City of Bothell

April 9, 2018

Jerome Cruz, Ecology Site Manager
Department of Ecology,
Northwest Regional Office Toxic Cleanup Program
3190 160th Avenue SE
Bellevue, Washington 98008-5452

Re: May 10 - Quarterly Progress Report for period ending April 2018

Site Name:	**BOTHELL SERVICE CENTER/ SIMON & SON**
Site Address:	18107 Bothell Way NE, Bothell WA 98011
Parcel Numbers:	237420-0065
Facility/Site No.:	33215922
Consent Decree No.:	18-2-02852-3 SEA (Effective date February 2, 2018)

Date submitted:	April 9, 2018
Reporting Period:	February 1 – March 30, 2018

Summary:

City of Bothell (PLP) continues to make progress on work being performed for the Bothell Service Center site (BSC), in accordance with the Consent Decree with the Department of Ecology.

Per the requirements of Section XI of the Consent Decree "Progress Reports", the attached quarterly progress report has been prepared for the three-month period preceding this submittal, to satisfy the terms described in the Consent Decree.

During this period much of the work has been geared towards installing the ERH system.

The attached progress report provides an update on work accomplished during the period ending March 30, 2018 for the Site. Please contact me if you have any questions.

Sincerely,

Nduta Mbuthia

Public Works Department
18415 101st Ave NE
Bothell, WA 98011
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City of Bothell™

Reporting Period:	February 1 – March 30, 2018
Date submitted (electronically):	April 9, 2018
Date mailed (certified w/return receipt):	April 10, 2018
Prepared by:	Nduta Mbuthia, Project Coordinator City of Bothell, Public Works Department Phone: 425.806.6829. Email: nduta.mbuthia@bothellwa.gov

CONTENTS

A. A list of on-site activities that have taken place during this quarter

The following activities have occurred this quarter –

i. Installation of Electrical Resistance Heating electrodes completed March 27, 2018

B. Detailed description of any deviations from required tasks not otherwise documented in project plans or amendment requests

There have been no deviations this quarter

C. Description of all deviations from the CAP (Exhibit C) and Schedule (Exhibit D) during the current quarter and any planned deviations in the upcoming quarter

There have been no deviations this quarter

For any deviations in schedule, a plan for recovering lost time and maintaining compliance with the schedule

There have been no deviations this quarter

D. All raw data (including laboratory analyses) received by Defendants during the past quarter and an identification of the source of the sample

Biological data from selected groundwater wells was received from laboratory and results have been included in this April 10, 2018 quarterly report to Ecology.

E. A list of deliverables for the upcoming quarter if different from the schedule.

Same as the schedule

Project photos

Attachments

Documentation Compliance Letter – Kane Environmental
Biological data from selected groundwater wells

KANE ENVIRONMENTAL INC

SEATTLE ▪ PHOENIX

April 4, 2018

Mr. Jerome Cruz
Washington State Department of Ecology
Northwest Regional Office
3190 160th Ave SE
Bellevue, Washington 98008-5452

RE: Consent Decree Documentation

Mr. Cruz:

The purpose of this letter is to document the completed tasks to date and on-going tasks for the Bothell Service Center Simon & Sons Site.

FINANCIAL OBLIGATIONS	
Provide a cost estimate to Ecology for the implementation of the CD requirements, including operation, maintenance, and compliance monitoring.	Provided in Feasibility Study
PRE-CLEANUP OBLIGATIONS	
Notify Ecology of selected contractor name and qualifications	Completed
Submit written monthly Progress Reports	To be submitted April 10, 2018
Submit draft Pre-Remedial Design Project Plans (included in draft EDR report)	Completed
Submit draft PRDI Data Report and draft Engineering Design Report	Completed
Submit final PRDI Data Report and EDR	Completed
Submit 90% plans and specs	Completed
Submit 100% plans and specs	Completed
FIELD CONSTRUCTION	
Complete construction procurement	Completed
ERH System Installation	In-progress
ERH Operation	In-progress
Start install and begin operation of bioremediation-groundwater recirculation /SVE systems	In-progress
Install compliance well monitoring network	In-progress
Complete construction	In-progress

Sincerely,
KANE ENVIRONMENTAL, INC.

John Kane
CEO / President

cc: Nduta Mbuthia, City of Bothell

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KANE ENVIRONMENTAL INC

SEATTLE ▪ PHOENIX▪ SAN FRANCISCO

April 5, 2018

Mr. Jerome Cruz, Ph.D.
Toxics Cleanup Program, Northwest Regional Office
Washington State Department of Ecology
Northwest Regional Office
3190 160th Ave SE
Bellevue, Washington 98008-5452

RE: Baseline Microbial Groundwater Sampling Results

Mr. Cruz:

Groundwater samples were collected from eight groundwater monitoring wells (MW-31, MW-30, MW-5, MW-21, MW-35, MW-12, MW-11, and MW-8) on February 12, 2018. The groundwater samples were collected to aid in the evaluation of the types of bacteria present in the areas where future in situ bioremediation is going to be conducted. The groundwater samples were submitted to Microbial Insights, Inc. (MI) of Nashville, Tennessee, for analyses of quantitative polymerase chain reaction (qPCR) to identify the concentration of Dehalococcoides (DHC) and other relevant bacteria present in the three groundwater bearing zones (shallow, intermediate, and deep) where in situ anaerobic bioremediation is being proposed as the remedial approach. In addition to qPCR, MI analyzed each groundwater sample for key functional genes responsible for the reductive dechlorination process.

These eight groundwater samples represent baseline conditions in these zones prior to biostimulation (i.e. addition of substrate/nutrients). Without the presence of a readily available carbon/energy source (i.e. substrate), the indigenous bacterial population will be at low concentrations in the aquifer. In addition, areas that contain low concentrations of chlorinated solvents (i.e. electron acceptors) may inhibit certain microbial growth. Upon biostimulation, these bacterial communities will likely reach higher concentrations during active remediation. These laboratory results are being used to initially evaluate the type of microbial community that can be detected in these three zones, and if biostimulation alone will be sufficient in the remedial process. If biostimulation isn't effective, then bioaugmentation (i.e. addition of specific bacteria or consortiums) will be required during full scale remediation.

Each sampling/well location results are discussed herein as presented in the attached laboratory report from MI. These biological/microbial results are compared to the historical groundwater data from each location to present the correlating solvent concentrations and associated biodegradation analysis.
MW-31: MW-31 has a screened interval from 40 to 50 ft bgs, which is within the deep zone. The concentrations of chlorinated solvents in MW-31 are low level detections of PCE (8-11 µg/L) and TCE (<

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1 ug/L). There are no significant detections of TCE, cis-DCE, and/or VC at MW-31 that indicate any significant dechlorination is occurring in this area. This is likely due to the lack of a carbon/energy source in the deep zone, which is expected. The MI report (Table 1) presents the results obtained from MW-31. Despite the lack of any significant chlorinated solvent mass or carbon/energy source, this location has a low detection of DHC (4.0E+00 cell/mL) and low to moderate detections of other key anaerobic bacteria ranging from 1.25E+02 to 4.48E+03 cells/mL. In addition, there was a high detection of sulfate reducing bacteria (APS) at 7.02E+04 cells/mL, and a low detection of methanogens (MGN) at 9.90E+00 cells/mL. These anaerobic bacteria are also indications of a robust and healthy microbial consortium around MW-31.

MW-30: MW-30 has a screened interval from 9 to 19 ft bgs, which is within the shallow zone. The concentrations of chlorinated solvents in MW-30 are high detections of PCE (92,000-130,000 µg/L), and no significant detections of TCE (BDL). There was only one significant detection of cis-DCE (1,300 µg/L), and no detections of VC at MW-30, which indicates very limited dechlorination is occurring in this area. This is likely due to the lack of a carbon/energy source in this part of the shallow zone, which is expected. The MI report (Table 1) presents the results obtained from MW-30. This location has no detection of DHC (<2.50+00 cell/mL) and moderate detections of other key anaerobic bacteria ranging from 6.71E+01 to 5.79E+03 cells/mL. While PCE doesn't appear to be dechlorinating to any significant degree at MW-30, there was a low-level detection of PCE Reductase (PCE-1) at 2.02E+01 (J) indicating the presence of some PCE dechlorinating bacteria. In addition, there was a high detection of sulfate reducing bacteria (APS) at 7.81E+04 cells/mL, and no significant detection of methanogens (MGN) at <2.50E+01 cells/mL (note: higher detection limit than MW-31 and others). The detections of these reductive dechlorinating and other bacteria are indications of a robust and healthy microbial consortium around MW-30, despite the lack of any dechlorination occurring in this area. Without a soluble and biodegradable substrate, no significant anaerobic dechlorination will occur and these bacteria will remain at suppressed concentrations.

MW-5: MW-5 has a screened interval from 10 to 25 ft bgs, which is within the shallow zone. The concentrations of chlorinated solvents in MW-5 are high detections of PCE (590-21,000 µg/L), and significant detections of TCE (15-660 µg/L), cis-DCE (20-630 µg/L), and one low-level detection of VC (4/4/14 sampling event, which also shows of negative ORP), which indicates significant anaerobic dechlorination is occurring in this area. This is likely due to the presence of a carbon/energy source in this part of the shallow zone. The MI report (Table 1) presents the results obtained from MW-5. This location has a low-level detection of DHC (1.54+01 cell/mL) and moderate detections of other key anaerobic bacteria ranging from 2.37E+02 to 2.45E+03 cells/mL. There were also low-level detections of PCE Reductase 1 and 2 (PCE-1 and PCE-2) at 2.0E-01 to 9.0E-01 cells/mL indicating the presence of some PCE dechlorinating bacteria. In addition, there was a high detection of sulfate reducing bacteria (APS) at 2.09E+04 cells/mL, and no significant detection of methanogens (MGN) at <5.00E+00 cells/mL.

The detections of these reductive dechlorinating and other bacteria are indications of a robust and healthy microbial consortium around MW-5, which correlates strongly with the dechlorination occurring in this area. The addition of a soluble and biodegradable substrate in this area would likely speed up the anaerobic dechlorination process.

MW-21: MW-21 has a screened interval from 10 to 15 ft bgs, which is within the shallow zone. The concentrations of chlorinated solvents in MW-21 are high detections of PCE (8,400-27,000 µg/L), and significant detections of TCE (210-540 µg/L), cis-DCE (190-360 µg/L), but no detections of VC, which indicates significant anaerobic dechlorination is occurring in this area. This is likely due to the presence of a carbon/energy source in this part of the shallow zone. The MI report (Table 1) presents the results obtained from MW-21. Despite the groundwater data showing some anaerobic dechlorination is occurring, this location has no significant detections of DHC (<5.00E-01 cell/mL) and no detections of other key anaerobic bacteria. The only bacteria detected was total Eubacteria (EBAC) at 1.06E+03 cells/mL. The lack of any anaerobic bacteria indicates this part of the site is lacking any significant microbial consortium around MW-21. However, the groundwater data (which is more representative of the microbial community in the subsurface) shows anaerobic dechlorinating bacteria are present around MW-21. The contradiction in the microbial data is likely due to groundwater samples not being as accurate as Bio-Trap samples.

MW-35: MW-35 has a screened interval from 48 to 58 ft bgs, which is within the deep zone. The concentrations of chlorinated solvents in MW-35 are low level detections of PCE (1.4-2.1 µg/L). There have been no significant detections of TCE, cis-DCE, and/or VC at MW-35 that indicate any significant dechlorination is occurring in this area. This is likely due to the lack of a carbon/energy source in the deep zone, which is expected. The MI report (Table 1) presents the results obtained from MW-35. Despite the lack of any significant chlorinated solvent mass or carbon/energy source, this location has a low detection of DHC (3.00E-01 cell/mL) and low to moderate detections of other key anaerobic bacteria ranging from 1.78E+01 to 1.85E+03 cells/mL. In addition, there was a moderate detection of sulfate reducing bacteria (APS) at 7.07E+03 cells/mL, and a low detection of methanogens (MGN) at 3.54E+01 cells/mL. These anaerobic bacteria are also indications of a robust and healthy microbial consortium around MW-35.

MW-12: MW-12 has a screened interval from 25 to 33 ft bgs, which is within the intermediate zone. The concentrations of chlorinated solvents in MW-12 are high detections of PCE (700-5,900 µg/L), and significant detections of TCE (5.1-390 µg/L), cis-DCE (29-1,600 µg/L), and two low-level detections of VC (3/20/13 and 4/4/14 sampling events, which also shows of negative ORPs), which indicates significant anaerobic dechlorination is occurring in this area. This is likely due to the presence of a carbon/energy source in this part of the intermediate zone. The MI report (Table 1) presents the results obtained from MW-12. This location has a low-level detection of DHC (8.06+01 cell/mL) and moderate detections of

other key anaerobic bacteria ranging from 2.38E+03 to 2.02E+04 cells/mL. There were also low-level detections of PCE Reductase 1 and 2 (PCE-1 and PCE-2) at 3.00E-01 to 6.69E+02 cells/mL indicating the presence of some PCE dechlorinating bacteria. In addition, there was a high detection of sulfate reducing bacteria (APS) at 1.39+04 cells/mL, and a significant detection of methanogens (MGN) at 5.37E+01 cells/mL. The detections of these reductive dechlorinating and other bacteria are indications of a robust and healthy microbial consortium around MW-12, which correlates strongly with the dechlorination occurring in this area. The addition of a soluble and biodegradable substrate in this area would likely speed up the anaerobic dechlorination process.

