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.

Results

Table 1: Summary of the QuantArray®-Chlor results obtained for samples LALF12-W-201908081111, LALF24-W-201908081345, and LALF09-W-201908150927.

Sample Name **Sample Date**	**LALF12-W-201908081111** **08/08/2019**	**LALF24-W-201908081345** **08/08/2019**	**LALF09-W-201908150927** **08/15/2019**
Reductive Dechlorination	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**4.16E+01**	**2.00E-01 (J)**	**3.63E+01**
tceA Reductase (TCE)	<5.00E-01	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<5.00E-01	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	<5.00E-01	<5.00E-01
Dehalobacter spp. (DHBt)	**1.74E+01**	<4.90E+00	**1.08E+02**
Dehalobacter DCM (DCM)	<4.90E+00	<4.90E+00	<4.80E+00
Dehalogenimonas spp. (DHG)	<4.90E+00	<4.90E+00	<4.80E+00
cerA Reductase (CER)	<4.90E+00	<4.90E+00	<4.80E+00
trans-1,2-DCE Reductase (TDR)	<4.90E+00	<4.90E+00	<4.80E+00
Desulfitobacterium spp. (DSB)	<4.90E+00	**1.37E+01**	**4.10E+02**
Dehalobium chlorocoercia (DECO)	**1.22E+01**	<4.90E+00	**1.17E+03**
Desulfuromonas spp. (DSM)	<4.90E+00	<4.90E+00	<4.80E+00
PCE Reductase (PCE-1)	<4.90E+00	<4.90E+00	<4.80E+00
PCE Reductase (PCE-2)	<4.90E+00	<4.90E+00	<4.80E+00
Chloroform Reductase (CFR)	<4.90E+00	<4.90E+00	<4.80E+00
1,1 DCA Reductase (DCA)	<4.90E+00	<4.90E+00	<4.80E+00
1,2 DCA Reductase (DCAR)	<4.90E+00	<4.90E+00	<4.80E+00
Aerobic (Co)Metabolic			
Soluble Methane Monooxygenase (SMMO)	<4.90E+00	<4.90E+00	<4.80E+00
Toluene Dioxygenase (TOD)	**8.50E+00**	**1.80E+00 (J)**	<4.80E+00
Phenol Hydroxylase (PHE)	**8.00E-01 (J)**	**3.30E+00 (J)**	**1.66E+01**
Trichlorobenzene Dioxygenase (TCBO)	<4.90E+00	<4.90E+00	<4.80E+00
Toluene Monooxygenase 2 (RDEG)	<4.90E+00	**4.99E+01**	<4.80E+00
Toluene Monooxygenase (RMO)	<4.90E+00	<4.90E+00	<4.80E+00
Ethene Monooxygenase (EtnC)	<4.90E+00	<4.90E+00	<4.80E+00
Epoxyalkane Transferase (EtnE)	<4.90E+00	<4.90E+00	<4.80E+00
Dichloromethane Dehalogenase (DCMA)	<4.90E+00	<4.90E+00	<4.80E+00
Other			
Total Eubacteria (EBAC)	**1.55E+04**	**1.14E+05**	**7.80E+04**
Sulfate Reducing Bacteria (APS)	**1.53E+02**	<4.90E+00	**7.18E+02**
Methanogens (MGN)	<4.90E+00	<4.90E+00	<4.80E+00

Legend:
NA = Not Analyzed
NS = Not Sampled
J = Estimated Gene Copies Below PQL but Above LQL
I = Inhibited
< = Result Not Detected

Table 2: Summary of the QuantArray®-Chlor results obtained for samples LALF10-W-201908151100, GWEX2-W-201908151257, and GWEX4-W-201908151440.

Sample Name	**LALF10-W-201908151100**	**GWEX2-W-201908151257**	**GWEX4-W-201908151440**
Sample Date	**08/15/2019**	**08/15/2019**	**08/15/2019**
Reductive Dechlorination	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**7.40E+00**	**4.00E-01 (J)**	**9.00E-01**
tceA Reductase (TCE)	<5.00E-01	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<5.00E-01	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	<5.00E-01	<5.00E-01
Dehalobacter spp. (DHBt)	<4.90E+00	**7.15E+02**	**8.47E+02**
Dehalobacter DCM (DCM)	<4.90E+00	<5.10E+00	<4.80E+00
Dehalogenimonas spp. (DHG)	**4.72E+01**	<5.10E+00	<4.80E+00
cerA Reductase (CER)	<4.90E+00	<5.10E+00	<4.80E+00
trans-1,2-DCE Reductase (TDR)	<4.90E+00	<5.10E+00	<4.80E+00
Desulfitobacterium spp. (DSB)	**1.38E+01**	<5.10E+00	**5.95E+02**
Dehalobium chlorocoercia (DECO)	**2.56E+02**	**1.20E+03**	**1.99E+02**
Desulfuromonas spp. (DSM)	<4.90E+00	**1.24E+02**	**2.60E+00 (J)**
PCE Reductase (PCE-1)	<4.90E+00	<5.10E+00	<4.80E+00
PCE Reductase (PCE-2)	<4.90E+00	<5.10E+00	<4.80E+00
Chloroform Reductase (CFR)	<4.90E+00	<5.10E+00	<4.80E+00
1,1 DCA Reductase (DCA)	<4.90E+00	<5.10E+00	<4.80E+00
1,2 DCA Reductase (DCAR)	<4.90E+00	<5.10E+00	<4.80E+00
Aerobic (Co)Metabolic			
Soluble Methane Monooxygenase (SMMO)	**1.42E+02**	**2.42E+02**	<4.80E+00
Toluene Dioxygenase (TOD)	<4.90E+00	<5.10E+00	<4.80E+00
Phenol Hydroxylase (PHE)	**1.01E+02**	**5.83E+02**	**1.66E+02**
Trichlorobenzene Dioxygenase (TCBO)	<4.90E+00	<5.10E+00	<4.80E+00
Toluene Monooxygenase 2 (RDEG)	**1.04E+02**	**5.65E+02**	**4.13E+02**
Toluene Monooxygenase (RMO)	<4.90E+00	<5.10E+00	**1.91E+02**
Ethene Monooxygenase (EtnC)	**5.30E+00**	**1.23E+01**	<4.80E+00
Epoxyalkane Transferase (EtnE)	<4.90E+00	<5.10E+00	<4.80E+00
Dichloromethane Dehalogenase (DCMA)	<4.90E+00	<5.10E+00	<4.80E+00
Other			
Total Eubacteria (EBAC)	**1.49E+05**	**7.86E+05**	**2.62E+05**
Sulfate Reducing Bacteria (APS)	**5.79E+02**	**7.60E+03**	**2.55E+03**
Methanogens (MGN)	<4.90E+00	<5.10E+00	<4.80E+00

Legend:
NA = Not Analyzed
NS = Not Sampled
J = Estimated Gene Copies Below PQL but Above LQL
I = Inhibited
< = Result Not Detected

