EA Engineering, P.C. and Its Affiliate
EA Science and Technology

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Syracuse, NY 13202
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21 August 2023

Ms. Jasmine Stefansky
Project Manager
New York State Department of Environmental Conservation
625 Broadway, 12th Floor
Albany, New York 12233

RE: Dinaburg Distributing, Inc (Site No. 828103)
Microbial Sampling
EA Project No. 1602534.18

Dear Ms. Stefansky:

EA Engineering, P.C. and its affiliate EA Science and Technology (EA) is interested in conducting microbial sampling at three (3) monitoring wells at the Dinaburg Distributing, Inc Site (No. 828103) to provide additional insight into the subsurface conditions. Specifically, EA would conduct QuantArray-Chlor analysis (**Attachment 1**) with a standard bio-trap sampling method. EA successfully conducted this analysis at other project sites.

SITE BACKGROUND

The Site property and buildings were reportedly used as an automobile repair shop from approximately 1950 through 1969. From 1971 to 1993, the Site was occupied by Dinaburg Distributing, which operated a dry-cleaning supply company selling chemical solvents to various dry cleaners in the area. Dinaburg Distributing stored TCE and PCE in above ground storage tanks located inside the north section of the site building. As a result of site operations, spills and or leaks of chlorinated solvents to the ground surface occurred, likely both inside and outside the site buildings. The property has been vacant since 1995 and currently consists of a parking lot. The on-site building and former house located at 350 Benton Street were demolished in 2004 (MACTEC Engineering and Geology, P.C. 2023)[1].

QUANTARRAY-CHLOR ANALYSIS

The purpose of the analysis is to provide simultaneous yet economic evaluation of potential biodegradation of a spectrum of common chlorinated contaminants through a multitude of anaerobic and aerobic (co)metabolic pathways and provide a clearer and more comprehensive view of contaminant biodegradation. QuantArray-Chlor simultaneously quantifies a broad spectrum of different microorganisms and key functional genes involved in a variety of pathways of chlorinated hydrocarbon biodegradation. QuantArray-Chlor analysis quantifies *Dehalococcoides*, the only know bacterial group capable of complete reductive dichlorination of PCE and TCE to ethene. This analysis may be an indispensable component of the site assessment, remedy selection, and monitoring. In addition, the analysis provides a quantification of functional

1 MACTEC Engineering and Geology, P.C. 2023. *Site Management Plan Dinaburg Distributing, Inc., Monroe County, Rochester, New York, NYSDEC Site Number 828103.* March.

genes involved in aerobic (co)metabolic pathways for biodegradation of chlorinated solvents and completing biological processes.

The QuantArray-Chlor analysis is used to quantify specific microorganisms and functional genes to evaluate the following:

- Anaerobic Reductive Dechlorination: Quantification of important halorespiring bacteria and key functional genes responsible for reductive dichlorination of a broad spectrum of chlorinated solvents.

- Aerobic Cometabolism: Quantification of functional genes capable of co-oxidation of chlorinated ethenes.

- Aerobic (Co)metabolism of Vinyl Chloride: Quantification of ethene oxidizing bacteria capable of cometabolism of vinyl chloride.

Microbial Insights will provide results including microbial population summary, summary tables, and comparison figures. An example of the report that is provided is included in **Attachment** 1.

COST ANALYSIS

The cost to perform the QuantArray-Chlor analysis with a standard bio-trap sampling method is $850.00 per sample. In total, the cost for the sampling event cost is approximately $2,550.00.

RECOMMENDATIONS

EA recommends conducting the QuantArray-Chlor analysis at three (3) monitoring wells on-site. These wells consist of MW-20S, MW-03CA and MW-13K (**Figure 1**). Each well was selected because of its proximity to the source area and actual screen depths. MW-20S and MW-03CA are located near the source area, and MW-13K is located downgradient of the source area. The direction of groundwater flow has been interpreted from previous investigations. By choosing these specified wells, EA expects to gain more insight into the contamination of this site. Locations of these wells are provided in **Figure 1**.

If you have any issues or questions, please do not hesitate to contact me using the contact information below.

Sincerely,

Joshua Oliver

Joshua Oliver, P.G.
Project Manager
EA Science and Technology
315.679.4856
joliver@eaest.com

Attachments

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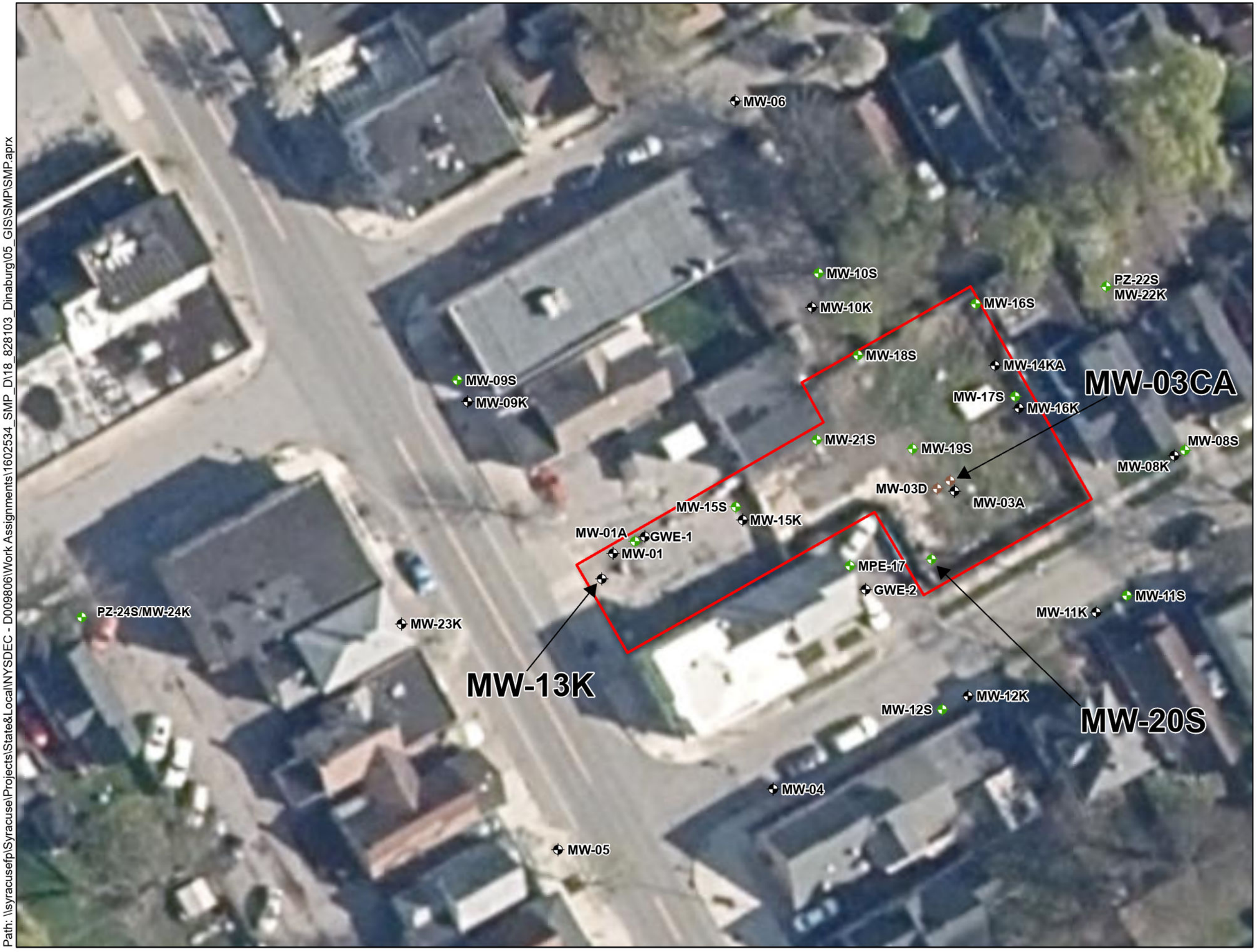
Figure

