APPENDIX G – MONITORED NATURAL ATTENUATION

FINAL FEASIBILITY STUDY
CASMALIA RESOURCES SUPERFUND SITE
CASMALIA, CALIFORNIA

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LIST OF ACRONYMS

BTA	Burial Trench Area
BTEX	benzene, toluene, ethylbenzene, and xylenes
CDA	Central Drainage Area
cm^2/s	centimeters squared per second
CVOCs	chlorinated volatile organic compounds
DGGE	Denaturing Gradient Gel Electrophoresis
Dhc	Dehaloccocoides bacteria
DNA	Deoxyribonucleic acid
DNAPL	dense non-aqueous phase liquid
FS	Feasibility Study
ft, bgs	feet below ground surface
g/g	grams per gram
HC	hydrocarbons
kg/L	kilograms per liter
L/kg	liters per kilogram
LNAPL	light non-aqueous phase liquid
MAC	methoxylated aromatic compounds
MCL	maximum contaminant level
MNA	monitored natural attenuation
NAPL	Non-aqueous phase liquid
ORP	oxidation-reduction potential (redox)
OSWER	Office of Solid Waste and Emergency Response
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PCE	tetrachloroethylene/tetrachloroethene
PCR	polymerase chain reaction
PCT	Perimeter Control Trench
PSCT	Perimeter Source Control Trench
RGMEW	Routine Groundwater Monitoring Element of Work
RI	Remedial Investigation
(the) site	Casmalia Resources Superfund Site
TCE	trichloroethylene/trichloroethene
TI	technical impracticability
TIE	technical impracticability evaluation
TOC	total organic carbon
TRFLP	terminal restriction fragment length polymorphism
USEPA	United States Environmental Protection Agency
VC	vinyl chloride
VOCs	volatile organic compounds

G1.0 Monitored Natural Attenuation

This appendix summarizes the natural attenuation processes that occur at the Casmalia Resources Superfund Site (site) to support a "monitored natural attenuation" (MNA) component of the overall remedy for the site. Natural attenuation processes are known to occur based on the data collected during the Remedial Investigation and the known physical and chemical characteristics of the hydrogeologic system and contaminants that occur at the site. Natural attenuation is the process whereby contaminant reduction occurs by one or more natural in-situ means through physical, chemical or biological interaction with the surrounding environment. Physical processes include dilution, dispersion, and volatilization; chemical processes include adsorption, absorption, and chemical transformation, and biological processes involve the metabolism of chemical contaminants by microbes. MNA is a viable remedy to achieve site-specific remediation objectives when the time frame to reach these goals is considered reasonable compared to that offered by more active methods.

The 1999 EPA guidance "Use of Monitored Natural Attenuation at Superfund, RCRA Corrective Action and Underground Storage Tanks Sites" outlines conditions for the application of MNA Office of Solid Waste and Emergency Response (OSWER) Directive 9200.4-17P). As stated in the guidance document, the United States Environmental Protection Agency (USEPA) supports MNA as a viable remediation method when source control measures have been implemented, impacted groundwater has a low potential for contaminant migration, and prefers processes that degrade or destroy the contaminants. While MNA is considered a "passive" approach, because remediation advances through natural means without human intervention, its use at a site does not preclude the implementation of "active" remediation or the application of supplementary compounds to enhance biological activity (USEPA 1999).

MNA would not be used as the only component of the remedy because active, long-term source control will be needed, particularly for Area 5 North. This area includes the most highly contaminated parts of the site, including the Capped Landfills, the PCB Landfill, the Burial Trench Area, and the Central Drainage Area. Light non-aqueous phase liquid (LNAPLs) and dense non-aqueous phase liquid (DNAPLs) are found within this area. A Technical Impracticability Evaluation (TIE) was performed for this area as documented in Appendix A of this Feasibility Study (FS) and a Technical Impracticability (TI) waiver may be invoked as part of the site remedy. Control of contaminant sources is an important aspect of USEPA's policy. OSWER Directive 9200.4-17P further states that *"Control of source materials is the most effective means of ensuring the timely attainment of remediation objectives (USEPA 1999a, page 22).*

Source control of organic and inorganic compounds at the site is provided by multiple clay barriers located at the toe of the landfill areas, and active removal of groundwater and NAPL at the Gallery Well and Sump 9B. Groundwater migration is controlled by the Perimeter Source Control Trenches (PSCT) and Perimeter Control Trenches (PCT), as well as groundwater extraction along these trenches. Groundwater flow is limited site-wide by the low hydraulic conductivities of native sediments and soil types.

During the Remedial Investigation (RI) (CSC 2011), an MNA investigation was performed that focused on finding supporting evidence for the degradation of chlorinated alkenes (i.e., tetrachloroethlyene (PCE) and its daughter compounds) via biological processes, and in

accordance with USEPA guidance documents (USEPA 1998). As part of the 2012 Spring Routine Groundwater Monitoring Element of Work (RGMEW) monitoring event additional samples were from select site features and wells and submitted the samples for identification of native bacteria. A discussion of the bacterial analysis is provided in the following Section, and their relevance is discussed herein. Microbial samples collected during the Spring 2012 RGMEW event, and collected in accordance with the procedures outlined in the RGMEW Revision Field Sampling Plan (CSC 2009). Samples were collected in unpreserved 1-liter poly bottles and chilled in an ice-chest while awaiting transportation to the laboratory. Laboratory data sheets and method descriptions are provided in Attachment G-1.

The RI study provided evidence that reductive dechlorination was occurring in the P/S Landfill, and areas within and immediately surrounding the Central Drainage Area (CDA) and Burial Trench Area (BTA). Supportive evidence for MNA included: the presence of hydrogen (the electron accepting compound for the reductive dechlorination process); low dissolved oxygen levels (generally >1 mg/L, indicative of anaerobic conditions); the presence of carbon dioxide and methane off-gases, which indicates the presence of ongoing biological activity is occurring, and; the presence of ethene and ethane, the final end products of PCE and trichloroethylene (TCE) reductive dechlorination chain.

To verify that reductive dechlorination was the result of biologically-driven processes, groundwater samples collected from four site locations (Gallery Well, Sump 9B, Lower HSU well RGPZ-7C, and Upper HSU well B3B) were analyzed for the presence of the Dehaloccocoides bacteria (Dhc), which is a known degrader of chlorinated alkenes (Maymo-Gatell et.al. 1997). The samples were collected on April 17 and 18, 2012. Testing for Dhc was not readily available at the time the RI/FS Workplan was prepared in 2004. Dhc sample collection locations were chosen based on the ease of sample collection at that location, the presence of NAPL, and the hydrostratigraphic unit associated with that location (shown below). Attachment G-1 provides a summary of the sampling and analysis performed for the Dehalococcoides bacteria, including the field and laboratory methods used.

Sample Location	**Hydrostratigraphy**	**NAPL Present**
Gallery Well	P/S Landfill waste	DNAPL
Sump-9B	Upper HSU	LNAPL
RGPZ-7C	Lower HSU	DNAPL
B3B	Upper HSU	Dissolved

The Dhc was found to be present in the Gallery Well, as well as samples from wells RGPZ-7C and B3B. The laboratory noted low biomass in samples from the NAPL wells, and the Dhc population count was highest in the well B3B, where historically, there have been only low concentrations of dissolved volatile organic compounds (VOCs) observed. These sample results suggest that the Dhc is somewhat ubiquitous and adapted to the wide range of environmental conditions displayed at the site, as it appears in the relatively clean groundwater from well B3B, as well as the Gallery Well, which may be considered a less hospitable environment for microorganisms, due to the presence of NAPL. USEPA (2006) provides several examples of Dhc population density and estimates of degradation (e.g., first order degradation rates) based on reported Dhc data from their Study sites, active dechlorination at

slow rates is anticipated for Dhc population of greater than 10^5 cells/L (e.g. sample B3B), with probably degradation rates (λ) between 0.01 to 0.1 per year. For the NAPL samples (Gallery Well, Sump 9B, RGPZ-7C) where Dhc population densities are measured in the $>10^3$ cells/L range, estimates of dechlorination are to be considered extremely slow, but active as the key factors (low nitrate, methane and negative ORP) for Dhc activity are favorable at the NAPL locations.

Several species of bacteria were identified that have the potential to degrade gasoline and more complex organic hydrocarbons compounds including PAHs, PCBs and phenols (see Section G-1.1). The presence carbon dioxide and methane, which are known end products of hydrocarbon degradation, suggest that these compounds are likely degrading via biological processes. The low dissolved oxygen values and absence of bionutrients (e.g., nitrate) suggest that optimum conditions for aerobic degradation of petroleum hydrocarbons may not exist (Chamberlain 2003), and enhancers may be required to foster additional aerobic microbial activity.

Several species of bacteria were identified that have the potential to degrade heavy metals, and anions (see Section G-1.1). Bacteria were identified that are capable of degrading or sorbing site contaminants of selenium, arsenic, cadmium, nickel and other heavy metals.

The volatilization of chlorinated and petroleum hydrocarbon compounds was confirmed by the detection of these compounds in the RI soil gas survey (Appendix C, RI Report, CSC 2011). Currently, capping of landfills with black top material (60-mil HDPE) has added to the potential of increasing subsurface temperatures and maintaining heat, which in turn promotes volatilization of organic compounds (particularly of NAPL). The planned capping of additional site areas will further act to maintain heat in the subsurface, and will likely increase volatilization across the site.

Limited vapor flux measurements were made in 1997 (CSC 2011) as part of the capping of the P/S Landfill. In total, 10 VOC compounds were detected at levels at the method detection limit for the individual compounds. The highest flux of a VOC that was detected during the assessment was also PCE (1.9 $\mu g/m^2$-min). Assuming the landfill capped areas cover 41 acres and PCE is being emitted equally from all landfill cells, then approximately 1 pound of PCE would be emitted by volatilization per day prior to landfill capping (CSC 2011).