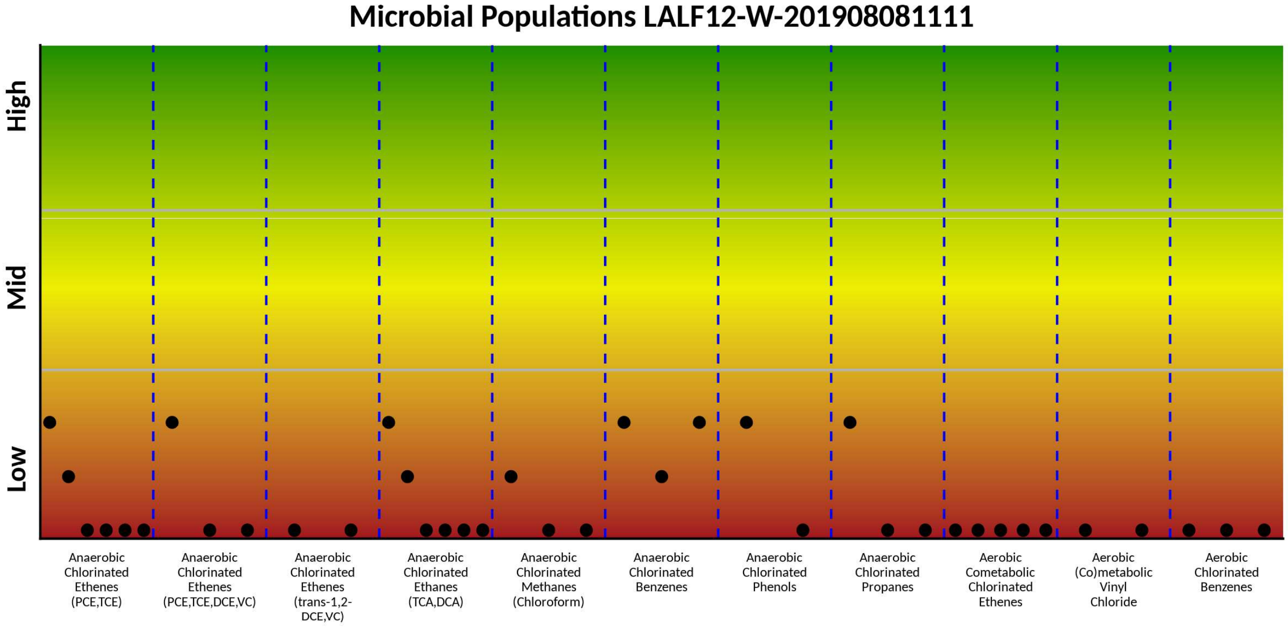
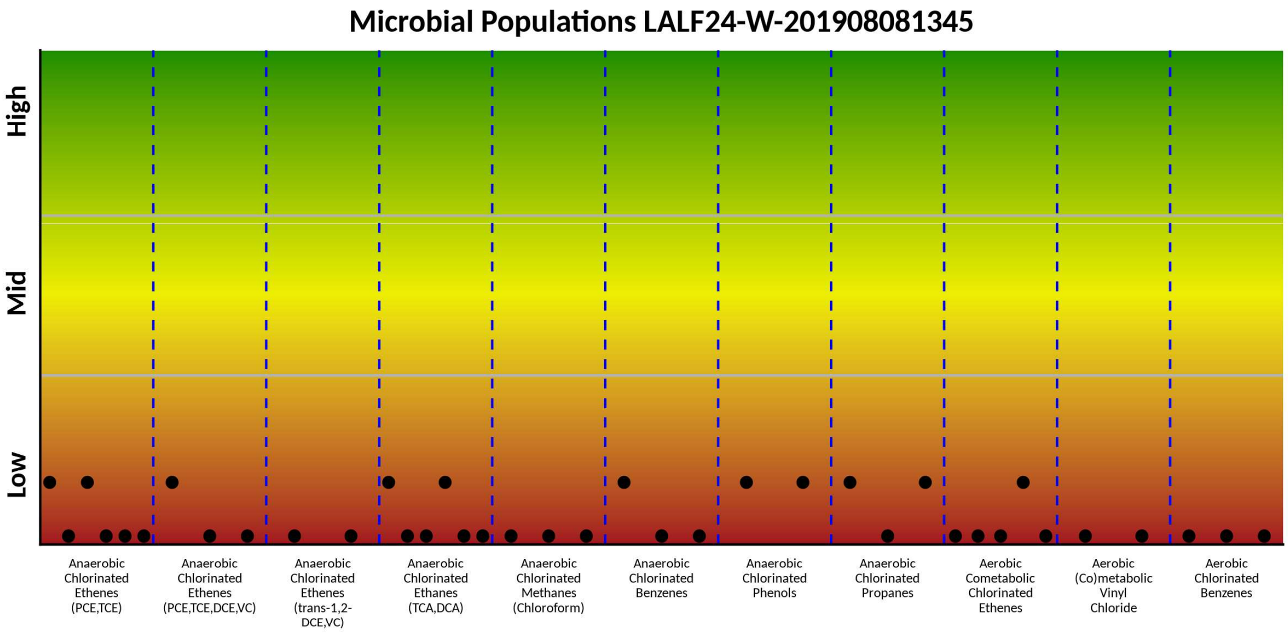


Figure 1: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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.



Microbial Populations LALF24-W-201908081345

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.

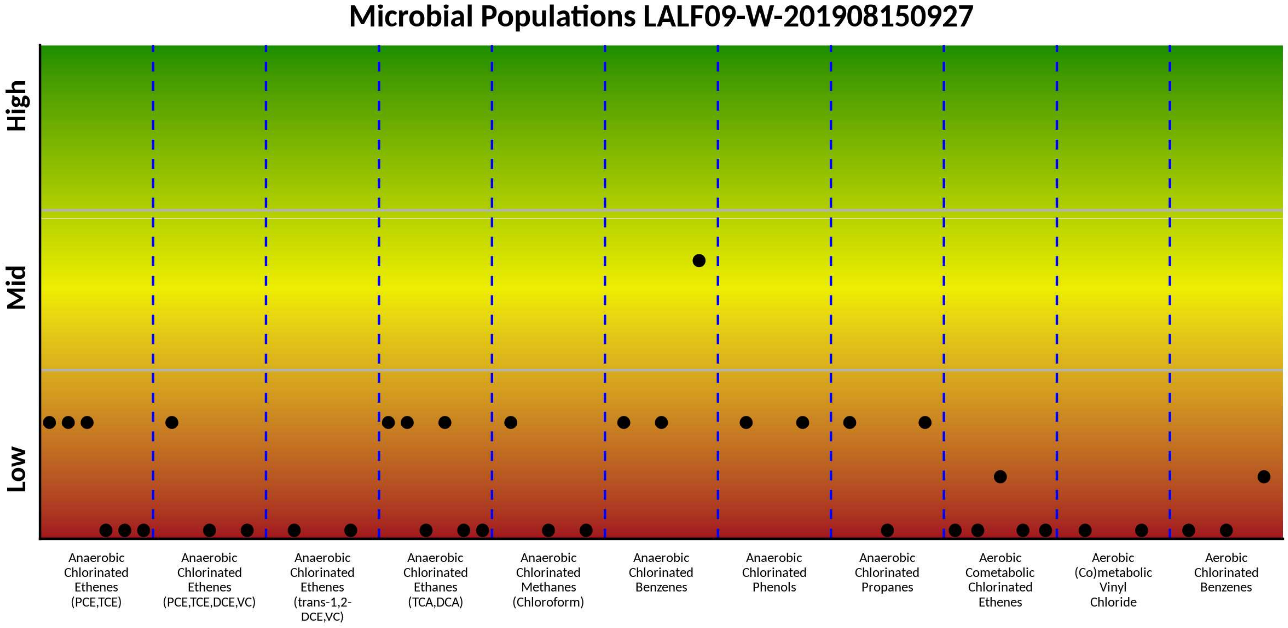


Figure 3: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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.

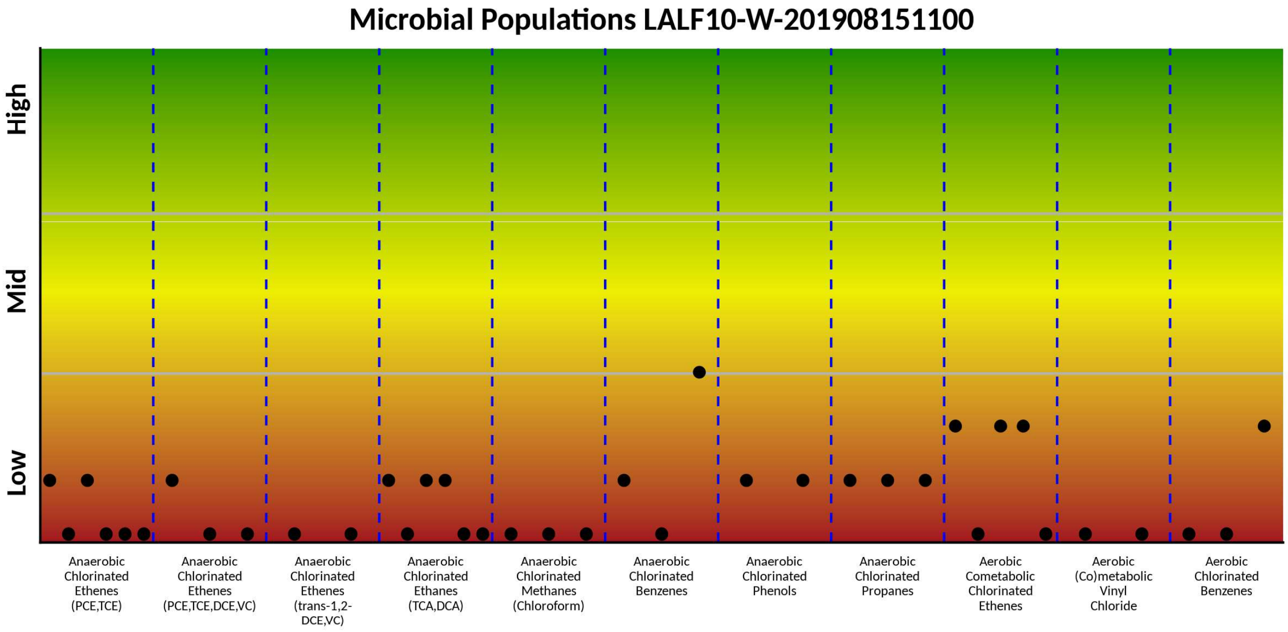


Figure 4: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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.