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Attachment 1

Microbial Insights Laboratory Report

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Legend

- Overburden Monitoring Well
- Overburden/Bedrock Interface Monitoring Well
- Bedrock Monitoring Well
- Site Boundary

N

0 30 60 Feet

Map Date: 8/10/2023 3:08 PM

NEW YORK STATE Department of Environmental Conservation

Figure 1
Selected Wells
Dinaburg Distributing, Inc. (828103)
Rochester, New York

Path: \\syracusefp\Syracuse\Projects\State&Local\NYSDEC - D009806\Work Assignments\1602534_SMP_D\18_828103_Dinaburg\05_GIS\SMP\SMP.aprx

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SITE LOGIC Report

QuantArray®-Chlor Study

Contact: Hilary Williams
Phone: (315) 565-6568
Address: EA Engineering
269 W. Jefferson Street
Syracuse, NY 13202
Email: hwilliams@eaest.com

MI Identifier: 003QG

Report Date: 07/15/2019

Project: Picatinny Arsenal-Area D, 6268609
Comments:

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The QuantArray®-Chlor Approach

Quantification of *Dehalococcoides*, the only known bacterial group capable of complete reductive dechlorination of PCE and TCE to ethene, has become an indispensable component of assessment, remedy selection, and performance monitoring at sites impacted by chlorinated solvents. While undeniably a key group of halorespiring bacteria, *Dehalococcoides* are not the only bacteria of interest in the subsurface because reductive dechlorination is not the only potential biodegradation pathway operative at contaminated sites, and chlorinated ethenes are not always the primary contaminants of concern. The QuantArray®-Chlor not only includes a variety of halorespiring bacteria (*Dehalococcoides*, *Dehalobacter*, *Dehalogenimonas*, etc.) to assess the potential for reductive dechlorination of chloroethenes, chloroethanes, chlorobenzenes, chlorophenols, and chloroform, but also provides quantification of functional genes involved in aerobic (co)metabolic pathways for biodegradation of chlorinated solvents and even competing biological processes. Thus, the QuantArray®-Chlor will give site managers the ability to simultaneously yet economically evaluate the potential for biodegradation of a spectrum of common chlorinated contaminants through a multitude of anaerobic and aerobic (co) metabolic pathways to give a much more clear and comprehensive view of contaminant biodegradation.

The QuantArray®-Chlor is used to quantify specific microorganisms and functional genes to evaluate the following:

Anaerobic Reductive Dechlorination	Quantification of important halorespiring bacteria (e.g. *Dehalococcoides*, *Dehalobacter*, *Dehalogenimonas*, *Desulfitobacterium* spp.) and key functional genes (e.g. vinyl chloride reductases, TCE reductase, chloroform reductase) responsible for reductive dechlorination of a broad spectrum of chlorinated solvents.
Aerobic Cometabolism	Several different types of bacteria including methanotrophs and some toluene/phenol utilizing bacteria can co-oxidize TCE, DCE, and vinyl chloride. The QuantArray®-Chlor quantifies functional genes like soluble methane monooxygenase encoding enzymes capable of co-oxidation of chlorinated ethenes.
Aerobic (Co)metabolism of Vinyl Chloride	Ethene oxidizing bacteria are capable of cometabolism of vinyl chloride. In some cases, ethenotrophs can also utilize vinyl chloride as a growth supporting substrate. The QuantArray®-Chlor targets key functional genes in ethene metabolism.

How do QuantArrays® work?

The QuantArray®-Chlor in many respects is a hybrid technology combining the highly parallel detection of microarrays with the accurate and precise quantification provided by qPCR into a single platform. The key to highly parallel qPCR reactions is the nanoliter fluidics platform for low volume, solution phase qPCR reactions.

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How are QuantArray® results reported?

One of the primary advantages of the QuantArray®-Chlor is the simultaneous quantification of a broad spectrum of different microorganisms and key functional genes involved in a variety of pathways for chlorinated hydrocarbon biodegradation. However, highly parallel quantification combined with the various metabolic and cometabolic capabilities of different target organisms can complicate data presentation. Therefore, in addition to Summary Tables, QuantArray® results will be presented as Microbial Population Summary and Comparison Figures to aid in data interpretation and subsequent evaluation of site management activities.

Types of Tables and Figures:

Microbial Population Summary	Figure presenting the concentrations of QuantArray®-Chlor target populations (e.g. *Dehalococcoides*) and functional genes (e.g. vinyl chloride reductase) relative to typically observed values.
Summary Tables	Tables of target population concentrations grouped by biodegradation pathway and contaminant type.
Comparison Figures	Depending on the project, sample results can be presented to compare changes over time or examine differences in microbial populations along a transect of the dissolved plume.

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Results

Table 1: Summary of the QuantArray®-Chlor results obtained for samples 9-H-Microbe, 112-7-Microbe, and 92-3-Microbe.

Sample Name Sample Date	9-H-Microbe 07/01/2019	112-7-Microbe 07/01/2019	92-3-Microbe 07/01/2019
Reductive Dechlorination	cells/bead	cells/bead	cells/bead
Dehalococcoides (DHC)	**4.89E+01**	**1.63E+03**	<2.50E+01
tceA Reductase (TCE)	<2.50E+01	<2.50E+01	<2.50E+01
BAV1 Vinyl Chloride Reductase (BVC)	<2.50E+01	<2.50E+01	<2.50E+01
Vinyl Chloride Reductase (VCR)	<2.50E+01	**6.78E+01**	<2.50E+01
Dehalobacter spp. (DHBt)	**2.01E+04**	**1.92E+04**	**1.92E+04**
Dehalobacter DCM (DCM)	<2.50E+02	<2.50E+02	<2.50E+02
Dehalogenimonas spp. (DHG)	<2.50E+02	<2.50E+02	<2.50E+02
cerA Reductase (CER)	<2.50E+02	**7.70E+01 (J)**	<2.50E+02
trans-1,2-DCE Reductase (TDR)	<2.50E+02	<2.50E+02	<2.50E+02
Desulfitobacterium spp. (DSB)	**2.35E+04**	<2.50E+02	<2.50E+02
Dehalobium chlorocoercia (DECO)	<2.50E+02	**4.23E+04**	<2.50E+02
Desulfuromonas spp. (DSM)	<2.50E+02	**4.48E+02**	**9.60E+00 (J)**
PCE Reductase (PCE-1)	<2.50E+02	<2.50E+02	<2.50E+02
PCE Reductase (PCE-2)	<2.50E+02	**3.55E+03**	<2.50E+02
Chloroform Reductase (CFR)	<2.50E+02	<2.50E+02	<2.50E+02
1,1 DCA Reductase (DCA)	<2.50E+02	<2.50E+02	<2.50E+02
1,2 DCA Reductase (DCAR)	<2.50E+02	<2.50E+02	<2.50E+02
Aerobic (Co)Metabolic			
Soluble Methane Monooxygenase (SMMO)	<2.50E+02	<2.50E+02	<2.50E+02
Toluene Dioxygenase (TOD)	<2.50E+02	<2.50E+02	<2.50E+02
Phenol Hydroxylase (PHE)	**6.69E+03**	**7.74E+04**	**6.72E+04**
Trichlorobenzene Dioxygenase (TCBO)	<2.50E+02	<2.50E+02	<2.50E+02
Toluene Monooxygenase 2 (RDEG)	**6.52E+03**	**4.23E+04**	**1.18E+04**
Toluene Monooxygenase (RMO)	**3.23E+04**	<2.50E+02	**1.28E+04**
Ethene Monooxygenase (EtnC)	<2.50E+02	<2.50E+02	<2.50E+02
Epoxyalkane Transferase (EtnE)	<2.50E+02	<2.50E+02	<2.50E+02
Dichloromethane Dehalogenase (DCMA)	<2.50E+02	**9.08E+02**	<2.50E+02
Other			
Total Eubacteria (EBAC)	**9.60E+06**	**2.03E+07**	**2.23E+07**
Sulfate Reducing Bacteria (APS)	**9.66E+03**	**4.53E+04**	**1.74E+05**
Methanogens (MGN)	**5.50E+00 (J)**	<2.50E+02	**9.30E+00 (J)**