Sorption of organic compounds, including long carbon chain compounds such as pesticides and PCBs, is recognized to occur in native organic carbon (e.g., charcoal) in the soil matrix of the site. Measurements of soil matrix total organic carbon (TOC) were performed as part of the RI soil investigation soil diffusion testing program (Appendix E, CSC 2011). A summary of soil diffusion test results is presented in Table 1 below.

Well Id.	Sample Depth	Hydrostratigraphic Unit	Total Porosity	Bulk Density	Chloride Matrix Diffusivity	Matrix Tortuosity Factor	TOC
--	(ft, bgs)	--	--	(kg/L)	(cm^2/s)	--	(%)
RIMW-2	9	Alluvium	0.36	1.66	3.00E-06	0.18	0.38
RIPZ-18	22.5	Alluvium	0.30	1.80	8.00E-06	0.48	0.18
RIMW-11	14	Upper HSU	0.46	2.61	3.00E-07	0.02	0.27
RIMW-8	58	Upper HSU	0.51	1.24	1.00E-07	0.01	0.09
RIMW-11	50	Lower HSU	0.49	2.48	5.00E-07	0.03	0.88
RIPZ-10B	58	Lower HSU	0.45	1.43	5.00E-07	0.03	2.34

Table G-1. Diffusivity Test Results.

Lower HSU TOC values of 1% or higher are consistent with fine–grained deep marine sediments, while the lower TOC content of the Upper HSU sediments reflects the weathering and oxidation of organic matter within these soils.

Sorption processes act to fix organic compounds within or onto a solid surface. The size of available surface area and the organic content of the soil matrix are important factors which determine adsorption capacity. The greater the soil's surface area and the higher the organic content, the greater the adsorption capacity of the soil will be (Nyer 1992). The fine-grained and moderate-to-high TOC of site soils are thought provide a large adsorption capacity for site contaminants.

In addition, adsorption acts to retard the movement of organic compounds in groundwater to the retention of contaminant mass onto native soil organic matter. The degree of chemical retardation is represented by the retardation factor (R), which within groundwater flow through a porous media is estimated by the following relationship:

$$R = 1 + (\rho_b/\theta_T)*K_d$$

Where, ρ_b = the soil bulk density, θ_T = the soil total porosity, and K_d = the partition coefficient for the organic compound. $K_d = K_{oc} * f_{oc}$, where K_{oc}= the chemical specific soil organic partition coefficient and f_{oc} = fraction of organic carbon in the soil sample. For TCE, a commonly-occurring site chlorinated hydrocarbon contaminant, Table 2 below presents representative retardation factors for site conditions.

Well Id.	Sample Depth	Retardation Factor (R)	Bulk Density	Total Porosity	Koc	foc
--	(ft, bgs)	--	(kg/L)	--	(L/kg)	(g/g)
RIMW-2	9	3.21	1.66	0.36	126	0.0038
RIPZ-18	22.5	2.36	1.80	0.30	126	0.0018
RIMW-11	14	2.93	2.61	0.46	126	0.0027
RIMW-8	58	1.28	1.24	0.51	126	0.0009
RIMW-11	50	6.61	2.48	0.49	126	0.0088
RIPZ-10B	58	10.37	1.43	0.45	126	0.0234

Table G-2. Retardation Factor for TCE within saturated site soils.

These estimates indicate that within the Alluvium and Upper HSU, retardation factors for TCE transport range from about 1.3 to 3.2. Within the Lower HSU, TCE transport is about 6 to 10 times slower than the rate of groundwater movement through this unit.

Dilution and dispersion act to lower the concentration of contaminants due to mixing of contaminated and clean groundwater (dilution), or non-uniform transport rates of contaminant mass through clean pore spaces of the host soil matrix (dispersion). At the site, two sources primarily control groundwater flux to site's water-bearing units: precipitation and groundwater underflow. Precipitation that falls on the site's surface will continue to percolate through the soil matrix and mix with groundwater to dilute contaminant concentrations (however, for those areas of the site where residual contamination exists in the vadose soil, percolating water may leach these compounds and add to the contaminant mass in groundwater). As contaminant mass moves downgradient with the flow of groundwater, the mass will disperse due to chemical

gradients (chemical diffusion) and soil tortuosity (mechanical dispersion). Collectively these processes are known as hydrodynamic dispersion (Freeze and Cherry 1979).

Within fine-grained soils, chemical diffusion may redistribute contaminant mass within soil pores from mobile to immobile zones, whereby the transport of contaminant mass is further retarded. Estimates of the fraction of immobile pore space within the soil can be obtained from derivation of the porous media tortuosity factor (Table 1). The porous media tortuosity factor (τ) is an empirical factor (where τ is between 0 and 1) that accounts for slower rates of diffusion in porous media compared to aqueous solutions due to longer, more tortuous diffusion pathways around solid particles in porous media, where they are relatively direct in aqueous solutions. The tortuosity factor is defined as the mean ratio of the actual to apparent path lengths within porous media, and provides an indication of the contact area between facture surfaces (Alder and Thovert 2010). In a porous media, molecular diffusion acts throughout the fluid and allows transfer of contaminants between pore spaces associated with mobile groundwater flow, and pore spaces of immobile stagnant water (Marsily 1986). The low tortuosity factors for Upper HSU and Lower HSU soils (Table 1) indicate that these claystone soils have a high proportion of immobile pore spaces into which contaminants have the potential to disperse and be retarded within this large volume of the soil matrix. Therefore, hydrodynamic dispersion plays an important role in the re-distribution of contaminants within the soil matrix, and the reduction of mobile contaminant mass in groundwater at the site.

The relative importance of diffusion processes in contaminant transport can be estimated by determination of the Peclet Number (Pe), which represents the ratio of advection to diffusion:

$$P_e = V*L/D$$

Where P_e = Peclet Number, V = seepage velocity, L = length of the fracture, and D = Diffusion Coefficient within the media. Assuming parameter values similar to the Fractran model of Appendix A Table A2-1, defining the velocity of groundwater in the fracture as $V_f = K_f * I$, where I = the hydraulic gradient, and assigning L equal to the fracture aperture width, then:

$$P_e = (3 *10^{-7} *0.1) * (60* 10^{-4}) / 0.5* 10^{-7} = 0.0036 << 1$$

The very low Peclet Number demonstrates that contaminant transport or re-distribution through the fracture network/soil matrix at the site is dominated by diffusion through the soil matrix rather than advective flow. Given the large volume of groundwater associated with the immobile pore space within the soil matrix as indicated by the diffusion testing, then the potential exists for retardation of contaminants within these immobile pore fluids.

The chemical transformation of inorganic compounds from the mobile dissolved phase to the immobile solid phase has not been a focus of investigations performed at the site. Chemical factors that control the precipitation of inorganic compounds are the ion species present in solution and the pH and Eh of the solution (EPA 2007a, b). Inorganic compounds (e.g., metals and oxy-anions) are capable of undoing biologically mediated and abiotic transformation. For metals, the transformation from a “toxic” compound to an inert compound typically involves a chemical reaction where the metal ion undergoes a change of state, e.g., the reduction of mobile arsenic III to immobile arsenic V. During the change of state, the metal ion (or oxy-anion) transforms from a soluble species to a solid species (i.e., precipitates out of solution to form an immobile solid).

Most metal ions are in a soluble state under acidic aqueous conditions and will precipitate under alkaline aqueous conditions. A review of groundwater sampling well purge data indicates that pH at the site varies within the "neutral range" (pH between 6 and 8) typical of most groundwater, and the Eh varies from positive (electron scarce, Eh>0) to negative (electron available, Eh<0). Therefore, the transformation of inorganic compounds (either precipitation or dissolution) will occur according to the variability of groundwater chemistry. As illustrated below in the form of Eh-pH diagrams, groundwater conditions favorable for the immobilization of arsenic are not identical to those favorable for the immobilization of selenium (USEPA 2007).

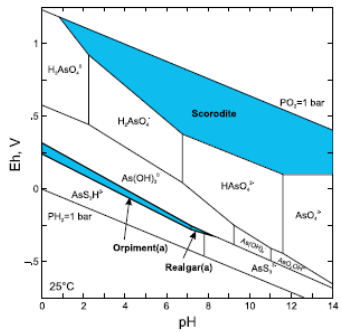

Figure 6.2 *Eh-pH diagram for arsenic at 25°C. System As-H_2O-Fe-S, with $\Sigma As=10^{-5}$ (750 µg L^{-1}), $\Sigma Fe=10^{-4}$ (5.6 mg L^{-1}), and $\Sigma S=10^{-3}$ (32 mg L^{-1}). Solid stability fields are shaded blue. Orpiment(a) and Realgar(a) are poorly crystalline forms of As_2S_3 and AsS, respectively. Arsenopyrite and crystalline forms of As_2S_3 and AsS are suppressed.*

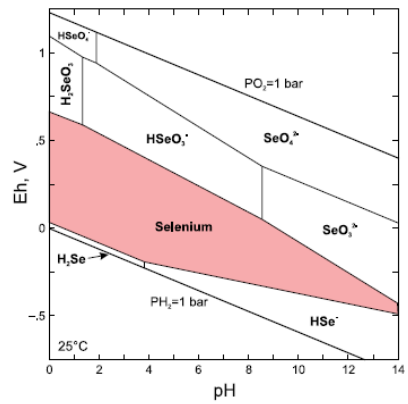

Figure 7.1 *Eh-pH diagram for selenium at 25 °C using thermodynamic data from Séby et al. (2001). $\Sigma Se=10^{-5}$ (790 µg L^{-1}). Solid stability field for elemental selenium is shaded pink.*

Reference (USEPA 2007a, b)

The precipitation and dissolution of minerals in groundwater are natural processes, and can lead to minerals exceeding regulatory standards. For example, the formation of uranium roll-front deposits, where oxidizing groundwater leaches uranium from source rocks and carries the dissolved uranium through the host rock until it reaches a reducing environment, where the uranium then precipitates out of solution (University of Arizona 2008). In these natural settings, dissolved uranium in groundwater often exceeds the 30 mg/L Federal MCL. Similar phenomena are likely to occur within groundwater at the site, and partial evidence for this has been observed as mineral precipitation within fractures due to changes in groundwater conditions across the site. Secondary mineralization appears most frequently as hematite staining on fracture and joint surfaces and well-developed gypsum infilling of fractures and joints (Woodward Clyde Consultants 1988).