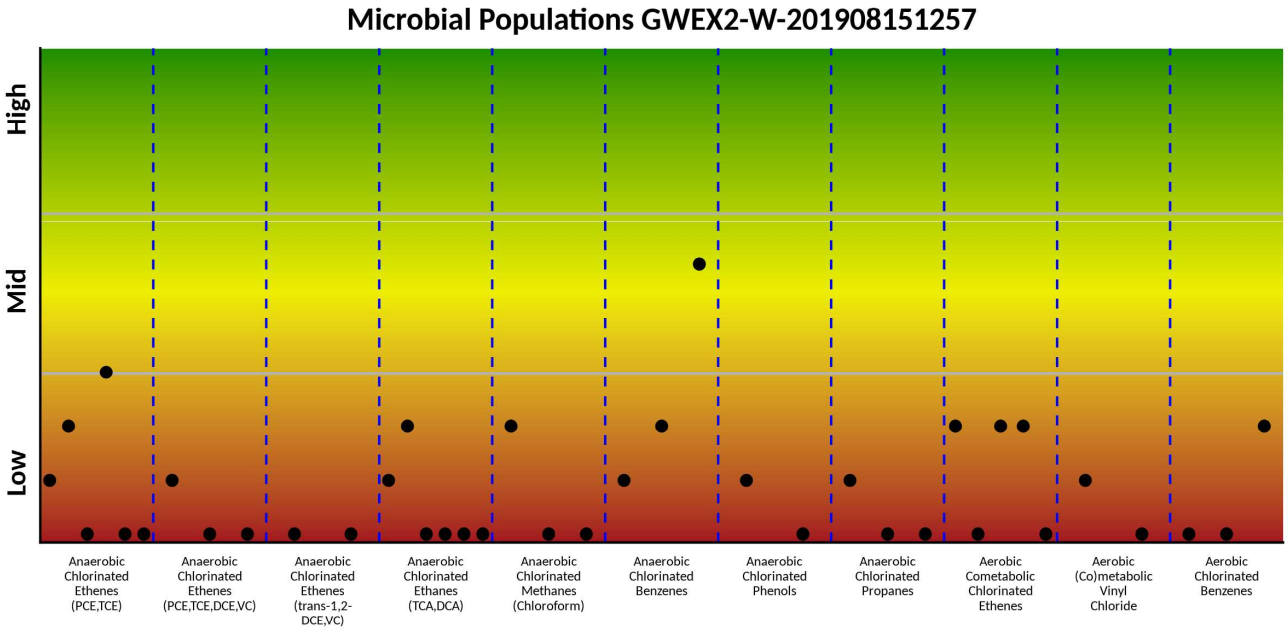


Figure 5: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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.

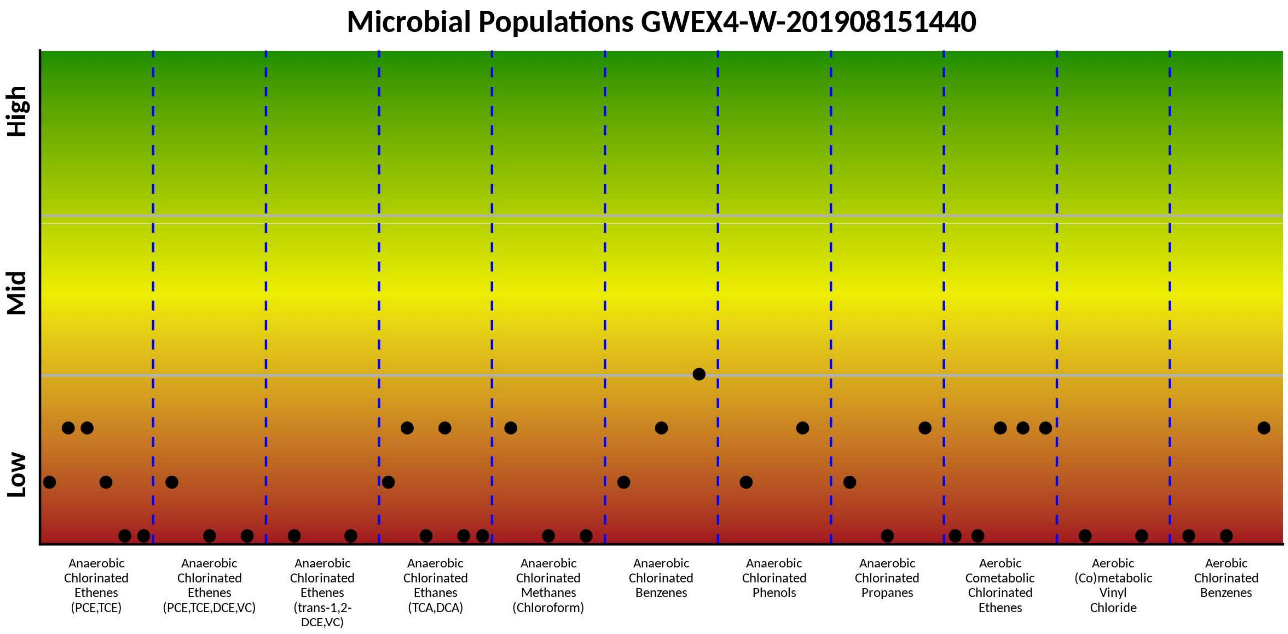


Figure 6: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

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.

Table 3: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples LALF12-W-201908081111, LALF24-W-201908081345, and LALF09-W-201908150927.

Sample Name **Sample Date**	**LALF12-W-201908081111** **08/08/2019**	**LALF24-W-201908081345** **08/08/2019**	**LALF09-W-201908150927** **08/15/2019**
Reductive Dechlorination	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**4.16E+01**	**2.00E-01 (J)**	**3.63E+01**
tceA Reductase (TCE)	<5.00E-01	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<5.00E-01	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	<5.00E-01	<5.00E-01
Dehalobacter spp. (DHBt)	**1.74E+01**	<4.90E+00	**1.08E+02**
Dehalobacter DCM (DCM)	<4.90E+00	<4.90E+00	<4.80E+00
Dehalogenimonas spp. (DHG)	<4.90E+00	<4.90E+00	<4.80E+00
Desulfitobacterium spp. (DSB)	<4.90E+00	**1.37E+01**	**4.10E+02**
Dehalobium chlorocoercia (DECO)	**1.22E+01**	<4.90E+00	**1.17E+03**
Desulfuromonas spp. (DSM)	<4.90E+00	<4.90E+00	<4.80E+00