MW-11: MW-11 has a screened interval from 25 to 33 ft bgs, which is within the intermediate zone. The concentrations of chlorinated solvents in MW-11 are low detections of PCE (2.0-27 µg/L), and low-level detections of TCE (0.18-0.53 µg/L), cis-DCE (0.26-0.42 µg/L), and no detections of VC, which indicates very limited anaerobic dechlorination is occurring in this area. This is likely due to the lack of a carbon/energy source in this part of the intermediate zone. The MI report (Table 1) presents the results obtained from MW-11. This location has a low-level detection of DHC (6.20E+00 cell/mL) and moderate to high detections of other key anaerobic bacteria ranging from 4.38E+03 to 1.82E+04 cells/mL. In addition, there was a high detection of sulfate reducing bacteria (APS) at 7.14+04 cells/mL, and a moderate detection of methanogens (MGN) at 1.32E+03 cells/mL. The detections of these reductive dechlorinating and other bacteria are indications of the presence of a good microbial consortium around MW-11. If significant chlorinated solvent mass was present in this area, then more TCE, cis-DCE, and VC would be detected. The addition of a soluble and biodegradable substrate in this area would likely speed up the anaerobic dechlorination process of this low-level solvent mass in this area.

MW-8: MW-8 has a screened interval from 45 to 50 ft bgs, which is within the deep zone. The concentrations of chlorinated solvents in MW-8 are low detections of PCE (.0.44-180 µg/L), and low-level detections of TCE (0.98-50 µg/L), cis-DCE (0.88-160 µg/L), and one low-level detection of VC (10/11/14 sampling event), which indicates some low-level anaerobic dechlorination is occurring in this area. This is likely due to the presence of a carbon/energy source in this part of the deep zone. The MI report (Table 1) presents the results obtained from MW-8. This location has a low-level detection of DHC (3.00E-01 cell/mL) and low to moderate detections of other key anaerobic bacteria ranging from 3.26E+01 to 1.06E+03 cells/mL. There was also a low-level detection of PCE Reductase 2 (PCE-2) at 1.50E+00 cells/mL indicating the presence of some PCE dechlorinating bacteria. In addition, there was a high detection of sulfate reducing bacteria (APS) at 1.02+04 cells/mL, but no detection of methanogens (MGN) at <5.00E+00 cells/mL. The detections of these reductive dechlorinating and other bacteria are indications of the presence of a microbial consortium around MW-8, which correlates strongly with the dechlorination occurring in this area. The addition of a soluble and biodegradable substrate in this area would likely speed up the anaerobic dechlorination process.

Overall, the majority of these sampling locations show the presence of DHC and many other bacteria capable of anaerobic dechlorination. These detections of these bacteria in all three zones indicate that biostimulation will be successful, and that bioaugmentation will not be required at this site. In addition, the historical groundwater data collected from MW-2 and MW-6 onsite, show when a substrate is added to this aquifer, robust and complete anaerobic reductive dechlorination of PCE, TCE, etc. occurs at this site.

Sincerely,
KANE ENVIRONMENTAL, INC.

John Kane
CEO / President

cc: Nduta Mbuthia, City of Bothell

ATTACHMENTS:
QuantArray Results
Chain of Custody
How to Use Estimated Percentile Ranks from the Microbial Insights Database
How to Retrieve and Use Estimated Percentile Ranks from the Microbial Insights Database

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SITE LOGIC Report

QuantArray®-Chlor Study

Contact: John Kane
Phone: 206-691-0476

Address: Kane Environmental, Inc.
3815 Woodland Park Ave. N.
Suite 102
Seattle, WA 98103

Email: jkane@kane-environmental.com

MI Identifier: 027PB

Report Date: 02/26/2018

Project: BSCSS Task 9.2, 82302-Task 9.2
Comments:

NOTICE: This report is intended only for the addressee shown above and may contain confidential or privileged information. If the recipient of this material is not the intended recipient or if you have received this in error, please notify Microbial Insights, Inc. immediately. The data and other information in this report represent only the sample(s) analyzed and are rendered upon condition that it is not to be reproduced without approval from Microbial Insights, Inc. Thank you for your cooperation.

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 MW-31, MW-30, MW-5, and MW-21.

Sample Name Sample Date	MW-31 02/12/2018	MW-30 02/12/2018	MW-5 02/13/2018	MW-21 02/13/2018
Reductive Dechlorination	cells/mL	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**4.00E+00**	<2.50E+00	**1.54E+01**	<5.00E-01
tceA Reductase (TCE)	<5.00E-01	<2.50E+00	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<2.50E+00	**4.10E+00**	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	<2.50E+00	**6.00E-01**	<5.00E-01
Dehalobacter spp. (DHBt)	**3.05E+03**	**1.92E+03**	<5.00E+00	<5.00E+00
Dehalobacter DCM (DCM)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Dehalogenimonas spp. (DHG)	**4.48E+03**	**5.79E+03**	**2.42E+03**	<5.00E+00
Desulfitobacterium spp. (DSB)	**2.70E+03**	<2.50E+01	**2.45E+03**	<5.00E+00
Dehalobium chlorocoercia (DECO)	**2.59E+03**	**1.47E+03**	**9.40E+02**	<5.00E+00
Desulfuromonas spp. (DSM)	**1.25E+02**	**6.71E+01**	**2.37E+02**	<5.00E+00
PCE Reductase (PCE-1)	<5.00E+00	**2.02E+01 (J)**	**2.00E-01 (J)**	<5.00E+00
PCE Reductase (PCE-2)	<5.00E+00	<2.50E+01	**9.00E-01 (J)**	<5.00E+00
Vinyl Chloride Reductase (CER)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
trans-1,2-DCE Reductase (TDR)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Chloroform Reductase (CFR)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
1,1 DCA Reductase (DCA)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
1,2 DCA Reductase (DCAR)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Aerobic (Co)Metabolic				
Soluble Methane Monooxygenase (SMMO)	**2.13E+02**	<2.50E+01	**6.06E+02**	<5.00E+00
Toluene Dioxygenase (TOD)	**5.27E+02**	**2.01E+03**	<5.00E+00	<5.00E+00
Phenol Hydroxylase (PHE)	**1.93E+02**	**7.36E+02**	**8.97E+01**	<5.00E+00
Trichlorobenzene Dioxygenase (TCBO)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Toluene Monooxygenase 2 (RDEG)	**2.04E+02**	**2.17E+03**	<5.00E+00	<5.00E+00
Toluene Monooxygenase (RMO)	**1.48E+02**	<2.50E+01	<5.00E+00	<5.00E+00
Ethene Monooxygenase (EtnC)	**7.76E+01**	<2.50E+01	**2.69E+01**	<5.00E+00
Epoxyalkane Transferase (EtnE)	<5.00E+00	<2.50E+01	**9.13E+01**	<5.00E+00
Dichloromethane Dehalogenase (DCMA)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Other				
Total Eubacteria (EBAC)	**3.38E+05**	**3.03E+06**	**1.37E+06**	**1.06E+03**
Sulfate Reducing Bacteria (APS)	**7.02E+04**	**7.81E+04**	**2.09E+04**	<5.00E+00
Methanogens (MGN)	**9.90E+00**	<2.50E+01	<5.00E+00	<5.00E+00

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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Table 2: Summary of the QuantArray®-Chlor results obtained for samples MW-35, MW-12, MW-11, and MW-8.

Sample Name Sample Date	MW-35 02/13/2018	MW-12 02/14/2018	MW-11 02/14/2018	MW-8 02/14/2018
Reductive Dechlorination	cells/mL	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**3.00E-01 (J)**	**8.06E+01**	**6.20E+00**	**3.00E-01 (J)**
tceA Reductase (TCE)	<5.00E-01	<5.00E-01	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<5.00E-01	<5.00E-01	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	**2.50E+00**	<5.00E-01	<5.00E-01
Dehalobacter spp. (DHBt)	**1.85E+03**	<5.00E+00	<5.00E+00	**1.06E+03**
Dehalobacter DCM (DCM)	**1.78E+01**	<5.00E+00	<5.00E+00	<5.00E+00
Dehalogenimonas spp. (DHG)	<5.00E+00	**2.02E+04**	**1.80E+04**	**1.99E+03**
Desulfitobacterium spp. (DSB)	**9.90E+02**	**2.38E+03**	**1.82E+04**	**1.75E+02**
Dehalobium chlorocoercia (DECO)	**5.01E+02**	**2.68E+03**	**1.18E+04**	**3.78E+02**
Desulfuromonas spp. (DSM)	**1.56E+02**	<5.00E+00	**4.38E+03**	**3.26E+01**
PCE Reductase (PCE-1)	<5.00E+00	**3.00E-01 (J)**	<5.00E+00	<5.00E+00
PCE Reductase (PCE-2)	<5.00E+00	**6.69E+02**	<5.00E+00	**1.50E+00 (J)**
Vinyl Chloride Reductase (CER)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
trans-1,2-DCE Reductase (TDR)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
Chloroform Reductase (CFR)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
1,1 DCA Reductase (DCA)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
1,2 DCA Reductase (DCAR)	<5.00E+00	**9.16E+01**	<5.00E+00	<5.00E+00
Aerobic (Co)Metabolic				
Soluble Methane Monooxygenase (SMMO)	**2.96E+01**	**3.86E+03**	**1.90E+00 (J)**	**7.90E+00**
Toluene Dioxygenase (TOD)	**9.33E+01**	**3.65E+03**	<5.00E+00	**3.44E+02**
Phenol Hydroxylase (PHE)	**3.60E+02**	**4.03E+02**	**2.73E+02**	**3.44E+02**
Trichlorobenzene Dioxygenase (TCBO)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
Toluene Monooxygenase 2 (RDEG)	**3.71E+02**	**7.33E+02**	**1.79E+03**	**1.81E+03**
Toluene Monooxygenase (RMO)	**2.16E+02**	**2.50E+03**	**1.25E+02**	**2.22E+01**
Ethene Monooxygenase (EtnC)	<5.00E+00	<5.00E+00	**1.09E+01**	**6.84E+01**
Epoxyalkane Transferase (EtnE)	**2.65E+01**	**1.12E+02**	**1.44E+02**	**4.29E+02**
Dichloromethane Dehalogenase (DCMA)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
Other				
Total Eubacteria (EBAC)	**1.53E+05**	**6.24E+05**	**1.21E+06**	**1.95E+05**
Sulfate Reducing Bacteria (APS)	**7.07E+03**	**1.39E+04**	**7.14E+04**	**1.02E+04**
Methanogens (MGN)	**3.54E+01**	**5.37E+01**	**1.32E+03**	<5.00E+00

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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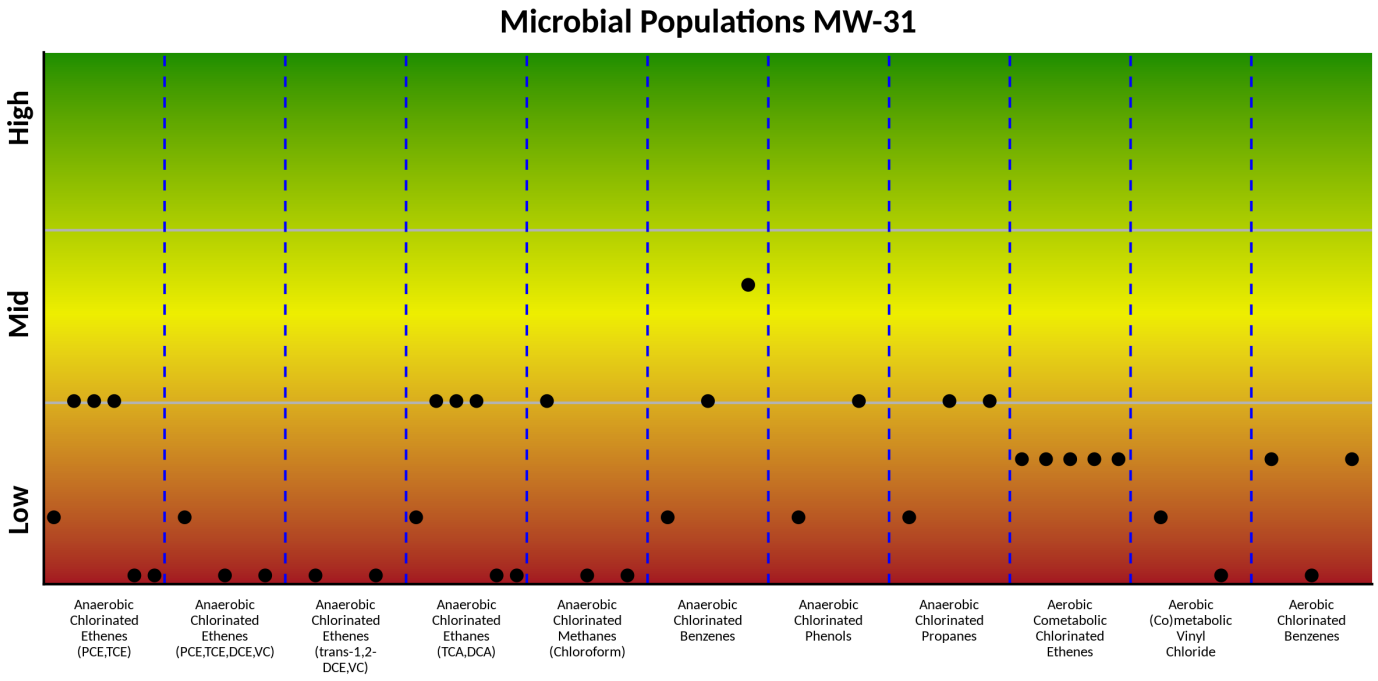