For those compounds moving through the Upper HSU, groundwater is captured by the PSCT, which contains most of the mobile organic compounds. The PCTs are located immediately downgradient from the ponds, to capture groundwater where inorganic contamination is prevalent. Thus, the trenches provide migration control and actively remove contaminant mass from the site. Within Zone 2, observed stable or declining VOC concentrations over time indicate that MNA process are ongoing at the site, and are decreasing contaminant mass within the groundwater, albeit slowly.

In summary, MNA processes at the site combined with actively implemented remedies (i.e., groundwater extraction) fulfill the criteria outlined in the 1999 USEPA guidance for

implementation of MNA at the site. MNA processes have been evaluated in accordance with USEPA MNA guidelines (USEPA 1999) and the following MNA processes have been identified to be active at the site:

- Mass loss and transform of DNAPL (primarily, PCE) and dissolved phase organics through biological mediated reduction dechlorination.
- Mass loss due to volatilization of organic compounds from soil and groundwater to the atmosphere.
- Sorption of organic compounds onto soil particles (native organic carbon)
- Containment of compounds within pore spaces filled with immobile groundwater.
- Very slow groundwater flow and transport rates primarily due to very low hydraulic conductivities of the Upper HSU and Lower HSU sediments.
- Precipitation of inorganic compounds to fracture surfaces.

G1.1 BACTERIAL SCREENING OF SITE GROUNDWATER

During the 2012 Spring RGMEW groundwater monitoring event, for the purpose of characterizing the site's bacterial population, samples were collected from the following locations:

Sample Location	Hydrostratigraphy	NAPL Present	Bacterial Testing
RCF pond	Surface water	Dissolved	TRFLP
RAP-1B	Containment Feature	Dissolved	TRFLP
B3B	Upper HSU	Dissolved	TRFLP, Dhc
Gallery Well	P/S Landfill waste	DNAPL	DGGE, Dhc
Sump-9B	Upper HSU	LNAPL	DGGE, Dhc
RGPZ-7C	Lower HSU	DNAPL	DGGE, Dhc

The samples were sent to Microbial Insight of Rockford, Tennessee for analysis. A description of the bacterial testing methods and the laboratory test results that identify bacteria enumerated from samples are provided in Attachment G-1.

The principal aim of the bacterial testing was to determine if viable bacterial populations capable of degrading contamination are present at the site, and what is the composition and character of these bacterial populations. In addition, four samples were specifically tested for Dhc, which is a known degrader of chlorinated alkenes (Maymo-Gatell et.al. 1997). Three of the samples were collected from areas where NAPL is known to be present and represent source area testing. Three samples were collected from the locations where dissolved contamination occurs and represent the greater portions of the site.

Dehalococcoides Results

Dhc was found to be present in three of the four for samples tested. Dhc was present in the Gallery Well, RGPZ-7C and B3B samples, and not present in the Sump 9B samples due to low biomass. The presence of the Dhc provides the mechanism for the reductive dechlorination process and explains the presence of reductive dechlorination byproducts observed during the RI MNA study (CSC 2011).

CENSUS - TRFLP and DGGE Results

All six samples were submitted for terminal restriction fragment length polymorphism (TRFLP) analysis. TRFLP is a DNA-based technique that can be used to identify microorganisms present in a sample. First, DNA is extracted from the sample and polymerase chain reaction (PCR) is used to generate a multitude of copies of a specific gene. The gene copied during the process (target gene) is determined by short segments of DNA called "primers" that contain a fluorescent label and are added to the reaction mixture. The net result of the PCR step is the generation of a mixture of fluorescently labeled DNA products that represent the microorganisms present in the sample. This mixture is digested with restriction enzymes that "cut" the DNA molecule at known sequences. The size and sequence of each resulting terminal restriction fragments are compared to a proprietary database to identify microorganisms present in the sample.

The three NAPL samples contained low biomass, and the available biomass could not be amplified for the TRFLP analysis. Instead the Denaturing Gradient Gel Electrophoresis (DGGE) method was employed to attempt to qualify the bacterial diversity of these samples. DGGE is a DNA-based technique which generates a genetic profile or “fingerprint” which can be used to identify the dominant members of the microbial community. The low biomass from the Gallery Well sample can be inferred from the “strength” of the genetic biomarkers when compared to Well RGPZ-7C (Attachment G-1). However, along with the Dhc bacteria, several other bacterial genera were identified that are recognized to facilitate the transformation of environmental pollutants, as indicated on the laboratory description of the identified bacterial genus.

A diverse group of site bacteria, including aerobic, anaerobic and fermentative bacteria, have been identified from the samples. Preliminary research on the functionality of these bacteria would indicate that many of the bacteria have the ability to degrade site contaminants, particularly those bacteria identified as facultative anaerobe (e.g., iron or sulfur reducing bacteria).

Table G-3 provides a summary (as reported in the literature) of the potential for site bacteria to degrade one or more environmental pollutants:

Pollutant Type	Bacteria	Comments	Reference
Chlorinated VOCs	Dehalococoides	CVOCs	Maymo-Gatell (1997)
	Nocoardiodes	VC	Coleman et.al (2002)
Aromatic Organics Petroleum Hydrocarbons	Deinococcus	BTEX	Mann (2009)
	Flexithrix	PCBs	Felix (2000)
	Holophaga	MAC	Kyrpides (2012)
	Acinetobacter	Phenol	El-Haleem (2003)

Pollutant Type	Bacteria	Comments	Reference
	Mycoplasma	HC, CVOCs	Dojka et.al. (2009)
	Sphingobacterium	BTEX	Jùrgensen (2000)
	Proteiniphilum	PAHs	Larsen (2009)
	Anthrobacter	PAHs, Herbicides	Kar (1997)
Anions	Ralstonia	Nitrate	Krauter (2004)
	Acinobacter	Phosphate	Haleem (2003)
	Desulfobacter	Sulfate	Doshi (2006)
	Candidatus	Perchlorate	SERDP (2010)
Heavy Metals Cations	Deinococcus	Cr(VI)	Mann (2009)
	Bacillus	Cu, Ni	Kumar et. al. (2010)
	Brevibacterium	Cr(VI)	Venkatesaperumal et.al. (2011)
	Phormidium	Cd(II), As (V),	Iqbal (2011), Matsuto (1984)
	Acinetobacter	Se(VI), Co(III)	
	Desulfobacter	Multiple	Doshi (2006)
Abbreviations: CVOCs = chlorinated volatile organic compounds; VC= vinyl chloride; BTEX= Benzene, toluene, ethylbenzene, xylenes; PCBs = polychlorinated biphenyls; MAC = methoxylated aromatic compounds; HC = hydrocarbons, PAHs – poly aromatic hydrocarbons			

Table G-3 Bacteria identified within site groundwater, and pollutant types that bacteria are recognized to degrade as published in the literature

The diversity of bacteria identified at the site is encouraging, as a healthy diverse population indicates that bionutrients are in ample supply and will support the MNA process.

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Bacterial Test Results - Identification

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Client: Warren B. Chamberlain
AMEC E & I, Inc.
2101 Webster St
Oakland, CA 94612

Phone: 510-663-3984

Fax:

Identifier: 037JD **Date Rec:** 04/18/2012 **Report Date:** 04/27/2012

Client Project #: **Client Project Name:** Casmalia

Purchase Order #:

Analysis Requested: CENSUS, TRFLP

Reviewed By:

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CENSUS

Client: **AMEC E & I, Inc.**
Project: Casmalia

MI Project Number: **037JD**
Date Received: 04/18/2012

Sample Information

Client Sample ID:		**Sump 9B-01**	**Gallery-01**	**RGPZ-7C-01**	**B3B-01**
Sample Date:		04/17/2012	04/17/2012	04/17/2012	04/18/2012
Units:		cells/mL	cells/mL	cells/mL	cells/mL
Analyst:		RW	RW	RW	RW
Dechlorinating Bacteria					
Dehalococcoides spp.	*DHC*	<5.00E-01 (I)	**5.80E+00**	**4.60E+00**	**1.19E+02**

Legend:
NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited
< = Result not detected

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Identifier: 037JD **Date Rec:** 04/18/2012 **Report Date:** 04/27/2012

Client Project #: **Client Project Name:** Casmalia

Purchase Order #:

Comments: Please note the total bacterial biomass on Sump 9B-01 was low and the sample may have PCR inhibition.

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Analysis Report

Contact: Warren Chamberlain
Address: AMEC Environmental & Infrastructure, Inc.
2101 Webster Street, 12th Floor
Oakland, CA 94612-3066

Phone: (510) 663-3984
Email: Warren.Chamberlain@amec.com

MI Identifier: 037JD

Report Date: 06/05/2012

Project: Casmalia

Comments:

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Table 1. Following table details the diversity index along with the percentage breakdown of phylogenetic classes identified.