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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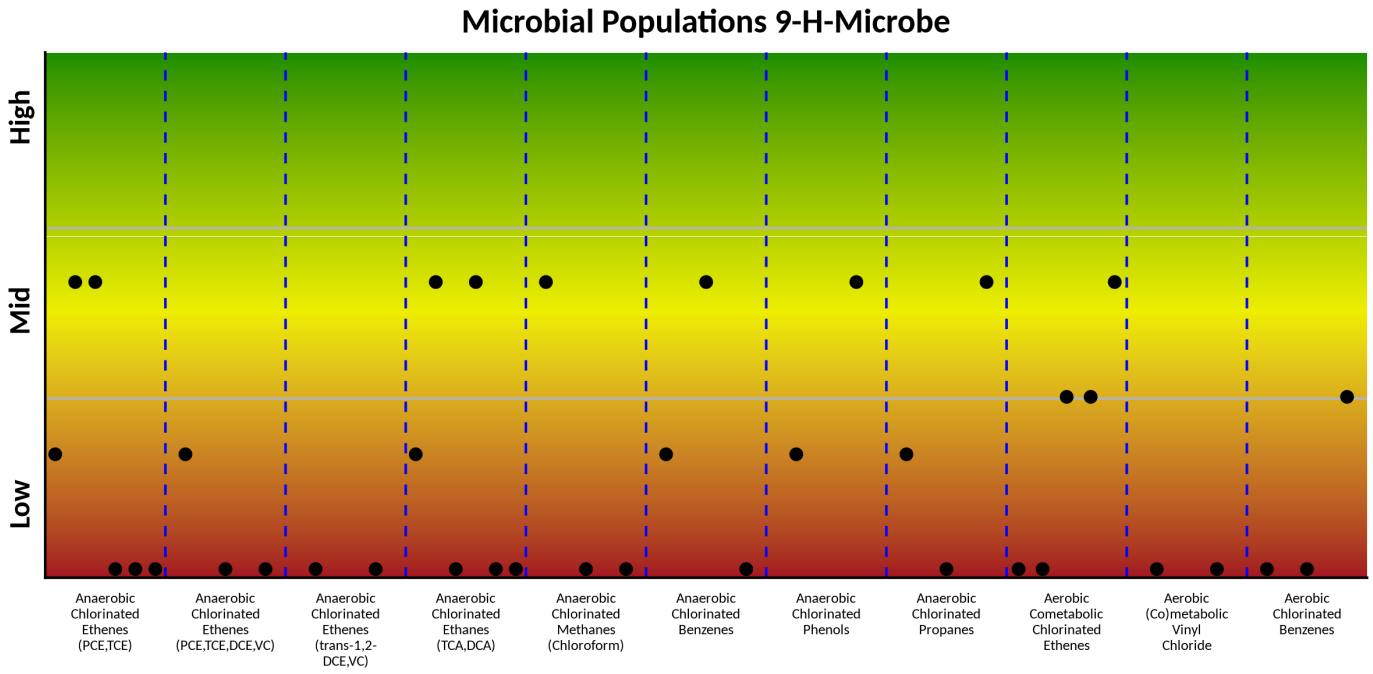


Figure 1: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Anaerobic - Reductive Dechlorination or Dichloroelimination		Aerobic - (Co)metabolism	
Chlorinated Ethenes (PCE, TCE)	DHC, DHBt, DSB, DSM, PCE-1, PCE-2	Chlorinated Ethenes (TCE,DCE,VC)	sMMO, TOD, PHE, RDEG, RMO
Chlorinated Ethenes (PCE, TCE, DCE, VC)	DHC, BVC, VCR	(Co)metabolic Vinyl Chloride	etnC, etnE
Chlorinated Ethenes (trans-1,2-DCE, VC)	TDR, CER	Chlorinated Benzenes	TOD, TCBO, PHE
Chlorinated Ethanes (TCA and 1,2-DCA)	DHC, DHBt, DHG, DSB[1], DCA, DCAR		
Chlorinated Methanes (Chloroform)	DHBt, DCM, CFR		
Chlorinated Benzenes	DHC, DHBt[2], DECO		
Chlorinated Phenols	DHC, DSB		
Chlorinated Propanes	DHC, DHG, DSB[1]		

[1] *Desulfitobacterium dichloroeliminans* DCA1. [2] Implicated in reductive dechlorination of dichlorobenzene and potentially chlorobenzene.

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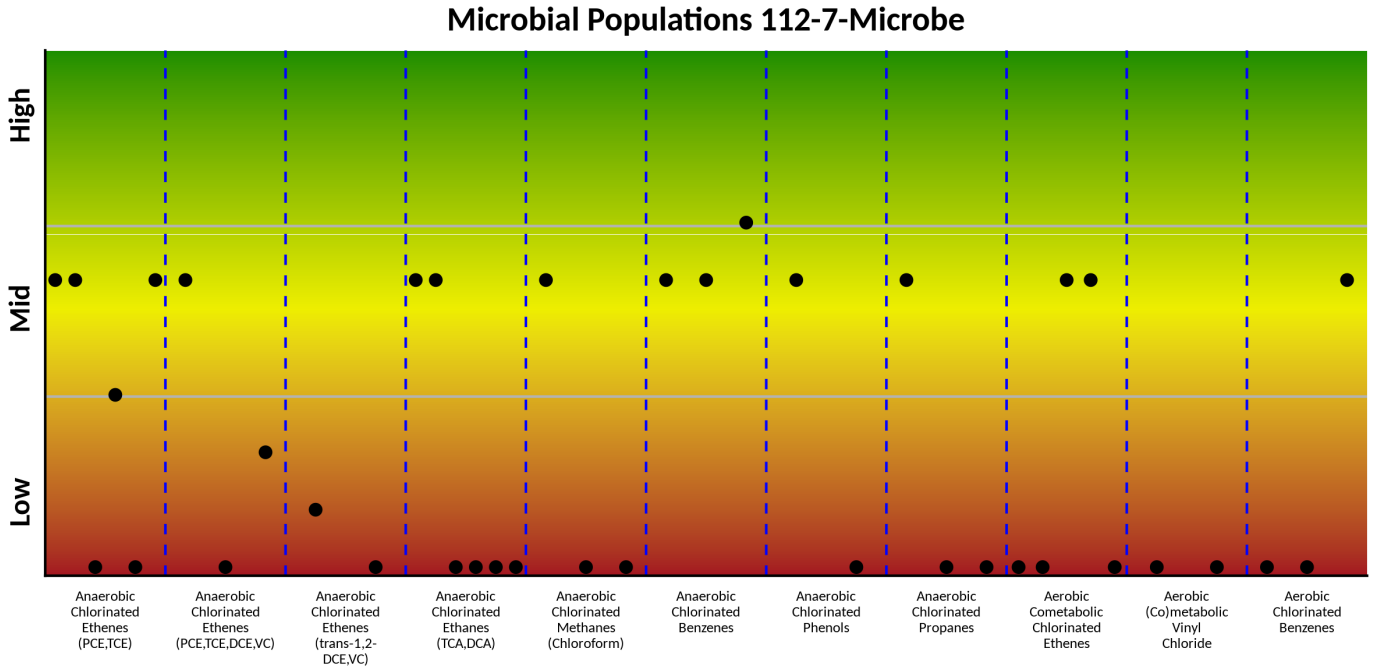