Figure 1: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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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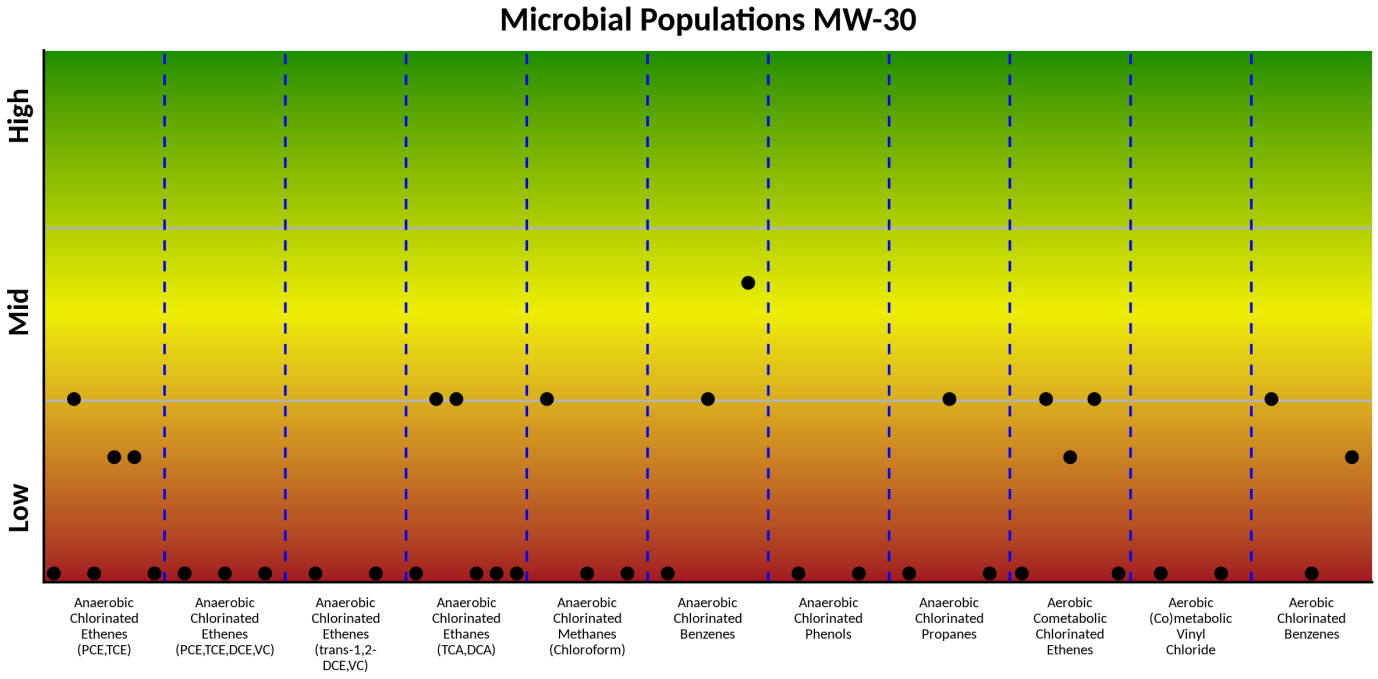

Figure 2: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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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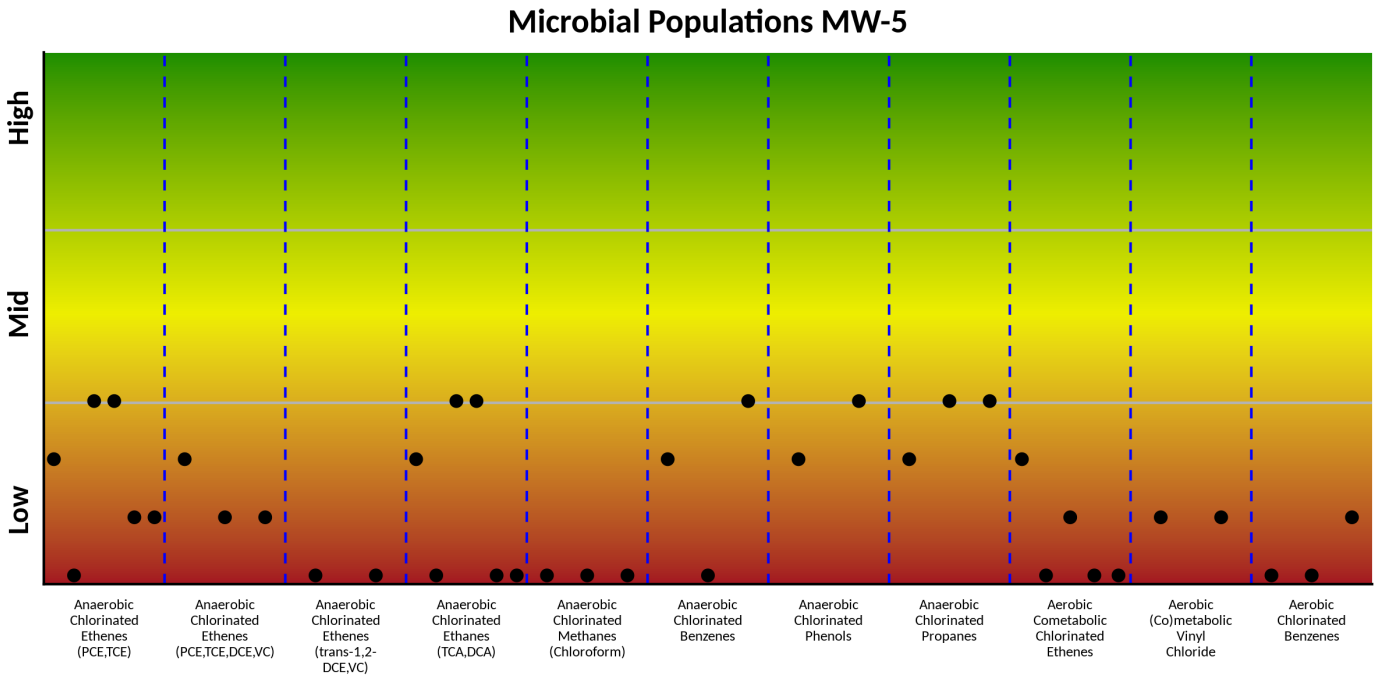

Figure 3: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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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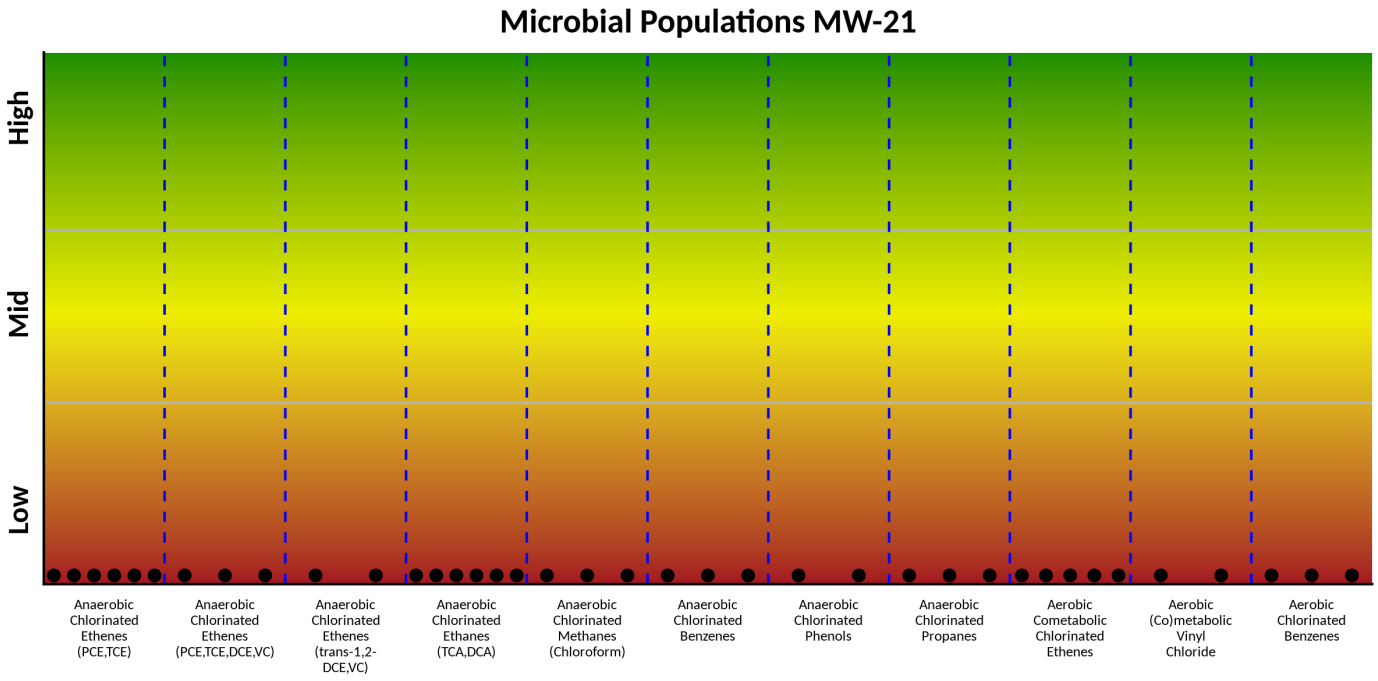

Figure 4: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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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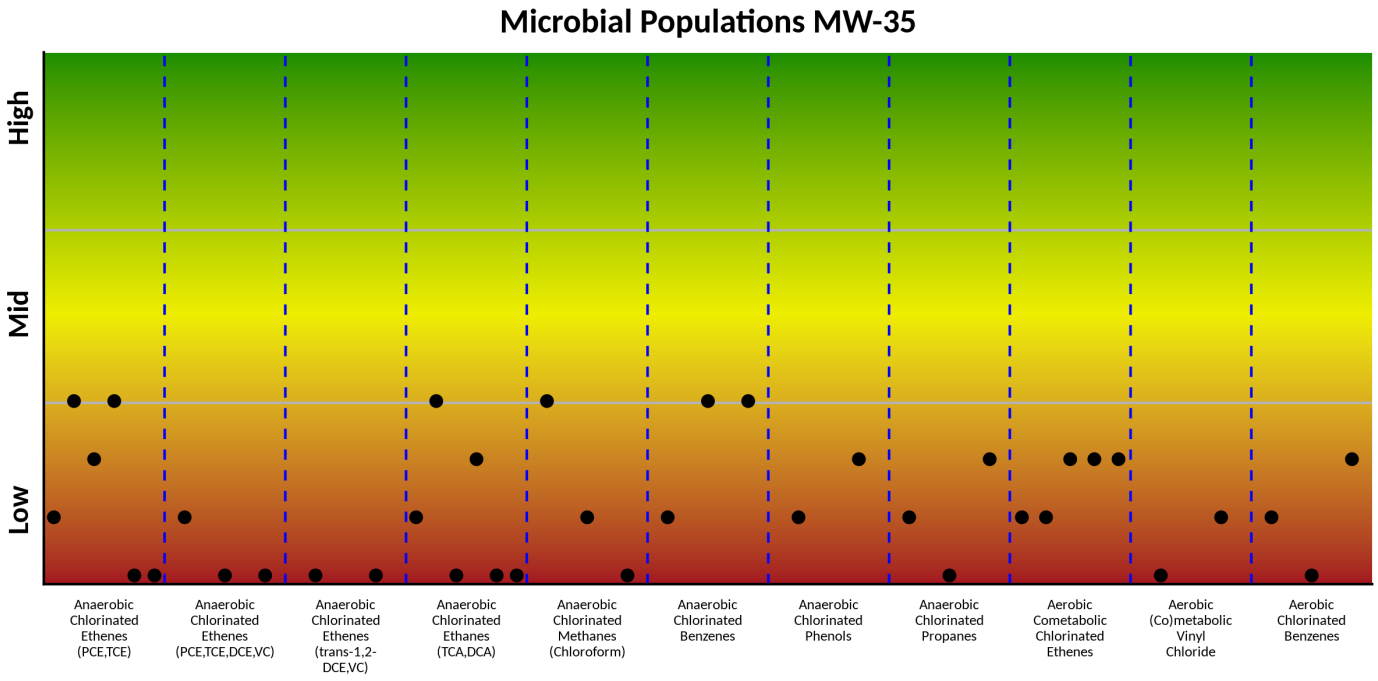