Sample	RCF Pond-01
Diversity	
Shannon Diversity Index	3.21
Simpson Diversity Index	0.09
Phylogenetic Class Distribution (% of Total)	
Actinobacteridae	10%
Bacilli	20%
Bacteroidia	20%
Cytophagia	10%
Deltaproteobacteria	10%
Holophagae	10%
Mollicutes	10%
Thermotogae	10%

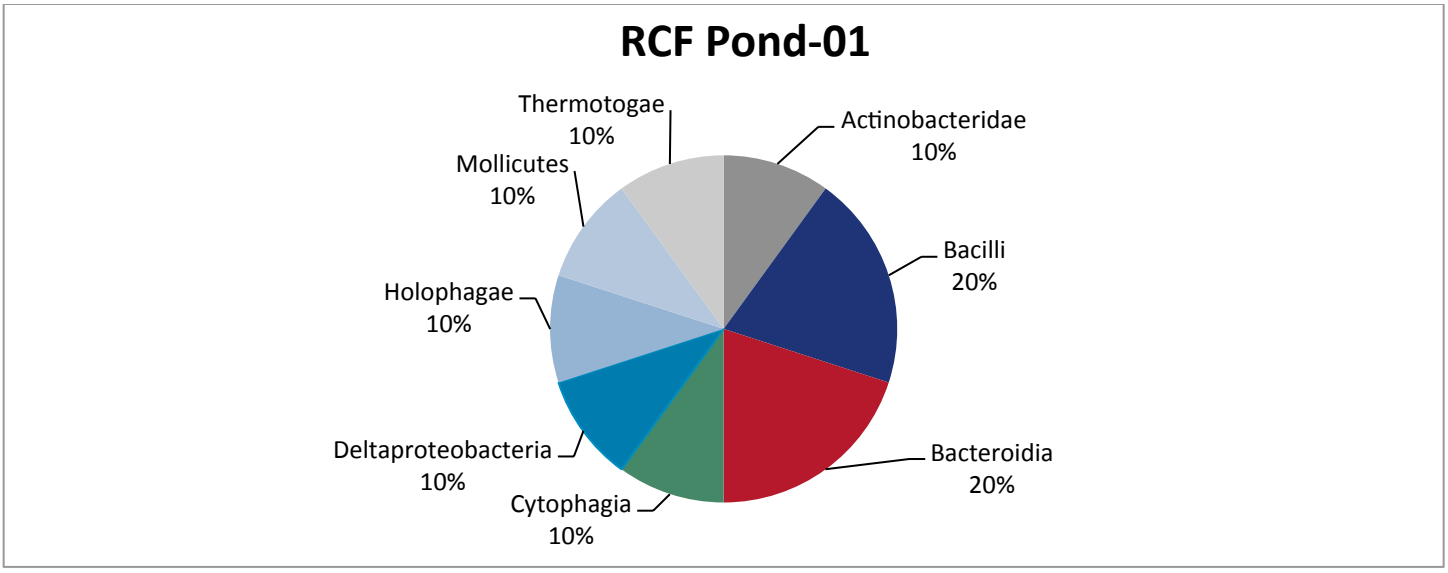

Figure 1. The diagram above represents the distribution of known phylogenetic classes. Identifications are obtained using a proprietary ribosomal database with over three million sequences that is comprised of both public and private sector sequences. Results are presented based upon the known bacterial species identified.

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Table 2. Detailed summary of the known genera identified within the predominant phylogenetic classes. Results shown below represent bacteria with at least one or more species identified within the genus

RCF Pond-01

Class / Genus	# species	% of Class	Description
Bacilli	**1**		
Bacillus spp.		100%	Metabolically diverse genus of bacteria that include heterotrophic nitrifiers, denitrifiers, nitrogen fixers, iron oxidizers, manganese oxidizers and managanese reducers.
Bacteroida	**1**		
Prevotella spp.	1	100%	This is a genus of bacteria found in the rumen of animals where they help the breakdown of protein and carbohydrate foods.
Cytophagia	1		
Flexithrix spp.	1	100%	A non-motile, gram-negative, chemoorganotrophic bacterium found in marine habitats.
Holophagae	**1**		
Holophaga spp.	1	100%	This genus is a homoacetogenic bacterium that can degrade methoxylated aromatic compounds.
Mollicutes	**1**		
Mycoplasma spp.	1	100%	These are pleomorphic, facultatively anaerobic and chemoorganotrophic. They require cholesterol or related sterols for growth.

Table 3. Following table details the diversity index along with the percentage breakdown of phylogenetic classes identified.

Sample	B3B-01
Diversity	
Shannon Diversity Index	3.65
Simpson Diversity Index	0.04
Phylogenetic Class Distribution (% of Total)	
Actinobacteridae	40%
Betaproteobacteria	20%
Deinococci	20%
Mollicutes	20%

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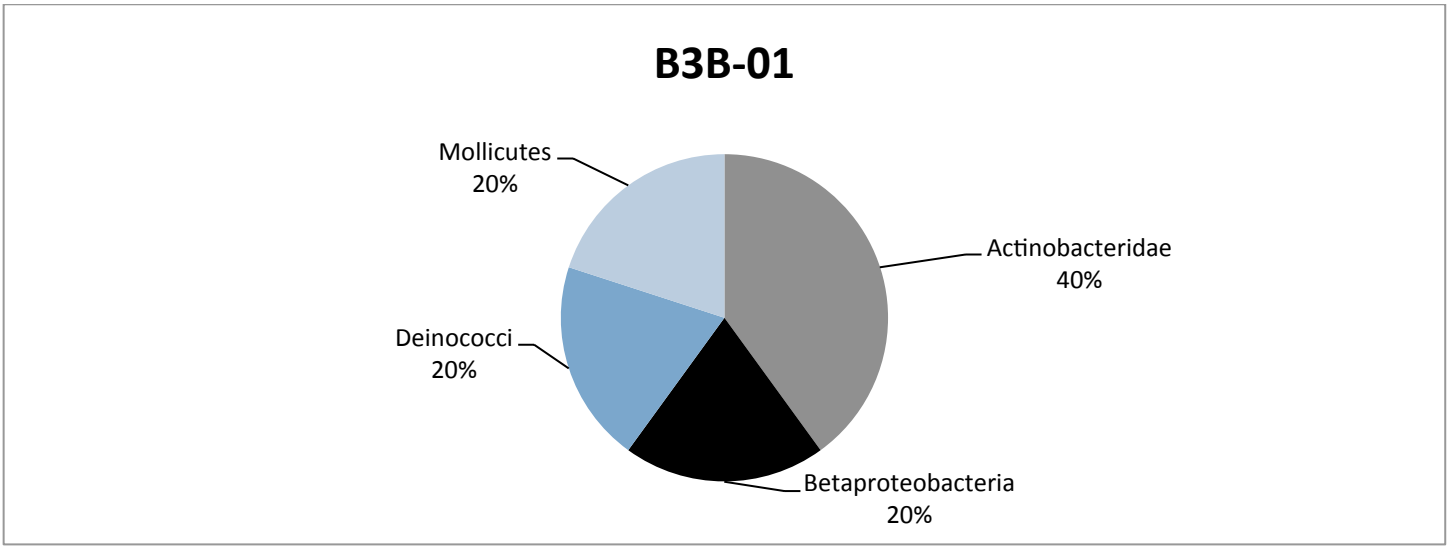

Figure 2. The diagram above represents the distribution of known phylogenetic classes. Identifications are obtained using a proprietary ribosomal database with over three million sequences that is comprised of both public and private sector sequences. Results are presented based upon the known bacterial species identified.

Table 4. Detailed summary of the known genera identified within the predominant phylogenetic classes. Results shown below represent bacteria with at least one or more species identified within the genus

B3B-01

Class / Genus	# species	% of Class	Description
Actinobacteria	**2**		
Brevibacterium spp.	1	50%	Aerobic chemoorganotrophic bacteria typically inhabiting saline environments.
Nocardioides spp.	1	50%	This genus can degrade crude oil. This bacterium is Gram-positive, aerobic, chemoorganotrophic organism that undergoes oxidative catabolism. It also uses both VC and ethene as carbon and energy sources.
Ralstonia spp.	1	100%	This aerobic genus has been found to facultatively reduce nitrate.

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Class / Genus	# species	% of Class	Description
Cyanophyceae	**1**		
Phormidium spp.	1	100%	A type of Cyanobacteria which usually forms unbranched, flat, slimy mats of tangled long cylindrical filaments.
Deinococci	**1**		
Deinococcus spp.	1	100%	Members of this genus are resistant to gamma and UV radiation. They are also recognized as being able to break down nuclear and other toxic waste, and survive the vacuum of space, extreme temperatures, dehydration and acid.

Table 5. Following table details the diversity index along with the percentage breakdown of phylogenetic classes identified.