Figure 2: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Anaerobic - Reductive Dechlorination or Dichloroelimination		Aerobic - (Co)metabolism	
Chlorinated Ethenes (PCE, TCE)	DHC, DHBt, DSB, DSM, PCE-1, PCE-2	Chlorinated Ethenes (TCE,DCE,VC)	sMMO, TOD, PHE, RDEG, RMO
Chlorinated Ethenes (PCE, TCE, DCE, VC)	DHC, BVC, VCR	(Co)metabolic Vinyl Chloride	etnC, etnE
Chlorinated Ethenes (trans-1,2-DCE, VC)	TDR, CER	Chlorinated Benzenes	TOD, TCBO, PHE
Chlorinated Ethanes (TCA and 1,2-DCA)	DHC, DHBt, DHG, DSB[1], DCA, DCAR		
Chlorinated Methanes (Chloroform)	DHBt, DCM, CFR		
Chlorinated Benzenes	DHC, DHBt[2], DECO		
Chlorinated Phenols	DHC, DSB		
Chlorinated Propanes	DHC, DHG, DSB[1]		

[1] *Desulfitobacterium dichloroeliminans* DCA1. [2] Implicated in reductive dechlorination of dichlorobenzene and potentially chlorobenzene.

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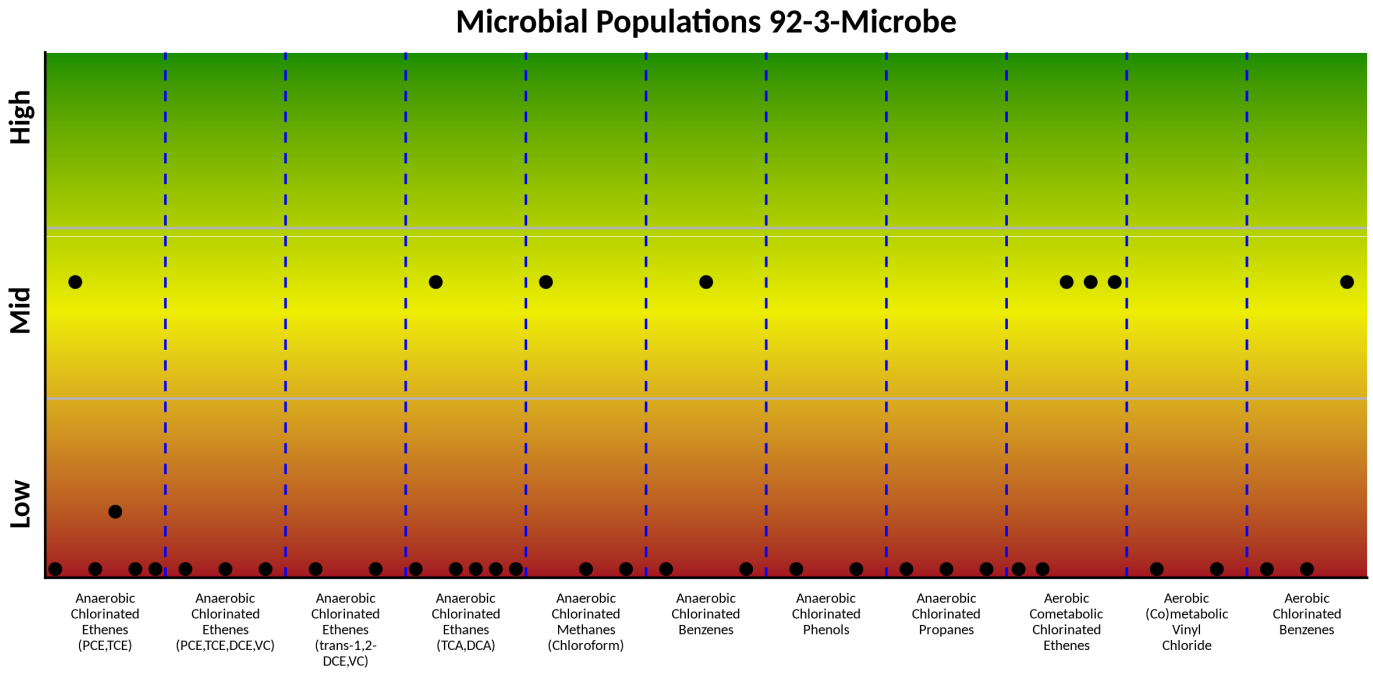


Figure 3: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Anaerobic - Reductive Dechlorination or Dichloroelimination		Aerobic - (Co)metabolism	
Chlorinated Ethenes (PCE, TCE)	DHC, DHBt, DSB, DSM, PCE-1, PCE-2	Chlorinated Ethenes (TCE,DCE,VC)	sMMO, TOD, PHE, RDEG, RMO
Chlorinated Ethenes (PCE, TCE, DCE, VC)	DHC, BVC, VCR	(Co)metabolic Vinyl Chloride	etnC, etnE
Chlorinated Ethenes (trans-1,2-DCE, VC)	TDR, CER	Chlorinated Benzenes	TOD, TCBO, PHE
Chlorinated Ethanes (TCA and 1,2-DCA)	DHC, DHBt, DHG, DSB[1], DCA, DCAR		
Chlorinated Methanes (Chloroform)	DHBt, DCM, CFR		
Chlorinated Benzenes	DHC, DHBt[2], DECO		
Chlorinated Phenols	DHC, DSB		
Chlorinated Propanes	DHC, DHG, DSB[1]		

[1] *Desulfitobacterium dichloroeliminans* DCA1. [2] Implicated in reductive dechlorination of dichlorobenzene and potentially chlorobenzene.

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Table 2: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples 9-H-Microbe, 112-7-Microbe, and 92-3-Microbe.

