APPENDIX K

EISB Treatability Study Reports for Deep Groundwater

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Laboratory Biotreatability Study to Evaluate Anaerobic Remediation of Benzene and Chlorobenzene in Groundwater

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

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LIST OF ABBREVIATIONS

%	percent
°C	degrees Celsius
°C/min	degrees Celsius per minute
µg/L	micrograms per liter
µL	microliter
abcA	benzene carboxylase
CB	chlorobenzene
CO_2	carbon dioxide
DAP	diammonium phosphate
drsA	dissimilatory sulfate reductase A
FID	flame ionization detector
g	grams
GC	gas chromatograph
gene copies/L	gene copies per liter
Geosyntec	Geosyntec Consultants Inc.
IC	ion chromatograph
ISA	ionic strength adjustment
mg/L	milligrams per liter
min	minutes
mL	milliliter
mL/min	milliliters per minute
mM	millimolar
mmol/bottle	millimoles per bottle
mV	millivolts
NRBC	nitrate reducing benzene culture
ORP	oxidation-reduction potential
ORP	oxidative-reduction potential
psi	pounds per square inch
QL	quantitation limit
qPCR	quantitative polymerase chain reaction
RPM	revolutions per minute
rRNA	ribosomal ribonucleic acid
SiREM	SiREM Laboratory
SRB	sulfate reducing bacteria
the Site	Brunswick site
VFA	volatile fatty acid
VOC	volatile organic compound
VOCs	volatile organic compounds

1. INTRODUCTION

Geosyntec Consultants Inc., (Geosyntec) retained SiREM Laboratory (SiREM) to perform a laboratory treatability study to evaluate the degradation of volatile organic compounds (VOCs) in the deep zone of upper surficial aquifer at the Brunswick site in Georgia (the Site). The purpose of the study was to assess the potential for anaerobic biodegradation of the target compounds, namely benzene and chlorobenzene (CB).

The groundwater was collected from MW-29D on 28 February 2020 by Geosyntec personnel and received by SiREM on 4 March 2020 at a temperature of 9 degrees Celsius (°C). The geologic material labelled TSB-02_MW29D (78-80', 80-82', 84-86', 86-88') was collected on 4 March 2020 by Geosyntec personnel and received by SiREM on 12 March 2020 at a temperature of 4 °C. Refer to Appendix A for the chain of custody documentation received with the materials.

The remainder of this report contains a summary of key biodegradation processes (Section 1.1), the experimental materials and methods (Section 2), the results and discussion of the microcosm study (Section 3), conclusions (Section 4) and report references (Section 5).

1.1 Summary of Biodegradation Processes

Benzene and chlorobenzene compounds can be biologically degraded under a variety of aerobic and anaerobic conditions (Wiedemeier *et al*. 1995). Under aerobic conditions the compounds are oxidized using atmospheric oxygen and carbon dioxide (CO_2) is produced. Under anaerobic conditions, natural attenuation processes can occur in situ and are often mediated by indigenous microbial populations present at sites containing benzene. Benzene can act as an electron donor for nitrate-reducing, iron-reducing, sulfate reducing, or methanogenic bacteria (Figure 1). In the process benzene is oxidized via anaerobic pathways to carbon dioxide (Ulrich *et al*., 2005). Enhanced biological remediation can in certain cases be achieved by stimulating the indigenous microbial populations through the addition of electron acceptors, such as nitrate.

Named in honour of anaerobic hydrocarbon degradation pioneer Dunja Grbić-Galić, DGG-B™ is an anaerobic mixed microbial consortium capable of degrading benzene. This mixed culture originated as an enrichment from a diverse natural microbial community chronically exposed to hydrocarbons (Nales *et al*., 1998), and has been maintained by the University of Toronto and SiREM for over 20 years (Burland and Edwards, 1999; Ulrich and Edwards, 2003; Mancini *et al*., 2008; Luo *et al*., 2016). Benzene is added as the sole carbon source and can couple hydrocarbon degradation to sulfate reduction, or fermentative (methanogenic) metabolism.

The DGG-B™ culture, grown fermentatively on benzene, has consistently been dominated by four microorganisms for more than 15 years (Ulrich and Edwards, 2003; Mancini *et al*., 2008; Luo *et al*., 2016). Benzene fermentation is first catalysed by a *Deltaproteobacteria* designated ORM2 (Luo *et al*., 2016), which typically comprises 14-32 percent (%) of the total microbial community composition at a concentration of 10^7-10^8 gene copies per liter (gene copies/L). The other organisms in the cultures are predominantly methanogens.

A research culture from the University of Toronto referred to as the nitrate reducing benzene culture (NRBC) was also tested in this study. This culture has similarly been maintained on benzene under nitrate reducing conditions for over 15 years at the University of Toronto (Burland and Edwards, 1999).

2. MATERIALS AND METHODS

The following sections describe the materials and methods used for microcosm construction and incubation (Section 2.1), and microcosm sampling and analysis (Section 2.2).

2.1 Microcosm Construction and Incubation

2.1.1 Microcosm Construction

Treatability microcosms were constructed in a disposable anaerobic glove bag containing the Site groundwater and geologic materials and all the materials required to construct the treatment and control microcosms. The glove bag was purged with nitrogen gas in order to create an anaerobic environment and to protect any microorganisms present in the site materials from oxygen exposure. Prior to microcosm construction, all of the Site geologic materials were thoroughly homogenized by hand.

Microcosms were constructed on 26 March 2020 (Day -25) by filling sterile 250 millilitre (mL) (nominal volume) screw cap Boston round clear glass bottles (Systems Plus, New Hamburg, ON) with 200 mL of Site groundwater and 60 grams (g) of geologic material. The DGG-B™ Bioaugmented treatment was added to the scope of the study and was set up using the spare bottles constructed on 26 March 2020 (Day -25). The DGG-B™ Bioaugmented treatment microcosms have a different Time 0 date (9 July 2020) than the rest of the study. The microcosms were capped with Mininert™ closures to allow repetitive sampling with minimal VOC loss. All treatment and control microcosms were constructed in triplicate. Table 1 summarizes the details of microcosm construction and the amendments used for the control and treatment microcosms.

Anaerobic sterile control microcosms were constructed to quantify potential abiotic and experimental volatile losses from the microcosms. The sterile controls were constructed by autoclaving the Site geologic materials at 121 °C and 15 pounds per square inch (psi) pressure for 45 to 60 minutes (min). After autoclaving, the sterile control microcosms were returned to the anaerobic chamber, filled with 200 mL of Site groundwater and amended with mercuric chloride and sodium azide as described in Table 1.

2.1.2 Microcosm Amendments and Incubation

All microcosms were sampled and incubated in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) filled with an atmosphere of approximately 80% nitrogen, 10% CO_2 and 10% hydrogen (Linde Gases, Guelph, ON). Hydrogen in the anaerobic chamber functions to scavenge trace oxygen via a palladium catalyst. Anaerobic conditions in the anaerobic chamber were verified using an indicator containing resazurin (Sigma, St. Louis, MO) in a mineral medium, which turns pink in the presence of oxygen. During quiescent incubation, all microcosms were covered

to minimize photodegradation, and stored horizontally to minimize volatile losses via the (submerged) Mininert™ closure. Microcosms were incubated for a period of up to 192 days for the main study and 112 days for the additional DGG-B™ Bioaugmented treatment microcosms at approximately 22°C (room temperature).

The initial benzene and chlorobenzene concentrations in the microcosms were 0.34 milligrams per liter (mg/L) and <0.020 mg/L respectively. Geosyntec confirmed that these concentrations were not representative of the Site conditions, therefore on 30 March 2020 (Day -19) the microcosms were spiked with benzene and chlorobenzene to target final concentrations of 2.3 mg/L and 0.8 mg/L respectively. Due to higher than targeted CB concentration, the microcosms were purged to remove the benzene and chlorobenzene and re-spiked on 20 April 2020 (Day 0) to the targeted concentrations. The DGG-B™ Bioaugmented microcosms were spiked with benzene and chlorobenzene on 9 July 2020 (Day 0) to target final benzene and chlorobenzene concentrations of 2.3 mg/L and 0.8 mg/L respectively. Saturated solutions of benzene and chlorobenzene were used to spike the microcosms. Details on spiking are outlined in Table 1.

In this study, nitrate was selected as the electron acceptor to be evaluated. Nitrate was provided in the form of sodium nitrate (BioShop Canada Inc., Burlington, ON). In one treatment nitrate amendment was tested in combination with NRBC (nitrate reducing culture) bioaugmentation. In another treatment nitrate amendment was tested in combination with nutrient amendment using diammonium phosphate (DAP) (BioShop Canada Inc., Burlington, ON) to potentially stimulate intrinsic nitrate reducing bacteria.

On 31 March 2020 (Day -20), Nitrate Amended microcosms were amended with sodium nitrate and Nitrate and DAP Amended microcosms were amended with sodium nitrate and diammonium phosphate. In consultation with Geosyntec, it was decided to target a nitrate concentration of 300 mg/L (as nitrogen) and a DAP concentration of 20 mg/L. The measured nitrate concentration was approximately 1,300 mg/L instead of the targeted 300 mg/L.

Bioaugmentation may improve the extent and rate of benzene and chlorobenzene degradation. Microcosms are typically bioaugmented after reducing conditions required by the DGG-B™/NRBC cultures are achieved. Suitable reducing conditions are assessed qualitatively by both changes in the resazurin indicator colour (from pink to clear), the onset of sulfate reduction, and observing negative oxidation reduction potential (ORP). Negative ORP measurements were observed on 20 April 2020 (Day 0) in the intrinsic control. Although sulfate reduction had not been confirmed, it was concluded (in consultation with Geosyntec) that these results indicated reducing conditions had been established. Therefore, microcosms in the NRBC bioaugmented treatment were bioaugmented with NCBC culture on 29 May 2020 (Day 39) and microcosms in the DGG-B™ bioaugmented treatment were bioaugmented with DGG-B™ on 10 July 2020 (Day 1). Details of the bioaugmentation are provided in Table 1.

The first microcosm of each treatment and control was amended with resazurin (Sigma, St. Louis, MO) to monitor redox conditions. Resazurin turns from pink to clear in the absence of oxygen and can be used to indicate the on-set of reducing conditions. Details of amendments are provided in Table 1 and Table 2.

2.2 Microcosm Sampling and Analysis

2.2.1 Microcosm Sampling Schedules

The sampling frequency for all parameters was determined in consultation with Geosyntec based on anticipated microbial activity. The microcosms were sampled using gas-tight 250 µL Hamilton glass syringes. Syringes were cleaned with acidified water (pH ~2) and rinsed 10 times with de-ionized water between samples to ensure that the VOCs and microorganisms were not transferred between different samples or treatments.

VOC, pH, ORP, and anion samples were collected the microcosms. Samples for ammonia were prepared from the Nitrate Amended/NRBC Bioaugmented microcosms as well as the Nitrate and DAP Amended microcosms. Samples for ammonia were prepared using a 5 mL plastic syringe (Fisher Scientific, Toronto).

2.2.2 Analysis of BTEX Compounds

This section describes the methods used to quantify the BTEX compounds. The quantitation limits (QL) for BTEX compounds are 10 micrograms per liter (µg/L) in the microcosms based on the sample dilution factor used and the lowest concentration standards that are included in the linear calibration trend.

Aqueous BTEX concentrations in the microcosms are measured using an Agilent 7890 gas chromatograph (GC) equipped with an Agilent G1888 headspace autosampler programmed to heat each sample vial to 75 °C for 45 min prior to headspace injection into a GSQ Plot column (0.53 millimeters x 30 meters, J&W) with a flame ionization detector (FID). Sample vials are heated to ensure that all VOCs in the aqueous sample partition into the headspace. The injector temperature was 200 °C, and the detector temperature was 250 °C. The oven temperature was programmed as follows: 35 °C for 2 min, increased to 100 °C at 30 degrees Celsius per minute (°C/min), then increased to 185 °C at 25 °C/min and held at 185 °C for 7 min and then increased to 225 °C at 25 °C/min and held at 225 °C for 10 min. The helium carrier gas was set to flow at a rate of 30 milliliters per minute (mL/min).

After withdrawing a sample (as described in Section 2.2.1) from the microcosms, the sample was injected into a 10 mL auto sampler vial containing acidified deionized water (pH ~2). The sample volume was added to the vial containing acidified deionized water to bring the total volume up to 6 mL. The water was acidified to inhibit microbial activity between microcosm sampling and GC analysis. The vial was sealed with an inert Teflon™-lined septum and aluminum crimp cap for automated injection of 3 mL of headspace onto the GC. One BTEX standard was analyzed with each set of samples to verify the instrument five-point calibration curve using methanolic stock solutions containing known concentrations of the target analytes. Calibration was performed using external standards purchased as standard solutions (Sigma, St Louis, Missouri), where known volumes of standard solutions were added to acidified water in auto sampler vials and analyzed as described above for microcosm samples. Data were integrated using ChemStation Software (Agilent Technologies, Santa Clara, California).

2.2.3 Analysis of Anions

Anion and total volatile fatty acids (VFA) analysis were performed by SIREM on a Thermo-Fisher ICS-2100 ion chromatograph (IC) equipped with a Thermo-Fisher AS-DV autosampler and an AS18 column, the sample loop volume was 25 µL. An isocratic separation was performed using 33 millimolar (mM) reagent grade sodium hydroxide eluent generator cartridge (Thermo Scientific, Burlington, ON) eluent for 13 min. One standard was analysed with each set of samples tested in order to verify the seven-point calibration using external standards of known concentrations. External standards were prepared gravimetrically using chemicals of the highest purity available (Sigma St Louis, MO or Bioshop, Burlington, ON). Data were integrated using Chromeleon 7® Chromatography software (Thermo-Fisher, Burlington, ON). The QLs were as follows: 0.07 mg/L total volatile fatty acid (VFA), 0.07 mg/L chloride, 0.09 mg/L nitrite, 0.09 mg/L nitrate, 0.07 mg/L sulfate, 0.07 mg/L phosphate and 0.08 mg/L bromide. The total VFA value includes lactate, formate, acetate, propionate, pyruvate and butyrate (valerate has not been confirmed).

A 0.5 mL sample was withdrawn (as described in section 2.2.1), after which the sample was placed in a 1.5 mL micro-centrifuge tube. Samples were centrifuged for five minutes at 13,000 revolutions per minute (RPM) to remove solids. The supernatant was removed, diluted 50-fold in deionized water and placed in a Thermo-Fisher autosampler vial with a cap that filters the sample during automated injection onto the IC.

2.2.4 Analysis of ORP

Oxidation-reduction potential (ORP) measurements were performed using an Omega PHH-127 Multi-Parameter Water Quality Monitor with ORP Probe (Omega, Laval, QC). A 1.5 mL sample was taken (as described in section 2.2.1) and placed in a 5 mL Thermo-Fisher vial. The ORP was measured on the lab bench immediately after sampling. A single point calibration of the meter was performed at each sampling event with Zobell ORP calibration solution (YSI Incorporated, Yellow Springs, OH).

2.2.5 Analysis of pH

The pH measurements were performed by SIREM using an Oakton pH spear with a combination pH electrode (Oakton, Vernon Hills, IL). A 0.5 mL sample was taken (as described in section 2.2.1), the vial was removed from the glove box and the pH was measured on the lab bench. The pH spear was calibrated at each sampling event according to the manufacturer's instructions using pH 4.0, 7.0 and 10 standards.

2.2.6 Gene-Trac® Testing

Gene-Trac® quantitative polymerase chain reaction (qPCR) testing was performed in this study to quantify and characterize sulfate reducing bacteria (SRB), ORM2, and *Peptococcaceae* microorganisms as well as the functional gene for anaerobic benzene carboxylase. SRB facilitate the reduction of sulfate to sulfide and are well known to promote the degradation of various petroleum hydrocarbons. The Gene-Trac® SRB test targets the *drsA* gene. ORM2 are benzene

degrading specialists and facilitate the oxidation of benzene to carbon dioxide. The Gene-Trac® ORM2 tests quantify the total ORM2 by targeting the 16S ribosomal ribonucleic acid (rRNA) gene. *Peptococcaceae* (now known more specifically as *Thermincola)* degrades benzene in the presence of nitrate. The functional gene anaerobic benzene carboxylase (abcA) is involved in the cleavage of the aromatic benzene ring.