Sample	RAP-1B-01
Diversity	
Shannon Diversity Index	3.55
Simpson Diversity Index	0.06
Phylogenetic Class Distribution (% of Total)	
Actinobacteria	10%
Bacilli	10%
Bacteroidia	20%
Betaproteobacteria	20%
Gammaproteobacteria	10%
Mollicutes	10%
Sphingobacteria	10%
Thermotogae	10%

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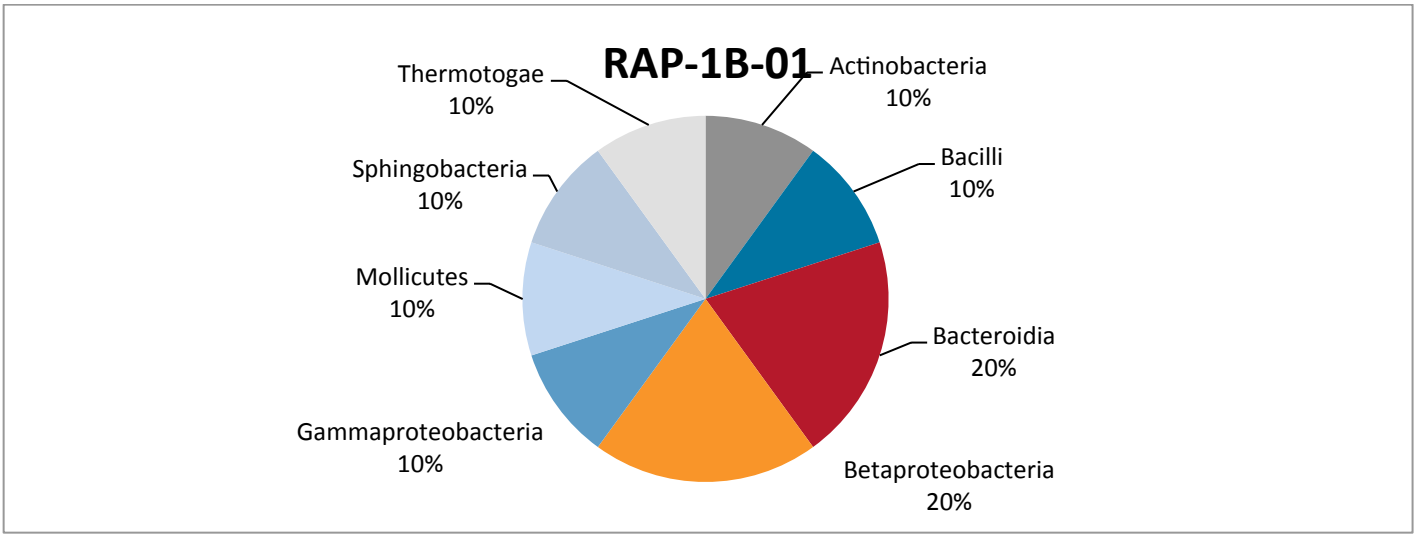

Figure 3. The diagram above represents the distribution of known phylogenetic classes. Identifications are obtained using a proprietary ribosomal database with over three million sequences that is comprised of both public and private sector sequences. Results are presented based upon the known bacterial species identified.

Table 6. Detailed summary of the known genera identified within the predominant phylogenetic classes. Results shown below represent bacteria with at least one or more species identified within the genus

RAP-1B-01

Class / Genus	# species	% of Class	Description
Actinobacteria	**1**		
Arthrobacter spp.	1	100%	Found in many environments including petroleum products. Hydrocarbon oxidation by *Arthrobacter* spp. has been found to support growth of sulfate-reducing bacteria.
Bacteroida	**1**		
Prevotella spp.	1	100%	This is a genus of bacteria found in the rumen of animals where they help the breakdown of protein and carbohydrate foods.
Gammaproteobacteria	1		
Acinetobacter spp.	1	100%	Shows dissimilatory Fe(III) reduction, is able to tolerate low O2 concentrations although it grows aerobically. Isolated from acidic mine drainage. Also capable of reducing nitrate, Co(III) and Se(VI).

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Class / Genus	# species	% of Class	Description
Mollicutes	**1**		
Candidatus spp.	1	100%	These are anaerobic, ammonia-oxidizing bacteria.
Sphingobacteriia	**1**		
Sphingobacterium spp.	1	100%	These bacteria are glucose-nonfermenting Gram-negative rods.
Thermotogae	**1**		
Petrotoga spp.	1	100%	A thermophilic, moderately halophilic, fermentative bacterium.

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Client: Warren B. Chamberlain
AMEC E & I, Inc.
2101 Webster St
Oakland, CA 94612

Phone: 510-663-3984

Fax:

Identifier: 037JD **Date Rec:** 04/18/2012 **Report Date:** 05/02/2012

Client Project #: **Client Project Name:** Casmalia

Purchase Order #:

Analysis Requested: CENSUS, DGGE, TRFLP

Reviewed By:

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DGGE

Client:	**AMEC E & I, Inc.**		**MI Project Number:**	037JD
Project:	Casmalia	affiliation	Date Received:	04/18/2012

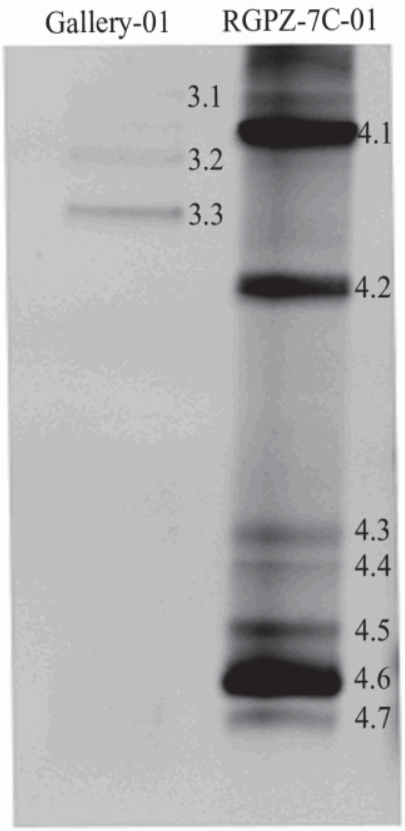

Figure 1. DGGE profile of amplified DNA from a portion of the 16S rRNA gene. Banding patterns and relative intensities of the recovered bands provide a means of comparing the communities. Bacteria must constitute at least 1-2% of the total bacterial community to form a visible band. Labeled bands were excised and sequenced. Results from sequencing can be found in the following table.

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DGGE

Client:	**AMEC E & I, Inc.**		**MI Project Number:**	037JD
Project:	Casmalia	affiliation	Date Received:	04/18/2012

Table 1. Sequence results from bands excised from Figure 1. Identifications are based on DNA sequences in the Ribosomal Database Project (RDP). Similarity indices above .900 are considered excellent, .700-.800 are good, and below .600 are considered to be unique sequences. Phylogenic affiliations are presented as phylum followed by family unless otherwise noted.

Band	Similar genus	Similarity Index	Affiliation	Description
3.1	*Acholeplasma spp.*	0.810	Mollicutes; Acholeplasmataceae	These facultative anaerobes include saprotrophic and pathogenic species.
3.2	*Uncultured bacterium*	0.840		
3.3	*Erysipelotrichaceae (Family)*	0.851	Erysipelotrichia	
4.1	*Proteiniphilum spp.*	0.966	Bacteroidia; Porphyromonadaceae	These organisms are gram negative proteolytic anaerobes.
4.2	*Desulfobacter spp.*	0.863	Deltaproteobacteria; Desulfobacteraceae	These anaerobic organisms are sulfate-reducers that occur in anaerobic parts of brackish water and marine habitats.
4.3	*Cloacibacillus spp.*	0.844	Synergistia; Synergistaceae	Members of Cloacibacillus are anaerobic organisms which are heterotrophic and proteolytic.
4.4	*Cloacibacillus spp.*	0.843	Synergistia; Synergistaceae	Members of Cloacibacillus are anaerobic organisms which are heterotrophic and proteolytic.
4.5	*Cloacibacillus spp.*	0.836	Synergistia; Synergistaceae	Members of Cloacibacillus are anaerobic organisms which are heterotrophic and proteolytic.
4.6	*Cloacibacillus spp.*	0.842	Synergistia; Synergistaceae	Members of Cloacibacillus are anaerobic organisms which are heterotrophic and proteolytic.
4.7	*Geoalkalibacter spp.*	0.987	Deltaproteobacteria; Geobacteraceae	These anaerobic organisms are iron reducers which are found in alkaline environments such as playa lakes and carbonate-rich soils.

Excised bands not included in this table did not produce phylogenetic matches.

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GBAN

Client:
Project: Casmalia

MI Project Number: 037JD
Date Received: 04/18/2012

Identifications are based on DNA sequences in the Ribosomal Database Project (RDP). Similarity indices above .900 are considered excellent, .700-.800 are good, and below .600 are considered to be unique sequences. Phylogenic affiliations are presented as phylum followed by family unless otherwise noted.

Band	Similar genus	Similarity Index	GenBank Accession Number
3.1	*Acholeplasma spp.*	0.810	FJ534963.1
3.2	*Uncultured bacterium*	0.840	EF582559.1
3.3	*Erysipelotrichaceae (Family)*	0.851	GUI96243
4.1	*Proteiniphilum spp.*	0.966	HM041958
4.2	*Desulfobacter spp.*	0.863	U12254
4.3	*Cloacibacillus spp.*	0.844	EU234149
4.4	*Cloacibacillus spp.*	0.843	EU234149
4.5	*Cloacibacillus spp.*	0.836	EU234149
4.6	*Cloacibacillus spp.*	0.842	EU234149
4.7	*Geoalkalibacter spp.*	0.987	AB514647

Excised bands not included in this report did not produce phylogenetic matches.

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Identifier: 037JD **Date Rec:** 04/18/2012 **Report Date:** 05/02/2012

Client Project #: **Client Project Name:** Casmalia

Purchase Order #:

Comments: Please note the total bacterial biomass on Sump 9B-01 was low and the sample may have PCR inhibition.

Amec
~~Mactec Engineering~~ CS

CHAIN OF CUSTODY RECORD

PROJ NO.	PROJECT NAME	NO. OF CONTAINERS	Lab: ~~Cirrus~~ microbial Insights CS Shipped on: 4/17/2012
~~47540-9100.07~~ CS	CASMALIA		
SDG NO.	SAMPLER (Signature) Chad Simpson CHS		

137jD

Lab ID No.	Sample ID	Sample Date	Sample Time	Matrix	Station Location	NO. OF CONTAINERS	Analyses	TAT
1	(1) 32574	4/17/2012	10:30	Ground water	RCF Pond-01	3	DGGE+5ID, TRFLP, DHC	
2 ☆	(2) 32575	4/17/2012	13:45	Ground water	Sump 9B-01	2	DGGE+5ID, TRFLP, DHC	
3 ☆	(2) 32576	4/17/2012	14:00	Ground water	Gallery-01	2	DGGE+5ID, TRFLP, DHC	
4 ☆	(1) 32577	4/17/2012	14:30	Ground water	RGPZ-7C-01	3	DGGE+5ID, TRFLP, DHC	

(1) Ran 500 mL. of water through filter, Sent additional Bottle
(2) Ran 1 Ltr of water through filter

☆ ~~Wells~~ CS Samples are Hot Use Caution Strong Odor!