Sample Name Sample Date	9-H-Microbe 07/01/2019	112-7-Microbe 07/01/2019	92-3-Microbe 07/01/2019
Reductive Dechlorination	cells/bead	cells/bead	cells/bead
Dehalococcoides (DHC)	**4.89E+01**	**1.63E+03**	<2.50E+01
tceA Reductase (TCE)	<2.50E+01	<2.50E+01	<2.50E+01
BAV1 Vinyl Chloride Reductase (BVC)	<2.50E+01	<2.50E+01	<2.50E+01
Vinyl Chloride Reductase (VCR)	<2.50E+01	**6.78E+01**	<2.50E+01
Dehalobacter spp. (DHBt)	**2.01E+04**	**1.92E+04**	**1.92E+04**
Dehalobacter DCM (DCM)	<2.50E+02	<2.50E+02	<2.50E+02
Dehalogenimonas spp. (DHG)	<2.50E+02	<2.50E+02	<2.50E+02
Desulfitobacterium spp. (DSB)	**2.35E+04**	<2.50E+02	<2.50E+02
Dehalobium chlorocoercia (DECO)	<2.50E+02	**4.23E+04**	<2.50E+02
Desulfuromonas spp. (DSM)	<2.50E+02	**4.48E+02**	**9.60E+00 (J)**

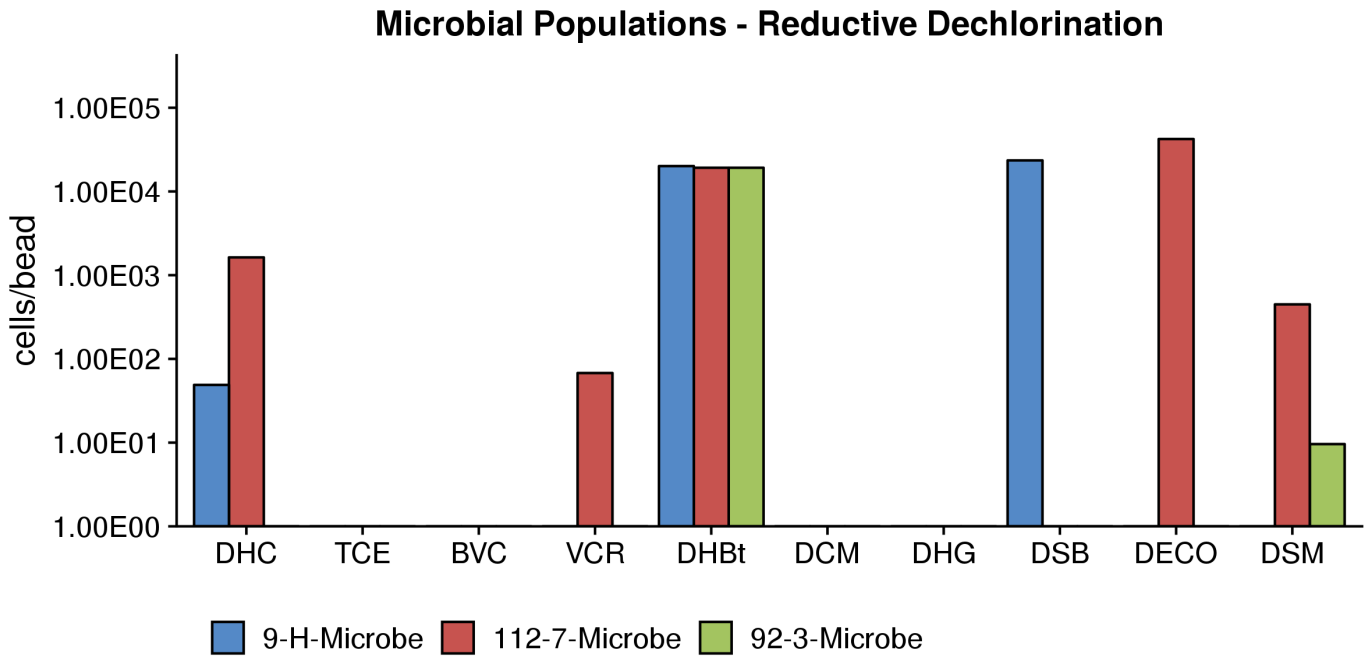


Figure 4: Comparison - microbial populations involved in reductive dechlorination.

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Table 3: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples 9-H-Microbe, 112-7-Microbe, and 92-3-Microbe.

Sample Name Sample Date	9-H-Microbe 07/01/2019	112-7-Microbe 07/01/2019	92-3-Microbe 07/01/2019
Reductive Dechlorination	cells/bead	cells/bead	cells/bead
Chloroform Reductase (CFR)	<2.50E+02	<2.50E+02	<2.50E+02
1,1 DCA Reductase (DCA)	<2.50E+02	<2.50E+02	<2.50E+02
1,2 DCA Reductase (DCAR)	<2.50E+02	<2.50E+02	<2.50E+02
PCE Reductase (PCE-1)	<2.50E+02	<2.50E+02	<2.50E+02
PCE Reductase (PCE-2)	<2.50E+02	**3.55E+03**	<2.50E+02
Dehalogenimonas trans-1,2-DCE Reductase (TDR)	<2.50E+02	<2.50E+02	<2.50E+02
Dehalogenimonas cerA Reductase (CER)	<2.50E+02	**7.70E+01 (J)**	<2.50E+02

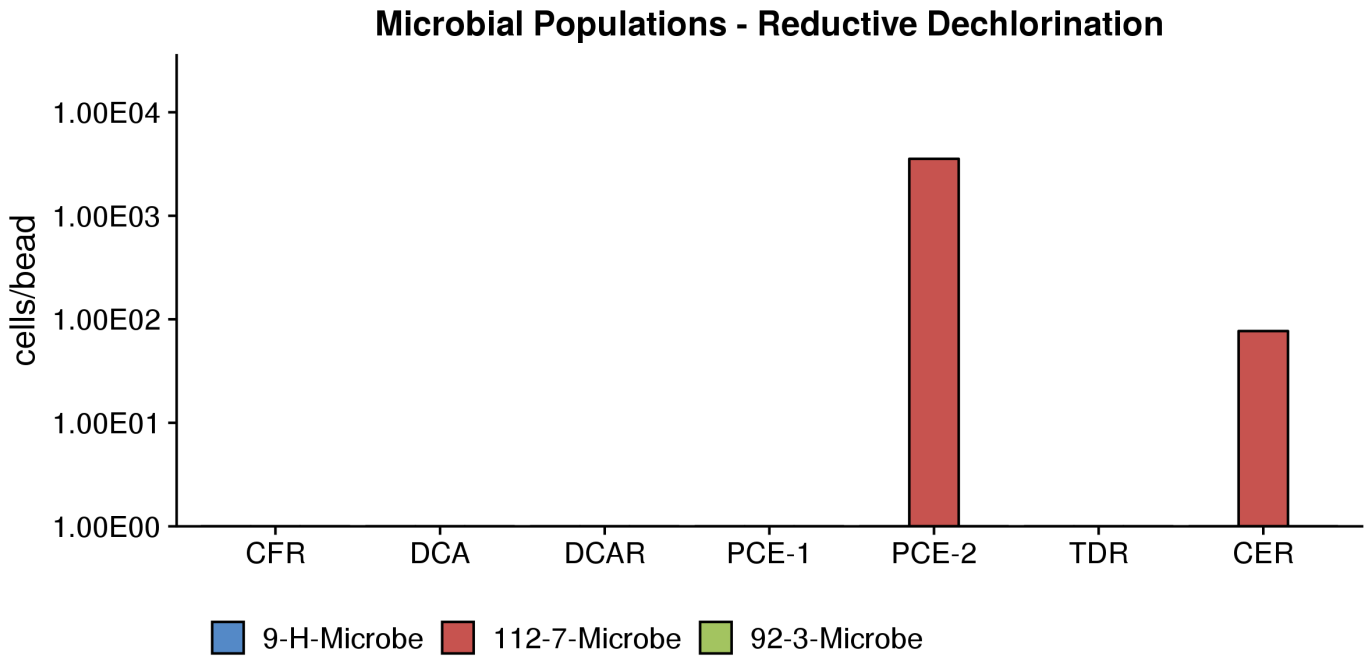


Figure 5: Comparison - microbial populations involved in reductive dechlorination.