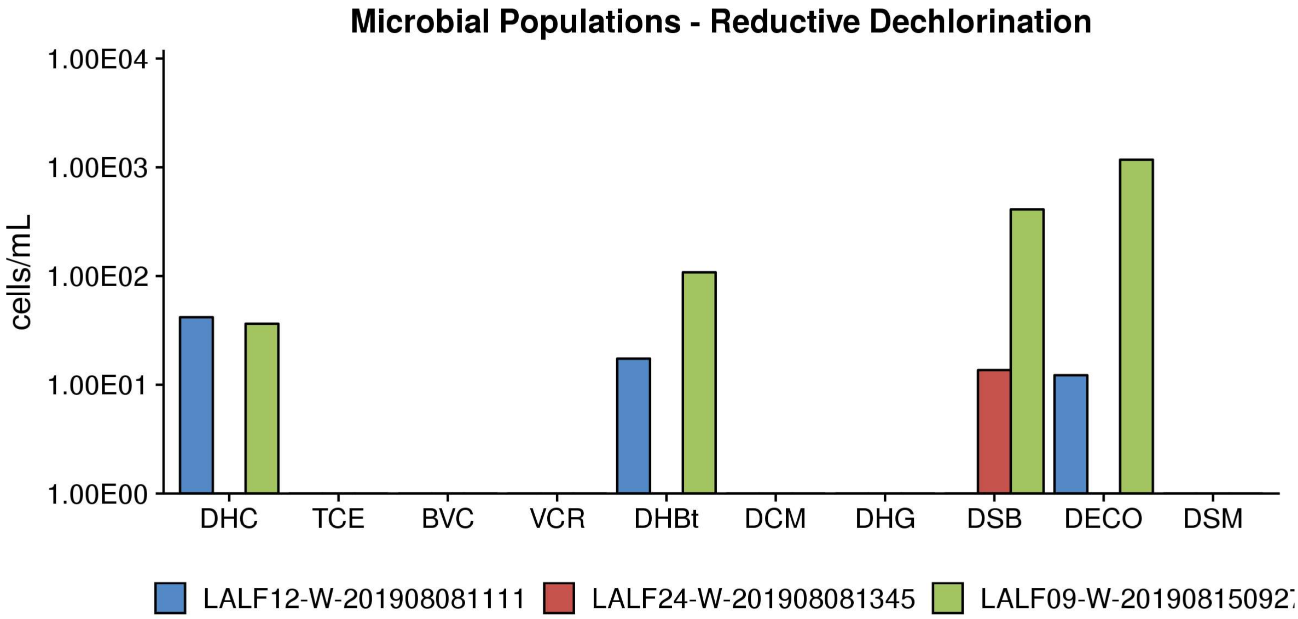


Figure 7: Comparison - microbial populations involved in reductive dechlorination.

Table 4: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples LALF12-W-201908081111, LALF24-W-201908081345, and LALF09-W-201908150927.

Sample Name **Sample Date**	**LALF12-W-201908081111** **08/08/2019**	**LALF24-W-201908081345** **08/08/2019**	**LALF09-W-201908150927** **08/15/2019**
Reductive Dechlorination	cells/mL	cells/mL	cells/mL
Chloroform Reductase (CFR)	<4.90E+00	<4.90E+00	<4.80E+00
1,1 DCA Reductase (DCA)	<4.90E+00	<4.90E+00	<4.80E+00
1,2 DCA Reductase (DCAR)	<4.90E+00	<4.90E+00	<4.80E+00
PCE Reductase (PCE-1)	<4.90E+00	<4.90E+00	<4.80E+00
PCE Reductase (PCE-2)	<4.90E+00	<4.90E+00	<4.80E+00
Dehalogenimonas trans-1,2-DCE Reductase (TDR)	<4.90E+00	<4.90E+00	<4.80E+00
Dehalogenimonas cerA Reductase (CER)	<4.90E+00	<4.90E+00	<4.80E+00

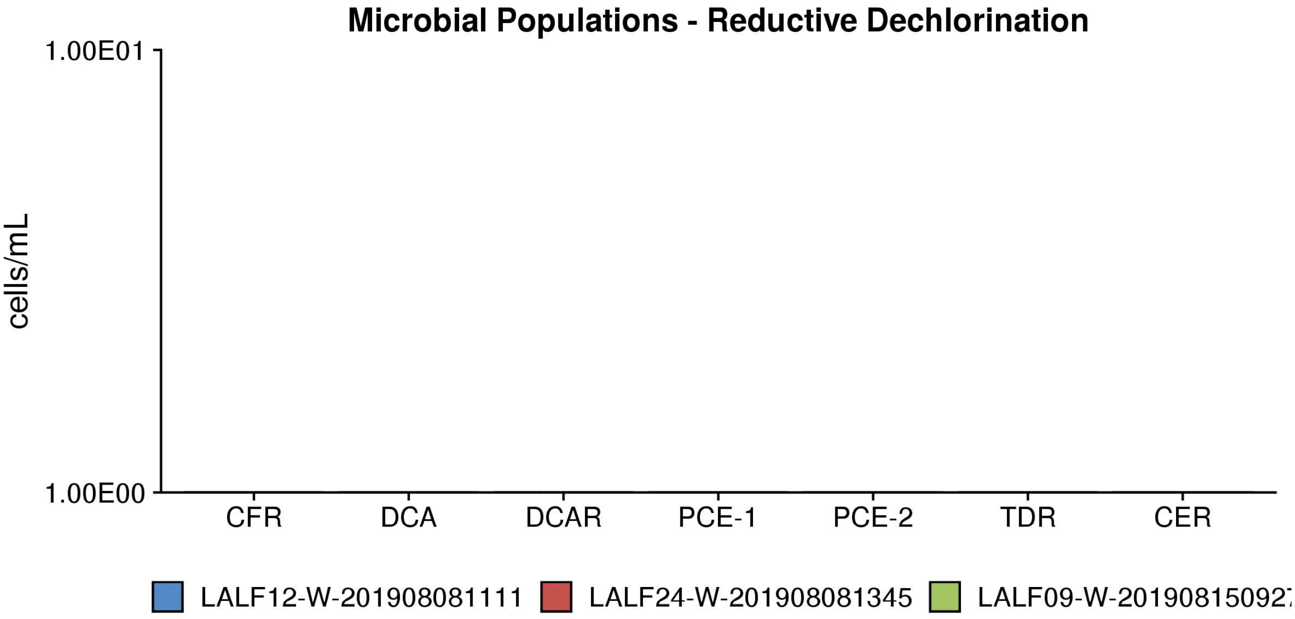


Figure 8: Comparison - microbial populations involved in reductive dechlorination.

Table 5: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples LALF10-W-201908151100, GWEX2-W-201908151257, and GWEX4-W-201908151440.

Sample Name **Sample Date**	**LALF10-W-201908151100** **08/15/2019**	**GWEX2-W-201908151257** **08/15/2019**	**GWEX4-W-201908151440** **08/15/2019**
Reductive Dechlorination	cells/mL	cells/mL	cells/mL
Dehalococcoides (DHC)	**7.40E+00**	**4.00E-01 (J)**	**9.00E-01**
tceA Reductase (TCE)	<5.00E-01	<5.00E-01	<5.00E-01
BAV1 Vinyl Chloride Reductase (BVC)	<5.00E-01	<5.00E-01	<5.00E-01
Vinyl Chloride Reductase (VCR)	<5.00E-01	<5.00E-01	<5.00E-01
Dehalobacter spp. (DHBt)	<4.90E+00	**7.15E+02**	**8.47E+02**
Dehalobacter DCM (DCM)	<4.90E+00	<5.10E+00	<4.80E+00
Dehalogenimonas spp. (DHG)	**4.72E+01**	<5.10E+00	<4.80E+00
Desulfitobacterium spp. (DSB)	**1.38E+01**	<5.10E+00	**5.95E+02**
Dehalobium chlorocoercia (DECO)	**2.56E+02**	**1.20E+03**	**1.99E+02**
Desulfuromonas spp. (DSM)	<4.90E+00	**1.24E+02**	**2.60E+00 (J)**

