A.7**.

For the bioaugmented microcosms, the KB-1 bioaugmentation culture from SiREM Lab was added as a source of *Dhc*.[29] The culture contains 10^8 copies *Dhc*/mL. To achieve a concentration of 10^6 cells/mL in the microcosms, 0.7 mL KB-1 was added,[28] as follows:

$$KB1\ Dose\ \left(\frac{mL\ KB1}{bottle}\right) = \frac{70\ mL\ GW}{bottle} \times \frac{10^6\ cells}{mL\ GW} \times \frac{mL\ KB1}{10^8\ cells} \quad (2.2)$$

Guidance on the amount of ZVI to add was obtained from the manufacturer and the literature.[30,31] A range of Ferox Flow ZVI (>95% ZVI, Hepure) doses was evaluated in a preliminary experiment (**Appendix A.10)**. On that basis, 0.16 g of Ferox Flow ZVI

were added to the designated microcosms. An additional set of Hepure ZVI microcosms were prepared with a dose of 0.38 g Ferox Flow ZVI per bottle. The second set of Hepure ZVI microcosms were prepared alongside microcosms with Regenesis ZVI, for comparative purposes (see below).

SmZVI is a glycerol solution with colloidal, sulfidated-ZVI particles. For the microcosms containing SmZVI (40% ZVI in glycerol, Regenesis), two doses were evaluated. The first dose was based on the initial mass of Hepure ZVI added in the first set of microcosms. These bottles were made with a dose of 0.16 g SmZVI per microcosm. The second set was based on a recommended dose of 2 g ZVI/L, provided by Regenesis, and was calculated as follows:

$$SmZVI\ Dose\ \left(\frac{g}{bottle}\right) = \frac{2\ g\ ZVI}{L} \times \frac{g\ SmZVI}{0.4\ g\ ZVI} \times \frac{0.076\ L\ GW}{bottle} \qquad (2.3)$$

This required addition of 0.38 g SmZVI per bottle. SmZVI is an injectable liquid so the volume of SmZVI added to the microcosms was determined based on a density of glycerol of 1.26 g/mL.

For the microcosms containing PlumeStop Liquid Activated Carbon (AC) (20% AC in water, Regenesis), the dose was based on a Freundlich adsorption isotherm using constants provided by Regenesis, as follows:

$$q_e = K_f C_e^{\,1/n} \qquad (2.4)$$

where *qe* is the amount adsorbed (mg TCE/g AC); K_f is the adsorption capacity (65.0 (mg/g)/(mg/L)$^{1/n}$); C_e is the equilibrium concentration in water, which was set to the MCL of 0.005 mg/L; and 1/n is the strength of adsorption (0.55).[32] PlumeStop is 20%

activated carbon. Assuming a specific gravity of 1, the dose of PlumeStop required was 150 μL per microcosm.

2.6 Microcosm Preparation and Time-Zero Measurements

Microcosms were prepared in 160-mL serum bottles according to the treatments shown in **Table 2.1** for the initial microcosms and **Table 2.2** for the additional abiotic microcosms. The microcosms were constructed in an anoxic chamber and contained 50 g aquifer material and 70 mL groundwater. Once removed from the anoxic chamber, the headspaces were purged with nitrogen (N_2) gas to remove hydrogen (H_2) from the glovebox atmosphere because H_2 is a preferred electron donor for reductive dechlorination.[11] The microcosms were sealed with Teflon-faced butyl rubber septa and aluminum crimp caps.

Table 2.1. Experimental design of the initial Lane Street microcosms. Each treatment was prepared in triplicate. The lactate and EVO amended microcosms were reamended periodically throughout the experiment; this table shows only the initial amendment.

Treatment	Solids (g)	GW (mL)	Lactate Stock (mL)	EVO Stock (mL)	KB-1 (mL)	Hepure ZVI (g)	TCE Saturated Water (μL)
Unamended	50	70	-	-	-	-	100
Lactate Amended	50	70	0.50	-	-	-	100
Lactate + KB-1	50	70	0.50	-	0.70	-	100
EVO Amended	50	70	-	0.50	-	-	100
EVO + KB-1	50	70	-	0.50	0.70	-	100
Hepure ZVI	50	70	-	0	-	0.16	100
Hepure ZVI + EVO	50	70	-	0.50	-	0.16	100
Autoclaved Controls	50	70	-	-	-	-	100
Water Controls	-	-	-	-	-	-	90

Table 2.2. Experimental design of the additional Lane Street microcosms with Hepure and Regenesis products. Each treatment was prepared in triplicates.

Treatment	Solids (g)	GW (mL)	Hepure ZVI (g)	Regenesis SmZVI (µL)	Regenesis PlumeStop (µL)	TCE Saturated Water (µL)
SMicro ZVI 1	50	70	-	125	-	100.00
SMicro ZVI 2	50	70	-	300	-	100.00
Hepure ZVI 2	50	70	0.38	-	-	100.00
PlumeStop Activated Carbon	50	70	-	-	150	100.00
Water Controls	-	-	-	-	-	100.00

Killed controls were prepared by autoclaving triplicate microcosms for one hour on three consecutive days.[33] TCE was added to these controls after the autoclaving was complete. Sterile equipment was used for the autoclaved microcosms to prevent contamination of the controls. Water controls were prepared on the bench top by adding 70 mL DDI water and enough glass beads to displace the same volume as the soil (**Appendix A.8**).

The constructed microcosms were incubated on a shaker table for 1 hour to promote equilibrium between the headspace and liquid before time-zero measurements. The average initial concentrations were ~1,200 µg TCE/L and *c*DCE <10 µg cDCE/L. Aqueous phase concentrations take into account partitioning of the VOCs between the aqueous phase and headspace based on Henry's Law.

The second set of microcosms were constructed at a later date and time-zero measurements for these microcosms revealed TCE concentrations of ~1,300 µg/L in the microcosms. PlumeStop was added to the designated microcosms after the time-zero

measurement. The microcosms were stored stationary, inverted, in the dark, and at room temperature.

For the microcosms amended with EVO and/or ZVI, the time-zero concentrations of TCE were lower because of rapid partitioning of TCE into EVO and adsorption to ZVI.[26,34] To more accurately represent the time zero concentrations in these treatments, the average time-zero measurements obtained for TCE in the lactate and KB1 amended microcosms was used. In the second set of microcosms, the time-zero measurements for the microcosms containing SmZVI were replaced with those from the microcosms amended with PlumeStop, before PlumeStop was added.

2.7 Analytical Techniques

Concentrations of TCE, cDCE, VC, methane, acetylene ethene, and ethane were measured by injecting 0.5 mL headspace samples onto a GC equipped with a flame ionization detector (FID).[4,35] Response factors were determined using standards with known amounts added (**Appendix A.21).** To ensure consistency, a mass balance for the chlorinated ethenes was calculated. The criterion used to assess consistency was a mass balance for TCE and its daughter products, i.e., the molar sum of TCE and products at the end of incubation should be within 20% of the initial molar amount of TCE added (**Appendix A.18**). Using the GC-FID, the detection limits for TCE, cDCE, and VC were at or below their maximum contaminant levels (**Appendix A.18**).[4] Headspace samples were analyzed until all VOCs were at or below MCLs for two consecutive measurements.

The concentration of lactate, formate, acetate, and propionate remaining at the end of incubation were determined using a high-performance liquid chromatograph

(HPLC).[36,37] The run time for the bioaugmented microcosms was 30 minutes. For the remaining microcosms, run times extended for 60 min, allowing for detection of pyruvate, lactate, formate, acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate. Response factors for lactate, formate, acetate, and propionate were determined using authentic material (**Appendix A.22).** Concentrations of sulfate and nitrate were analyzed using an ion chromatograph (IC).[36,35] An initial sample of groundwater was taken to the Clemson University Agricultural Service Laboratory (ASL) for nitrate and sulfate measurements. These samples were rerun on the IC in Rich Lab to confirm the results (**Appendix A.3**). Response factors using nitrate and sulfate standards (**Appendix A.23).**

For the initial set of microcosms, at the end of the incubation, one bottle from each triplicate was sent to Microbial Insights for characterization of the microbial community and dechlorination enzymes using the qPCR QuantArray-Clor analysis. qPCR quantified the specific genera and functional genes responsible for contaminant biodegradation.[38,39] More details on these analytical techniques can be found in **Appendix 9.**

2.8 Determination of TCE Dechlorination and Degradation Rates

In reductive dechlorination, a chlorine molecule is replaced with hydrogen at each transformation step from TCE to cDCE to VC to ethene. To assess the extent of dechlorination, the amount of chlorine removed was determined using the following equation:

$$\Sigma\left(Cl^{-}_{removed,t}\right) = (cDCE * 1) + (VC * 2) + ((ethene + ethane + acetylene) * 3) \quad (2.5)$$

where $Cl^{-}_{removed,t}$ is the sum of the chlorine removed at time t. Each dechlorination step removes an additional mole of chlorine.

To demonstrate the differences in dechlorination rates between the treatment methods, dechlorination rates were calculated for the treatment methods that showed dechlorination activity. Dechlorination rates were determined based on the amount of chlorine remaining over time using the following equation:

$$Cl^{-}Remaining = 1 - \left(\frac{\Sigma(Cl^{-}_{removed,t})}{\Sigma(Cl^{-}_{removed,f})}\right) \quad (2.6)$$

where $Cl^{-}_{removed,t\backslash f}$ is the sum of the chlorine removed at the final time (i.e., end of incubation). The overall rate of dechlorination in each microcosm was determined using the slope of the linear regression line for the chlorine remaining (μmol/bottle) over time. The final value and outliers were excluded.

The average TCE degradation rates were calculated as an additional method of comparison between the treatments that showed dechlorination activity. These rates were determined using the total incubation time and amount of TCE degraded in each microcosm. The average TCE degradation rates were calculated using the following equation:

$$Average\ Degradation\ Rate\ for\ TCE = \frac{(C_i - C_f)}{incubation\ time\ (days)} \quad (2.7)$$

where C_i is the initial concentration of TCE at the start of the incubation period and C_f is the final concentration of TCE in the microcosms in μM. For this calculation, the incubation period for the bioaugmented microcosms began once the KB-1 addition was made.

3.0 RESULTS AND DISCUSSION

3.1 Lane Street Microcosm Results

The Lane Street microcosms were incubated for up to 242 days. All of the microcosms experienced an initial decrease in TCE, likely due to adsorption.[11,26] Analysis of a soil sample revealed an organic carbon content of 0.28%. Based on a K_{OC} of 126 mL/g,[40] that is too low to account for the magnitude of TCE decrease by partitioning into the organic phase. Consequently, most of the TCE adsorption is presumed to be to the mineral phase.[41,42]

The unamended and autoclaved microcosms exhibited no dechlorination activity throughout the incubation period. The average amounts of TCE in the unamended microcosms, autoclaved controls, and water controls are shown in **Figure 3.1**. The water controls are meant to ensure accuracy of the GC throughout the experiment. Following the initial decline in TCE due to adsorption, the amount of TCE in the unamended microcosms remained stable. These results further indicate that there is no dechlorination activity, biotic or abiotic, currently occurring at this Site; consequently, monitored natural attenuation is not a viable option.

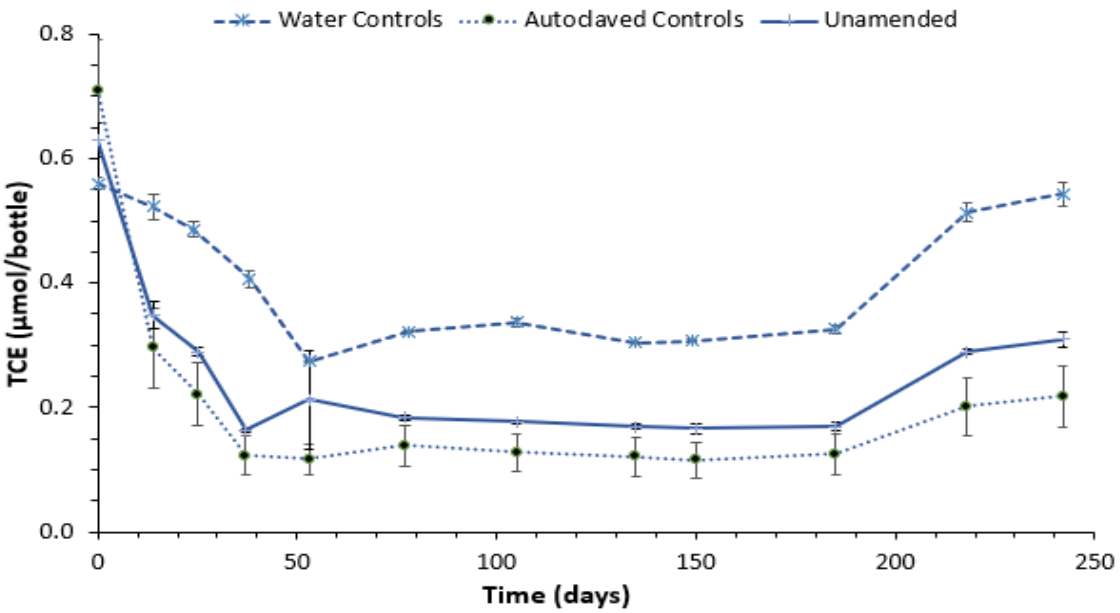

Figure 3.1. Average TCE amounts for the water control, autoclaved control, and unamended microcosms. In this and subsequent figures, error bars represent the standard deviation or triplicate bottles.

The bioaugmented microcosms completely dechlorinated TCE to ethene within 20 days of adding KB-1. The average amounts of VOCs in the bioaugmented microcosms are shown in **Figure 3.2**. These microcosms were bioaugmented with KB-1 on day 24, after the resazurin had turned clear. Dechlorination activity was observed immediately following the KB-1 addition. The lactate and KB-1 bottles completely reduced TCE to ethene by day 37. The EVO and KB-1 bottles reached the clean-up goal after the lactate and KB-1 bottles most likely because lactate is fermented to hydrogen more quickly than EVO.[5] The bioaugmented microcosms produced VC at concentrations up to 126 µg/L, but the accumulation period was only a few days. All of the chlorinated ethenes in these microcosms were reduced to ethene, which was observed at a stoichiometric amount.

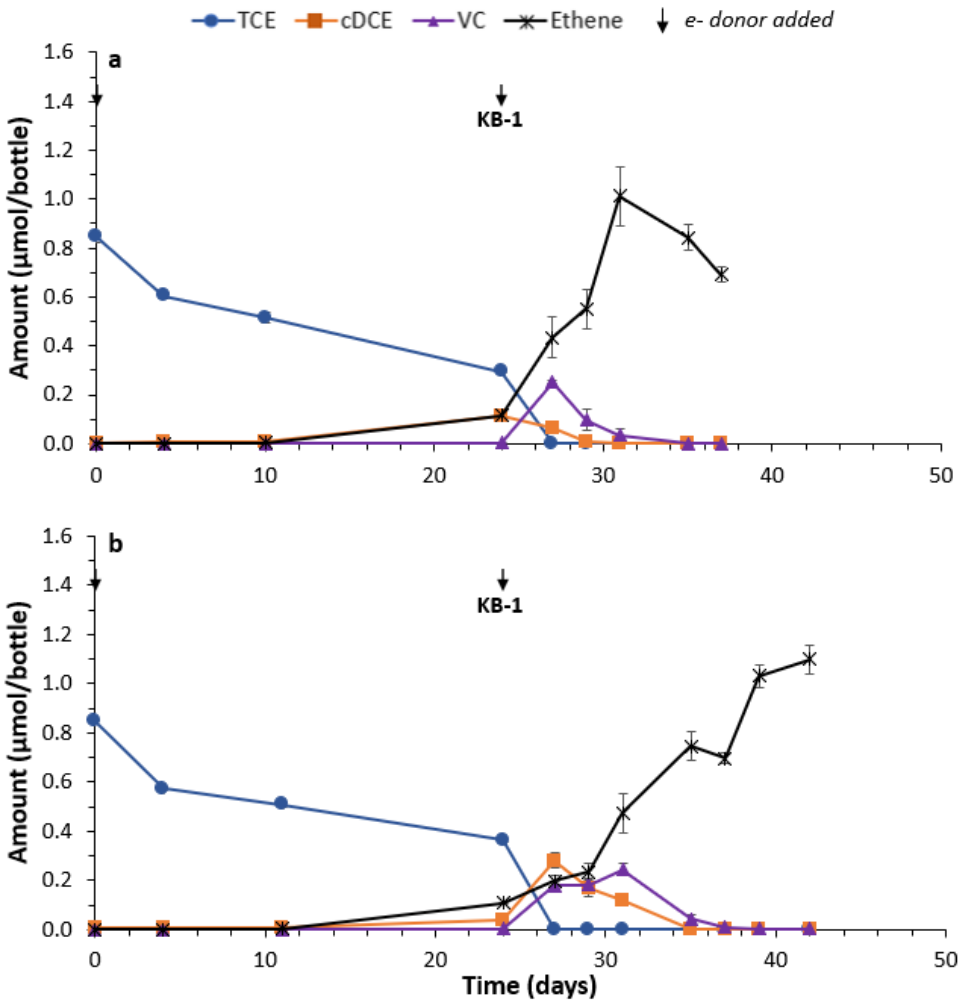

Figure 3.2. Average VOCs for a) the lactate + KB-1 treatment; and b) the EVO + KB-1 treatment. KB-1 was added on day 24, once it was evident that the redox level was sufficiently low.

The lactate amended microcosms revealed dechlorination activity despite the limited evidence of anaerobic reductive dechlorination occurring at this Site. They first showed signs of reductive dechlorination activity on day 52. The three lactate amended microcosms dechlorinated TCE at inconsistent rates, as shown in **Figures 3.3a-c.** The first bottle fully reduced the TCE to ethene within 169 days, but produced a high

concentration of VC, at a maximum of 300 μg/L. The second bottle completely dechlorinated TCE within 88 days, with a small amount of VC and *c*DCE observed. VC accumulation was persistent in the third microcosm and remained above the MCL by the end of the incubation period. These microcosms were reamended with lactate throughout the experiment to promote dechlorination activity and to ensure the reactions were not donor limited. The third lactate bottle received an additional dose of donor in an attempt to further promote VC reduction. These results demonstrate the unpredictability of biostimulation alone at this Site, as well as the risk of accumulating more dangerous products, especially VC.

There was a lack of response to biostimulation in the EVO amended microcosms, as shown in **Figures 3.3d-f**. EVO has proven to be an effective electron donor in similar microcosm studies with TCE contamination.[5] The lack of activity in the EVO amended microcosms indicates the absence of fermenters required for long chain fatty acids and eliminates biostimulation with EVO as a viable treatment for this Site.

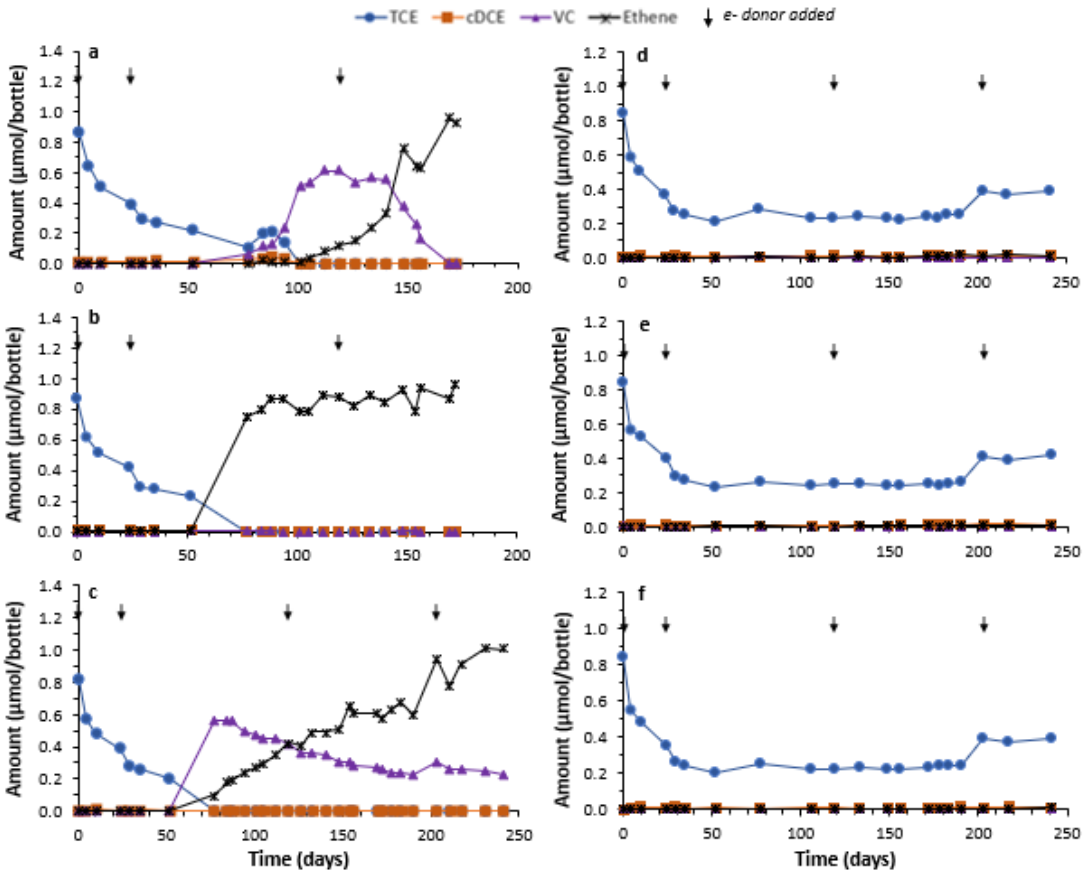

Figure 3.3. a-c) VOC measurements for the lactate amended microcosms; d-f) VOC measurements for the EVO amended microcosms. Lactate and EVO were added on the same days.

The microcosms that received 0.16 g Hepure ZVI and 0.16 g Hepure ZVI with EVO in the initial set of microcosms were effective in lowering the amount of TCE by more than 90% in the first 6 weeks of incubation, as demonstrated in **Figure 3.4**. However, the rate of degradation slowed after that and TCE remained above the MCL for the remainder of incubation. The MCL for TCE is indicated with a dotted line in **Figure 3.4**. These results revealed that the initial dose of ZVI was not high enough to completely dechlorinate all of the TCE in these microcosms. The microcosms amended with ZVI and

EVO did not prove to be significantly more effective in reducing TCE than ZVI alone. The main byproducts observed in these microcosms were ethene and ethane, at stoichiometric amounts. There was no VC accumulation observed in any of the ZVI or ZVI and EVO amended microcosms.

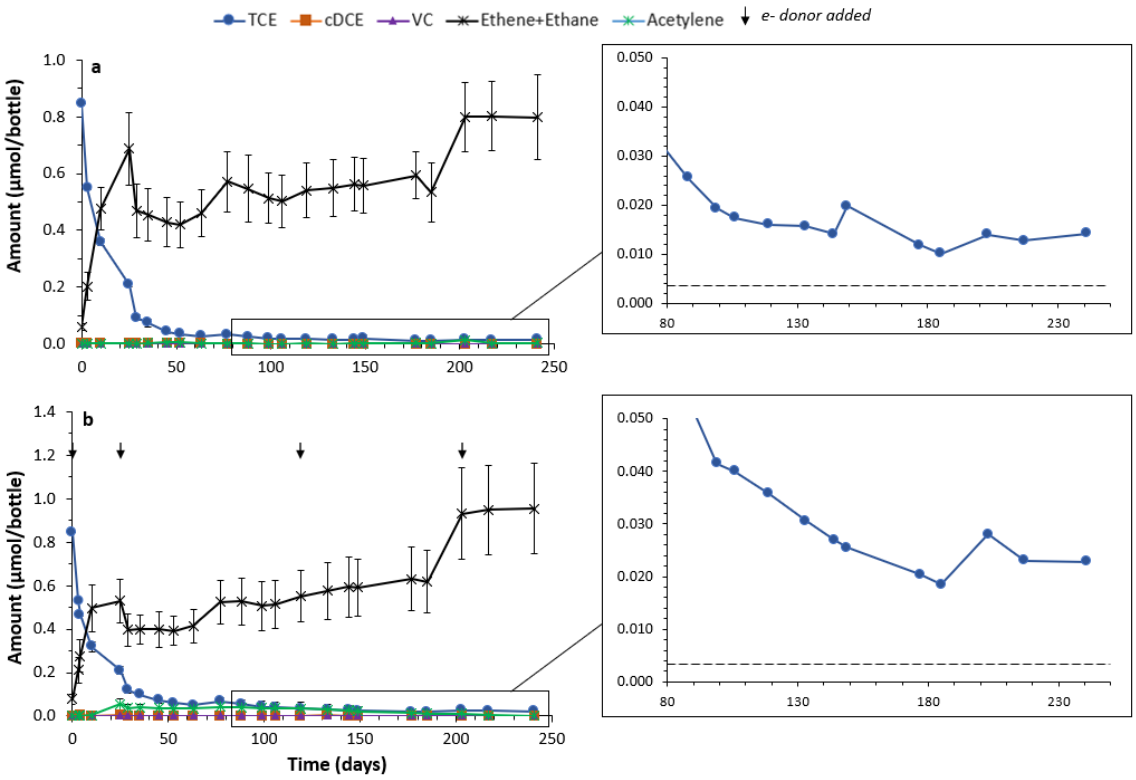

Figure 3.4. Average VOC measurements for the a) 0.16 g Hepure ZVI and b) 0.16 g Hepure ZVI and EVO microcosms. The dotted line indicates the MCL for TCE at 0.0035 µmol/bottle.

At the end of the incubation period, groundwater pH was measured in one replicate from the triplicate unamended, lactate, EVO, lactate and KB-1, EVO and KB-1, and ZVI and EVO amended microcosms. *Dhc* require a circumneutral pH, between 6 and 8, for dechlorination and growth.[43] The treatments analyzed in this study may have affected the pH in the microcosms, possibly hindering reductive dechlorination activity.

The results of the pH measurements are provided in **Appendix A.15.** The microcosms with ZVI and EVO had the highest pH, at 8.77. The addition of ZVI has been shown to increase the pH of groundwater as the dissolution of ZVI forms hydroxide ions.[44] The pH of the ZVI and EVO microcosms is outside the ideal pH range for *Dhc*, and the increase in pH may have inhibited *Dhc* in these microcosms. The remaining microcosms had pH measurements within the pH range favorable for *Dhc*.

3.2. Hepure and Regenesis Microcosm Results

The initial set of microcosms analyzed in this study demonstrated the effectiveness of ZVI in reducing TCE concentrations without the accumulation of cDCE or VC. However, the dose used in the initial microcosms was not adequate in reducing TCE to meet the clean-up goal, which prompted the additional set of microcosms, including a higher dose of Hepure, two doses of SmZVI, and PlumeStop liquid activated carbon.

The microcosms with the recommended dose of 0.38 g SmZVI per bottle were the first to reduce TCE below the MCL. In these microcosms, TCE concentrations were lowered to the clean-up goal within 30 days. At a lower dose of 0.16 g SmZVI per bottle, this goal has been reached by one of the three bottles by day 65. Increasing the dose of Hepure ZVI also proved to be more effective in reducing the concentration of TCE than the initial dose analyzed. However, by day 65, the concentration of TCE is still above the MCL. **Figure 3.5** compares the average amounts of TCE in the SmZVI microcosms, the higher dose of Hepure ZVI, and the initial ZVI and ZVI and EVO microcosms. All the

microcosms exhibited an initial decline in TCE followed by a tail of persistent, lesser amounts of TCE.

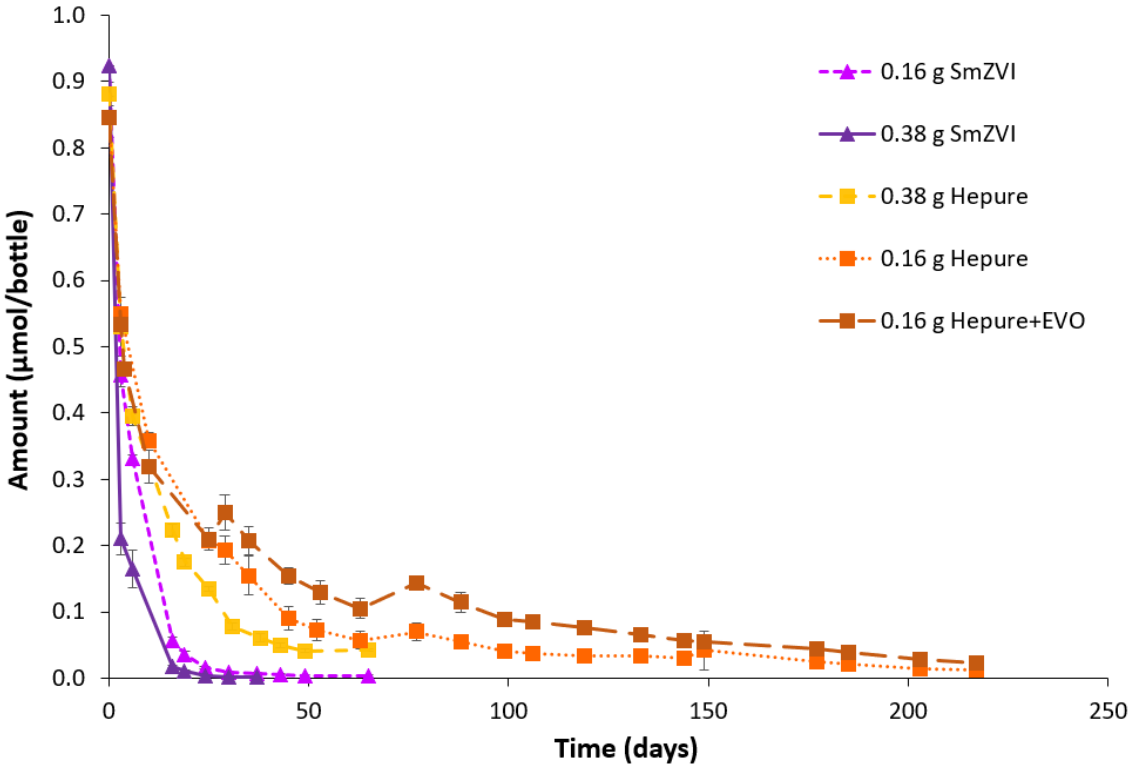

Figure 3.5. Average amount of TCE in the ZVI amended microcosms. The microcosms amended with SmZVI and 0.38 g Hepure ZVI were constructed in the second set of microcosms and had a shorter incubation time than the 0.16 g Hepure ZVI and 0.16 g Hepure ZVI and EVO microcosms which were constructed in the initial set.

The production of acetylene and its transformation to ethene were observed with both doses of SmZVI. A small amount of cDCE was also observed, but the concentrations of cDCE remained below the MCL, and there was no generation of VC. The main byproducts in all of the ZVI amended microcosms were ethene and ethane. The microcosms with Hepure ZVI were more reactive and generated more ethene and ethane compared to the SmZVI or PlumeStop microcosms. The dechlorination products formed

within the various ZVI and activated carbon amended microcosms are shown in **Figure 3.6**. This figure compares the average amounts of byproducts observed at the final time measurement in each treatment. The unaccounted µmol of products demonstrates the difference between the initial µmol of TCE in each microcosm compared to the sum of products at the final measurement. There may have been more adsorption in the SmZVI microcosms than the Hepure because the SmZVI particles are suspended in glycerol.

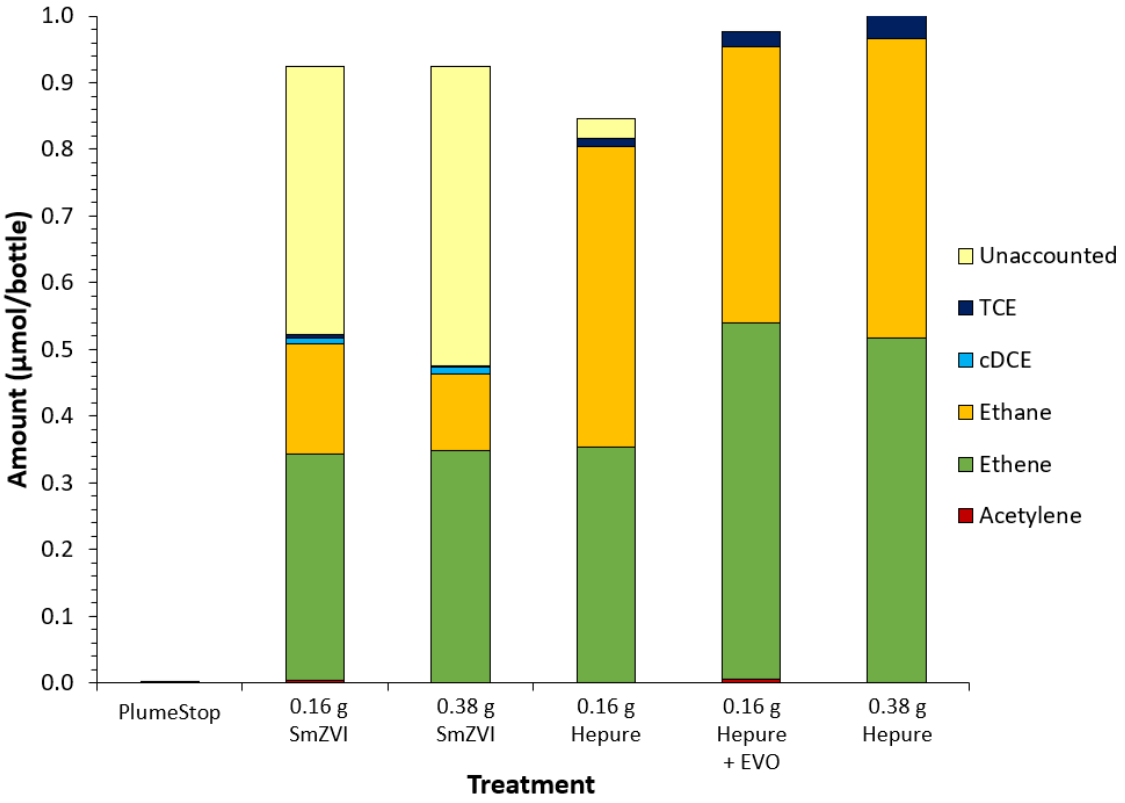

Figure 3.6. Average dechlorination products at the end of incubation. Unaccounted for is the difference between the TCE added and the sum of products accounted for at the end of incubation.

The microcosms amended with PlumeStop activated carbon sequestered and reduced the amount of TCE immediately. TCE concentrations were lowered to meet the clean-up goal within 24 hours. Activated carbon primarily works through adsorption, and

the TCE contamination adsorbed to the activated carbon very quickly. However, minimal production of degradation products was observed in microcosms with this treatment.

3.3 Dechlorination Rates

Dechlorination rates were calculated for the microcosms that showed dechlorination activity, including the three lactate amended microcosms, lactate and KB-1, EVO and KB-1, Hepure ZVI, Hepure ZVI and EVO, and SmZVI amended microcosms. These rates were calculated using equations 2.5 and 2.6 as described in **Section 2.8.** The dechlorination rates for representative EVO and KB-1 and lactate and KB-1 microcosms are shown in **Figures 3.7a and 3.7b** to demonstrate how the dechlorination rates were developed. The dechlorination rates for the averages of these microcosms, and the individual lactate microcosms, are compared in **Figure 3.7c.** It is important to note that by the end of the incubation period, the 0.16 g Hepure ZVI, 0.16 g Hepure ZVI and EVO, 0.16 g SmZVI, 0.38 g Hepure ZVI, and third lactate amended microcosm did not completely reduce TCE or VC to ethene. These results revealed that microcosms bioaugmented with KB-1 have the highest rate of dechlorination. The lactate and KB-1 amended microcosms have a faster dechlorination rate than the EVO and KB-1 microcosms or any of the other treatments. The lactate and KB-1 microcosms have an average rate of dechlorination of 39.57 μmol/year compared to the average of the EVO and KB-1 microcosms at 21 μmol/year.

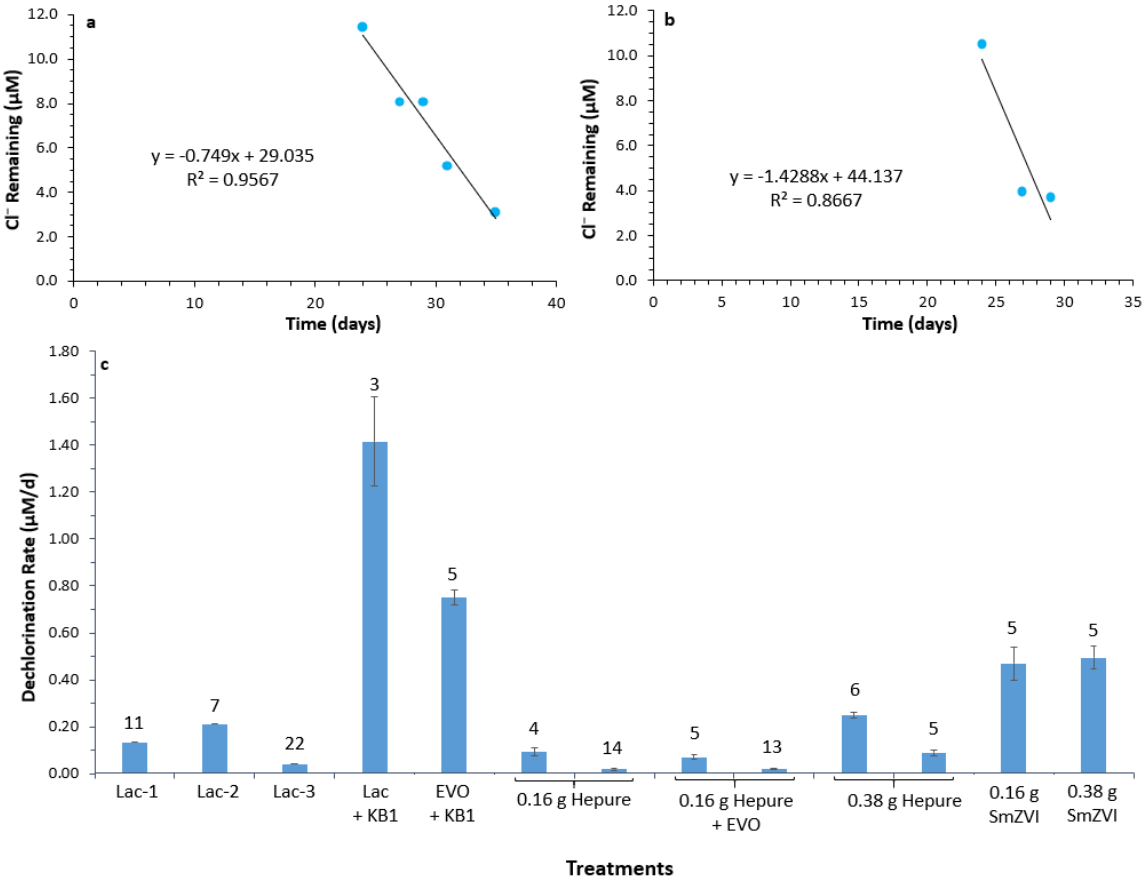

Figure 3.7. a) Dechlorination rate for a representative EVO and KB-1 amended microcosm (EVO+KB1-3); b) Dechlorination rate for a representative lactate + KB-1 amended microcosm (Lac+KB1-3); and c) Comparison of zero order dechlorination rates among the treatments that showed dechlorination activity. Lag periods prior to the onset of dechlorination are not included in the estimates for lactate or KB-1 amended treatments. The average number of data points used to arrive at these rates is indicated with the numbers above each bar.

The higher doses of Hepure ZVI and SmZVI, at 0.38 g per bottle, revealed higher dechlorination rates than the lower doses of Hepure ZVI, Hepure ZVI with EVO, and SmZVI. The effectiveness of these treatments is dose-dependent and the smaller dose of 0.16 g/bottle has proven to be too low for Hepure ZVI. The microcosms amended with Hepure ZVI exhibited an initial decline in TCE followed by a long trail of persistent, decreasing TCE. To differentiate between the two stages observed in the Hepure

microcosms, two rates were calculated, and both are shown in **Figure 3.7c**. The entirety of the dechlorination rates and associated figures are provided in **Appendix A.17.**

As another method of comparison between the treatments, the average TCE degradation rates were calculated using equation 2.7 as described in **Section 2.8.** These rates account for the total amount of TCE reduced in each microcosm over the incubation period. A comparison of the average TCE degradation rates for each treatment are shown in **Figure 3.8.** The average number of days of incubation used to arrive at these rates is indicated with the numbers above each bar. The asterisk indicates treatments which did not reach the clean-up goal by the end of the incubation period. For the KB-1 amended microcosms, the incubation period began once the KB-1 addition was made. The lower dose of Hepure has a much lower rate than the higher dose because of the difference in incubation times. The degradation rate for the higher dose of Hepure is expected to decrease further with additional incubation. The bioaugmented microcosms have the highest rate of TCE degradation, followed by the recommended dose of SmZVI.

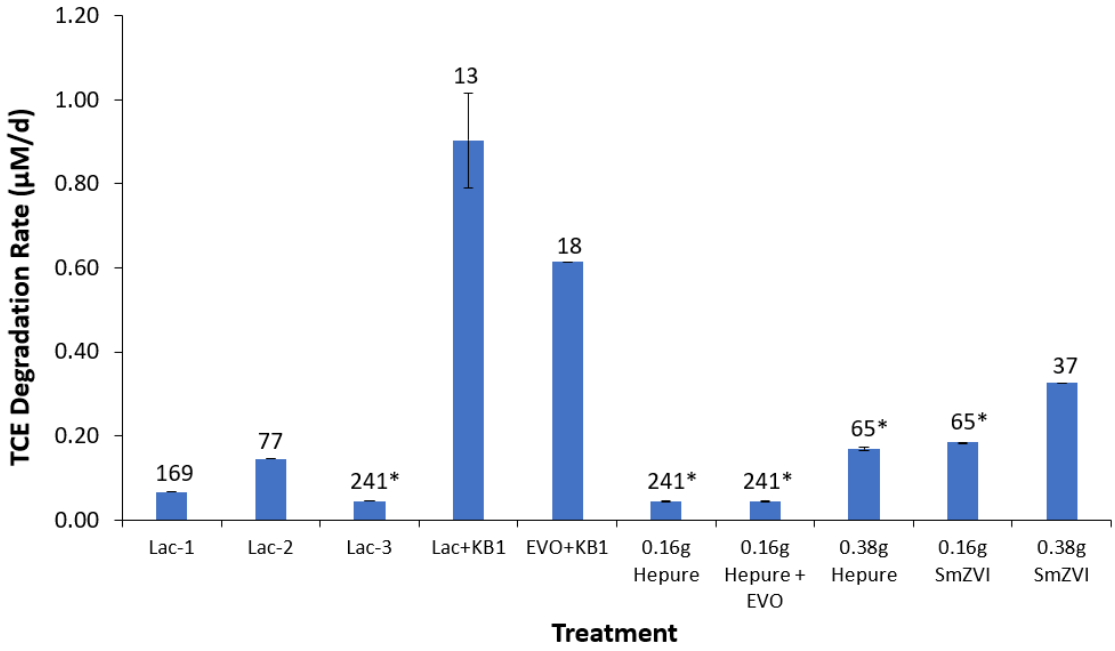

Figure 3.8. Comparison of average TCE degradation rates among the treatments that showed dechlorination activity. The average number of days of incubation used to arrive at these rates is indicated with the numbers above each bar. Asterisks indicate treatments in which TCE, cDCE and/or VC were above their MCL at the end of incubation.

3.3 Microbiological Analysis of Lane Street Microcosms

One triplicate from the lactate and KB-1, EVO and KB-1, unamended, EVO amended, and ZVI and EVO amended, and two lactate amended microcosms were sent to Microbial Insights for a qPCR analysis. The qPCR assay (QuantArray-Chlor) from Microbial Insights quantified key dechlorinating, sulfate reducing, and methanogenic microbes as well as those responsible for aerobic cometabolism. This analysis also provided information on the total number of eubacteria detected. The entirety of the results provided by Microbial Insights can be found in **Appendix A.19** and a description of each microorganism and gene quantified is provided in **Appendix A.20** for further clarification.

A time-zero microcosm with no amendments was initially analyzed for the presence of dechlorinating microbes and to gain an overall understanding of the microbial activity occurring at this Site. The time-zero microcosm revealed the presence of sulfate reducing bacteria and microorganisms responsible for aerobic cometabolism of TCE with methanol and phenol. Most importantly, there was no detectable *Dhc* or associated reductive dechlorination genes within the sample. The detection limit for *Dhc* in these microcosms was 1,000 cells/mL. The concentration of *Dhc* was also below the detection limit in the unamended, EVO, and ZVI and EVO microcosms. These microcosms had a detection limit of 500 cells/mL. *Desulfitobacterium, Dehalobium chlorocoercia, and Desulfuromonas* are microbes capable of reducing TCE to cDCE and were found in many of the microcosms that also revealed *Dhc*.