Figure 5: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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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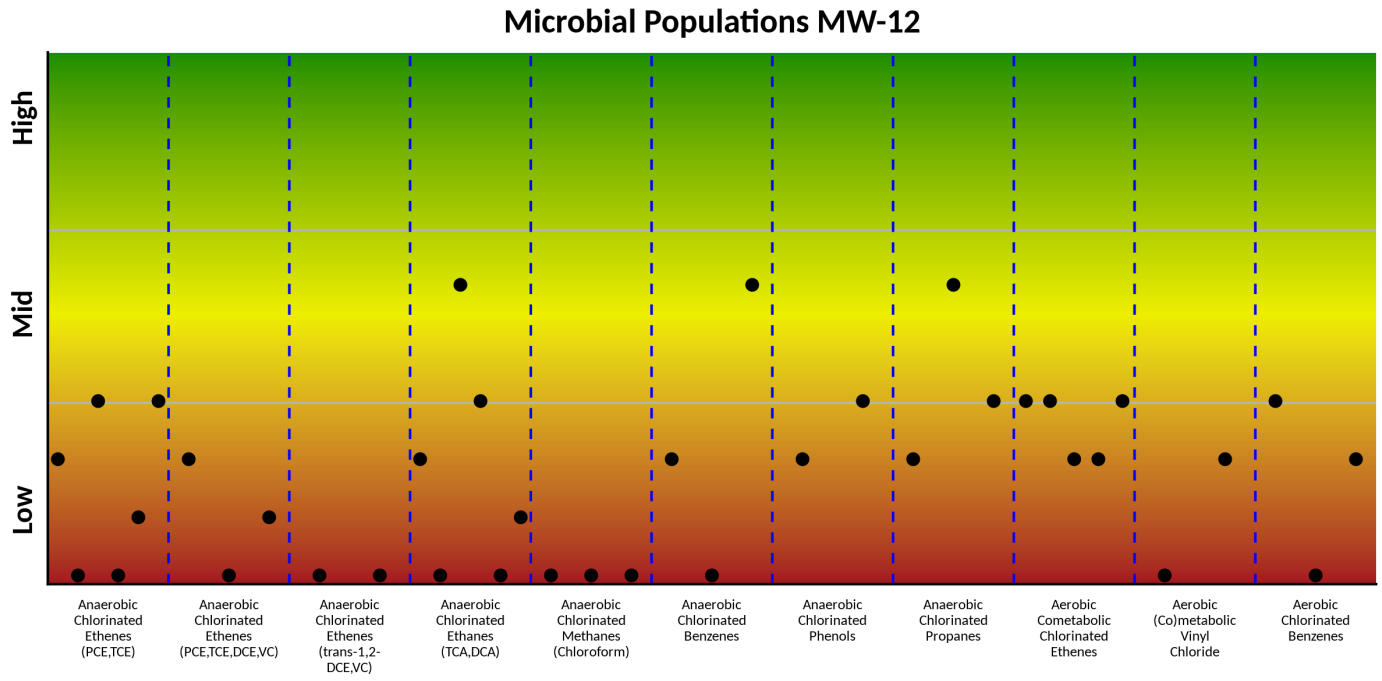

Figure 6: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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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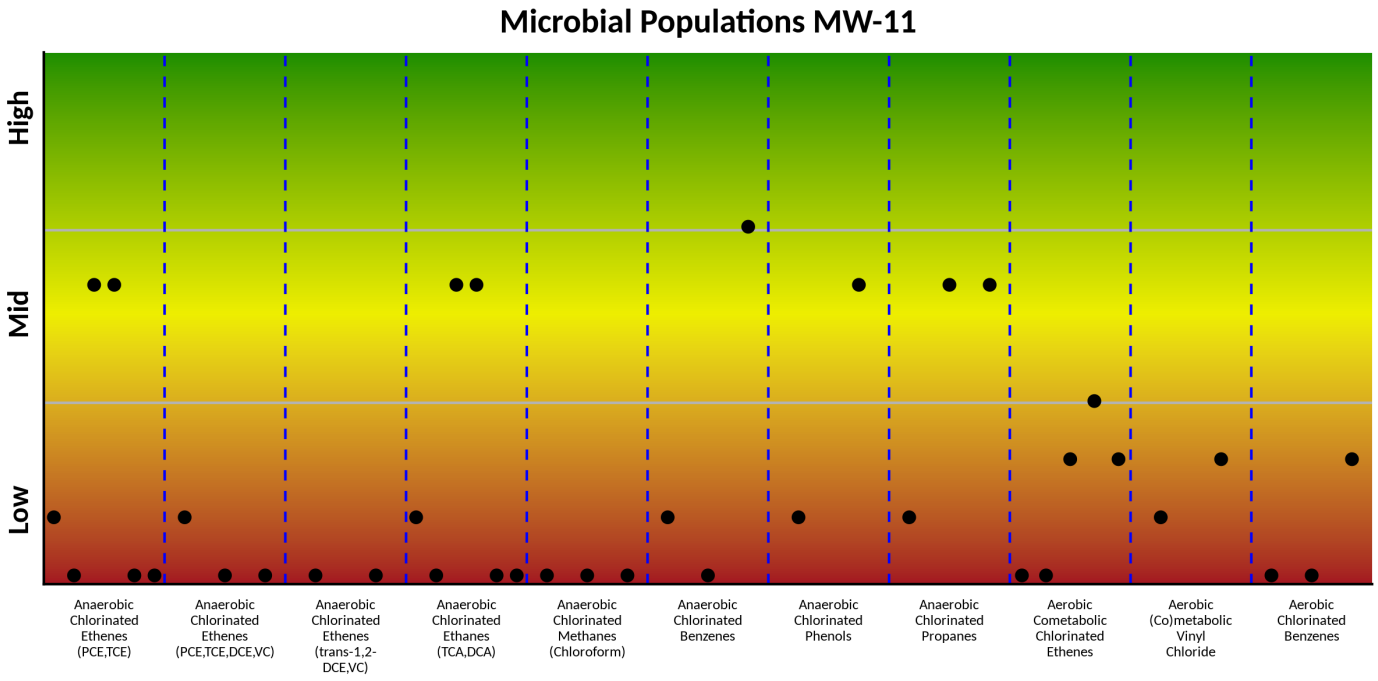

Figure 7: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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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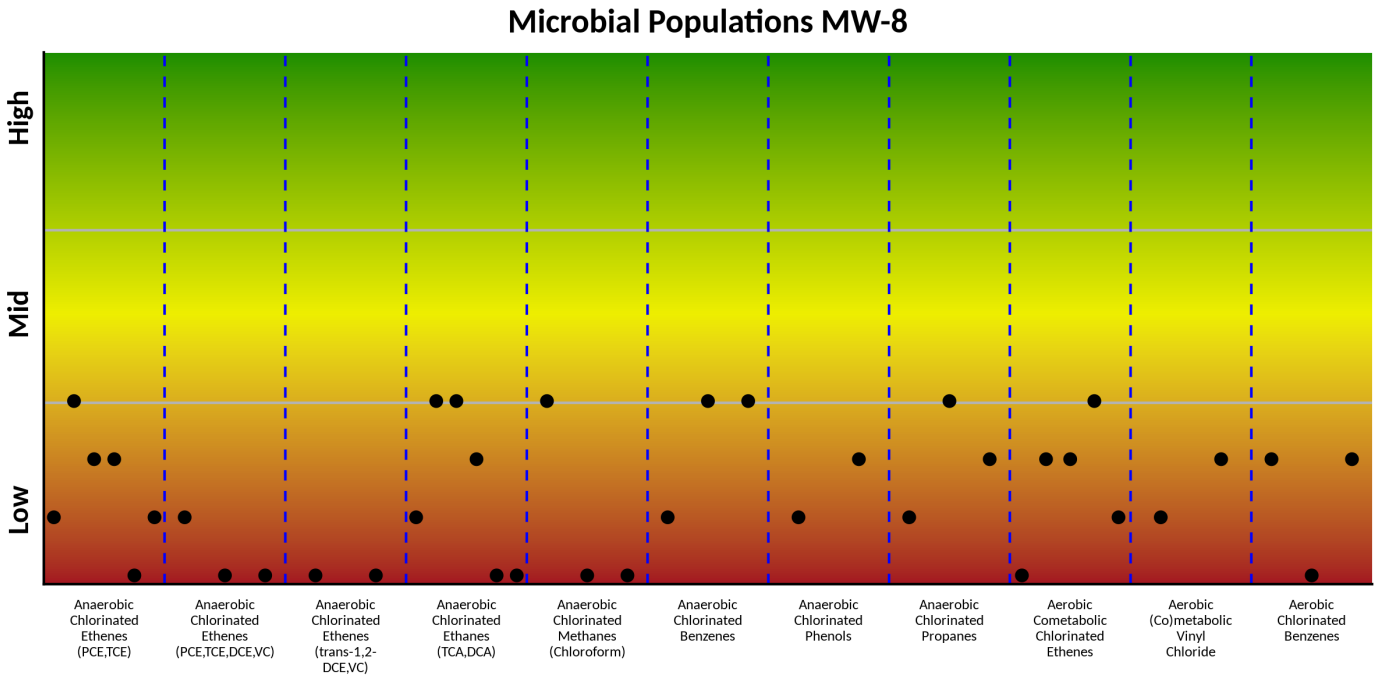

Figure 8: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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 3: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples MW-31, MW-30, MW-5, and MW-21.

Sample Name Sample Date	MW-31 02/12/2018	MW-30 02/12/2018	MW-5 02/13/2018	MW-21 02/13/2018
Reductive Dechlorination	cells/mL	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**4.00E+00**	<2.50E+00	**1.54E+01**	<5.00E-01
tceA Reductase (TCE)	<5.00E-01	<2.50E+00	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<2.50E+00	**4.10E+00**	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	<2.50E+00	**6.00E-01**	<5.00E-01
Dehalobacter spp. (DHBt)	**3.05E+03**	**1.92E+03**	<5.00E+00	<5.00E+00
Dehalobacter DCM (DCM)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Dehalogenimonas spp. (DHG)	**4.48E+03**	**5.79E+03**	**2.42E+03**	<5.00E+00
Desulfitobacterium spp. (DSB)	**2.70E+03**	<2.50E+01	**2.45E+03**	<5.00E+00
Dehalobium chlorocoercia (DECO)	**2.59E+03**	**1.47E+03**	**9.40E+02**	<5.00E+00
Desulfuromonas spp. (DSM)	**1.25E+02**	**6.71E+01**	**2.37E+02**	<5.00E+00

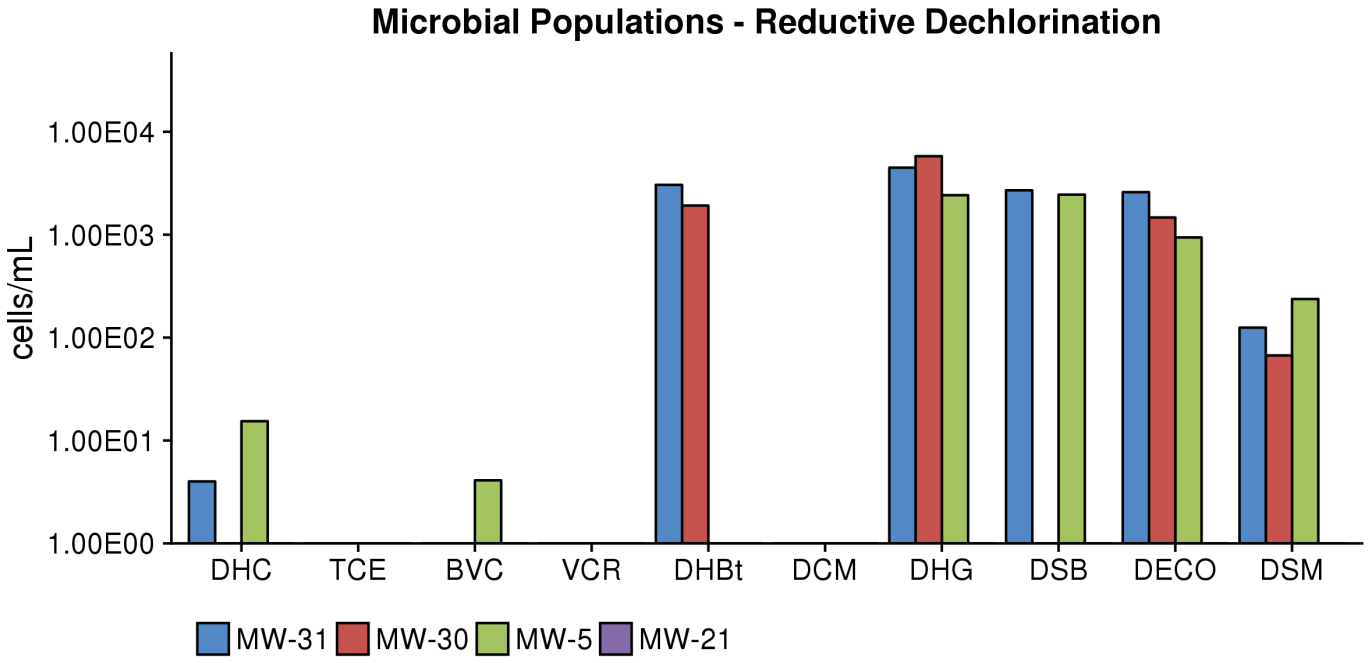

Figure 9: Comparison - microbial populations involved in reductive dechlorination.