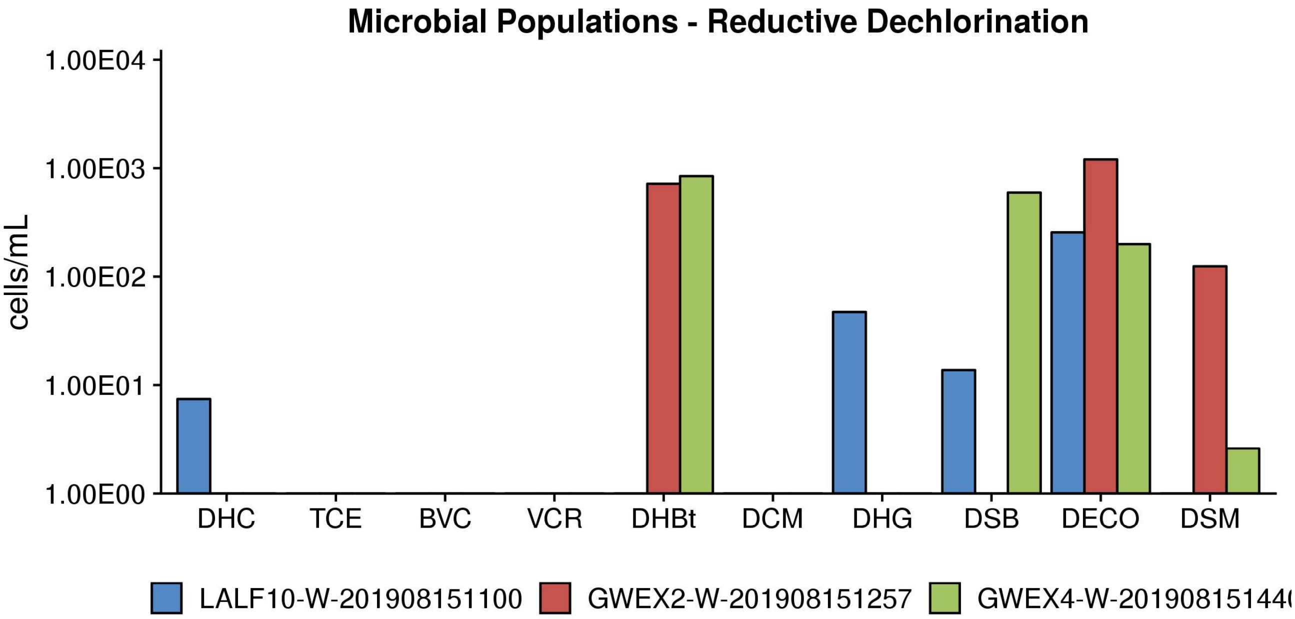


Figure 9: Comparison - microbial populations involved in reductive dechlorination.

Table 6: Summary of the QuantArray®-Chlor results for microorganisms responsible for reductive dechlorination for samples LALF10-W-201908151100, GWEX2-W-201908151257, and GWEX4-W-201908151440.

Sample Name **Sample Date**	**LALF10-W-201908151100** **08/15/2019**	**GWEX2-W-201908151257** **08/15/2019**	**GWEX4-W-201908151440** **08/15/2019**
Reductive Dechlorination	cells/mL	cells/mL	cells/mL
Chloroform Reductase (CFR)	<4.90E+00	<5.10E+00	<4.80E+00
1,1 DCA Reductase (DCA)	<4.90E+00	<5.10E+00	<4.80E+00
1,2 DCA Reductase (DCAR)	<4.90E+00	<5.10E+00	<4.80E+00
PCE Reductase (PCE-1)	<4.90E+00	<5.10E+00	<4.80E+00
PCE Reductase (PCE-2)	<4.90E+00	<5.10E+00	<4.80E+00
Dehalogenimonas trans-1,2-DCE Reductase (TDR)	<4.90E+00	<5.10E+00	<4.80E+00
Dehalogenimonas cerA Reductase (CER)	<4.90E+00	<5.10E+00	<4.80E+00

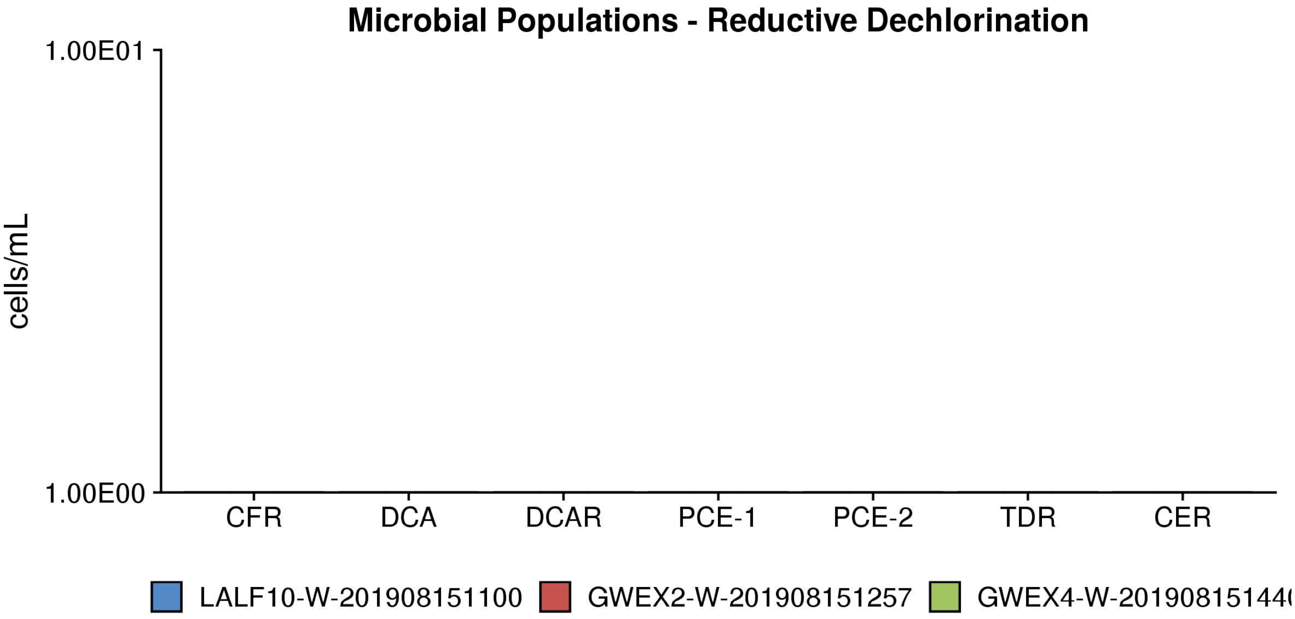


Figure 10: Comparison - microbial populations involved in reductive dechlorination.

Table 7: Summary of the QuantArray®-Chlor results for microorganisms responsible for aerobic (co)metabolism for samples LALF12-W-201908081111, LALF24-W-201908081345, and LALF09-W-201908150927.

Sample Name **Sample Date**	**LALF12-W-201908081111** **08/08/2019**	**LALF24-W-201908081345** **08/08/2019**	**LALF09-W-201908150927** **08/15/2019**
Aerobic (Co)Metabolic	cells/mL	cells/mL	cells/mL
Soluble Methane Monooxygenase (SMMO)	<4.90E+00	<4.90E+00	<4.80E+00
Toluene Dioxygenase (TOD)	**8.50E+00**	**1.80E+00 (J)**	<4.80E+00
Phenol Hydroxylase (PHE)	**8.00E-01 (J)**	**3.30E+00 (J)**	**1.66E+01**
Trichlorobenzene Dioxygenase (TCBO)	<4.90E+00	<4.90E+00	<4.80E+00
Toluene Monooxygenase 2 (RDEG)	<4.90E+00	**4.99E+01**	<4.80E+00
Toluene Monooxygenase (RMO)	<4.90E+00	<4.90E+00	<4.80E+00
Ethene Monooxygenase (EtnC)	<4.90E+00	<4.90E+00	<4.80E+00
Epoxyalkane Transferase (EtnE)	<4.90E+00	<4.90E+00	<4.80E+00
Dichloromethane Dehalogenase (DCMA)	<4.90E+00	<4.90E+00	<4.80E+00