The bioaugmented microcosms contained very high concentrations of *Dhc*, as anticipated from the KB-1 addition. The EVO and KB-1 amended microcosms showed more diversity in dechlorinating microbes than the lactate and KB-1 amended microcosms. The lactate and KB-1 microcosms only had a detectable amount of *Dhc* and at a much lower concentration than the EVO and KB-1 microcosm. This is likely due to the fact that the lactate and KB-1 microcosm was stored in the fridge for multiple days before it was shipped to Microbial Insights, while the EVO and KB-1 microcosm was shipped much quicker. An effort was made for the remaining microcosms to ship them as soon as possible following the end of the incubation period.

Two lactate amended microcosms, Lac-1 and Lac-3, were sent to Microbial Insights for qPCR analysis to gain a better understanding of the activity occurring within

these microcosms. The qPCR analysis revealed the presence of *Dhc* in both of the lactate amended microcosms, despite the time-zero measurement being below detection. The presence of *Dhc* in the lactate amended microcosms was one of the key outcomes of this study. Similar results have recently been observed in a microcosm study for a contamination site in East Palestine, OH. In this study, the concentration of *Dhc* was initially below detection, but *Dhc* were identified in microcosms following an addition of lactate.[45,46] Inconsistencies in the lactate amended microcosms may be due to the concentrations of *Dhc* being heterogenous in the soil sample, despite the effort made to homogenize it. The quantities of anaerobic reductive dechlorinating microbes identified in the microcosms are shown in **Figure 3.9a.** These results are shown on a log scale because the amount of *Dhc* constitutes only a small percent of the total eubacteria quantified in these microcosms. **Figure 3.9b** shows the amount of each dechlorinating microbe normalized to the sum of the dechlorinating microbes quantified in the microcosms that revealed concentrations of *Dhc*. This figure demonstrates the microbial diversity found in the EVO and KB-1 microcosm and in the lactate amended microcosms, but *Dhc* was found at the highest concentration in these microcosms.

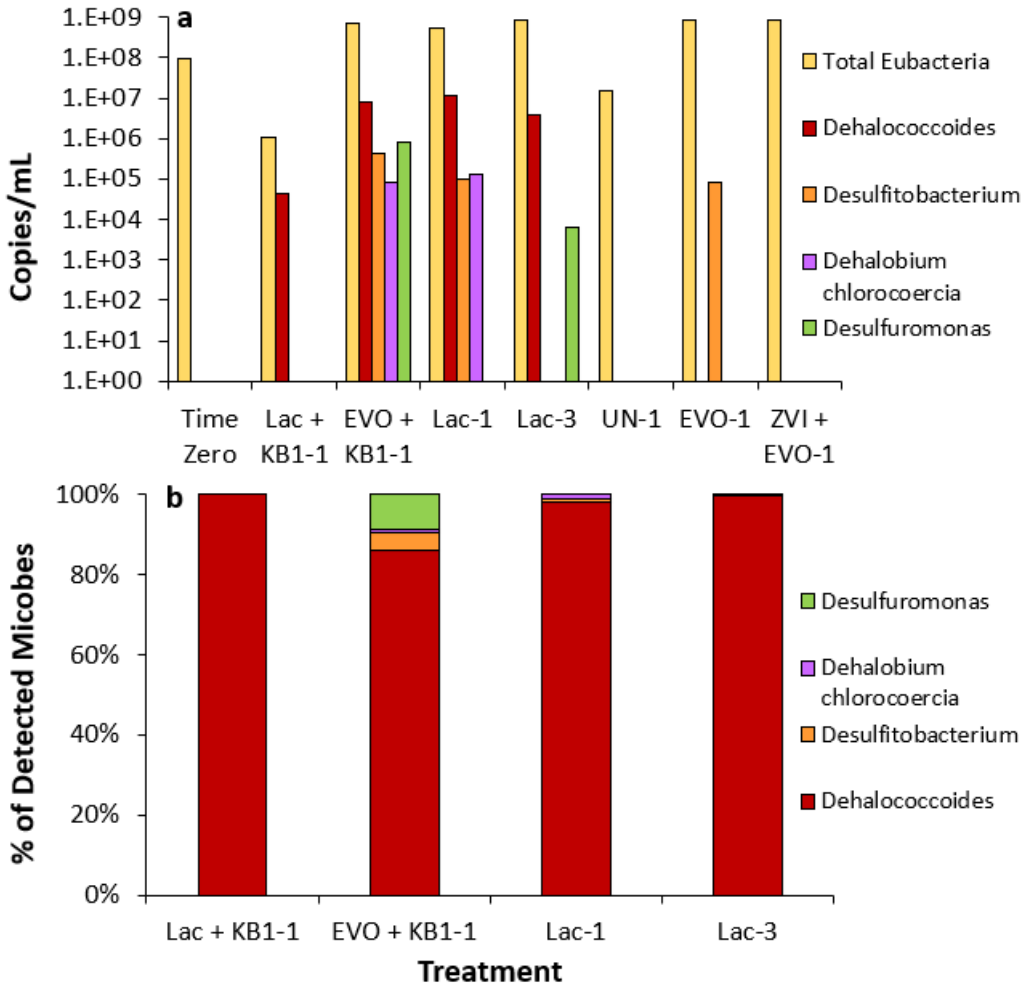

Figure 3.9. a) The concentrations of total eubacteria and anaerobic reductive dechlorinating microorganisms in each of the microcosms; b) Percentages of the reductive dechlorinating microorganisms normalized to the sum identified in each microcosm. These microorganisms include *Dhc*, *Dhgm*, *Desulfitobacterium*, *Dehalobium chlorocoercia*, and *Desulfuromonas*.

The quantities of reductive dechlorinating genes found in the microcosms are shown in **Figure 3.10a.** The time zero microcosm and final unamended, EVO, and ZVI and EVO amended microcosms did not reveal any reductive dechlorination genes above the detection limit. However, key reductive dechlorination genes vinyl chloride reductase (vcr), BAV1 vinyl chloride reductase (bvc), and tceA reductase (tceA) are found in the

two bioaugmented microcosms and the Lac-1 microcosm. **Figure 3.10b** shows the quantities of the reductive dechlorinating genes normalized to the sum of the genes quantified. This figure reveals that the bioaugmented microcosms are mostly comprised of vcr and bvc genes while the lactate amended microcosms contained primarily tceA genes. Lac-3 revealed only tceA genes, with vcr and bvc genes possibly below detection. The vcr and bvc genes are capable of reducing VC to ethene, while tceA is only responsible for the transformation of TCE to VC.[11] These results explain the lag in complete dechlorination and the persistence of VC observed in the lactate amended microcosm.

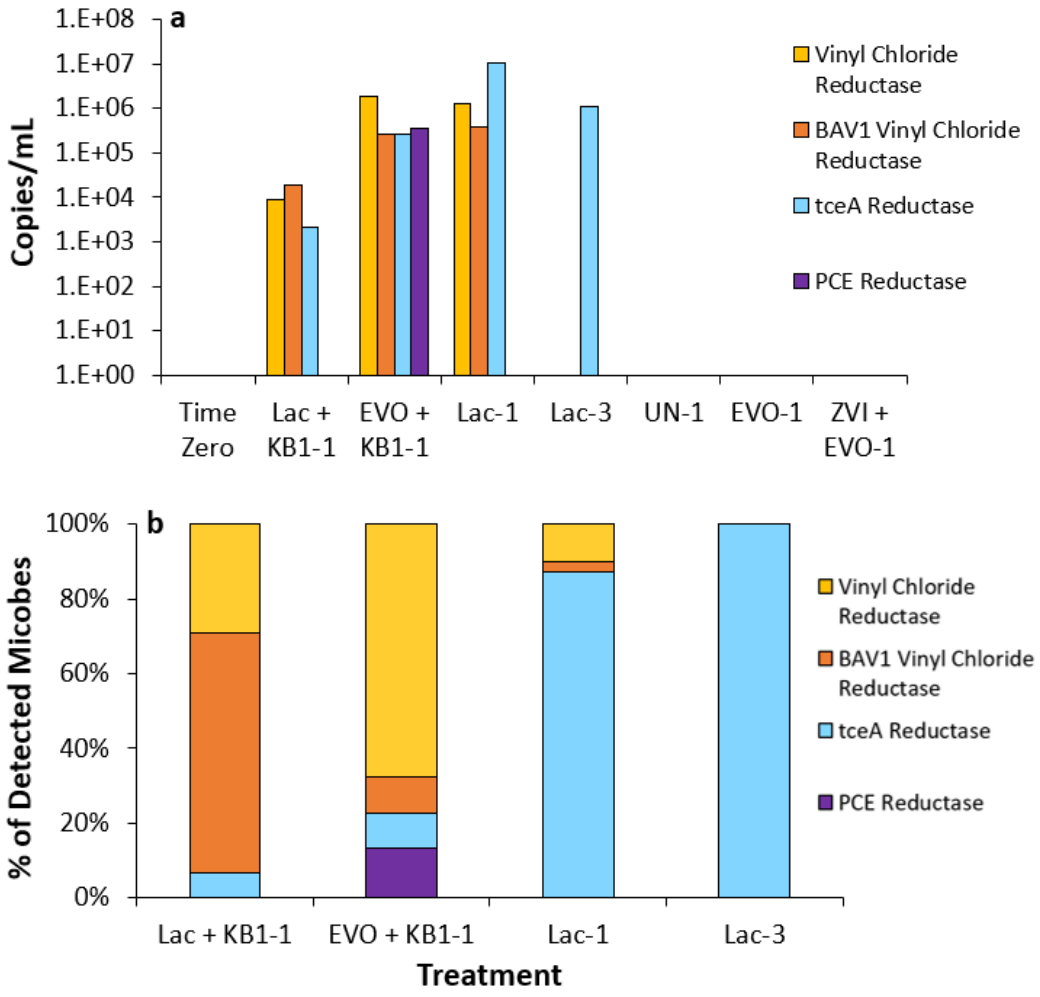

Figure 3.10. a) The concentrations of reductase genes identified in each of the microcosms; b) Percentages of the reductase genes normalized to the sum quantified in each microcosm. These genes include *vcr*, *bvc*, *tceA*, and *pce* reductase.

There were sporadic detections of genes capable of aerobic cometabolism of contaminants in the microcosms. Genes capable of cometabolically degrading TCE, DCE, or VC and methane, phenol, toluene, or ethene were detected infrequently in the microcosms (**Appendix A.19)**. The detection of these genes suggests that the addition of oxygen to the aquifer could potentially stimulate aerobic cometabolism.[47] This remediation method is especially useful for Sites with various contaminants, which may

be the case at the Lane Street Superfund Site, considering the contamination likely stemmed from multiple sources. Aerobic cometabolism could potentially be considered for lingering contamination at low concentrations at this Site.

3.4 Evaluation of Electron Balance and Methane Production

In bioremediation, *Dhc* rely on hydrogen for the electron flow necessary for reductive dechlorination. However, this process only accounts for a small portion of the electrons required for bioremediation. There are many other processes taking place in the environment that consume a larger portion of the electrons available. These processes include methanogenesis, sulfate-reduction, nitrate-reduction, and the formation of organic acids.[43,6] These processes have proven to compete with TCE reducing microbes for hydrogen and inhibit the rate of reductive dechlorination.[48,15] Electron donor additions were made multiple times throughout the experiment to ensure the reactions within the microcosms were not electron donor limited. Amendments were made in excess to promote biological reductive dechlorination and to account for reactions that not thoroughly analyzed, such as iron reduction.[43]

An electron mass balance was used in this experiment to track how many electrons are being consumed by each process in the initial set of microcosms. Lactate and EVO were used as electron donors and provided approximately 4 and 102 meq/mol when fermented to acetate, respectively. An electron balance was done by comparing the total amount of electron donors added to the number of electrons used in methane

production, sulfate reduction, nitrate reduction, organic acid production, and reductive dechlorination. More details on this electron balance can be found in **Appendix A.16**.

The results of the electron balance are shown in **Figure 3.11.** This figure demonstrates the very small amount of electron donor required for reductive dechlorination compared to the more considerable amount that went towards methane production and organic acid formation. 62% of the electron equivalence added to the third lactate amended microcosm went towards methane production before VC was reduced (**Appendix A.16**). Similar studies with mass balances have also observed high percentages of available electron equivalents being consumed by methanogens in lactate-amended microcosms.[6] This can be compared to the bioaugmented microcosms, which used less than 1% of the electrons available for methane production. There were some electrons unaccounted for in each of the microcosms, which could have gone to another process that was not fully evaluated within the microcosm.[6,43] Butyrate, isovalerate and valerate were identified in HPLC analyses of microcosms that received donor amendment. These organic acids were not quantified but it is likely that a portion of the unaccounted electron donor went towards the generation of these fermentation products.

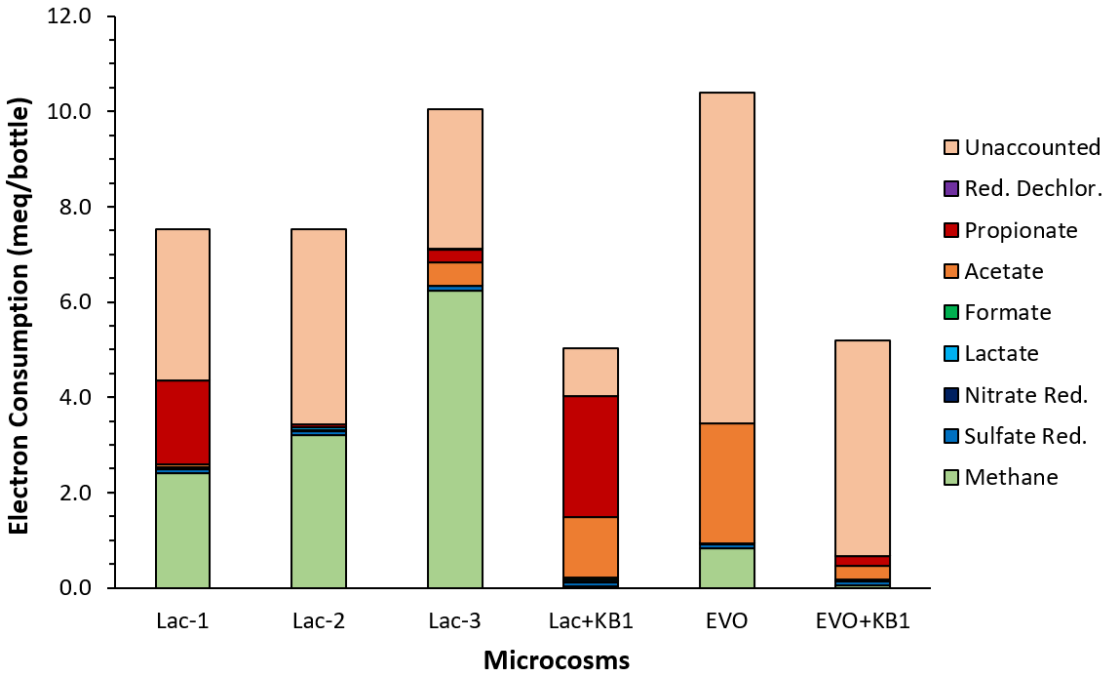

Figure 3.11. Electron mass balance to compare the amount of electron donor available in the lactate, bioaugmented, and EVO amended microcosms to the amount used for methane production, sulfate reduction, nitrate reduction, organic acid formation, and reductive dechlorination. All three lactate amended microcosms are shown because of the variability in dechlorination activity and amount of lactate added. For other treatments, the average of the triplicate bottles is shown.

The microcosms amended with only lactate generated a significantly higher amount of methane compared to the other treatment methods. The lactate amended bottles generated up to 800 µmol methane/bottle while the bioaugmented microcosms produced less than 11 µmol/bottle. The lower amount of methane production in the bioaugmented microcosms may have also be due to sulfate inhibition in these microcosms.[43] The average amount of methane produced from each treatment method is shown in **Figure 3.12** to illustrate the difference in methane production amongst the treatment options. The lactate and EVO amended bottles were reamended 3 or 4 times throughout the experiment to promote activity and a large quantity of the added donor

went to methane production. Each amendment was meant to provide 10x the electron equivalence required to fully reduce TCE to ethene, sulfate to sulfide, and nitrate to nitrogen gas.

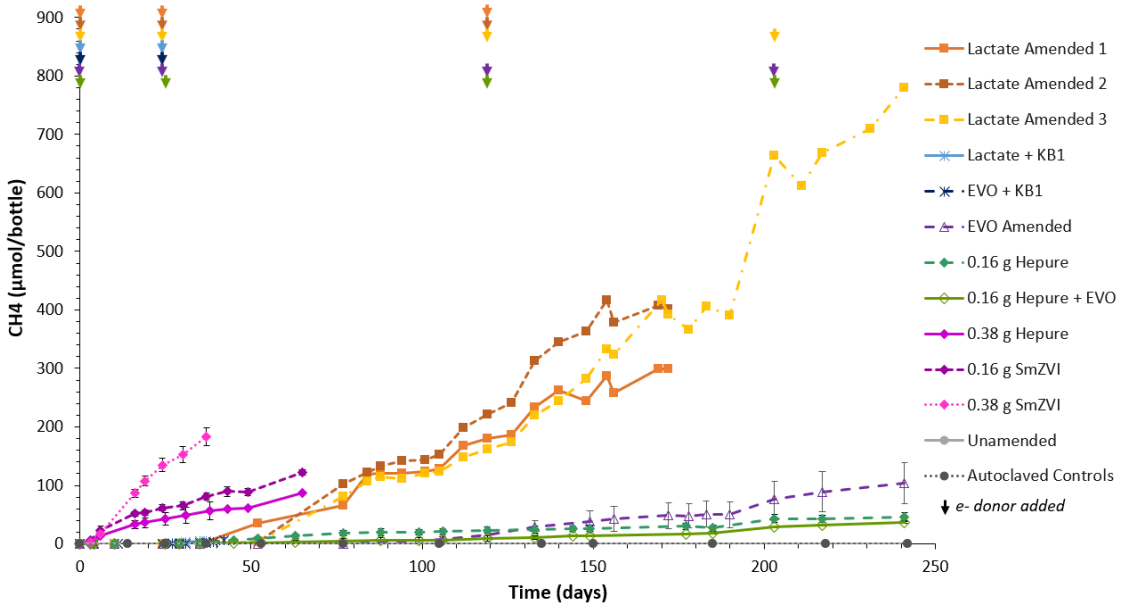

Figure 3.12. The average methane amount for each treatment. The three lactate amended microcosms are plotted individually to demonstrate the differences in their activity and incubation times. The colors of the arrows indicate when each electron donor was added to the corresponding microcosms.

The microcosms amended with the higher dose of Hepure and both doses of SmZVI generated methane very quickly and at a higher concentration than the lower dose of Hepure ZVI and ZVI and EVO microcosms. The microcosms with 0.38 g SmZVI produced approximately 180 µmol methane/bottle by the end of the incubation period, which is notably less than the amount of methane generated by the lactate amended microcosms. SmZVI is approximately 50% glycerol, which serves as an electron donor. In the microcosms amended with 0.16 g SmZVI, approximately 8% of the donor provided from the glycerol went to methane production. In the microcosms amended with

0.38 g SmZVI, approximately 5% of the donor was used for methane production (**Appendix A.16**). The SmZVI microcosms with the recommended dose of ZVI produced more methane than the bioaugmented microcosms but completely reduced TCE to ethene in a shorter time.

4.0 CONCLUSIONS AND RECOMMENDATIONS

Based on the results of this study, the following conclusions have been reached.

1) Microcosms bioaugmented with KB-1 completely reduced TCE to ethene within 50 days and at higher rates compared to the other treatments. These microcosms exhibited consistent results with lower methane production and less need for electron donor. These microcosms produced VC, but the peak amount was relatively low and the accumulation period was relatively short. There were no signs of inhibition due to toxicity caused by unidentified contaminants in the groundwater or soil.

2) In spite of the fact that *Dhc* and *Dhgm* were below detection in the time zero samples, the lactate amended microcosms revealed dechlorination activity after ~2 months of incubation. These results reiterate the importance and value of microcosm studies when evaluating remediation methods to implement at a contaminated site. However, there was a high level of variability in rates of dechlorination among the replicates, suggesting low concentrations and heterogenous distribution of the indigenous dechlorinators. These microcosms also produced a large amount of methane and required more electron donor compared to the bioaugmented microcosms. Two of the three lactate amended microcosms accumulated VC at much higher concentrations than the bioaugmented microcosms and VC persisted in one of the lactate amended microcosms through the end of the incubation period.

3) The lactate amended microcosms contained primarily of tceA genes, compared to the bioaugmented microcosms which contained a larger percentage of vcr and bvc genes.

4) There was a lack of activity in the EVO amended microcosms which suggests that the fermenters required for long chain fatty acids were absent. The EVO amended microcosms did not demonstrate the same dechlorinating activity as the lactate amended microcosms.

5) The ZVI amended microcosms were effective at TCE dechlorination but at a lower rate than the bioaugmented microcosms. The ZVI amended microcosms showed no accumulation of VC.

6) Adding EVO to the Hepure ZVI amendment did not enhance the TCE dechlorination.

7) Increasing the dose of Hepure ZVI increased the rate of dechlorination with the main products being ethene and ethane.

8) SmZVI from Regenesis was effective at TCE dechlorination and met the clean-up goal within ~1 month at the recommended dose. SmZVI microcosms did not accumulate VC and the main products were ethene and ethane.

9) Microcosms with higher doses of SmZVI and Hepure ZVI generated more methane than the bioaugmented microcosms but significantly less than the lactate amended microcosms.

10) PlumeStop activated carbon sequestered TCE almost immediately and reduced the concentration with minimal byproduct formation.

The results and conclusions reached in this study have led to the following recommendations for remediation of the Lane Street Superfund Site

1) Bioaugmentation or SmZVI are recommended for use at this site. Bioaugmentation achieved complete dechlorination without prolonged accumulation of VC, limited methane production, and lower demand for electron donor compared to biostimulation alone. Bioaugmented microcosms provided consistent dechlorination results at the highest rates. The aquifer at the Lane Street Superfund Site is conducive to bioaugmentation, with well buffered groundwater and sufficient porosity to permit distribution of the inoculum and electron donor.

2) Effective dechlorination of TCE was also achieved at the recommended dose of SmZVI (5 g SmZVI/L). Microcosms with SmZVI exhibited more methane production than the bioaugmented microcosms but TCE was completely dechlorinated without the accumulation of VC. The use of ZVI is dose dependent and the dose of SmZVI recommended by Regenesis is effective in reducing TCE concentrations at a higher rate.

3) It is also recommended to consider the use of bioaugmentation in combination with PlumeStop. PlumeStop is capable of rapidly adsorbing TCE and in combination with bioaugmentation, the adsorbed TCE is expected to degrade to environmentally sound byproducts.

4) It is also recommended to evaluate combining ZVI with PlumeStop. There are commercially available products available that combine activated carbon with ZVI and it is expected that this combination would effectively adsorb and reduce TCE to meet the clean-up goal.

Overall, SmZVI and bioaugmentation have proved to be the most effective remediation methods for reaching the clean-up goals at this Site. However, additional

considerations, especially regarding cost, will need to be evaluated before arriving at a final recommendation for remediation of the Lane Street Superfund Site.

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APPENDIX

A.1. Maps of the Lane Street Superfund Site

Figure A.1. Overview of the Lane Street Superfund Site and site boundary. This site consists of active and inactive industrial, commercial, and residential areas. The site consists of a small drainage ditch located in the industrial portion of the Site. Groundwater has potentially been intersecting the drainage ditch during the spring and summer.[23]

Figure A.2. Overview of the Lane Street Superfund Site including the site boundary and maximum extent of the PCE and TCE contamination plumes. One plume is located in the northeast industrial area and the second plume is larger, located in the central industrial area, and extends into the residential areas of the Site, along Lane Street.[23]

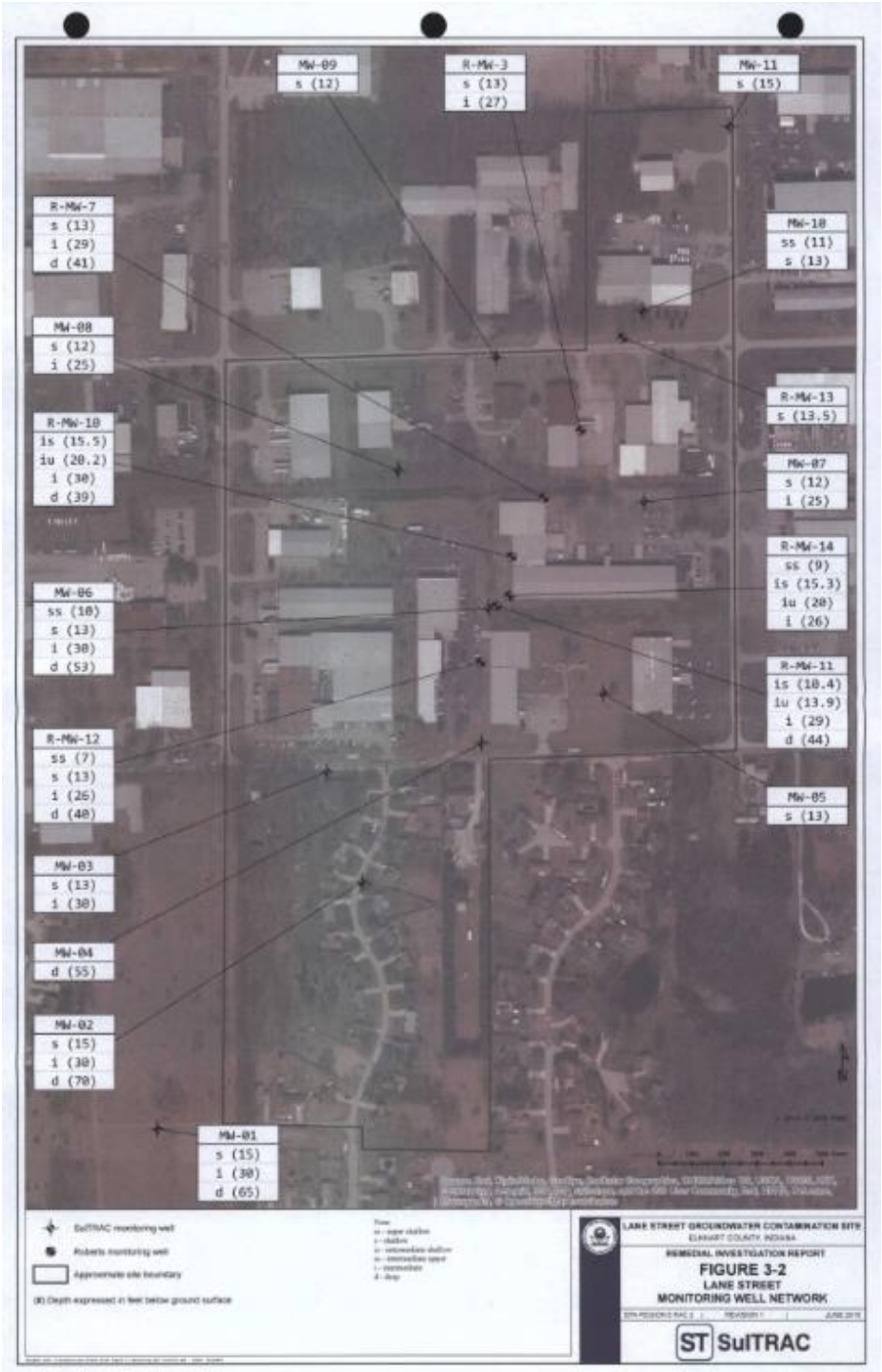

Figure A.3. Overview of the Lane Street Superfund Site including the site boundary and monitoring wells. Groundwater samples from monitoring wells R-MW-14i, R-MW-12s, R-MW-12i, MW-04d, MW-02s, MW-02i, MW-01i, and MW-01d were analyzed in this study. Groundwater from wells R-MW-14i, R-MW-12s, and R-MW-12i were composited and used in the microcosms.[23]

A.2. Sediment and Groundwater Samples

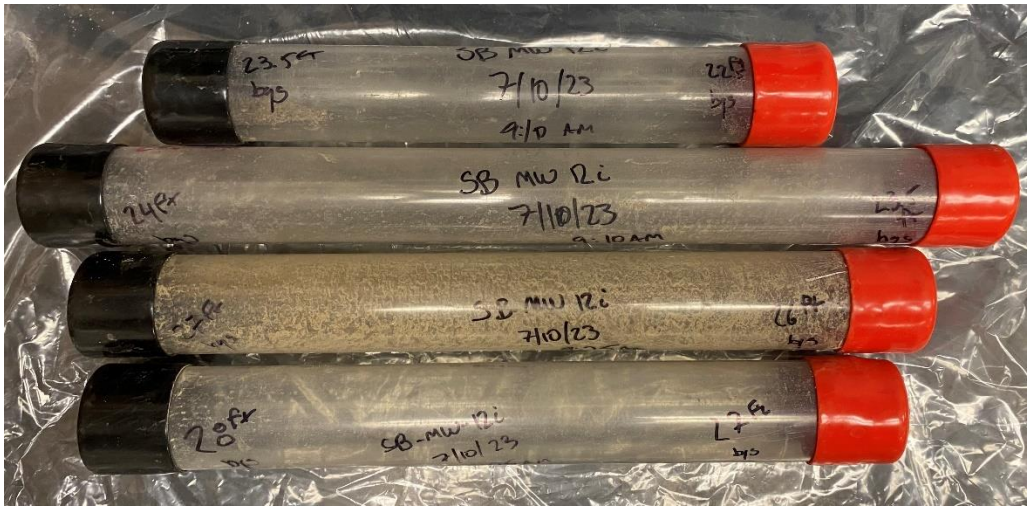

Figure A.4. Empty cores used for sediment samples from the Lane Street Superfund Site. The cores arrived on July 11, 2023. The four columns are from SB-MW-12i at depths from 22.5-24 and 26-28 ft bgs.

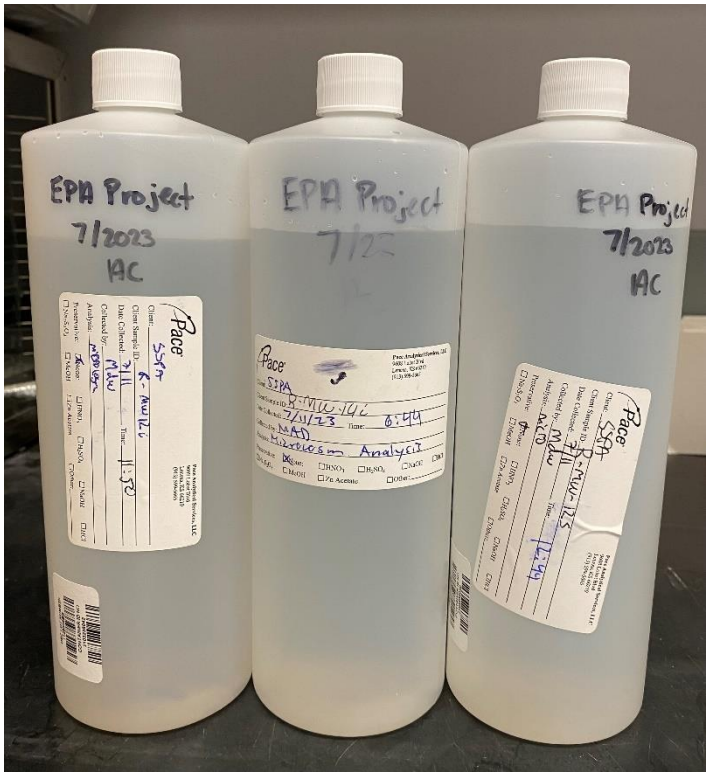

Figure A.5. Groundwater samples from the Lane Street Superfund Site. The water arrived on July 12, 2023. Groundwater from wells R-MW-14i, R-MW-12s, and R-MW-12i were composited and used in the microcosms.

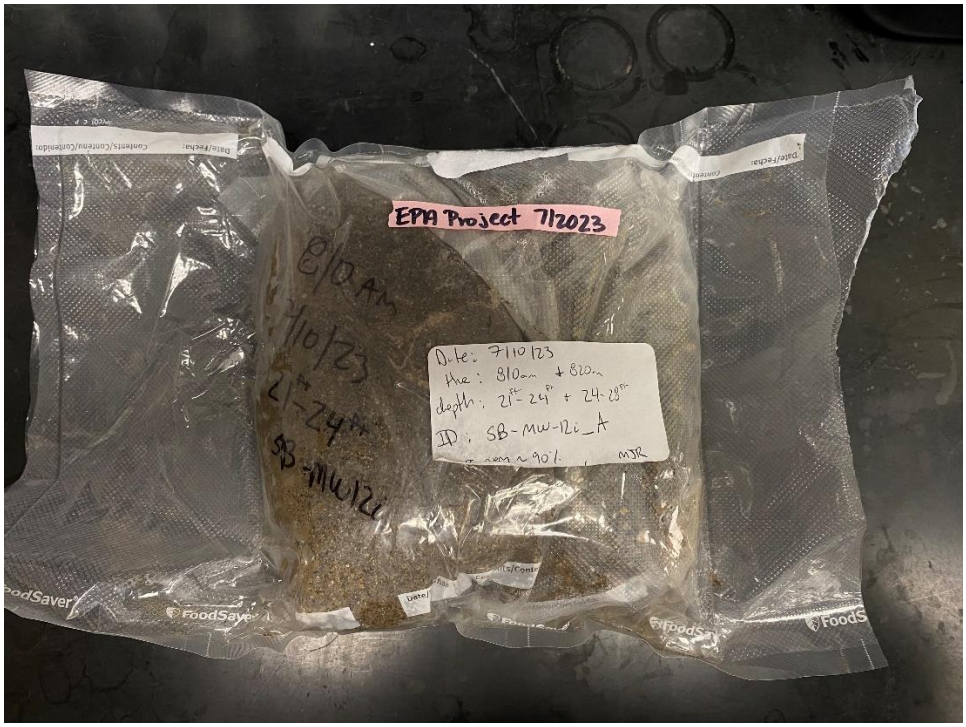

Figure A.6. Vacuum sealed bag containing a sediment sample from the Lane Street Superfund Site. This sample arrived on July 11, 2023. The sediment is from well SB-MW-12i_A at a depth of 21-24 ft bgs. This sample was used in the preliminary PlumeStop microcosms.

A.3. Initial Groundwater Sampling

Groundwater samples (~2 L) were collected from the following monitoring wells: R-MW-14i, R-MW-12s, R-MW-12i, MW-04d, MW-02s, MW-02i, MW-01i, and MW-01d. The groundwater samples were analyzed for VOCs upon arrival. To analyze the VOCs in the groundwater samples, 100 mL of cold groundwater from each sample was poured into 160-mL serum bottles which were sealed with butyl rubber slotted septa and placed on a shaker table. These bottles were incubated on the shaker table for about an hour, until the temperature of the water reached room temperature. 0.5 mL headspace samples were then removed from each groundwater sample and injected into a GC-FID to measure the initial concentrations of VOCs in each monitoring well, as shown in

Table A.1. Groundwater from monitoring wells R-MW-14i, R-MW-12s, and R-MW-12i contain detectable concentrations of TCE. Monitoring wells R-MW-14i, R-MW-2i, MW-02i, and MW-01i show detectable levels of *c*DCE.

Table A.1. Initial measurements of VOCs in each well. These measurements were taken using the GC-FID upon arrival. Only wells R-MW-14i, R-MW-12s, and R-MW-12i showed detectable levels of TCE.

GW Sample	Concentration (µg/L)		
	Methane	**cDCE**	**TCE**
R-MW-14i	0.1	7.1	54.0
R-MW-12s	0.7	0.0	12.0
R-MW-12i	0.6	16.3	61.5
MW-04d	1.5	0.0	0.0
MW-02s	0.0	0.0	0.0
MW-02i	0.8	83.3	0.0
MW-01i	0.8	29.0	0.0
MW-01d	1.9	0.0	0.0

Triplicate microcosms were prepared alongside the initial set of Lane Street microcosms, but without the addition of TCE saturated water. They were prepared in the same 160 mL serum bottles with 50 g sediment and 70 mL groundwater and are identified as UN-DNA in **Appendix A.12**. These microcosms represent what is present in the microcosms prior to the addition of TCE saturated water or any of the amendments made in the remainder of the microcosms. The results of the VOC analysis are shown in **Table A.2.** One of the triplicate microcosms was sent to Microbial Insights for a time-zero qPCR assay. The time-zero qPCR analysis revealed that concentrations of *Dhc* and *Dhgm* are below detection (**Appendix A.19).**

Table A.2. Time-zero measurements of VOCs in microcosms prepared with only 50 g of sediment and 70 mL of groundwater.

Identifier	Concentration (µg/L)		
	Methane	**cDCE**	**TCE**
UN-DNA-1	0.1	4.9	71.2
UN-DNA-2	0.1	8.1	66.2
UN-DNA-3	0.1	8.7	65.5
Average	0.1	7.0	67.6

Initial groundwater samples were also analyzed for the presence of nitrate, sulfate, and organic acids. There was no indication of organic acids in the groundwater sample, which is to be expected. The time-zero groundwater sample was initially brought to the Clemson ASL for analysis on their IC. This initial sample indicated a sulfate concentration of 13.66 mg/L and nitrate below detection. These values were used for the initial calculations of amounts of each electron donor required in the microcosms. However, these samples were later rerun on the IC in Rich Laboratory and it was revealed that the groundwater has a sulfate concentration of 14.90 mg/L and a nitrate concentration of 0.53 mg/L. These are very minor differences, but they are accounted for in the final electron balance.

A.4. Summary of Water Quality Parameters

Table A.3. Summary of water quality parameters from each well sampled in this microcosm study.

Well ID	Microcosm Sample	Date	Time	Purge Volume (L)	pH (su) ± 0.1 su	Temp (°C) ± 10%
MW-01i	Y	7/13/2023	12:07	13.2	7	15.25
MW-01d	Y	7/13/2023	14:03	25.6	7.37	17.52
MW-02s	Y	7/11/2023	15:39	5.7	7.34	18.92
MW-02i	Y	7/11/2023	14:56	7.6	7.35	18.28
MW-04d	Y	7/11/2023	13:47	10.6	7.29	19.29
R-MW-12s	Y	7/11/2023	12:33	8.6	7.53	20.09
R-MW-12i	Y	7/11/2023	11:40	7.6	7.37	19.75
R-MW-14i	Y	7/11/2023	6:27	15.3	6.72	17.24
Averages (12s, 12i, 14i)	-	-	-	-	7.21	19.03

Well ID	Conductivity (mS/cm) ± 10%	Turbidity (NTU) < 10 NTU	ORP (mV)	Dissolved Oxygen (DO) (mg/L)	Purge Rate (L/min)	Depth to Water (ft)
MW-01i	0.703	7.4	1	0	300	10.71
MW-01d	0.68	6.5	-65	0	320	10.46
MW-02s	0.453	4.5	43	2.43	300	9.14
MW-02i	0.679	4	-30	0	300	9.07
MW-04d	0.667	1.3	-33	0	300	5.21
R-MW-12s	0.313	4.3	27	0	280	5.7
R-MW-12i	0.632	3.2	21	0	260	5.63
R-MW-14i	0.658	2.6	121	0	320	7.02
Averages (12s, 12i, 14i)	0.53	3.37	56.33	0	286.67	6.12

A.5. Chemicals

Hydrogen (99.99%) and air (dry grade) were obtained from National Welders. Methane (99.5%), ethane (99.9%), ethene (99.9%), and acetylene (99.5%) were obtained from National Welders and VC (99.5%) was obtained from Matheson. These gases were used in the preparation of analytical standards.

Sodium acetate (Mallinckrodt Baker Inc., 99.7%), propionic acid (Aldrich Chemical Co., 99%), sodium formate (J.T.Baker, 99.3%), sodium lactate (Sigma Aldrich, 60%), sodium sulphate (EMD chemicals, 99%), potassium nitrate (EM Science, 99%), and cDCE (Fujifilm Wako Pure Chemical Corp., 99%) were used in the preparation of analytical standards.

A.6. Sample Preparation and Characterization

To measure the dry weight of the sediment, an aluminum dish was weighed and filled with ~20g of wet sediment. The aluminum dish with sediment was reweighed to determine the weight of the wet sediment. The dish was placed in a 103 °C oven overnight. The dish was then cooled in a desiccator before being reweighed. This procedure was done in triplicate. The results determined that the soil used in this microcosm study is 86.8% dry. The percent moisture of the sediment was calculated with the following equation:

$$\%\, moisture = \frac{(dish\ and\ soil_{initial}\ (g)) - (dish\ and\ soil_{dried}\ (g))}{(dish\ and\ soil_{initial}\ (g)) - (dish\ (g))} \qquad (A.6.1)$$

To determine the volume of water displaced by 50 g of sediment, a 100 mL graduated cylinder was filled with 50 mL of water. 50 g of soil was added to the graduated cylinder, and the new volume was recorded. A 100 mL graduated cylinder was then filled with 50 mL of water and glass beads were added to displace the same volume of water. This procedure was done in triplicate to determine the volume of water displaced by 50 g of sediment.

Duplicate samples of sediment were taken to the ASL to obtain the fraction of organic carbon in the sample. The fraction of organic carbon is 0.28%. The fraction of organic carbon is useful for predicting the amount of TCE that may have initially adsorbed to the sediment. However, the fraction of organic carbon in this sediment was very low and does not completely account for the loss of TCE that was observed.

A.7. Additions to Microcosms

For the microcosms receiving electron donor amendments, the donor demand for the microcosms was calculated to determine the dose required to ensure there would be enough donor to completely reduce TCE to ethene. This was influenced by the demand of sulfate and nitrate reducers based on the sulfate and nitrate present. Sulfate and nitrate reducers can compete with organohalide respiring microbes as the terminal electron acceptor in anaerobic conditions.[6]

For the lactate and EVO amendments, stock solutions were made to provide 10-fold more electron equivalents than needed to reduce TCE to ethene in a 0.5 mL injection. To calculate the amount of donor required to provide 10x the amount of

electron equivalents needed to reduce TCE to ethene, the following equation was used. For this calculation, 5 meq/mmol are required for nitrate reduction, 8 meq/mmol are required for sulfate reduction, and 6 meq/mmol are required for complete dechlorination of TCE to ethene.

$$e^{-}demand = \{(mmol\ NO_3^- \times 5) + (mmol\ SO_4^{2-} \times 8) + (mmol\ TCE \times 6)\} \times 10 \quad \textit{(A.7.1)}$$

Once the amount of donor required was calculated, the amount of lactate and EVO required to meet the donor demand was determined. Lactate provides 4 meq/mmol and EVO provides 102 meq/mmol. This assumed that acetate formed during fermentation is not usable as an electron donor. The amount of sodium lactate required for the stock solution was calculated using the following equation:

Sodium lactate has a molecular weight of 112 mg/mmol and the sodium lactate syrup used is 60% sodium lactate. The concentration of Newman 55 EVO was calculated in the same manner with a molecular weight of 879.4 mg/mmol and the product being 55% EVO. The stock solution was added (0.5 mL) to the designated microcosms.

$$\frac{g\ sodium\ lactate}{100\ mL\ DDI} = meq\ e^{-}demand \times \left(\frac{mmol}{4\ meq}\right) \times \left(\frac{112\ mg}{mmol}\right) \times \left(\frac{1}{0.6}\right) \times \left(\frac{1\ bottle}{0.5\ mL}\right) \quad \textit{(A.7.1)}$$

The three lactate stock solutions were made by diluting 7.8111, 7.7518, and 7.7795 g of 60% sodium lactate syrup (Sigma-Aldrich) in 100 mL DDI water. Various lactate stock solutions were made throughout the experiment because lactate is reactive and lactate was reamended multiple times. The EVO stock solution was made by diluting

2.6453 g of Newman 55 EVO (>55% soybean oil, 4% sodium lactate, RNAS Remediation Products) in 100 mL DDI water. The same EVO stock solution was used throughout the experiment.