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Table 4: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples MW-31, MW-30, MW-5, and MW-21.

Sample Name Sample Date	MW-31 02/12/2018	MW-30 02/12/2018	MW-5 02/13/2018	MW-21 02/13/2018
Reductive Dechlorination	cells/mL	cells/mL	cells/mL	cells/mL
Chloroform Reductase (CFR)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
1,1 DCA Reductase (DCA)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
1,2 DCA Reductase (DCAR)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
PCE Reductase (PCE-1)	<5.00E+00	**2.02E+01 (J)**	**2.00E-01 (J)**	<5.00E+00
PCE Reductase (PCE-2)	<5.00E+00	<2.50E+01	**9.00E-01 (J)**	<5.00E+00
trans-1,2-DCE Reductase (TDR)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Vinyl Chloride Reductase (CER)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00

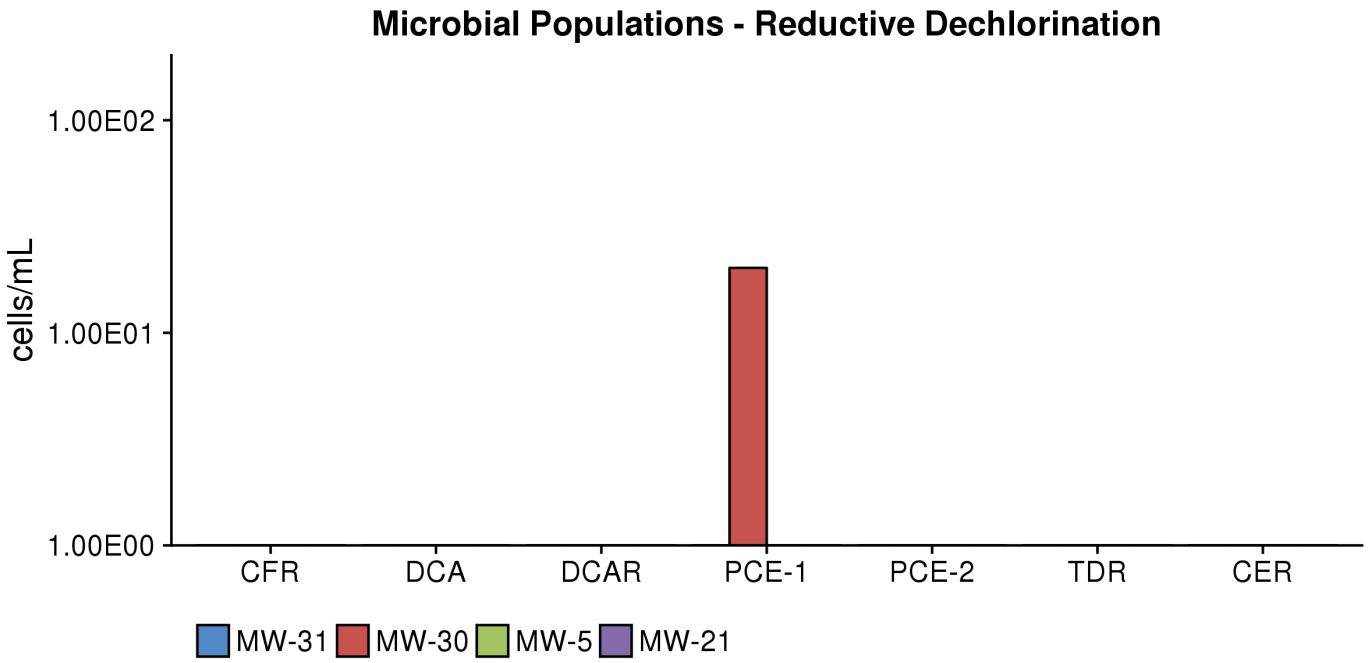

Figure 10: Comparison - microbial populations involved in reductive dechlorination.

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Table 5: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples MW-35, MW-12, MW-11, and MW-8.

Sample Name Sample Date	MW-35 02/13/2018	MW-12 02/14/2018	MW-11 02/14/2018	MW-8 02/14/2018
Reductive Dechlorination	cells/mL	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**3.00E-01 (J)**	**8.06E+01**	**6.20E+00**	**3.00E-01 (J)**
tceA Reductase (TCE)	<5.00E-01	<5.00E-01	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<5.00E-01	<5.00E-01	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	**2.50E+00**	<5.00E-01	<5.00E-01
Dehalobacter spp. (DHBt)	**1.85E+03**	<5.00E+00	<5.00E+00	**1.06E+03**
Dehalobacter DCM (DCM)	**1.78E+01**	<5.00E+00	<5.00E+00	<5.00E+00
Dehalogenimonas spp. (DHG)	<5.00E+00	**2.02E+04**	**1.80E+04**	**1.99E+03**
Desulfitobacterium spp. (DSB)	**9.90E+02**	**2.38E+03**	**1.82E+04**	**1.75E+02**
Dehalobium chlorocoercia (DECO)	**5.01E+02**	**2.68E+03**	**1.18E+04**	**3.78E+02**
Desulfuromonas spp. (DSM)	**1.56E+02**	<5.00E+00	**4.38E+03**	**3.26E+01**

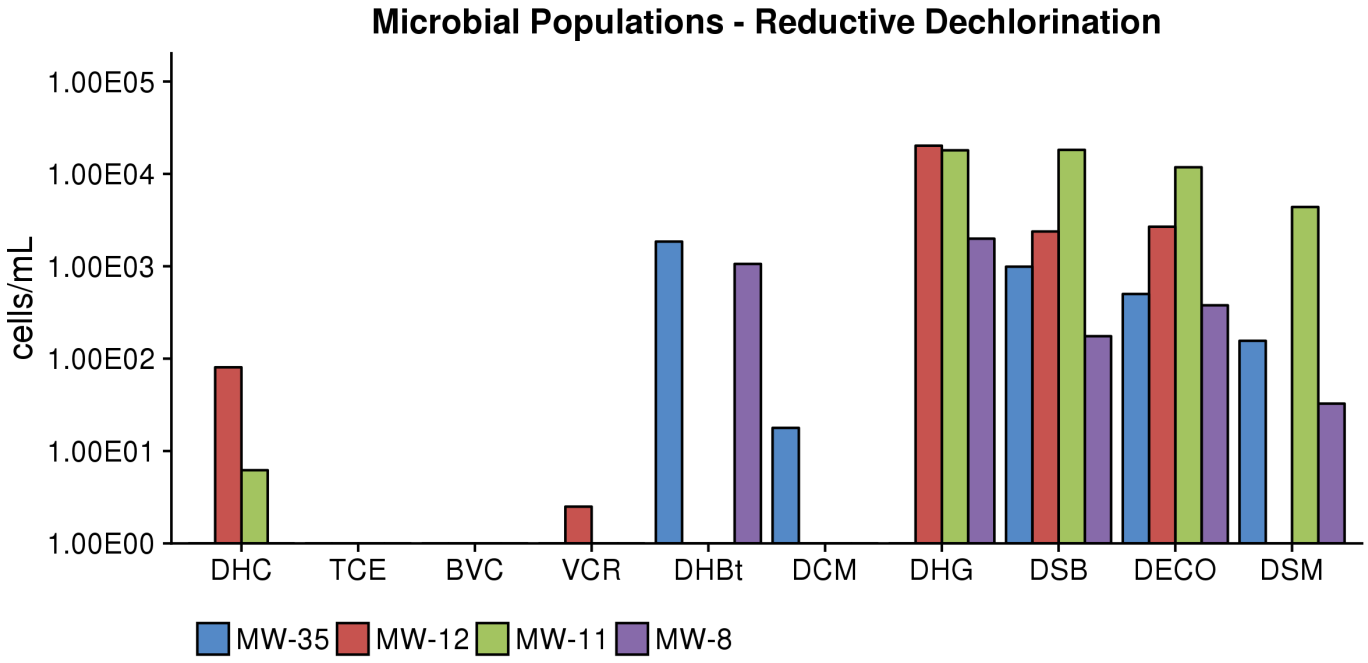

Figure 11: Comparison - microbial populations involved in reductive dechlorination.

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Table 6: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples MW-35, MW-12, MW-11, and MW-8.

Sample Name Sample Date	MW-35 02/13/2018	MW-12 02/14/2018	MW-11 02/14/2018	MW-8 02/14/2018
Reductive Dechlorination	cells/mL	cells/mL	cells/mL	cells/mL
Chloroform Reductase (CFR)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
1,1 DCA Reductase (DCA)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
1,2 DCA Reductase (DCAR)	<5.00E+00	**9.16E+01**	<5.00E+00	<5.00E+00
PCE Reductase (PCE-1)	<5.00E+00	**3.00E-01 (J)**	<5.00E+00	<5.00E+00
PCE Reductase (PCE-2)	<5.00E+00	**6.69E+02**	<5.00E+00	**1.50E+00 (J)**
trans-1,2-DCE Reductase (TDR)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
Vinyl Chloride Reductase (CER)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00

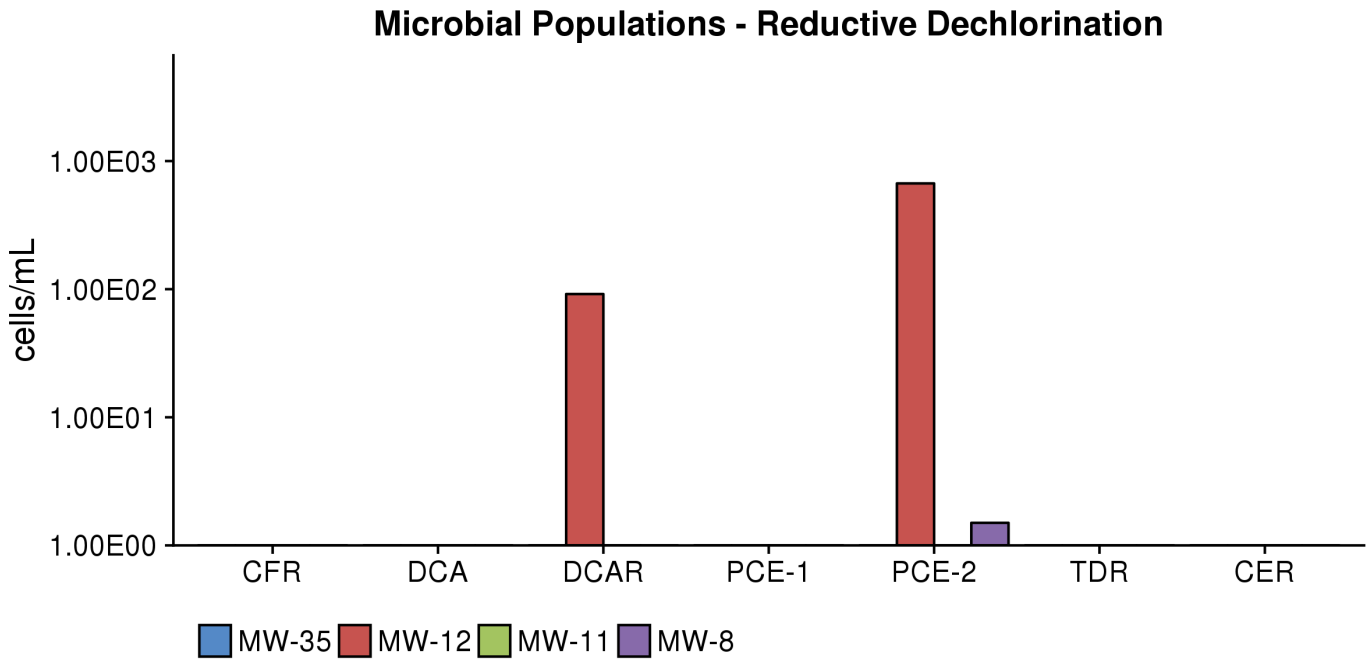

Figure 12: Comparison - microbial populations involved in reductive dechlorination.