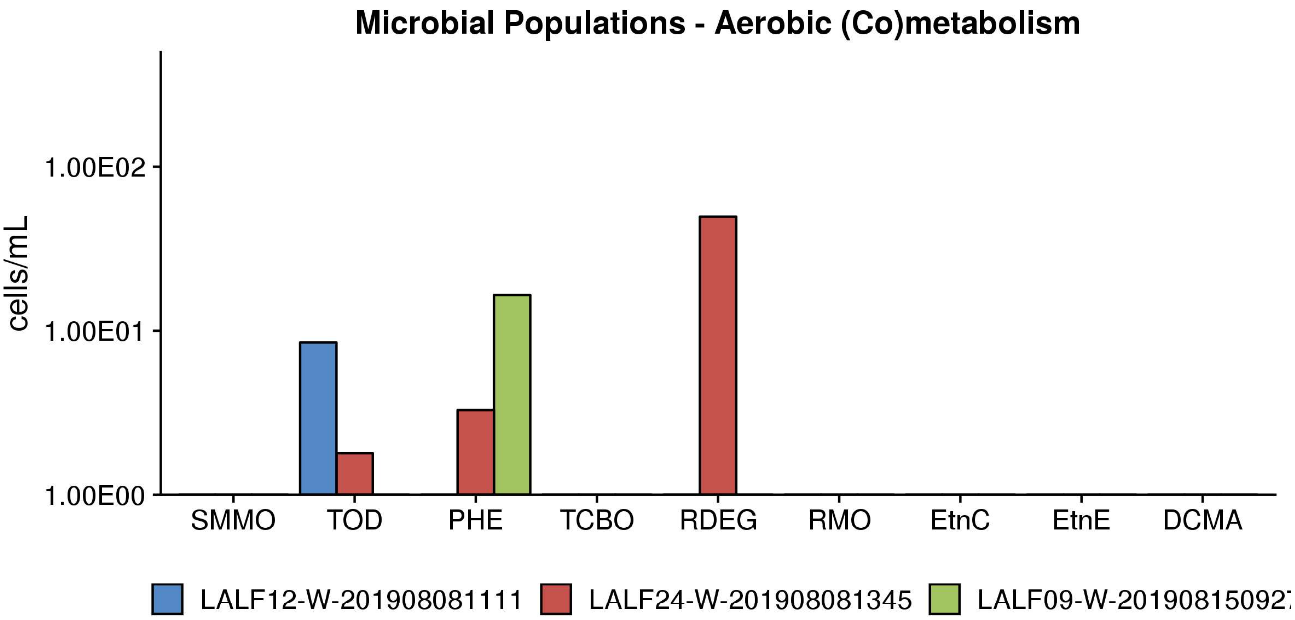


Figure 11: Comparison - microbial populations involved in aerobic (co)metabolism.

Table 8: Summary of the QuantArray®-Chlor results for microorganisms responsible for aerobic (co)metabolism for samples LALF10-W-201908151100, GWEX2-W-201908151257, and GWEX4-W-201908151440.

Sample Name	**LALF10-W-201908151100**	**GWEX2-W-201908151257**	**GWEX4-W-201908151440**
Sample Date	**08/15/2019**	**08/15/2019**	**08/15/2019**
Aerobic (Co)Metabolic	cells/mL	cells/mL	cells/mL
Soluble Methane Monooxygenase (SMMO)	**1.42E+02**	**2.42E+02**	<4.80E+00
Toluene Dioxygenase (TOD)	<4.90E+00	<5.10E+00	<4.80E+00
Phenol Hydroxylase (PHE)	**1.01E+02**	**5.83E+02**	**1.66E+02**
Trichlorobenzene Dioxygenase (TCBO)	<4.90E+00	<5.10E+00	<4.80E+00
Toluene Monooxygenase 2 (RDEG)	**1.04E+02**	**5.65E+02**	**4.13E+02**
Toluene Monooxygenase (RMO)	<4.90E+00	<5.10E+00	**1.91E+02**
Ethene Monooxygenase (EtnC)	**5.30E+00**	**1.23E+01**	<4.80E+00
Epoxyalkane Transferase (EtnE)	<4.90E+00	<5.10E+00	<4.80E+00
Dichloromethane Dehalogenase (DCMA)	<4.90E+00	<5.10E+00	<4.80E+00

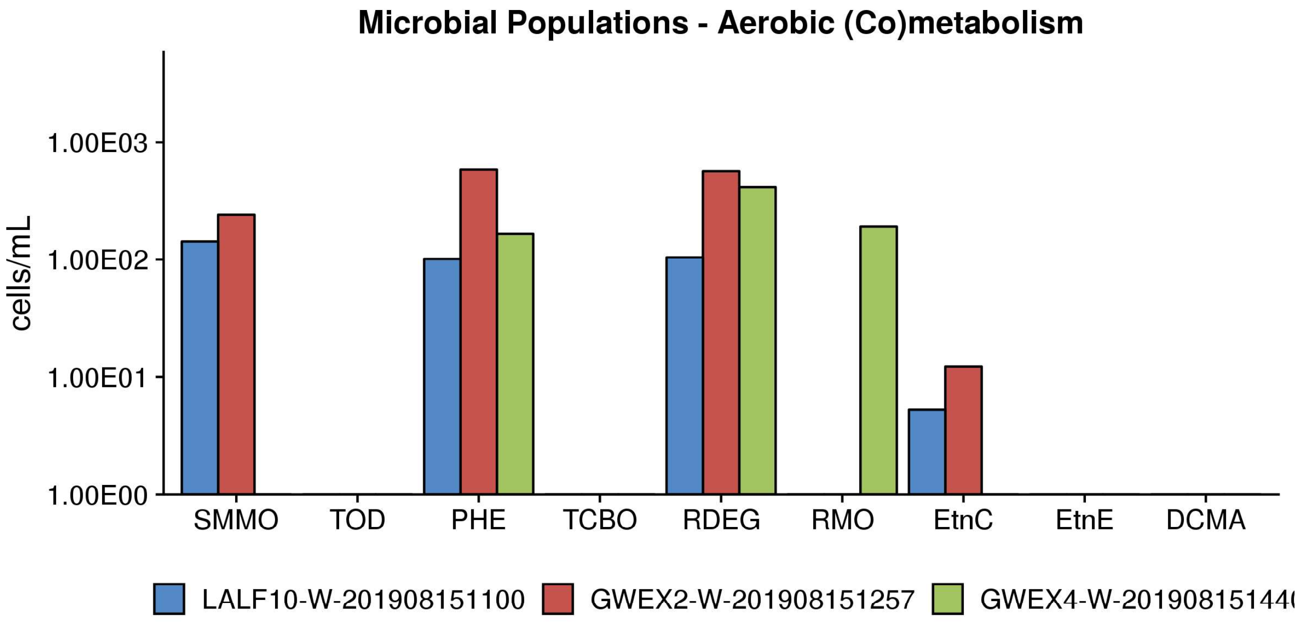


Figure 12: Comparison - microbial populations involved in aerobic (co)metabolism.

Table 9: Summary of the QuantArray® results for total bacteria and other populations for samples LALF12-W-201908081111, LALF24-W-201908081345, and LALF09-W-201908150927.

Sample Name **Sample Date**	**LALF12-W-201908081111** **08/08/2019**	**LALF24-W-201908081345** **08/08/2019**	**LALF09-W-201908150927** **08/15/2019**
Other	cells/mL	cells/mL	cells/mL
Total Eubacteria (EBAC)	**1.55E+04**	**1.14E+05**	**7.80E+04**
Sulfate Reducing Bacteria (APS)	**1.53E+02**	<4.90E+00	**7.18E+02**
Methanogens (MGN)	<4.90E+00	<4.90E+00	<4.80E+00

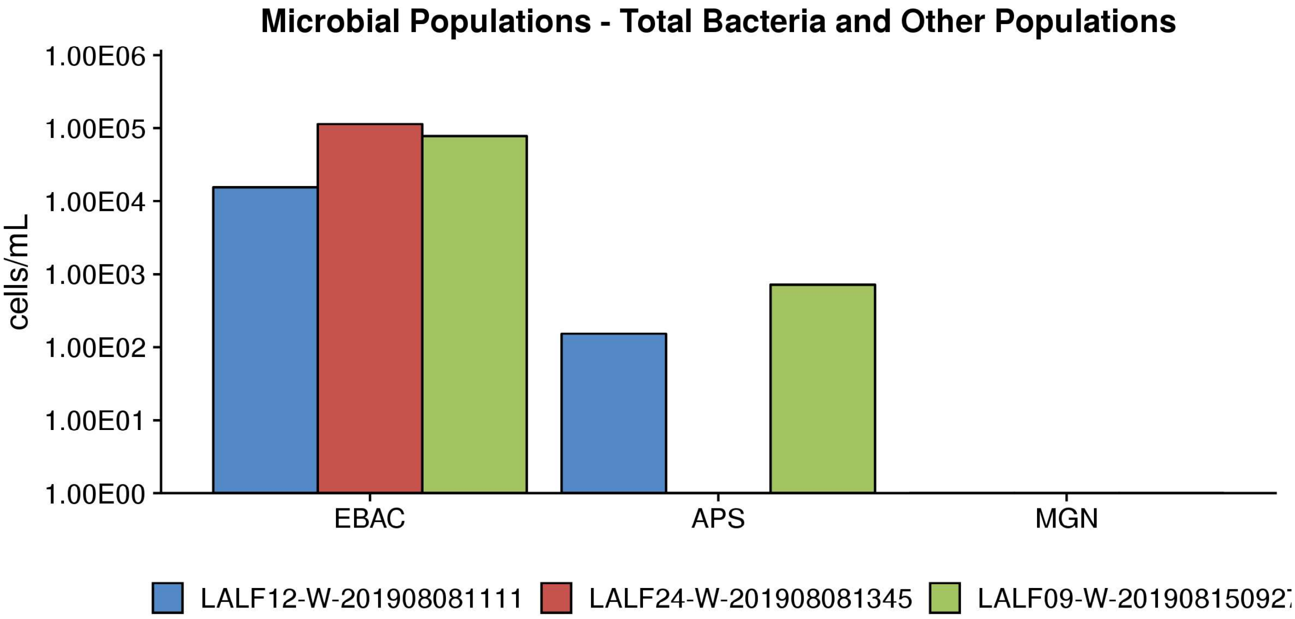


Figure 13: Comparison - microbial populations.