For the bioaugmented microcosms, the KB-1 bioaugmentation culture from SiREM Lab was added as a source of *Dhc*.[29] This culture is non-pathogenic and is meant to establish a microbial community capable of completely reducing TCE contamination to ethene. KB-1 is an aqueous solution of mineral salts and nutrients that contains *Dhc, Geobacter sp., and Methanomethylovorans sp.*[49] *Dhc* are capable of completely reducing TCE to ethene and the other microbes in this culture assist with this process. The culture contains 10^8 copies *Dhc*/mL. To achieve a concentration of 10^6 cells/mL in the microcosms, 0.7 mL KB-1 was added,[28] as follows:

$$KB1\ Dose\ \left(\frac{mL\ KB1}{bottle}\right) = \frac{70\ mL\ GW}{bottle} \times \frac{10^6\ cells}{mL\ GW} \times \frac{mL\ KB1}{10^8\ cells} \qquad (A.7.2)$$

Ferox Flow ZVI from Hepure is a powder ZVI product at a particle size of 125 microns. This product is 95% pure ZVI with small amounts of carbon, sulfur, silicon, and iron.[50] Guidance on the amount of Hepure ZVI to add was obtained from the manufacturer and the literature.[30,31] A range of Ferox Flow ZVI (>95% ZVI, Hepure) doses was evaluated in a preliminary experiment (**Appendix A.10**). On that basis, 0.16 g of Ferox Flow ZVI were added to the designated microcosms. An additional set of Hepure ZVI microcosms were prepared with a dose of 0.38 g Ferox Flow ZVI per bottle. The second set of Hepure ZVI microcosms were prepared alongside microcosms with Regenesis ZVI, for comparative purposes (see below).

SmZVI is a glycerol solution with colloidal, sulfidated-ZVI particles. The ZVI particles are less than 5 μm in diameter and are coated with a thin iron sulfide layer to enhance the reaction rate with chlorinated ethenes and to minimize the reaction rate with water.[20] For the microcosms containing SmZVI (40% ZVI in glycerol, Regenesis), two doses were evaluated. The first dose was based on the initial mass of Hepure ZVI added in the first set of microcosms. These bottles were made with a dose of 0.16 g SmZVI per microcosm. The second set was based on a recommended dose of 2 g ZVI/L, provided by Regenesis, and was calculated as follows:

$$SmZVI\ Dose\ \left(\frac{g}{bottle}\right) = \frac{2\ g\ ZVI}{L} \times \frac{g\ SmZVI}{0.4\ g\ ZVI} \times \frac{0.076\ L\ GW}{bottle} \qquad (A.7.3)$$

This required addition of 0.38 g SmZVI per bottle. SmZVI is an injectable liquid so the volume of SmZVI added to the microcosms was determined based on a density of glycerol of 1.26 g/mL. The glycerol in SmZVI can be fermented and produce hydrogen which serves as an electron donor.

PlumeStop Liquid AC consists of colloidal activated carbon particles less than 2.5 μm in water. This product works primarily through adsorption and upon injection, contaminants partition out of the aqueous phase and sorb to the activated carbon particles. For the microcosms containing PlumeStop (20% AC in water, Regenesis), the dose was based on a Freundlich adsorption isotherm using constants provided by Regenesis, as follows:

$$q_e = K_f {C_e}^{1/n} \qquad (A.7.4)$$

where *qe* is the amount adsorbed (mg TCE/g AC); K_f is the adsorption capacity (65.0 (mg/g)/(mg/L)$^{1/n}$); C_e is the equilibrium concentration in water, which was set to the MCL of 0.005 mg/L; and 1/n is the strength of adsorption (0.55).[32] PlumeStop is 20% activated carbon. Assuming a specific gravity of 1, the dose of PlumeStop required was 150 μL per microcosm.

A.8. Microcosm Preparation

Water controls were prepared on the bench top. The first set of controls were prepared in 160 mL serum bottles containing 70 mL DDI water and 57.82 g glass beads to displace the same volume as the soil. The second set of controls were prepared in the same manner with 56.83 g glass beads. The remaining microcosms were prepared in the anoxic glovebox using 160 mL serum bottles. These microcosms contained 70 mL of groundwater and 50 g sediment from the Lane Street Superfund Site. **Figure A.7** shows the construction of the microcosms in the glovebox.

Figure A.7. Construction of microcosms in the anoxic glovebox.

Hepure Ferox Flow ZVI powder was added to the designated microcosms in the anoxic chamber. Ferox Flow ZVI was added to the microcosms at 2 and 5 g/L ZVI. SmZVI is a liquid ZVI product and was injected into the designated microcosms in the anoxic chamber. SmZVI was added to the microcosms as doses of 2 and 5 g/L product.

Once removed from the anaerobic chamber, the headspaces were purged with nitrogen (N_2) gas to remove hydrogen (H_2) because H_2 is a preferred electron donor for reductive dechlorination.[11] The septum on each microcosm was replaced with sterile Teflon-faced septa and an aluminum crimp cap while sparging. Treatments with designated electron donor amendments were injected with an initial dose of approximately 10-fold the electron equivalents to reduce TCE to ethene. The microcosms were periodically re-amended with electron donor as needed to supplement the initial amounts added.

Autoclaved controls were prepared by autoclaving triplicate microcosms for one hour on three consecutive days.[33] TCE was added to the autoclaved controls after the autoclaving was complete because autoclaving reduces the amount of TCE initially present in the microcosms. After the autoclaving was complete, the septa were replaced with sterile Teflon-faced septa and an aluminum crimp cap while sparging with filter sterilized N_2. The tubing and canula after the filter are also sterile. Sterile equipment was used for the autoclaved microcosms to prevent contamination of the controls. **Figure A.8** shows the autoclaved microcosms after autoclaving. The resazurin is still a bright pink.

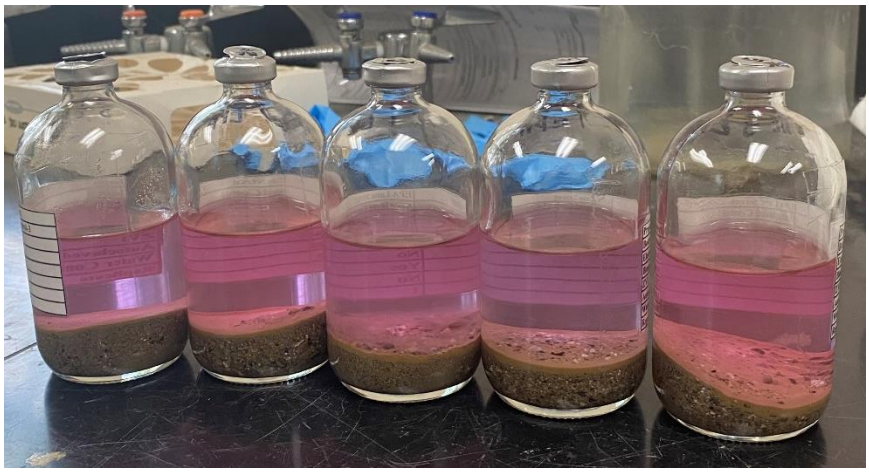

Figure A.8. Autoclaved microcosms after autoclaving.

PlumeStop is a liquid activated carbon product and was injected into the designated microcosms at a dose of 150 μL/bottle following the initial time-zero measurements. These injections were done on the bench top using an air-tight liquid syringe.

The constructed microcosms were incubated on a shaker table for 1 hour to promote equilibrium in the microcosms before time-zero measurements were taken. The

initial set of microcosms were all constructed on the same day, which is when time-zero measurements were made. Time-zero measurements were made for the EVO amended and EVO and KB-1 amended microcosms the following day. Time-zero measurements revealed TCE concentrations of ~1,200 µg/L and *c*DCE concentrations <10 µg/L in the microcosms. Water controls had a lower initial TCE concentration of ~800 µg/L because less TCE saturated water was added to these microcosms.

The second set of microcosms were constructed at a later date and time-zero measurements were made on the same day. Time-zero measurements for these microcosms revealed TCE concentrations of ~1,300 µg/L in the microcosms. The addition of PlumeStop was made to the designated microcosms after the time-zero measurement. Following the time-zero measurements, all microcosms were stored stationary, inverted, and in the dark at room temperature throughout this experiment.

Once the resazurin turned clear, the designated microcosms were bioaugmented. This ensures that the microcosm environment is low in redox to allow the growth of dechlorinating microbes.[4] The dose of bioaugmentation cultures was based on a concentration of *Dhc* of ~10^6 cells/mL.[28] The microcosms were bioaugmented using KB-1 from SiREM.[29] The bioaugmentation culture was injected into the microcosms using an air-tight syringe in the anoxic chamber.

A CENSUS qPCR assay by Microbial Insights was done at the beginning of this study to produce a snapshot of the microbial community existing at the Site (**Appendix A.19**). Microbes and genes evaluated in this procedure are described in **Appendix A.20**. CENSUS qPCR provided an opportunity to observe the initial concentration of *Dhc* and

how the community responds to biostimulation and bioaugmentation at the end of the experiment.

For the initial set of microcosms, once the concentrations of contaminants in each microcosm fell below their designated MCLs, the triplicate microcosms were moved into the glovebox. The microcosms were then placed upright and settled overnight so 10 mL of groundwater could be removed, filtered using a 13 mm syringe filter with 0.2 μm PTFE membrane (VWR, 28145-491), and placed in a scintillation vial. These samples were used for quantification of nitrate, sulfate, and organic acids at the end of the incubation period. The microcosms were then resealed with gray butyl rubber slotted septa and aluminum crimp caps. One replicate from each triplicate was sent to Microbial Insights to repeat the qPCR procedure.

A.9. Analytical Techniques

Concentrations of TCE, *c*DCE, VC, methane, acetylene, ethene, and ethane were measured by injecting 0.5 mL headspace samples onto a GC (Hewlett Packard 5890 Series II) equipped with an FID and a stainless-steel column packed with 1% SP-1000 on and a 30 m column with a 0.25 mm inside diameter. A gradient temperature program starts at 60 °C for 2 minutes, ramps the temperature up to 150 °C at a rate of 20 °C/min. The temperature then increases at a rate of 10 °C/min to 200 °C where it is held for the final 10 minutes.[4,35] This method is able to detect the previously listed VOCs as well as 1,1,1-TCA and its biotic and abiotic degradation products (1,1-DCE, 1-1-dischloroethane, and chloroethane).[51] Response factors for TCE, *c*DCE, VC, methane, acetylene, ethene,

and ethane were determined using the GC-FID. Response factors for these gases were determined multiple times throughout this experiment. Sets I, II, III, and IIII of response factors are described in **Appendix A.21.**

Concentrations of lactate, formate, acetate, and propionate were analyzed using the HPLC.[36,37] 1 mL samples were analyzed using a Dionex/Thermo UltiMate 3000 HPLC system or an Agilent 1100 Series HPLC. Both systems were equipped with an Aminex HPX-87H ion exclusion organic analysis column (300 x 7.8 mm, Bio-Rad) and a micro guard cation H cartridge in the guard column (30 x 4.6 mm, Bio-Rad). This system used a mobile phase of 0.005 M H_2SO_4. The method for the HPLC includes a gradient flow rate program that starts at 0 mL/min and increases 10 mL/min every 10 minutes until the flow reaches 0.6 mL/min. The time zero and KB-1 microcosms were run on the Dionex HPLC with a run time of 30 minutes to detect pyruvate, lactate, formate, acetate, propionate, isobutyrate, butyrate, and isovalerate. The remaining samples were run on the Agilent HPLC with a run time extended for 60 minutes, allowing for detection of pyruvate, lactate, formate, acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate. Response factors for lactate, formate, acetate, and propionate were determined using the HPLC. Response factors for these organic acids were determined multiple times throughout this experiment. The response factors are described in **Appendix A.22**.

Concentrations of sulfate and nitrate were analyzed using an IC.[36] 5 mL samples were analyzed using a Dionex Aquion IC System equipped with a Dionex IonPac AS9-HC analytical column (4 x 250 mm, Thermo Scientific) and Dionex IonPac AG9-HC guard column (4 x 50 mm, Thermo Scientific). This system used a 9 mM $NaCO_3$ mobile

phase.[35] An initial sample of groundwater was taken to the Clemson University ASL for nitrate and sulfate measurements. These samples were rerun on the IC in Rich Lab further into the experiment to confirm the results (**Appendix A.3**). Response factors for nitrate and sulfate were determined using the IC. Response factors for nitrate and sulfate were determined multiple times throughout this experiment and are described in **Appendix A.23**.

For the initial set of microcosms, at the end of the incubation, one bottle from each triplicate was sent to Microbial Insights to gain insight on the microbial activity through qPCR. qPCR uses DNA to quantify specific microorganisms and functional genes present in the samples. In qPCR, target genes are amplified and produce fluorescent signals which can then be measured and used to quantify the amount of that target gene present in a sample.[39] This process is useful in quantifying microbes and genes specifically responsible for contaminant biodegradation to assess the potential of monitored natural attenuation or biostimulation.[38] *Dhc* and *Dhgm* are the only two microorganisms known capable of completely reducing TCE to ethene.[11,52,53] *Dhc* has three genes that play major roles in the reductive dechlorination of TCE: tceA, bvc, and vcr.[11,12]

A.10. Preliminary Hepure Ferox Flow ZVI Microcosms

In preparation for adding ZVI to the microcosms produced in this experiment, preliminary microcosms were made to observe the concentration and transformations of TCE over time with different doses of Hepure Ferox Flow ZVI. This is the ZVI product

that was added to the initial Lane Street microcosms. The doses of ZVI used in this preliminary experiment were determined using calculations performed by the Naval Facilities Engineering Command in a ZVI application for TCE remediation at Hunter's Point Shipyard in California.[30] These calculations were based on a ZVI:TCE ratio and a ZVI:soil ratio. The microcosms constructed will each have a TCE concentration of ~1,000 μg/L and will contain 50 g of soil. Using a ZVI:TCE ratio of 1100 and a ZVI:soil ratio of 0.004, the amount of ZVI required is 0.31 g per bottle.[30] The equation used for this calculation is shown here:

$$ZVI\left(\frac{g}{bottle}\right) = \left(1100\frac{g\ ZVI}{g\ TCE} \times \left(9.67 \times 10^{-5}\frac{g\ TCE}{bottle}\right)\right) + \left(0.004\frac{g\ ZVI}{g\ soil} \times 50\frac{g\ soil}{bottle}\right) \quad \textit{(A.10.1)}$$

A range of doses from 10x higher to 10x lower than the required dose was used to analyze the effects of the ZVI product on TCE concentrations. The microcosms constructed contained 3.1, 1.5, 0.31, 0.15, and 0.03 g of ZVI per bottle. There was also a control microcosm which was prepared in the same way but will contain no ZVI.

To prepare these microcosms, 6 160 mL serum bottles were cleaned and sterilized, along with their respective Teflon-faced septa (SUN-SRI, 20 mm Pharma 0.130 Butyl, 14234-854). Microcosms were constructed by adding 50 g of sandy soil and the designated amount of ZVI into each serum bottle. About 0.5 L of unfiltered groundwater from Phoenix was poured and placed in the anaerobic glovebox along with the serum bottles. The groundwater and serum bottles were left in the glovebox overnight with the tops open to deoxygenate. In the glovebox, 70 mL of groundwater was added to

each microcosm. The bottles were then capped and removed from the glovebox. The headspace of each microcosm was sparged with N_2 for 1 minute. Without allowing any introduction of air, the slotted grey butyl rubber septum was replaced with the Teflon-faced septum inside the aluminum crimp cap and crimped closed. 90 μL of TCE saturated water (~1.1 mg/mL) was added to each bottle to bring the initial concentrations to ~1,000 μg/L. The bottles were then inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The amount of TCE and other VOCs were determined by injecting 0.5 mL of headspace samples into the GC-FID. Using the response factors described in **Appendix A.21**, the PAUs were recorded and converted to concentrations of TCE and other VOCs. The amount of TCE in each of the microcosms are shown in **Figure A.9a**. The sums of products generated by each microcosm were calculated and plotted over time. These products include acetylene, ethene, ethane, VC, and cDCE. The results from this summation are shown in **Figure A.9b.** Outliers were removed from these figures.

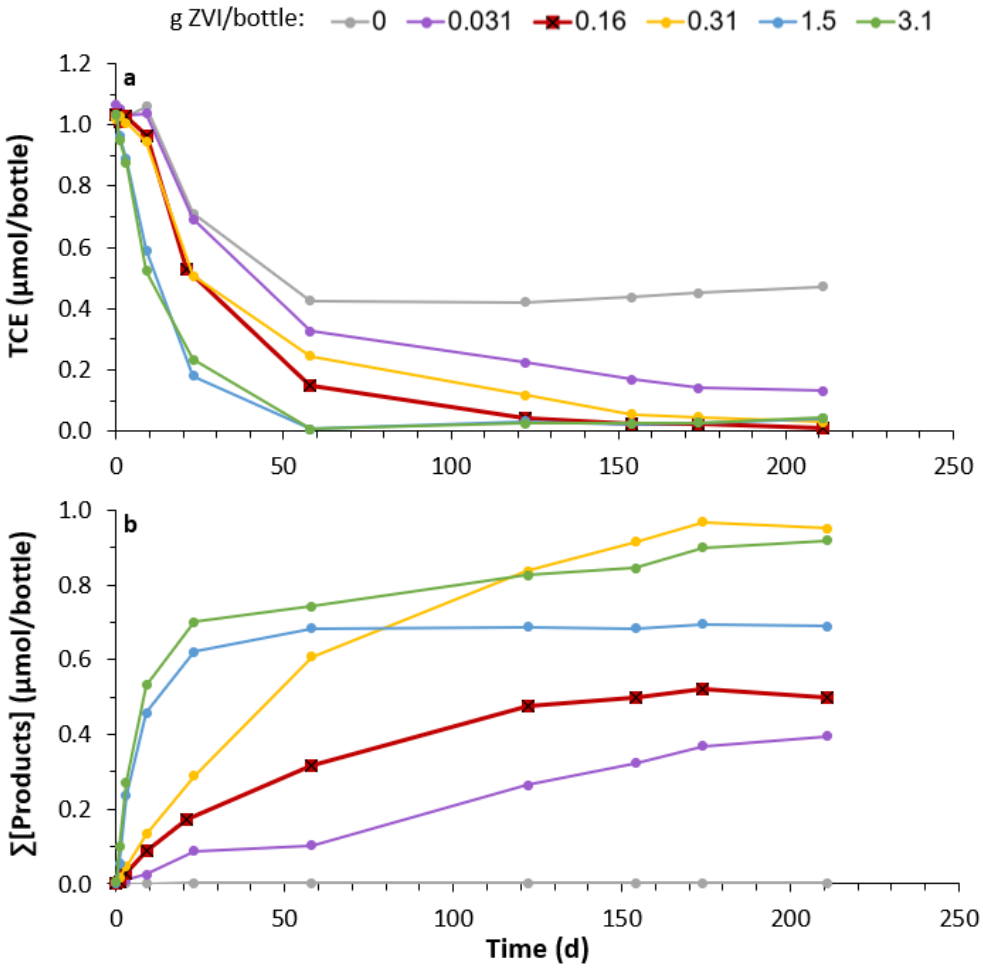

Figure A.9. a) The amounts of TCE in the preliminary Hepure ZVI microcosms; b) The sum of the products generated by the preliminary Hepure ZVI microcosms. The products include acetylene, ethene, ethane, VC, and cDCE. The microcosm with the ZVI dose used in the Lane Street Superfund Site microcosm study is highlighted in red.

As TCE degrades in these microcosms, the sum of the products increased as dechlorination byproducts were formed at varying rates. The microcosms with the higher doses of ZVI reduced TCE and formed products more rapidly than the microcosms with less ZVI. The dose selected for use in the Lane Street Superfund Site microcosm study was 0.1579 g per bottle. The microcosm with 0.1579 g of ZVI closely followed the trend of TCE degradation and product formation shown by the microcosm with 0.3155 g of

ZVI but with half the mass of ZVI. This decision was made very early into this preliminary experiment, around day 9.

A.11. Preliminary Regenesis PlumeStop Microcosms

In preparation for adding Regenesis PlumeStop to the microcosms in this study, preliminary microcosms were prepared to observe the concentration and transformations of TCE over time with different doses of PlumeStop activated carbon. PlumeStop activated carbon was used in the second set of microcosms in this study and is be compared to different abiotic methods of remediation. For this experiment, the doses were based on Freundlich adsorption isotherm constants provided by Regenesis. The dose of PlumeStop required for the designated microcosms was derived using the following equation:

$$q_e = K_f C_e^{\,1/n}$$

The adsorption capacity at unit concentration (Kf) for TCE is 97.0 $(mg/g)/(mg/L)^{1/n}$, the strength of adsorption (1/n) is 0.429, and the equilibrium concentration of TCE in the water (Ce) is 1.1 (mg TCE/L).[32] The Kf and 1/n values were derived empirically for TCE and PlumeStop. PlumeStop is 20% activated carbon. These microcosms contained 70 mL of groundwater so the lowest dose of PlumeStop used in this experiment was 6 μL per bottle. The microcosms constructed contained 6, 25, 50, and 100 μL per bottle. Results from this experiment were meant to be used to determine the dose of PlumeStop activated carbon used in the second set of microcosms.

To prepare these microcosms, 6 160 mL serum bottles were cleaned and sterilized, along with their respective Teflon-faced septa (SUN-SRI, 20 mm Pharma 0.130 Butyl, 14234-854). Microcosms were constructed by adding 50 g of sediment and 70 mL of groundwater from the Site. The sediment, groundwater, and serum bottles were placed in the anaerobic glovebox overnight to deoxygenate. Sediment used in this experiment was collected from well SB-MW012i at a depth of 21-24 ft bgs. This sediment was opened in the glovebox and homogenized before use. Groundwater used in this experiment was composited from wells R-MW-14i, R-MW-12s, and R-MW-12i. In the glovebox. 50 g of sediment and 70 mL of groundwater were added to each microcosm in the glovebox. The bottles were capped and removed from the glovebox. The headspace of each microcosm was sparged with N_2 for 1 minute. Without allowing any introduction of air, the slotted grey butyl rubber septum was replaced with Teflon-faced septum inside the aluminum crimp cap and crimped closed. 100 μL of TCE saturated water (~1.1 mg/mL) was added to each bottle to bring the initial concentration of TCE to ~1,000 μg/L. The bottles were then inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. Time-zero measurements of TCE and other VOCs were taken using the GC-FID. PlumeStop activated carbon was then injected into the septum of the bottles at the designated doses. The bottles were incubated on the shaker table for approximately 10 minutes before being moved to a stationary and dark incubation and were kept inverted. The microcosms with varying doses of PlumeStop are shown in **Figure A.10.** The amount of TCE and other VOCs were determined by injecting 0.5 mL of headspace samples into the GC-FID. Using the response factors

described in **Appendix A.21**, the PAUs were recorded and converted to concentrations of TCE and other VOCs.

Figure A.10. Preliminary Regenesis PlumeStop microcosms containing different doses of PlumeStop activated carbon. The microcosms have 50 g sediment, 70 mL groundwater, and 100, 50, 25, and 6 uL PlumeStop.

A few days into this experiment, it was clear that the initial doses of PlumeStop were not enough to lower the TCE concentration below the MCL. Regenesis provided updated Freundlich adsorption isotherm constants to recalculate an adequate dose. The new adsorption capacity at unit concentration (Kf) for TCE is 65.0 $(mg/g)/(mg/L)^{1/n}$, the strength of adsorption (1/n) is 0.55, and the goal for the equilibrium concentration of TCE in the water (Ce) is 0.005 (mg TCE/L). The newly calculated dose of PlumeStop was 150 µL of product per bottle. On day 6, the constructed microcosms were injected with another addition of PlumeStop, so each bottle contained 150 µL of PlumeStop.

The amount of TCE present in each microcosm was plotted over time in **Figure A.11**. The time zero-measurement was taken from the microcosms before the

introduction of PlumeStop. The PlumeStop adsorbed the TCE very quickly with very little production of by-products. These results indicated that the updated Freundlich adsorption isotherm constants provided by Regenesis were suitable to calculate an adequate dose for use in these microcosms. The dose selected for use in the Lane Street Superfund Site microcosm study was 150 µL per bottle. The microcosms with 150 µL of PlumeStop adsorbed and lowered the amount of TCE in the aqueous phase below the MCL within a few days. An additional dose of PlumeStop was added to the microcosms on day 6, as indicated by the black arrow shown in the figure.

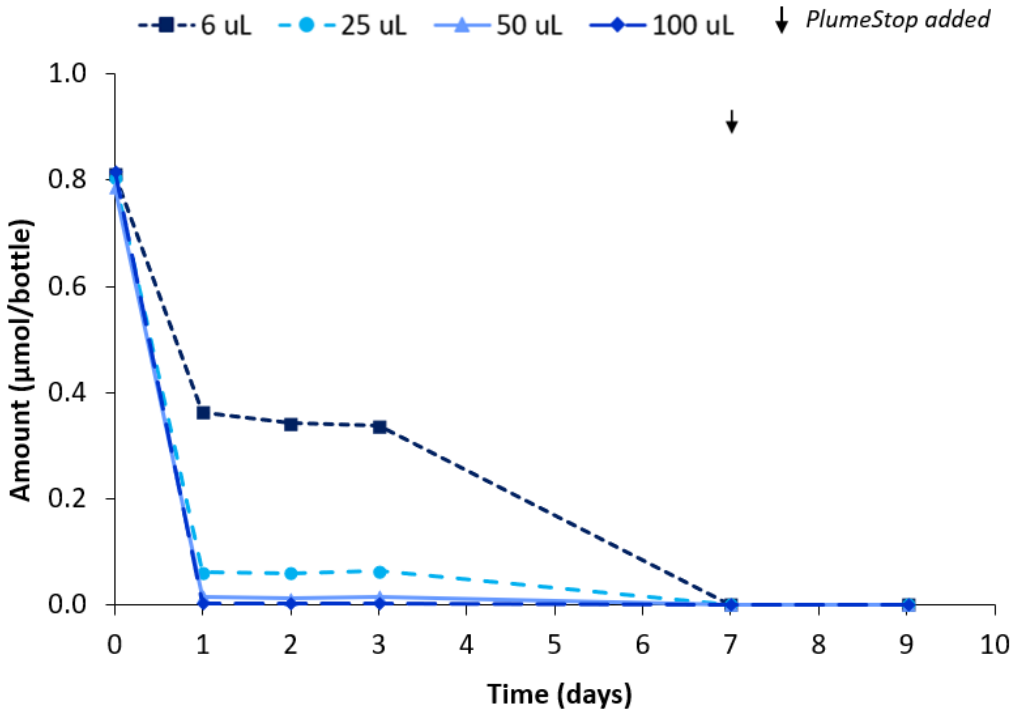

Figure A.11. The concentration of TCE in microcosms containing initial doses of 6, 25, 50, and 100 µL of PlumeStop per bottle. An additional dose of PlumeStop was added on day 6 so each microcosm contained 150 µL of PlumeStop. This addition is indicated by the black arrow.

A.12. Step-by-Step Protocol for Initial Microcosms

The purpose of this microcosm study is to evaluate various treatments that may be employed to remediate the Lane Street Superfund Site.

Preliminary: Bottle Preparation

1. Clean and sterilize 31 (27 + 4 extra) 160 mL serum bottles, along with their respective Teflon-faced septa (SUN-SRI, 20 mm Pharma 0.130 Butyl, 14234-854) and 150 gray butyl rubber slotted septa (VWR, 89426-904).
2. Label all bottles according to the following treatments in **Table A.4**.
3. Move the bottles to the glove box; keep the foil on.

Triplicates of the treatments in **Table A.4** will be prepared.

Table A.4. Treatments to prepare for the microcosm study.

Treatment Identifier	Solids (g)	Groundwater (mL)	Lactate Stock (mL)	EVO Stock (mL)	KB-1 (mL)	ZVI (g)	TCE Saturated Water (µL)
UN	50	70	0	0	0	0	100.00
UN-DNA	50	70	0	0	0	0	0
Lac	50	70	0.50	0	0	0	100.00
Lac + KB-1	50	70	0.50	0	0.70	0	100.00
EVO	50	70	0	0.50	0	0	100.00
EVO + KB-1	50	70	0	0.50	0.70	0	100.00
ZVI	50	70	0	0	0	0.16	100.00
ZVI + EVO	50	70	0	0.50	0	0.16	100.00
AC	50	70	0	0	0	0	100.00
WC	0	0	0	0	0	0	90.00

Preliminary: Soil Preparation

4. Clean a Tupperware container large enough to contain all of the aquifer material sample. Do this by wiping the container with ethanol and placing it in the 103 °C oven for ~30 minutes to evaporate the ethanol. The top should be covered with aluminum foil that is also wiped with ethanol.
5. Transfer the solids into the container and homogenize it with a sterile spoon. Transfer ~400g to a beaker; this subsample will be used to determine the percent moisture and the volume of water displaced by 50 g.
    a. To measure the dry weight of the solids, determine the weight of an aluminum dish. Add ~50 g of wet soil. Reweigh. Place in a 103 °C oven

overnight. Cool in a desiccator. Reweigh. Calculate the percent moisture. Do this with triplicate samples.

b. To measure the volume displaced by the solids, fill a 100 mL graduate cylinder with ~50 mL of water; record the exact volume of water. Weigh the cylinder with the water. Add ~50 g of wet soil. Record the new volume and weight. Calculate the density of the wet soil and the dry soil (knowing the percent moisture). Do this with triplicate samples.

Preliminary: Groundwater

6. Transfer 3 L of groundwater into the glovebox. Allow it to warm and deoxygenate overnight.

Microcosms: Inside the Glove Box

7. Record the weight of each empty serum bottle. Add the designated amount of aquifer solids to all of the serum bottles (50 ± 1 g) and reweigh each to determine the exact amount of soil added. Cap these bottles with grey butyl rubber septa and set aside. It is not necessary to seal them with aluminum crimp caps.
8. For the treatments with ZVI, add 0.16 g per bottle.
9. Using a sterile graduated cylinder, add 70 mL of groundwater. Again, record the weights to determine the exact amount of groundwater. Cap these bottles with grey butyl rubber septa and make sure the stopper is firmly inserted.

Microcosms: Outside the Glove Box

10. Remove the serum bottles from the glovebox.
11. For all the treatments except the autoclaved controls, sparge the headspace with N_2 for 1 minute. Without allowing any introduction of air, replace the slotted grey butyl rubber septum with the Teflon-faced septum inside the aluminum crimp cap; crimp closed.
12. Prepare triplicate water controls containing 70 mL DDI water and glass beads that displace the same volume as the soil, these will be prepared on the bench top.
13. Crimp the 5 autoclave bottles closed with the slotted septa and autoclave them for 1 hour on the long cycle for three consecutive days. Once that is accomplished, replace the septa with sterile Teflon-faced septa while sparging with filter sterilized N_2. The tubing and canula after the filter also need to be sterile. 5 bottles will be prepared in case of breakage.

Microcosms: Adding TCE and Amendments

14. Considering the concentration of TCE in groundwater in the microcosms after sparging (based on headspace samples from UN-DNA bottles), add 100 µL of TCE-saturated water to raise the initial aqueous phase TCE concentration to ~1,000 µg/L, taking into account Henry's Law constant at 23 °C (presumed room temperature). TCE saturated water is ~1.1 µg/mL. Add the TCE to all of the bottles.
15. Prepare a stock solution of sodium lactate so that adding 50 µL will result in 10X more eeq than is needed to reduce the TCE to ethene.
16. Prepare a stock solution of EVO so that adding 50 µL will result in 10X more eeq than is needed to reduce the TCE to ethene.
17. Add a stock solution of resazurin (1 g/L) to achieve 1 mg/L in the serum bottles.
18. Incubate on the shaker table for ~1 hour and then measure the time zero VOCs on the GC/FID. Approximately twice per month, remove one microcosm at a time and measure the VOCs in a headspace sample. Immediately return the bottles to the incubator. The incubation period will depend on if enough data has been collected to measure rate constants.
19. When the resazurin turns clear in the bottles to be bioaugmented, add 0.70 mL of 10^{11} cfu/L KB-1 culture, with a goal to achieve 10^{6} cells *Dhc* per mL.
20. VOCs in the headspace will be measured until concentrations of VC, cDCE, and TCE are below their respective maximum contaminant levels.

A.13. Step-by-Step Protocol for Regenesis/Hepure Microcosms

The purpose of these microcosms is to evaluate various treatments from Hepure and Regenesis that may be employed to remediate the Lane Street Superfund Site.

Preliminary: Bottle preparation

1. Clean and sterilize 12 160 mL serum bottles, along with their respective Teflon-faced septa (SUN-SRI, 20 mm Pharma 0.130 Butyl, 14234-854) and 150 gray butyl rubber slotted septa (VWR, 89426-904).
2. Label all bottles according to the following treatments in **Table A.5**.
3. Move the bottles to the glove box; keep the foil on.

Triplicates of the treatments in **Table A.5** will be prepared.

Table A.5. Treatments to prepare for the microcosm study.

Treatment Identifier	Solids (g)	Groundwater (mL)	Hepure ZVI (g)	Regenesis S-mZVI (µL)	Regenesis PlumeStop (µL)	ZVI (g)	TCE Saturated Water (µL)
HP	50	70	0.383	0	0	0	100.00
PS	50	70	0	0	150	0	100.00
SM-1	50	70	0	127	0	0	100.00
SM-2	50	70	0	304	0	0	100.00
WC	0	0	0	0	0	0	100.00

Preliminary: Soil preparation

4. Use the same sediment prepared in the initial microcosm study. This sediment was prepared following these steps:
    a. Clean a Tupperware container large enough to contain all of the aquifer material sample. Do this by wiping the container with ethanol and placing it in the 103 °C oven for ~30 minutes to evaporate the ethanol. The top should be covered with aluminum foil that is also wiped with ethanol.
    b. Transfer the solids into the container and homogenize it with a sterile spoon.

Preliminary: Groundwater

5. Composite equal amounts of groundwater from wells R-MW-14i, R-MW-12i, and R-MW-12s.
6. Transfer 1 L of GW into the glovebox. Allow it to warm and deoxygenate overnight.

Microcosms: Inside the Glove Box

7. Record the weight of each empty serum bottle. Add the designated amount of aquifer solids to all of the serum bottles (50 ± 1 g), and reweigh each to determine the exact amount of soil added. Cap these bottles with grey butyl rubber septa and set aside. It is not necessary to seal them with aluminum crimp caps.
8. For the treatments with Ferox Flow ZVI powder, add the designated dose to each bottle.
9. Using a graduated cylinder, add 70 mL of GW; the cylinder should be sterile. Again, record the weights to determine the exact amount of GW added.
10. For the treatments with S-Micro ZVI, inject the designated dose to each bottle.

11. Cap these bottles with grey butyl rubber septa. No need to crimp them closed, but be certain that the stopper is firmly inserted.

Microcosms: Outside the Glove Box

12. Remove the serum bottles from the glove box.
13. Sparge the headspace with N_2 for 1 minute. Without allowing any introduction of air, replace the slotted grey butyl rubber septum with the Teflon-faced septum inside the aluminum crimp cap; crimp closed.
14. Prepare triplicate water controls containing 70 mL DDI water and glass beads that displace the same volume as the soil, these will be prepared on the bench top.

Microcosms: Adding TCE and Amendments

15. Add 100 µL of TCE-saturated water to raise the initial aqueous phase TCE concentration to ~1,000 µg/L, taking into account Henry's Law constant at 23 °C (presumed room temperature). TCE saturated water is ~1.1 µg/mL. Add the TCE to all of the bottles.
16. Incubate on the shaker table for ~1 hour and then measure the time zero VOCs on the GC/FID.
17. Add the designated amounts of PlumeStop to all of the bottles.
18. Throughout the duration of the experiment, remove one microcosm at a time and measure the VOCs in a headspace sample. Immediately return the bottles to the incubator. The incubation period will depend on if enough data have been collected to measure rate constants.

A.14. Complete VOC Results in Microcosms

Initial Lane Street Microcosms

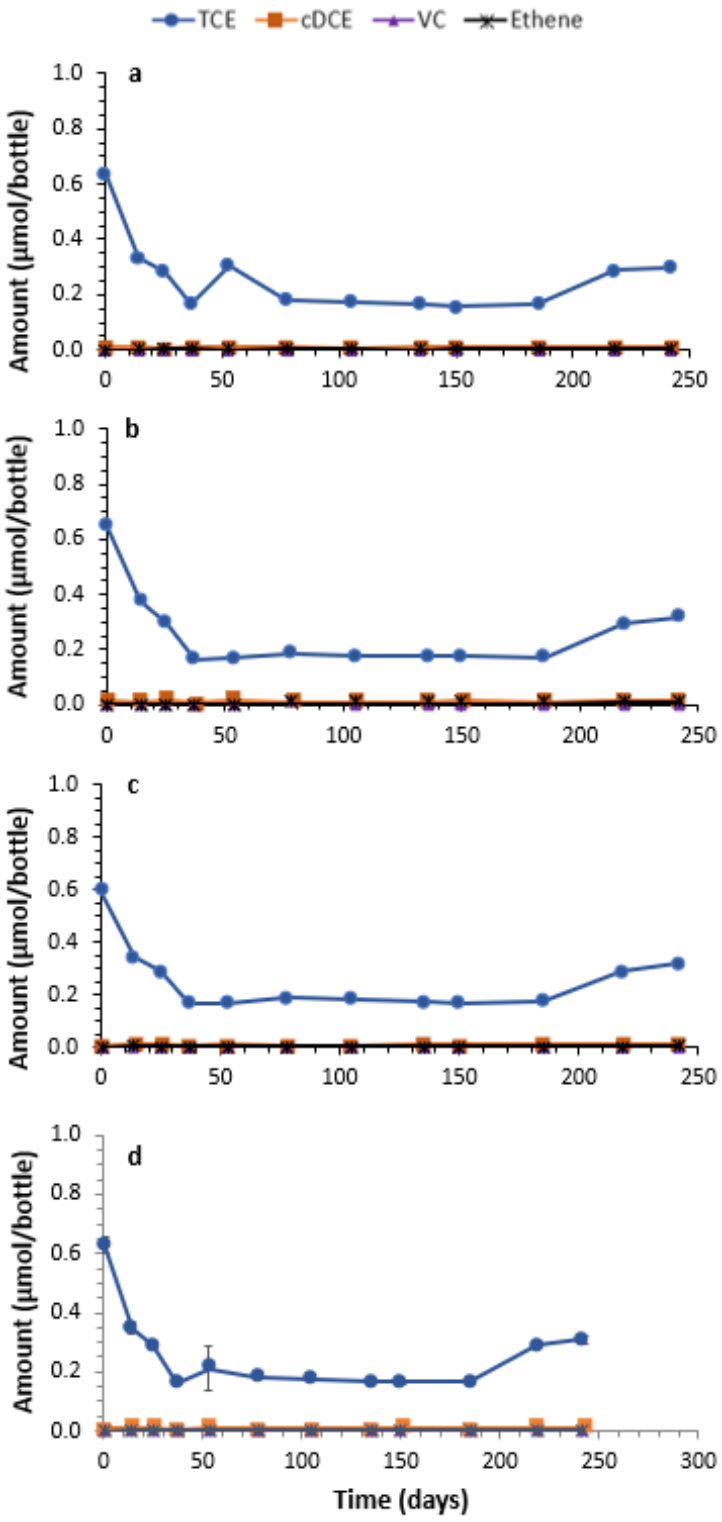

Figure A.12. a-c) VOC measurements for the individual unamended microcosms; d) average VOC measurements for the unamended microcosms.

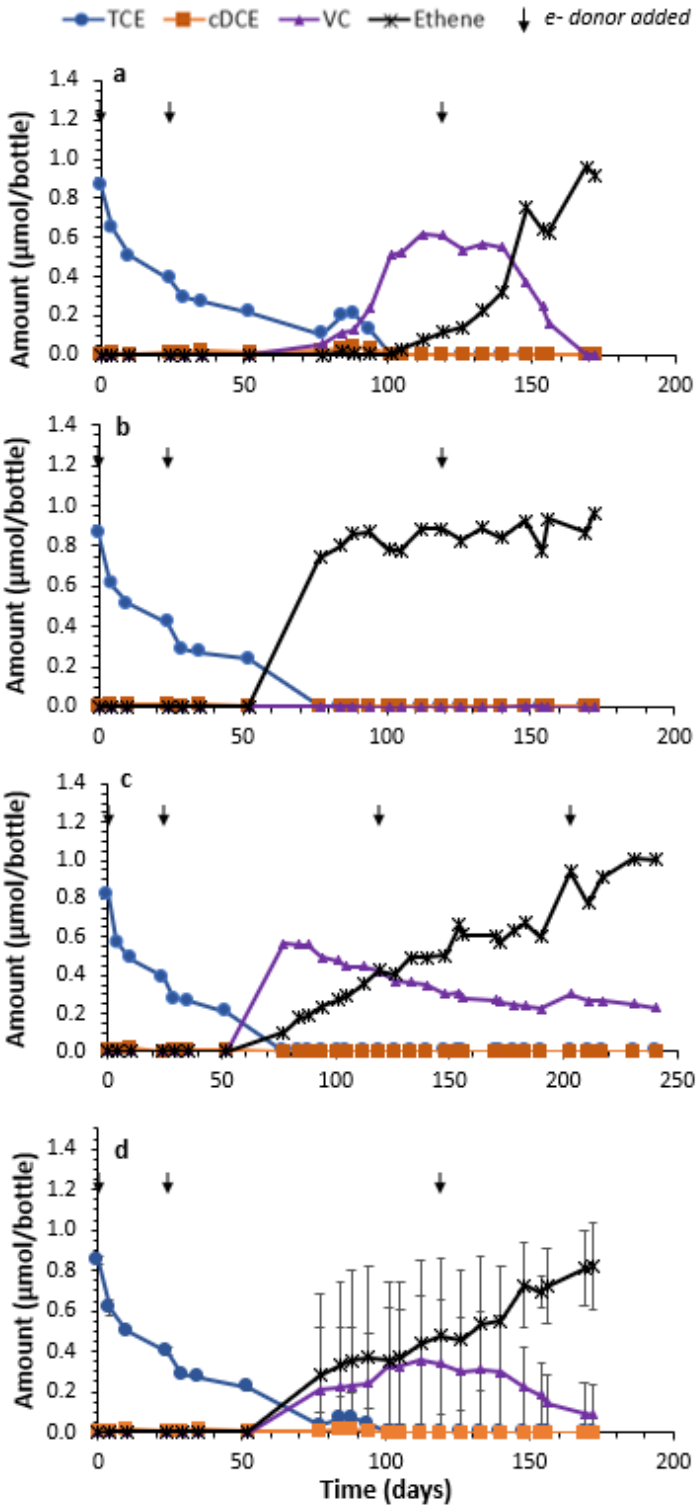

Figure A.13. a-c) VOC measurements for the individual lactate amended microcosms; d) average VOC measurements for the lactate amended microcosms.

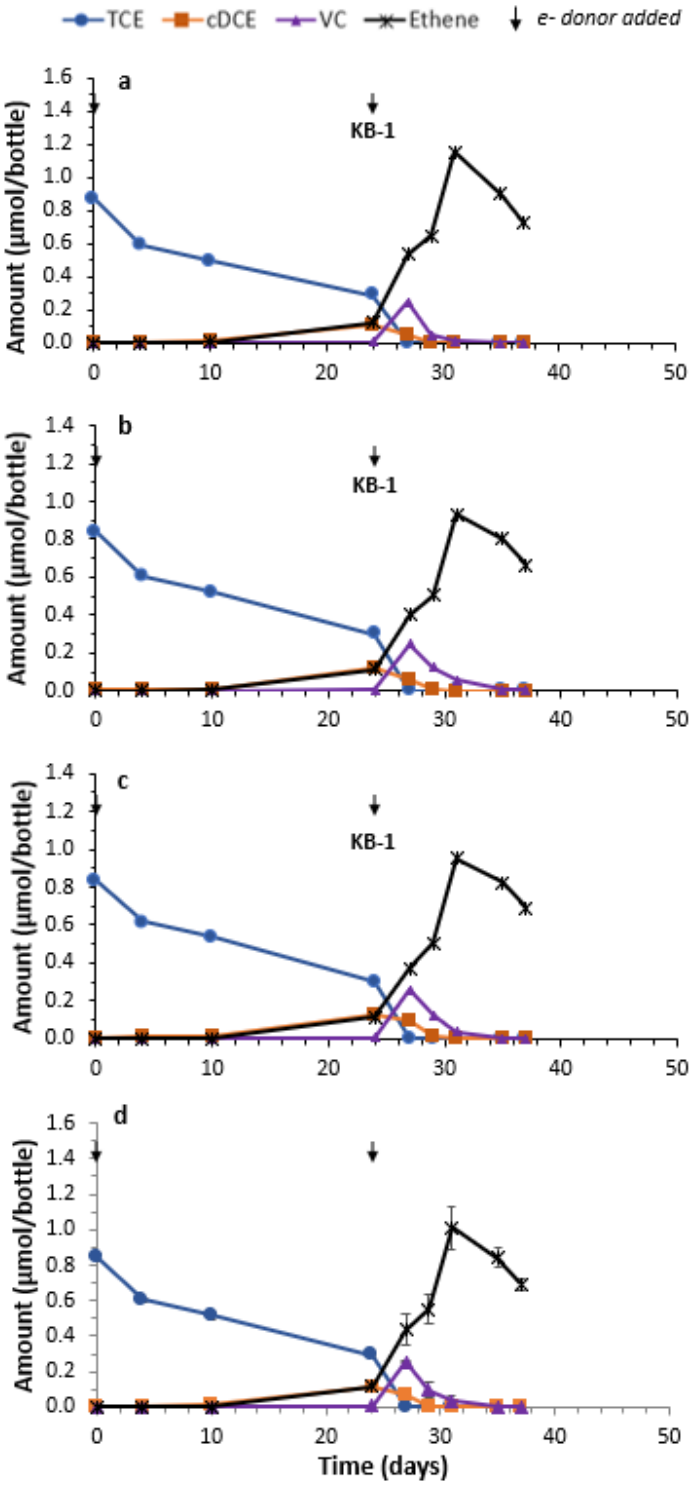

Figure A.14. a-c) VOC measurements for the individual lactate and KB-1 amended microcosms; d) average VOC measurements for the lactate and KB-1 amended microcosms.