Samples for Gene-Trac® analysis were collected from a sacrificial microcosm at the beginning of the study (30 March 2020), the Nitrate Amended/NRBC Bioaugmented treatment on 19 June 2020 (Day 60) and at the end of the study from the Intrinsic control and all treatments. Samples for Gene-Trac® analysis were prepared by removing a 5 mL sample from triplicate microcosms of each treatment to create a 15 mL composite sample.

2.2.7 Analysis of Ammonia

Ammonia analysis was completed using a HACH HQ30d meter with a HACH Intellical™ Ammonia probe. A 3 mL sample was removed with a 5 mL plastic syringe and diluted to 25 mL. The solution was then amended with a HACH Ammonia ionic strength adjustment (ISA) powder pillow. After dissolving the ISA powder, the probe was inserted into solution and the ammonia concentration determined. The probe was calibrated against a 3-point calibration curve prepared from HACH standards at every sampling event according to the manufacturer's instructions.

Ammonia in water is either un-ionized ammonia or the ammonium ion. Typically, the value reported from analysis is the sum of both forms and is reported as total ammonia as nitrogen (ammonia-N). Ammonia-N is concentration of ammonia and ammonium present in a sample reported as the concentration of nitrogen in the sample that is from ammonia and ammonium. To convert from ammonia to ammonia-N, the concentration, in mg/L, is divided by the molar mass of ammonia and multiplied by the molar mass of nitrogen. The detection limit for ammonia analysis is 0.5 mg/L.

3. RESULTS AND DISCUSSION

The following sections present and discuss the results of the biotreatability study:

- Gene-Trac® Results (Section 3.1),
- Redox processes (Section 3.2),
- VOC Biodegradation Results (Section 3.3)

Tables 2, 3, 4, 5, and 6 provide VOC, methane, anion, pH, ORP, Gene-Trac®, and half-life data. All VOC and DHG concentrations are presented in units of mg/L and millimoles per microcosm bottle (mmol/bottle) to demonstrate mass balances on a molar basis. Concentrations were converted from mg/L to mmol/bottle using Henry's Law as demonstrated in Appendix B. Anion concentrations are reported in mg/L. ORP is reported in millivolts (mV). Gene-Trac® data is reported in gene copies/L. VOC half-life data is reported in days. Figures 3-7 present trends in

the concentrations of VOCs in the control and treatment microcosms over the incubation period. Gene-Trac® reports are provided in Appendix C.

3.1 Gene-Trac® Results

The Gene-Trac® results from the microcosm groundwater are presented in Table 5. Baseline samples had very low or non-detect concentrations of ORM, dsrA, *Peptococcaceae* and abcA. On Day 60 (June 19, 2002) At Day 60, ORM2 and SRB were detected at 10^4 gene copies/L except in the Nitrate and DAP amended microcosms where the SRB increased from an estimated value of 4 x 10^3 to 2 x 10^7 *dsrA* (dissimilatory sulfate reductase A) gene copies/L. *Peptococcaceae* and benzene carboxylase (*abcA*) were not detected. These results suggest that at Day 60 indigenous nitrate reducing benzene degrading organisms may have been stimulated by the addition of DAP in combination with the higher than targeted nitrate (1,300 mg/L instead of 300 mg/L).

Endpoint samples were taken from the microcosms on November 12, 2020. No increases in biomarker targets were observed. SRB decreased to non-detect (10^4 dsrA Gene-copies/L) from the increased counts observed at Day 60. This may be due to initial trace sulfate being consumed and then in the absence of any additional sulfate, the SRB populations decline due to lack of their preferred electron acceptor.

These results may also suggest that low concentrations of indigenous sulfate SRB were present and potentially benzene degrading organisms may be present.

3.2 Redox Processes

The presence of electron donors, including benzene and other organic compounds, and electron acceptors (i.e., nitrate), typically stimulates microbial activity that promotes increasingly reduced conditions in groundwater.

The sequence of redox reactions in groundwater is well known (Appelo and Postma, 1994). Oxygen is first consumed, followed by nitrate (denitrification), iron, manganese, and sulfate reduction producing sulfides. The final step is CO_2 reduction producing methane (methanogenesis). The consumption of each species in sequence indicates that conditions are becoming increasingly reducing. Benzene degrades readily under aerobic conditions and can also be degraded anaerobically in the range of nitrate reducing to methanogenic conditions.

Nitrate decreased from 1,344 mg/L (calculated as nitrate) to 1,132 mg/L in the Nitrate Amended/NRBC bioaugmented treatment and from 1,327 mg/L to 1,217 mg/L in the Nitrate and DAP amended treatment (Table 3). Ammonia increased in the Nitrate Amended/NRBC Bioaugmented treatment from 0.18 mg/L to 3.86 mg/L by the end of the incubation period and decreased from 3.35 mg/L to 1.29 mg/L in the Nitrate and DAP Amended treatment. Sulfate concentrations in the both the Nitrate Amended/NRBC and Nitrate and DAP Amended treatments remained relatively stable during the incubation period (Table 3).

These results indicate that the reducing conditions necessary for anaerobic benzene degradation to occur were slowly being established over the 192-day incubation period of the study in both the nitrate amended treatments and that following bioaugmentation with NRBC.

For the DGG-B™ Bioaugmented treatment, nitrate at the start of the study was <0.07 mg/L and sulfate decreased from 16 mg/L on Day 22 to <0.07 mg/L on Day 50 (Table 3). These results suggest that the reducing conditions required for anaerobic benzene degradation were promoted with bioaugmentation with DGG-B™.

3.3 VOC Biodegradation Results

3.3.1 Half Lives

Laboratory half-lives were calculated based on the average dechlorination observed in the treatment microcosms. First order reaction kinetics was assumed for all calculations as described in Newell *et al*, 2002. The half-lives were calculated using the following relationship:

$$Half - life = \frac{\ln(2)}{\left[\frac{\ln\left(\frac{C_2}{C_1}\right)}{t_2 - t_1}\right]}$$

where,

C_1 is the concentration at first time (t_1 days)

C_2 is the concentration at second time (t_2 days)

Half-lives were not calculated if net degradation of the compound was not detected during the study period (Table 6).

3.3.2 VOC Biodegradation Results

All VOC results discussed in this section are presented in Table 2 and Figures 3-7. Half-life data are presented in Table 6.

Benzene concentrations remained stable in both the Sterile Control and the Intrinsic Control resulting in long half-lives of 3,991 and 1,218 days respectively (Figures 3 and 4, Table 6). Chlorobenzene concentrations in the Sterile Control remained stable (no half-life was calculated) and for the Intrinsic Control, the chlorobenzene half-life was 2,062 days (Table 6).

In the Nitrate Amended/NRBC Bioaugmented treatment, benzene remained stable in replicate 1 and replicate 3 while replicate 2 decreased from 2.1 mg/L on Day 0 to <0.020 mg/L by Day 102 resulting in an average benzene half-life of 328 days (Figure 5, Table 2). Chlorobenzene decreased from 1.0 mg/L to 0.72 mg/L by the end of the incubation period resulting in a half-life

of 409 days. The greatest decrease in benzene was measured in replicate 3 that decreased to 0.78 mg/L from a starting concentration of 1.6 mg/L.

In the Nitrate and DAP Amended treatment the benzene and chlorobenzene concentrations remained stable and no half-lives could be calculated (Figure 6, Table 2).

In the DGG-B™ treatment, benzene decreased from 2.9 mg/L to 2.3 mg/L and chlorobenzene decreased from 0.88 to 0.82 mg/L resulting in half-lives of 596 days and 1,857 days respectively (Figure 7 and Table 2).

These results suggest that nitrate and DAP amendments did not stimulate intrinsic degradation of benzene or chlorobenzene over the incubation period of the study. NRBC bioaugmentation, with addition of nitrate, increased benzene and chlorobenzene degradation rates. It is expected that with a longer incubation period (at least 1 year in total) that additional degradation of the key compounds may occur. DGG-B™ bioaugmentation was evaluated over a shorter incubation period (112 days) and this time period may not have been long enough to evaluate its effects.

4. CONCLUSIONS

The study was conducted with a primary objective to assess the potential for anaerobic degradation of benzene and chlorobenzene using nitrate amendment and nutrient amendment. Bioaugmentation with NRBC and DGG-B™ was added to the scope midway through the study. The laboratory biotreatability study results suggest the following conclusions:

1. Biomarkers for known benzene degrading organisms were not detected in the baseline Site groundwater. Sulfate reducing bacteria were detected and may indicate that potential benzene degrading populations could be present, albeit at low concentrations, at the Site.

2. Benzene degradation was beginning to occur in the DGG-B™ treatment at Day 112, the shorter incubation period many have not been long enough to allow benzene degradation activity to get fully established.

3. Although benzene degradation was observed in one replicate of triplicate microcosms in the Nitrate Amended/NRBC treatment, additional incubation and benzene degradation in all 3 replicates would need to be confirmed.

4. Chlorobenzene degradation may be occurring in the Nitrate Amended/NRBC Bioaugmented treatment, but additional incubation time is needed to confirm this trend.

The results of this study indicate that nitrate and nutrient amendment alone may not be capable of stimulating degradation of benzene via nitrate reduction under the specific conditions studied and incubation period. Evaluation of the impact of bioaugmentation would benefit from a longer incubation period using DGG-B™ and NRBC.

5. REFERENCES

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TABLES

TABLE 1: SUMMARY OF MICROCOSM CONTROLS, TREATMENTS AND AMENDMENTS
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

Treatment/Control	Assigned Microcosm Number	Number of Microcosms	Geological Material (g)	Groundwater (mL)	Headspace (mL)	Sodium Azide	Mercuric Chloride	Benzene and Chlorobenzene	Resazurin	Bioaugmentation	Nitrate	DAP
Anaerobic Sterile Control	13 to 15	3	60	200	20	Amended with 0.5 mL of a 5% solution on Day -25.	Amended with 2.8 mL of a 2.7% solution on Day -25.	Spiked with 190 µL of saturated benzene and 345 µL of saturated CB to target final concentrations of 2 mg/L and 0.8 mg/L respectively.	Amended first replicate with 100 µL of a 1,000 mg/L solution on Day -2.	--	--	--
Intrinsic Control	16 to 18	3	60	200	20	--	--			--	--	--
Nitrate Amended/NRBC Bioaugmented	19 to 21	3	60	200	20	--	--			Amended with 8 mL of NRBC on Day 39.	Amend with 600 µL of a 100 g/L sodium nitrate solution to target a final nitrate concentration of 300 mg/L.	267 uL of a 15 g/L sodium nitrate stock to target 20 mg/L DAP on Day 142.
Nitrate and DAP Amended	22 to 24	3	60	200	20	--	--			--		267 uL of a 15 g/L sodium nitrate stock to target 20 mg/L DAP.
DGG-B™ Bioaugmented	25-27	3	60	200	20	--	--	Spiked with 212 µL of saturated benzene and 301 µL of saturated CB to target final concentrations of 2.3 mg/L and 0.8 mg/L respectively.	Amended first replicate with 100 µL of a 1,000 mg/L solution on Day 0.	Amended with 5 mL of DGG-B™ on Day 0.	--	--

Notes:

-- - not applicable
% - percent
µL - microliter
DAP - diammonium phosphate
g - grams
g/L - grams per liter
mg/L - milligrams per liter
mL - milliliters
NRBC - nitrate reducing benzene culture

TABLE 2: SUMMARY OF MICROCOSM BENZENE, CB AND METHANE RESULTS SiREM

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	Benzene	CB	Methane	Comments
				mg/L	mg/L	mg/L	
Anaerobic Sterile Control	26-Mar-20	-25					Poisoned with mecuric chloride and sodium azide.
							Amended the first replicate with resazurin.
	20-Apr-20	0					Spiked with benzene and CB to target concentrations of 2.3 mg/L and 0.8 mg/L respectively.
			ANSC-1	2.2	0.91	<0.10	
			ANSC-2	2.4	0.84	<0.10	
			ANSC-3	2.4	1.3	<0.10	
			Average Concentration (mg/L)	2.3	1.0	ND	
			Standard Deviation (mmoles)	2.4E-04	4.4E-04	0.0E+00	
			Average Total mmoles	**0.0061**	**0.0018**	**ND**	
	11-May-20	21	ANSC-1	2.6	1.1	<0.10	
			ANSC-2	2.7	1.0	<0.10	
			ANSC-3	2.6	1.4	<0.10	
			Average Concentration (mg/L)	2.6	1.2	ND	
			Standard Deviation (mmoles)	1.8E-04	3.7E-04	0.0E+00	
			Average Total mmoles	**0.0069**	**0.0022**	**ND**	
	19-Jun-20	60	ANSC-1	1.9	0.78	<0.10	
			ANSC-2	2.2	0.73	<0.10	
			ANSC-3	2.2	1.1	<0.10	
			Average Concentration (mg/L)	2.1	0.86	ND	
			Standard Deviation (mmoles)	3.4E-04	3.5E-04	0.0E+00	
			Average Total mmoles	**0.0055**	**0.0016**	**ND**	
	31-Jul-20	102	ANSC-1	2.1	0.88	<0.10	
			ANSC-2	2.3	0.82	<0.10	
			ANSC-3	2.5	1.3	<0.10	
			Average Concentration (mg/L)	2.3	1.0	ND	
			Standard Deviation (mmoles)	5.2E-04	5.1E-04	0.0E+00	
			Average Total mmoles	**0.0060**	**0.0018**	**ND**	
	24-Sep-20	157	ANSC-1	2.0	0.88	<0.10	
			ANSC-2	2.2	0.81	<0.10	
			ANSC-3	2.4	1.3	<0.10	
			Average Concentration (mg/L)	2.2	0.99	ND	
			Standard Deviation (mmoles)	4.3E-04	4.5E-04	0.0E+00	
			Average Total mmoles	**0.0058**	**0.0018**	**ND**	
	29-Oct-20	192	ANSC-1	2.0	0.89	<0.10	
			ANSC-2	2.3	0.85	<0.10	
			ANSC-3	2.4	1.3	<0.10	
			Average Concentration (mg/L)	2.2	1.0	ND	
			Standard Deviation (mmoles)	4.8E-04	4.6E-04	0.0E+00	
			Average Total mmoles	**0.0059**	**0.0018**	**ND**	
Anaerobic Intrinsic Control	26-Mar-20	-25					Amended the first replicate with resazurin.
	20-Apr-20	0					Spiked with benzene and CB to target concentrations of 2.3 mg/L and 0.8 mg/L respectively.
			ANIC-1	2.3	0.90	<0.10	
			ANIC-2	2.2	0.79	<0.10	
			ANIC-3	2.2	0.9	<0.10	
			Average Concentration (mg/L)	2.2	0.86	ND	
			Standard Deviation (mmoles)	1.4E-04	1.1E-04	0.0E+00	
			Average Total mmoles	**0.0058**	**0.0016**	**ND**	