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Table 7: Summary of the QuantArray®-Chlor results for microorganisms responsible for aerobic (co)metabolism for samples MW-31, MW-30, MW-5, and MW-21.

Sample Name Sample Date	MW-31 02/12/2018	MW-30 02/12/2018	MW-5 02/13/2018	MW-21 02/13/2018
Aerobic (Co)Metabolic	cells/mL	cells/mL	cells/mL	cells/mL
Soluble Methane Monooxygenase (SMMO)	**2.13E+02**	<2.50E+01	**6.06E+02**	<5.00E+00
Toluene Dioxygenase (TOD)	**5.27E+02**	**2.01E+03**	<5.00E+00	<5.00E+00
Phenol Hydroxylase (PHE)	**1.93E+02**	**7.36E+02**	**8.97E+01**	<5.00E+00
Trichlorobenzene Dioxygenase (TCBO)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00
Toluene Monooxygenase 2 (RDEG)	**2.04E+02**	**2.17E+03**	<5.00E+00	<5.00E+00
Toluene Monooxygenase (RMO)	**1.48E+02**	<2.50E+01	<5.00E+00	<5.00E+00
Ethene Monooxygenase (EtnC)	**7.76E+01**	<2.50E+01	**2.69E+01**	<5.00E+00
Epoxyalkane Transferase (EtnE)	<5.00E+00	<2.50E+01	**9.13E+01**	<5.00E+00
Dichloromethane Dehalogenase (DCMA)	<5.00E+00	<2.50E+01	<5.00E+00	<5.00E+00

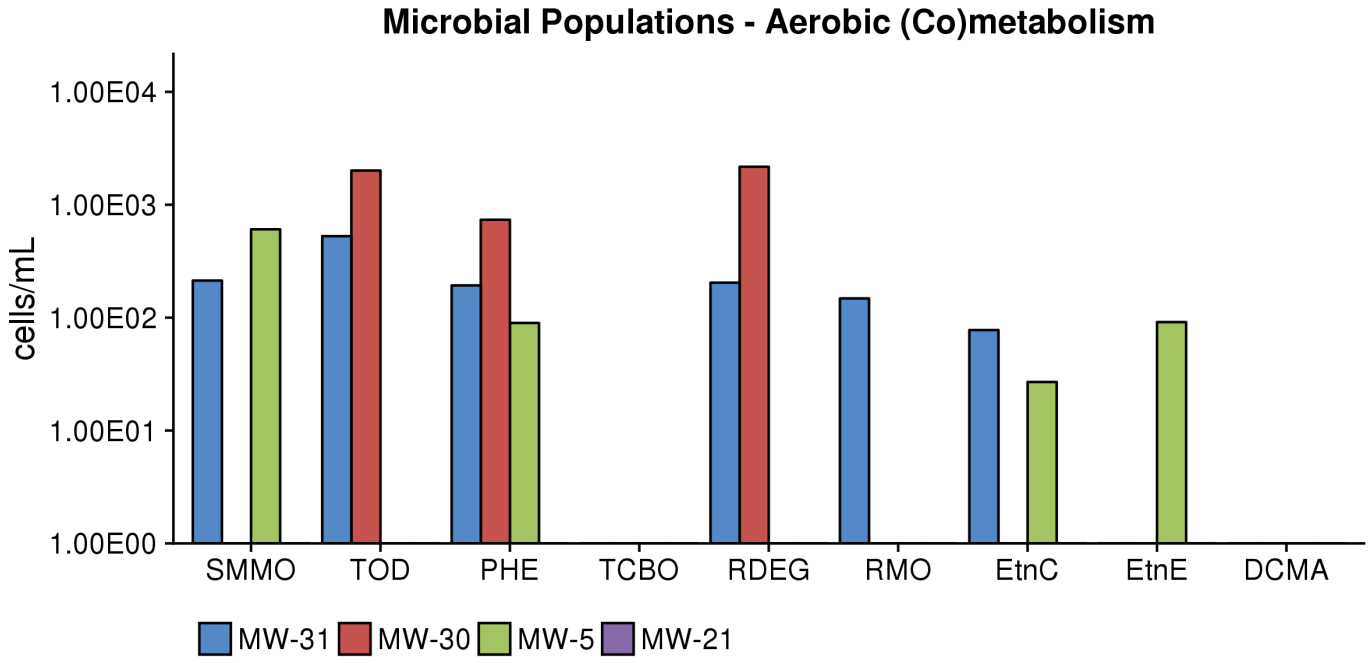

Figure 13: Comparison - microbial populations involved in aerobic (co)metabolism.

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Table 8: Summary of the QuantArray®-Chlor results for microorganisms responsible for aerobic (co)metabolism for samples MW-35, MW-12, MW-11, and MW-8.

Sample Name Sample Date	MW-35 02/13/2018	MW-12 02/14/2018	MW-11 02/14/2018	MW-8 02/14/2018
Aerobic (Co)Metabolic	cells/mL	cells/mL	cells/mL	cells/mL
Soluble Methane Monooxygenase (SMMO)	**2.96E+01**	**3.86E+03**	**1.90E+00 (J)**	**7.90E+00**
Toluene Dioxygenase (TOD)	**9.33E+01**	**3.65E+03**	<5.00E+00	**3.44E+02**
Phenol Hydroxylase (PHE)	**3.60E+02**	**4.03E+02**	**2.73E+02**	**3.44E+02**
Trichlorobenzene Dioxygenase (TCBO)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00
Toluene Monooxygenase 2 (RDEG)	**3.71E+02**	**7.33E+02**	**1.79E+03**	**1.81E+03**
Toluene Monooxygenase (RMO)	**2.16E+02**	**2.50E+03**	**1.25E+02**	**2.22E+01**
Ethene Monooxygenase (EtnC)	<5.00E+00	<5.00E+00	**1.09E+01**	**6.84E+01**
Epoxyalkane Transferase (EtnE)	**2.65E+01**	**1.12E+02**	**1.44E+02**	**4.29E+02**
Dichloromethane Dehalogenase (DCMA)	<5.00E+00	<5.00E+00	<5.00E+00	<5.00E+00

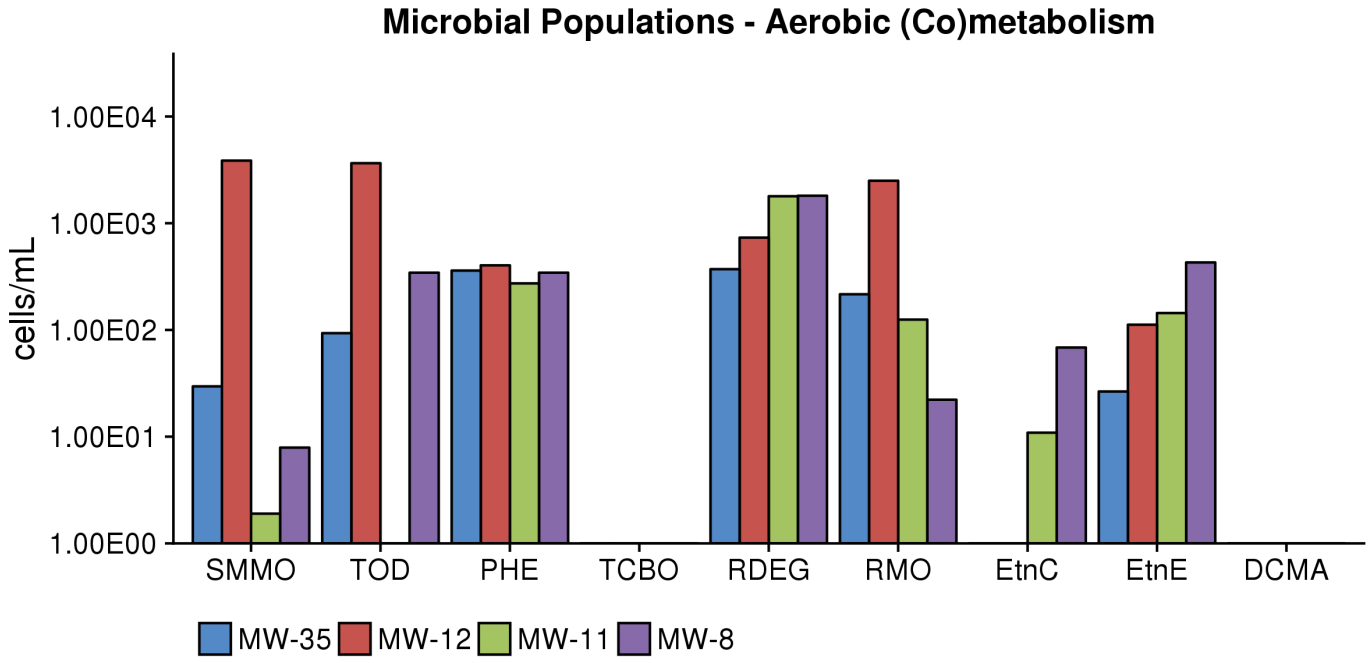

Figure 14: Comparison - microbial populations involved in aerobic (co)metabolism.

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Table 9: Summary of the QuantArray® results for total bacteria and other populations for samples MW-31, MW-30, MW-5, and MW-21.

Sample Name Sample Date	MW-31 02/12/2018	MW-30 02/12/2018	MW-5 02/13/2018	MW-21 02/13/2018
Other	cells/mL	cells/mL	cells/mL	cells/mL
Total Eubacteria (EBAC)	**3.38E+05**	**3.03E+06**	**1.37E+06**	**1.06E+03**
Sulfate Reducing Bacteria (APS)	**7.02E+04**	**7.81E+04**	**2.09E+04**	<5.00E+00
Methanogens (MGN)	**9.90E+00**	<2.50E+01	<5.00E+00	<5.00E+00

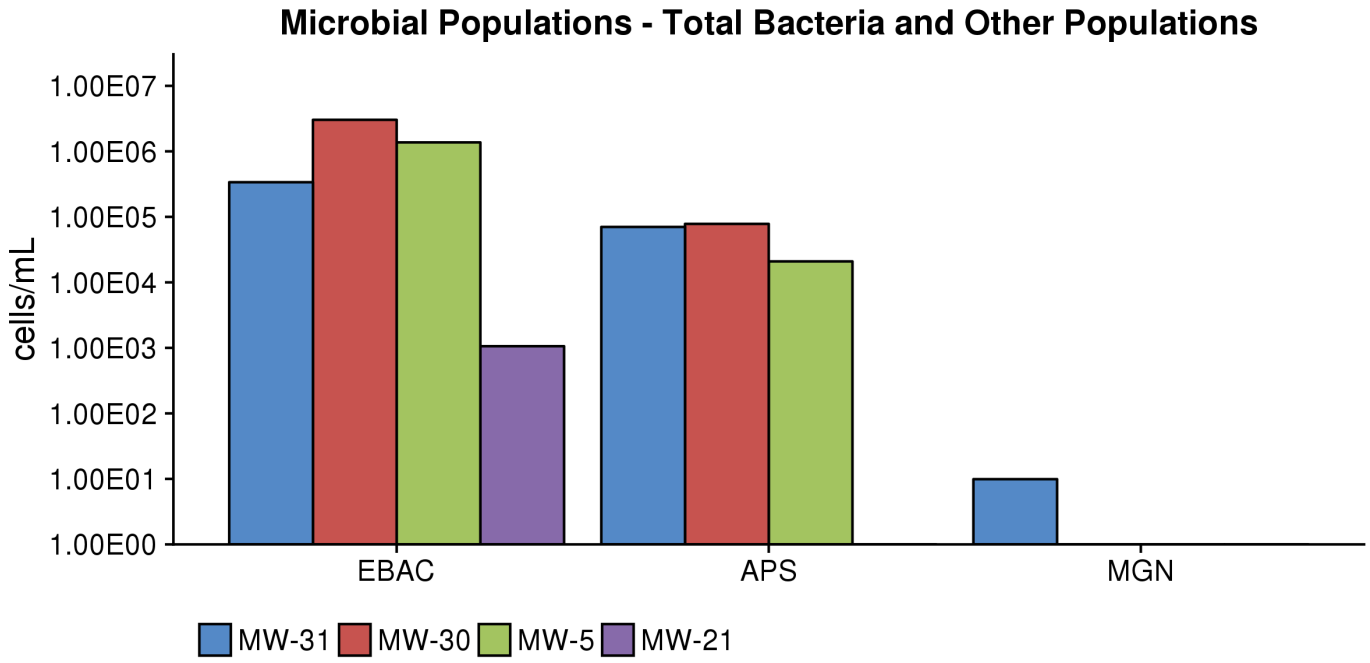

Figure 15: Comparison - microbial populations.