Contact Name: Warren Chamberlin (510) 663-3984

Relinquished by: (Signature)	Date / Time	Received by (Signature)	Relinquished by: (Signature)	Date / Time	Received by (Signature)	Date / Time
CHS / Amec		Jennifer Cruzan 4/19/11 0650				
Relinquished by: (Signature)	Date / Time	Received by (Signature)	Relinquished by: (Signature)	Date / Time	Received by (Signature)	Date / Time
Relinquished by: (Signature)	Date / Time	Received by (Signature)	Relinquished by: (Signature)	Date / Time	Received by (Signature)	Date / Time

Mactec Engineering

CHAIN OF CUSTODY RECORD

PROJ NO.	PROJECT NAME
~~47540-9100.07~~ CS	CASMALIA
SDG NO.	SAMPLER (Signature) CHS

Lab: ~~Cirrus~~

Shipped on: 4/18/2012

037jD

Lab ID No.	Sample ID	Sample Date	Sample Time	Matrix	Station Location	NO. OF CON-TAINERS	Analyses	TAT
5	32578	4/18/2012	14:20	Ground water	B3B-01	2	DGGE+5ID, TRFLP	2 wk
6	32579	4/18/2012	14:45	Ground water	RAP-1B-01	2	DGGE+5ID, TRFLP	2 wk

Ran 1 Ltr. of water Through filters

Relinquished by: (Signature)	Date / Time	Received by (Signature)	Relinquished by: (Signature)	Date / Time	Received by (Signature)	Date / Time
CHS	4/18/12 1600	Jennifer Chason	4/19/12 0640			
Relinquished by: (Signature)	Date / Time	Received by (Signature)	Relinquished by: (Signature)	Date / Time	Received by (Signature)	Date / Time
Relinquished by: (Signature)	Date / Time	Received by (Signature)	Relinquished by: (Signature)	Date / Time	Received by (Signature)	Date / Time

Bacterial Test Method Description

CENSUS® Overview

mi CENSUS®
MOLECULAR BIOLOGICAL TOOL

Rapidly detect and quantify specific microbial populations and processes

CENSUS® allows site managers to cost effectively quantify targeted members of the microbial community deemed critical for site remediation. At a site impacted by chlorinated solvents like PCE or TCE for example, quantification of *Dehalococcoides* spp. (DHC), a key dechlorinating bacteria, permits project managers to address the following:

- Directly evaluate the feasibility of monitored natural attenuation
- Evaluate the efficacy of enhanced bioremediation approaches
- Assess the need for bioaugmentation

Currently, Microbial Insights offers over 30 targets for a wide variety of functions ranging from reductive dechlorination of chlorinated solvents to BTEX and MTBE biodegradation that can provide direct evidence of the biological processes occurring at your site.

CENSUS® Advantages:

- Accurate — Direct analysis of sample removes the need to grow the bacteria thus eliminating biases associated with more traditional based approaches (i.e. plate counts).
- Specific — Target either the specific bacterial group (e.g. *Dehalococcoides* spp.) or a specific gene encoding a desired function (e.g. reductive dechlorination).
- Rapid — Results are available within days (7–10 standard TAT) * Rush service available.
- Sensitivity — Practical Detection Limits (PDL) are as low as 100 cells per sample with a dynamic range over seven orders of magnitude.

Targets available for a wide range of pollutants including:

Chlorinated Compounds:
- PCE, TCE, DCE, VC
- TCA, DCA
- PCP
- Perchlorate

And more!

Petroleum Hydrocarbons:
- BTEX
- MTBE
- Diesel
- Naphthalene
- Alkanes

Approaches include:

CENSUS® is offered in a variety of formats to meet the objectives of your particular project. Please choose from the following:

CENSUS® — *Are organisms present that have the potential to degrade…?*

Our standard DNA based approach provides quantification of bacteria with the genetic potential to degrade a particular contaminant.

CENSUS®-Expression — *Are organisms actively expressing a desired function?*

RNA as opposed to DNA is extracted and used to quantify metabolically active bacteria of interest expressing the desired function.

CENSUS®-Store — *What were the baseline results before treatment?*

Collect those valuable points in time and store them for potential future analysis. Allows the collection of more data points at a lower cost. Samples can be stored and processed even years down the road.

Need the ability to quantify a unique population or function? MI can develop custom CENSUS® targets for your contaminant of concern. For more information, please call us at (865) 573-8188.

microbialinsights
www.microbe.com

Targets available for a wide range of organisms including:

Dechlorinating Bacteria

- *Dehalococcoides* spp.
- *Desulfuromonas* spp.
- *Dehalobacter* spp.
- *Desulfitobacterium* spp.
- And more!

Bacterial groups involved in remedial processes

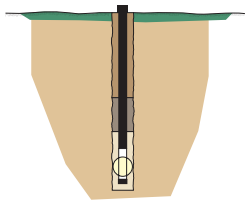
- Methanogens
- Sulfate/iron reducing bacteria
- *Geobacter* spp.
- Methane oxidizing bacteria
- Propane oxidizing bacteria
- Denitrifying bacteria
- Ammonia oxidizers
- BTEX utilizing bacteria
- MTBE utilizing PM1
- Acetogens
- Total bacteria
- Fungi
- Anaerobic ammonia oxidizing bacteria (Anammox)
- And more

How does CENSUS® work?

CENSUS® is based on a technique called quantitative polymerase chain reaction (qPCR) whereby many copies of a specific gene are generated. As each gene copy is made, a fluorescent marker is released, measured, and used to quantify the number of target genes present in the sample. The gene copied during the process (target gene) is determined by short segments of DNA called "primers" which are added to the reaction mixture. In essence, qPCR is like a copy machine with a counter. The "primers" select which pages (target gene) of the book (DNA) are copied and the counter keeps a running total of how many pages were copied (number of target genes in the sample).

Traditionally, culture-based methods such as plate counts or most probable number (MPN) analyses have been used to estimate bacterial populations in environmental samples. However, cultivation based approaches detect less than 10% of the targeted bacterial group thus severely underestimating the total population.

Sample Collection

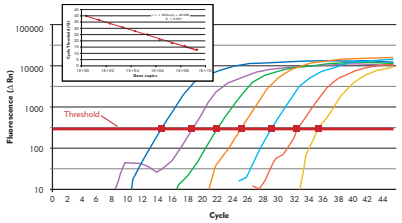


Groundwater, soil, or Bio-Trap® Sampler collected and shipped overnight on ice (4°c)

DNA Extraction

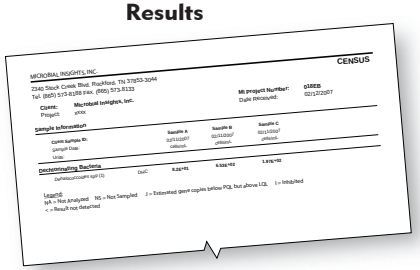
DNA is extracted from samples upon arrival

Amplification



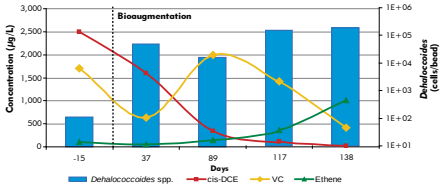
Quantitative Real-Time PCR is used to detect and quantify targets of interest (i.e. *Dehalococcoides* spp.)

Results



Results are emailed to project contact

Assessment



Results are integrated with other site parameters to evaluate site management decisions

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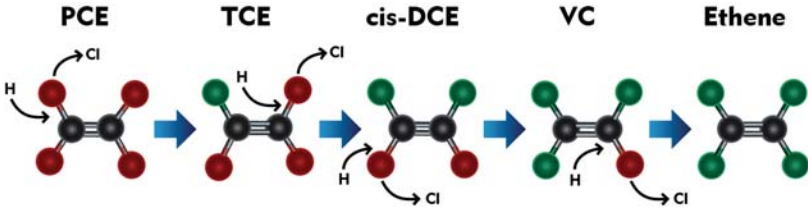
DHC Interpretation

Dehalococcoides 16S rRNA gene (qDHC)

Under anaerobic conditions, tetrachloroethene (PCE) and trichloroethene (TCE) can undergo sequential reductive dechlorination through the daughter products *cis*-dichloroethene (*cis*-DCE) and vinyl chloride to nontoxic ethene (1,2).



While a number of bacterial cultures capable of utilizing PCE and TCE as growth supporting electron acceptors have been isolated (3-7), *Dehalococcoides* spp. may be the most important because they are the only bacterial group that has been isolated to date which is capable of complete reductive dechlorination of PCE to ethene (8). In fact, the presence of *Dehalococcoides* spp. has been associated with complete dechlorination to ethene at sites across North America and Europe (9).

Status	*Dehalococcoides spp.*	Observation
	≥ 10^4 (cells/mL)	Lu et al. proposed that a concentration of 1 x 10^4 DHC cells/mL could be used as a screening criterion to identify sites where reductive dechlorination will yield a generally useful biodegradation rate (10). Similarly, in an internal study conducted with nearly 1000 groundwater samples obtained from sites across the US, ethene production was observed in approximately 80% of samples in which CENSUS® qDHC results were greater than or equal to 10^4 DHC cells/mL.
	10^1 to < 10^4 (cells/mL)	When vinyl chloride reductase genes (See DHC functional genes discussion below) are also detected, complete reductive dechlorination of PCE and TCE to ethene may still occur even with moderate DHC concentrations. When the DHC population is below the 10^4 cells/mL criterion proposed by Lu et al. (10), project managers should carefully consider other site-specific data to determine whether subsurface conditions may be limiting reductive dechlorination. For example, the addition of an electron donor may be able to stimulate DHC growth and enhance anaerobic bioremediation.
	< 10^1 (cells/mL)	DHC concentrations are low suggesting that complete reductive dechlorination of PCE and TCE to ethene is unlikely to occur under existing conditions. Enhanced anaerobic bioremediation options (biostimulation or bioaugmentation) may need to be considered.