TABLE 2: SUMMARY OF MICROCOSM BENZENE, CB AND METHANE RESULTS SiREM

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	Benzene	CB	Methane	Comments
				mg/L	mg/L	mg/L	
Anaerobic Intrinsic Control Continued	11-May-20	21	ANIC-1	2.4	0.98	<0.10	
			ANIC-2	2.3	0.89	<0.10	
			ANIC-3	2.4	1.0	<0.10	
			Average Concentration (mg/L)	2.4	0.97	ND	
			Standard Deviation (mmoles)	1.9E-04	1.3E-04	0.0E+00	
			Average Total mmoles	**0.0062**	**0.0018**	**ND**	
	19-Jun-20	60	ANIC-1	1.9	0.73	<0.10	
			ANIC-2	1.6	0.57	<0.10	
			ANIC-3	2.1	0.80	<0.10	
			Average Concentration (mg/L)	1.9	0.70	ND	
			Standard Deviation (mmoles)	6.6E-04	2.0E-04	0.0E+00	
			Average Total mmoles	**0.0049**	**0.0013**	**ND**	
	31-Jul-20	102	ANIC-1	2.1	0.86	<0.10	
			ANIC-2	1.7	0.66	<0.10	
			ANIC-3	2.3	0.94	<0.10	
			Average Concentration (mg/L)	2.0	0.82	ND	
			Standard Deviation (mmoles)	7.6E-04	2.5E-04	0.0E+00	
			Average Total mmoles	**0.0053**	**0.0015**	**ND**	
	24-Sep-20	157	ANIC-1	2.0	0.84	<0.10	
			ANIC-2	1.7	0.67	<0.10	
			ANIC-3	2.2	0.92	<0.10	
			Average Concentration (mg/L)	2.0	0.81	ND	
			Standard Deviation (mmoles)	7.1E-04	2.4E-04	0.0E+00	
			Average Total mmoles	**0.0051**	**0.0015**	**ND**	
	29-Oct-20	192	ANIC-1	2.1	0.86	<0.10	
			ANIC-2	1.6	0.65	<0.10	
			ANIC-3	2.2	0.95	<0.10	
			Average Concentration (mg/L)	2.0	0.82	ND	
			Standard Deviation (mmoles)	8.5E-04	2.7E-04	0.0E+00	
			Average Total mmoles	**0.0052**	**0.0015**	**ND**	
Nitrate Amended/NRBC Bioaugmented	26-Mar-20	-25					Amended the first replicate with resazurin.
	31-Mar-20	-20					Amended with sodium nitrate to target a final nitrate-N concentration of 300 mg/L.
	20-Apr-20	0					Spiked with benzene and CB to target concentrations of 2.3 mg/L and 0.8 mg/L respectively.
			Nitrate-1	2.3	0.79	<0.10	
			Nitrate-2	2.1	0.69	<0.10	
			Nitrate-3	2.4	1.6	<0.10	
			Average Concentration (mg/L)	2.3	1.0	ND	
			Standard Deviation (mmoles)	3.8E-04	8.8E-04	0.0E+00	
			Average Total mmoles	**0.0060**	**0.0018**	**ND**	
	11-May-20	21	Nitrate-1	2.4	0.82	<0.10	
			Nitrate-2	2.3	0.76	<0.10	
			Nitrate-3	2.3	1.7	<0.10	
			Average Concentration (mg/L)	2.3	1.1	ND	
			Standard Deviation (mmoles)	9.5E-05	9.0E-04	0.0E+00	
			Average Total mmoles	**0.0061**	**0.0019**	**ND**	

TABLE 2: SUMMARY OF MICROCOSM BENZENE, CB AND METHANE RESULTS SiREM

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	Benzene	CB	Methane	Comments
				mg/L	mg/L	mg/L	
Nitrate Amended/NRBC Bioaugmented Continued	29-May-20	39	Nitrate-1	2.2	0.59	<0.10	
			Nitrate-2	2.3	0.62	<0.10	
			Nitrate-3	2.3	1.3	<0.10	
			Average Concentration (mg/L)	2.3	0.85	ND	
			Standard Deviation (mmoles)	2.2E-04	7.5E-04	0.0E+00	
			Average Total mmoles	**0.0059**	**0.0015**	**ND**	
							Bioaugmented with 8 mL of NRBC.
	19-Jun-20	60	Nitrate-1	1.9	0.59	<0.10	
			Nitrate-2	1.9	0.57	<0.10	
			Nitrate-3	2.1	1.3	<0.10	
			Average Concentration (mg/L)	2.0	0.83	ND	
			Standard Deviation (mmoles)	2.2E-04	7.7E-04	0.0E+00	
			Average Total mmoles	**0.0051**	**0.0015**	**ND**	
	31-Jul-20	102	Nitrate-1	2.1	0.7	<0.10	
			Nitrate-2	<0.020	0.67	<0.10	
			Nitrate-3	2.1	1.2	<0.10	
			Average Concentration (mg/L)	1.4	0.95	ND	
			Standard Deviation (mmoles)	2.7E-03	5.4E-04	0.0E+00	
			Average Total mmoles	**0.0037**	**0.0017**	**ND**	
	05-Aug-20	107		<0.020	0.62	<0.10	
	28-Aug-20	130	Nitrate-1	2.1	0.70	<0.10	
			Nitrate-2	<0.020	0.67	<0.10	
			Nitrate-3	2.4	1.7	<0.10	
			Average Concentration (mg/L)	1.5	1.0	ND	
			Standard Deviation (mmoles)	3.4E-03	1.1E-03	0.0E+00	
			Average Total mmoles	**0.0039**	**0.0019**	**ND**	
	09-Sep-20	142					Amended with DAP to target 20 mg/L DAP.
	24-Sep-20	157	Nitrate-1	2.0	0.68	<0.10	
			Nitrate-2	<0.020	0.66	<0.10	
			Nitrate-3	2.3	1.7	<0.10	
			Average Concentration (mg/L)	1.4	1.0	ND	
			Standard Deviation (mmoles)	3.3E-03	1.0E-03	0.0E+00	
			Average Total mmoles	**0.0038**	**0.0018**	**ND**	
	29-Oct-20	192	Nitrate-1	2.2	0.78	<0.10	
			Nitrate-2	<0.020	0.67	<0.10	
			Nitrate-3	2.3	0.71	<0.10	
			Average Concentration (mg/L)	1.5	0.72	ND	
			Standard Deviation (mmoles)	3.4E-03	1.0E-04	0.0E+00	
			Average Total mmoles	**0.0040**	**0.0013**	**ND**	
Nitrate and DAP Amended	26-Mar-20	-25					Amended the first replicate with resazurin.
	31-Mar-20	-20					Amended with sodium nitrate to target a final nitrate-N concentration of 300 mg/L.
							Amended with DAP to target 20 mg/L DAP.
	20-Apr-20	0					Spiked with benzene and CB to target concentrations of 2.3 mg/L and 0.8 mg/L respectively.
			Nitrate/DAP-1	2.2	0.76	<0.10	
			Nitrate/DAP-2	2.1	0.71	<0.10	
			Nitrate/DAP-3	2.3	0.75	<0.10	
			Average Concentration (mg/L)	2.2	0.74	ND	
			Standard Deviation (mmoles)	2.7E-04	4.6E-05	0.0E+00	
			Average Total mmoles	**0.0058**	**0.0013**	**ND**	

TABLE 2: SUMMARY OF MICROCOSM BENZENE, CB AND METHANE RESULTS SiREM

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	Benzene	CB	Methane	Comments
				mg/L	mg/L	mg/L	
Nitrate and DAP Amended Continued	11-May-20	21	Nitrate/DAP-1	2.1	0.73	<0.10	
			Nitrate/DAP-2	2.1	0.72	<0.10	
			Nitrate/DAP-3	2.3	0.75	<0.10	
			Average Concentration (mg/L)	2.2	0.73	ND	
			Standard Deviation (mmoles)	2.6E-04	3.1E-05	0.0E+00	
			Average Total mmoles	**0.0057**	**0.0013**	**ND**	
	19-Jun-20	60	Nitrate/DAP-1	1.9	0.64	<0.10	
			Nitrate/DAP-2	2.0	0.62	<0.10	
			Nitrate/DAP-3	1.9	0.57	<0.10	
			Average Concentration (mg/L)	1.9	0.61	ND	
			Standard Deviation (mmoles)	5.0E-05	6.9E-05	0.0E+00	
			Average Total mmoles	**0.0051**	**0.0011**	**ND**	
	31-Jul-20	102	Nitrate/DAP-1	2.1	0.71	<0.10	
			Nitrate/DAP-2	2.0	0.72	<0.10	
			Nitrate/DAP-3	2.2	0.67	<0.10	
			Average Concentration (mg/L)	2.1	0.7	ND	
			Standard Deviation (mmoles)	1.7E-04	4.1E-05	0.0E+00	
			Average Total mmoles	**0.0055**	**0.0013**	**ND**	
	24-Sep-20	157	Nitrate/DAP-1	2.1	0.73	<0.10	
			Nitrate/DAP-2	2.0	0.74	<0.10	
			Nitrate/DAP-3	2.2	0.66	<0.10	
			Average Concentration (mg/L)	2.1	0.71	ND	
			Standard Deviation (mmoles)	1.8E-04	7.8E-05	0.0E+00	
			Average Total mmoles	**0.0055**	**0.0013**	**ND**	
	29-Oct-20	192	Nitrate/DAP-1	2.4	0.87	0.76	
			Nitrate/DAP-2	2.3	0.80	0.86	
			Nitrate/DAP-3	2.3	0.79	0.70	
			Average Concentration (mg/L)	2.3	0.82	0.77	
			Standard Deviation (mmoles)	1.5E-04	8.5E-05	3.8E-03	
			Average Total mmoles	**0.0060**	**0.0015**	**0.036**	
DGG-B Bioaugmented	26-Mar-20	-104					Microcosms constructed and stored in anaerobic box.
	09-Jul-20	0					Amended the first replicate with resazurin.
							Spiked with benzene and CB to target concentrations of 2.3 mg/L and 0.8 mg/L respectively.
			DGG-1	2.9	0.97	0.85	
			DGG-2	2.8	0.83	0.79	
			DGG-3	3.0	0.84	0.90	
			Average Concentration (mg/L)	2.9	0.88	0.85	
			Standard Deviation (mmoles)	1.1E-01	7.5E-02	5.4E-02	
			Average Total mmoles	**0.0076**	**0.0016**	**0.039**	
	10-Jul-20	1					Bioaugmented with 5 mL of DGG-B™.
	31-Jul-20	22	DGG-1	2.5	0.81	0.81	
			DGG-2	2.7	0.71	0.88	
			DGG-3	2.6	0.76	0.71	
			Average Concentration (mg/L)	2.6	0.76	0.80	
			Standard Deviation (mmoles)	8.4E-02	4.8E-02	8.3E-02	
			Average Total mmoles	**0.0068**	**0.0014**	**0.037**	

TABLE 2: SUMMARY OF MICROCOSM BENZENE, CB AND METHANE RESULTS SiREM

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	Benzene	CB	Methane	Comments
				mg/L	mg/L	mg/L	
DGG-B Bioaugmented Continued	28-Aug-20	50	DGG-1	2.6	0.92	0.85	
			DGG-2	2.7	0.80	0.92	
			DGG-3	2.6	0.79	0.73	
			Average Concentration (mg/L)	2.6	0.83	0.83	
			Standard Deviation (mmoles)	9.1E-02	7.2E-02	9.8E-02	
			Average Total mmoles	**0.0069**	**0.0015**	**0.039**	
	24-Sep-20	77	DGG-1	2.5	0.84	0.78	
			DGG-2	2.4	0.71	0.62	
			DGG-3	2.5	0.76	0.70	
			Average Concentration (mg/L)	2.4	0.77	0.70	
			Standard Deviation (mmoles)	7.6E-02	6.8E-02	8.2E-02	
			Average Total mmoles	**0.0064**	**0.0014**	**0.033**	
	29-Oct-20	112	DGG-1	2.4	0.87	0.76	
			DGG-2	2.3	0.80	0.86	
			DGG-3	2.3	0.79	0.69	
			Average Concentration (mg/L)	2.3	0.82	0.77	
			Standard Deviation (mmoles)	6.3E-02	4.6E-02	8.6E-02	
			Average Total mmoles	**0.0061**	**0.0015**	**0.036**	

Notes:

< - the compound is not detected, the associated value is the detection limit
ANIC - anaerobic instrinsic control
ANSC - anaerobic sterile control
CB - chlorobenzene
DAP - diammonium phosphate
mg/L - milligrams per liter
mmoles - millimoles
ND - not detected
NRBC - nitrate reducing bacterial culture

TABLE 3: SUMMARY OF MICROCOSM ANION RESULTS SiREM

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	Total VFAs	Chloride	Nitrite-N	Nitrate-N	Sulfate	Phosphate	Calculated Nitrate
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Anaerobic Sterile Control	31-Mar-20	-25	ANSC-1	<0.07	2,902	<0.09	<0.09	86	<0.07	<0.09
			ANSC-2	<0.07	2,496	<0.09	<0.09	56	<0.07	<0.09
			ANSC-3	<0.07	1,978	<0.09	<0.09	46	<0.07	<0.09
			Average	**ND**	**2,458**	**ND**	**ND**	**63**	**ND**	**ND**
	20-Apr-20	0	ANSC-1	<0.07	2,414	<0.09	<0.09	62	<0.07	<0.09
			ANSC-2	<0.07	2,952	<0.09	<0.09	87	<0.07	<0.09
			ANSC-3	<0.07	2,185	<0.09	<0.09	50	<0.07	<0.09
			Average	**ND**	**2,517**	**ND**	**ND**	**66**	**ND**	**ND**
	11-May-20	21	ANSC-1	<0.07	2,676	<0.09	<0.09	64	<0.07	<0.09
			ANSC-2	<0.07	2,316	<0.09	<0.09	61	<0.07	<0.09
			ANSC-3	<0.07	2,372	<0.09	<0.09	58	<0.07	<0.09
			Average	**ND**	**2,455**	**ND**	**ND**	**61**	**ND**	**ND**
	19-Jun-20	60	ANSC-1	<0.07	3,469	<0.09	<0.09	50	<0.07	<0.09
			ANSC-2	<0.07	3,401	<0.09	<0.09	57	<0.07	<0.09
			ANSC-3	<0.07	2,506	<0.09	<0.09	46	<0.07	<0.09
			Average	**ND**	**3,125**	**ND**	**ND**	**51**	**ND**	**ND**
	31-Jul-20	102	ANSC-1	<0.07	3,346	<0.09	<0.09	104	<0.07	<0.09
			ANSC-2	<0.07	2,693	<0.09	<0.09	69	<0.07	<0.09
			ANSC-3	<0.07	2,755	<0.09	<0.09	76	<0.07	<0.09
			Average	**ND**	**2,931**	**ND**	**ND**	**83**	**ND**	**ND**
	24-Sep-20	157	ANSC-1	<0.07	2,719	<0.09	<0.09	76	<0.07	<0.09
			ANSC-2	<0.07	2,560	<0.09	<0.09	66	<0.07	<0.09
			ANSC-3	<0.07	2,652	<0.09	<0.09	73	<0.07	<0.09
			Average	**ND**	**2,644**	**ND**	**ND**	**72**	**ND**	**ND**
	29-Oct-20	192	ANSC-1	<0.07	Pending	<0.09	<0.09	64	<0.07	<0.09
			ANSC-2	<0.07	2,657	<0.09	<0.09	77	<0.07	<0.09
			ANSC-3	<0.07	Pending	<0.09	<0.09	74	<0.07	<0.09
			Average	**ND**	**886**	**ND**	**ND**	**72**	**ND**	**ND**
Anaerobic Intrinsic Control	31-Mar-20	-25	ANIC-1	<0.07	2,497	<0.09	<0.09	19	<0.07	<0.09
			ANIC-2	<0.07	2,180	<0.09	<0.09	15	<0.07	<0.09
			ANIC-3	<0.07	2,378	<0.09	<0.09	26	<0.07	<0.09
			Average	**ND**	**2,352**	**ND**	**ND**	**20**	**ND**	**ND**
	20-Apr-20	0	ANIC-1	<0.07	2,106	<0.09	<0.09	18	<0.07	<0.09
			ANIC-2	<0.07	2,042	<0.09	<0.09	23	<0.07	<0.09
			ANIC-3	<0.07	2,232	<0.09	<0.09	19	<0.07	<0.09
			Average	**ND**	**2,126**	**ND**	**ND**	**20**	**ND**	**ND**
	11-May-20	21	ANIC-1	<0.07	2,223	<0.09	<0.09	21	<0.07	<0.09
			ANIC-2	<0.07	2,314	<0.09	<0.09	22	<0.07	<0.09
			ANIC-3	<0.07	2,210	<0.09	<0.09	20	<0.07	<0.09
			Average	**ND**	**2,249**	**ND**	**ND**	**21**	**ND**	**ND**
	19-Jun-20	60	ANIC-1	<0.07	2,671	<0.09	<0.09	19	<0.07	<0.09
			ANIC-2	<0.07	2,425	<0.09	<0.09	12	<0.07	<0.09
			ANIC-3	<0.07	2,493	<0.09	<0.09	16	<0.07	<0.09
			Average	**ND**	**2,530**	**ND**	**ND**	**16**	**ND**	**ND**
	31-Jul-20	102	ANIC-1	<0.07	2,668	<0.09	<0.09	29	<0.07	<0.09
			ANIC-2	<0.07	2,925	<0.09	<0.09	16	<0.07	<0.09
			ANIC-3	<0.07	2,881	<0.09	<0.09	25	<0.07	<0.09
			Average	**ND**	**2,824**	**ND**	**ND**	**23**	**ND**	**ND**
	24-Sep-20	157	ANIC-1	<0.07	2,147	<0.09	<0.09	<0.07	<0.07	<0.09
			ANIC-2	<0.07	2,539	<0.09	<0.09	<0.07	<0.07	<0.09
			ANIC-3	<0.07	2,219	<0.09	<0.09	<0.07	<0.07	<0.09
			Average	**ND**	**2,302**	**ND**	**ND**	**ND**	**ND**	**ND**
	29-Oct-20	192	ANIC-1	<0.07	2,451	<0.09	<0.09	11	<0.07	<0.09
			ANIC-2	<0.07	2,619	<0.09	<0.09	3.5	<0.07	<0.09
			ANIC-3	<0.07	2,880	<0.09	<0.09	15	<0.07	<0.09
			Average	**ND**	**2,650**	**ND**	**ND**	**10**	**ND**	**ND**