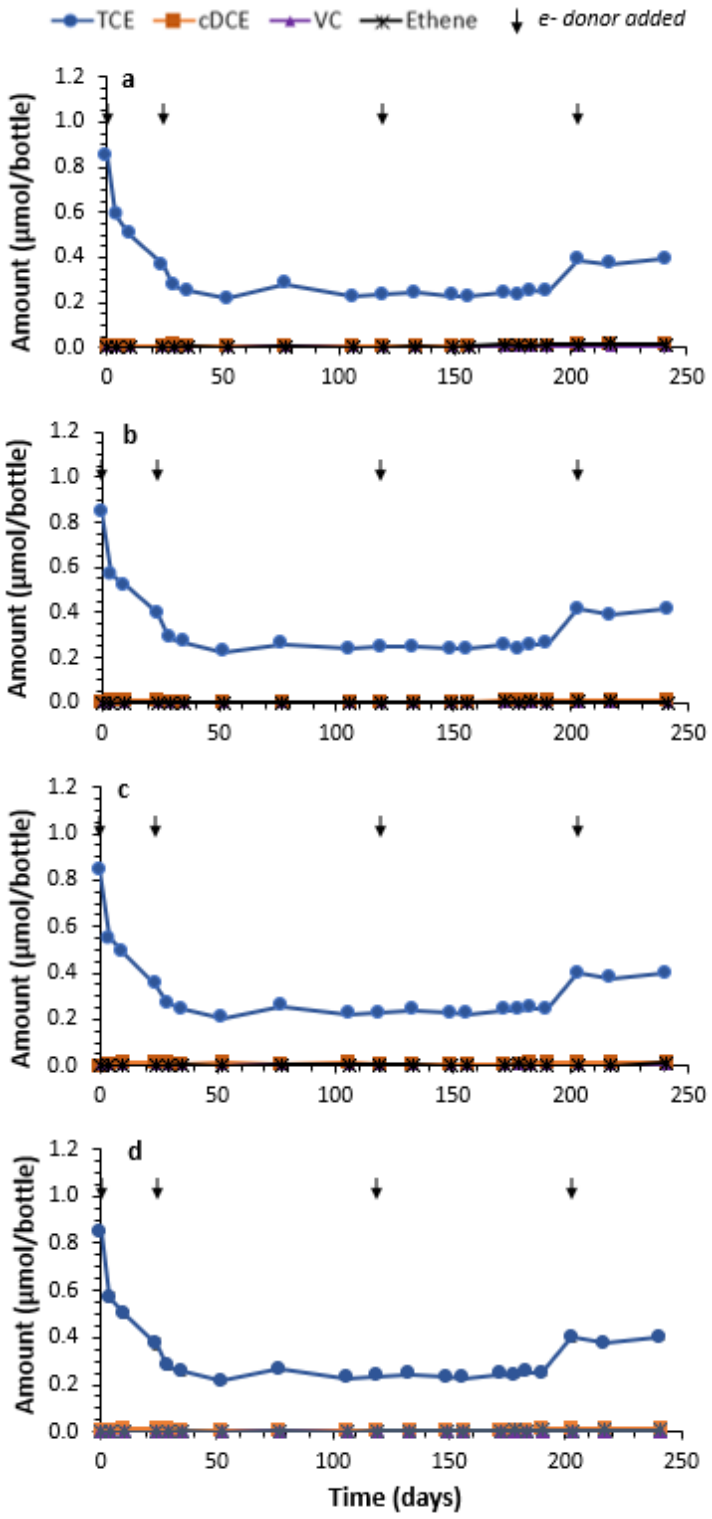

Figure A.15. a-c) VOC measurements for the individual EVO amended microcosms; d) average VOC measurements for the EVO amended microcosms.

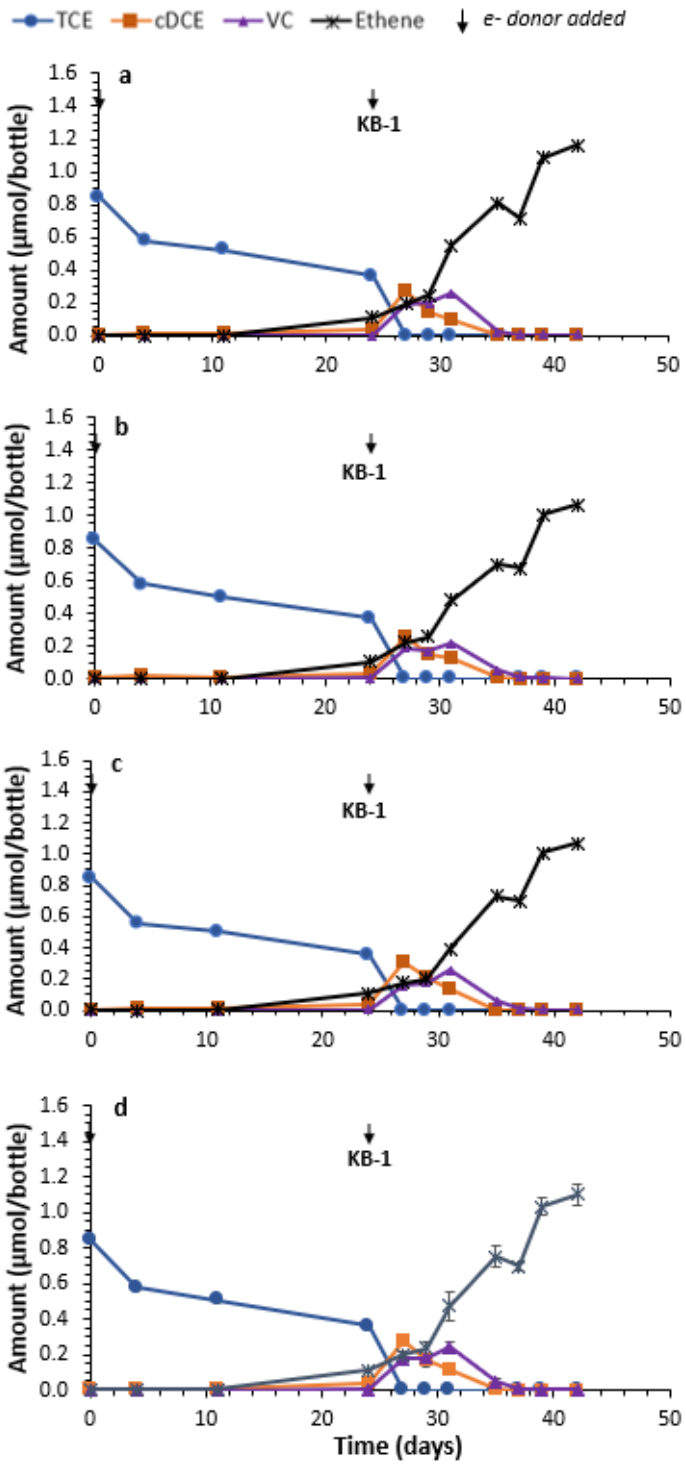

Figure A.16. a-c) VOC measurements for the individual EVO and KB-1 amended microcosms; d) average VOC measurements for the EVO and KB-1 amended microcosms.

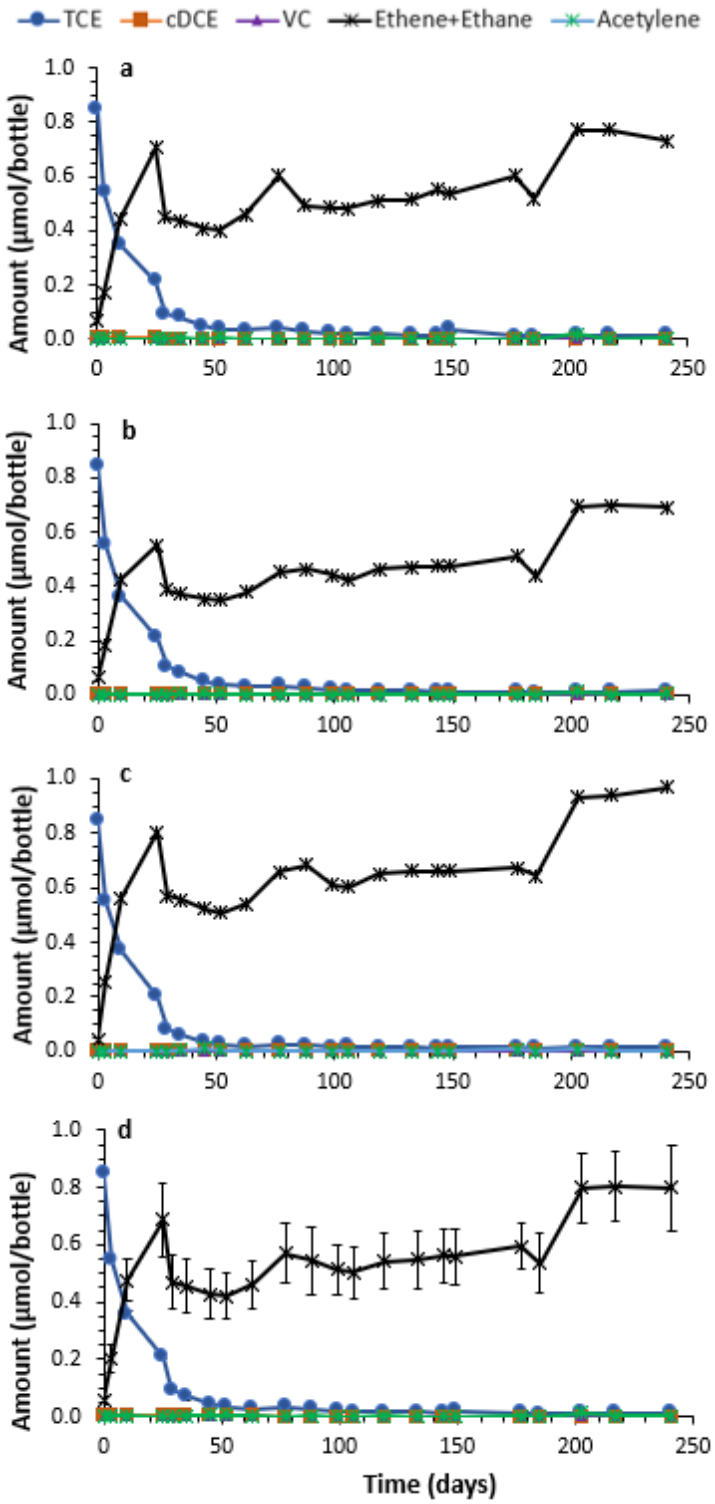

Figure A.17. a-c) VOC measurements for the individual Hepure ZVI amended microcosms; d) average VOC measurements for the Hepure ZVI amended microcosms.

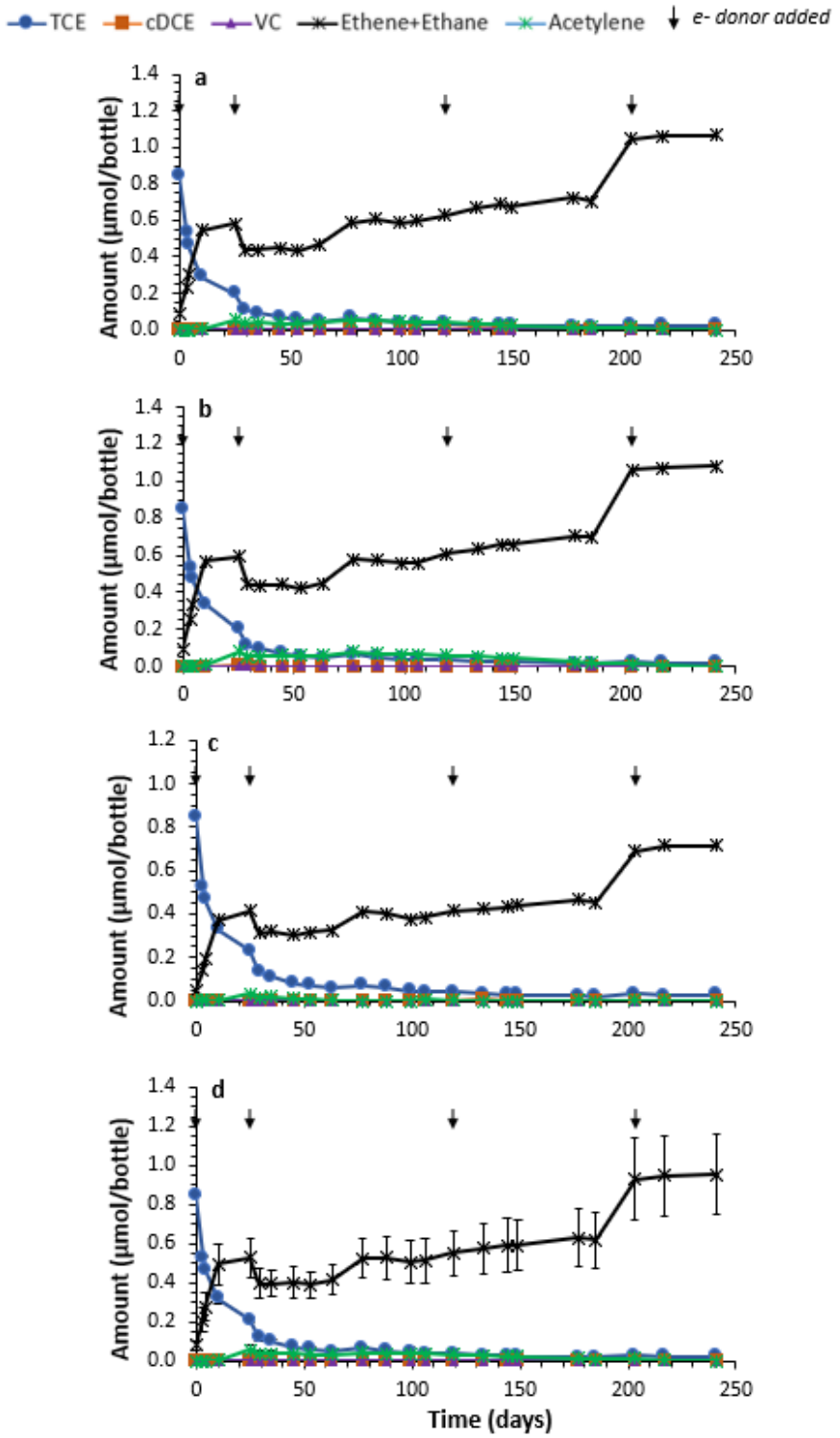

Figure A.18. a-c) VOC measurements for the individual Hepure ZVI and EVO amended microcosms; d) average VOC measurements for the Hepure ZVI and EVO amended microcosms.

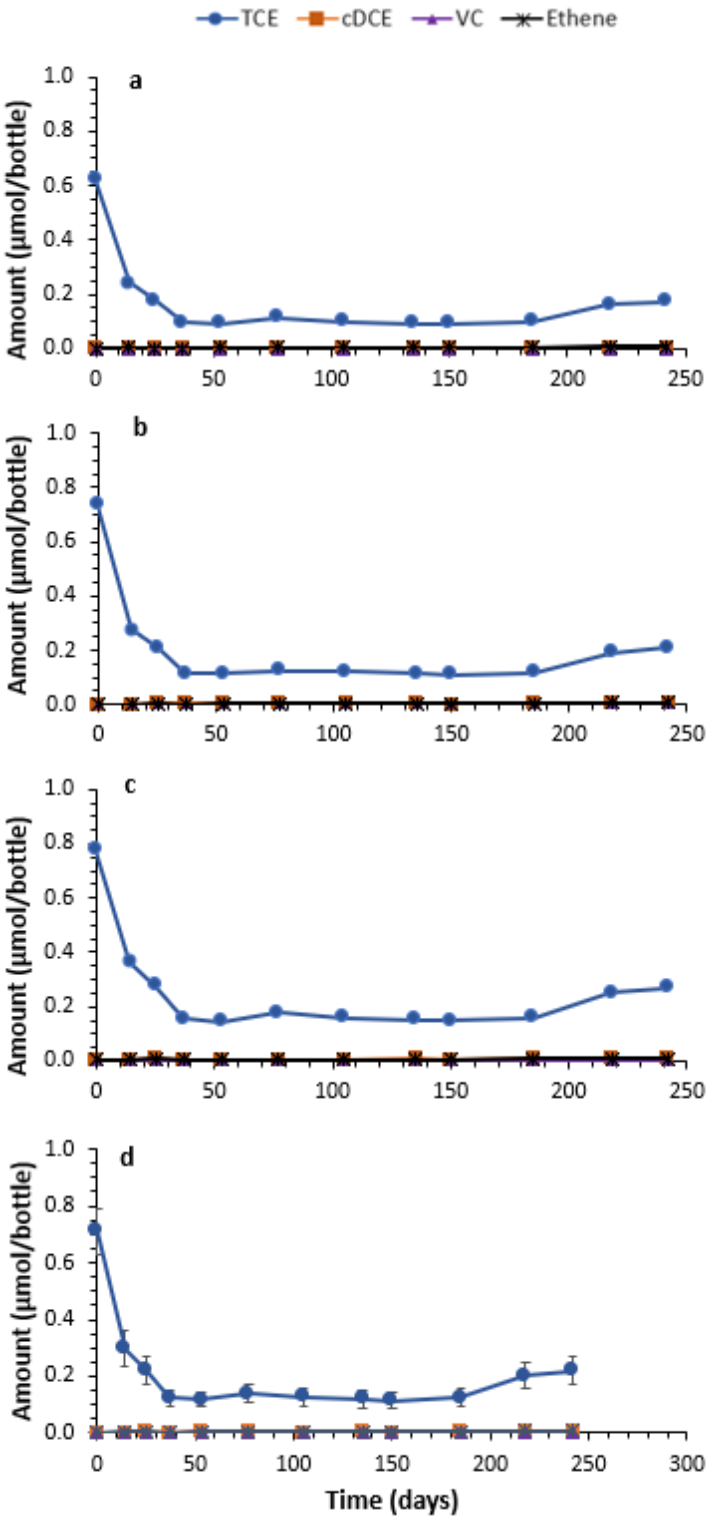

Figure A.19. a-c) VOC measurements for the individual autoclaved control microcosms; d) average VOC measurements for the autoclaved control microcosms.

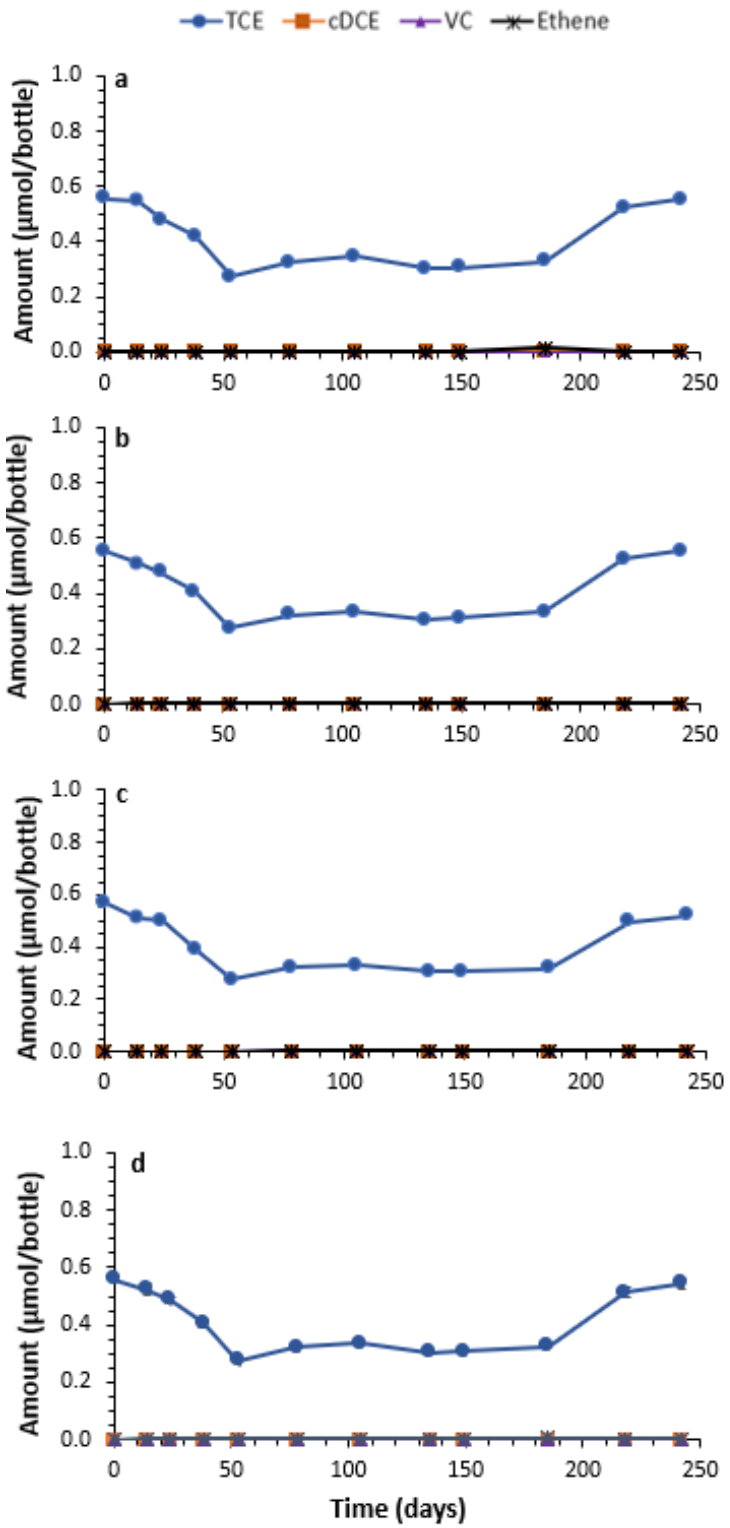

Figure A.20. a-c) VOC measurements for the individual water control microcosms; d) average VOC measurements for the water control microcosms.

Additional Abiotic Lane Street Microcosms

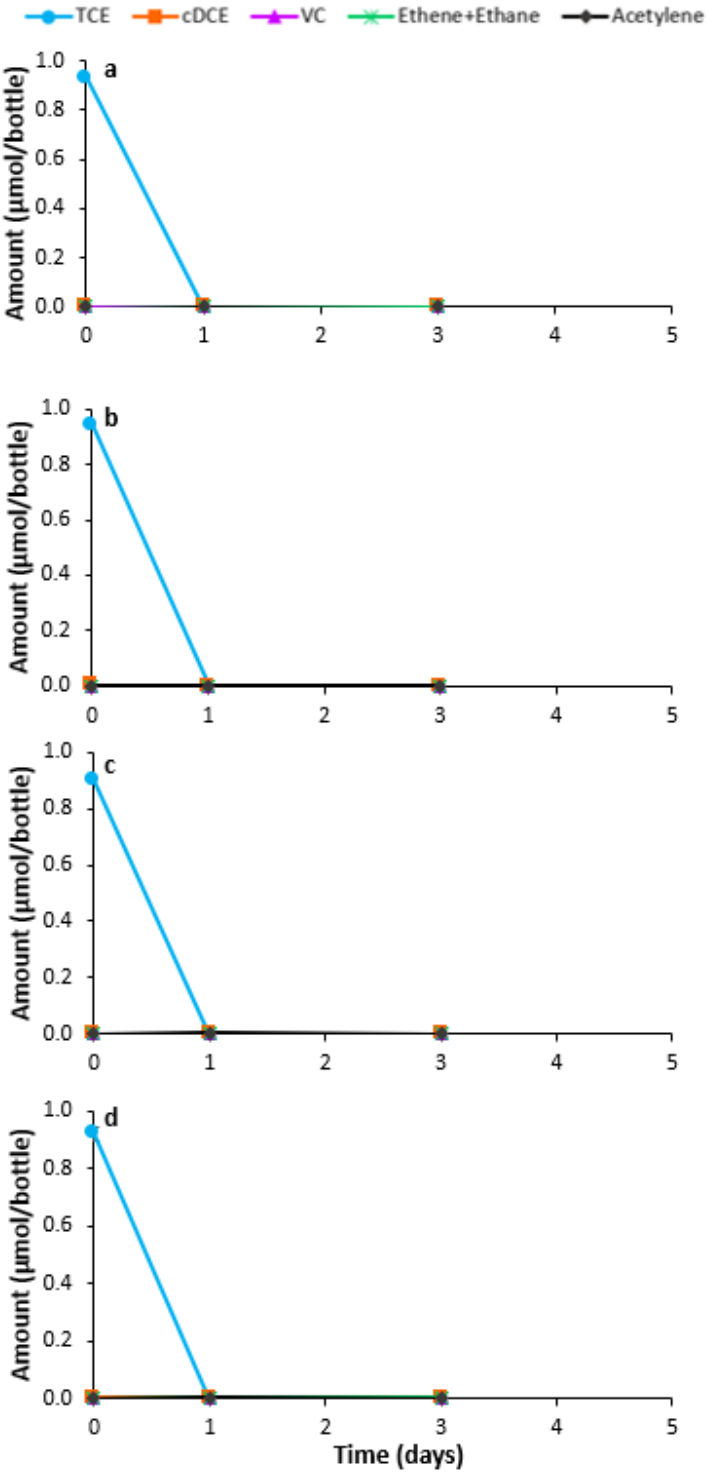

Figure A.21. a-c) VOC measurements for the individual PlumeStop amended microcosms; d) average VOC measurements for the PlumeStop amended microcosms.

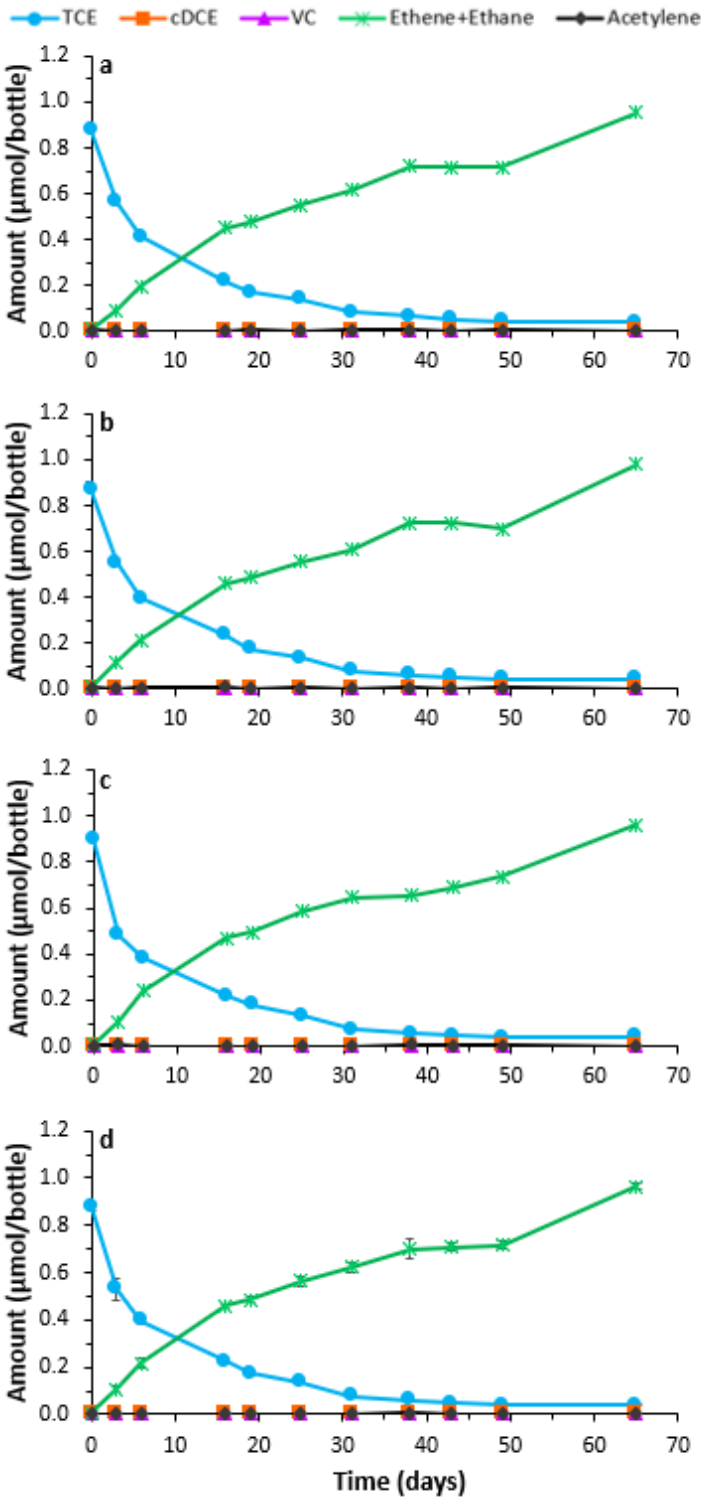

Figure A.22. a-c) VOC measurements for the individual microcosms with 0.38 g Hepure; d) average VOC measurements for the microcosms with 0.38 g Hepure.

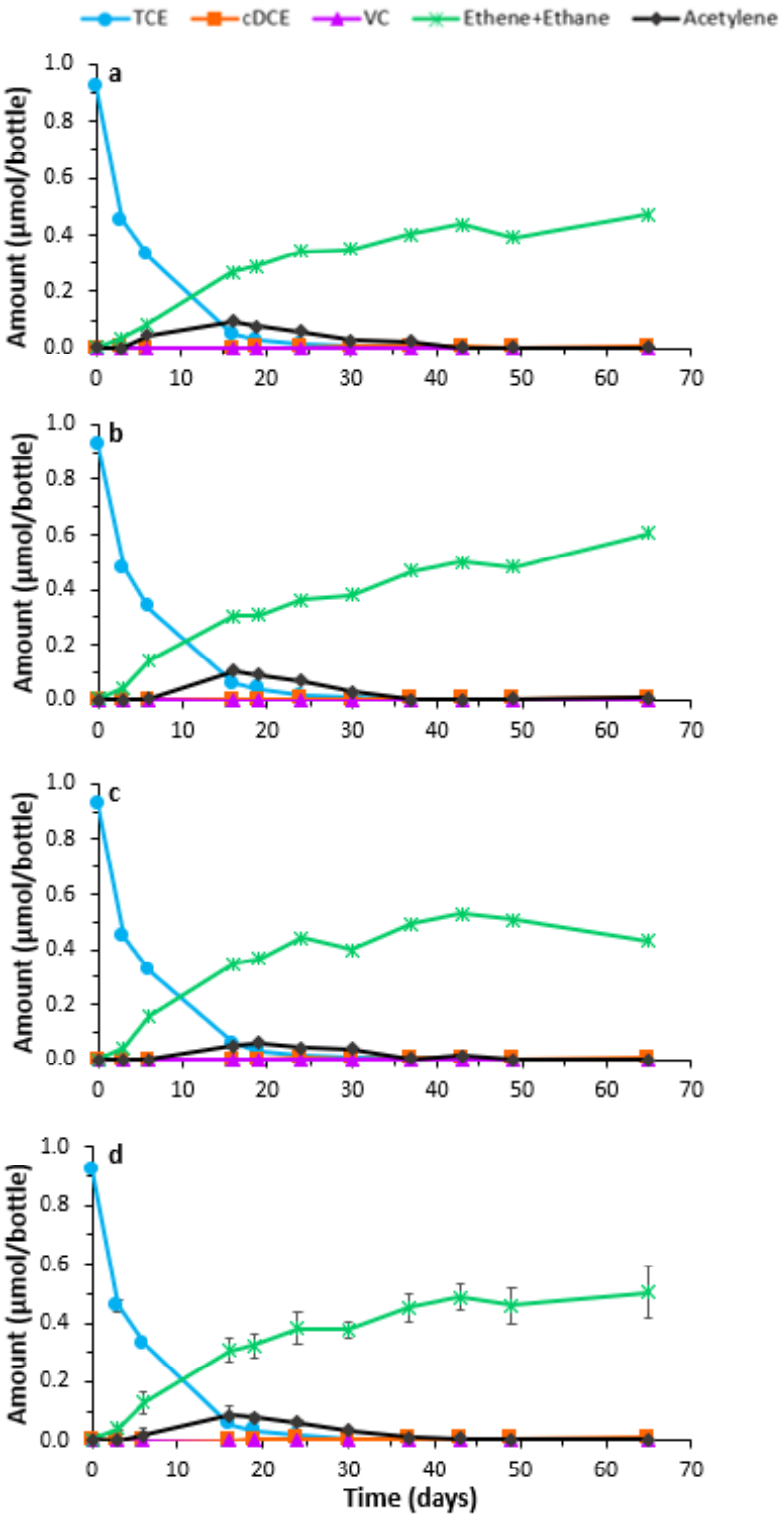

Figure A.23. a-c) VOC measurements for the individual microcosms with 0.16 g SmZVI; d) average VOC measurements for the microcosms with 0.16 g SmZVI.

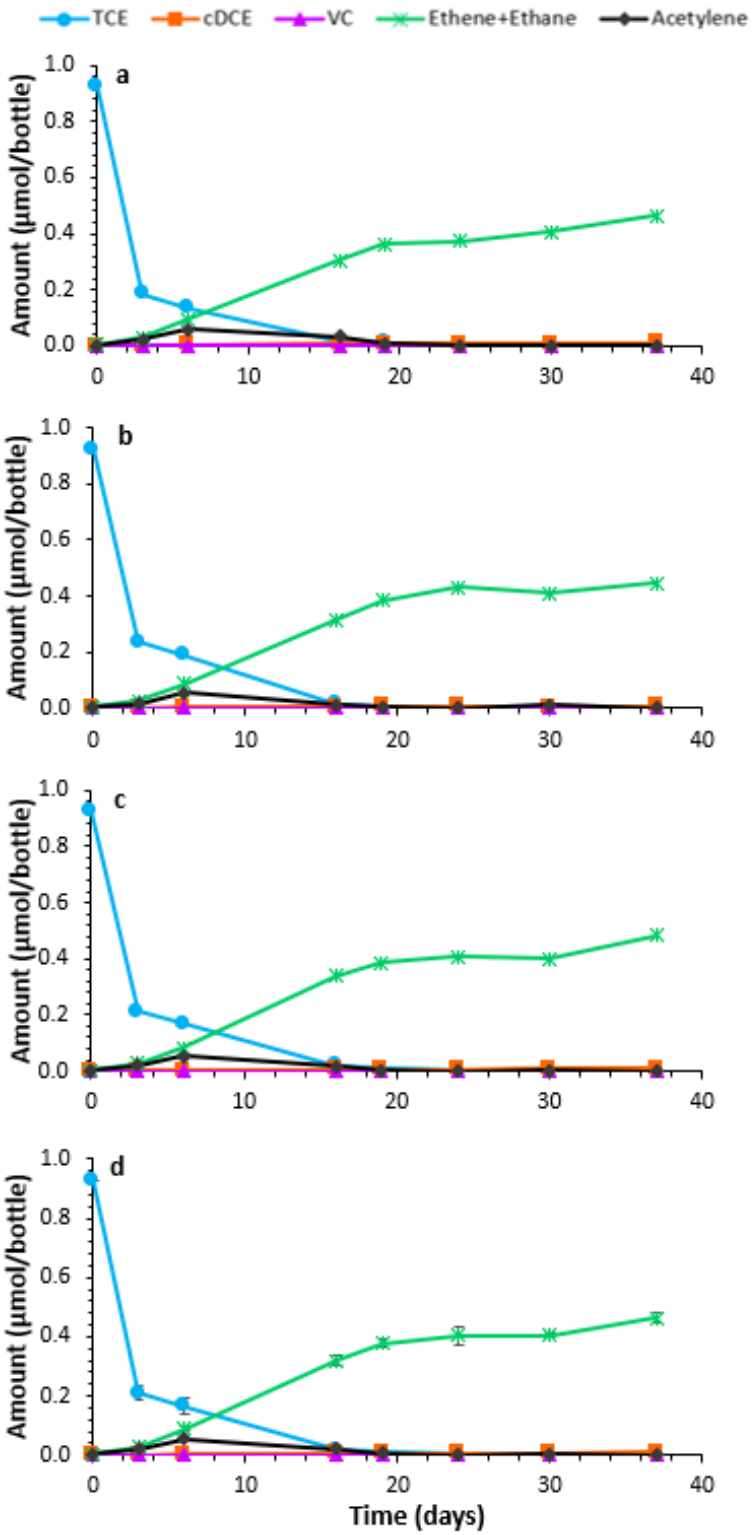

Figure A.24. a-c) VOC measurements for the individual microcosms with 0.38 g SmZVI; d) average VOC measurements for the microcosms with 0.38 g SmZVI.

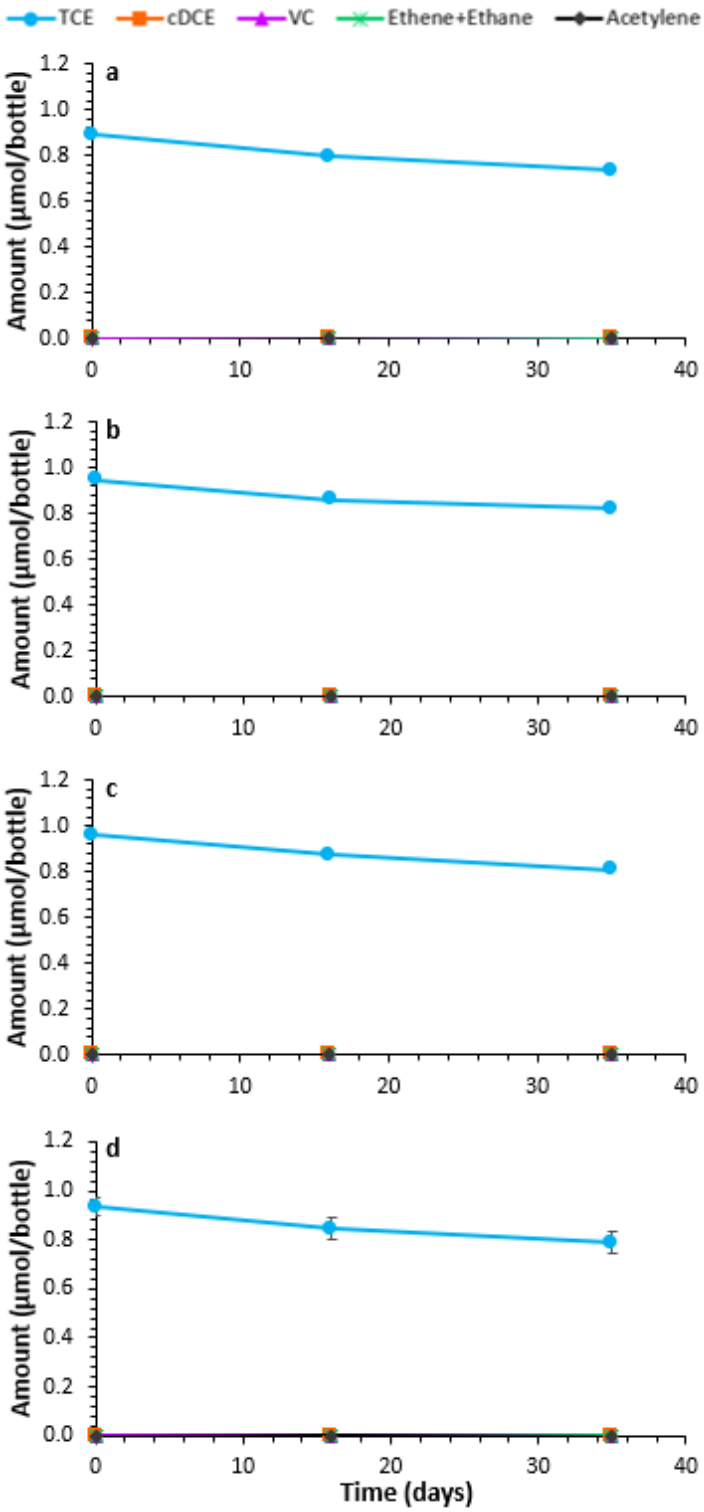

Figure A.25. a-c) VOC measurements for the individual water control microcosms; d) average VOC measurements for the water control microcosms.

A.15. Microcosm pH Measurements

At the end of the incubation period, groundwater pH was measured in one of the triplicate unamended, lactate, EVO, lactate and KB-1, EVO and KB-1, and ZVI and EVO microcosms. Microcosms were moved to the anoxic glovebox where they were allowed to settle before they were opened to remove groundwater samples. Approximately 10 mL of groundwater was removed from each microcosm and transferred to a scintillation vial, without filtering. In the anoxic glovebox, a pH probe was used to measure the pH of each groundwater sample. The results of this experiment are provided in **Table A.6.** *Dhc* require a circumneutral pH, between 6 and 8, for dechlorination and growth. The ideal range for these microbes is between 6.9 and 7.5.[43] An initial analysis of groundwater this Site, described in **Appendix A.4** indicated that the initial pH of the groundwater used in this experiment was approximately 7.2.

Table A.6. Microcosm pH measurements at the end of incubation.

Treatment	Bottle no.	pH
Unamended	2	7.58
Lactate	2	7.25
EVO	2	7.43
Lactate + KB-1	2	7.46
EVO + KB-1	2	8.02
ZVI + EVO	2	8.77

A.16. Complete Electron Mass Balance

Table A.7. Summary of the overall electron mass balance.