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DHC Functional Genes (*tce*A, *bvc*A, *vcr*A)

A "stall" where daughter products *cis*-DCE and vinyl chloride accumulate can occur at PCE- and TCE-impacted sites especially under MNA conditions. The accumulation of vinyl chloride, generally considered more carcinogenic than the parent compounds, is particularly problematic. Although elevated *Dehalococcoides* concentrations correspond to ethene production in numerous studies, the range of chlorinated ethenes metabolized and cometabolized varies among species and strains within the *Dehalococcoides* genus. For example, *Dehalococcoides ethenogenes* str. 195 metabolizes PCE, TCE, and *cis*-DCE and cometabolizes vinyl chloride (8) to produce ethene. Conversely, *Dehalococcoides* sp. CBDB1 utilizes PCE and TCE but does not cometabolize additional chloroethenes (11). Other *Dehalococcoides* strains, such as BAV1, GT and VS, are known to fully dechlorinate cis-DCE and VC to ethene (14,16,19). Quantification of reductive dehalogenase genes is used to more definitively confirm the potential for reductive dechlorination of TCE, cis-DCE, and vinyl chloride (12-15).

Functional Gene	Observation
TCE Reductase	
***tce*A gene**	The *tce*A gene encodes the enzyme responsible for reductive dechlorination of TCE to *cis*-DCE in some strains of *Dehalococcoides*.
	Absence of *tce*A does not preclude the potential for reductive dechlorination of TCE in the field since the *tce*A gene is not universally distributed among all DHC and is not present in other microorganisms capable of reductive dechlorination of TCE (e.g. *Dehalobacter*).
	Detection of the *tce*A gene provides an additional line of evidence indicating the potential for dechlorination of TCE.
Vinyl Chloride Reductase	
***bvc*A gene**	The *bvc*A gene encodes the vinyl chloride reductase enzyme responsible for reductive dechlorination of vinyl chloride to ethene by *Dehalococcoides* sp. str. BAV1 (16).
	Presence of *bvc*A gene indicates the potential for reductive dechlorination of VC to ethene.
	Absence of both *bvc*A and *vcr*A genes suggests VC may accumulate.
	An internal study with ~1,000 samples showed ethene production was observed in 80% of the samples that the DHC population was greater than or equal to 10^4 cells/mL. The *bvc*A gene was detected in over 50% of these samples.
	Van Der Zaan et al (17) noted that the bvcA gene was the only VC reductase gene detected at three of their sites.
	Alfred Spormann's laboratory at Stanford University (18) reported that the *bvc*A gene was the most abundant and active at the outflow of a PCE fed column study. This section of the column was in the DCE to VC stages of reductive dechlorination thus confirming the importance of the *bvc*A gene for complete reductive dechlorination.
***vcr*A gene**	The *vcr*A gene encodes the vinyl chloride reductase enzyme responsible for reductive dechlorination of *cis*-DCE and vinyl chloride by *Dehalococcoides* sp. strain VS (14).
	Presence of *vcr*A gene indicates the potential for reductive dechlorination of DCE and/or VC to ethene.
	Absence of both *bvc*A and *vcr*A genes suggest VC may accumulate.
	As with the *bvc*A gene, detection of the *vcr*A gene is associated with ethene production in internal studies (67%) and vinyl chloride reduction in independent studies (14, 17).

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Reporting

Microbial Insights can provide a variety of data packages and reporting levels to suit the needs of any project. Data packages range from simple analytical reports with results only to more complex data packages that include a report narrative, analytical results, QC data, and supporting materials including all raw data and chain-of-custody documentation. The figure below shows our standard report and explains the way values are reported.

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CENSUS

Client: Company Name
Project: Your Project Name

MI Project Number: Unique Laboratory Identifier
Date Received: Date Samples Arrived

Sample Information

Client Sample ID:		Sample A	Sample B	Sample C
Sample Date:		00/00/0000	00/00/0000	00/00/0000
Units:		cells/mL	cells/mL	cells/mL
Analyst:		Intials	Intials	Intials
Dechlorinating Bacteria				
Dehalococcoides spp.	DHC	**1.84E+05**	**2.76E+02**	**2.28E+01 (J)**
Functional Genes				
tceA Reductase	TCE	**6.00E+01**	**3.23E+01**	<4.00E-01
bvcA Reductase	BVC	**1.17E+04**	**1.81E+01**	<4.00E-01
vcrA Reducatase	VCR	**8.42E+04**	**1.74E+02**	<4.00E-01

Legend:
NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited
< = Result not detected

"J" value
Result is an estimated value. This data qualifier (flag) is used when the target gene is detected but at a concentration or abundance below the practical quantification limit (PQL).

< value
The target gene was not detected at the limit of quantitation (LOQ) reported for that sample.

"I" value
QA Procedure indicated that the sample may have exhibited PCR inhibition. Although relatively rare, PCR inhibition can occur due to the presence of metals or humic acids at high concentrations in the sample.

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Quality Assurance

Microbial Insights' comprehensive Quality Assurance (QA) Program is the foundation of all laboratory analyses, ensuring that our clients receive high-quality analytical services that are timely, reliable, and meet their intended purpose in a cost effective manner. MI is committed to providing quality data that surpasses regulatory and industry standards, thus enabling the client to make well-informed decisions. MI maintains strict standard operating procedures and QA/QC measures throughout all of the analyses offered. The following Table details specific QA/QC procedures that are used for CENSUS.

QA/QC	Description
Date of Extraction	DNA and RNA extractions are performed the day the samples are received by MI to minimize the possibility of any changes to the microbial community prior to analysis.
Laboratory Method Blanks	An extraction blank (no sample added) is processed alongside each set of field samples from DNA extraction through CENSUS® analysis to ensure that cross contamination has not occurred. Although MI has never experienced this issue, the detection of the CENSUS® target (e.g. *Dehalococcoides*) in an extraction blank is direct evidence of cross contamination with a sample or contamination of a reagent and would invalidate the results. If this were to occur, MI would re-extract the sample. If not possible to re-extract, MI would contact the client immediately and notate it on the laboratory report.
Laboratory Control Samples (LCS)	A laboratory control sample (LCS) or positive control (target DNA) is included with each CENSUS® plate to confirm amplification and as a continuing calibration check.
Negative Controls	A negative control (no DNA) is included with each CENSUS plate to ensure that cross contamination has not occurred during amplification. As with the extraction blank, detection of CENSUS target (e.g. DHC) in a negative control is direct evidence of contamination and would invalidate the results. If this were to occur, MI would rerun the analysis.

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Analysis Report

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Address: Company
Address 1
Address 2
City, State Zip Code

Phone: (xxx) xxx-xxxx

Email: xxx@xxxx.com

MI Identifier: 001XX

Report Date: xx/xx/xxxx

Project: Project Reference

Comments:

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Table 1. Following table details the diversity index along with the percentage breakdown of phylogenetic classes identified.

Sample	Sample A
Diversity	
Shannon Diversity Index	4.49
Phylogenetic Class Distribution (% of Total)	
Acidobacteria	0.8
Actinobacteria	7.0
Actinobacteridae	1.4
Alphaproteobacteria	7.0
Anaerolineae	0.3
Bacilli	12.2
Bacteroidia	0.8
Betaproteobacteria	11.4
Chloroflexi	0.3
Chrysiogenetes	0.5
Clostridia	9.2
Cytophagia	2.7
Deferribacteres	0.5
Dehalococcoidetes	0.0
Deinococci	0.3
Deltaproteobacteria	7.0
Dictyoglomia	0.0
Epsilonproteobacteria	4.6
Erysipelotrichi	0.0
Flavobacteria	0.8
Fusobacteria	0.3
Gammaproteobacteria	26.8
Mollicutes	1.6
Nitrospira	0.8
Planctomycetacia	2.7
Spartobacteria	0.5
Sphingobacteria	0.0
Thermotogae	0.3
Verrucomicrobiae	0.3

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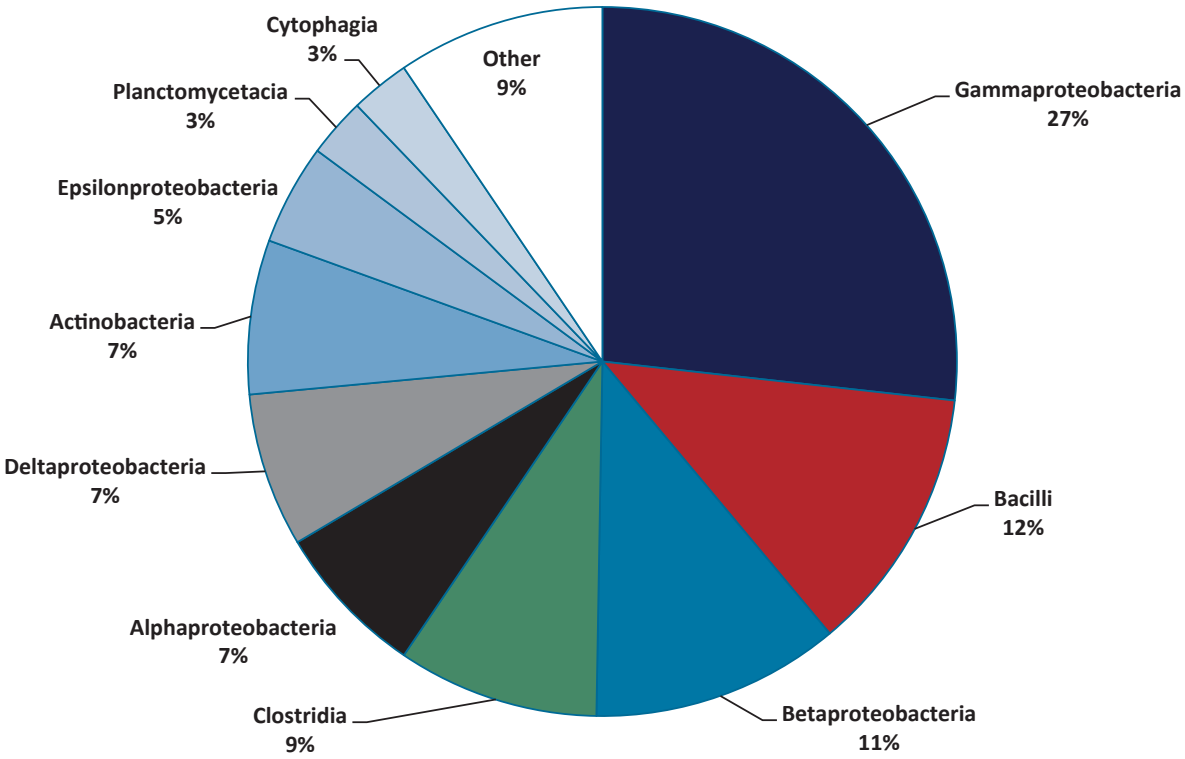