TABLE 3: SUMMARY OF MICROCOSM ANION RESULTS

SiREM

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	Total VFAs	Chloride	Nitrite-N	Nitrate-N	Sulfate	Phosphate	Calculated Nitrate
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Nitrate Amended/NRBC Bioaugmented	31-Mar-20	-25	**Amended with nitrate to target a concentration of 300 mg/L nitrate-N.**							
			Nitrate-1	<0.07	2,182	<0.09	301	13	<0.07	1,333
			Nitrate-2	<0.07	2,136	<0.09	293	11	<0.07	1,296
			Nitrate-3	<0.07	2,295	<0.09	317	15	<0.07	1,402
			Average	**ND**	**2,204**	**ND**	**304**	**13**	**ND**	**1,344**
	20-Apr-20	0	Nitrate-1	<0.07	2,419	10	302	40	<0.07	1,335
			Nitrate-2	<0.07	2,250	<0.09	271	26	<0.07	1,198
			Nitrate-3	<0.07	2,553	10	313	45	<0.07	1,384
			Average	**ND**	**2,407**	**6.8**	**295**	**37**	**ND**	**1,306**
	11-May-20	21	Nitrate-1	<0.07	2,184	<0.09	264	28	<0.07	1,169
			Nitrate-2	<0.07	2,370	<0.09	278	26	<0.07	1,232
			Nitrate-3	<0.07	2,372	<0.09	278	40	<0.07	1,231
			Average	**ND**	**2,309**	**ND**	**274**	**31**	**ND**	**1,211**
	29-May-20	39	Nitrate-1	<0.07	2,153	<0.09	259	29	<0.07	1,145
			Nitrate-2	<0.07	2,060	<0.09	246	29	<0.07	1,090
			Nitrate-3	<0.07	2,355	<0.09	276	27	<0.07	1,220
			Average	**ND**	**2,189**	**ND**	**260**	**28**	**ND**	**1,152**
			Bioaugmented with 8 mL of NRBC.							
			Nitrate-1	<0.07	2,343	<0.09	273	27	<0.07	1,207
			Nitrate-2	<0.07	2,149	10	251	33	<0.07	1,113
			Nitrate-3	<0.07	2,412	<0.09	286	37	<0.07	1,266
			Average	**ND**	**2,301**	**3.4**	**270**	**32**	**ND**	**1,195**
	19-Jun-20	60	Nitrate-1	<0.07	2,418	11	290	30	<0.07	1,285
			Nitrate-2	<0.07	2,633	<0.09	312	29	<0.07	1,379
			Nitrate-3	<0.07	3,068	11	369	44	<0.07	1,631
			Average	**ND**	**2,706**	**7.2**	**324**	**34**	**ND**	**1,432**
	31-Jul-20	102	Nitrate-1	<0.07	2,438	<0.09	291	33	<0.07	1,290
			Nitrate-2	<0.07	2,318	<0.09	268	27	<0.07	1,187
			Nitrate-3	<0.07	2,511	<0.09	297	38	<0.07	1,316
			Average	**ND**	**2,422**	**ND**	**286**	**33**	**ND**	**1,264**
	28-Aug-20	130	Nitrate-1	<0.07	2,119	<0.09	253	30	<0.07	1,119
			Nitrate-2	<0.07	2,345	<0.09	273	27	<0.07	1,208
			Nitrate-3	<0.07	1,993	<0.09	235	29	<0.07	1,042
			Average	**ND**	**2,152**	**ND**	**254**	**28**	**ND**	**1,123**
	24-Sep-20	157	Nitrate-1	<0.07	2,249	<0.09	265	30	<0.07	1,173
			Nitrate-2	<0.07	2,353	<0.09	271	25	<0.07	1,200
			Nitrate-3	<0.07	1,976	<0.09	231	28	<0.07	1,022
			Average	**ND**	**2,193**	**ND**	**256**	**28**	**ND**	**1,132**
	29-Oct-20	192	Nitrate-1	<0.07	2,284	<0.09	271	28	<0.07	1,200
			Nitrate-2	<0.07	2,300	<0.09	266	24	<0.07	1,178
			Nitrate-3	<0.07	2,600	<0.09	308	39	<0.07	1,362
			Average	**ND**	**2,395**	**ND**	**282**	**30**	**ND**	**1,247**
Nitrate and DAP Amended	31-Mar-20	-25	**Amended with nitrate to target a concentration of 300 mg/L nitrate-N.**							
			Amended with DAP to a target concentration of 20 mg/L.							
			Nitrate/DAP-1	<0.07	2,404	<0.09	327	20	<0.07	1,448
			Nitrate/DAP-2	<0.07	2,076	<0.09	291	14	<0.07	1,286
			Nitrate/DAP-3	<0.07	2,080	<0.09	282	16	<0.07	1,246
			Average	**ND**	**2,187**	**ND**	**300**	**16**	**ND**	**1,327**
	20-Apr-20	0	Nitrate/DAP-1	<0.07	2,342	13	281	41	<0.07	1,246
			Nitrate/DAP-2	<0.07	2,340	12	286	38	<0.07	1,267
			Nitrate/DAP-3	<0.07	2,272	<0.09	276	36	<0.07	1,221
			Average	**ND**	**2,318**	**8.3**	**281**	**39**	**ND**	**1,245**
	11-May-20	21	Nitrate/DAP-1	<0.07	1,930	<0.09	223	25	<0.07	988
			Nitrate/DAP-2	<0.07	2,310	<0.09	275	29	<0.07	1,217
			Nitrate/DAP-3	<0.07	2,261	<0.09	269	33	<0.07	1,192
			Average	**ND**	**2,167**	**ND**	**256**	**29**	**ND**	**1,132**

TABLE 3: SUMMARY OF MICROCOSM ANION RESULTS
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

Treatment	Date	Day	Replicate	Total VFAs	Chloride	Nitrite-N	Nitrate-N	Sulfate	Phosphate	Calculated Nitrate
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Nitrate and DAP Amended Continued	19-Jun-20	60	Nitrate/DAP-1	<0.07	2,270	10	268	29	<0.07	1,187
			Nitrate/DAP-2	<0.07	2,190	<0.09	260	23	<0.07	1,150
			Nitrate/DAP-3	<0.07	2,210	<0.09	264	30	<0.07	1,169
			Average	**ND**	**2,223**	**ND**	**264**	**27**	**ND**	**1,169**
	31-Jul-20	102	Nitrate/DAP-1	<0.07	2,534	11	301	40	<0.07	1,332
			Nitrate/DAP-2	<0.07	2,861	<0.09	343	40	<0.07	1,519
			Nitrate/DAP-3	<0.07	2,592	<0.09	312	38	<0.07	1,382
			Average	**ND**	**2,662**	**ND**	**319**	**39**	**ND**	**1,411**
	24-Sep-20	157	Nitrate/DAP-1	<0.07	2,276	<0.09	268	31	<0.07	1,188
			Nitrate/DAP-2	<0.07	2,259	<0.09	269	29	<0.07	1,190
			Nitrate/DAP-3	<0.07	2,406	<0.09	288	34	<0.07	1,273
			Average	**ND**	**2,313**	**ND**	**275**	**31**	**ND**	**1,217**
	29-Oct-20	192	Nitrate/DAP-1	<0.07	2,486	<0.09	295	35	<0.07	1,304
			Nitrate/DAP-2	<0.07	2,599	<0.09	312	33	<0.07	1,380
			Nitrate/DAP-3	<0.07	2,482	<0.09	299	35	<0.07	1,325
			Average	**ND**	**2,522**	**ND**	**302**	**34**	**ND**	**1,336**
DGG-B™ Bioaugmented	26-Mar-20	-104	**Microcosms constructed and stored in anaerobic box.**							
	09-Jul-20	0	**Spiked with benzene and CB to target concentrations of 2.3 mg/L and 0.8 mg/L respectively.**							
	10-Jul-20	1	**Bioaugmented with 5 mL of DGG-B™.**							
	31-Jul-20	22	DGG-1	<0.07	2,504	<0.09	<0.09	11	<0.07	<0.09
			DGG-2	<0.07	2,497	<0.09	<0.09	20	<0.07	<0.09
			DGG-3	<0.07	2,411	<0.09	<0.09	16	<0.07	<0.09
			Average	**ND**	**2,471**	**ND**	**ND**	**16**	**ND**	**ND**
	28-Aug-20	50	DGG-1	<0.07	2,335	<0.09	<0.09	31	<0.07	<0.09
			DGG-2	<0.07	2,348	<0.09	<0.09	<0.07	<0.07	<0.09
			DGG-3	<0.07	2,276	<0.09	<0.09	<0.07	<0.07	<0.09
			Average	**ND**	**2,320**	**ND**	**ND**	**10.2**	**ND**	**ND**
	24-Sep-20	77	DGG-1	<0.07	2,290	<0.09	<0.09	<0.07	<0.07	<0.09
			DGG-2	<0.07	2,329	<0.09	<0.09	<0.07	<0.07	<0.09
			DGG-3	<0.07	2,324	<0.09	<0.09	<0.07	<0.07	<0.09
			Average	**ND**	**2,314**	**ND**	**ND**	**ND**	**ND**	**ND**
	29-Oct-20	192	DGG-1	<0.07	2,754	<0.09	<0.09	2.1	<0.07	<0.09
			DGG-2	<0.07	2,561	<0.09	<0.09	8.6	<0.07	<0.09
			DGG-3	<0.07	2,394	<0.09	<0.09	7.6	<0.07	<0.09
			Average	**ND**	**2,570**	**ND**	**ND**	**6.1**	**ND**	**ND**

Notes:

< - compound not detected, the associated value is the detection limit
ANIC - anaerobic intrinsic control
ANSC - anaerobic sterile control
CB - chlorobenzene
DAP - diammonium phosphate
mg/L - milligrams per liter
mV - millivolts
ND - not detected
NRBC - nitrate reducing bacterial culture
VFAs - total volatile fatty acids, calibrated as lactate but may include other VFAs such as formate, acetate, propionate, pyruvate and butyrate

TABLE 4: SUMMARY OF MICROCOSM pH, ORP and AMMONIA RESULTS

Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

Treatment	Date	Day	Replicate	pH	ORP	Ammonia-N
					mV	mg/L
Anaerobic Sterile Control	31-Mar-20	-25	ANSC-1	6.30	--	--
			ANSC-2	6.32	--	--
			ANSC-3	6.35	--	--
			Average	**6.32**	**--**	**--**
	20-Apr-20	0	ANSC-1	6.43		--
			ANSC-2	6.48	124	--
			ANSC-3	6.39		--
			Average	**6.43**	**124**	**--**
	11-May-20	21	ANSC-1	6.77	--	--
			ANSC-2	6.81	--	--
			ANSC-3	6.72	--	--
			Average	**6.77**	**--**	**--**
	19-Jun-20	60	ANSC-1	6.51	--	--
			ANSC-2	6.47	--	--
			ANSC-3	6.43	--	--
			Average	**6.47**	**--**	**--**
	28-Aug-20	130	ANSC-1	6.58	--	--
			ANSC-2	6.61	--	--
			ANSC-3	6.56	--	--
			Average	**6.58**	**--**	**--**
	24-Sep-20	157	ANSC-1	6.64	--	--
			ANSC-2	6.69	--	--
			ANSC-3	6.66	--	--
			Average	**6.66**	**--**	**--**
	29-Oct-20	192	ANSC-1	6.65	--	--
			ANSC-2	6.71	--	--
			ANSC-3	6.65	--	--
			Average	**6.67**	**--**	**--**
Anaerobic Intrinsic Control	31-Mar-20	-25	ANIC-1	6.41	--	--
			ANIC-2	6.37	--	--
			ANIC-3	6.42	--	--
			Average	**6.40**	**--**	**--**
	20-Apr-20	0	ANIC-1	6.39		--
			ANIC-2	6.44	-25	--
			ANIC-3	6.48		--
			Average	**6.44**	**-25**	**--**
	11-May-20	21	ANIC-1	6.74	--	--
			ANIC-2	6.79	--	--
			ANIC-3	6.79	--	--
			Average	**6.77**	**--**	**--**
	19-Jun-20	60	ANIC-1	6.43	--	
			ANIC-2	6.42	--	0.43
			ANIC-3	6.44	--	
			Average	**6.43**	**--**	**0.43**
	28-Aug-20	130	ANIC-1	6.62	--	--
			ANIC-2	6.58	--	--
			ANIC-3	6.61	--	--
			Average	**6.60**	**--**	**--**
	24-Sep-20	157	ANIC-1	6.62	--	--
			ANIC-2	6.63	--	--
			ANIC-3	6.63	--	--
			Average	**6.63**	**--**	**--**
	29-Oct-20	192	ANIC-1	6.63	--	--
			ANIC-2	6.64	--	--
			ANIC-3	6.67	--	--
			Average	**6.65**	**--**	**--**
	12-Nov-20	206	ANIC-1	--	--	
			ANIC-2	--	--	<1.0
			ANIC-3	--	--	
			Average	**--**	**--**	**<1.0**
Nitrate Amended/NRBC Bioaugmented	31-Mar-20	-25	Nitrate-1	6.35	--	
			Nitrate-2	6.31	--	0.18
			Nitrate-3	6.35	--	
			Average	**6.34**	**--**	**0.18**
	20-Apr-20	0	Nitrate-1	6.44		--
			Nitrate-2	6.40	26	--
			Nitrate-3	6.28		--
			Average	**6.37**	**26**	**--**
	11-May-20	21	Nitrate-1	6.81	--	--
			Nitrate-2	6.83	--	--
			Nitrate-3	6.76	--	--
			Average	**6.80**	**--**	**--**
	29-May-20	39	Nitrate-1	6.99	--	--
			Nitrate-2	7.02	--	--
			Nitrate-3	6.98	--	--
			Average	**7.00**	**--**	**--**
	19-Jun-20	60	Nitrate-1	6.57	--	
			Nitrate-2	6.58	--	2.92
			Nitrate-3	6.51	--	
			Average	**6.55**	**--**	**2.92**
	31-Jul-20	102	Nitrate-1	6.76	--	--
			Nitrate-2	6.74	--	--
			Nitrate-3	6.68	--	--
			Average	**6.73**	**--**	**--**
	28-Aug-20	130	Nitrate-1	6.77	--	--
			Nitrate-2	6.73	--	--
			Nitrate-3	6.74	--	--
			Average	**6.75**	**--**	**--**
	24-Sep-20	157	Nitrate-1	6.82	--	--
			Nitrate-2	6.79	--	--
			Nitrate-3	6.81	--	--
			Average	**6.81**	**--**	**--**