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Table 10: Summary of the QuantArray® results for total bacteria and other populations for samples MW-35, MW-12, MW-11, and MW-8.

Sample Name Sample Date	MW-35 02/13/2018	MW-12 02/14/2018	MW-11 02/14/2018	MW-8 02/14/2018
Other	cells/mL	cells/mL	cells/mL	cells/mL
Total Eubacteria (EBAC)	**1.53E+05**	**6.24E+05**	**1.21E+06**	**1.95E+05**
Sulfate Reducing Bacteria (APS)	**7.07E+03**	**1.39E+04**	**7.14E+04**	**1.02E+04**
Methanogens (MGN)	**3.54E+01**	**5.37E+01**	**1.32E+03**	<5.00E+00

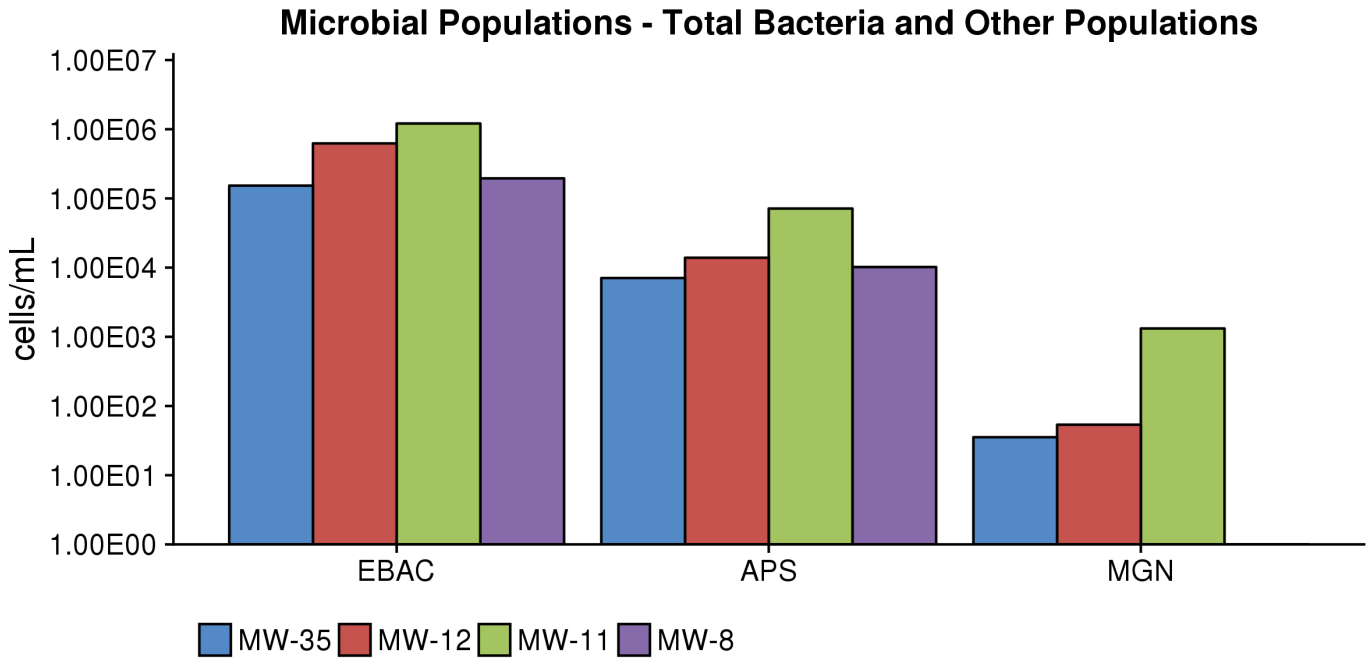

Figure 16: Comparison - microbial populations.

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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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Project Manager: John Kane
Project Name: BSCSS
Project No.: 82302 - Task 9.2

Purchase Order No.
Subcontract No.
MI Quote No.

Please Check One:
☒ More samples to follow
☐ No Additional Samples

Report Type: ☑ Standard (default) ☐ Microbial Insights Level III raw data (15% surcharge) ☐ Microbial Insights Level IV (25% surcharge) ☐ Comprehensive Interpretive (15%) ☐ Historical Interpretive (35%)

EDD type: ☐ Microbial Insights Standard (default) ☐ All other available EDDs (5% surcharge) Specify EDD Type:

Please contact us with any questions about the analyses or filling out the COC at (865) 573-8188 (9:00 am to 5:00 pm EST, M-F). After hours email: customerservice@microbe.com

Sample Information						Analyses				CENSUS: Please select the target organism/gene																				
MI ID (Laboratory Use Only)	Sample Name	Date Sampled	Time Sampled	Matrix	Total Number of Containers	PLFA	NGS	QuantArray Chlor	QuantArray Petro	DHC (Dehalococcoides)	DHC Functional genes (bvc, tce, vcr)	DHBt (Dehalobacter)	DHG (Dehalogenimonas)	DSM (Desulfuromonas)	DSB (Desulfitobacterium)	EBAC (Total)	SRB (Sulfate Reducing Bacteria-APS)	MGN (Methanogens)	MOB (Methanotrophs)	SMMO	DNF (Dentrifiers-nirS and nirK)	AMO (ammonia oxidizing bacteria)	PM1 (MTBE aerobic)	RMO (Toluene Monooxygenase)	RDEG (Toluene Monooxygenase)	PHE (Phenol Hydroxylase)	NAH (Napthalene-aerobic)	BSSA (Toluene/Xylene-Anaerobic)	add. qPCR:	RNA (Expression Option)*	Other:	Other:	Other:
D27PB1	MW-31	2-12-18	11:30	GW	1			X																								
2	MW-30	2-12-18	13:00	GW	1			X																								

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* additional cost and sample preservation are associated with RNA samples.

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Project Manager: J. Kane
Project Name: BSC55 Task 9.2
Project No.: 82302 Task 9.2

Purchase Order No.
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Please Check One:
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Report Type: ☑ Standard (default) ☐ Microbial Insights Level III raw data(15% surcharge) ☐ Microbial Insights Level IV (25% surcharge) ☐ Comprehensive Interpretive(15%) ☐ Historical Interpretive (35%)
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027PB 3	MW-5	2/13/18	1405					X																									
4	MW-21	↓	1505					X																									
5	MW-35	↓	1610					X																									
6	MW-12	2/14/18	0945					X																									
7	MW-11	↓	1115					X																									
8	MW-8	↓	1220					X																									

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How to Use Estimated Percentile Ranks from the Microbial Insights Database

The MI Database and Client Portal

The Microbial Insights Database is the largest collection of field concentrations of key microorganisms and functional genes currently containing qPCR and QuantArray results for more than 32,000 unique groundwater, soil, and sediment samples from all 50 states and 33 countries worldwide. Driven by field samples, the database reflects the impacts of common contaminants, geochemical conditions, and site management practices on critical microbial populations.

With your report, you received a passcode enabling you to retrieve estimates of the percentile ranks of your results based on those compiled in the MI database at no additional charge. When accessing the database, you will be asked to provide background information about the sample (e.g. contaminant concentrations) to aid in understanding the links between environmental conditions and microbial populations. As with all client information provided to MI, site specific data will be treated as confidential.

Is that low, medium or high?

In practice, biodegradation depends not just on the presence but the actual concentrations of the contaminant degrading microorganisms. Simply put, qPCR and QuantArray results demonstrating high concentrations of target microorganisms or functional genes suggest in situ selection, enrichment and growth of those specific contaminant degraders and therefore a greater probability that monitored natural attenuation (MNA) or bioremediation will be successful.

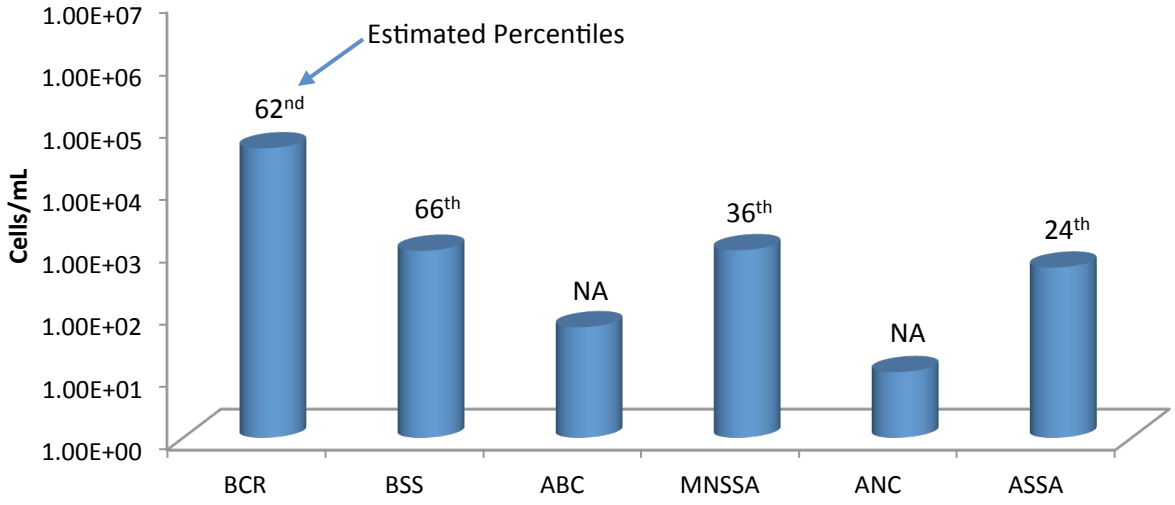
Is that a low, medium, or high concentration? The estimated percentile ranks retrieved from the MI Database answer that question by comparing your qPCR and QuantArray results to those of the literally thousands of other environmental samples submitted to MI for analysis over the last 20+ years.

Using the Estimated Percentile - Interpretation Examples

MNA Assessment – Petroleum Hydrocarbon Site:

Whenever possible, interpretation of qPCR and QuantArray results should include comparisons between samples obtained from background and impacted wells. The estimated percentile ranks however provide an additional avenue for comparison and evaluation of treatment options as shown below.

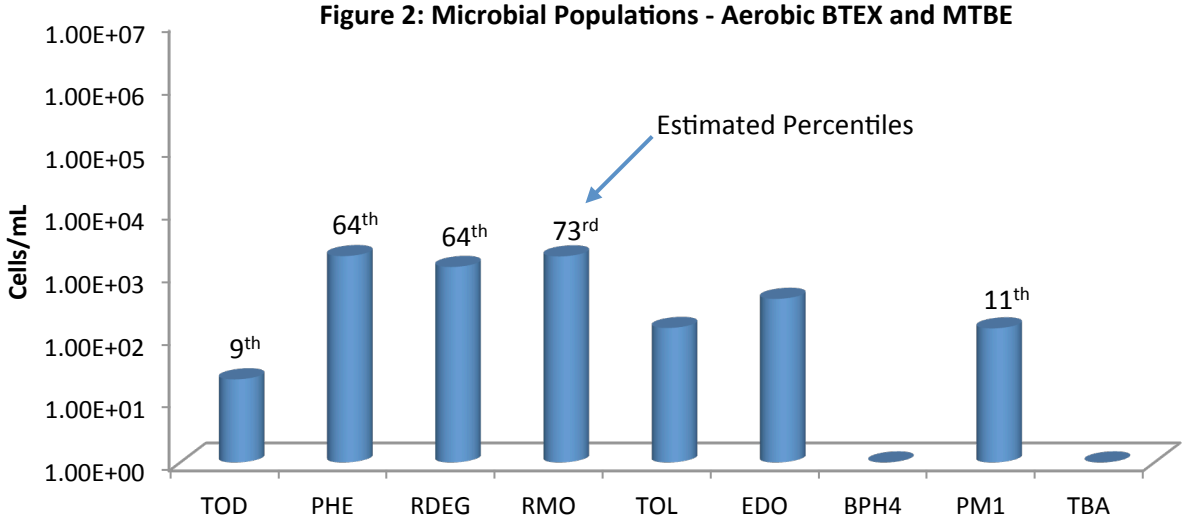
Figure 1: Microbial Populations - Anaerobic BTEX and PAHs



Anaerobic BTEX and PAH Biodegradation (Figure 1):