	Electron Milli-Equivalents Per Bottle								
Treatment	**Methane**	**Sulfate Reduction**	**Nitrate Reduction**	**Lactate**	**Formate**	**Acetate**	**Propionate**	**TCE Reduction**	**Unaccounted for**
UN-1	0	0	0.013	0	0	0	0	0	0
UN-2	0	0.005	0.013	0	0	0	0	0	0
UN-3	0	0.003	0.013	0	0	0	0	0	0
Lac-1	2.399	0.087	0.013	0.030	0	0.053	1.770	0.005	3.174
Lac-2	3.209	0.087	0.013	0.054	0.006	0	0.060	0.005	4.098
Lac-3	6.243	0.087	0.013	0	0	0.483	0.279	0.004	2.933
Lac + KB1-1	0.040	0.081	0.013	0.034	0.036	1.187	2.220	0.005	1.403
Lac + KB1-2	0.039	0.086	0.012	0.044	0.025	1.562	2.554	0.005	0.694
Lac + KB1-3	0.023	0.086	0.013	0.048	0.037	1.105	2.807	0.005	0.898
EVO-1	1.019	0.087	0.013	0	0	3.107	0	0	6.163
EVO-2	0.513	0.087	0.013	0	0	1.653	0	0	8.123
EVO-3	0.965	0.087	0.013	0	0	2.817	0	0	6.508
EVO + KB1-1	0.084	0.086	0.013	0	0.033	0.479	0.164	0.005	4.329
EVO + KB1-2	0.036	0.087	0.013	0	0.045	0.233	0.228	0.005	4.548
EVO + KB1-3	0.018	0.087	0.013	0	0.033	0.115	0.210	0.005	4.713
ZVI + EVO-1	0.304	0.087	0.013	0	0	0.257	0	0.005	9.724
ZVI + EVO-2	0.309	0.087	0.013	0	0	0.078	0	0.005	9.898
ZVI + EVO-3	0.280	0.087	0.013	0	0	0.315	0.269	0.005	9.421

Table A.8. Summary of the amount of electron donor added to each microcosm and the fraction of available donor that was consumed for methane production

Treatment	Number Doses of Donor	Donor Added (meq/bottle)	Potential Methane (µmol/bottle)	% of meq Used for Methanogenesis
Lac-1	3	7.532	942	32%
Lac-2	3	7.532	942	43%
Lac-3	4	10.043	1255	62%
Lac + KB1-1	2	5.021	628	0.8%
Lac + KB1-2	2	5.021	628	0.8%
Lac + KB1-3	2	5.021	628	0.4%
EVO-1	4	10.390	1299	10%
EVO-2	4	10.390	1299	5%
EVO-3	4	10.390	1299	9%
EVO + KB1-1	2	5.195	649	2%
EVO + KB1-2	2	5.195	649	0.7%
EVO + KB1-3	2	5.195	649	0.4%
ZVI + EVO-1	4	10.390	1299	3%
ZVI + EVO-2	4	10.390	1299	3%
ZVI + EVO-3	4	10.390	1299	3%
0.16 g SmZVI-1	1	12.16	1520	8%
0.16 g SmZVI-2	1	12.16	1520	8%
0.16 g SmZVI-3	1	12.16	1520	8%
0.38 g SmZVI-1	1	28.88	3611	5%
0.38 g SmZVI-2	1	28.88	3611	5%
0.38 g SmZVI-3	1	28.88	3611	6%

A.17. Complete Dechlorination and Degradation Rate Results

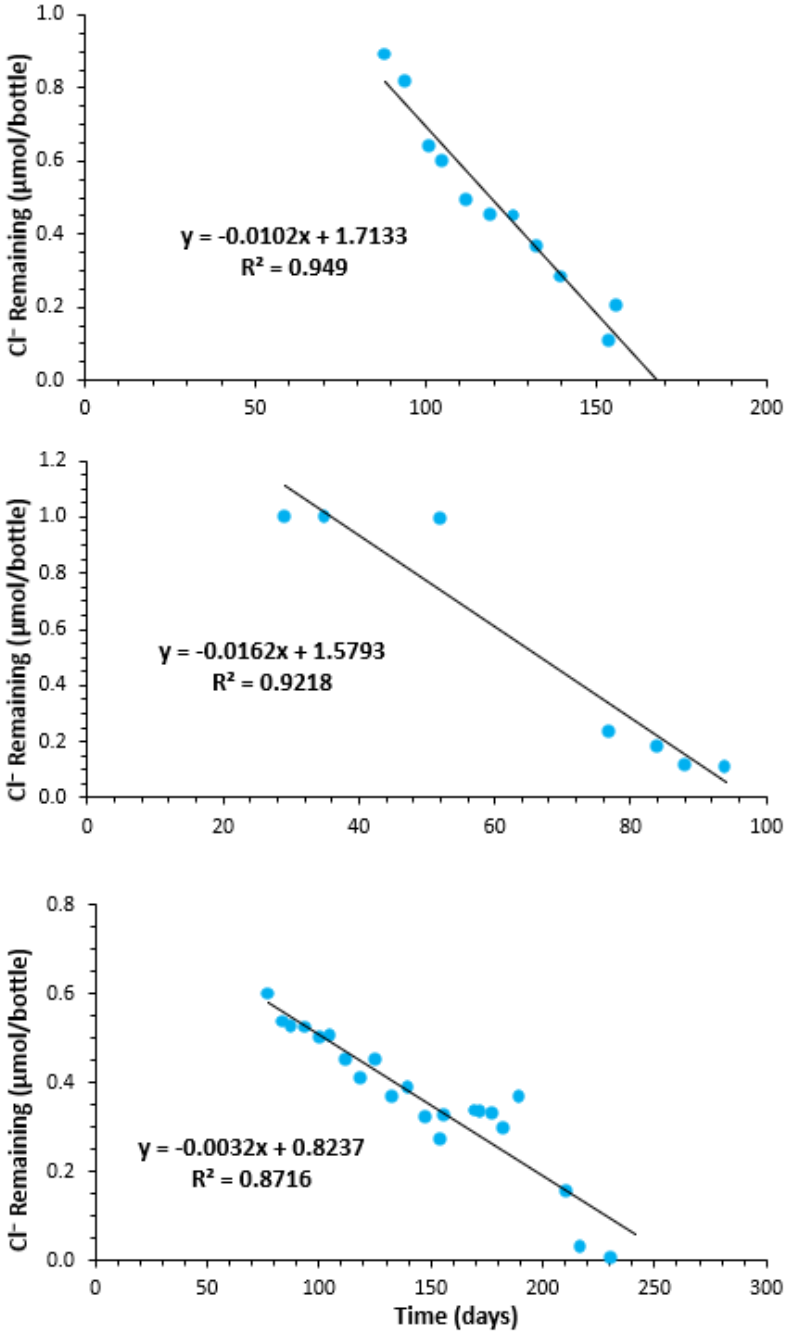

Figure A.26. Dechlorination rates for the triplicate lactate amended microcosms.

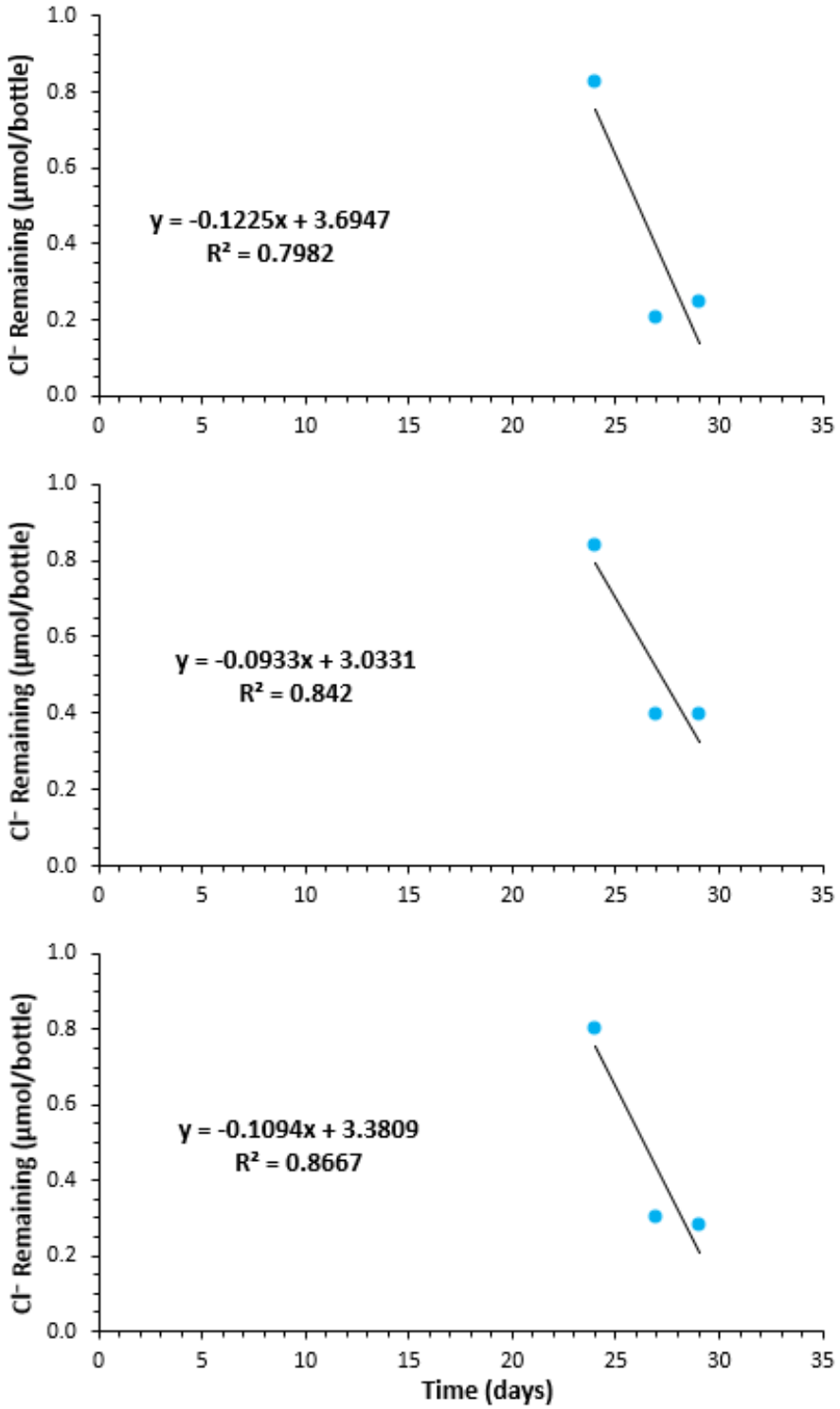

Figure A.27. Dechlorination rates for the triplicate lactate and KB-1 amended microcosms.

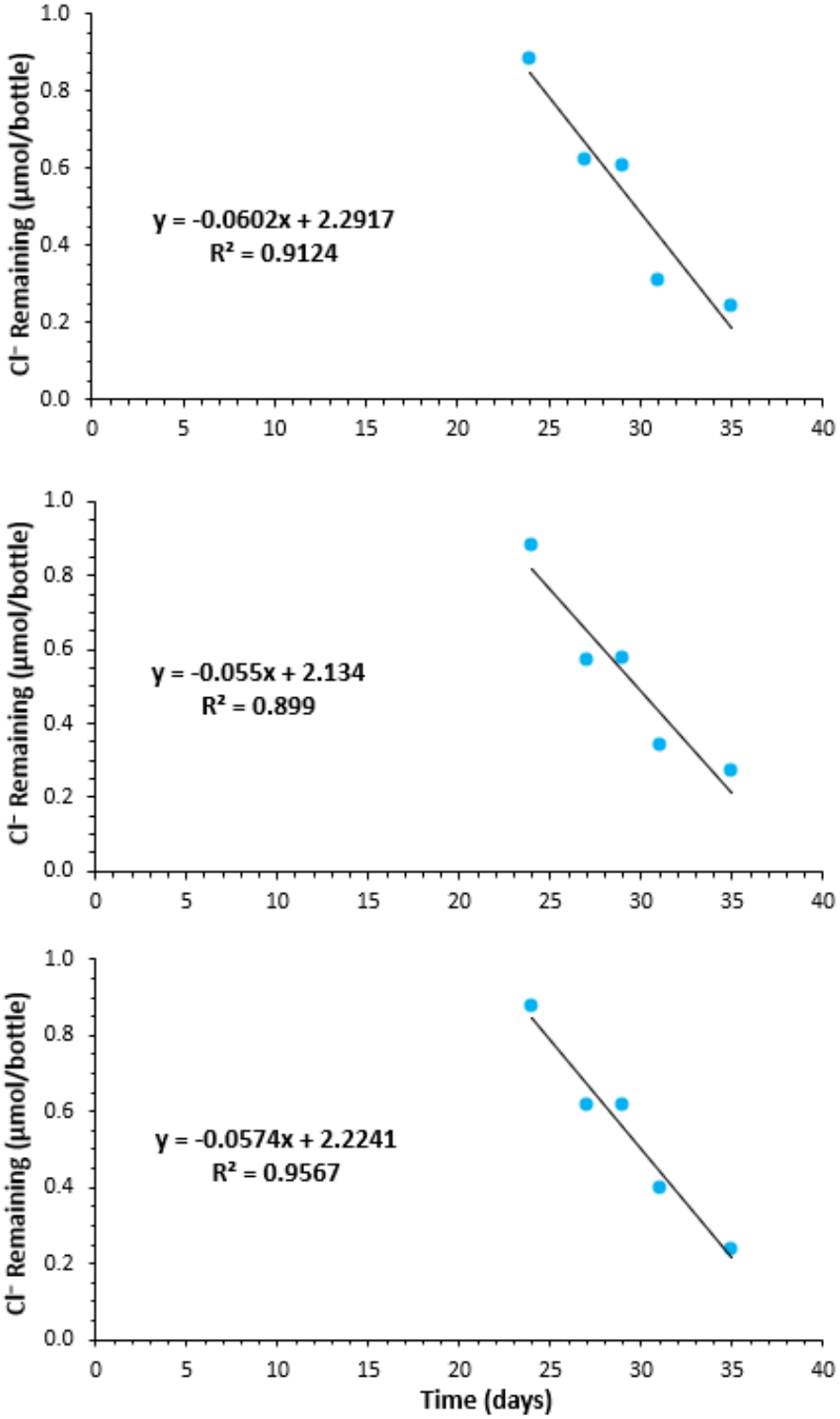

Figure A.28. Dechlorination rates for the triplicate EVO and KB-1 amended microcosms.

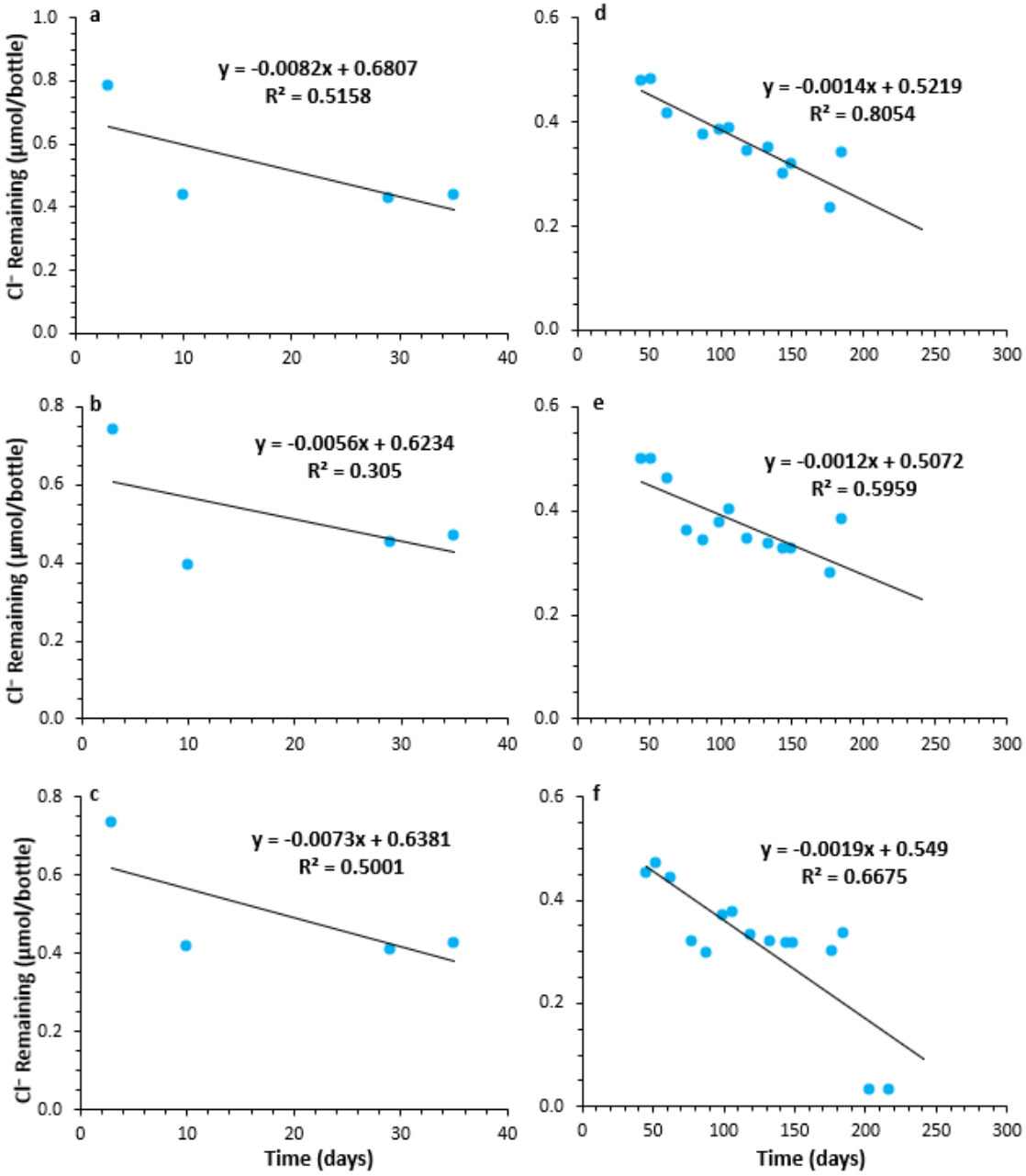

Figure A.29. a-c) Dechlorination rates for the initial decline in the microcosms with 0.16 g Hepure; d-f) dechlorination rates for the tail end of the microcosms with 0.16 g Hepure.

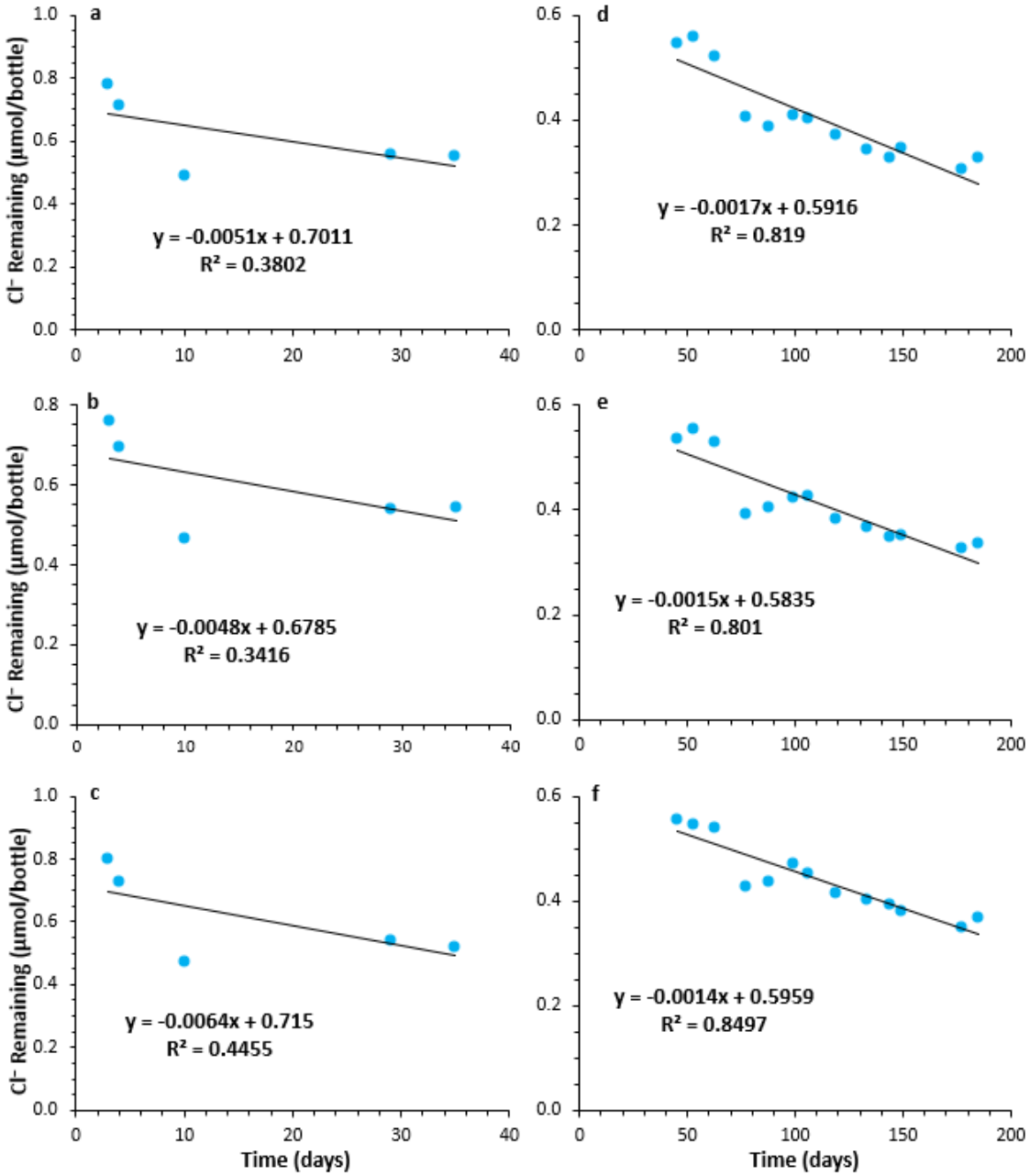

Figure A.30. a-c) Dechlorination rates for the initial decline in the microcosms with 0.16 g Hepure and EVO; d-f) dechlorination rates for the tail end of the microcosms with 0.16 g Hepure and EVO.

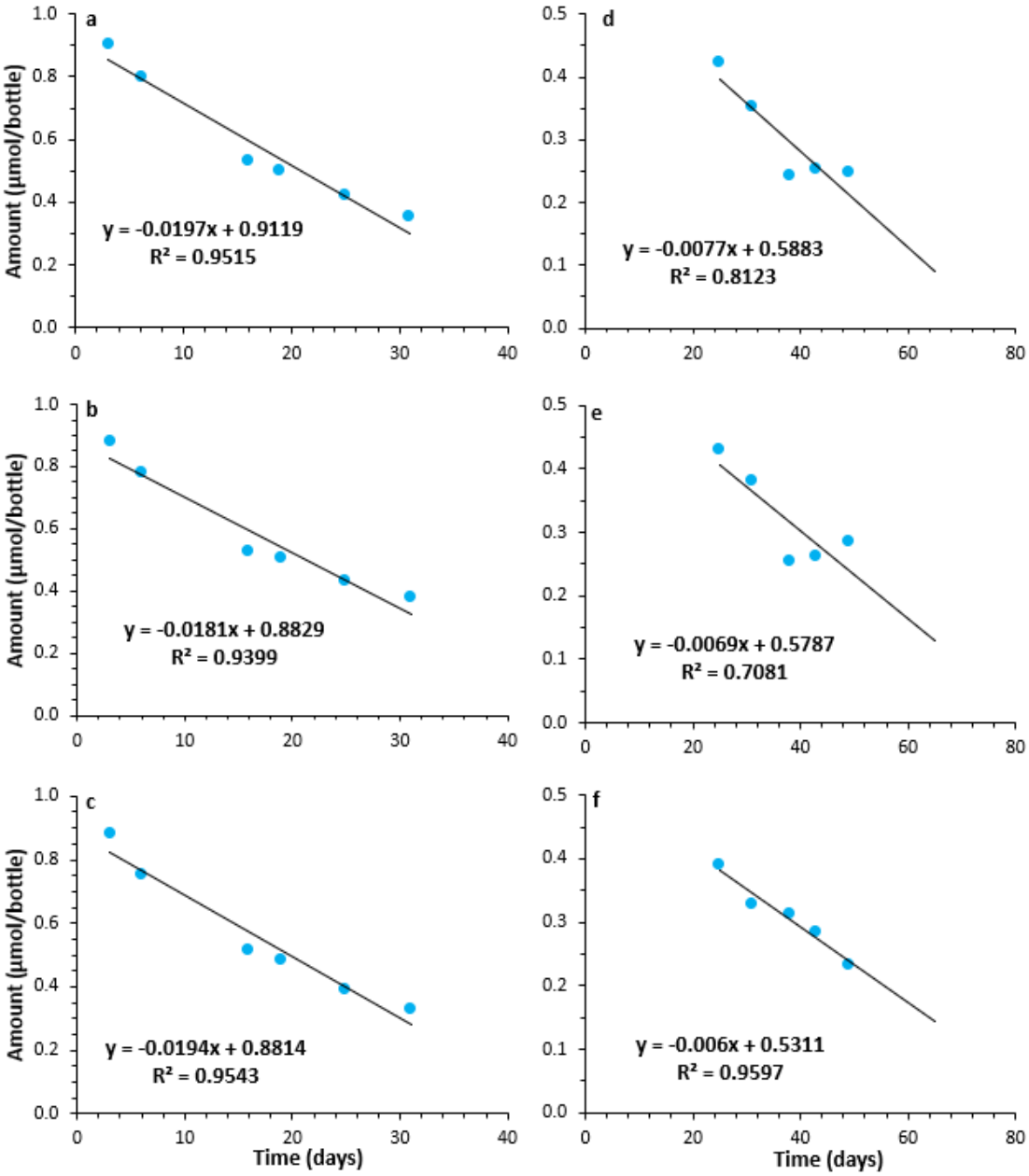

Figure A.31. a-c) Dechlorination rates for the initial decline in the microcosms with 0.38 g Hepure d-f) dechlorination rates for the tail end of the microcosms with 0.38 g Hepure.

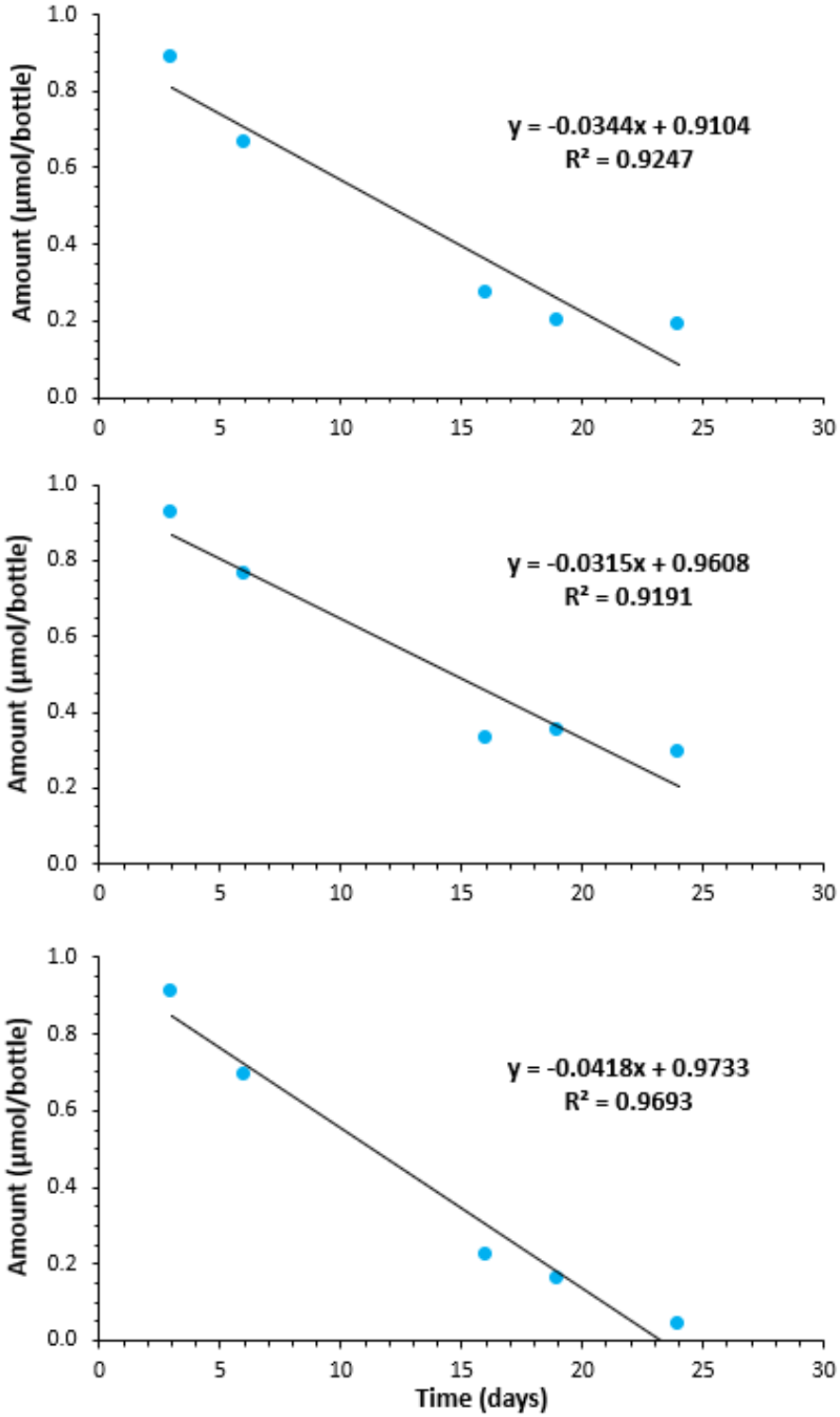

Figure A.32. Dechlorination rates for the triplicate microcosms amended with 0.16 g SmZVI.

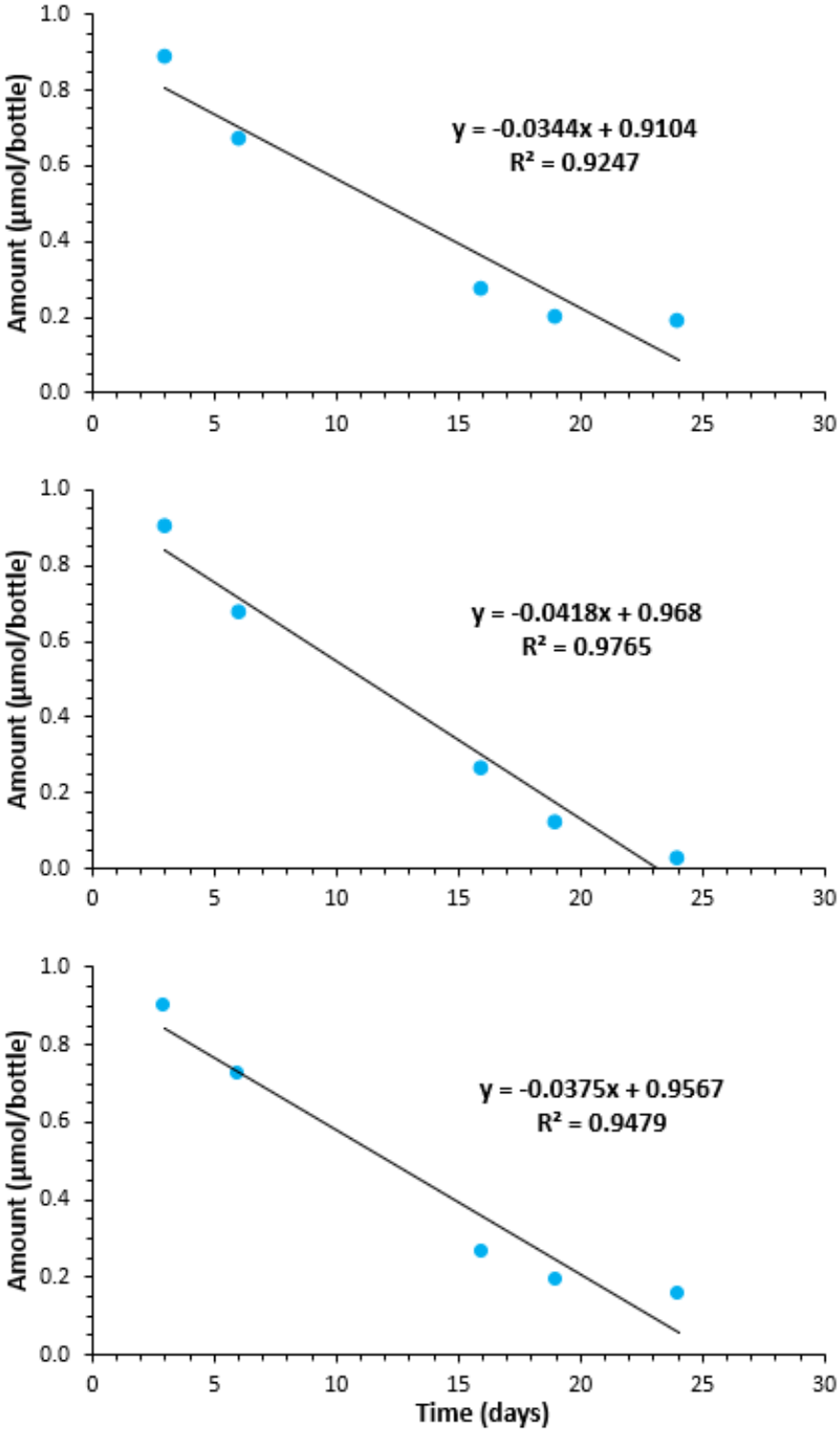

Figure A.33. Dechlorination rates for the triplicate microcosms amended with 0.38 g SmZVI.

Table A.9. Summary of the dechlorination rates determined for each microcosm.

Treatment	Average Rate (umol/d)	Average Rate (uM/d)	n points	Std
Lac-1	0.0102	0.1332	11	-
Lac-2	0.0162	0.2115	7	-
Lac-3	0.0032	0.0418	22	-
Lac + KB1	0.1084	1.4151	3	0.1909
EVO + KB1	0.0575	0.7511	5	0.0340
0.16 g $Hepure_{initial}$	0.0070	0.0918	4	0.0172
0.16 g $Hepure_{tail}$	0.0015	0.0196	14	0.0047
0.16 g Hepure + $EVO_{initial}$	0.0054	0.0709	5	0.0111
0.16 g Hepure + EVO_{tail}	0.0017	0.0222	13	0.0026
0.38 g $Hepure_{initial}$	0.025	0.2489	6	0.0111
0.38 g $Hepure_{tail}$	0.009	0.0896	5	0.0111
0.16 g SmZVI	0.041	0.4687	5	0.0693
0.38 g SmZVI	0.038	0.4948	5	0.0485

Table A.10. Summary of the average TCE degradation rates determined for each treatment.

Treatment	MCL Reached	Average Deg. Rate (µM/d)	Standard Deviation
Lac-1	yes	0.067	-
Lac-2	yes	0.146	-
Lac-3	no	0.045	-
Lac + KB1	yes	0.903	0.11
EVO + KB1	yes	0.614	0.00
0.16 g Hepure	no	0.045	0.00
0.16 g Hepure + EVO	no	0.045	0.00
0.38 g Hepure	no	0.169	0.00
0.16 g SmZVI	no	0.185	0.00
0.38 g SmZVI	yes	0.326	0.00

A.18. Quality Assurance Project Plan

A Quality Assurance Project Plan (QAPP) Addendum has been provided as part of the contract with the USEPA Region 5 and S.S. Papadopulous & Associates, Inc. The QAPP is in place for assistance with field activities and the microcosm study. Quality

Assurance/Quality Control (QA/QC) procedures were set for the QAAP Addendum to be performed according to professional technical standards, USEPA requirements and guidelines, and specific project goals and requirements.[54]

It is stated on page 21 of the QAPP that the main quality control metric for VOC measurements is the maintenance of a mass balance based on the amount of TCE at time zero. In this mass balance, the sum of the amounts of TCE, cDCE, VC, ethene, and ethane should remain within 80-120% of the initial amount of TCE present, in μmol/bottle.[54] To ensure that this standard has been met, a mass balance was performed as instructed for each microcosm. The averages of each triplicate where calculated for each treatment and are shown in **Table A.11.** This protocol did not take adsorption into account, which has impacted the mass balances of the unamended, EVO amended, and autoclaved controls. It is likely that TCE adsorbed to the EVO addition before the time zero was made, so the initial amount of TCE for the EVO and EVO+KB1 microcosms was replaced with the average initial amount measured in the Lac+KB1 microcosms.[26] It can also be noted that the mass balance is not met for the lactate amended or EVO+KB1 microcosms. Two of the three triplicates of the lactate amended microcosms are within the 80-120% range but it is currently unknown why the third lactate triplicate and the EVO+KB1 microcosms fall outside of the range.

Table A.11. A mass balance was performed for each microcosm using the sum of the final amount of TCE, cDCE, VC, ethene, and ethane for each microcosm compared to the initial amount of TCE. Mass balances for individual microcosms as well as the averages for each treatment are shown.

	Final Sum/Initial TCE			
Treatment	**Bottle #1**	**Bottle #2**	**Bottle #3**	**Average**
Unamended	0.49	0.51	0.56	0.52
Lactate Amended	1.11	1.13	1.53	1.26
Lactate + KB-1	0.83	0.80	0.83	0.82
EVO Amended	0.49	0.51	0.51	0.50
EVO + KB-1	1.38	1.26	1.26	1.30
ZVI	0.89	0.84	1.16	0.96
ZVI+EVO	1.29	1.30	0.88	1.16
Autoclave Control	0.30	0.31	0.38	0.33
Water Control	1.00	1.01	0.92	0.98

Page 21 also states that monitoring will be considered complete once the concentrations of TCE, cDCE, and VC are below their designated MCLs or when eight months of monitoring data is complete.[54] The bioaugmented microcosms and lactate amended microcosms 1 and 2 exhibited complete dechlorination and contaminants reached concentrations below their MCLs within the incubation period. The remaining bottles were incubated for 242 days, roughly eight months.

Worksheet 12 of the QAPP describes measurement performance criteria that must be provided.[54] The QAPP requires laboratory control samples, analysis of blanks, and duplicate samples. The laboratory control samples must demonstrate the current lab control limits. To demonstrate the current lab control limits, the lowest detected concentrations of TCE, cDCE, and VC were compiled. The lowest detectable TCE, cDCE, and VC were 0.981, 0.85, and 0.6 μg/L which were observed in microcosms 0.38g

SmZVI-1, PlumeStop-3, and Lac+KB1-3, respectively. All of these concentrations are well below the contaminants respective MCLs, as specified in QAPP Worksheet 15.[54] The requirement regarding method blanks states that these blanks must show no result greater than the quantification limit except common lab contaminants that may be 3x the quantification limit. DDI blanks were run throughout the experiment on the IC and HPLC to ensure there was not contamination or interference with the results.

Microcosms for this experiment were prepared in triplicate and the standard deviation error bars for these measurements can be found in **Appendix A.14**. The lactate amended microcosms were the only microcosms which showed significantly inconsistent results. However, this inconsistency was one of the most notable results of the microcosm study. The QAPP requires duplicate samples which should have a relative percent difference (RPD) less than 30. Duplicate samples were taken for nitrate, sulfate, and organic acid measurements for one triplicate from the unamended, EVO amended, and ZVI and EVO amended microcosms. These results of the RPD calculations are shown in **Table A.12.**

Table A.12. RPDs for duplicate measurements taken of the unamended, EVO amended, and ZVI+EVO amended microcosms to demonstrate that these results met the RPD requirement.

		RPD					
Treatment	**Bottle No.**	**Nitrate**	**Sulfate**	**Lactate**	**Formate**	**Acetate**	**Propionate**
UN	3	0%	1%	0%	0%	0%	0%
EVO	3	0%	0%	0%	0%	12%	0%
ZVI+EVO	3	0%	0%	0%	0%	3%	13%

QAPP Worksheet 19 describes the analytical and preparation method, sample volume, container size, preservation requirements, and maximum holding time for VOC, nitrate, sulfate, volatile fatty acids, and microbial community analyses.[54] The analytical and preparation procedures were performed as described in the QAPP. The sample volumes for VOCs were the same as stated in the QAPP but at least 10 mL of groundwater was removed from each microcosm at the end of incubation to measure nitrate, sulfate, and volatile fatty acids. These measurements were taken as soon as possible following removal from the microcosm. However, in the cases were the samples had to be preserved, the groundwater was stored in the fridge in a scintillation vial. For microbial community analysis, the samples were stored in the fridge before shipping them to Microbial Insights in a cooler with ice packs.

QAPP Worksheet 24 describes the requirements for analytical instrument calibration, specifically for the GC/FID, IC, and HPLC. Calibration standards were made for each instrument at the beginning of the study and were repeated as deemed necessary, as described in the QAPP. The acceptance criteria for these calibration curves includes a correlation coefficient ≥ 0.995 for the listed analyses groups, which has been met. Response factors and coefficients of determination for measurements on the GC/FID, IC, and HPLC are described in **Appendices A.21, A.22, and A.23.**

QAPP Worksheet 28 goes further into the analytical control and corrective action requirements. This Worksheet reemphasizes the need of calibration standards and repeating the standards as deemed fit.[54] There have been multiple sets of standards measured on the GC/FID, IC, and HPLC which can be found in **Appendices A.21, A.22,**

and A.23. This Worksheet also specifies that a microcosm sample should be sent to an outside lab for a measurement of nitrate and sulfate. This measurement was done in the beginning of the experiment and is described in **Appendix A.3**. The initial groundwater was brought to the Clemson ASL. The ASL produced very similar results to those measured on the IC at Clemson's Rich Lab. The ASL determined that nitrate was below detection, but it is unclear what the detection limit of their IC is. Worksheet 28 also reemphasizes the importance of replicate samples for nitrate, sulfate, and volatile fatty acid measurements. Duplicate measurements were made of one triplicate from the unamended, EVO amended, and ZVI+EVO amended microcosms, as previously described.

Worksheet 30 of the QAPP specifies the laboratory/organization responsible for various analytical groups. The Freedman laboratory at Clemson University is responsible for VOC measurements through microcosm headspace measurements and nitrate, sulfate, and volatile fatty acid measurements through groundwater measurements. These measurements are done following the standard operating procedures provided within the QAPP.[54]

A.19. Microbial Insights Results

Table A.13. Microbial Insights qPCR results for identification and quantification of reductive dechlorinating microorganisms and genes in microcosms.

			Final Time						
Type	**Acronym**	**Time Zero**	**Lac + KB1-1**	**EVO + KB1-1**	**Lac-1**	**Lac-3**	**UN-1**	**EVO-1**	**ZVI + EVO-1**
Dehalococcoides	DHC	ND[a]	4.19E+04	7.96E+06	1.16E+07	3.90E+06	ND[c]	ND[c]	ND[c]
tceA Reductase	TCE	ND[a]	2.04E+03	2.65E+05	1.08E+07	1.10E+06	ND[c]	ND[c]	ND[c]
BAV1 Vinyl Chloride Reductase	BVC	ND[a]	1.95E+04	2.67E+05	3.68E+05	ND[c]	ND[c]	ND[c]	ND[c]
Vinyl Chloride Reductase	VCR	ND[a]	8.85E+03	1.85E+06	1.24E+06	ND[c]	ND[c]	ND[c]	ND[c]
Dehalobacter spp.	DHBt	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
Dehalobacter DCM	DCM	ND[b]	ND[b]	ND[b]	6.28E+04	ND[c]	ND[c]	ND[c]	ND[c]
Dehalogenimonas spp.	DHG	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
cerA Reductase	CER	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
trans-1,2-DCE Reductase	TDR	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
			Final Time						

Type	Acronym	Time Zero	Lac + KB1-1	EVO + KB1-1	Lac-1	Lac-3	UN-1	EVO-1	ZVI + EVO-1
Dehalobium chlorocoercia	DECO	ND[b]	ND[b]	8.00E+04	1.32E+05	ND[c]	ND[c]	ND[c]	ND[c]
Desulfuromonas spp.	DSM	ND[b]	ND[b]	8.00E+05	ND[b]	6.30E+03	ND[c]	ND[c]	ND[c]
PCE Reductase	PCE-1	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
PCE Reductase	PCE-2	ND[b]	ND[b]	3.60E+05	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
Chloroform Reductase	CFR	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
1,1-DCA Reductase	DCA	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
1,2-DCA Reductase	DCAR	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]

ND = results are nondetectable and below the detection limit.
J = idk what this means – check microbial insights

[a] = detection limit of 1.00E+03 cells/mL
[b] = detection limit of 1.00E+04 cells/mL
[c] = detection limit of 5.00E+02 cells/mL

Table A.14. Microbial Insights qPCR results for identification and quantification of aerobic (co)metabolic microorganisms and genes in microcosms.

Type	Acronym	Time Zero	Final Time						
			Lac + KB1-1	EVO + KB1-1	Lac-1	Lac-3	UN-1	EVO-1	ZVI + EVO-1
Soluble Methane Monooxygenase	SMMO	7.90E+03[J]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
Toluene Dioxygenase	TOD	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
Phenol Hydroxylase	PHE	4.98E+03[J]	ND[b]	9.48E+02[J]	4.44E+04	ND[c]	ND[c]	ND[c]	ND[c]
Trichlorobenzene Dioxygenase	TCBO	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
Toluene Monooxygenase 2	RDEG	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	1.90E+05	ND[c]
Toluene Monooxygenase	RMO	ND[b]	ND[b]	ND[b]	1.49E+05	ND[c]	ND[c]	ND[c]	ND[c]
Ethene Monooxygenase	EthC	ND[b]	ND[b]	8.13E+04	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
Epoxyalkane Transferase	EtnE	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]
Dichloromethane Dehalogenase	DCMA	ND[b]	ND[b]	ND[b]	ND[b]	ND[c]	ND[c]	ND[c]	ND[c]

ND = results are nondetectable and below the detection limit.
J = idk what this means – check microbial insights

[a] = detection limit of 1.00E+03 cells/mL
[b] = detection limit of 1.00E+04 cells/mL
[c] = detection limit of 5.00E+02 cells/mL

Table A.15. Microbial Insights qPCR results for identification and quantification of total eubacteria, sulfate reducing bacteria, and methanogenic bacteria.

			Final Time						
Type	**Acronym**	**Time Zero**	**Lac + KB1-1**	**EVO + KB1-1**	**Lac-1**	**Lac-3**	**UN-1**	**EVO-1**	**ZVI + EVO-1**
Total Eubacteria	EBAC	9.34E+07	1.06E+06	7.26E+08	5.27E+08	8.35E+08	1.50E+07	8.20E+08	8.45E+08
Sulfate Reducing Bacteria	APS	4.84E+05	ND[b]	2.32E+06	4.62E+07	7.00E+04	8.90E+02	4.00E+04	2.10E+04
Methanogens	MGN	ND[b]	ND[b]	5.18E+06	2.87E+05	4.70E+06	1.40E+03	2.90E+04	4.60E+05

ND = results are nondetectable and below the detection limit.
J = idk what this means – check microbial insights

[a] = detection limit of 1.00E+03 cells/mL
[b] = detection limit of 1.00E+04 cells/mL
[c] = detection limit of 5.00E+02 cells/mL

A.20. Interpreting Microbial Insights Results

At the end of the incubation period for the initial set of microcosms, one bottle from each triplicate was sent to Microbial Insights for a qPCR analysis of the microbial activity in each microcosm. These results are used to evaluate the effects of different treatment methods on microbial activity in the microcosms. The results provided by Microbial Insights are divided into three categories: reductive dechlorination, aerobic co(metabolic), and other.