TABLE 4: SUMMARY OF MICROCOSM pH, ORP and AMMONIA RESULTS
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment	Date	Day	Replicate	pH	ORP	Ammonia-N
					mV	mg/L
Nitrate Amended/NRBC Bioaugmented Continued	29-Oct-20	192	Nitrate-1	6.82	--	--
			Nitrate-2	6.83	--	--
			Nitrate-3	6.75	--	--
			Average	**6.80**	**--**	**--**
	12-Nov-20	206	Nitrate-1	6.82	--	
			Nitrate-2	6.83	--	3.86
			Nitrate-3	6.75	--	
			Average	**6.80**	**--**	**3.86**
Nitrate and DAP Amended	31-Mar-20	-25	Nitrate/DAP-1	6.37	--	
			Nitrate/DAP-2	6.39	--	3.35
			Nitrate/DAP-3	6.37	--	
			Average	**6.38**	**--**	**3.35**
	20-Apr-20	0	Nitrate/DAP-1	6.44		--
			Nitrate/DAP-2	6.45	31	--
			Nitrate/DAP-3	6.43		--
			Average	**6.44**	**31**	**--**
	11-May-20	21	Nitrate/DAP-1	6.81	--	--
			Nitrate/DAP-2	6.87	--	--
			Nitrate/DAP-3	6.85	--	--
			Average	**6.84**	**--**	**--**
	19-Jun-20	60	Nitrate/DAP-1	6.51	--	
			Nitrate/DAP-2	6.51	--	2.41
			Nitrate/DAP-3	6.49	--	
			Average	**6.50**	**--**	**2.41**
	31-Jul-20	102	Nitrate/DAP-1	6.67	--	--
			Nitrate/DAP-2	6.65	--	--
			Nitrate/DAP-3	6.67	--	--
			Average	**6.66**	**--**	**--**
	24-Sep-20	157	Nitrate/DAP-1	6.74	--	--
			Nitrate/DAP-2	6.74	--	--
			Nitrate/DAP-3	6.77	--	--
			Average	**6.75**	**--**	**--**
	29-Oct-20	192	Nitrate/DAP-1	6.74	--	--
			Nitrate/DAP-2	6.75	--	--
			Nitrate/DAP-3	6.72	--	--
			Average	**6.74**	**--**	**--**
	12-Nov-20	206	Nitrate/DAP-1	6.74	--	
			Nitrate/DAP-2	6.75	--	1.29
			Nitrate/DAP-3	6.72	--	
			Average	**6.74**	**--**	**1.29**
DGG-B™ Bioaugmented	31-Jul-20	22	DGG-1	6.61	--	--
			DGG-2	6.62	--	--
			DGG-3	6.64	--	--
			Average	**6.62**	**--**	**--**
	28-Aug-20	50	DGG-1	6.68	--	--
			DGG-2	6.68	--	--
			DGG-3	6.68	--	--
			Average	**6.68**	**--**	**--**
	24-Sep-20	77	DGG-1	6.68	--	--
			DGG-2	6.67	--	--
			DGG-3	6.66	--	--
			Average	**6.67**	**--**	**--**
	29-Oct-20	192	DGG-1	6.70	--	--
			DGG-2	6.70	--	--
			DGG-3	6.71	--	--
			Average	**6.70**	**--**	**--**

Notes:

ANIC - anaerobic intrinsic control
ANSC - anaerobic sterile control
DAP - diammonium phosphate
mg/L - milligrams per liter
mV - millivolts
NRBC - nitrate reducing bacterial culture
ORP - oxidation reduction potential

TABLE 5: SUMMARY OF MICROCOSMS GENE-TRAC® RESULTS
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

Treatment	Date	Molecular Analysis			
		ORM2	SRB	*Peptococcaceae*	Benzene Carboxylase
		16S rRNA gene copies/L	*dsr* A gene copies/L	16S rRNA gene copies/L	*abc* A gene copies/L
Microcosm MW-29D	30-Mar-20	4×10^3 J	4×10^3 J	6×10^3 U	6×10^3 U
Anaerobic Intrinsic Control	19-Jun-20	--	--	--	--
	12-Nov-20	Pending	Pending	Pending	Pending
Nitrate Amended/NRBC Bioaugmented	19-Jun-20	9×10^4 U, I	9×10^4 U,I	9×10^4 U,I	9×10^4 U,I
	12-Nov-20	Pending	Pending	Pending	Pending
Nitrate and DAP Amended	19-Jun-20	9×10^4 U	2×10^7	9×10^4 U	9×10^4 U
	12-Nov-20	Pending	Pending	Pending	Pending
DGG-B™ Bioaugmented	19-Jun-20	--	--	--	--

Notes:

-- - not applicable
abcA - benzene carboxylase
DAP - diammonium phosphate
DNA - deoxyribonucleic acid
dsrA - dissimilatiory sulfate reductase A
gene copies/L - gene copies of functional gene per liter
I - sample inhibited the test reaction based on inability to PCR amplify extracted DNA with universal primers
J - the associalted value is an estimated quantity between the method detection limit and th quantification limit
NRBC - nitrate reducing bacterial culture
PCR - polymerase chain reaction
rRNA - ribosomal ribonucleic acid
SRB - sulfate reducing bacteria
U - not detected, the associated value is the quantitation limit

TABLE 6: HALF-LIVES (DAYS) OF BENZENE AND CHLOROBENZENE

SiREM

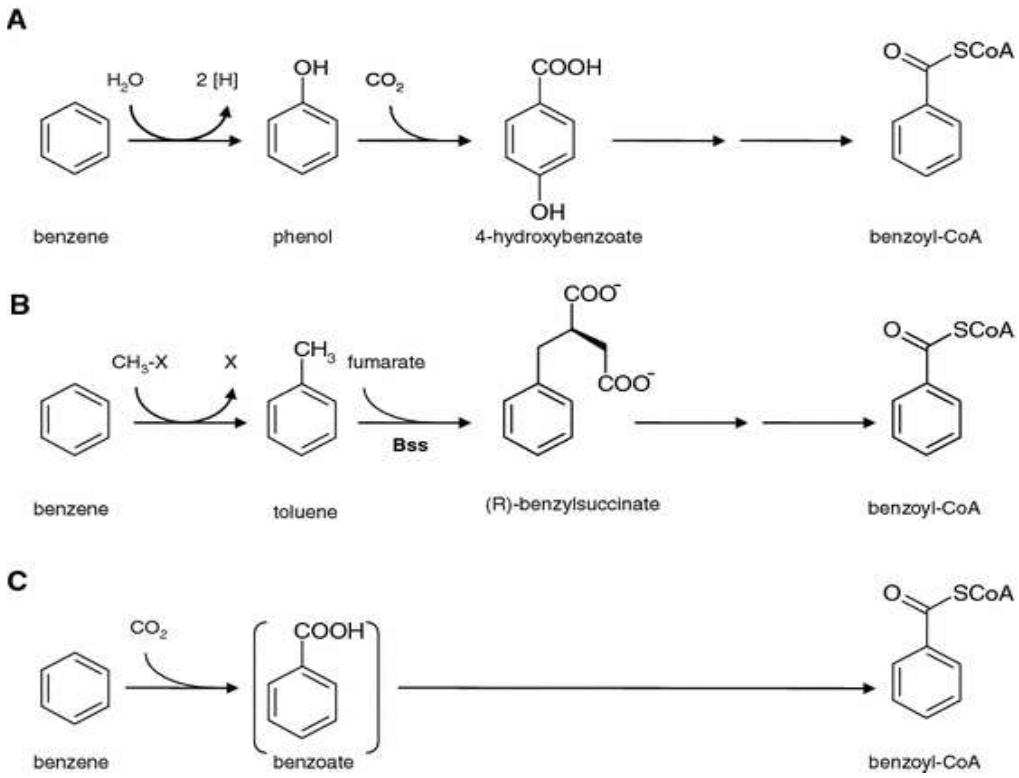
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

Treatment/Control	Benzene			Chlorobenzene		
	Half Life (Days)	T_1 (Day)	T_2 (Days)	**Half Life (Days)**	T_1 (Day)	T_2 (Days)
Anaerobic Sterile Control	3,991	0	192	~	0	192
Intrinsic Control	1,218	0	192	2,062	0	192
Nitrate Amended/NRBC Bioaugmented	328	0	192	409	0	192
Nitrate and DAP Amended	~	0	192	~	0	192
DGG-B™ Bioaugmented	596	0	192	1,857	0	192

Notes:

~ - net degradation of compound was not detected over duration of study
DAP - diammonium phosphate
NRBC - nitrate reducing benzene culture

FIGURES



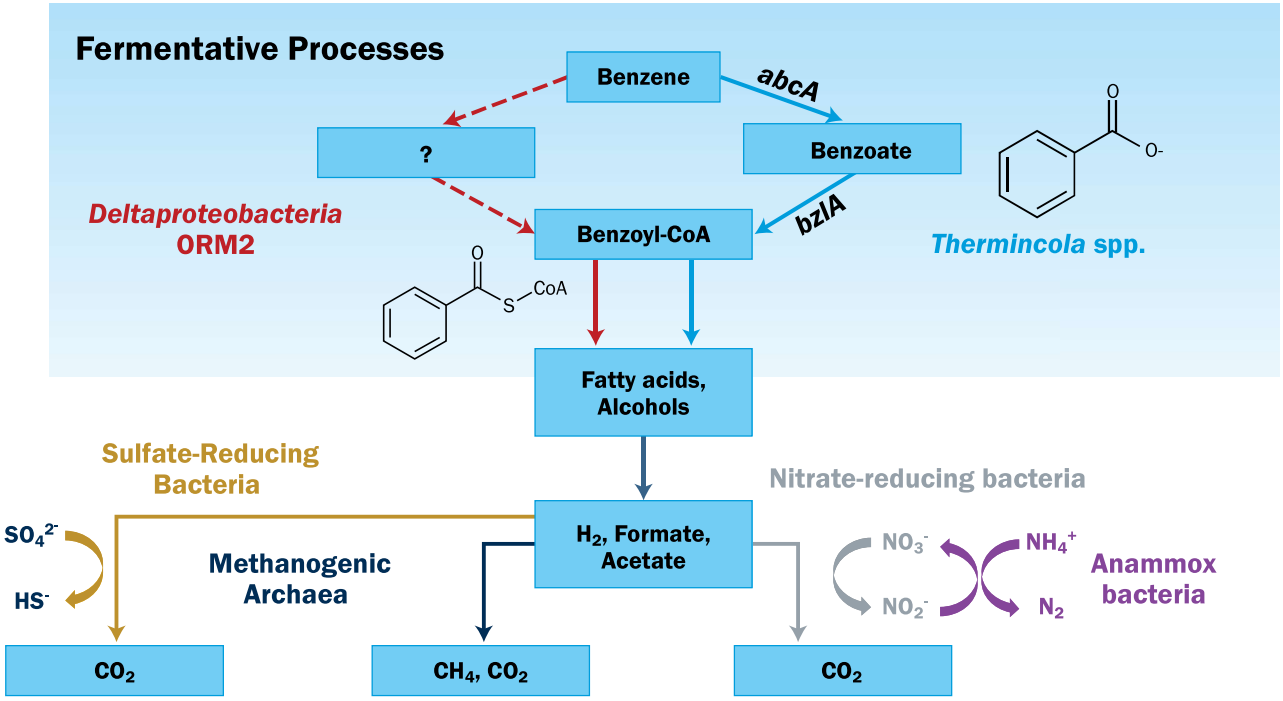
Potential Anaerobic Pathway for the Biodegradation of Benzene

SiREM

January 2021

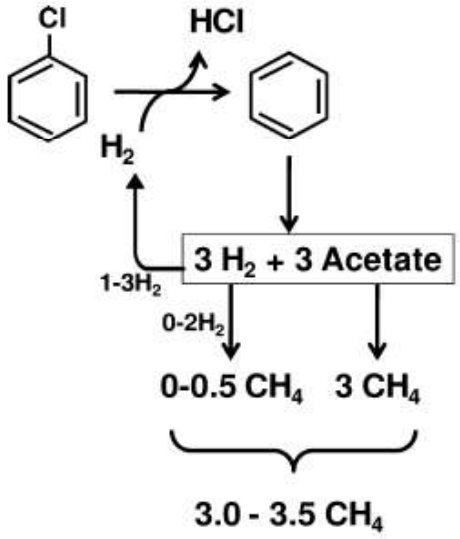
Figure: **1a**

Sander *et al* ., 2010



Overview of Anaerobic Benzene Degradation.ai

Overview of ~~Fermentative~~ Anaerobic Benzene Degradation	
SiREM	January 2021
	Figure: **1b**



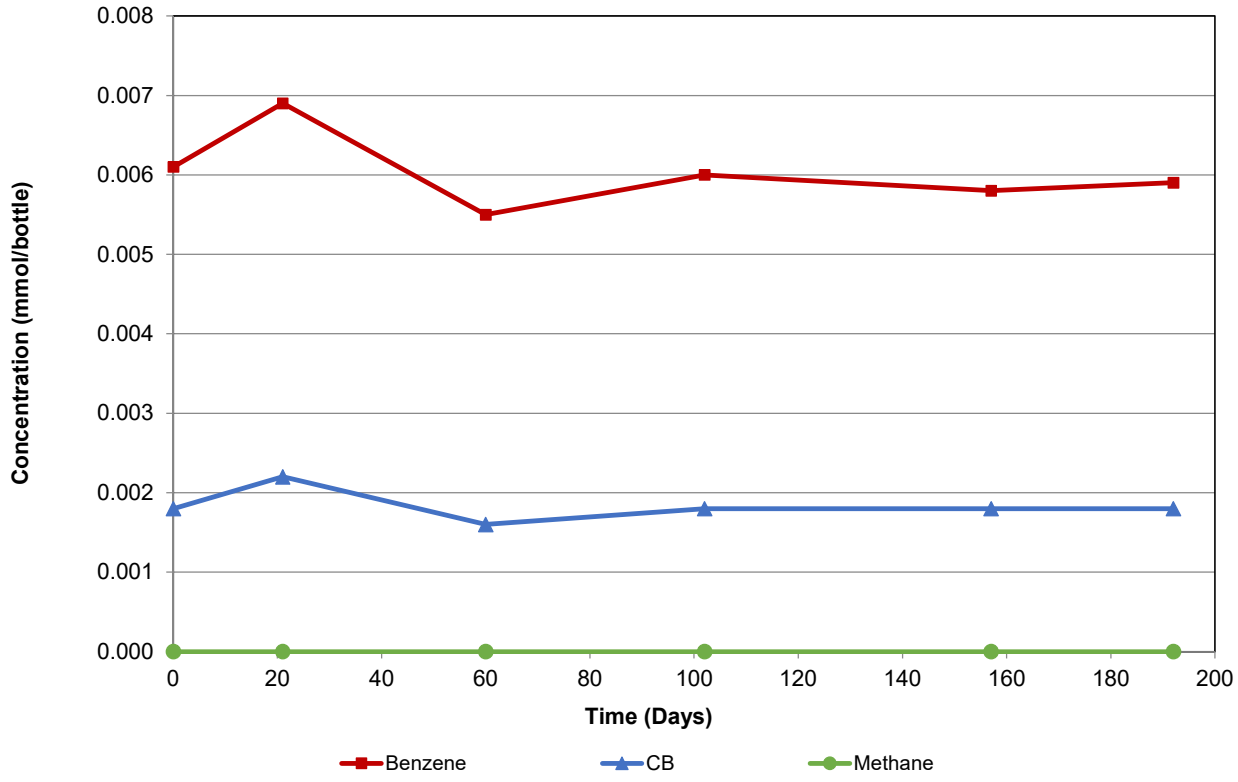
Potential Anaerobic Pathway for the Biodegradation of Chlorobenzene

SiREM

January 2021

Figure: **2**

Liang et al., 2013



Notes:

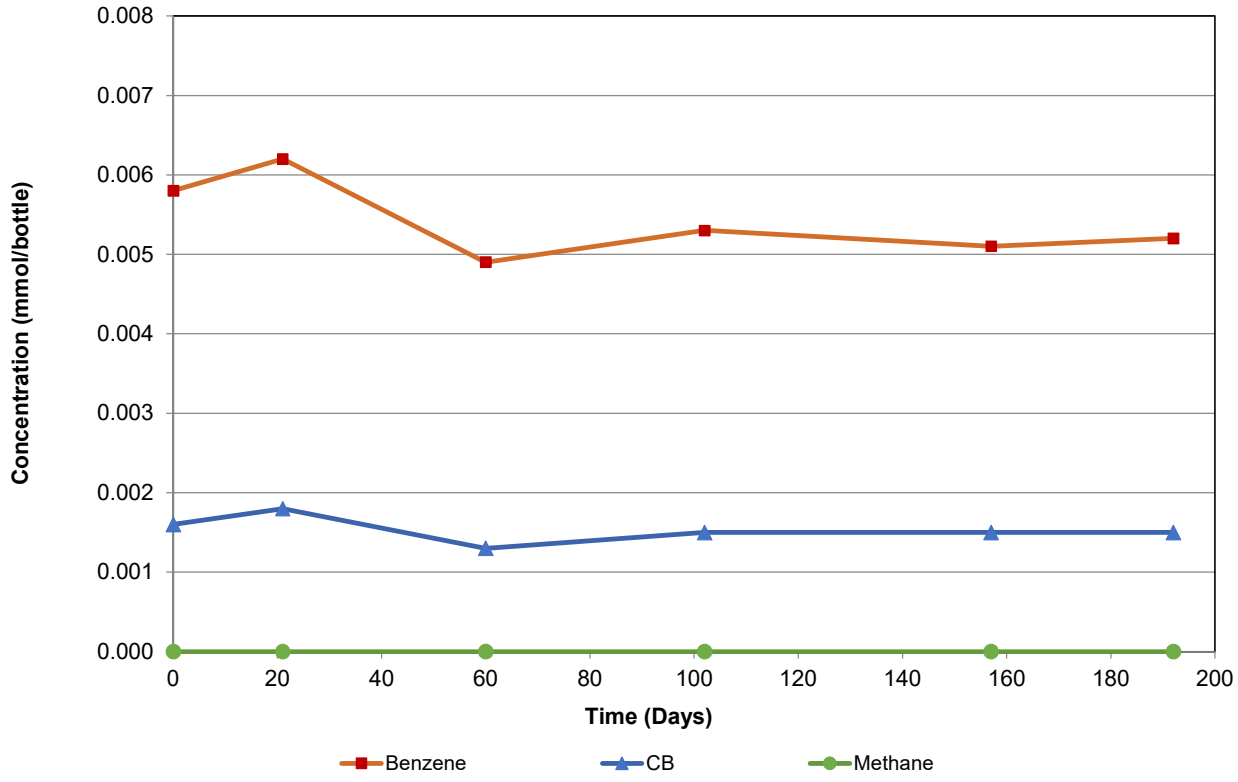
CB - chlorobenzene
mmoles/bottle - millimoles per bottle