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Table 4: Summary of the QuantArray®-Chlor results for microorganisms responsible for aerobic (co)metabolism for samples 9-H-Microbe, 112-7-Microbe, and 92-3-Microbe.

Sample Name Sample Date	9-H-Microbe 07/01/2019	112-7-Microbe 07/01/2019	92-3-Microbe 07/01/2019
Aerobic (Co)Metabolic	cells/bead	cells/bead	cells/bead
Soluble Methane Monooxygenase (SMMO)	<2.50E+02	<2.50E+02	<2.50E+02
Toluene Dioxygenase (TOD)	<2.50E+02	<2.50E+02	<2.50E+02
Phenol Hydroxylase (PHE)	**6.69E+03**	**7.74E+04**	**6.72E+04**
Trichlorobenzene Dioxygenase (TCBO)	<2.50E+02	<2.50E+02	<2.50E+02
Toluene Monooxygenase 2 (RDEG)	**6.52E+03**	**4.23E+04**	**1.18E+04**
Toluene Monooxygenase (RMO)	**3.23E+04**	<2.50E+02	**1.28E+04**
Ethene Monooxygenase (EtnC)	<2.50E+02	<2.50E+02	<2.50E+02
Epoxyalkane Transferase (EtnE)	<2.50E+02	<2.50E+02	<2.50E+02
Dichloromethane Dehalogenase (DCMA)	<2.50E+02	**9.08E+02**	<2.50E+02

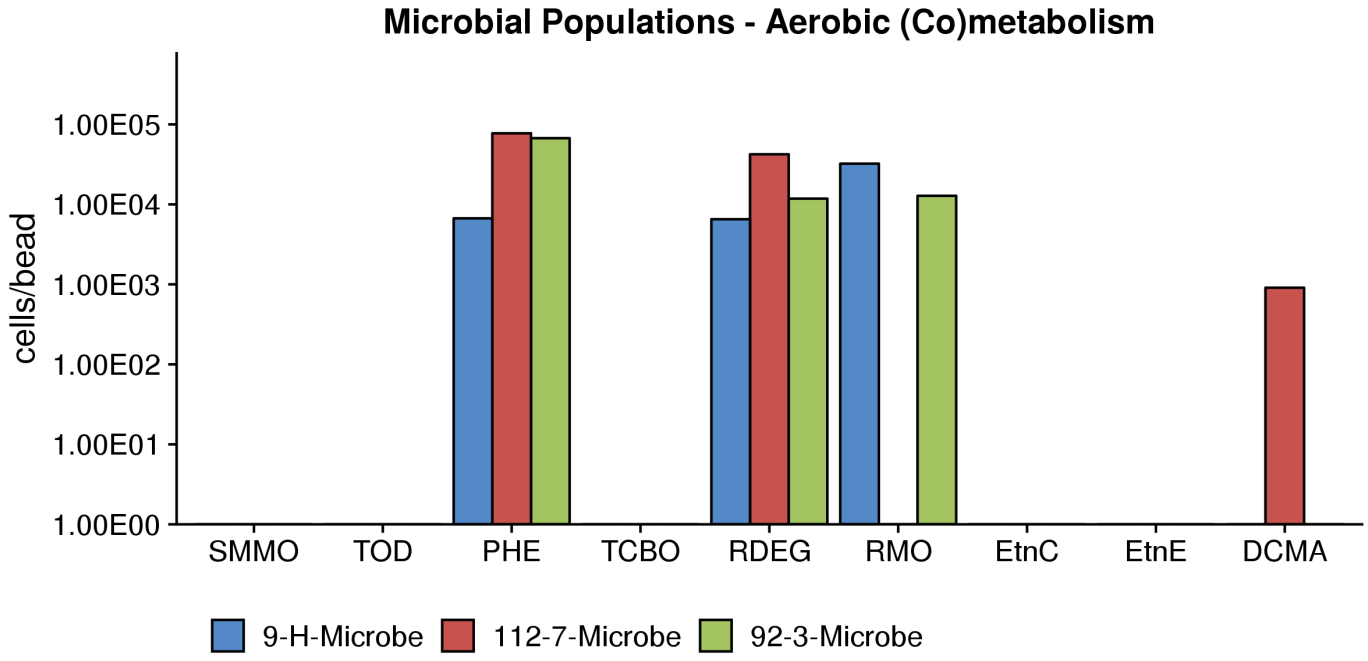


Figure 6: Comparison - microbial populations involved in aerobic (co)metabolism.

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Table 5: Summary of the QuantArray® results for total bacteria and other populations for samples 9-H-Microbe, 112-7-Microbe, and 92-3-Microbe.

Sample Name Sample Date	9-H-Microbe 07/01/2019	112-7-Microbe 07/01/2019	92-3-Microbe 07/01/2019
Other	cells/bead	cells/bead	cells/bead
Total Eubacteria (EBAC)	**9.60E+06**	**2.03E+07**	**2.23E+07**
Sulfate Reducing Bacteria (APS)	**9.66E+03**	**4.53E+04**	**1.74E+05**
Methanogens (MGN)	**5.50E+00 (J)**	<2.50E+02	**9.30E+00 (J)**