Table 10: Summary of the QuantArray® results for total bacteria and other populations for samples LALF10-W-201908151100, GWEX2-W-201908151257, and GWEX4-W-201908151440.

Sample Name **Sample Date**	**LALF10-W-201908151100** **08/15/2019**	**GWEX2-W-201908151257** **08/15/2019**	**GWEX4-W-201908151440** **08/15/2019**
Other	cells/mL	cells/mL	cells/mL
Total Eubacteria (EBAC)	**1.49E+05**	**7.86E+05**	**2.62E+05**
Sulfate Reducing Bacteria (APS)	**5.79E+02**	**7.60E+03**	**2.55E+03**
Methanogens (MGN)	<4.90E+00	<5.10E+00	<4.80E+00

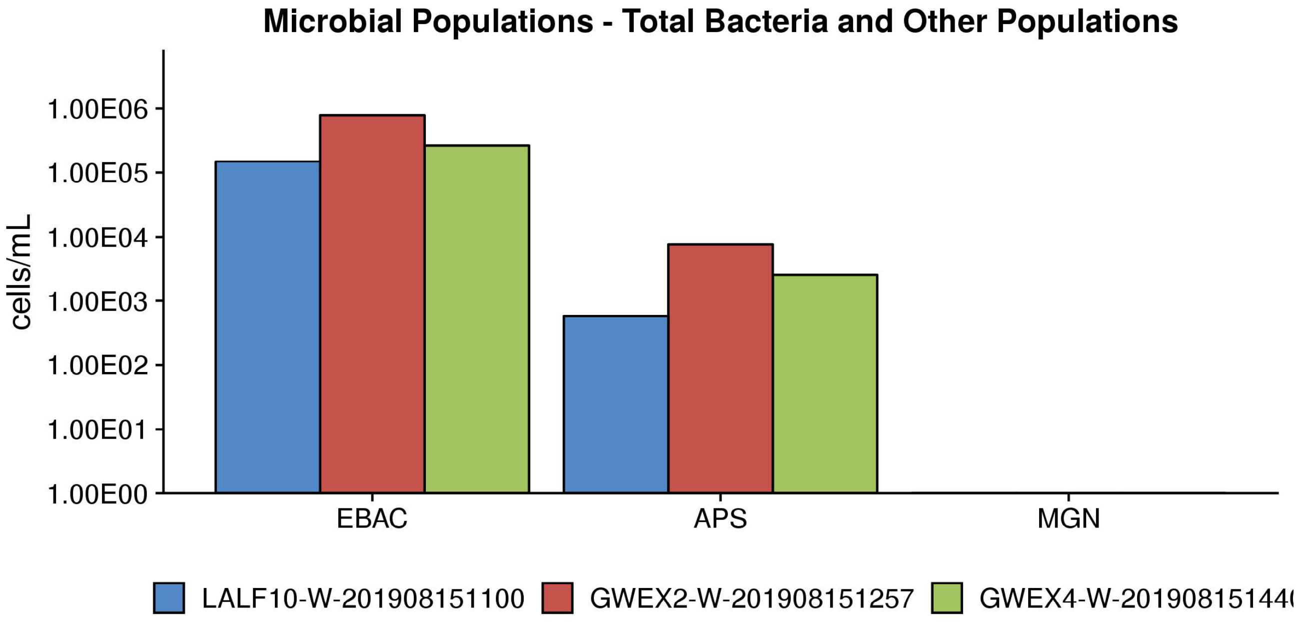


Figure 14: Comparison - microbial populations.

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), 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

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].

References

1. Gerritse, J. *et al.* Influence of different electron donors and acceptors on dehalorespiration of tetrachloroethene by *Desulfitobacterium frappieri* TCE1. *Applied and Environmental Microbiology* **65,** 5212–5221 (1999).
2. Gerritse, J. *et al.* *Desulfitobacterium* sp. strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho-chlorinated phenols. *Archives of Microbiology* **165,** 132–140 (1996).
3. Holliger, C., Schraa, G., Stams, A. & Zehnder, A. A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. *Applied and Environmental Microbiology* **59,** 2991–2997 (1993).
4. Krumholz, L. R., Sharp, R. & Fishbain, S. S. A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation. *Applied and Environmental Microbiology* **62,** 4108–4113 (1996).
5. Loffler, F. E., Sanford, R. A. & Tiedje, J. M. Initial Characterization of a Reductive Dehalogenase from *Desulfitobacterium chlororespirans* Co23. *Applied and Environmental Microbiology* **62,** 3809–3813 (1996).
6. Maymó-Gatell, X., Anguish, T. & Zinder, S. H. Reductive dechlorination of chlorinated ethenes and 1, 2-dichloroethane by *"Dehalococcoides ethenogenes"* 195. *Applied and Environmental Microbiology* **65,** 3108–3113 (1999).
7. Hendrickson, E. R. *et al.* Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. *Applied and Environmental Microbiology* **68,** 485–495 (2002).
8. Lu, X., Wilson, J. T. & Kampbell, D. H. Relationship between *Dehalococcoides* DNA in ground water and rates of reductive dechlorination at field scale. *Water Research* **40,** 3131–3140 (2006).
9. Adrian, L., Szewzyk, U., Wecke, J. & Görisch, H. Bacterial dehalorespiration with chlorinated benzenes. *Nature* **408,** 580–583 (2000).
10. Amos, B. K., Suchomel, E. J., Pennell, K. D. & Löffler, F. E. Spatial and temporal distributions of Geobacter lovleyi and Dehalococcoides spp. during bioenhanced PCE-NAPL dissolution. *Environmental Science & Technology* **43,** 1977–1985 (2009).
11. Duhamel, M. & Edwards, E. A. Growth and yields of dechlorinators, acetogens, and methanogens during reductive dechlorination of chlorinated ethenes and dihaloelimination of 1, 2-dichloroethane. *Environmental Science & Technology* **41,** 2303–2310 (2007).
12. Duhamel, M. *et al.* Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, /textitcis-dichloroethene and vinyl chloride. *Water Research* **36,** 4193–4202 (2002).
13. Grostern, A. & Edwards, E. A. A 1, 1, 1-trichloroethane-degrading anaerobic mixed microbial culture enhances biotransformation of mixtures of chlorinated ethenes and ethanes. *Applied and Environmental Microbiology* **72,** 7849–7856 (2006).
14. Huang, D. & Becker, J. G. Determination of intrinsic monod kinetic parameters for two heterotrophic tetrachloroethene (PCE)-respiring strains and insight into their application. *Biotechnology and Bioengineering* **104,** 301–311 (2009).
15. Mayer-Blackwell, K. *et al.* 1, 2-Dichloroethane exposure alters the population structure, metabolism, and kinetics of a trichloroethene-dechlorinating dehalococcoides mccartyi consortium. *Environmental Science & Technology* **50,** 12187–12196 (2016).
16. Krajmalnik-Brown, R. *et al.* Genetic identification of a putative vinyl chloride reductase in Dehalococcoides sp. strain BAV1. *Applied and Environmental Microbiology* **70,** 6347–6351 (2004).
17. Müller, J. A. *et al.* Molecular identification of the catabolic vinyl chloride reductase from *Dehalococcoides* sp. strain VS and its environmental distribution. *Applied and Environmental Microbiology* **70,** 4880–4888 (2004).
18. Ritalahti, K. M. *et al.* Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. *Applied and Environmental Microbiology* **72,** 2765–2774 (2006).