Figure 1. The diagram above represents the distribution of known phylogenetic classes. These results are based upon data identified to a known bacterial species.

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Table 2. Detailed summary of the known genera identified within the predominant phylogenetic classes. Results shown below represent bacteria with at least two or more species identified within the genus.

Class / Genus	# species	% of Class	Description
Gammaproteobacteria	**99**		
Pseudomonas spp.	15	15%	Pseudomonas can grow very rapidly to take advantage when carbon and oxygen is made available. It is gram-negative, chemoorganotrophic, and aerobic, which nitrate can be used as an alternated electron acceptor.
Shewanella spp.	4	4%	unknown
Buchnera spp.	3	3%	These bacteria are mutualistic intracellular symbionts of aphids, possibly providing essential nutrients lacking in the aphid diet.
Halomonas spp.	3	3%	Most species can grow under anaerobic conditions in the presence of nitrate.
Serratia spp.	3	3%	butanediol fermenter
Spongiobacter spp.	3	3%	unknown
Vibrio spp.	3	3%	These are facultatively anaerobic, gram-negative bacilli that typically are found in salt water. Several species are important human pathogens. They can perform both fermentative and respiratory metabolism. Their growth is stimulated by sodium.
Achromatium spp.	2	2%	unknown
Methylophaga spp.	2	2%	unknown
Photobacterium spp.	2	2%	unknown
Bacilli	**45**		
Bacillus spp.	11	24%	These bacteria are obligate aerobes or facultative anaerobes found in soil. Several species have industrial uses, and some can degrade methylparathion, phenol or petroleum.
Streptococcus spp.	7	16%	Streptococcus is found in the human mouth, skin, and feces, as well as in soil, living by aerobic respiration or fermentation.
Lactobacillus spp.	6	13%	Gram-positive nonsporulated, and anaerobic bacteria. They are normal inhabitants of the oral cavity and the digestive tract.
Staphylococcus spp.	4	9%	Pathogenic, produce slime resulting in bioflim formation
Alicyclobacillus spp.	2	4%	This soil-dwelling, strictly aerobic, acidophilic, thermophile causes food spoilage especially in fruit juices.
Paenibacillus spp.	2	4%	Facultative anaerobe
Betaproteobacteria	**42**		
Comamonas spp.	3	7%	Aerobic
Burkholderia spp.	2	5%	Burkholderia species are found in consortia of microorganisms that degrade polycyclic hydrocarbons and other environmental pollutants. They are found in soil, water, the rhizosphere, animals and humans.
Gallionella spp.	2	5%	Iron-oxidizer

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Clostridia	**34**		
Clostridium spp.	5	15%	Obligate anaerobe, spore-formation, no sulfate reduction, and Gram positive cell walls. Clostridia live by fermentation.
Desulfotomaculum spp.	3	9%	Desulfotomaculum is a Gram positive, spore-forming sulfate reducer.
Desulfitobacterium spp.	2	6%	Desulfobacterium autotrophicum ferments pyruvate.
Moorella spp.	2	6%	unknown
Alphaproteobacteria	**26**		
Brevundimonas spp.	2	8%	Brevundimonas is an aerobic chemoorganotroph found in groundwaters and surface waters. Some species can degrade naphthalene.
Hyphomicrobium spp.	2	8%	Aerobic
Deltaproteobacteria	**26**		
Desulfovibrio spp.	2	8%	Halophilic sulfate reducer found in sediment of lakes, anaerobe, can accumulate large amounts of NaCl (can survive at sea depth in hypersaline environment)
Actinobacteria	**26**		
Mycobacterium spp.	2	8%	Gram-positive nonsporulated, and anaerobic bacteria. They are normal inhabitants of the oral cavity and the digestive tract.

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mi DGGE

MOLECULAR BIOLOGICAL TOOL

Profile and identify dominant members within a microbial community

Denaturing Gradient Gel Electrophoresis (DGGE) is a DNA-based technique which generates a genetic profile or "fingerprint" which can be used to identify the dominant members of the microbial community. DGGE has been used to investigate microbial responses in a wide variety of applications including:

- Bioremediation assessment
- Wastewater treatment
- Drinking water treatment
- Biofilm formation
- Microbial induced corrosion
- Identification of microbial contaminants in commercial/industrial products
- And more!

For bioremediation assessment, DGGE profiles and sequence analysis are commonly used for evaluating the similarities/differences in the microbial community composition (dominant bacterial or fungal groups). DGGE highlights differences between samples and changes or "shifts" in microbial community composition over time or following a treatment. For example, DGGE can be used to determine the differences in the dominant bacterial groups in contaminated versus non-contaminated groundwater monitoring wells to evaluate which groups are enriched in impacted zones. Likewise, DGGE can be utilized to determine which bacterial groups are stimulated following a corrective action such as addition of a growth substrate or nutrient.

DGGE fingerprints can be produced and dominant microorganisms can be identified for a variety of target groups:

Target Group	Level	Examples
Bacteria (DGGE-BAC)	Bacterial Community	*Bacteroidetes*, *Clostridia*, *Pseudomonas*, Proteobacteria, among many others
Fungi (DGGE-FGI)	Fungal Community	*Acremonium*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Saccharomyces*, and others
Sulfate Reducing Bacteria (DGGE-SRB)	Specifically targets sulfate reducing bacteria which are functionally important but may represent less than 1% of the total bacterial community	*Desulfobulbus*, *Desulfomonas*, *Desulfuromonas*, *Desulfobacter*, and others
Dehalococcoides (DGGE-DHC)	Specifically targets *Dehalococcoides* spp. which even under conditions favorable for growth may represent less than 1% of the total bacterial population	Allows separation of different strains of *Dehalococcoides*

mi DGGE
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How does DGGE work?

Denaturing Gradient Gel Electrophoresis (DGGE) separates mixtures of amplified 16S rRNA gene segments, which are all the same size, based on nucleotide sequence.

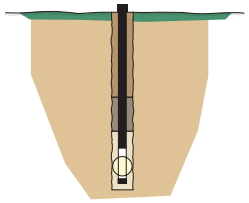
Denaturing—breaking apart the two strands of the DNA molecule.
Gradient Gel—gel with an increasing concentration of a chemical (denaturant) which breaks apart the DNA molecule.

Electrophoresis—application of an electric current across a gel. In response to the current, double-stranded DNA migrates (moves down) the gel. Denaturing the DNA molecule forms Y- and T-shaped structures greatly slowing migration.

DNA contains four nucleotide bases which bond across the two strands of the molecule - "G" forms three hydrogen bonds with "C"; "A" forms 2 hydrogen bonds with "T". Thus, DNA segments with more GC base pairs (high GC content) form stronger bonds between the DNA strands than those with less GC base pairs. Consequently, high GC content DNA segments require a greater concentration of the denaturing chemical before the DNA strands break apart.

The mixture of amplified DNA segments is loaded at the top of the gel. DNA migrates from the top (low denaturant concentration) toward the bottom of the gel (high denaturant concentration). DNA segments with low GC content denature near the top of gel and stop migrating. DNA segments with higher GC content denature further down the gel. DNA with the identical sequences migrate the same distance forming a "band". Individual bands are excised for sequencing and results are compared to a database of 16S rRNA genes to identify the dominant organisms.

Sample Collection

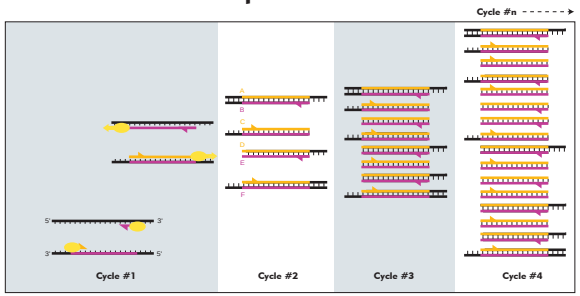


Groundwater, soil, or Bio-Trap® Sampler collected and shipped overnight on ice (4°c)

DNA Extraction

DNA is extracted from samples upon arrival

Amplification



Variable region of 16S rRNA gene amplified by PCR

Results

DGGE Profile

GCCAGCCGTGTTTACAGA… *Acinetobacter sp.*

GCCTTAAGCAGGCCTTCG… *Geobacter sp.*

GCCAGCCGTGTTTACAGA… *Unknown sp X.*

GCCGGCTCTAGCTTCCGT… *Methylobacterium sp.*

Dominant bands excised and sequenced

Dominant organisms identified

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