Benzene and Chlorobenzene Concentration Trends in Sterile Control Microcosms
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

January 2021

Figure: **3**



Notes:

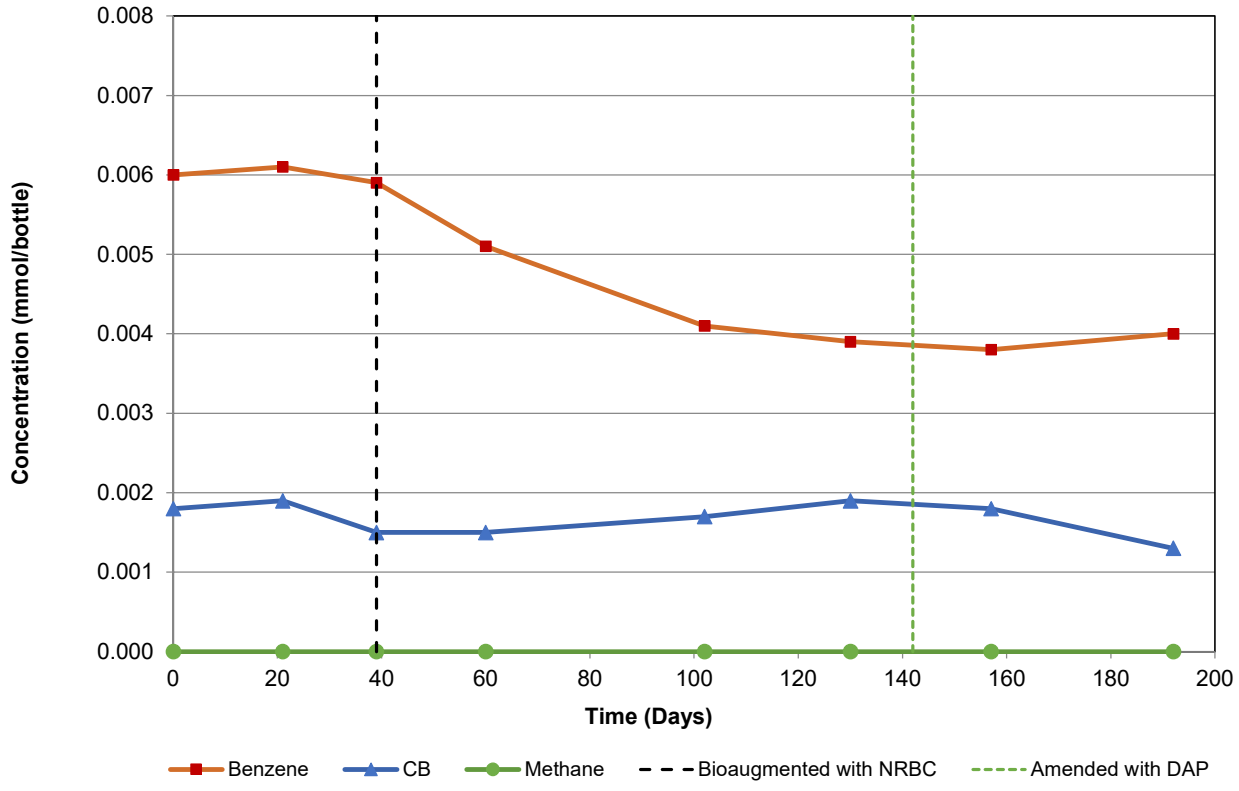
CB - chlorobenzene
mmoles/bottle - millimoles per bottle

Benzene and Chlorobenzene Concentration Trends in Intrinsic Control Microcosms
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

January 2021

Figure: **4**



Notes:

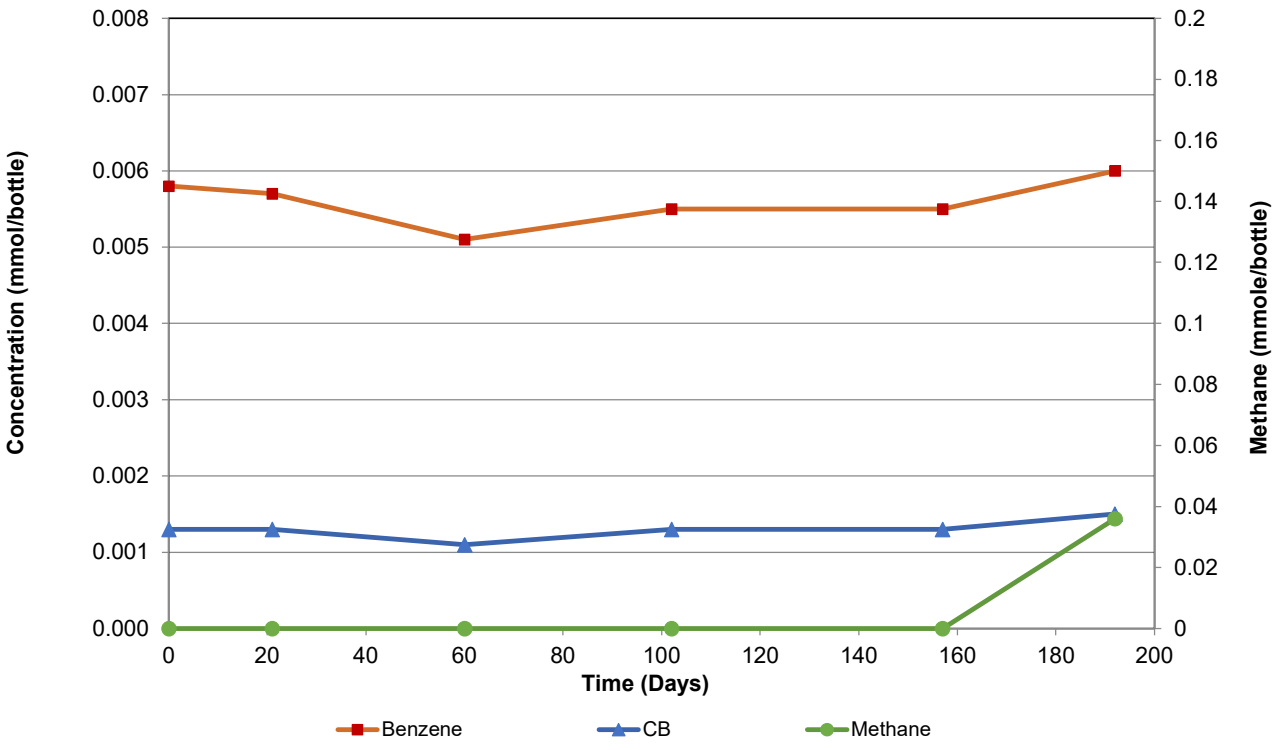
CB - chlorobenzene
DAP - diammonium phosphate
mmoles/bottle - millimoles per bottle
NRBC - nitrate reducing bacterial culture

Benzene and Chlorobenzene Concentration Trends in Nitrate Amended/NRBC Bioaugmented Microcosms
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

January 2021

Figure: **5**



Notes:

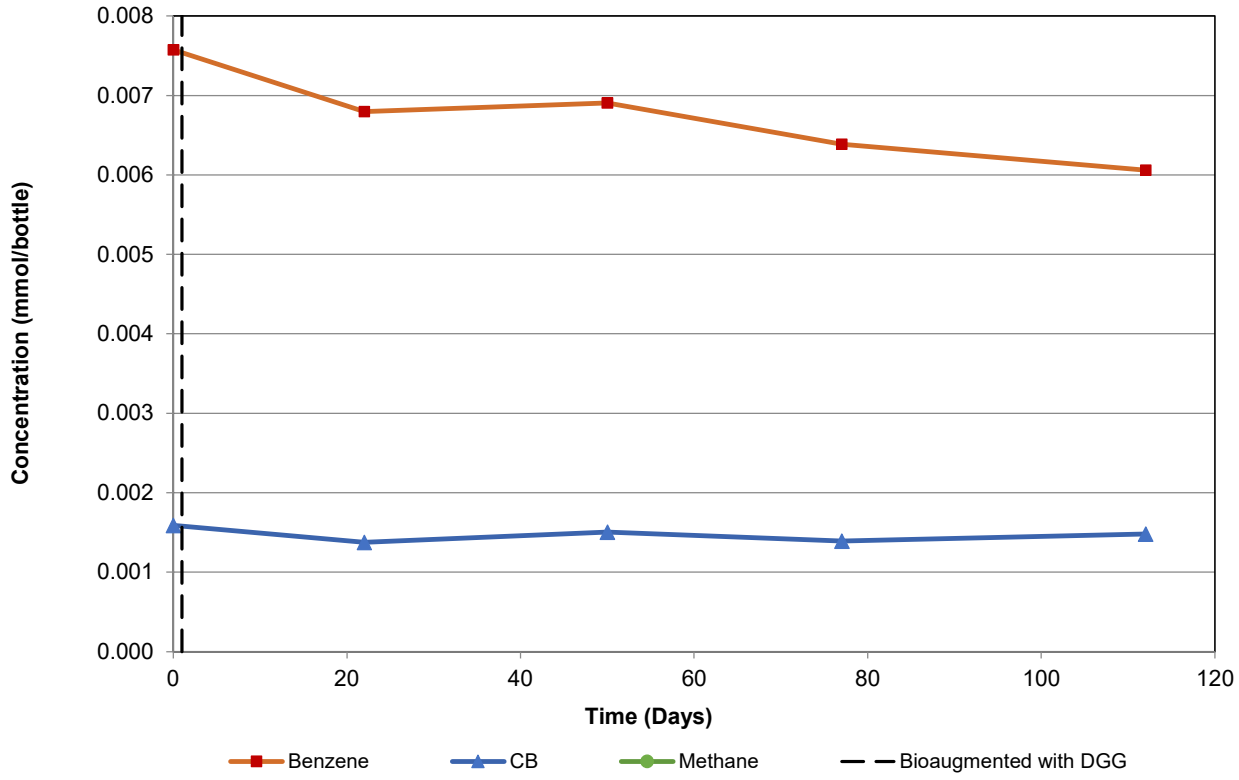
CB - chlorobenzene
DAP - diammonium phosphate
mmoles/bottle - millimoles per bottle

Benzene and Chlorobenzene Concentration Trends in Nitrate and DAP Amended Microcosms
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

January 2021

Figure: **6**



Notes:

CB - chlorobenzene
mmoles/bottle - millimoles per bottle

Benzene and Chlorobenzene Concentration Trends in DGG-B™ Bioaugmented Microcosms
Deep Zone of Upper Surficial Aquifer - Brunswick, GA

SiREM

January 2021

Figure: **7**

APPENDIX A: Chain of Custody Documentation

SiREM

Chain-of-Custody Form

siremlab.com

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Lab # S-5735

*Project Name: Brunswick Hercules/Pinova	*Project #: GR6381
*Project Manager: Adrian Reimer	*Company: Geosyntec
*Email Address: areimer@geosyntec.com	
Address (Street): 1255 Roberts Boulevard	
City: Kennesaw	State/Province: GA
Country: USA	
*Phone #: +1 470-367-7557	
*Sampler's Signature	*Sampler's Printed Name

Analysis

Preservative Key: O

0. None
1. HCL
2. Other
3. Other
4. Other
5. Other
6. Other

Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Gene-Trac DHC	Gene-Trac FGA (vcrA, bvcA, tceA)	Gene-Trac DHB	Gene-Trac DHG	Gene-Trac SRB	Volatile Fatty Acids	Dissolved hydrocarbon gases	Treatability Study	Other Information
MW-28D*	2/28/20	1240	water	2								X	2 x 4L plastic bottles
MW-29D**			water	5								X	5 x 4L plastic bottles
													* MW28D: chloroform, Area and benzene (chlorobenzene)
													** As MW28D plus inhibition of nitrate mediated benzene remed.

Billing Information

P.O. #:

*Bill To:

Turnaround Time Requested

Normal ☐
Rush ☐

For Lab Use Only

Cooler Condition: Good

Cooler Temperature: 9°C

Custody Seals: Yes ☐ No ☑

For Lab Use Only

Proposal #: Si-4402-013120 / Si-4384-013020

Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature: [signature]	Signature: [signature]	Signature: [signature]	Signature: [signature]	Signature:	Signature:
Printed Name: Christine Hug	Printed Name: Ben Weinmann	Printed Name: Ben Weinmann	Printed Name: Rachel Hallman	Printed Name:	Printed Name:
Firm: Geosyntec Cons.	Firm: Geosyntec Consultants	Firm: Geosyntec Consultants	Firm: SiREM	Firm:	Firm:
Date/Time: 2/28/2020 1300	Date/Time: 2/29/2020 1305	Date/Time: 3/2/20	Date/Time: 3/4/20 1430	Date/Time:	Date/Time:

Distribution: White - return to Originator; Yellow - Lab Copy; Pink - Retained by Client

* Mandatory Fields

SiREM

Chain-of-Custody Form

sirem.com

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Lab # S-5734

*Project Name: Brunswick Treatability	*Project #:
*Project Manager: Adria Reimer	*Company: Geosyntec
*Email Address: areimer@geosyntec.com	
Address (Street): 1255 Roberts Boulevard Suite 200	
City: Kennesaw	State/Province: GA	Country: USA
*Phone #: 678 202 9564	
*Sampler's Signature:	*Sampler's Printed Name:

Analysis

Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Gene-Trac DHC	Gene-Trac VC	Gene-Trac DHB	Gene-Trac DHG	Gene-Trac tceA	Volatile Fatty Acids	Dissolved hydrocarbon gases	Treatability Study	Other Information
MW-29D	2/25/20		GW	5								X	20L total. for CB/CE study ~~and E~~

Preservative Key
0. None
1. HCL
2. Other _____
3. Other _____
4. Other _____
5. Other _____
6. Other _____

Billing Information	Turnaround Time Requested	For Lab Use Only	For Lab Use Only
P.O. #:	Normal ☐	Cooler Condition: GOOD	
*Bill To:	Rush ☐	Cooler Temperature: 0°C.	
		Custody Seals: Yes ☐ ~~No~~ ☒	Proposal #:

Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature	Signature	Signature	Signature	Signature	Signature
Printed Name	Printed Name: D. Nespoli	Printed Name	Printed Name	Printed Name	Printed Name
Firm	Firm: SiREM	Firm	Firm	Firm	Firm
Date/Time	Date/Time: MAR 3 '20 1:30pm	Date/Time	Date/Time	Date/Time	Date/Time

Distribution: White - return to Originator. Yellow - Lab Copy. Pink - Retained by Client

* Mandatory Fields

Chain-of-Custody Form

Lab # S-5746

*Project Name: Brunswick Hercules/Pinova	*Project #: GRG881B
*Project Manager: Adria Reimer	*Company: Geosyntec
*Email Address: areimer@geosyntec.com	
Address (Street): 1255 Roberts Blvd	
City: Kennesaw	State/Province: GA	Country: USA
*Phone #: 678-202-9564	
*Sampler's Signature: Ben W	*Sampler's Printed Name: Ben Weinmann