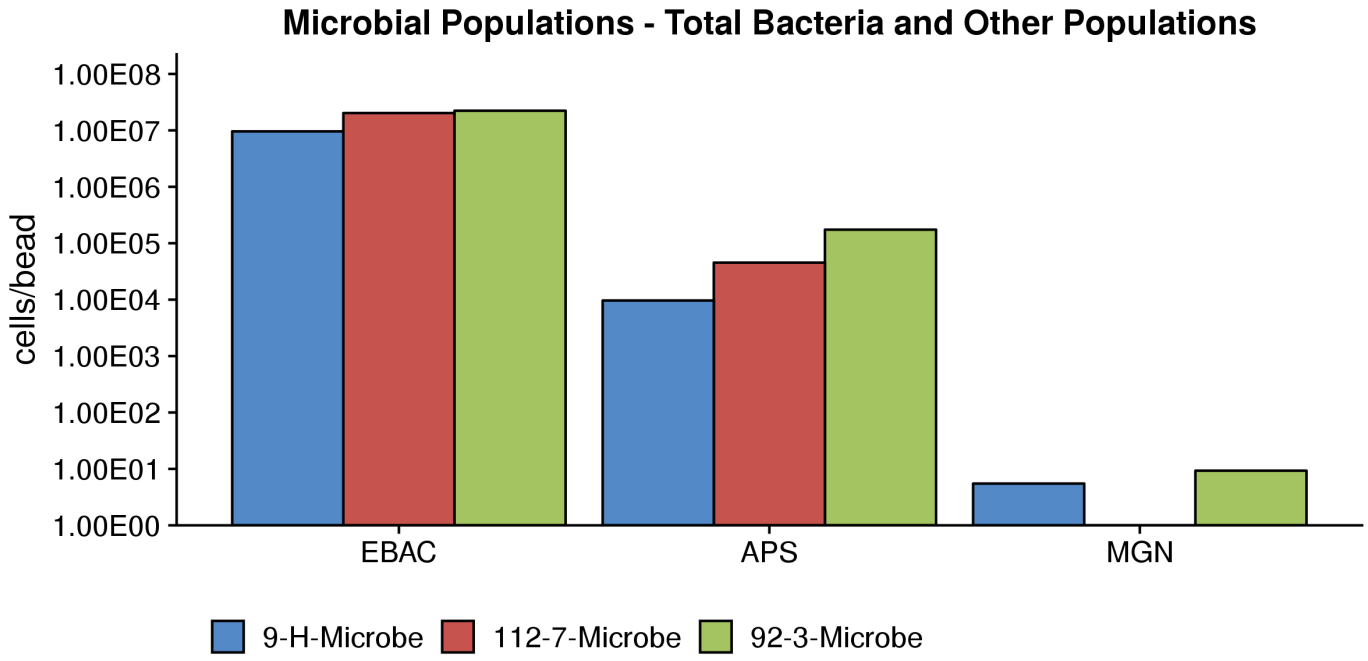


Figure 7: Comparison - microbial populations.

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Interpretation

The overall purpose of the QuantArray®-Chlor is to give site managers the ability to simultaneously yet economically evaluate the potential for biodegradation of a spectrum of common chlorinated contaminants through a multitude of anaerobic and aerobic (co)metabolic pathways in order to provide a clearer and more comprehensive view of contaminant biodegradation. The following discussion describes the interpretation of results in general terms and is meant to serve as a guide.

Reductive Dechlorination - Chlorinated Ethenes: While a number of bacterial cultures including *Dehalococcoides*, *Dehalobacter*, *Desulfitobacterium*, and *Desulfuromonas* spp. capable of utilizing PCE and TCE as growth-supporting electron acceptors have been isolated [1–5], *Dehalococcoides* 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 [6]. In fact, the presence of *Dehalococcoides* has been associated with complete reductive dechlorination to ethene at sites across North America and Europe [7], and Lu et al. [8] have proposed using a *Dehalococcoides* concentration of 1 x 10^4 cells/mL as a screening criterion to identify sites where biological reductive dechlorination is predicted to proceed at "generally useful" rates.

At chlorinated ethene sites, any "stall" leading to the accumulation of daughter products, especially vinyl chloride, would be a substantial concern. While *Dehalococcoides* concentrations greater than 1 x 10^4 cells/mL correspond to ethene production and useful rates of dechlorination, the range of chlorinated ethenes degraded varies by strain within the *Dehalococcoides* genus [6, 9], and the presence of co-contaminants and competitors can have complex impacts on the halorespiring microbial community [10–15]. Therefore, QuantArray®-Chlor also provides quantification of a suite of reductive dehalogenase genes (PCE, TCE, BVC, VCR, CER, and TDR) to more definitively confirm the potential for reductive dechlorination of all chlorinated ethene compounds including vinyl chloride.

Perhaps most importantly, QuantArray®-Chlor quantifies TCE reductase (TCE) and both known vinyl chloride reductase genes (BVC, VCR) from *Dehalococcoides* to conclusively evaluate the potential for complete reductive dechlorination of chlorinated ethenes to non-toxic ethene [16–18]. In addition, the analysis also includes quantification of reductive dehalogenase genes from *Dehalogenimonas* spp. capable of reductive dechlorination of chlorinated ethenes. More specifically, these are the trans-1,2-DCE dehalogenase gene (TDR) from strain WBC-2 [19] and the vinyl chloride reductase gene (CER) from GP, the only known organisms other than *Dehalococcoides* capable of vinyl chloride reduction [20]. Finally, PCE reductase genes responsible for sequential reductive dechlorination of PCE to *cis*-DCE by *Sulfurospirillum* and *Geobacter* spp. are also quantified. In mixed cultures, evidence increasingly suggests that partial dechlorinators like *Sulfurospirillum* and *Geobacter* may be responsible for the majority of reductive dechlorination of PCE to TCE and *cis*-DCE while *Dehalococcoides* functions more as *cis*-DCE and vinyl chloride reducing specialists [10, 21].

Reductive Dechlorination - Chlorinated Ethanes: Under anaerobic conditions, chlorinated ethanes are susceptible to reductive dechlorination by several groups of halorespiring bacteria including *Dehalobacter*, *Dehalogenimonas*, and *Dehalococcoides*. While the reported range of chlorinated ethanes utilized varies by genus, species, and sometimes at the strain level, several general observations can be made regarding biodegradation pathways and daughter product formation. *Dehalobacter* spp. have been isolated that are capable of sequential reductive dechlorination of 1,1,1-TCA through 1,1-DCA to chloroethane [13]. Biodegradation of 1,1,2-TCA by several halorespiring bacteria including *Dehalobacter* and *Dehalogenimonas* spp. proceeds via dichloroelimination producing vinyl chloride [22–24]. Similarly, 1,2-DCA biodegradation by *Dehalobacter*, *Dehalogenimonas*, and *Dehalococcoides* occurs via dichloroelimination producing ethene. While not utilized by many *Desulfitobacterium* isolates, at least one strain, *Desulfitobacterium dichloroeliminans* strain DCA1, is also capable of dichloroelimination of 1,2-DCA [25]. The 1,2-dichloroethane reductive dehalogenase gene (DCAR) from members of *Desulfitobacterium* and *Dehalobacter* is known to dechlorinate 1,2-DCA to ethene, while the 1,1-dichloroethane reductive dehalogenase (DCA) targets the gene responsible for 1,1-DCA dechlorination in some strains of *Dehalobacter*. In addition to chloroform,chloroform reductase (CFR) has also been shown to be responsible for reductivedechlorination of 1,1,1-TCA [26].

Reductive Dechlorination - Chlorinated Methanes: Chloroform is a common co-contaminant at chlorinated solvent sites and can inhibit reductive dechlorination of chlorinated ethenes. Grostern et al. demonstrated that a *Dehalobacter* population was capable of reductive dechlorination of chloroform to produce dichloromethane [27]. The *cfrA* gene encodes the reductase which catalyzes this initial step in chloroform biodegradation [26]. Justicia-Leon et al. have since shown that dichloromethane can support growth of a distinct group of *Dehalobacter* strains via fermentation [28]. The *Dehalobacter* DCM assay targets the 16S rRNA gene of these strains.

Reductive Dechlorination - Chlorinated Benzenes: Chlorinated benzenes are an important class of industrial solvents and chemical intermediates in the production of drugs, dyes, herbicides, and insecticides. The physical-chemical properties of chlorinated benzenes as well as susceptibility to biodegradation are functions of their degree of chlorination and the positions of chlorine substituents. Under anaerobic conditions, reductive dechlorination of higher chlorinated benzenes including hexachlorobenzene (HCB),

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pentachlorobenzene (PeCB), tetrachlorobenzene (TeCB) isomers, and trichlorobenzene (TCB) isomers has been well documented [29], although biodegradation of individual compounds and isomers varies between isolates. For example, *Dehalococcoides* strain CBDB1 reductively dechlorinats HCB, PeCB, all three TeCB isomers, 1,2,3-TCB, and 1,2,4-TCB [9, 30]. *Dehalobium chlorocoercia* DF-1 has been shown to be capable of reductive dechlorination of HCB, PeCB, and 1,2,3,5-TeCB [31]. The dichlorobenzene (DCB) isomers and chlorobenzene (CB) were considered relatively recalcitrant under anaerobic conditions. However, new evidence has demonstrated reductive dechlorination of DCBs to CB and CB to benzene [32] with corresponding increases in concentrations of *Dehalobacter* spp. [33].