Reductive Dechlorination

Reductive dechlorination is the replacement of a chlorine atom with a hydrogen atom. TCE reductive dechlorination sequentially transforms TCE to *c*DCE, VC, and ethene. There are multiple organohalide respiring bacteria capable of reducing TCE to *c*DCE and VC. However, *Dhc* and *Dhgm* are the only known microorganisms capable of completely reducing TCE to ethene. Ethene is a benign and environmentally suitable end product, while *c*DCE and VC are not.[11] In the qPCR analysis, the following bacteria have been analyzed due to their potential role in reductive dechlorination of TCE.

Dhc are capable of completely reducing PCE and TCE past VC and *c*DCE to ethene, a non-toxic end product.[55] There are multiple strains of *Dhc* which interact with chlorinated ethenes differently, not all strains are capable of complete reductive dechlorination. The reductive dechlorination capabilities of individual strands are dependent on the presence of *Dhc* functional genes, including tceA, bvcA, and vcrA.[11] Each of the functional genes have different roles, as shown in **Figure A.34**. The tceA gene is a reductive dehalogenase gene that encodes the tceA reductase enzyme which is

responsible for the reductive dechlorination of TCE to *c*DCE.[56] tceA is not present in every strand of *Dhc* but it provides an indication that biological dechlorination of TCE is possible. The bvcA gene encodes the BAV1 VC reductase enzyme. This enzyme drives reductive dechlorination of VC to ethene by *Dhc* sp. str. BAV1. The presence of this enzyme indicates the potential for complete reduction of TCE to ethene. The vcrA gene encodes the VC reductase enzyme. Similar to the BAV1 VC reductase enzyme, this enzyme also drives the reductive dechlorination of *c*DCE and VC to ethene by *Dhc* sp. str. VS. If neither the bvcA or vcrA genes are present, TCE reduction may not proceed past VC and VC may accumulate.[55]

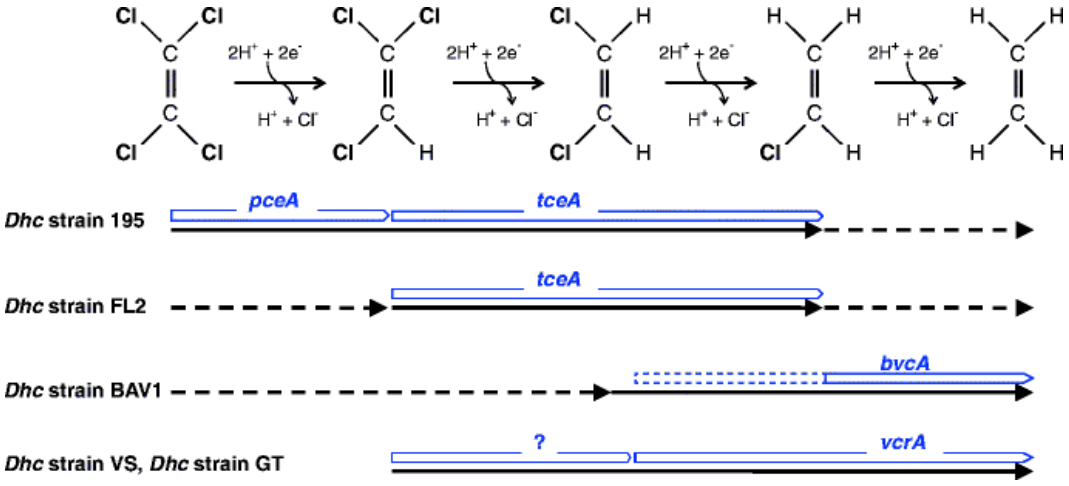

Figure A.34. *Dhc* reductase genes implicated in reductive dechlorination of chlorinated ethenes.[11]

Dehalobacter spp. are anaerobic bacteria that are capable of reductive dehalogenation of PCE and TCE to *c*DCE. *Dehalobacter* is not capable of complete reductive dechlorination to ethene. Various strains of *Dehalobacter* can reduce chloroform and 1,1,1-TCA to dichloromethane which is not an environmentally-suitable end product. *Dehalobacter* DCM is a strain of *Dehalobacter* capable of degrading

chloroform and 1,1,1-TCA past the daughter product dichloromethane (DCM) and to acetate, methane, and chloride. This strain is able to degrade DCM through fermentative dehalogenation using a chloroform reductase enzyme.[57,58]

Dhgm are anaerobic bacteria that include reductive dehalogenase genes capable of reductive dechlorination of TCE to DCE isomers, VC, and finally ethene, which is environmentally benign. Certain strains of *Dhgm* are capable of using TCE and other chlorinate ethenes as electron acceptors and can completely reduce TCE to ethene.[52,53] The cerA (chloroethane reductase) gene encodes the VC reductase enzyme responsible for the dechlorination of VC to ethene. This gene belongs to *Dhgm* sp. str. GP. Strains GP and WBC-2 are the only organisms other than *Dhc* that can completely reduce vinyl chloride to ethene.[53]

Desulfitobacterium, Dehalobium chlorocoercia, and Desulfuromonas are anaerobic bacteria capable of reducing TCE and TCE to DCE.[59,11,60] This reduction is not complete and TCE and DCE are not environmentally suitable end products. *Desulfuromonas* are also capable of reducing sulfur and exhibit iron respiration with acetate oxidation. Certain strains of *Desulfuromonas* are capable of reductively dechlorinating PCE and TCE to *c*DCE using acetate as the electron donor.[60] DCE and TCE are toxic products that cannot be released into the environment. PCE-1 is a strain of *Desulfitobacterium* capable of reductively dechlorinating PCE to TCE and small amounts of *c*DCE and *trans*-DCE.[59] Chloroform reductase is also found in *Desulfitobacterium* and can be used to reduce chloroform to DCM.[58]

1,1 DCA reduction can be accomplished with the 1,1 DCA reductase enzyme. This enzyme activates the 1,1 DCA reductive dehalogenase gene in some strains of *Dehalobacter*. The reduction of 1,2 DCA is capable with the 1,2 DCA reductive dehalogenase gene from strains of *Desulfitobacterium* and *Dehalobacter*. These genes can dechlorinate 1,1 DCA and 1,2 DCA to ethene.[38]

Aerobic (Co)Metabolic

Aerobic cometabolism is the transformation of a co-metabolized substrate by microorganisms using a different growth supporting substrate. Cometabolism is especially useful in contamination sites with combined pollution where the degradation of one contaminant can attribute to the cometabolism of another.[61] Cometabolism does not support microbial growth but it can transform substrates, such as TCE, into various products that can potentially be utilized by the microorganisms.[9] TCE, DCE, and VC can be aerobically co-metabolized.[47] TCE has been show to degrade aerobically to carbon dioxide as well as other products, such as dichloroacetate and formate.[47,62] There are various pathways for TCE cometabolism, depending on the primary substrate being reduced.

TCE can be aerobically degraded by methanotrophic cometabolism with the use of the methane monooxygenase enzyme. This enzyme activates the methane monooxygenase gene that is responsible for methane oxidation and contributes to the aerobic cometabolism of TCE, DCE, and chloroform. In this reaction, TCE oxidation produces epoxide and ultimately carbon dioxide.[9] Toluene dioxygenase and monooxygenase are genes capable of metabolizing toluene but are also active genes in

the cometabolism of TCE.[61] Phenol hydroxylase is a gene in phenol-degrading microorganisms that is capable of degrading TCE.[63] Trichlorobenzene dioxygenase is gene that initiates aerobic biodegradation of 1,2,4-trichlorobenzene.[38] Ethene monooxygenase is involved in ethene utilization and VC (co)metabolism. This gene is responsible for converting ethene and VC to their respective epoxyalkanes.[64] Epoxyalkane transferase is a gene that plays a large role in the aerobic degradation of ethene, propene, and VC.[65] Dichloromethane dehalogenase is a gene responsible for the degradation of DCM to formaldehyde and inorganic chloride.[66]

Other

The total eubacteria are the total bacteria identified by the qPCR analysis. Eubacteria are prokaryotic, single cell microorganisms. These bacteria may contain function genes that play important roles in the processes discussed above. Other microbes may be associated with processes that inhibit TCE dechlorination. Sulfate reducing bacteria can act as an inhibitor in TCE dechlorination. Sulfate reducing bacteria are hydrogen consuming and compete with organohalide respiring bacteria for hydrogen.[67] Methanogens are another inhibitor of TCE dechlorination. Methanogens compete with dechlorinating microbes for hydrogen and may decrease the rate and efficiency of TCE dechlorination.[68,4] Reductive dechlorination can still occur under sulfate-reducing and methanogenic conditions but the dechlorinating rates may decrease.

A.21. Response Factors – VOCs

Set I

An initial response factor for TCE was determined using a methanol stock solution containing 0.10 mL of neat TCE dissolved in 50 mL of reagent grade methanol. Gravimetric analysis was done to determine the concentration of TCE in the methanol stock solution. Four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. Increasing volumes of stock solution (10, 50, 100, and 200 μL) were injected into the serum bottles with DDI water and glass beads. The moles of TCE in each serum bottle was determined based on the mass of the methanol stock solution added and the mass of each compound per mass of stock solution. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The amount of TCE was determined by injecting 0.5 mL of headspace samples into the GC-FID. The peak areas at the corresponding retention times were recorded. The response factor for TCE was determined using the slope of the regression line for the μmol gas per bottle vs. the peak area units (PAUs), forced through the origin. This analysis determined that TCE has a response factor of 0.09665 μmol/PAU (**Figure A.35**). The response factor for cDCE could not be measured at this point in time due to a lack of neat cDCE. For Set I, an old reference factor is 0.216 μmol/PAU will be used for cDCE.

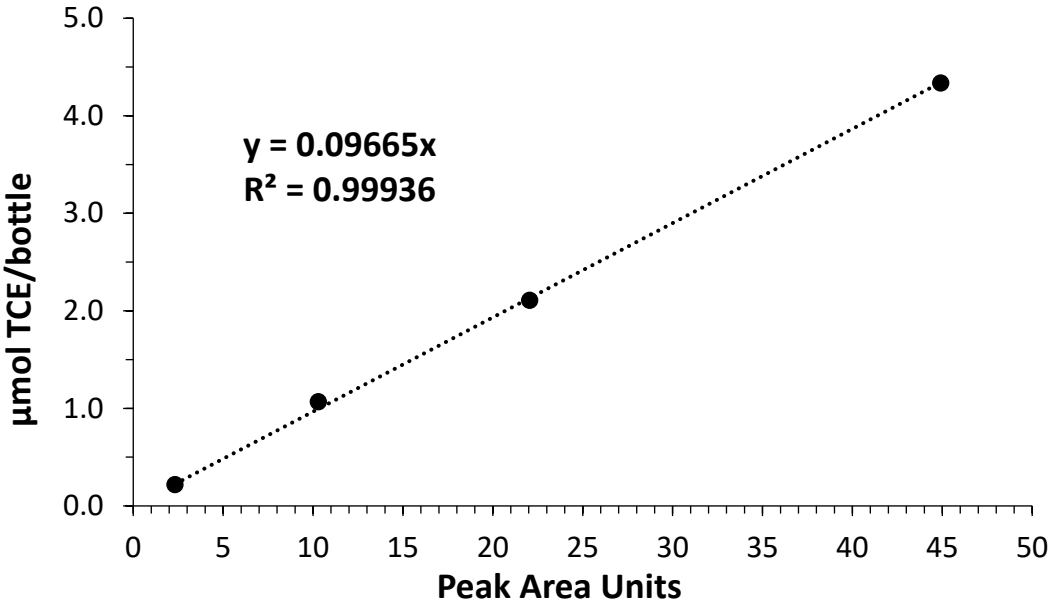

Figure A.35. The amount of TCE per bottle in Set I was calculated using gravimetric analysis and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Response factors for methane, acetylene, ethene, ethane, and VC were determined using the GC-FID. Two sets of four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. 0.05, 0.10, 0.15, and 0.20 mL of acetylene and ethane were injected into the first set of bottles with DDI water and glass beads. 0.10, 0.20, 0.50, and 1.00 mL of methane, 0.10, 0.25, 0.50, and 1.00 mL of ethene, and 0.10, 0.20, 0.35, and 0.50 mL of VC were injected into the second set of bottles with DDI water and glass beads. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The moles of each gas added to each bottle was calculated using the ideal gas law. The barometric pressure and room temperature were recorded to be used in this calculation. The amount of methane, acetylene, ethene, ethane, and VC was determined by injecting 0.5 mL of headspace

samples into the GC-FID. The peak areas at the corresponding retention times were recorded. The response factors for each gas were determined using the slope of the regression line for the μmol gas per bottle vs. the PAUs, forced through the origin. This analysis determined that methane has a response factor of 0.04488 μmol/PAU (**Figure A.36**), acetylene has a response factor of 0.04004 μmol/PAU (**Figure A.37**), ethene has a response factor of 0.02567 μmol/PAU (**Figure A.38**), ethane has a response factor of 0.02245 μmol/PAU (**Figure A.39**), and VC has a response factor of 0.05118 μmol/PAU (**Figure A.40**).

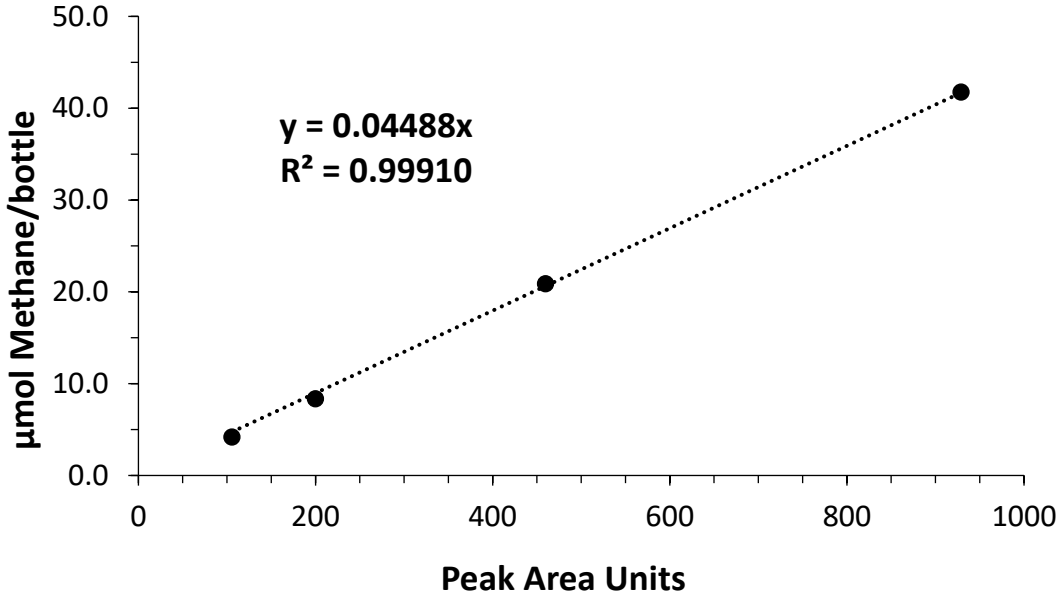

Figure A.36. The amount of methane per bottle in Set I was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

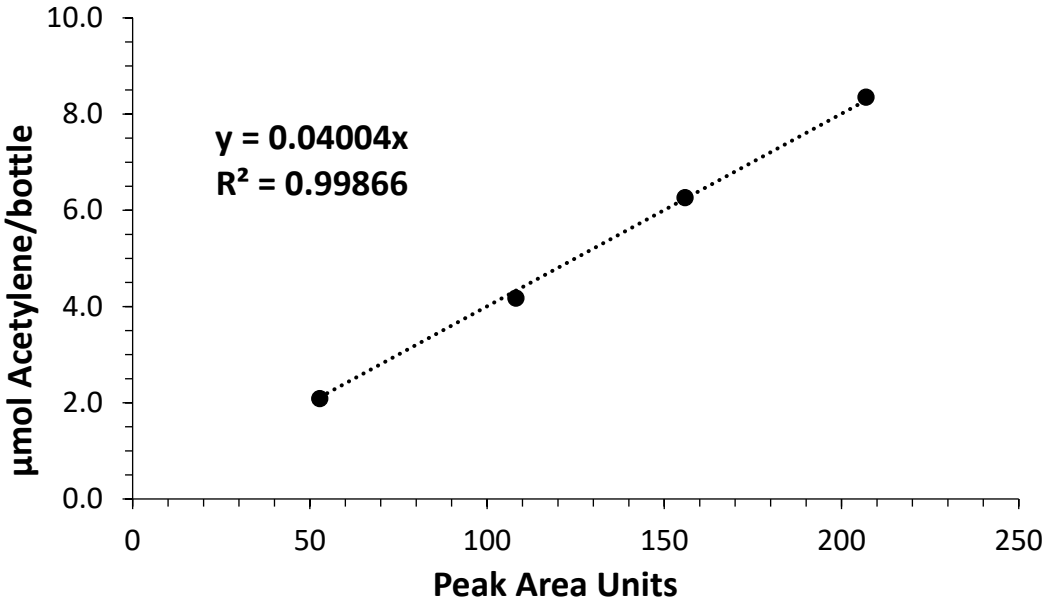

Figure A.37. The amount of acetylene per bottle in Set I was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

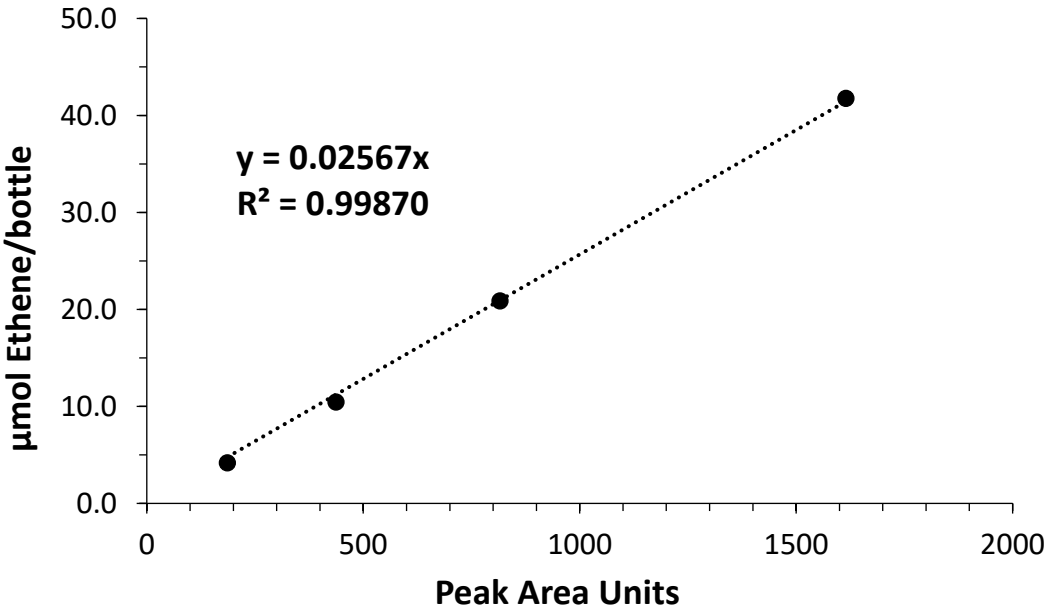

Figure A.38 The amount of ethene per bottle in Set I was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

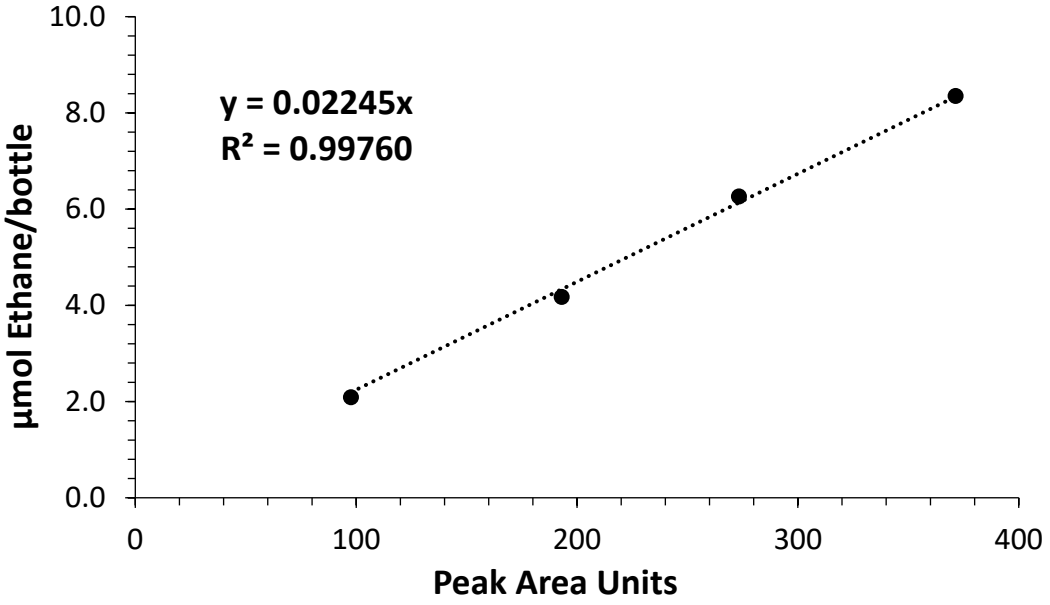

Figure A.39. The amount of ethane per bottle in Set I was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

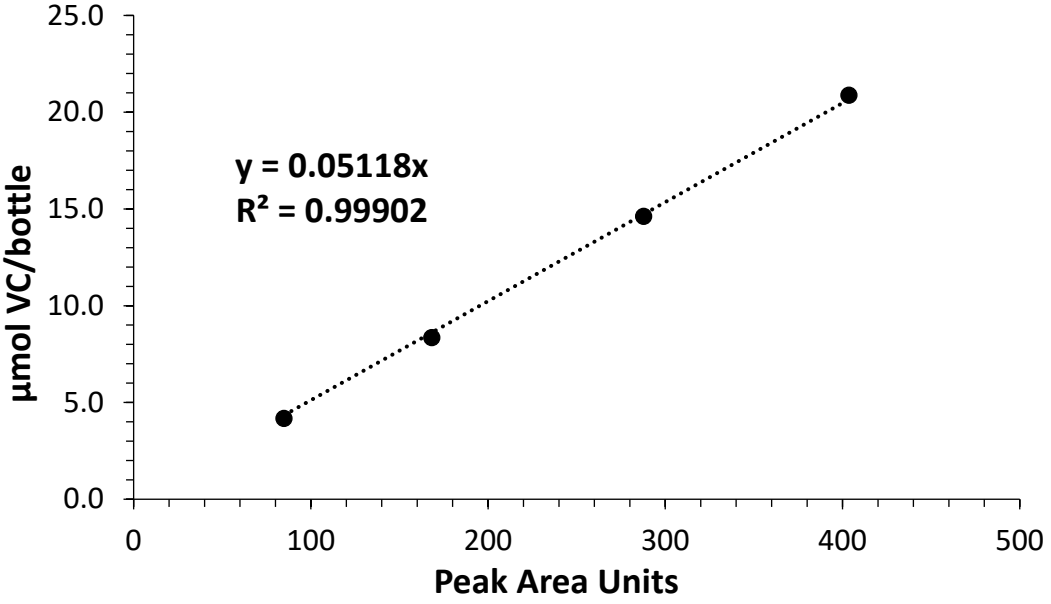

Figure A.40. The amount of VC per bottle in Set I was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Set II

The second set of response factors for TCE and cDCE was determined using a methanol stock solution containing 0.10 mL of neat TCE and 0.10 mL of neat cDCE dissolved in 50 mL of reagent grade methanol. Gravimetric analysis was done to determine the concentrations of TCE and cDCE in the methanol stock solution. Four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. Increasing volumes of stock solution (10, 50, 100, and 200 μL) were injected into the serum bottles with DDI water and glass beads. The moles of TCE and cDCE in each serum bottle was determined based on the mass of the methanol stock solution added and the mass of each compound per mass of stock solution. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The amount of TCE and cDCE was determined by injecting 0.5 mL of headspace samples into the GC-FID. The peak areas at the corresponding retention times were recorded. The response factors for TCE and cDCE were determined using the slope of the regression line for the μmol gas per bottle vs. the peak area units (PAUs), forced through the origin. This analysis determined that TCE has a response factor of 0.1348 μmol/PAU (**Figure A.41**) and cDCE has a response factor of 0.29007 μmol/PAU (**Figure A.42**).

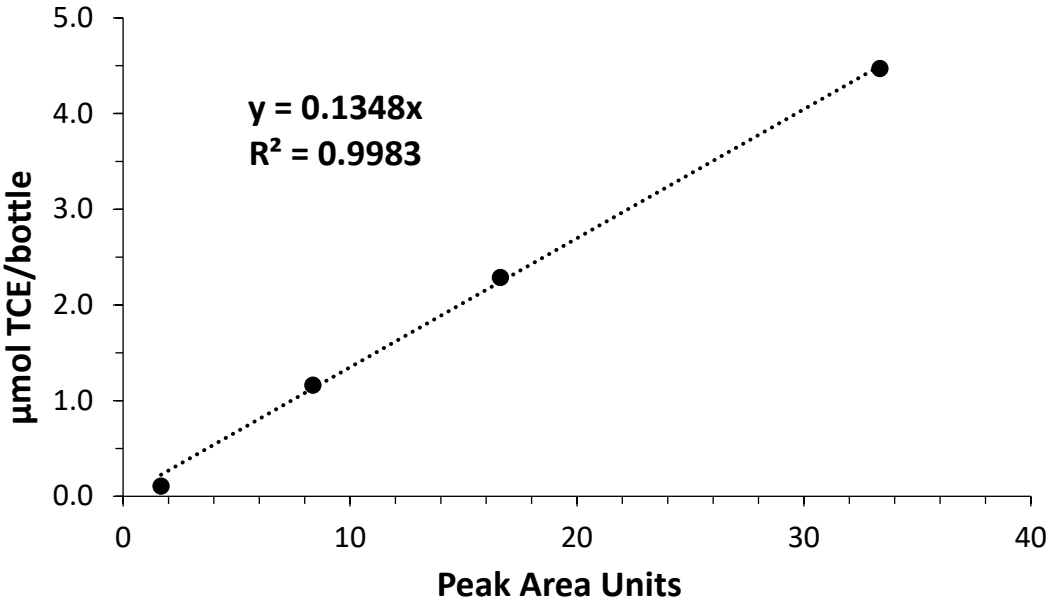

Figure A.41. The amount of TCE per bottle in Set II was calculated using gravimetric analysis and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

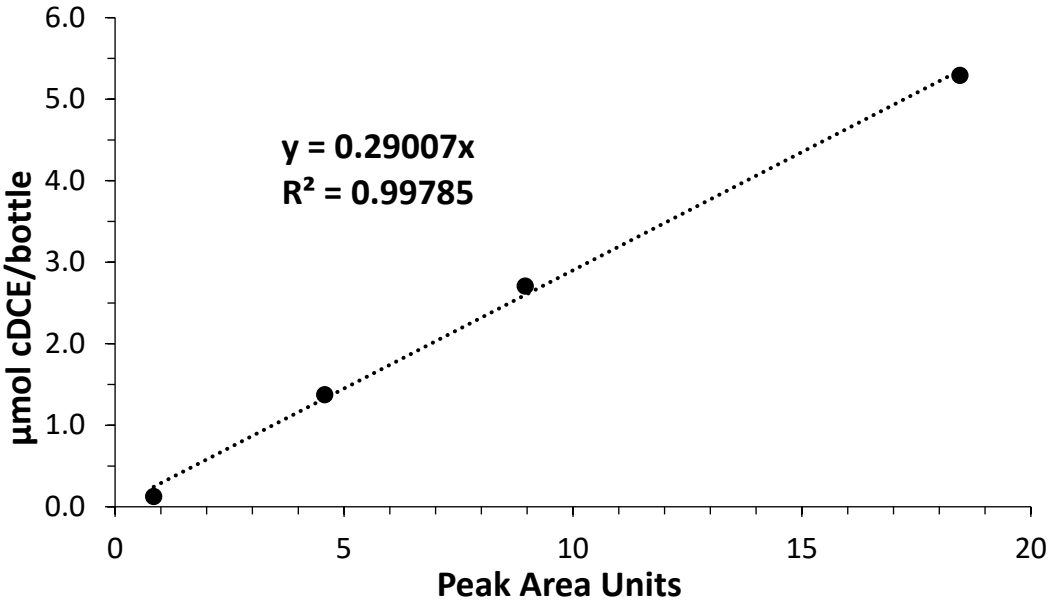

Figure A.42. The amount of cDCE per bottle in Set II was calculated using gravimetric analysis and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Response factors for methane, acetylene, ethene, ethane, and VC were determined using the GC-FID. Two sets of four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. 0.05, 0.10, 0.15, and 0.20 mL of acetylene and ethane were injected into the first set of bottles with DDI water and glass beads. 0.10, 0.20, 0.50, and 1.00 mL of methane and VC and 0.10, 0.25, 0.50, and 1.00 mL of ethene were injected into the second set of bottles with DDI water and glass beads. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The moles of each gas added to each bottle was calculated using the ideal gas law. The barometric pressure and room temperature were recorded to be used in this calculation. The amount of methane, acetylene, ethene, ethane, and VC was determined by injecting 0.5 mL of headspace samples into the GC-FID. The peak areas at the corresponding retention times were recorded. The response factors for each gas were determined using the slope of the regression line for the μmol gas per bottle vs. the PAUs, forced through the origin. This analysis determined that methane has a response factor of 0.0668 μmol/PAU (**Figure A.43**), acetylene has a response factor of 0.0601 μmol/PAU (**Figure A.44**), ethene has a response factor of 0.0387 μmol/PAU (**Figure A.45**), ethane has a response factor of 0.0337 μmol/PAU (**Figure A.46**), and VC has a response factor of 0.0732 μmol/PAU (**Figure A.47**).

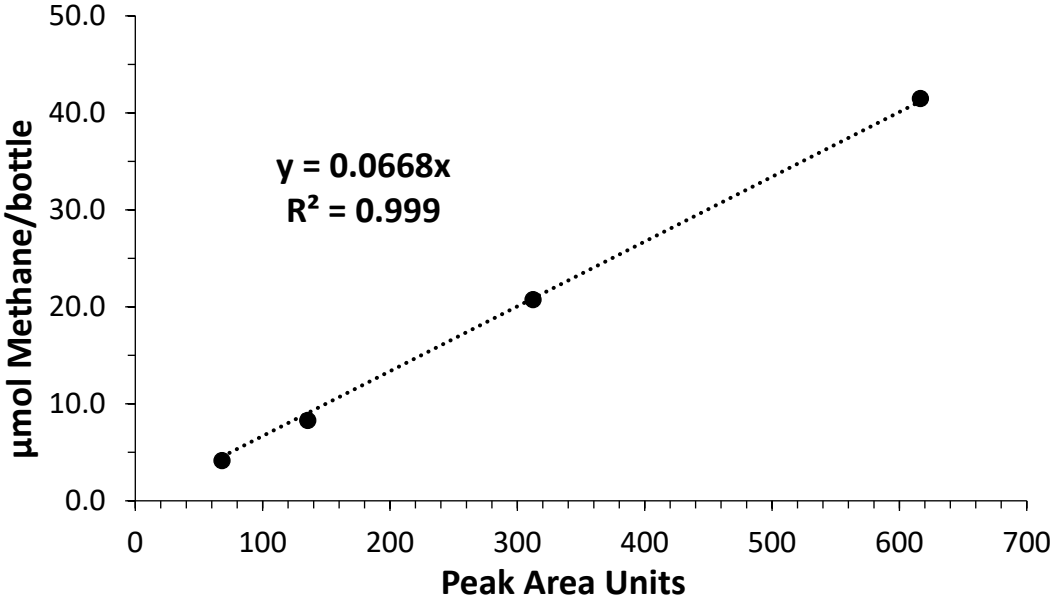

Figure A.43. The amount of methane per bottle in Set II was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

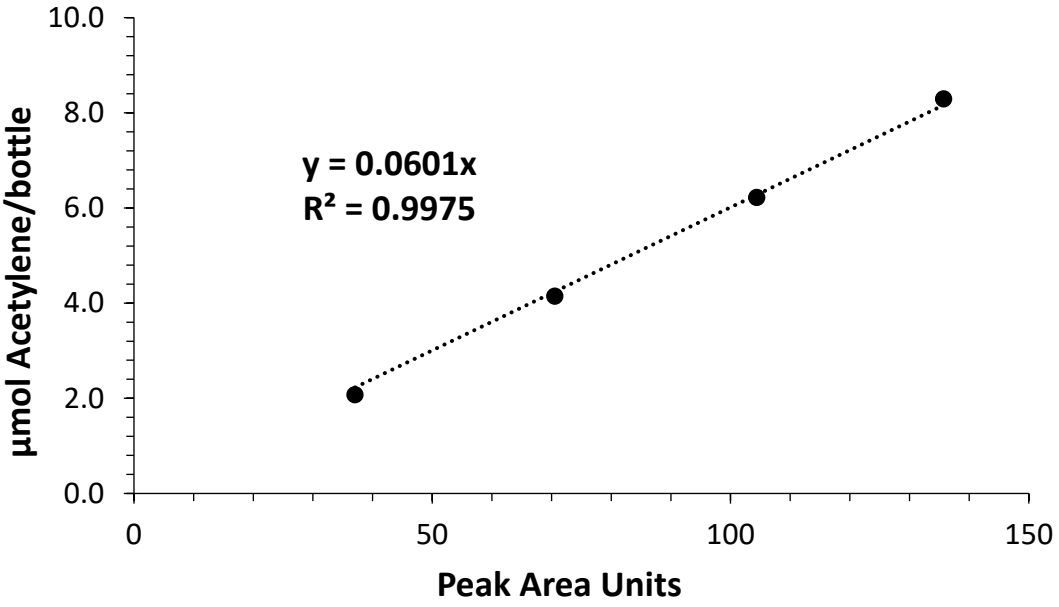

Figure A.44. The amount of acetylene per bottle in Set II was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

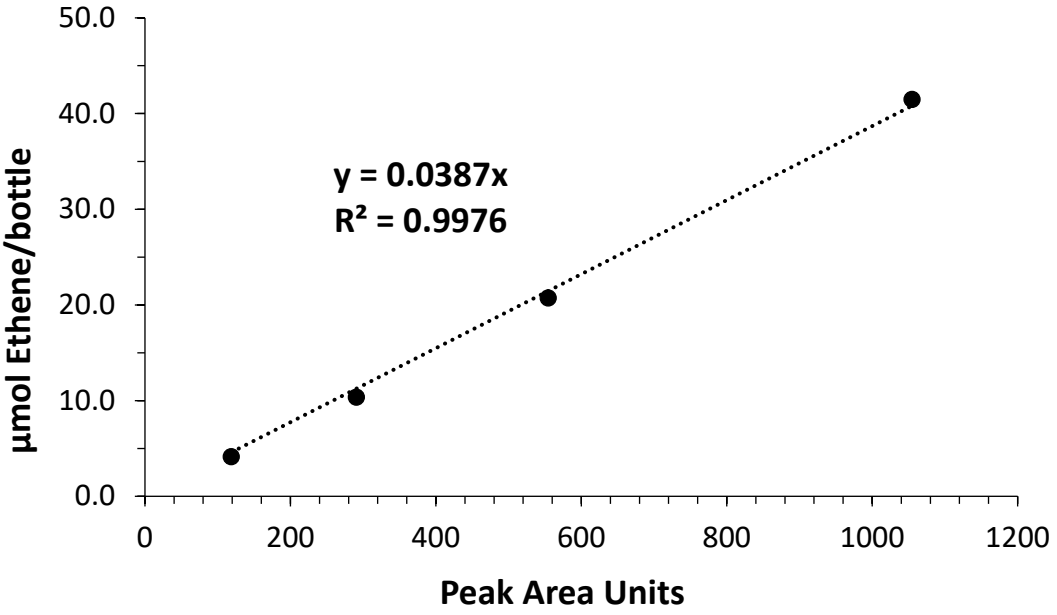

Figure A.45. The amount of ethene per bottle in Set II was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

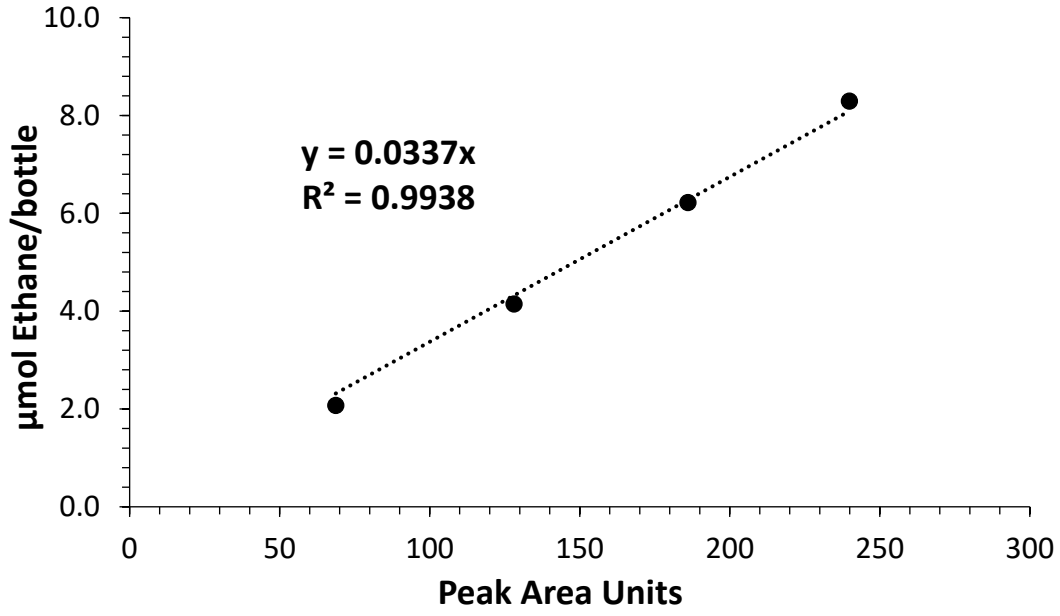

Figure A.46. The amount of ethane per bottle in Set II was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

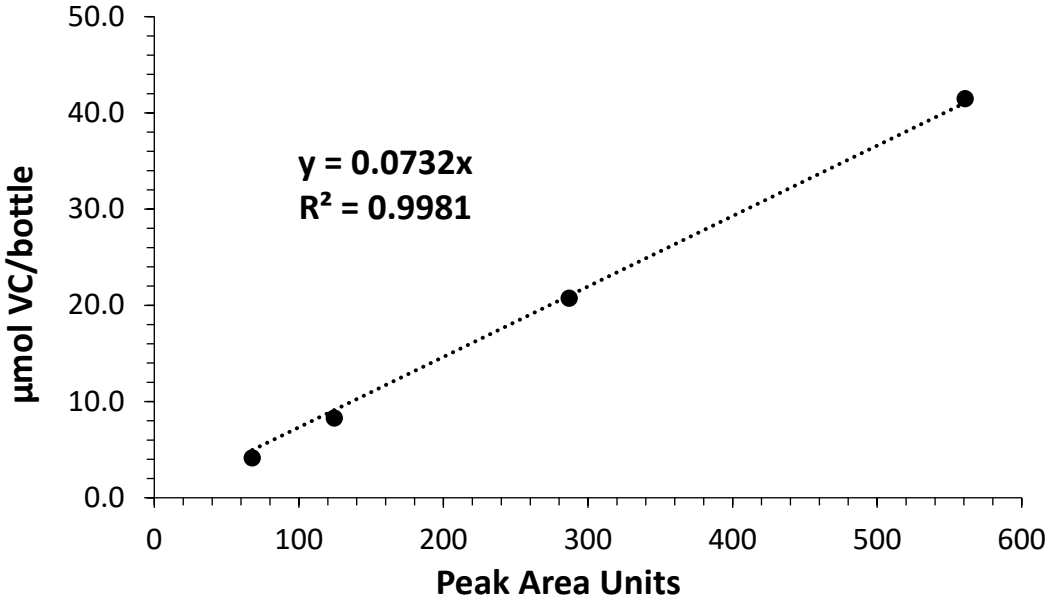

Figure A.47. The amount of VC per bottle in Set II was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Set III

The third set of standards was not used for analytical purposes because there were clear outliers within the results and the response factors were noticeably different from the other sets of response factors. The third set of response factors for TCE and cDCE was determined using a methanol stock solution containing 0.10 mL of neat TCE and 0.10 mL of neat cDCE dissolved in 50 mL of reagent grade methanol. Gravimetric analysis was done to determine the concentrations of TCE and cDCE in the methanol stock solution. Four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. Increasing volumes of stock solution (10, 50, 100, and 200 µL) were injected into the serum bottles with DDI water and glass

beads. The moles of TCE and cDCE in each serum bottle was determined based on the mass of the methanol stock solution added and the mass of each compound per mass of stock solution. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The amount of TCE and cDCE was determined by injecting 0.5 mL of headspace samples into the GC-FID. The peak areas at the corresponding retention times were recorded. The response factors for TCE and cDCE were determined using the slope of the regression line for the μmol gas per bottle vs. the peak area units (PAUs), forced through the origin. This analysis determined that TCE has a response factor of 0.2854 μmol/PAU (**Figure A.48**) and cDCE has a response factor of 0.5572 μmol/PAU (**Figure A.49**).

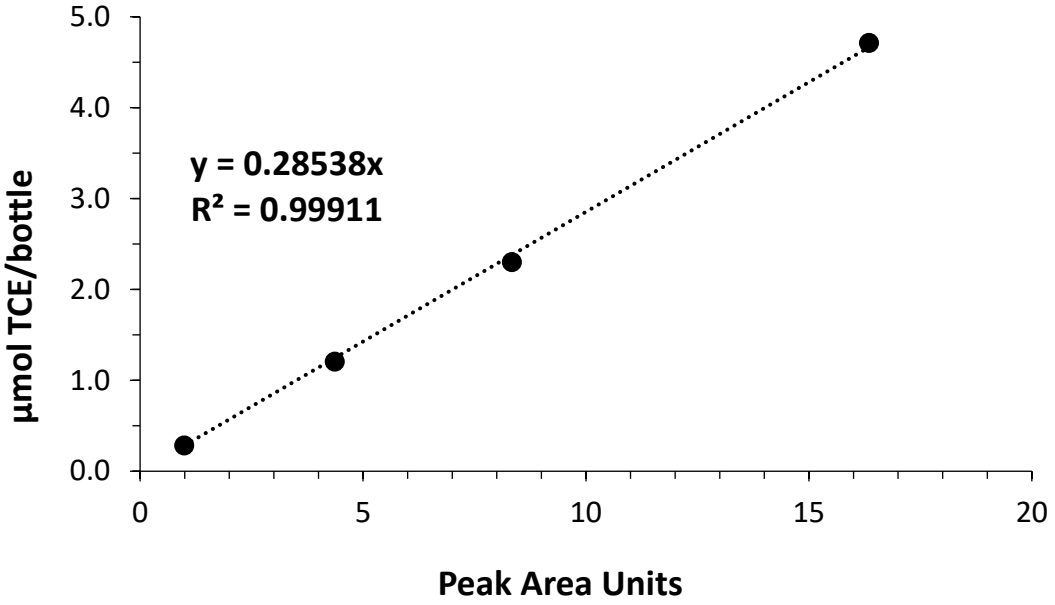

Figure A.48. The amount of TCE per bottle in Set III was calculated using gravimetric analysis and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

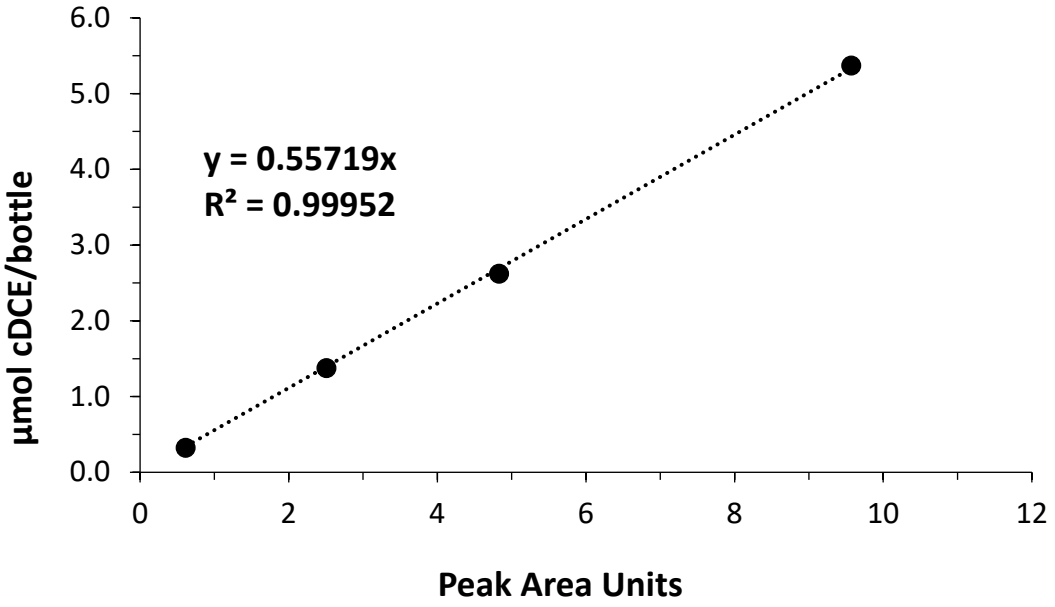

Figure A.49. The amount of cDCE per bottle in Set II was calculated using gravimetric analysis and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Response factors for methane, acetylene, ethene, ethane, and VC were determined using the GC-FID. Two sets of four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. 0.05, 0.10, 0.15, and 0.20 mL of acetylene and ethane were injected into the first set of bottles with DDI water and glass beads. 0.10, 0.20, 0.50, and 1.00 mL of methane, ethene, and VC were injected into the second set of bottles with DDI water and glass beads. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The moles of each gas added to each bottle was calculated using the ideal gas law. The barometric pressure and room temperature were recorded to be used in this calculation. The amount of methane, acetylene, ethene, ethane, and VC was determined by injecting 0.5 mL of headspace samples into the GC-FID. The peak areas at the

corresponding retention times were recorded. The response factors for each gas were determined using the slope of the regression line for the μmol gas per bottle vs. the PAUs, forced through the origin. This analysis determined that methane has a response factor of 0.09783 μmol/PAU (**Figure A.50**), acetylene has a response factor of 0.10304 μmol/PAU (**Figure A.51**), ethene has a response factor of 0.05810 μmol/PAU (**Figure A.52**), ethane has a response factor of 0.04970 μmol/PAU (**Figure A.53**), and VC has a response factor of 0.11239 μmol/PAU (**Figure A.54**).