- With moderate concentrations of functional genes involved in anaerobic BTEX metabolism detected, the QuantArray-Petro® results were encouraging in terms of evaluating biodegradation potential under existing site conditions.
- More specifically, benzylsuccinate synthase (BSS) was detected on the order of nearly 10^3 cells/mL indicating the presence of a substantial population (66^{th} percentile) capable of anaerobic biodegradation of toluene and other alkyl substituted benzenes.
- Naphthyl-2-methylsuccinate synthase (MNSSA) and alkylsuccinate synthase (ASSA) genes were also detected indicating the potential for anaerobic biodegradation of 2-methylnaphthalene and normal alkanes.
- The concentration of MNSSA genes would be considered modest with an estimated percentile of 36^{th}.
- While the percentile rank for MNSSA would be "below average", a number of additional factors should be considered.
    - First, anaerobic hydrocarbon degraders are less prevalent than aerobic BTEX degraders and overall detection frequencies for many genes involved in anaerobic hydrocarbon biodegradation are less than 50%.
    - Therefore, the detection of genes like BSS, MNSSA, ASSA, anaerobic benzene carboxylase (ABC), and anaerobic naphthalene carboxylase (ANC) even at low concentrations is certainly noteworthy and inherently "better than average".
    - The estimated percentiles for all assays are based only on samples where the concentration of the target gene was greater than the practical quantitation limit (PQL).
    - For less commonly detected targets like many of the genes involved in anaerobic hydrocarbon biodegradation this is an especially important consideration.
    - Excluding samples where a gene target is below the PQL ensured that the median concentrations of less commonly detected targets would not be unduly biased low by the fact that the gene is not detected in most samples.
- Anaerobic benzene carboxylase (ABC) and naphthalene carboxylase (ANC) genes were also detected indicating the presence of bacterial populations capable of anaerobic biodegradation of benzene and naphthalene.
- For newly identified genes like ABC and ANC, estimated percentile ranks are not yet available due to the limited number of field samples that have been analyzed to date.
- However, like MNSSA and other genes involved in anaerobic hydrocarbon biodegradation, ABC and ANC detection frequencies are relatively low so the detection of these genes even at low concentrations should be considered when evaluating biodegradation potential under existing site conditions.

Figure 2: Microbial Populations - Aerobic BTEX and MTBE



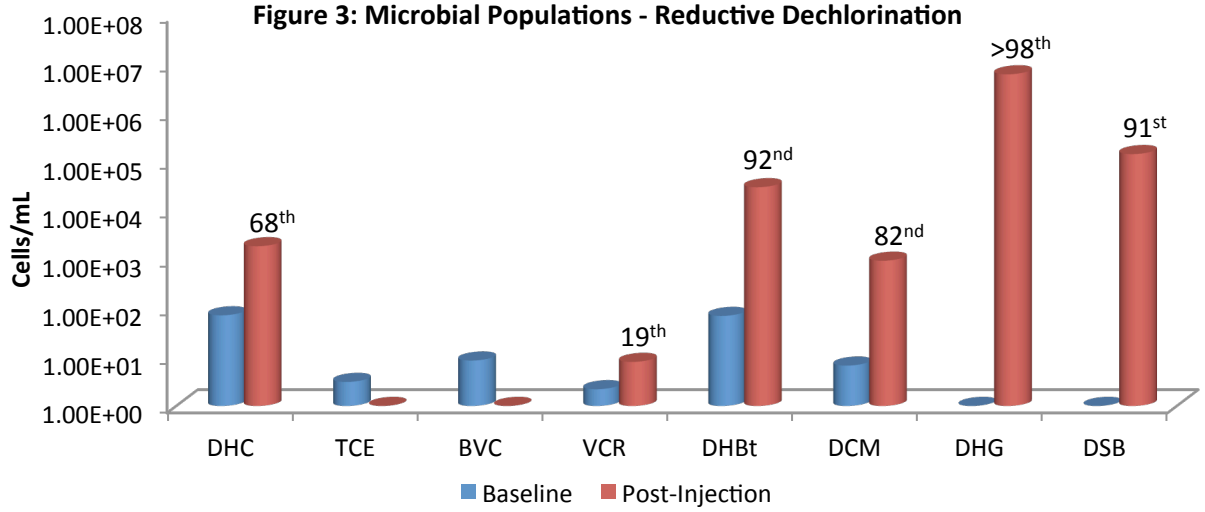
Aerobic BTEX and MTBE Biodegradation (Figure 2):

- With growing evidence that aromatic oxygenases function at low dissolved oxygen concentrations, aerobic BTEX biodegradation pathways should also be evaluated when considering MNA.
- Again, the QuantArray-Petro results were encouraging – genes encoding the first step in multiple pathways for aerobic BTEX biodegradation were detected indicating the presence of a diverse population of aerobic BTEX degraders.
- However, aerobic BTEX degraders are often considered ubiquitous. Therefore answering the question "Is that low, medium or high?" becomes especially important when evaluating aerobic BTEX biodegradation at petroleum hydrocarbon sites.
- In this case, the estimated percentile ranks of the concentrations of toluene/benzene monooxygenase (RMO and RDEG) and phenol hydroxylase (PHE) genes ranged from the 64th to 73rd percentile.
- In other words, the concentrations of RMO, RDEG, and PHE detected in this groundwater sample were greater than the concentrations detected in 64% to 73% of all other groundwater samples where these genes were analyzed and detected above the PQL.
- Aerobic BTEX degraders are common in the environment, but in this sample concentrations of toluene/benzene monooxygenase genes could be viewed as "better than average" when compared to the MI Database.

Biostimulation – Chlorinated Solvent Site:

Whenever possible, interpretation of qPCR and QuantArray results should include comparisons between baseline and post-injection monitoring events as shown below (Figure 3). The estimated percentile ranks however provide an additional avenue for comparison and evaluation of remedy performance.

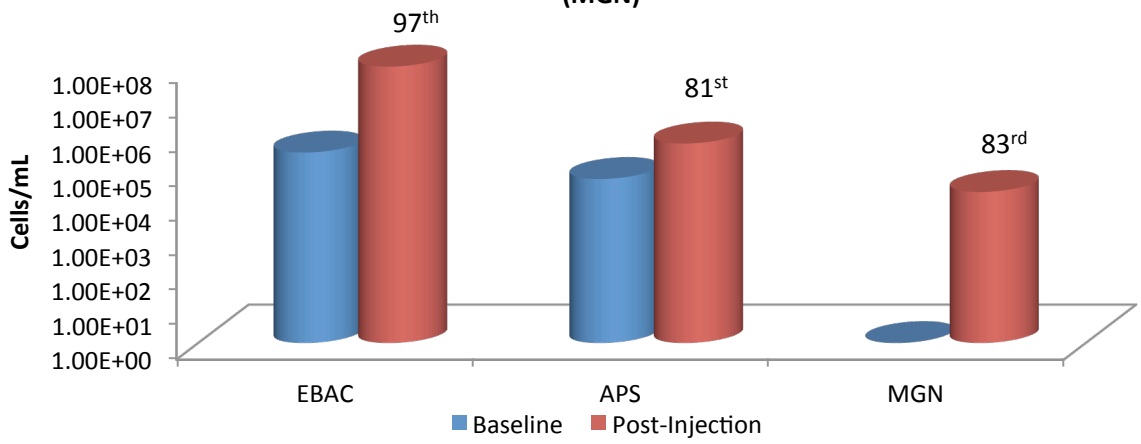
Figure 3: Microbial Populations - Reductive Dechlorination



- During the baseline groundwater sampling event, *Dehalococcoides* and vinyl chloride reductase genes were detected indicating the potential for complete reductive dechlorination of PCE and TCE to ethene.
- However, the *Dehalococcoides* concentration was well below the 10^4 cells/mL recommended by Lu et al. (2006) for generally effective rates of reductive dechlorination.
- Based on qPCR results as well as traditional groundwater monitoring, biostimulation with electron donor addition was selected as the site management plan.
- By the first monitoring event after injection, populations of halorespiring bacteria had increased substantially in response to electron donor addition.
    - *Dehalobacter* populations increased by more than two orders of magnitude to post-injection concentrations greater than 10^4 cells/mL (92^{nd} percentile).
    - *Dehalogenimonas* (10^6 cells/mL) and *Desulfitobacterium* (10^5 cells/mL) which had not been detected prior electron donor addition were present at concentrations greater than observed in over 90% of other groundwater samples where these halorespiring bacteria were detected.
- After injection, *Dehalococcoides* populations increased by more than an order of magnitude to a concentration of over 10^3 cells/mL (68^{th} percentile) demonstrating growth of this key group of halorespiring bacteria.
- Despite a substantial increase and a "better than average" concentration, the *Dehalococcoides* population was still below the 10^4 cells/mL threshold and vinyl chloride reductase gene copies were low (19^{th} percentile).
    - In terms of electron donors and acceptors, the metabolic capabilities of *Dehalococcoides* are rather specialized (hydrogen utilizing obligate halorespiring bacteria) so the median concentration is low. With a low median concentration across the database, a "better than average" *Dehalococcoides* concentration in a given sample may not exceed the 10^4 cells/mL threshold established for effective reductive dechlorination (Lu et al. 2006) and ethene production (Microbial Insights, unpublished data).

- In this case, the initial growth of *Dehalococcoides* was substantial but may have been somewhat hindered by competition with sulfate reducing bacteria (Figure 4 below).
    - The baseline population of sulfate reducing bacteria was moderate (10^4 cells/mL; 63rd percentile). Consistent with an observed decreased in dissolved sulfate concentrations, populations of sulfate reducing bacteria increased and were detected at a relatively high concentration (81st percentile) after electron donor addition.
    - After injection, methanogen populations also increased to a relatively high concentration (83rd percentile) suggesting generation of methanogenic conditions.
- With sulfate depletion and generation of highly anaerobic conditions more conducive to reductive dechlorination, *Dehalococcoides* populations may continue to increase and exceed the 10^4 *Dehalococcoides* cells/mL threshold in subsequent monitoring events.
- Overall, QuantArray analysis conclusively demonstrated that electron donor addition stimulated growth of halorespiring bacteria with the estimated percentiles retrieved from the MI Database providing the "low, medium or high" perspective to the observed changes in microbial populations.

Figure 4: Total Bacteria (EBAC), Sulfate Reducering Bacteria (APS) and Methanogens (MGN)



References

Lu, X., J.T. Wilson, and D.H. Kampbell. 2006. Relationship between *Dehalococcoides* DNA in ground water and rates of reductive dechlorination at field scale. *Water Research* 40 no. 16: 3131-3140.

How to Retrieve and Use Estimated Percentile Ranks from the Microbial Insights Database

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Is that low, medium or high?

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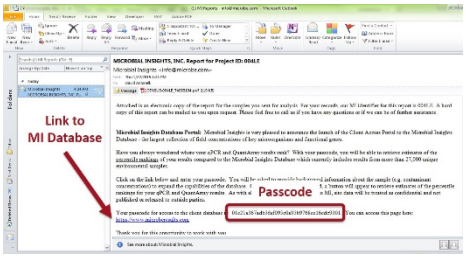
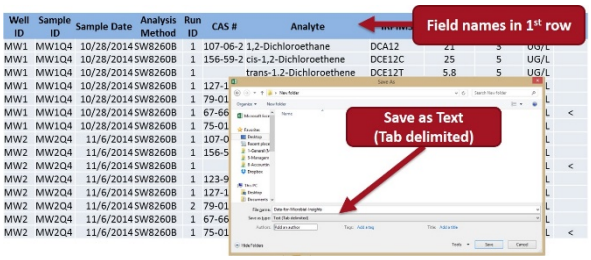
Retrieving Estimated Percentile Ranks

With your report, you were emailed a passcode and link enabling you to login to the Client Portal. Just enter basic information about the sample (e.g. contaminant concentrations) to aid in understanding the links between environmental conditions and microbial populations and you can retrieve estimates of the percentile ranks of your results based on those compiled in the MI database at no additional charge.





All site specific data will be treated as confidential and uploading is easy.

You can even upload chemical and geochemical data from EDDs. Just save as a Tab Delimited text file.

Example - Using Estimated Percentile for MNA Assessment at an MGP Site

CENSUS® qPCR was performed to quantify anaerobic naphthalene carboxylase (ANC) and naphthyl-2-methylsuccinate synthase (MNSSA) to assess anaerobic biodegradation of naphthalene and methyl-naphthalene under existing site conditions.

- Not only were ANC and MNSSA genes detected, but these functional genes responsible for anaerobic biodegradation of PAHs were present at concentrations "far better than average" based on the estimated percentile ranks.
- Demonstrating high concentrations of ANC and MNSSA gave an additional line of evidence indicating growth substantial populations of anaerobic PAH degraders and suggested a greater probability that monitored natural attenuation (MNA) will be successful.