Reductive Dechlorination - Chlorinated Phenols: Pentachlorophenol (PCP) was one of the most widely used biocides in the U.S. and despite residential use restrictions, is still extensively used industrially as a wood preservative. Along with PCP, the tetrachlorophenol and trichlorophenol isomers were also used as fungicides in wood preserving formulations. 2,4-Dichlorophenol and 2,4,5-TCP were used as chemical intermediates in herbicide production (e.g. 2,4-D) and chlorophenols are known byproducts of chlorine bleaching in the pulp and paper industry. While the range of compounds utilized varies by strain, some *Dehalococcoides* isolates are capable of reductive dechlorination of PCP and other chlorinated phenols. For example, *Dehalococcoides* strain CBDB1 is capable of utilizing PCP, all three tetrachlorophenol (TeCP) congeners, all six trichlorophenol (TCP) congeners, and 2,3-dichlorophenol (2,3-DCP). PCP dechlorination by strain CBDB1 produces a mixture of 3,5-DCP, 3,4-DCP, 2,4-DCP, 3-CP, and 4-CP [34]. In the same study, however, *Dehalococcoides* strain 195 dechlorinated a more narrow spectrum of chlorophenols which included 2,3-DCP, 2,3,4-TCP, and 2,3,6-TCP, but no other TCPs or PCP. Similar to *Dehalococcoides*, some species and strains of *Desulfitobacterium* are capable of utilizing PCP and other chlorinated phenols. *Desulfitobacterium hafniense* PCP-1 is capable of reductive dechlorination of PCP to 3-CP [35]. However, the ability to biodegrade PCP is not universal among *Desulfitobacterium* isolates. *Desulfitobacterium* sp. strain PCE1 and *D. chlororespirans* strain Co23, for example, can utilize some TCP and DCP isomers, but not PCP for growth [2, 36].

Reductive Dechlorination - Chlorinated Propanes: *Dehalogenimonas* is a recently described bacterial genus of the phylum Chloroflexi which also includes the well-known chloroethene-respiring *Dehalococcoides* [23]. The *Dehalogenimonas* isolates characterized to date are also halorespiring bacteria, but utilize a rather unique range of chlorinated compounds as electron acceptors including chlorinated propanes (1,2,3-TCP and 1,2-DCP) and a variety of other vicinally chlorinated alkanes including 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, and 1,2-dichloroethane [23].

Aerobic - Chlorinated Ethene Cometabolism: Under aerobic conditions, several different types of bacteria including methane-oxidizing bacteria (methanotrophs), and many benzene, toluene, ethylbenzene, xylene, and (BTEX)-utilizing bacteria can cometabolize or co-oxidize TCE, DCE, and vinyl chloride [37]. In general, cometabolism of chlorinated ethenes is mediated by monooxygenase enzymes with "relaxed' specificity that oxidize a primary (growth supporting) substrate (*e.g.* methane) and co-oxidize the chlorinated compound (*e.g.*TCE). QuantArray®-Chlor provides quantification of a suite of genes encoding oxygenase enzymes capable of co-oxidation of chlorinated ethenes including soluble methane monooxygenase (sMMO). Soluble methane monooxygenases co-oxidize a broad range of chlorinated compounds [38–41] including TCE, *cis*-DCE, and vinyl chloride. Furthermore, soluble methane monooxygenases are generally believed to support greater rates of aerobic cometabolism [40]. QuantArray®-Chlor also quantifies aromatic oxygenase genes encoding ring hydroxylating toluene monooxygenase genes (RMO, RDEG), toluene dioxygenase (TOD) and phenol hydroxylases (PHE) capable of TCE co-oxidation [42–46]. TCE or a degradation product has been shown to induce expression of toluene monooxygenases in some laboratory studies [43, 47] raising the possibility of TCE cometabolism with an alternative (non-aromatic) growth substrate. Moreover, while a number of additional factors must be considered, recent research under ESTCP Project 201584 has shown positive correlations between concentrations of monooxygenase genes (soluble methane monooxygenase, ring hydroxylating monooxygenases, and phenol hydroxylase) and the rate of TCE degradation [48].

Aerobic - Chlorinated Ethane Cometabolism: While less widely studied than cometabolism of chlorinated ethenes, some chlorinated ethanes are also susceptible to co-oxidation. As mentioned previously, soluble methane monooxygenases (sMMO) exhibit very relaxed specificity. In laboratory studies, sMMO has been shown to co-oxidize a number of chlorinated ethanes including 1,1,1-TCA and 1,2-DCA [38, 40].

Aerobic - Vinyl Chloride Cometabolism: Beginning in the early 1990s, numerous microcosm studies demonstrated aerobic oxidation of vinyl chloride under MNA conditions without the addition of exogenous primary substrates. Since then, strains of

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Mycobacterium, *Nocardioides*, *Pseudomonas*, *Ochrobactrum*, and *Ralstonia* species have been isolated which are capable of aerobic growth on both ethene and vinyl chloride (see Mattes et al. [49] for a review). The initial steps in the pathway are the monooxygenase (*etn*ABCD) catalyzed conversion of ethene and vinyl chloride to their respective epoxyalkanes (epoxyethane and chlorooxirane), followed by epoxyalkane:CoM transferase (*etn*E) mediated conjugation and breaking of the epoxide [50].

Aerobic - Chlorinated Benzenes: In general, chlorobenzenes with four or less chlorine groups are susceptible to aerobic biodegradation and can serve as growth-supporting substrates. Toluene dioxygenase (TOD) has a relatively relaxed substrate specificity and mediates the incorporation of both atoms of oxygen into the aromatic ring of benzene and substituted benzenes (toluene and chlorobenzene). Comparison of TOD levels in background and source zone samples from a CB-impacted site suggested that CBs promoted growth of TOD-containing bacteria [51]. In addition, aerobic biodegradation of some trichlorobenzene and even tetrachlorobenzene isomers is initiated by a group of related trichlorobenzene dioxygenase genes (TCBO). Finally, phenol hydroxylases catalyze the continued oxidation and in some cases, the initial oxidation of a variety of monoaromatic compounds. In an independent study, significant increases in numbers of bacteria containing PHE genes corresponded to increases in biodegradation of DCB isomers [51].

Aerobic - Chlorinated Methanes: Many aerobic methylotrophic bacteria, belonging to diverse genera (*Hyphomicrobium*, *Methylobacterium*, *Methylophilus*, *Pseudomonas*, *Paracoccus*, and *Alibacter*) have been isolated which are capable of utilizing dichloromethane (DCM) as a growth substrate. The DCM metabolic pathway in methylotrophic bacteria is initiated by a dichloromethane dehalogenase (DCMA) gene. DCMA is responsible for aerobic biodegradation of dichloromethane by methylotrophs by first producing formaldehyde which is then further oxidized [52]. As discussed in previous sections, soluble methane monooxygenase (sMMO) exhibits relaxed specificity and co-oxidizes a broad spectrum of chlorinated hydrocarbons. In addition to chlorinated ethenes, sMMO has been shown to co-oxidize chloroform in laboratory studies [38, 41].

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