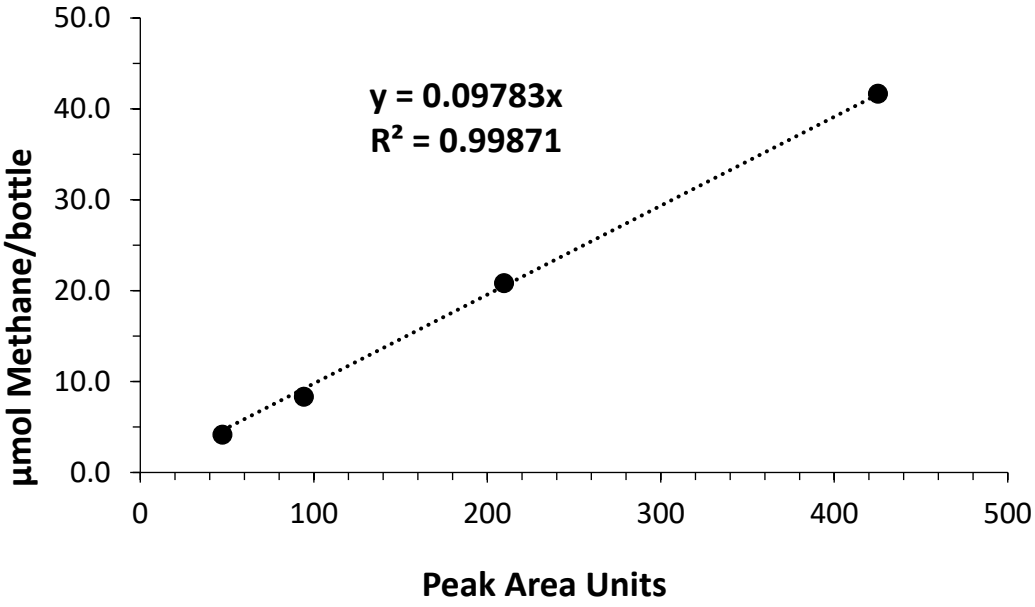

Figure A.50. The amount of methane per bottle in Set III was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

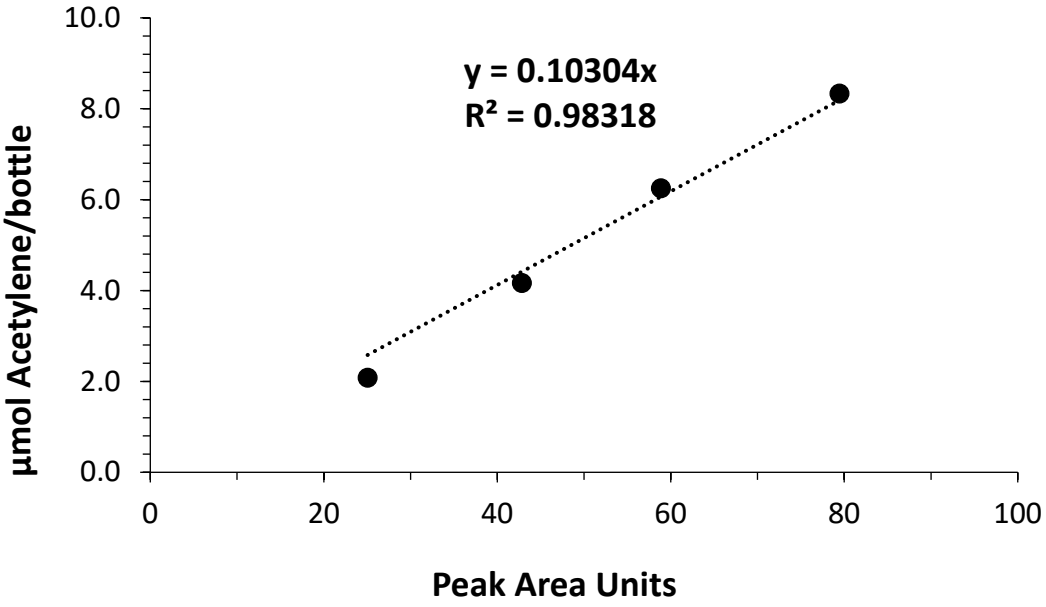

Figure A.51. The amount of acetylene per bottle in Set III was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

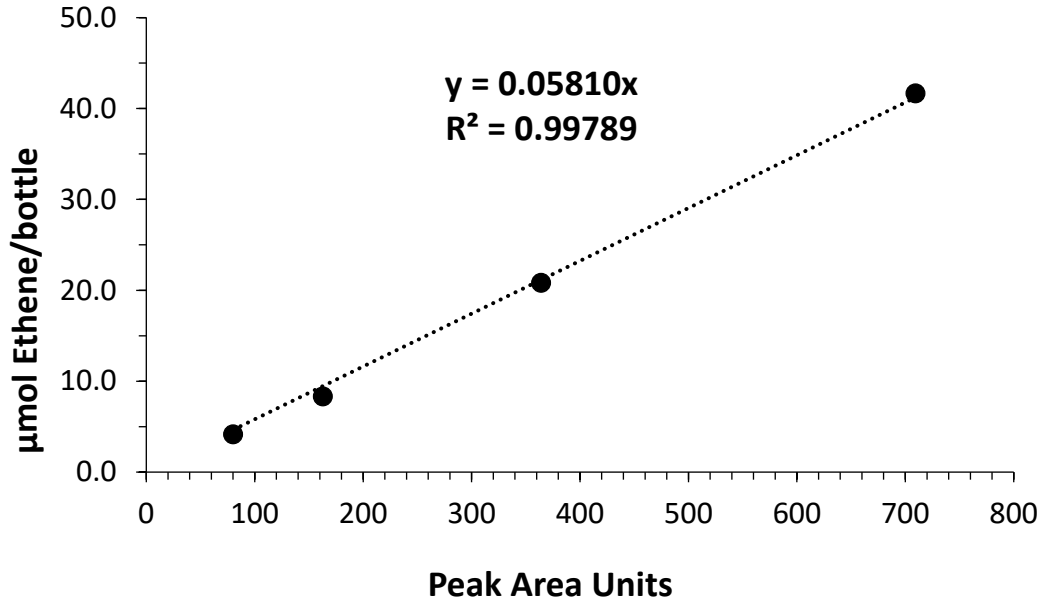

Figure A.52. The amount of ethene per bottle in Set III was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

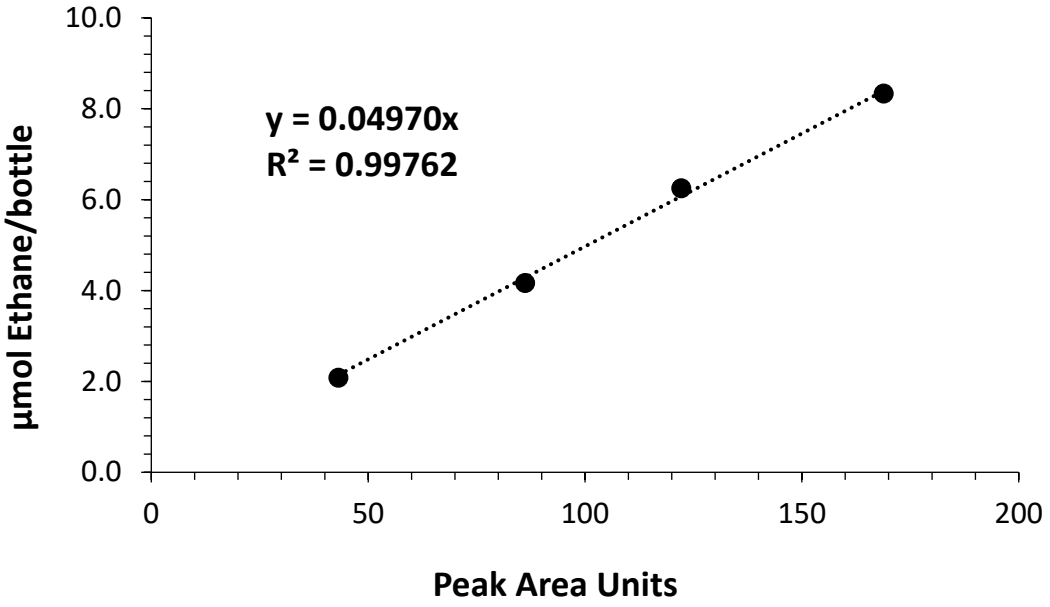

Figure A.53. The amount of ethane per bottle in Set III was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

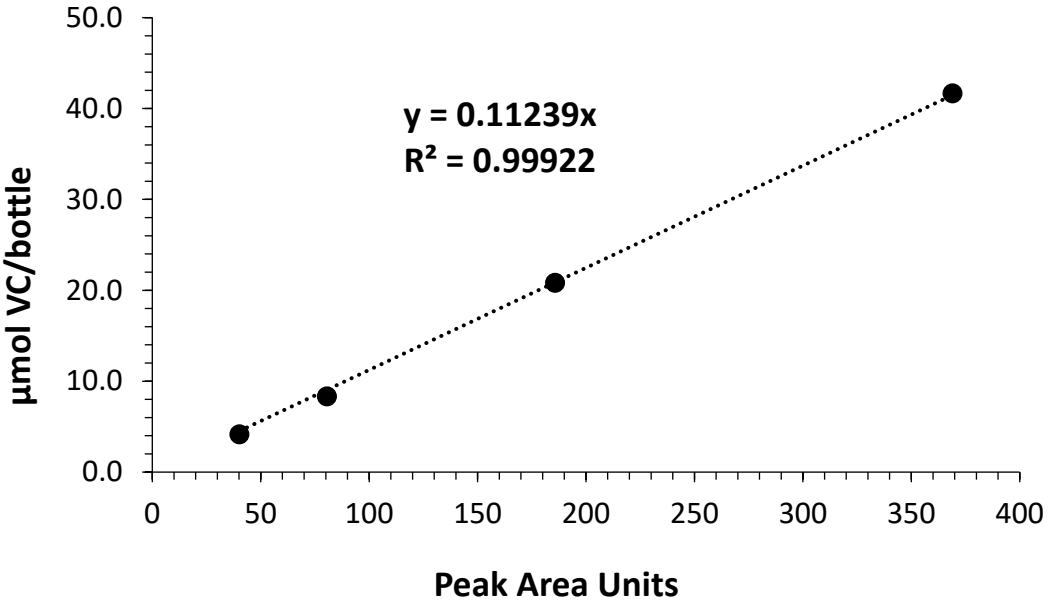

Figure A.54. The amount of VC per bottle in Set III was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Set IV

The fourth set of response factors for TCE and cDCE was determined using a methanol stock solution containing 0.10 mL of neat TCE and 0.10 mL of neat cDCE dissolved in 50 mL of reagent grade methanol. Gravimetric analysis was done to determine the concentrations of TCE and cDCE in the methanol stock solution. Four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. Increasing volumes of stock solution (10, 50, 100, and 200 μL) were injected into the serum bottles with DDI water and glass beads. The moles of TCE and cDCE in each serum bottle was determined based on the mass of the methanol stock solution added and the mass of each compound per mass of stock solution. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The amount of TCE and cDCE was determined by injecting 0.5 mL of headspace samples into the GC-FID. The peak areas at the corresponding retention times were recorded. The response factors for TCE and cDCE were determined using the slope of the regression line for the μmol gas per bottle vs. the peak area units (PAUs), forced through the origin. This analysis determined that TCE has a response factor of 0.1371 μmol/PAU (**Figure A.55**) and cDCE has a response factor of 0.2788 μmol/PAU (**Figure A.56**).

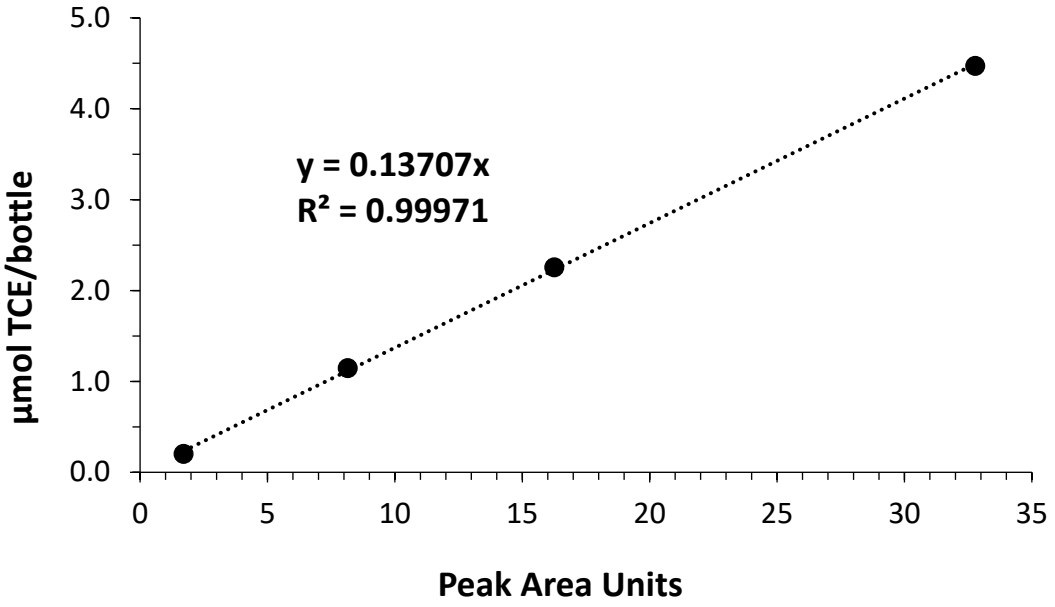

Figure A.55. The amount of TCE per bottle in Set IV was calculated using gravimetric analysis and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

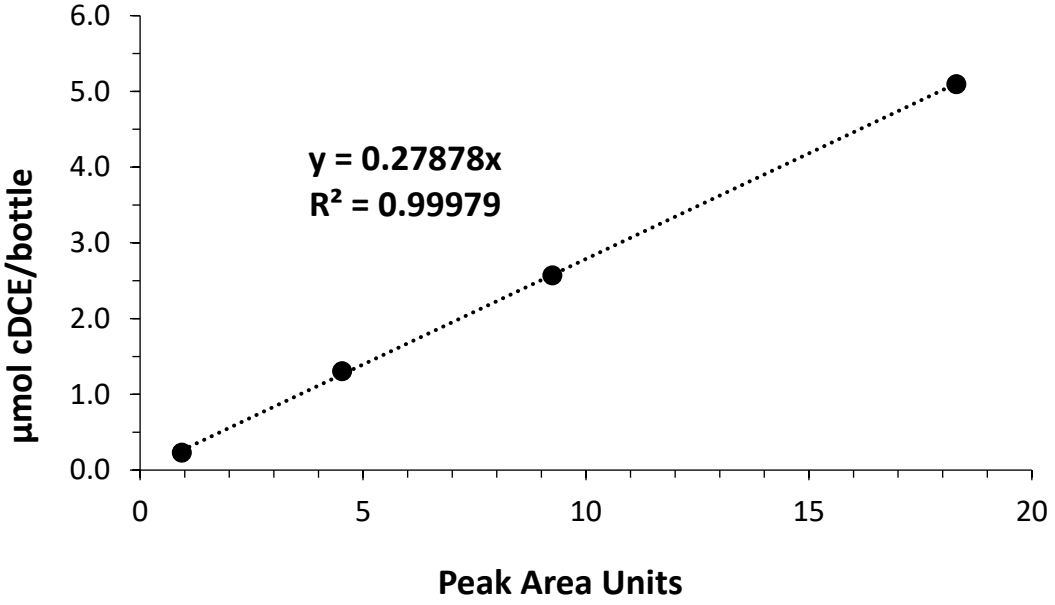

Figure A.56. The amount of cDCE per bottle in Set IV was calculated using gravimetric analysis and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Response factors for methane, acetylene, ethene, ethane, and VC were determined using the GC-FID. Two sets of four 160 mL serum bottles were filled with enough glass beads to displace 21 mL of water and 78 mL of DDI water. 0.05, 0.10, 0.15, and 0.20 mL of acetylene and ethane were injected into the first set of bottles with DDI water and glass beads. 0.10, 0.20, 0.50, and 1.00 mL of methane and ethene and 0.10, 0.20, 0.35, and 0.5 mL of VC were injected into the second set of bottles with DDI water and glass beads. The serum bottles were inverted and placed on a shaker table to ensure equilibrium between the headspace and liquid phases. The moles of each gas added to each bottle was calculated using the ideal gas law. The barometric pressure and room temperature were recorded to be used in this calculation. The amount of methane, acetylene, ethene, ethane, and VC was determined by injecting 0.5 mL of headspace samples into the GC-FID. The peak areas at the corresponding retention times were recorded. The response factors for each gas were determined using the slope of the regression line for the μmol gas per bottle vs. the PAUs, forced through the origin. This analysis determined that methane has a response factor of 0.06362 μmol/PAU (**Figure A.57**), acetylene has a response factor of 0.0545 μmol/PAU (**Figure A.58**), ethene has a response factor of 0.03678 μmol/PAU (**Figure A.59**), ethane has a response factor of 0.0305 μmol/PAU (**Figure A.60**), and VC has a response factor of 0.07224 μmol/PAU (**Figure A.61**).

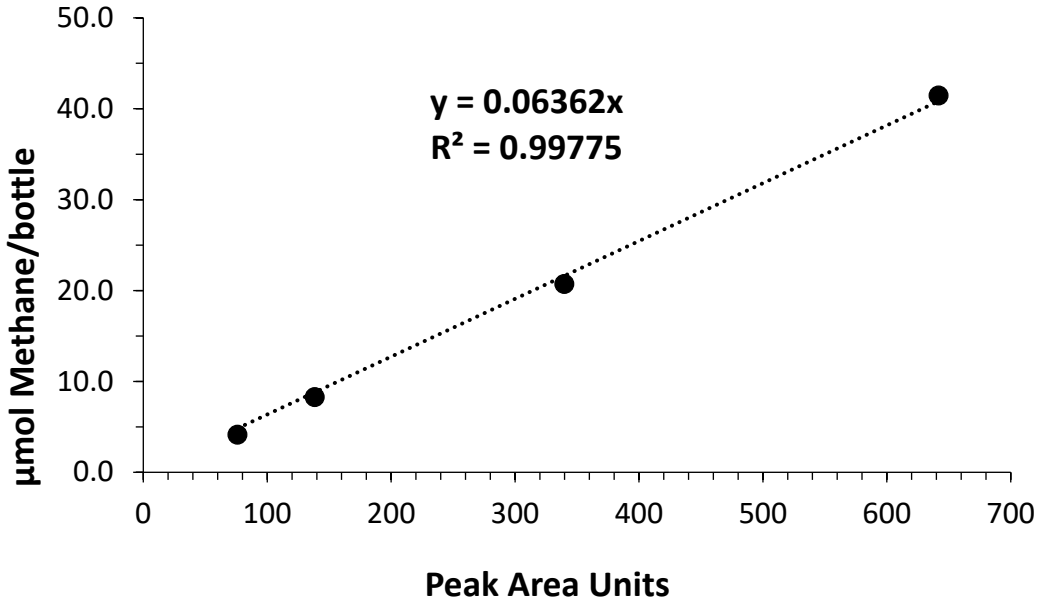

Figure A.57. The amount of methane per bottle in Set IV was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

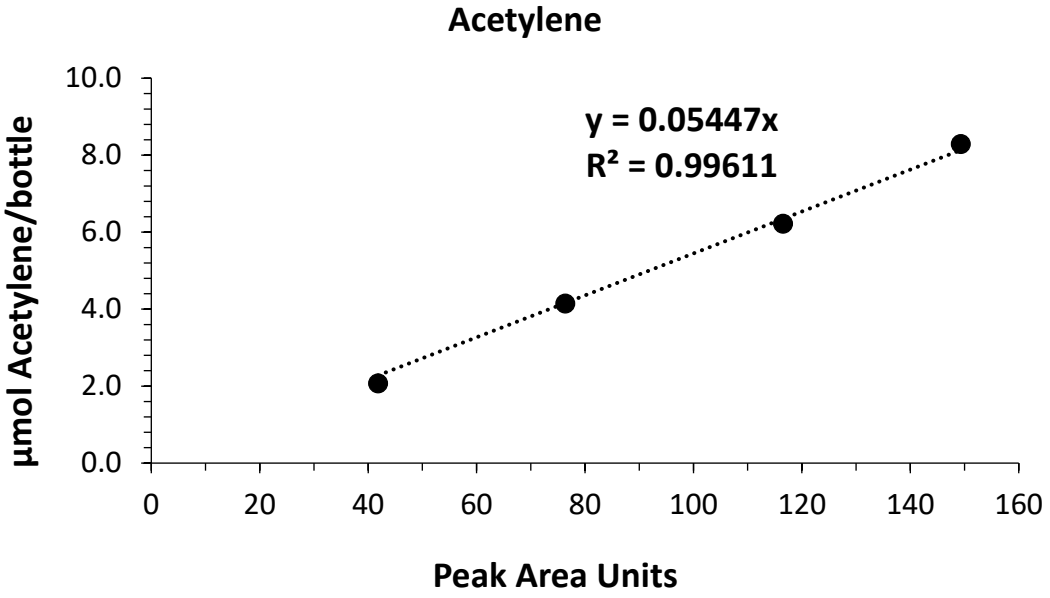

Figure A.58. The amount of acetylene per bottle in Set IV was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

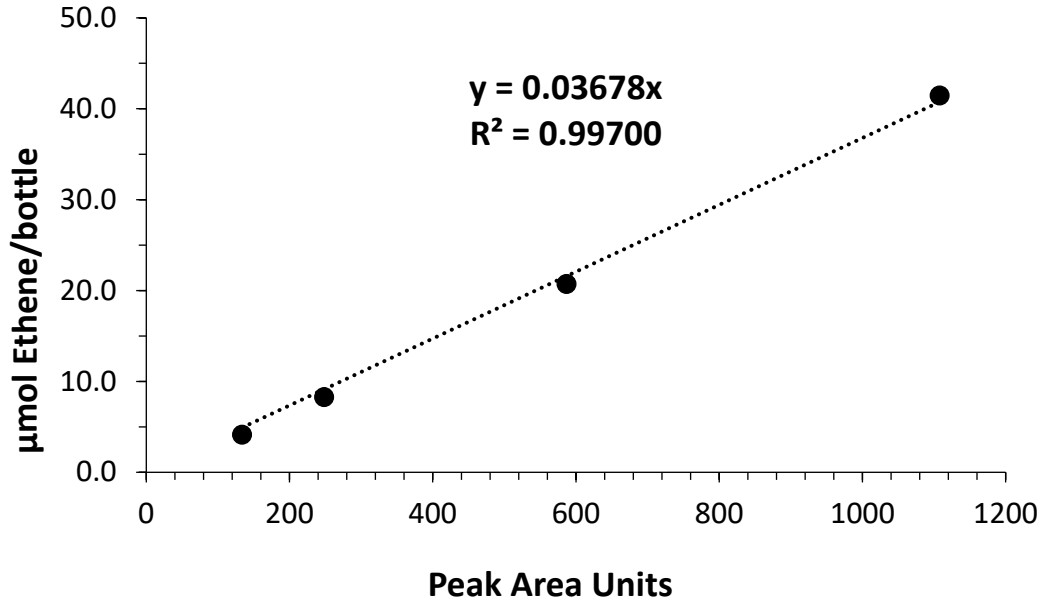

Figure A.59. The amount of ethene per bottle in Set IV was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

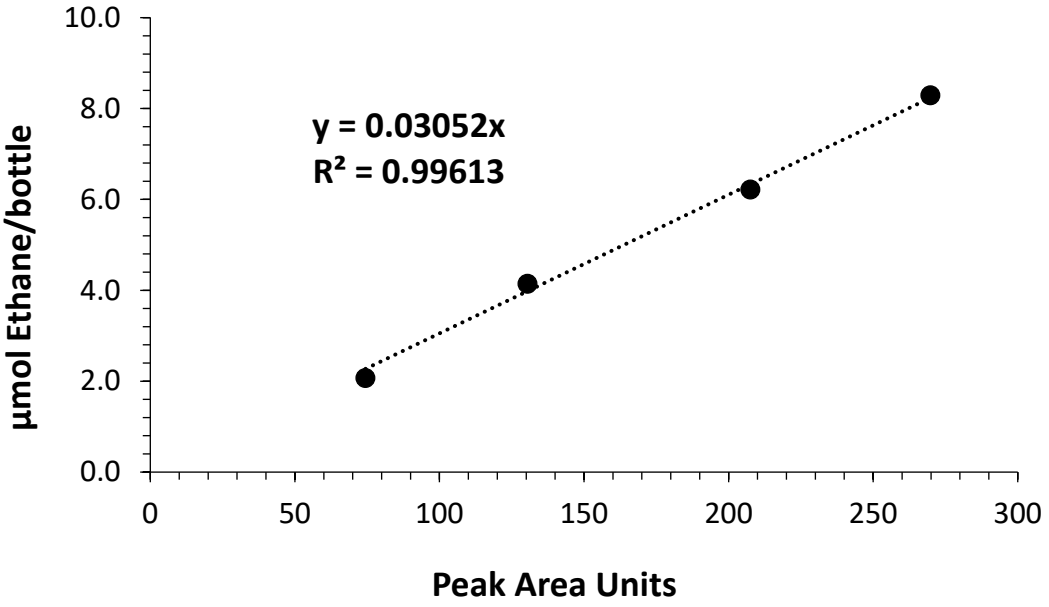

Figure A.60. The amount of ethane per bottle in Set IV was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

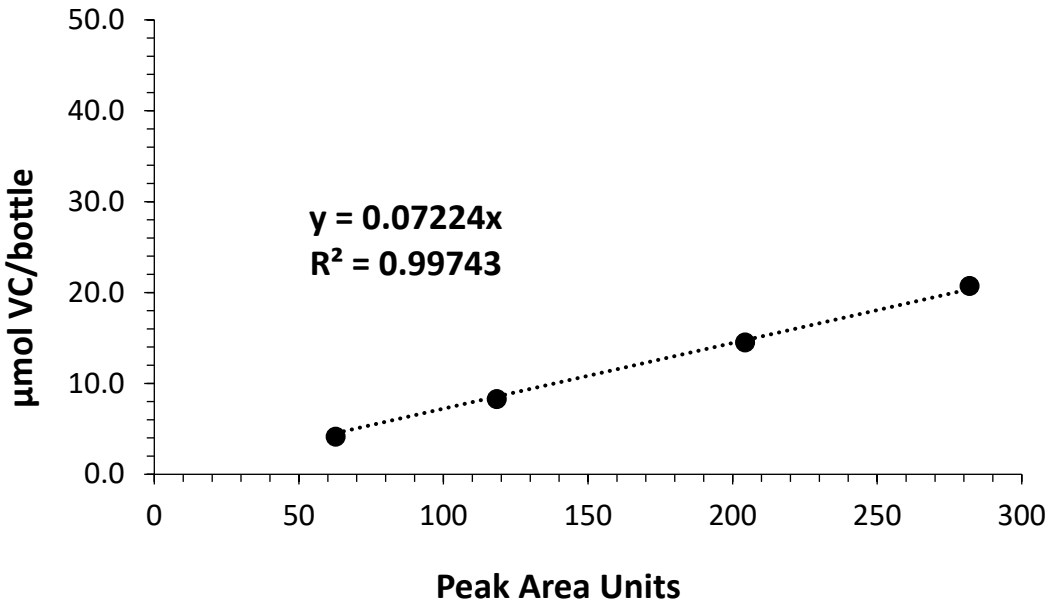

Figure A.61. The amount of VC per bottle in Set IV was calculated using the ideal gas law and is plotted against peak area units from the GC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 µmol/bottle.

Summary Tables

Table A.16. Summary of Set I response factors and coefficients of determination for VOC measurements taken using the GC.

			Response Factor	
Compound	**GC RT (min)**	**H_c**	**(µmol/bottle)**	**R^2**
Methane	0.576	28.130	0.0448	99.99%
Acetylene	0.744	0.905	0.0400	99.97%
Ethene	0.869	7.240	0.0257	99.94%
Ethane	1.005	17.300	0.0225	99.95%
VC	3.324	0.846	0.0512	100.00%
*c*DCE	7.178	0.113	0.2160[a]	99.79%
TCE	10.373	0.269	0.0966	99.85%

[a] the response factor for *c*DCE could not be measured at the same time as all of the other chemicals. An old response factor will be used until it is measured in the next set.

Table A.17. Summary of Set II response factors and coefficients of determination for VOC measurements taken using the GC.

			Response Factor	
Compound	**GC RT (min)**	**H_c**	**(µmol/bottle)**	**R^2**
Methane	0.576	28.130	0.0668	99.99%
Acetylene	0.744	0.905	0.0601	99.97%
Ethene	0.869	7.240	0.0387	99.94%
Ethane	1.005	17.300	0.0337	99.95%
VC	3.324	0.846	0.0732	100.00%
*c*DCE	7.178	0.113	0.2901	99.79%
TCE	10.373	0.269	0.1348	99.85%

Table A.18. Summary of Set III response factors and coefficients of determination for VOC measurements taken using the GC.

			Response Factor	
Compound	**GC RT (min)**	**H_c**	**(µmol/bottle)**	**R^2**
Methane	0.591	28.130	0.0978	99.87%
Acetylene	0.789	0.905	0.1030	98.32%
Ethene	0.894	7.240	0.0581	99.79%
Ethane	1.022	17.300	0.0497	99.76%
VC	3.324	0.846	0.1124	99.92%
*c*DCE	7.198	0.113	0.5572	99.95%
TCE	10.473	0.269	0.2854	99.91%

Table A.19. Summary of Set IV response factors and coefficients of determination for VOC measurements taken using the GC.

			Response Factor	
Compound	**GC RT (min)**	**H_c**	**(µmol/bottle)**	**R^2**
Methane	0.582	28.130	0.0636	99.92%
Acetylene	0.773	0.905	0.0545	99.94%
Ethene	0.889	7.240	0.0368	99.89%
Ethane	1.018	17.300	0.0305	99.94%
VC	3.332	0.846	0.0722	99.94%
*c*DCE	7.200	0.113	0.2788	99.99%
TCE	10.460	0.269	0.1371	99.99%

A.22. Response Factors – Organic Acids

Set I

Response factors for lactate, acetate, formate, and propionate were determined using a stock solution of 5 mM sodium lactate syrup, sodium acetate, sodium formate, and propionic acid. Dilutions of 1, 0.5 and 0.1 mM were made by diluting 20, 10, and 2 mL of the 5 mM stock solution into 100 mL of DDI water. These dilutions and the remaining 5 mM stock solution were stored in serum bottles sealed with slotted grey butyl rubber caps and aluminum crimp caps. The moles of sodium lactate, sodium acetate, sodium formate, and propionic acid in each serum bottle was determined based on the amount of the stock solution added and the mass of each compound added to the stock solution. The amount of each compound in each serum bottle was determined by placing 1 mL samples of each dilution into the HPLC. The peak areas at the corresponding retention times were recorded. The response factors for each fatty acid were determined using the slope of the regression line for the mmol per

bottle vs the PAUs, forced through the origin. This analysis determined that lactate has a response factor of 0.2074 mM/PAU (**Figure A.62**), acetate has a response factor of 0.4125 mM/PAU (**Figure A.63**), formate has a response factor of 0.2839 mM/PAU (**Figure A.64**), and propionate has a response factor of 0.3091 (**Figure A.65)**.

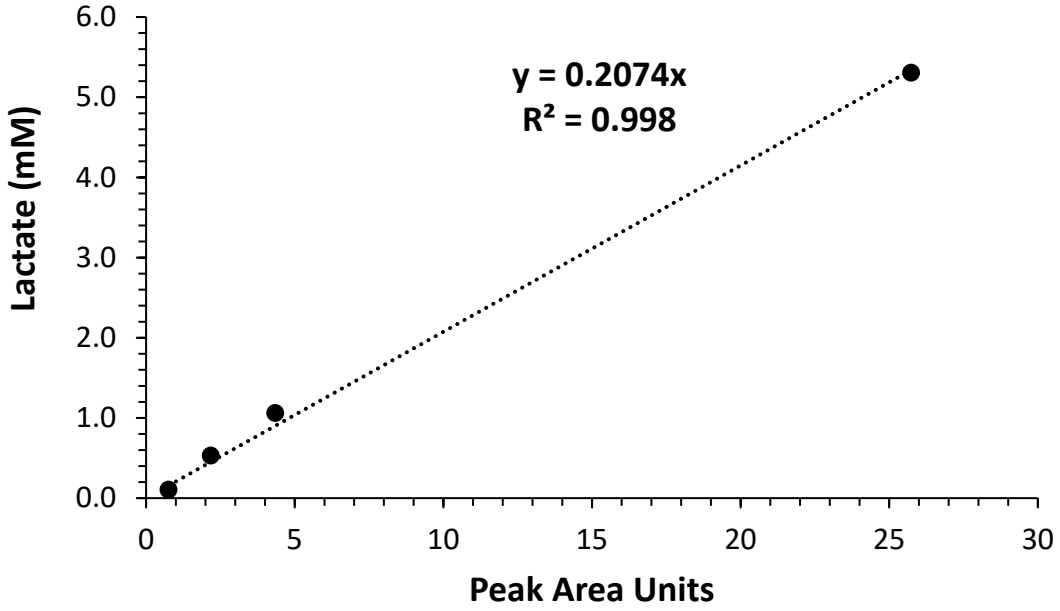

Figure A.62. The amount of lactate per bottle was calculated by diluting a stock solution and is plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

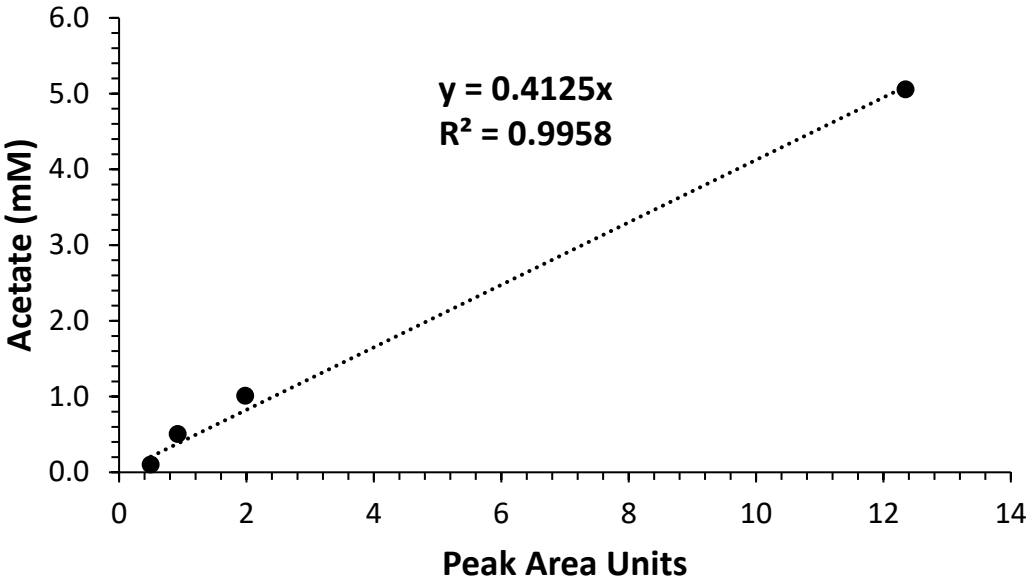

Figure A.63. The amount of acetate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

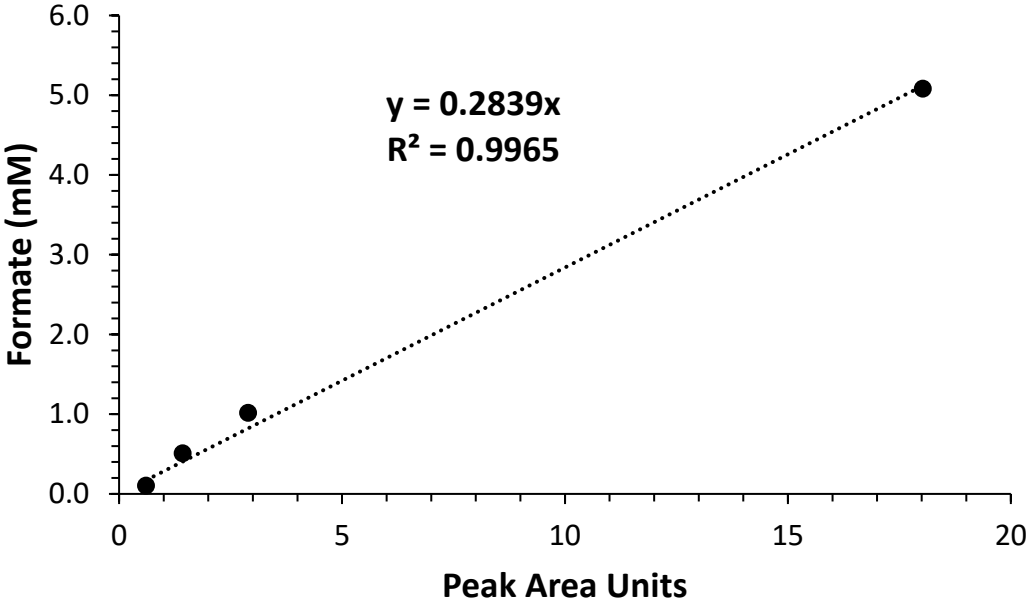

Figure A.64. The amount of formate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

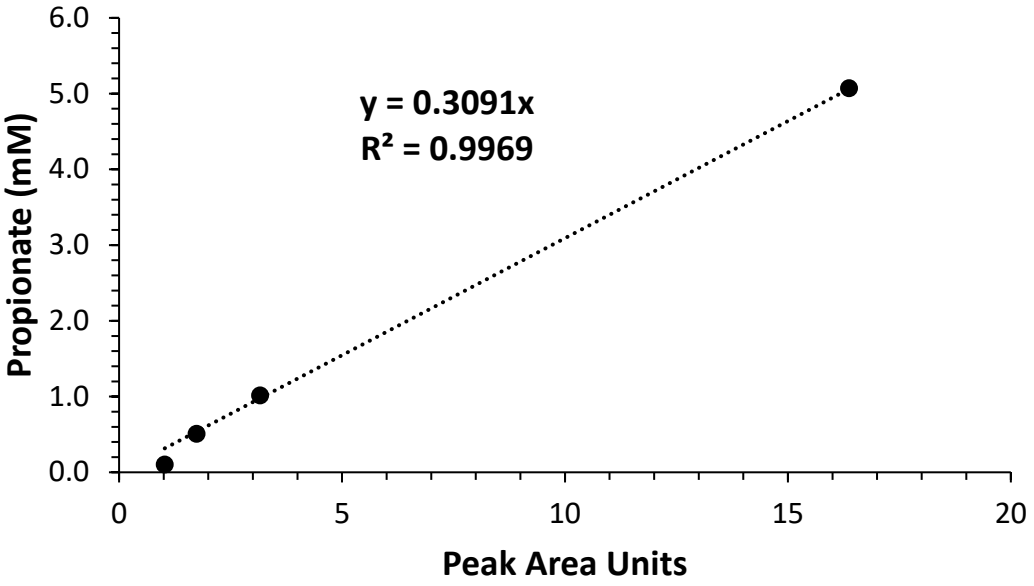

Figure A.65. The amount of propionate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

Set II

Response factors for lactate, acetate, formate, and propionate were determined using a stock solution of 5 mM sodium lactate syrup, sodium acetate, sodium formate, and propionic acid. Dilutions of 1, 0.5 and 0.1 mM were made by diluting 20, 10, and 2 mL of the 5 mM stock solution into 100 mL of DDI water. These dilutions and the remaining 5 mM stock solution were stored in serum bottles sealed with slotted grey butyl rubber caps and aluminum crimp caps. The moles of sodium lactate, sodium acetate, sodium formate, and propionic acid in each serum bottle was determined based on the amount of the stock solution added and the mass of each compound added to the stock solution. The amount of each compound in each serum bottle was determined by placing 1 mL samples of each dilution into the HPLC. The peak areas at the

corresponding retention times were recorded. The response factors for each fatty acid were determined using the slope of the regression line for the mmol per bottle vs the PAUs, forced through the origin. This analysis determined that lactate has a response factor of 0.2118 mM/PAU (**Figure A.66**), acetate has a response factor of 0.4133 mM/PAU (**Figure A.67**), formate has a response factor of 0.2909 mM/PAU (**Figure A.68**), and propionate has a response factor of 0.3175 (**Figure A.69)**.