19. Molenda, O., Quaile, A. T. & Edwards, E. A. Dehalogenimonas sp. strain WBC-2 genome and identification of its trans-dichloroethene reductive dehalogenase, TdrA. *Applied and Environmental Microbiology* **82,** 40–50 (2016).
20. Yang, Y. *et al.* Grape pomace compost harbors organohalide-respiring Dehalogenimonas species with novel reductive dehalogenase genes. *The ISME Journal* **11,** 2767 (2017).
21. Maillard, J. *et al.* Reductive dechlorination of tetrachloroethene by a stepwise catalysis of different organohalide respiring bacteria and reductive dehalogenases. *Biodegradation* **22,** 949–960 (2011).
22. Grostern, A. & Edwards, E. A. Growth of Dehalobacter and Dehalococcoides spp. during degradation of chlorinated ethanes. *Applied and Environmental Microbiology* **72,** 428–436 (2006).
23. Moe, W. M., Yan, J., Nobre, M. F., da Costa, M. S. & Rainey, F. A. *Dehalogenimonas lykanthroporepellens* gen. nov., sp. nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater. *International Journal of Systematic and Evolutionary Microbiology* **59,** 2692–2697 (2009).
24. Yan, J., Rash, B., Rainey, F. & Moe, W. Isolation of novel bacteria within the Chloroflexi capable of reductive dechlorination of 1, 2, 3-trichloropropane. *Environmental Microbiology* **11,** 833–843 (2009).
25. De Wildeman, S., Diekert, G., Van Langenhove, H. & Verstraete, W. Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes. *Applied and Environmental Microbiology* **69,** 5643–5647 (2003).
26. Tang, S. & Edwards, E. A. Identification of *Dehalobacter* reductive dehalogenases that catalyse dechlorination of chloroform, 1,1,1-trichloroethane and 1,1-dichloroethane. *Phil. Trans. R. Soc. B* **368,** 20120318 (2013).
27. Grostern, A., Duhamel, M., Dworatzek, S. & Edwards, E. A. Chloroform respiration to dichloromethane by a *Dehalobacter* population. *Environmental Microbiology* **12,** 1053–1060 (2010).
28. Justicia-Leon, S. D., Ritalahti, K. M., Mack, E. E. & Löffler, F. E. Dichloromethane fermentation by a *Dehalobacter* sp. in an enrichment culture derived from pristine river sediment. *Applied and Environmental Microbiology* **78,** 1288–1291 (2012).
29. Field, J. A. & Sierra-Alvarez, R. Microbial degradation of chlorinated benzenes. *Biodegradation* **19,** 463–480 (2008).
30. Jayachandran, G., Görisch, H. & Adrian, L. Dehalorespiration with hexachlorobenzene and pentachlorobenzene by *Dehalococcoides* sp. strain CBDB1. *Archives of Microbiology* **180,** 411–416 (2003).
31. Wu, Q. *et al.* Dechlorination of chlorobenzenes by a culture containing bacterium DF-1, a PCB dechlorinating microorganism. *Environmental Science & Technology* **36,** 3290–3294 (2002).
32. Fung, J. M. *et al.* Reductive dehalogenation of dichlorobenzenes and monochlorobenzene to benzene in microcosms. *Environmental Science & Technology* **43,** 2302–2307 (2009).
33. Nelson, J. L., Fung, J. M., Cadillo-Quiroz, H., Cheng, X. & Zinder, S. H. A role for *Dehalobacter* spp. in the reductive dehalogenation of dichlorobenzenes and monochlorobenzene. *Environmental Science & Technology* **45,** 6806–6813 (2011).
34. Adrian, L., Hansen, S. K., Fung, J. M., Görisch, H. & Zinder, S. H. Growth of *Dehalococcoides* strains with chlorophenols as electron acceptors. *Environmental Science & Technology* **41,** 2318–2323 (2007).
35. Bouchard, B. *et al.* Isolation and characterization of *Desulfitobacterium frappieri* sp. nov., an anaerobic bacterium which reductively dechlorinates pentachlorophenol to 3-chlorophenol. *International Journal of Systematic and Evolutionary Microbiology* **46,** 1010–1015 (1996).
36. Sanford, R. A., Cole, J. R., Löffler, F. & Tiedje, J. M. Characterization of *Desulfitobacterium chlororespirans* sp. nov., which grows by coupling the oxidation of lactate to the reductive dechlorination of 3-chloro-4-hydroxybenzoate. *Applied and Environmental Microbiology* **62,** 3800–3808 (1996).
37. Field, J. & Sierra-Alvarez, R. Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds. *Reviews in Environmental Science and Biotechnology* **3,** 185–254 (2004).

38. Chang, H.-L. & Alvarez-Cohen, L. Biodegradation of individual and multiple chlorinated aliphatic hydrocarbons by methane-oxidizing cultures. *Applied and Environmental Microbiology* **62,** 3371–3377 (1996).
39. Colby, J., Stirling, D. I. & Dalton, H. The soluble methane mono-oxygenase of Methylococcus capsulatus (Bath). Its ability to oxygenate n-alkanes, n-alkenes, ethers, and alicyclic, aromatic and heterocyclic compounds. *Biochemical Journal* **165,** 395–402 (1977).
40. Oldenhuis, R., Oedzes, J. Y., Van der Waarde, J. & Janssen, D. B. Kinetics of chlorinated hydrocarbon degradation by Methylosinus trichosporium OB3b and toxicity of trichloroethylene. *Applied and Environmental Microbiology* **57,** 7–14 (1991).
41. Van Hylckama, V. J., De Koning, W. & Janssen, D. B. Transformation kinetics of chlorinated ethenes by Methylosinus trichosporium OB3b and detection of unstable epoxides by on-line gas chromatography. *Applied and Environmental Microbiology* **62,** 3304–3312 (1996).
42. Futamata, H., Harayama, S. & Watanabe, K. Group-specific monitoring of phenol hydroxylase genes for a functional assessment of phenol-stimulated trichloroethylene bioremediation. *Applied and Environmental Microbiology* **67,** 4671–4677 (2001).
43. McClay, K., Streger, S. H. & Steffan, R. J. Induction of toluene oxidation activity in Pseudomonas mendocina KR1 and Pseudomonas sp. strain ENVPC5 by chlorinated solvents and alkanes. *Applied and Environmental Microbiology* **61,** 3479–3481 (1995).
44. Newman, L. M. & Wackett, L. P. Trichloroethylene oxidation by purified toluene 2-monooxygenase: products, kinetics, and turnover-dependent inactivation. *Journal of Bacteriology* **179,** 90–96 (1997).
45. Byrne, A. M. & Olsen, R. H. Cascade regulation of the toluene-3-monooxygenase operon (tbuA1UBVA2C) of *Burkholderia pickettii* PKO1: role of the tbuA1 promoter (PtbuA1) in the expression of its cognate activator, TbuT. *Journal of Bacteriology* **178,** 6327–6337 (1996).
46. Wackett, L. P. & Gibson, D. T. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with Pseudomonas putida F1. *Applied and Environmental Microbiology* **54,** 1703–1708 (1988).
47. Leahy, J. G., Byrne, A. M. & Olsen, R. H. Comparison of factors influencing trichloroethylene degradation by toluene-oxidizing bacteria. *Applied and Environmental Microbiology* **62,** 825–833 (1996).
48. Wiedemeier, T. H., Wilson, J. T., Freedman, D. L. & Lee, B. *Providing Additional Support for MNA by Including Quantitative Lines of Evidence for Abiotic Degradation and Co-metabolic Oxidation of Chlorinated Ethylenes* tech. rep. (TH Wiedemeier and Associates, Inc. Sedalia United States, 2017).
49. Mattes, T. E., Alexander, A. K. & Coleman, N. V. Aerobic biodegradation of the chloroethenes: pathways, enzymes, ecology, and evolution. *FEMS Microbiology Reviews* **34,** 445–475 (2010).
50. Coleman, N. V. & Spain, J. C. Epoxyalkane: coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of *Mycobacterium* strain JS60. *Journal of Bacteriology* **185,** 5536–5545 (2003).
51. Dominguez, R. F. *et al.* Aerobic bioremediation of chlorobenzene source-zone soil in flow-through columns: performance assessment using quantitative PCR. *Biodegradation* **19,** 545–553 (2008).
52. La Roche, S. D. & Leisinger, T. Sequence analysis and expression of the bacterial dichloromethane dehalogenase structural gene, a member of the glutathione S-transferase supergene family. *Journal of Bacteriology* **172,** 164–171 (1990).