Analysis

Preservative Key
0. None
1. HCL
2. Other ____
3. Other ____
4. Other ____
5. Other ____
6. Other ____

	Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Gene-Trac DHC	Gene-Trac VC	Gene-Trac DHB	Gene-Trac DHG	Treatability Study	Other Information
1	ISB-01_MW28D_83-84	3/4/20	0945	S	1					X	
2	ISB01_MW28D_84-85	3/4/20	0950	S	1					X	
3	ISB-01_MW28D_85-86	3/4/20	0955	S	1					X	
4	ISB-01_MW28D-86-87	3/4/20	1000	S	1					X	
5	ISB-02_MW29D_78-80	3/4/20	1200	S	1					X	
6	ISB-02_MW29D_80-82	3/4/20	1205	S	1					X	
7	ISB-02_MW29D_84-86	3/4/20	1210	S	1					X	
8	ISB-02_MW29D_86-88	3/4/20	1215	S	1					X	
9	ISB-01_MW29D_89-90	3/4/20	1220	S	1					X	

Billing Information
P.O. #:
*Bill To:

Turnaround Time Requested
Normal ☐
Rush ☐

For Lab Use Only
Cooler Condition: Good
Cooler Temperature: 4°C
Custody Seals: Yes ☑ No ☐

For Lab Use Only
Proposal #: ____

Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature: Ben W	Signature: Rachel Hallman	Signature:	Signature:	Signature:	Signature:
Printed Name: Ben Weinmann	Printed Name: Rachel Hallman	Printed Name:	Printed Name:	Printed Name:	Printed Name:
Firm: Geosyntec	Firm: SiREM	Firm:	Firm:	Firm:	Firm:
Date/Time: 3/9/21 1530	Date/Time: 3-12-20 10:00	Date/Time:	Date/Time:	Date/Time:	Date/Time:

Distribution: White - return to Originator; Yellow - Lab Copy; Pink - Retained by Client
* Mandatory Fields

APPENDIX B: Henry's Law Calculation

The following Henry's Law calculation was used to convert aqueous concentrations (Table 2) to total mmoles of each analyte per microcosm bottle (Figures 3 to 7):

$$Total\ mmoles = \frac{C_{liq} \cdot \left(V_{liq} + H \cdot V_{gas}\right)}{Molecular\ Weight\ (\frac{mg}{mmol})}$$

Where for the 250 mL microcosms:

C_{liq} = liquid concentration (mg/L)
V_{liq} = liquid volume (0.200 L) per bottle
V_{gas} = headspace volume (0.020 L) per bottle
H = Henry's Law constant (dimensionless)

The Henry's Law constants used are summarized in the table below.

Analyte	Henry's Law Constant [a] (dimensionless)
Benzene	0.222
Chlorobenzene	0.161
Methane	27.3

[a] Source: Montgomery, J.H. 2000. *Groundwater Chemicals Desk Reference, Third Edition.* CRC Press LLC, Boca Raton, FL.

APPENDIX C: Gene-Trac® Laboratory Reports

SiREM
Leading Science · Lasting Solutions

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Certificate of Analysis: Gene-Trac® ORM-2, Assay

Customer: Duane Graves, Geosyntec Consultants

Project: Brunswick Deep Benzene Treatability Study

Customer Reference: SC4384

SiREM Reference: S-5775

Report Date: 12-May-20

Data Files: iQ5C-ORM2-QPCR-0134
iQ5C-ORM2-DB-QPCR-0134

Table 1a: Test Results

Sample ID	Deltaproteobacterium ORM-2	
	Percent ORM-2[(1)]	ORM-2 16S rRNA Gene Copies/Liter
Microcosm MW-29D	0.0002 - 0.0007 %	4×10^3 J

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Certificate of Analysis: Gene-Trac® SRB, Sulfate Reducing Bacteria (*dsrA*) Assay

Customer: Duane Graves, Geosyntec Consultants

Project: Brunswick Deep Benzene Treatability Study

Customer Reference: SC4384

SiREM Reference: S-5775

Report Date: 12-May-20

Data Files: iQ5B-SRB-QPCR-0057
iQ5B-DB-SRB-QPCR-0057

Table 1b: Test Results

Sample ID	Sulfate Reducing Bacteria (*dsrA*)	
	Percent *dsrA* (1)	*dsrA* Gene Copies/Liter
Microcosm MW-29D	0.0002 - 0.0007 %	4×10^3 J

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

Certificate of Analysis: Gene-Trac® abcA Benzene Carboxylase Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-5775
Report Date: 12-May-20
Data Files: iQ5A-abcA-QPCR-0113
iQ5A-DB-abcA-QPCR-0113

Table 1c: Test Results

Sample ID	Benzene Carboxylase (*abcA*)	
	Percent *abcA* (1)	*abcA* Gene Copies/Liter
Microcosm MW-29D	NA	6×10^3 U

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

Certificate of Analysis: Gene-Trac® Pepto-ben *Peptococcaceae* Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-5775
Report Date: 12-May-20
Data Files: iQ5B-Pepto-QPCR-0112
iQ5B-DB-Pepto-QPCR-0112

Table 1d: Test Results

Sample ID	*Peptococcaceae*	
	Percent *Peptococcaceae* (1)	*Peptococcaceae* 16S rRNA Gene Copies/Liter
S-4500	NA	6×10^3 U

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

Table 2: Detailed Test Parameters, Test Reference S-5775

Customer Sample ID	Microcosm MW-29D
SiREM ORM-2 Test ID	ORM-0188
SiREM SRB Test ID	SRB-0330
SiREM *abcA* Test ID	ABC-0156
SiREM Pepto-ben Test ID	PEP-0139
Date Sampled [(2)]	30-Mar-20
Matrix	Microcosm
Date Received [(2)]	30-Mar-20
Sample Temperature	NA
Filtration Date [(2)]	30-Mar-20
Volume Used for DNA Extraction	200 mL
DNA Extraction Date	1-Apr-20
DNA Concentration in Sample (extractable)	3428 ng/L
PCR Amplifiable DNA	Detected
ORM-2 qPCR Date Analyzed	2-Apr-20
SRB qPCR Date Analyzed	2-Apr-20
***abcA* qPCR Date Analyzed**	3-Apr-20
Pepto-ben qPCR Date Analyzed	3-Apr-20
Laboratory Controls (see Tables 3, 4, 5 & 6)	Passed
Comments	- -

See final page for notes.

Table 3: Gene-Trac ORM-2 Control Results, Test Reference S-5775

Laboratory Control	Analysis Date	Control Description	ORM-2		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	2-Apr-20	Genomic DNA (CSLO-0134)	5.2×10^8	3.9×10^8	Passed
Positive Control High Concentration	2-Apr-20	Genomic DNA (CSHO-0134)	9.1×10^9	7.9×10^9	Passed
DNA Extraction Blank	2-Apr-20	Sterile Water (FB-3519)	0	2.6×10^3 U	Passed
Negative Control	2-Apr-20	Test Reagent Blank (TBO-0134)	0	2.6×10^3 U	Passed

See final page for notes.

Table 4: Gene-Trac SRB Control Results, Test Reference S-5775

Laboratory Control	Analysis Date	Control Description	*dsrA*		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	2-Apr-20	Genomic DNA (CSLSR-0057)	8.6×10^5	1.1×10^6	Passed
Positive Control High Concentration	2-Apr-20	Genomic DNA (CSHSR-0057)	4.8×10^7	4.2×10^7	Passed
DNA Extraction Blank	2-Apr-20	Sterile Water (FB-3519)	0	2.6×10^3 U	Passed
Negative Control	2-Apr-20	Test Reagent Blank (TBSR-0057)	0	2.6×10^3 U	Passed

See final page for notes.

Table 5: Gene-Trac abcA Control Results, Test Reference S-5775

Laboratory Control	Analysis Date	Control Description	*abcA*		Comments
			Spiked Gene Copies per Reaction	Recovered Gene Copies per Reaction	
Positive Control Low Concentration	3-Apr-20	Plasmid DNA (CSLAB-0113)	3.5×10^4	2.5×10^4	Passed
Positive Control High Concentration	3-Apr-20	Plasmid DNA (CSHAB-0113)	3.5×10^6	3.3×10^6	Passed
DNA Extraction Blank	3-Apr-20	Sterile Water (FB-3519)	0	2.0×10^1 U	Passed
Negative Control	3-Apr-20	Test Reagent Blank (TBAB-0113)	0	2.0×10^1 U	Passed

See final page for notes.

Table 6: Gene-Trac Pepto-ben Control Results, Test Reference S-5775

Laboratory Control	Analysis Date	Control Description	Pepto-ben		Comments
			Spiked Gene Copies per Reaction	Recovered Gene Copies per Reaction	
Positive Control Low Concentration	3-Apr-20	Genomic DNA (CSLPE-0112)	3.2×10^4	$1.5 \times 10^{4\ (3)}$	See Note 3
Positive Control High Concentration	3-Apr-20	Genomic DNA (CSHPE-0112)	3.2×10^6	1.7×10^6	Passed
DNA Extraction Blank	3-Apr-20	Sterile Water (FB-3519)	0	2.0×10^1 U	Passed
Negative Control	3-Apr-20	Test Reagent Blank (TBPE-0112)	0	2.0×10^1 U	Passed

See final page for notes.

Notes:
ORM-2 = Deltaproteobacterium ORM-2
dsrA = *dissimilatory sulfate reductase A*
abcA = Benzene Carboxylase
J The associated value is an estimated quantity between the method detection limit and quantitation limit.
U Not detected, associated value is the quantitation limit.
B Analyte was detected in the method blank within an order of magnitude of the test sample.
E Extracted genomic DNA was not detected in the sample.
I Sample inhibited the test reaction based on inability to PCR amplify extracted DNA with universal primers.
ng/L = nanograms per liter
mL = milliliter
NA = not applicable
ND = not detected
DNA = deoxyribonucleic acid
16S rRNA = 16S ribosomal ribonucleic acid
PCR = polymerase chain reaction
qPCR = quantitative PCR
°C = degrees Celsius

[1]Percent ORM-2 *Deltaproteobacterium* (ORM-2), *Peptococcaceae, dsrA,* or *abcA* in microbial population. This value is calculated by dividing the number of specific gene copies by the total number of bacteria as estimated by the mass of DNA extracted from the sample. Range represents normal variation in enumeration.

[2]Samples are stabilized by freezing at -80 °C upon sample reception (field filters) or in-lab filtration (groundwater). Hold time not exceeded if sampling date is within 14 days of date received or filtration date.

[3]Control was outside recovery limit guidelines (+/- 50%), however, test results are deemed acceptable if one of two positive controls falls within the recovery limit.

SiREM

Chain-of-Custody Form

sirem.com

140 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-4849

Lab # S-5975

Part of a treedability study S-5734.

*Project Name: Brunswick Deep Benzene	*Project #: SC-4384.
*Project Manager: Duane Graves	*Company: Geosyntec
*Email Address: dgraves@geosyntec.com	
Address (Street): 1020A Market Place Blvd.	
City: Knoxville / State/Province: TN 37922 / Country: USA	
*Phone #:	
*Sampler's Signature: J Webb	*Sampler's Printed Name: Jen Webb

Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Gene-Trac DHC	Gene-Trac VC	Gene-Trac DHB	Gene-Trac DHG	Gene-Trac tceA	Volatile Fatty Acids	Dissolved hydrocarbon gases	Treatability Study abcA	ORM2	SRB	peptococcaceae	Other Information
Microcosm MW-290	30/6/20	15:00	aqu	1								✓	✓	✓	✓	

Analysis

Preservative Key
0. None
1. HCL
2. Other
3. Other
4. Other
5. Other
6. Other

Billing Information	Turnaround Time Requested	For Lab Use Only	For Lab Use Only
P.O. #:	Normal ☒	Cooler Condition: NA	
*Bill To:	Rush ☐	Cooler Temperature: NA	
		Custody Seals: Yes ☐ No ☐	Proposal #:

Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature: J Webb	Signature:	Signature:	Signature:	Signature:	Signature:
Printed Name: Jen Webb	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:
Firm: SiREM	Firm:	Firm:	Firm:	Firm:	Firm:
Date/Time: 30/6/20 15:00	Date/Time:	Date/Time:	Date/Time:	Date/Time:	Date/Time:

Distribution: White - return to Originator. Yellow - Lab Copy. Pink - Retained by Client.
* Mandatory Fields

SiREM
Leading Science · Lasting Solutions

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Certificate of Analysis: Gene-Trac® ORM-2, Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-5939
Report Date: 21-Jul-20
Data Files: iQ5C-ORM2-QPCR-0141
iQ5C-ORM2-DB-QPCR-0141

Table 1a: Test Results

Sample ID	Deltaproteobacterium ORM-2	
	Percent ORM-2[(1)]	ORM-2 16S rRNA Gene Copies/Liter
Brunswick-DB-nitrate +DAP	NA	9×10^4 U
Brunswick-DB-nitrate	NA	9×10^4 U, I

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Certificate of Analysis: Gene-Trac® SRB, Sulfate Reducing Bacteria (*dsrA*) Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-5939
Report Date: 21-Jul-20
Data Files: iQ5B-SRB-QPCR-0063
iQ5B-DB-SRB-QPCR-0063

Table 1b: Test Results

Sample ID	Sulfate Reducing Bacteria (*dsrA*)	
	Percent *dsrA* (1)	*dsrA* Gene Copies/Liter
Brunswick-DB-nitrate +DAP	0.03 - 0.09 %	2×10^7
Brunswick-DB-nitrate	NA	9×10^4 U, I

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

Certificate of Analysis: Gene-Trac® abcA Benzene Carboxylase Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-5939
Report Date: 21-Jul-20
Data Files: iQ5A-abcA-QPCR-0116
iQ5A-DB-abcA-QPCR-0116

Table 1c: Test Results

Sample ID	Benzene Carboxylase (*abcA*)	
	Percent *abcA* (1)	*abcA* Gene Copies/Liter
Brunswick-DB-nitrate +DAP	NA	9×10^4 U
Brunswick-DB-nitrate	NA	9×10^4 U, I

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

Certificate of Analysis: Gene-Trac® Pepto-ben *Peptococcaceae* Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-5939
Report Date: 21-Jul-20
Data Files: iQ5B-Pepto-QPCR-0115
iQ5B-DB-Pepto-QPCR-0115

Table 1d: Test Results

Sample ID	*Peptococcaceae*	
	Percent *Peptococcaceae* (1)	*Peptococcaceae* 16S rRNA Gene Copies/Liter
Brunswick-DB-nitrate +DAP	NA	9×10^4 U
Brunswick-DB-nitrate	NA	9×10^4 U, I

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

Table 2: Detailed Test Parameters, Test Reference S-5939

Customer Sample ID	Brunswick-DB-nitrate+DAP	Brunswick-DB-nitrate
SiREM ORM-2 Test ID	ORM-0215	ORM-0216
SiREM SRB Test ID	SRB-0342	SRB-0343
SiREM *abcA* Test ID	ABC-0163	ABC-0164
SiREM Pepto-ben Test ID	PEP-0143	PEP-0144
Date Sampled [(2)]	19-Jun-20	19-Jun-20
Matrix	Microcosm	Microcosm
Date Received [(2)]	19-Jun-20	19-Jun-20
Sample Temperature	NA	NA
Filtration Date [(2)]	19-Jun-20	19-Jun-20
Volume Used for DNA Extraction	15 mL	15 mL
DNA Extraction Date	30-Jun-20	30-Jun-20
DNA Concentration in Sample (extractable)	121000 ng/L	112500 ng/L
PCR Amplifiable DNA	Detected	ND
ORM-2 qPCR Date Analyzed	2-Jul-20	2-Jul-20
SRB qPCR Date Analyzed	1-Jul-20	1-Jul-20
***abcA* qPCR Date Analyzed**	6-Jul-20	6-Jul-20
Pepto-ben qPCR Date Analyzed	16-Jul-20	16-Jul-20
Laboratory Controls (see Tables 3, 4, 5 & 6)	Passed	Passed
Comments	- -	- -

See final page for notes.

Table 3: Gene-Trac ORM-2 Control Results, Test Reference S-5939

Laboratory Control	Analysis Date	Control Description	ORM-2		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	2-Jul-20	Genomic DNA (CSLO-0141)	5.2×10^{8}	7.8×10^{7} (3)	See Note 3
Positive Control High Concentration	2-Jul-20	Genomic DNA (CSHO-0141)	9.1×10^{9}	6.6×10^{9}	Passed
DNA Extraction Blank	2-Jul-20	Sterile Water (FB-3579)	0	2.6×10^{3} U	Passed
Negative Control	2-Jul-20	Test Reagent Blank (TBO-0141)	0	2.6×10^{3} U	Passed

See final page for notes.