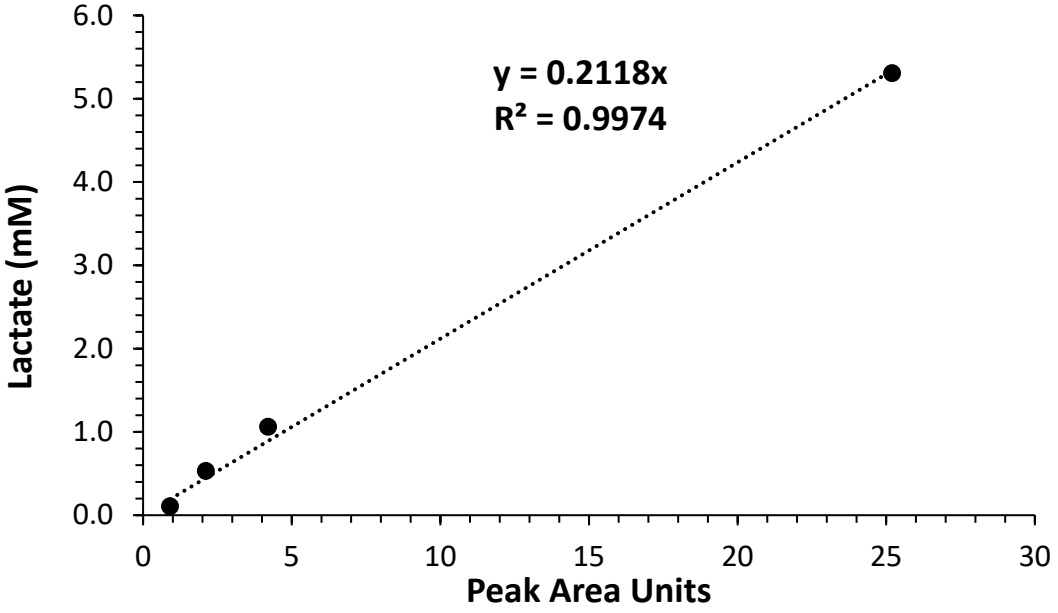

Figure A.66. The amount of lactate per bottle was calculated by diluting a stock solution and is plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

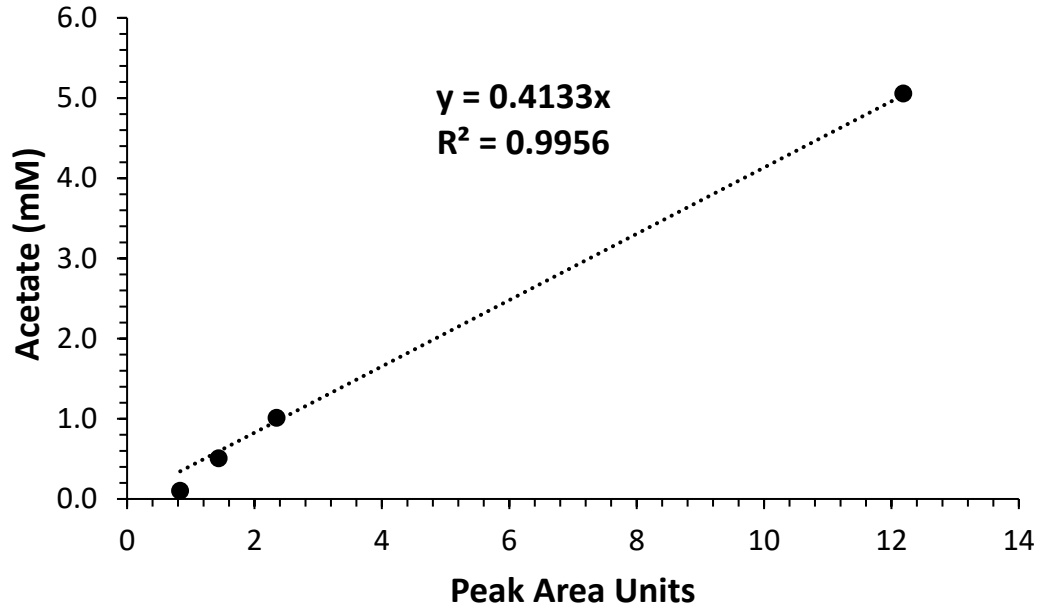

Figure A.67. The amount of acetate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

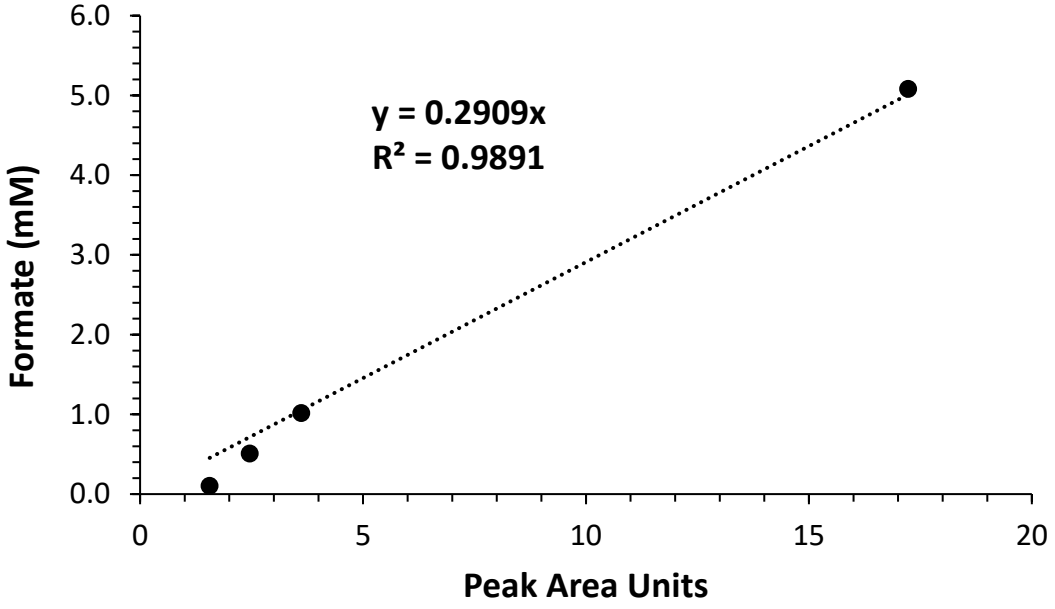

Figure A.68. The amount of formate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

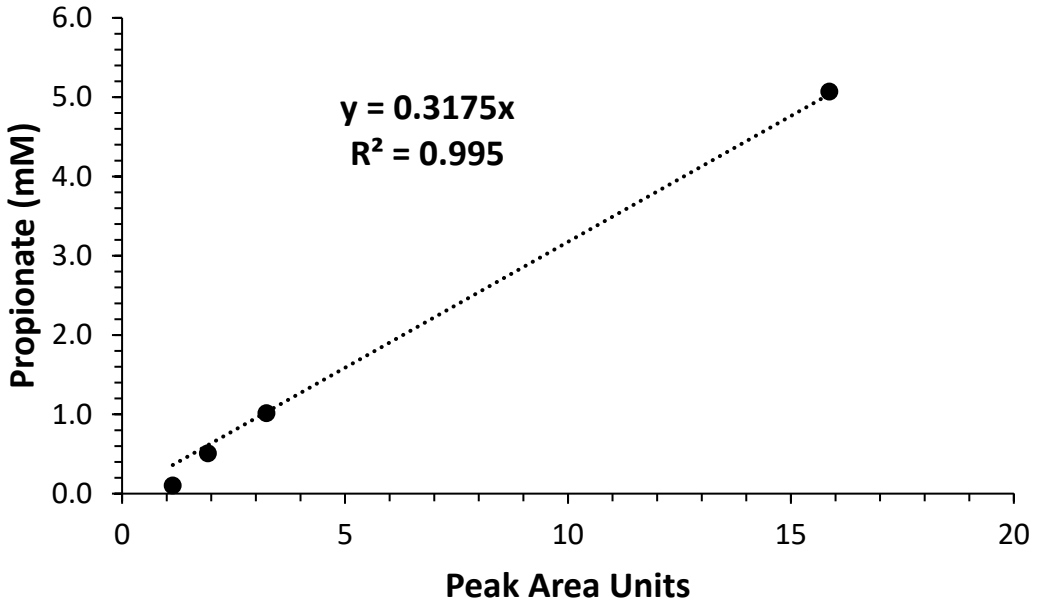

Figure A.69. The amount of propionate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

Set III

Set III of response factors were done on a different HPLC than the first two sets because of issues with the first HPLC. Response factors for lactate, acetate, formate, and propionate were determined using a stock solution of 5 mM sodium lactate syrup, sodium acetate, sodium formate, and propionic acid. Dilutions of 1, 0.5 and 0.1 mM were made by diluting 20, 10, and 2 mL of the 5 mM stock solution into 100 mL of DDI water. These dilutions and the remaining 5 mM stock solution were stored in serum bottles sealed with slotted grey butyl rubber caps and aluminum crimp caps. The moles of sodium lactate, sodium acetate, sodium formate, and propionic acid in each serum bottle was determined based on the amount of the stock solution added and the mass of each compound added to the stock solution. The amount of each compound in each serum

bottle was determined by placing 1 mL samples of each dilution into the HPLC. The peak areas at the corresponding retention times were recorded. The response factors for each fatty acid were determined using the slope of the regression line for the mmol per bottle vs the PAUs, forced through the origin. This analysis determined that lactate has a response factor of 0.0035 mM/PAU (**Figure A.70**), acetate has a response factor of 0.0064 mM/PAU (**Figure A.71**), formate has a response factor of 0.0053 mM/PAU (**Figure A.72**), and propionate has a response factor of 0.0049 (**Figure A.73)**.

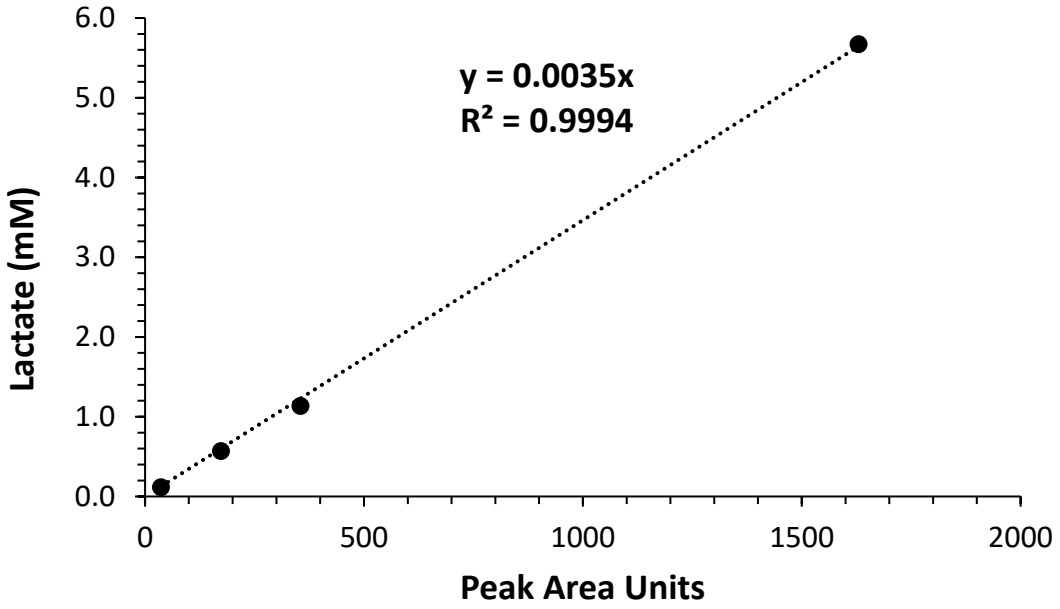

Figure A.70. The amount of lactate per bottle was calculated by diluting a stock solution and is plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

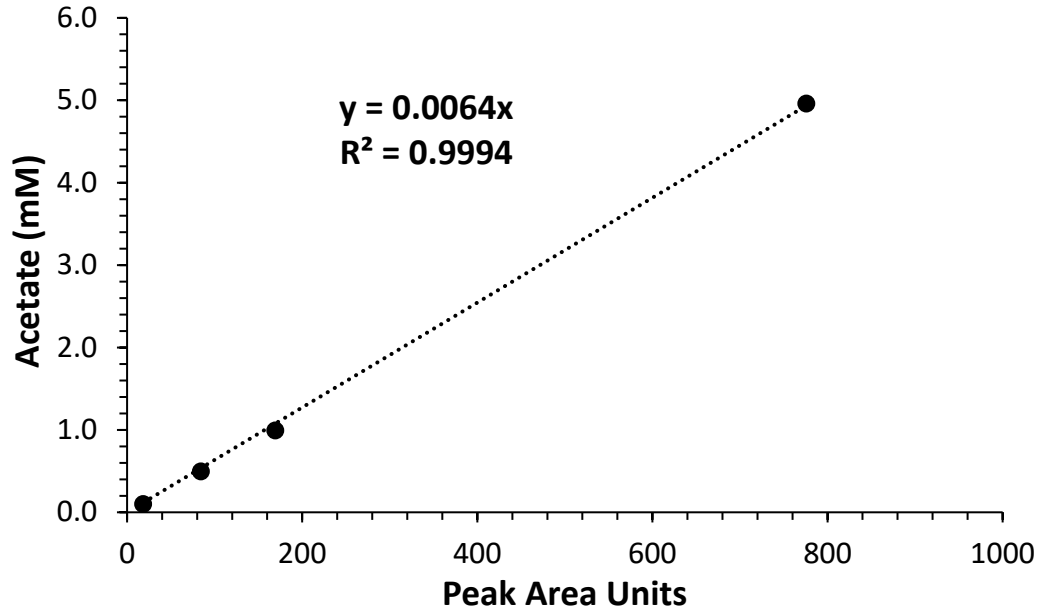

Figure A.71. The amount of acetate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

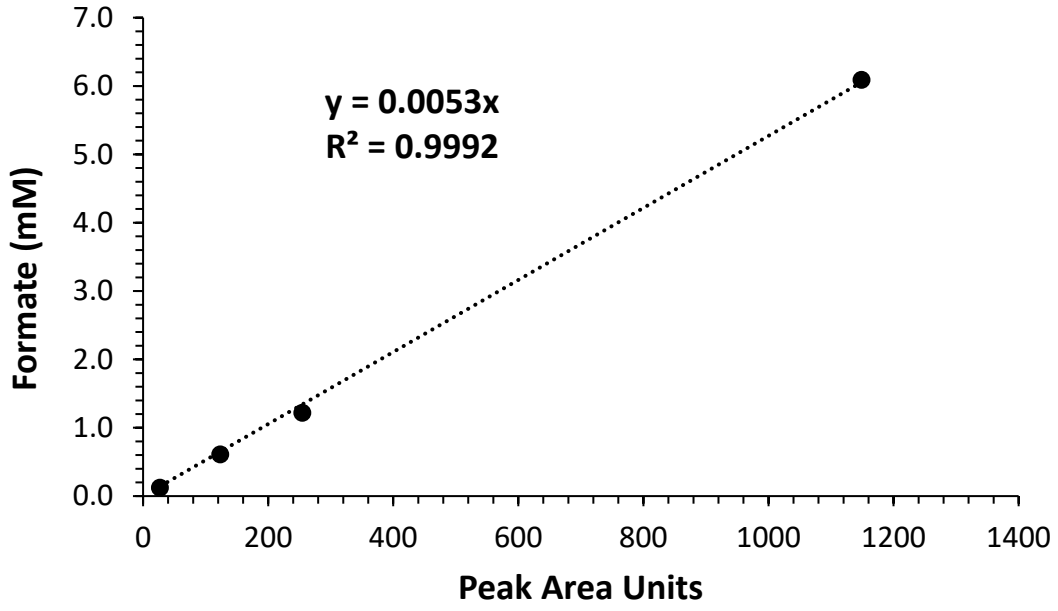

Figure A.72. The amount of formate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

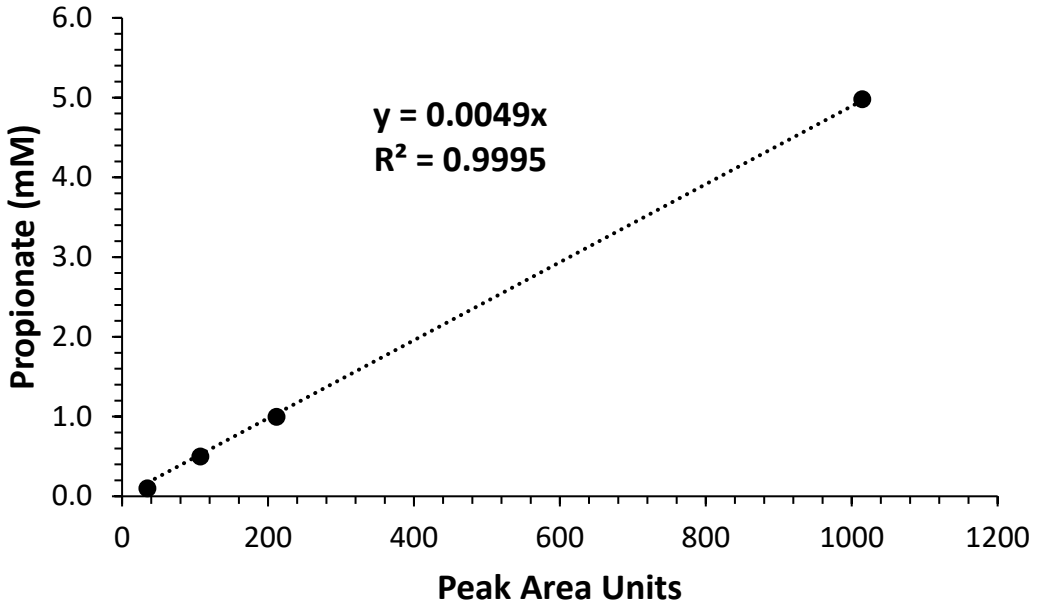

Figure A.73. The amount of propionate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

Set IV

Set IV of response factors were done using the same HPLC as Set III. Response factors for lactate, acetate, formate, and propionate were determined using a stock solution of 5 mM sodium lactate syrup, sodium acetate, sodium formate, and propionic acid. Dilutions of 1, 0.5 and 0.1 mM were made by diluting 20, 10, and 2 mL of the 5 mM stock solution into 100 mL of DDI water. These dilutions and the remaining 5 mM stock solution were stored in serum bottles sealed with slotted grey butyl rubber caps and aluminum crimp caps. The moles of sodium lactate, sodium acetate, sodium formate, and propionic acid in each serum bottle was determined based on the amount of the stock solution added and the mass of each compound added to the stock solution. The amount of each compound in each serum bottle was determined by placing 1 mL samples of each

dilution into the HPLC. The peak areas at the corresponding retention times were recorded. The response factors for each fatty acid were determined using the slope of the regression line for the mmol per bottle vs the PAUs, forced through the origin. The 1mM dilutions were measured incorrectly and were not used in determining the response factors. This analysis determined that lactate has a response factor of 0.0049 mM/PAU (**Figure A.74**), acetate has a response factor of 0.0095 mM/PAU (**Figure A.75**), formate has a response factor of 0.0075 mM/PAU (**Figure A.76**), and propionate has a response factor of 0.0068 (**Figure A.77)**.

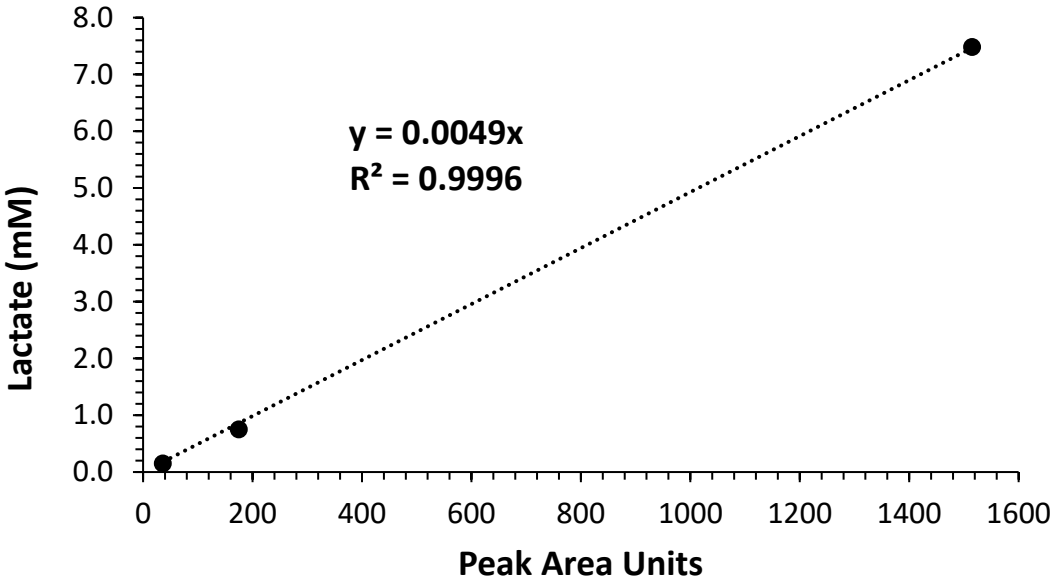

Figure A.74. The amount of lactate per bottle was calculated by diluting a stock solution and is plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

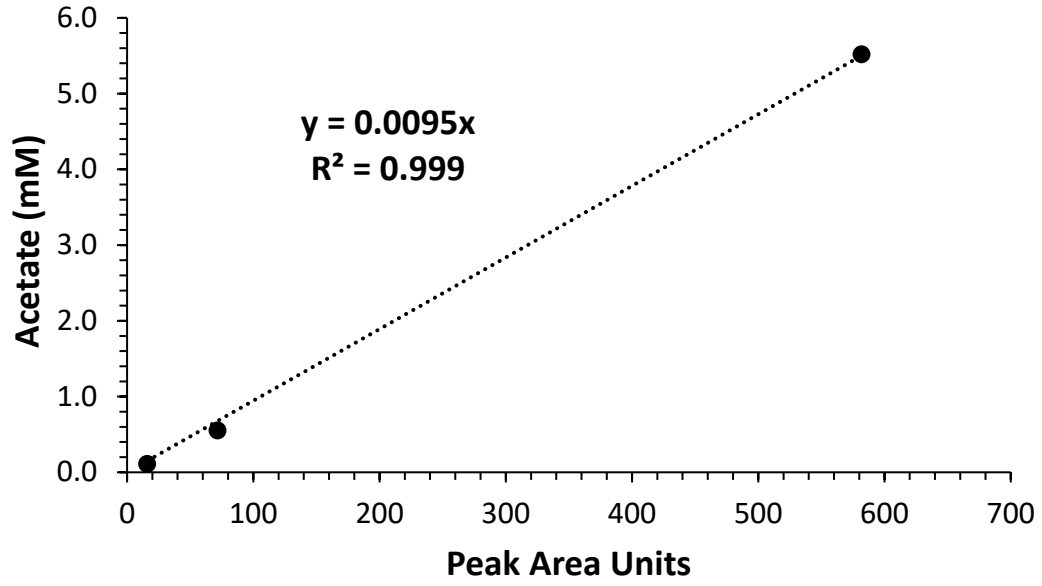

Figure A.75. The amount of acetate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

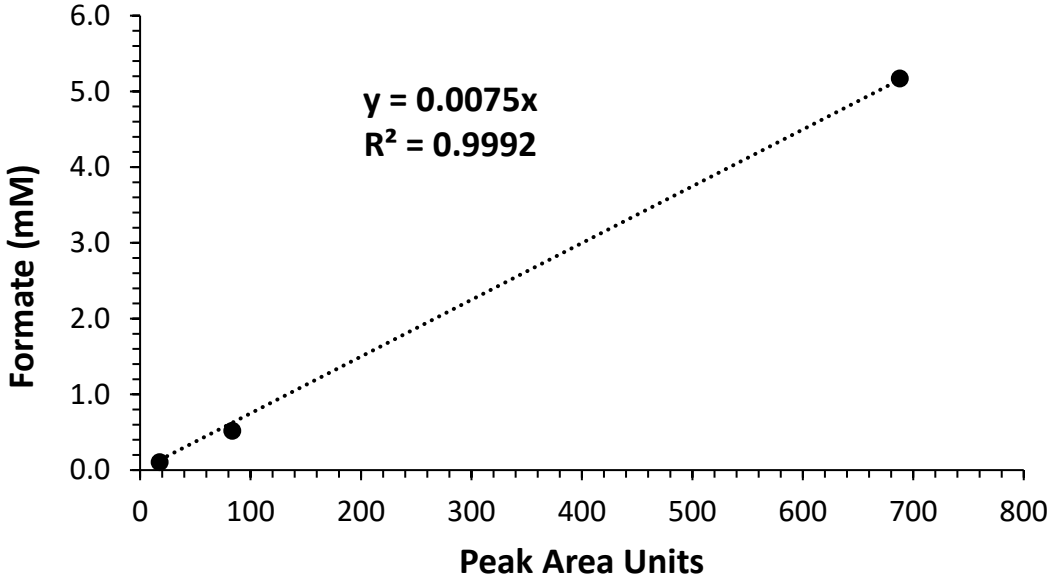

Figure A.76. The amount of formate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

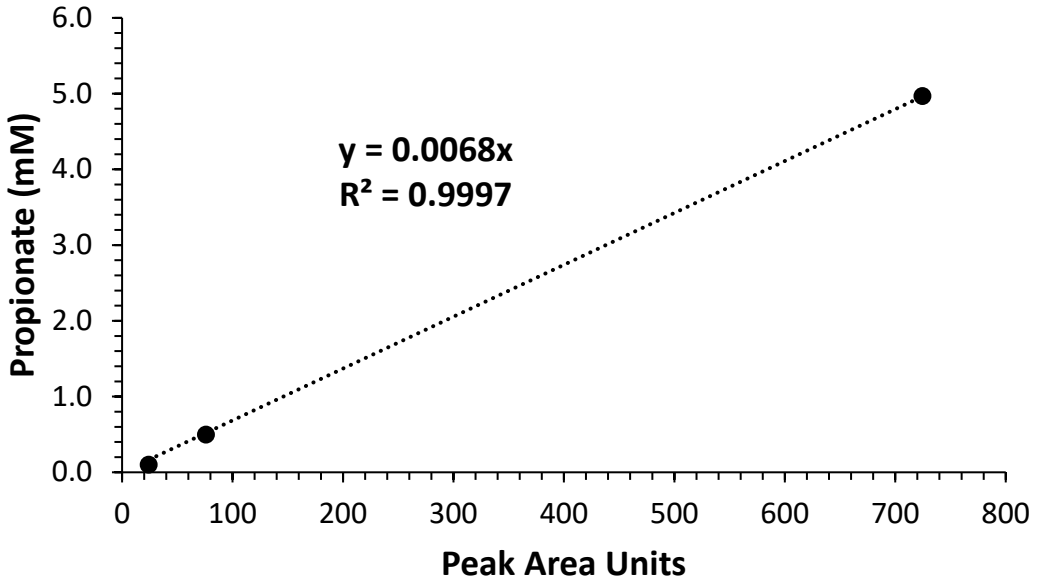

Figure A.77. The amount of propionate per bottle was calculated by diluting a stock solution and plotted against peak area units from the HPLC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

Summary Tables

Table A.20. Summary of Set I response factors and coefficients of determination for organic acid measurements taken using the HPLC.

		Response Factor	
Compound	**GC RT (min)**	**(μmol/bottle)**	**R^2**
Lactate	12.86	0.207	99.80%
Formate	14.33	0.284	99.64%
Acetate	15.54	0.413	99.58%
Propionate	18.37	0.309	99.69%

Table A.21. Summary of Set II response factors and coefficients of determination for organic acid measurements taken using the HPLC.

		Response Factor	
Compound	**GC RT (min)**	**(µmol/bottle)**	**R^2**
Lactate	13.17	0.212	99.85%
Formate	14.73	0.291	98.36%
Acetate	15.97	0.413	99.74%
Propionate	18.80	0.318	99.71%

Table A.22. Summary of Set III response factors and coefficients of determination for organic acid measurements taken using the HPLC.

		Response Factor	
Compound	**GC RT (min)**	**(µmol/bottle)**	**R^2**
Lactate	12.68	0.004	99.97%
Formate	14.11	0.005	99.95%
Acetate	15.45	0.006	99.96%
Propionate	18.34	0.005	99.97%

Table A.23. Summary of Set IV response factors and coefficients of determination for organic acid measurements taken using the HPLC.

		Response Factor	
Compound	**GC RT (min)**	**(µmol/bottle)**	**R^2**
Lactate	12.68	0.005	99.96%
Formate	14.17	0.008	99.92%
Acetate	15.47	0.010	99.90%
Propionate	18.38	0.07	99.97%

A.23. Response Factors – Nitrate and Sulfate

Set I

A response factor for sulfate was determined using a stock solution of 500 mg/L sulfate using sodium sulfate. Sodium sulfate (0.7394 g) was diluted to 1.0 L using DDI water. Standards of 50, 25, 20, 15, 10, and 5 mg/L sulfate were made by diluting 10, 5, 4, 3, 2, and 1 mL of stock to 100 mL of DDI water. A response factor for nitrate was determined using a stock solution of 100 mg N/L nitrate using potassium nitrate. Potassium nitrate (0.7212 g) was diluted to 1.0 L using DDI water. Standards of 10, 5, 2, and 1 mg/L nitrate-N were made by diluting 10, 5, 2, and 1 mL of stock to 100 mL of DDI water. These dilutions were stored in 160 mL serum bottles sealed with slotted grey butyl rubber caps and aluminum crimp caps. The amount of sulfate and nitrate was determined by placing 5 mL samples of each dilution into the IC. The peak areas at the corresponding retention times were recorded. The response factors for sulfate and nitrate were determined using the slope of the regression line for the mg per liter vs. the PAUs, forced through the origin. This analysis determined that sulfate has a response factor of 0.6879 mg/L/PAU (**Figure A.78**) and nitrate-N has a response factor of 0.1974 mg/L/PAU (**Figure A.79**).

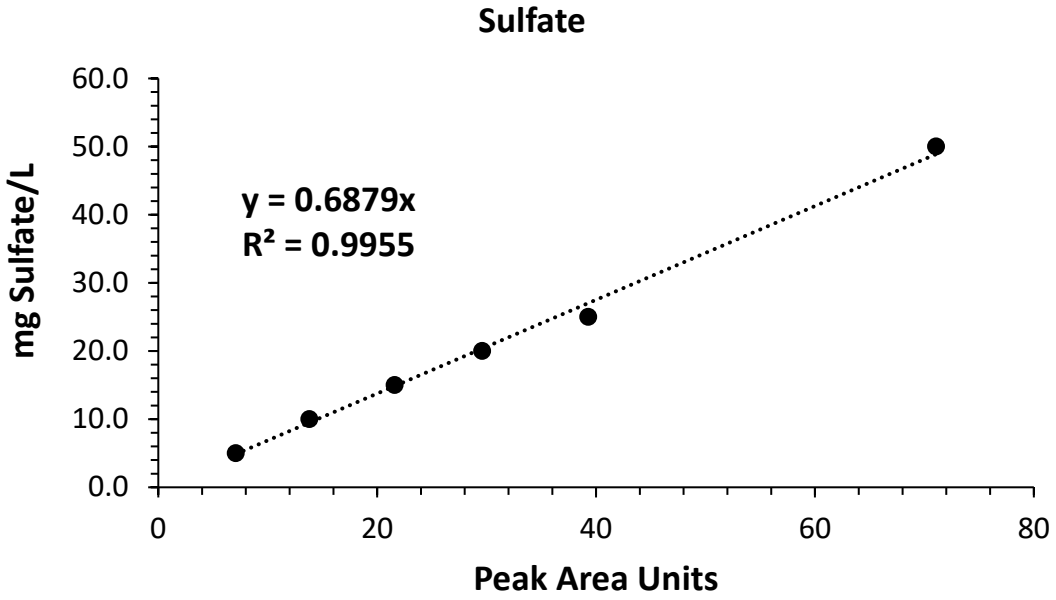

Figure A.78. The amount of sulfate per bottle in Set I was calculated by diluting a sodium sulfate stock solution and is plotted against peak area units from the IC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

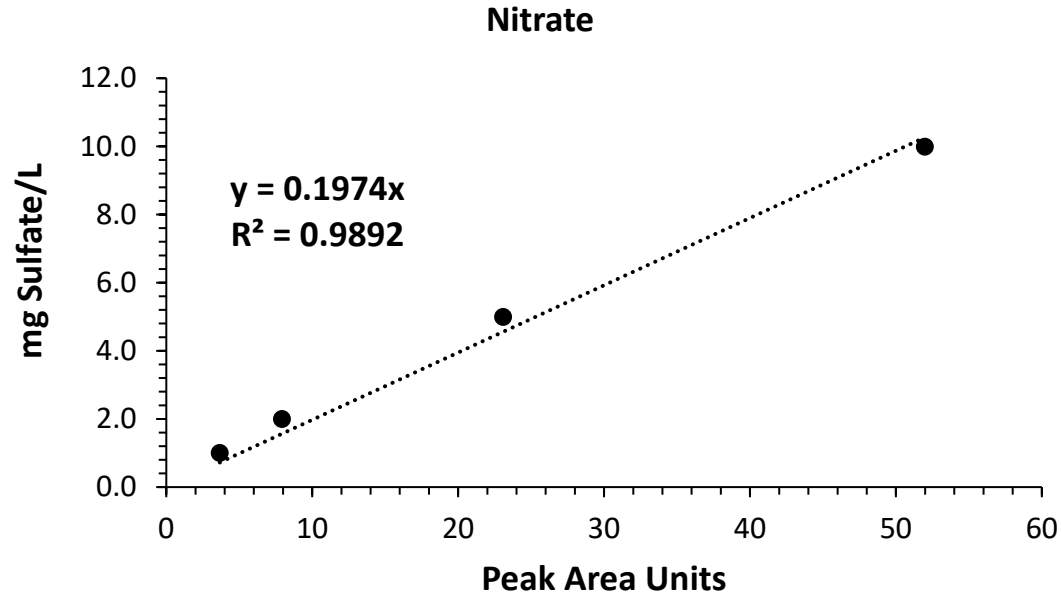

Figure A.79. The amount of nitrate-N per bottle for Set I was calculated by diluting a potassium nitrate stock solution and is plotted against peak area units from the IC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

Set II

Response factors for sulfate and nitrate were determined using a stock solution of 500 mg/L sulfate and 100 mg/L nitrate using sodium sulfate and potassium nitrate. Sodium sulfate (0.7398 g) and potassium nitrate (0.7220 g) were diluted to 1.0 L using DDI water. Standards of 50, 25, 10, and 5 mg/L sulfate and 10, 5, 2, and 1 mg/L nitrate were made by diluting 10, 5, 2, and 1 mL of stock to 100 mL of DDI water. These dilutions were stored in 160 mL serum bottles sealed with slotted grey butyl rubber caps and aluminum crimp caps. The amount of sulfate and nitrate was determined by placing 5 mL samples of each dilution into the IC. The peak areas at the corresponding retention times were recorded. The response factors for sulfate and nitrate were determined using the slope of the regression line for the mg per liter vs. the PAUs, forced through the origin. This analysis determined that sulfate has a response factor of 0.6698 mg/L/PAU (**Figure A.80**) and nitrate-N has a response factor of 0.1958 mg/L/PAU (**Figure A.81**).

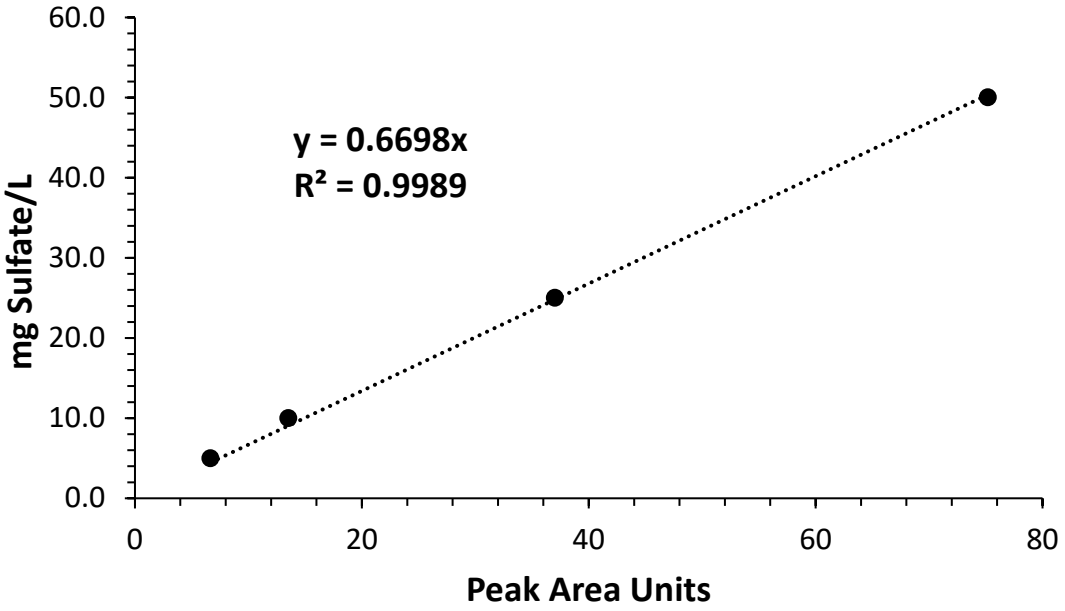

Figure A.80. The amount of sulfate per bottle in Set II was calculated by diluting a sodium sulfate stock solution and is plotted against peak area units from the IC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

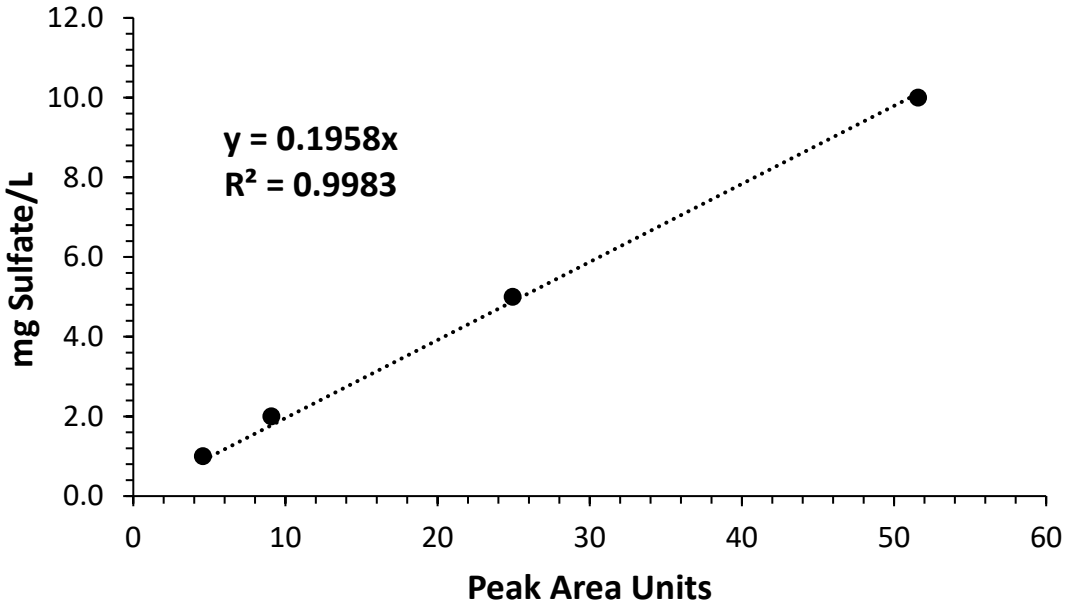

Figure A.81. The amount of nitrate-N per bottle for Set II was calculated by diluting a potassium nitrate stock solution and is plotted against peak area units from the IC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

Set III

Response factors for sulfate and nitrate were determined using a stock solution of 500 mg/L sulfate and 100 mg/L nitrate using sodium sulfate and potassium nitrate. Sodium sulfate (0.7393 g) and potassium nitrate (0.718 g) were diluted to 1.0 L using DDI water. Standards of 50, 25, 10, and 5 mg/L sulfate and 10, 5, 2, and 1 mg/L nitrate were made by diluting 10, 5, 2, and 1 mL of stock to 100 mL of DDI water. These dilutions were stored in 160 mL serum bottles sealed with slotted grey butyl rubber caps and aluminum crimp caps. The amount of sulfate and nitrate was determined by placing 5 mL samples of each dilution into the IC. The peak areas at the corresponding retention times were recorded. The response factors for sulfate and nitrate were determined using the slope of the regression line for the mg per liter vs. the PAUs, forced through the origin. This analysis determined that sulfate has a response factor of 0.6319 mg/L/PAU (**Figure A.82**) and nitrate-N has a response factor of 0.1853 mg/L/PAU (**Figure A.83**).

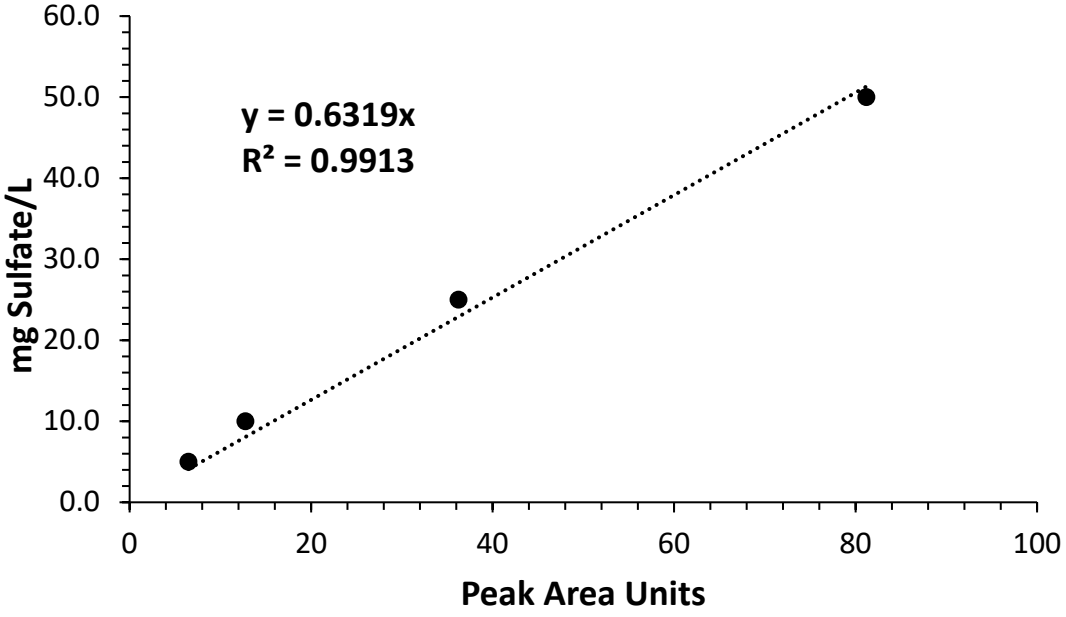

Figure A.82. The amount of sulfate per bottle in Set III was calculated by diluting a sodium sulfate stock solution and is plotted against peak area units from the IC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

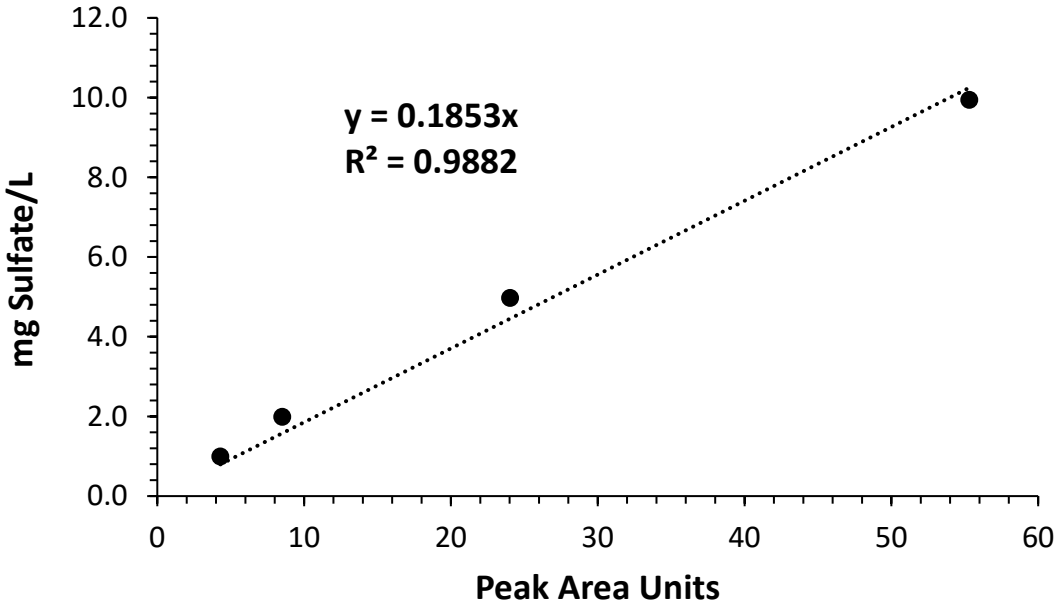

Figure A.83. The amount of nitrate-N per bottle for Set III was calculated by diluting a potassium nitrate stock solution and is plotted against peak area units from the IC computer software. The response factor was determined using the slope of the regression line with the y-intercept set to 0 μmol/bottle.

Summary Tables

Table A.24. Summary of Set I response factors and coefficients of determination for nitrate and sulfate measurements taken using the IC.

		Response Factor	
Compound	**GC RT (min)**	**(mg/L/PAU)**	**R^2**
Nitrate	8.5	0.1974	99.59%
Sulfate	11.4	0.688	99.85%

Table A.25. Summary of Set II response factors and coefficients of determination for nitrate and sulfate measurements taken using the IC.

		Response Factor	
Compound	**GC RT (min)**	**(mg/L/PAU)**	**R^2**
Nitrate	7.57	0.196	99.94%
Sulfate	11.35	0.670	99.96%

Table A.26. Summary of Set III response factors and coefficients of determination for nitrate and sulfate measurements taken using the IC.

		Response Factor	
Compound	**GC RT (min)**	**(mg/L/PAU)**	**R^2**
Nitrate	7.54	0.185	98.82%
Sulfate	11.22	0.632	99.13%