Table 4: Gene-Trac SRB Control Results, Test Reference S-5939

Laboratory Control	Analysis Date	Control Description	*dsrA*		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	1-Jul-20	Genomic DNA (CSLSR-0063)	1.7×10^6	2.3×10^6	Passed
Positive Control High Concentration	1-Jul-20	Genomic DNA (CSHSR-0063)	8.2×10^7	8.3×10^7	Passed
DNA Extraction Blank	1-Jul-20	Sterile Water (FB-3579)	0	2.6×10^3 U	Passed
Negative Control	1-Jul-20	Test Reagent Blank (TBSR-0064)	0	2.6×10^3 U	Passed

See final page for notes.

Table 5: Gene-Trac abcA Control Results, Test Reference S-5939

Laboratory Control	Analysis Date	Control Description	*abcA*		Comments
			Spiked Gene Copies per Reaction	Recovered Gene Copies per Reaction	
Positive Control Low Concentration	6-Jul-20	Plasmid DNA (CSLAB-0116)	7.1×10^5	5.1×10^5	Passed
Positive Control High Concentration	6-Jul-20	Plasmid DNA (CSHAB-0116)	1.7×10^8	1.9×10^8	Passed
DNA Extraction Blank	6-Jul-20	Sterile Water (FB-3579)	0	2.0×10^1 U	Passed
Negative Control	6-Jul-20	Test Reagent Blank (TBAB-0116)	0	2.0×10^1 U	Passed

See final page for notes.

Table 6: Gene-Trac Pepto-ben Control Results, Test Reference S-5939

Laboratory Control	Analysis Date	Control Description	Pepto-ben		Comments
			Spiked Gene Copies per Reaction	Recovered Gene Copies per Reaction	
Positive Control Low Concentration	16-Jul-20	Genomic DNA (CSLPE-0115)	3.3×10^{5}	4.3×10^{4} (3)	See Note 3
Positive Control High Concentration	16-Jul-20	Genomic DNA (CSHPE-0115)	3.7×10^{7}	3.8×10^{7}	Passed
DNA Extraction Blank	16-Jul-20	Sterile Water (FB-3579)	0	2.0×10^{1} U	Passed
Negative Control	16-Jul-20	Test Reagent Blank (TBPE-0115)	0	2.0×10^{1} U	Passed

See final page for notes.

Notes:
ORM-2 = Deltaproteobacterium ORM-2
dsrA = *dissimilatory sulfate reductase A*
abcA = Benzene Carboxylase
J The associated value is an estimated quantity between the method detection limit and quantitation limit.
U Not detected, associated value is the quantitation limit.
B Analyte was detected in the method blank within an order of magnitude of the test sample.
E Extracted genomic DNA was not detected in the sample.
I Sample inhibited the test reaction based on inability to PCR amplify extracted DNA with universal primers.
ng/L = nanograms per liter
mL = milliliter
NA = not applicable
ND = not detected
DNA = deoxyribonucleic acid
16S rRNA = 16S ribosomal ribonucleic acid
PCR = polymerase chain reaction
qPCR = quantitative PCR
°C = degrees Celsius

[1]Percent ORM-2 *Deltaproteobacterium* (ORM-2), *Peptococcaceae, dsrA,* or *abcA* in microbial population. This value is calculated by dividing the number of specific gene copies by the total number of bacteria as estimated by the mass of DNA extracted from the sample. Range represents normal variation in enumeration.

[2]Samples are stabilized by freezing at -80 °C upon sample reception (field filters) or in-lab filtration (groundwater). Hold time not exceeded if sampling date is within 14 days of date received or filtration date.

[3]Control was outside recovery limit guidelines (+/- 50%), however, test results are deemed acceptable if one of two positive controls falls within the recovery limit.

SiREM

Chain-of-Custody Form

Lab # S5939

Part of a treatability study

*Project Name: Brunswick Deep Benzene	*Project #:
*Project Manager: Duane Groves	*Company:
*Email Address: dgroves@geosyntec.com	
Address (Street):	
City:	State/Province: Country:
*Phone #:	
*Sampler's Signature: J Webb	*Sampler's Printed Name: Jen Webb

Analysis

Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Genie-Trac D+C	Genie-Trac VC	Genie-Trac D+B	Genie-Trac D+G	Genie-Trac EtA	Volatile Fatty Acids	Dissolved Hydrocarbon gases	Terminal Electron Acceptors QR43	SRB	peptococcus	DCA	Other Information
Brunswick - DB - nitrate + DAP	19Jun20	3:00		1								✓	✓	✓	✓	15ml filtered
Brunswick - DB - nitrate	19Jun20	3:00		1								✓	✓	✓	✓	15ml filtered.

Preservative Key
0. None
1. HCl
2. Other _____
3. Other _____
4. Other _____
5. Other _____
6. Other _____

Billing Information
P.O. #:
*Bill To:

Turnaround Time Requested
Normal ☐
Rush ☐

For Lab Use Only
Cooler Condition: NA
Cooler Temperature: NA
Custody Seals: Yes ☐ No ☐

For Lab Use Only
Proposal #: _____

Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature: J Webb	Signature:	Signature:	Signature:	Signature:	Signature:
Printed Name: Jen Webb	Printed Name: Rachel Hallman	Printed Name:	Printed Name:	Printed Name:	Printed Name:
Firm: SiREM	Firm: SiREM	Firm:	Firm:	Firm:	Firm:
Date/Time: 19 June 20 3:00	Date/Time: 19 Jun 20 4:15	Date/Time:	Date/Time:	Date/Time:	Date/Time:

Distribution: White - return to Originator; Yellow - Lab Copy; Pink - Retained by Client
* Mandatory Fields

SiREM
Leading Science · Lasting Solutions

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Certificate of Analysis: Gene-Trac® ORM-2, Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-6653
Report Date: 30-Nov-20
Data Files: iQ5C-ORM2-QPCR-0146
iQ5C-ORM2-DB-QPCR-0146

Table 1a: Test Results

Sample ID	Deltaproteobacterium ORM-2	
	Percent ORM-2[(1)]	ORM-2 16S rRNA Gene Copies/Liter
Intrinsic Control	NA	9×10^4 U
Nitrate Amended/NRBC	NA	9×10^4 U
Nitrate and DAP Amended	NA	9×10^4 U

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Certificate of Analysis: Gene-Trac® SRB, Sulfate Reducing Bacteria (*dsrA*) Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-6653
Report Date: 30-Nov-20
Data Files: iQ5B-SRB-QPCR-0074
iQ5B-DB-SRB-QPCR-0074

Table 1b: Test Results

Sample ID	Sulfate Reducing Bacteria (*dsrA*)	
	Percent *dsrA* (1)	*dsrA* Gene Copies/Liter
Intrinsic Control	0.002 - 0.008 %	5×10^4 J
Nitrate Amended/NRBC	NA	9×10^4 U
Nitrate and DAP Amended	NA	9×10^4 U

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

Certificate of Analysis: Gene-Trac® abcA Benzene Carboxylase Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-6653
Report Date: 30-Nov-20
Data Files: iQ5A-abcA-QPCR-0118
iQ5A-DB-abcA-QPCR-0118

Table 1c: Test Results

Sample ID	Benzene Carboxylase (*abcA*)	
	Percent *abcA* (1)	*abcA* Gene Copies/Liter
Intrinsic Control	NA	9×10^4 U
Nitrate Amended/NRBC	0.00004 - 0.0001 %	3×10^3 J
Nitrate and DAP Amended	NA	9×10^4 U

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

SiREM
Leading Science · Lasting Solutions

Certificate of Analysis: Gene-Trac® Pepto-ben *Peptococcaceae* Assay

Customer: Duane Graves, Geosyntec Consultants
Project: Brunswick Deep Benzene Treatability Study
Customer Reference: SC4384

SiREM Reference: S-6653
Report Date: 30-Nov-20
Data Files: iQ5B-Pepto-QPCR-0117
iQ5B-DB-Pepto-QPCR-0117

Table 1d: Test Results

Sample ID	*Peptococcaceae*	
	Percent *Peptococcaceae* (1)	*Peptococcaceae* 16S rRNA Gene Copies/Liter
Intrinsic Control	NA	9×10^4 U
Nitrate Amended/NRBC	0.00004 - 0.0001 %	3×10^3 J
Nitrate and DAP Amended	NA	9×10^4 U

See final page for notes.

Analyst: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician II

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

Table 2: Detailed Test Parameters, Test Reference S-6653

Customer Sample ID	Intrinsic Control	Nitrate Amended/NRBC	Nitrate and DAP Amended
SiREM ORM-2 Test ID	ORM-0248	ORM-0249	ORM-0250
SiREM SRB Test ID	SRB-0384	SRB-0385	SRB-0386
SiREM *abcA* Test ID	ABC-0170	ABC-0171	ABC-0172
SiREM Pepto-ben Test ID	PEP-0145	PEP-0146	PEP-0147
Date Sampled [(2)]	12-Nov-20	12-Nov-20	12-Nov-20
Matrix	Microcosm	Microcosm	Microcosm
Date Received [(2)]	13-Nov-20	13-Nov-20	13-Nov-20
Sample Temperature	NA	NA	NA
Filtration Date [(2)]	16-Nov-20	16-Nov-20	16-Nov-20
Volume Used for DNA Extraction	15 mL	15 mL	15 mL
DNA Extraction Date	18-Nov-20	18-Nov-20	18-Nov-20
DNA Concentration in Sample (extractable)	4000 ng/L (J)	13000 ng/L (J)	12000 ng/L (J)
PCR Amplifiable DNA	Detected	Detected	Detected
ORM-2 qPCR Date Analyzed	24-Nov-20	24-Nov-20	24-Nov-20
SRB qPCR Date Analyzed	24-Nov-20	24-Nov-20	24-Nov-20
***abcA* qPCR Date Analyzed**	23-Nov-20	23-Nov-20	23-Nov-20
Pepto-ben qPCR Date Analyzed	26-Nov-20	26-Nov-20	26-Nov-20
Laboratory Controls (see Tables 3, 4, 5 & 6)	Passed	Passed	Passed
Comments	- -	- -	- -

See final page for notes.

Table 3: Gene-Trac ORM-2 Control Results, Test Reference S-6653

Laboratory Control	Analysis Date	Control Description	ORM-2		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	24-Nov-20	Genomic DNA (CSLO-0146)	6.7×10^6	9.4×10^6	Passed
Positive Control High Concentration	24-Nov-20	Genomic DNA (CSHO-0146)	5.8×10^8	6.6×10^8	Passed
DNA Extraction Blank	24-Nov-20	Sterile Water (FB-3685)	0	2.6×10^3 U	Passed
Negative Control	24-Nov-20	Test Reagent Blank (TBO-0146)	0	2.6×10^3 U	Passed

See final page for notes.

Table 4: Gene-Trac SRB Control Results, Test Reference S-6653

Laboratory Control	Analysis Date	Control Description	*dsrA*		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	24-Nov-20	Genomic DNA (CSLSR-0074)	2.6×10^{7}	4.6×10^{6} (3)	See Note 3
Positive Control High Concentration	24-Nov-20	Genomic DNA (CSHSR-0074)	2.7×10^{9}	2.3×10^{9}	Passed
DNA Extraction Blank	24-Nov-20	Sterile Water (FB-3685)	0	2.6×10^{3} U	Passed
Negative Control	24-Nov-20	Test Reagent Blank (TBSR-0074)	0	2.6×10^{3} U	Passed

See final page for notes.

Table 5: Gene-Trac abcA Control Results, Test Reference S-6653

Laboratory Control	Analysis Date	Control Description	*abcA*		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	23-Nov-20	Plasmid DNA (CSLAB-0118)	4.3×10^{7}	4.2×10^{7}	Passed
Positive Control High Concentration	23-Nov-20	Plasmid DNA (CSHAB-0118)	1.0×10^{10}	6.8×10^{9}	Passed
DNA Extraction Blank	23-Nov-20	Sterile Water (FB-3685)	0	2.6×10^{3} U	Passed
Negative Control	23-Nov-20	Test Reagent Blank (TBAB-0118)	0	2.6×10^{3} U	Passed

See final page for notes.

Table 6: Gene-Trac Pepto-ben Control Results, Test Reference S-6653

Laboratory Control	Analysis Date	Control Description	Pepto-ben		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	
Positive Control Low Concentration	26-Nov-20	Genomic DNA (CSLPE-0117)	2.0×10^{7}	9.3×10^{5} (3)	See Note 3
Positive Control High Concentration	26-Nov-20	Genomic DNA (CSHPE-0117)	2.3×10^{9}	1.5×10^{9}	Passed
DNA Extraction Blank	26-Nov-20	Sterile Water (FB-3685)	0	2.6×10^{3} U	Passed
Negative Control	26-Nov-20	Test Reagent Blank (TBPE-0117)	0	2.6×10^{3} U	Passed

See final page for notes.

Notes:
ORM-2 = Deltaproteobacterium ORM-2
dsrA = *dissimilatory sulfate reductase A*
abcA = Benzene Carboxylase
J The associated value is an estimated quantity between the method detection limit and quantitation limit.
U Not detected, associated value is the quantitation limit.
B Analyte was detected in the method blank within an order of magnitude of the test sample.
E Extracted genomic DNA was not detected in the sample.
I Sample inhibited the test reaction based on inability to PCR amplify extracted DNA with universal primers.
ng/L = nanograms per liter
mL = milliliter
NA = not applicable
ND = not detected
DNA = deoxyribonucleic acid
16S rRNA = 16S ribosomal ribonucleic acid
PCR = polymerase chain reaction
qPCR = quantitative PCR
°C = degrees Celsius

[1]Percent ORM-2 *Deltaproteobacterium* (ORM-2), *Peptococcaceae, dsrA,* or *abcA* in microbial population. This value is calculated by dividing the number of specific gene copies by the total number of bacteria as estimated by the mass of DNA extracted from the sample. Range represents normal variation in enumeration.

[2]Samples are stabilized by freezing at -80 °C upon sample reception (field filters) or in-lab filtration (groundwater). Hold time not exceeded if sampling date is within 14 days of date received or filtration date.

[3]Control was outside recovery limit guidelines (+/- 50%), however, test results are deemed acceptable if one of two positive controls falls within the recovery limit.

S-16653

SiREM

Chain-of-Custody Form

siremlab.com

130 Stone Road West
Guelph ON, Canada N1G 3Z2
(519) 822-2265

Lab # ~~S-16645~~

* Part of a treatability study

*Project Name: Brunswick Deep Benzene	*Project #:
*Project Manager: Duane Graves	*Company:
*Email Address: dgraves@geosyntec.com	
Address (Street):	
City	State/Province	Country
*Phone #:	
*Sampler's Signature: J Webb	*Sampler's Printed Name: Jen Webb

Analysis

Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Gene-Trac DHC	Gene-Trac VC	Gene-Trac DHB	Gene-Trac DHG	Treatability Study	ORH2	SRB	Peptococcaceae	abc A			Other Information
Intrinsic Control	12/4/2020	4:00	agu	1						✓	✓	✓	✓			
Nitrate Amended/NRBC	↓	↓	↓	1						✓	✓	✓	✓			
Nitrate and DAP Amended	↓	↓	↓	1						✓	✓	✓	✓			

Preservative Key
0. None
1. HCL
2. Other
3. Other
4. Other
5. Other
6. Other

Billing Information
P.O. #:
*Bill To:

Turnaround Time Requested
Normal ☐
Rush ☐

For Lab Use Only
Cooler Condition: N/A
Cooler Temperature: N/A
Custody Seals: Yes ☐ No ☐

For Lab Use Only
Proposal #:

Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature: J Webb	Signature: J. Wilkinson	Signature	Signature	Signature	Signature
Printed Name: Jen Webb	Printed Name: J. Wilkinson	Printed Name	Printed Name	Printed Name	Printed Name
Firm: SiREM	Firm: SiREM	Firm	Firm	Firm	Firm
Date/Time: 12Nov20 4:00pm	Date/Time: 13Nov20.	Date/Time	Date/Time	Date/Time	Date/Time

Distribution: White - return to Originator; Yellow - Lab Copy; Pink - Retained by Client

* Mandatory Fields