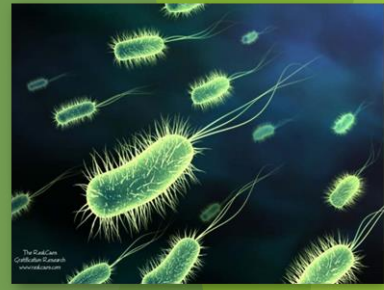


# Enhanced *In Situ* Biodegradation Evaluation of Chlorinated Ethenes in Low Permeability Groundwater

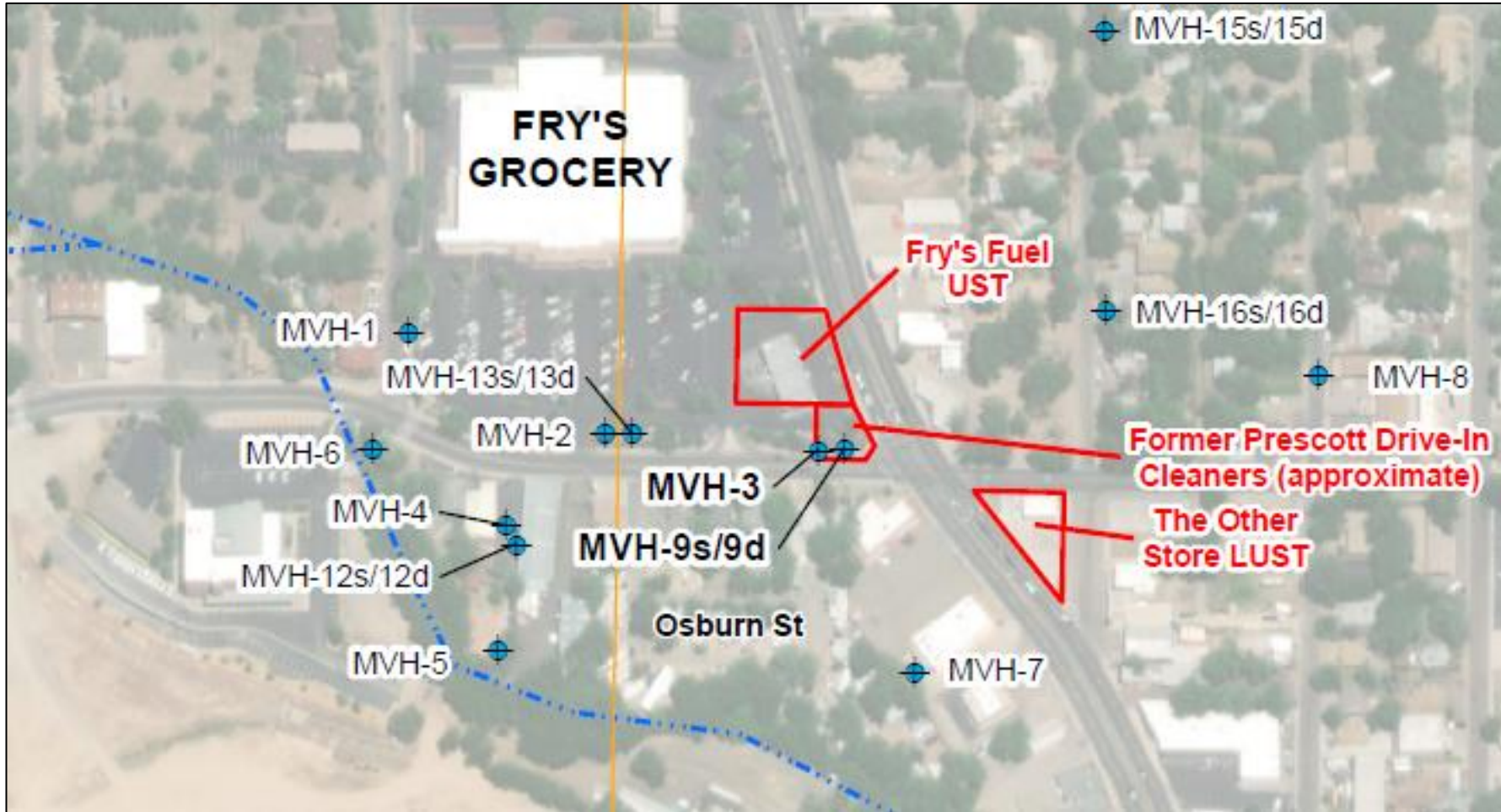
Bridget Hoagland, Matrix New World Engineering  
Laurie LaPat-Polasko, PhD, QEP, Matrix New World Engineering  
Jeremy Gniffke, RG, Matrix New World Engineering  
Georgia Waters, RG, Matrix New World Engineering  
Hazel Cox, PhD, Arizona Dept. of Environmental Quality  
Adam Nagle, Arizona Dept. of Environmental Quality



# Site Background Information

- ▶ Site located in Prescott, Arizona
- ▶ Major contaminants include chlorinated volatile organic compounds (CVOCs): tetrachloroethene (PCE), trichloroethene (TCE) and *cis*-1,2-dichloroethene (*cis*-1,2-DCE).
- ▶ Contamination first detected in 2002. Listed on WQARF registry on December 12, 2016.
- ▶ Potential sources include former dry cleaners, fuel island, and former Other Store.
- ▶ Soil-gas: PCE contamination exceeding Soil Vapor Screening Levels located near former dry cleaners
- ▶ 24 soil borings drilled and sampled to define the horizontal and vertical extent of CVOC contamination.
- ▶ 23 groundwater monitor wells installed.

# Site Location Map



# Site Geology Information

- ▶ Three geologic units are present at the Site
- ▶ The surface unit or Quaternary Alluvium (Qal) extends to depths of 15 to 30 feet below ground surface (ft bgs).
  - ▶ The Qal unit includes unconsolidated, unsorted, poorly bedded clay, silt, sand, pebbles, cobbles, and trace levels of well-rounded boulders.
- ▶ Tertiary Sediments (Ths) are beneath the Qal unit.
  - ▶ Mixture of colluvial/alluvial deposits including silty sand, clayey sand, and decomposed granitic material. Extends from about 30 ft bgs to depths of 140 to 192 ft bgs with alternating wet and dry zones.
- ▶ Below the Ths unit is fractured granodiorite bedrock (Xpr).
- ▶ Miller Creek (ephemeral drainage) is south of the Site. Hydraulic evaluation indicates that the groundwater is not connected to Miller Creek.

# Site Hydrogeology Information

- ▶ Intermittent perched groundwater zone at approximately 16 ft bgs is present in some areas.
- ▶ Groundwater below the perched zone: three separate depth intervals (aquifers) based on lithology and hydrogeologic characteristics:
  - ▶ 1) a shallow depth interval from approximately 29 to 50 ft bgs,
  - ▶ 2) an intermediate depth interval from approximately 80 to 115 ft bgs, and
  - ▶ 3) a deep interval from approximately 165 to 200 ft bgs.
- ▶ A significant undersaturated zone extends from about 50 to 80 ft bgs between the shallow and intermediate depth interval groundwater.

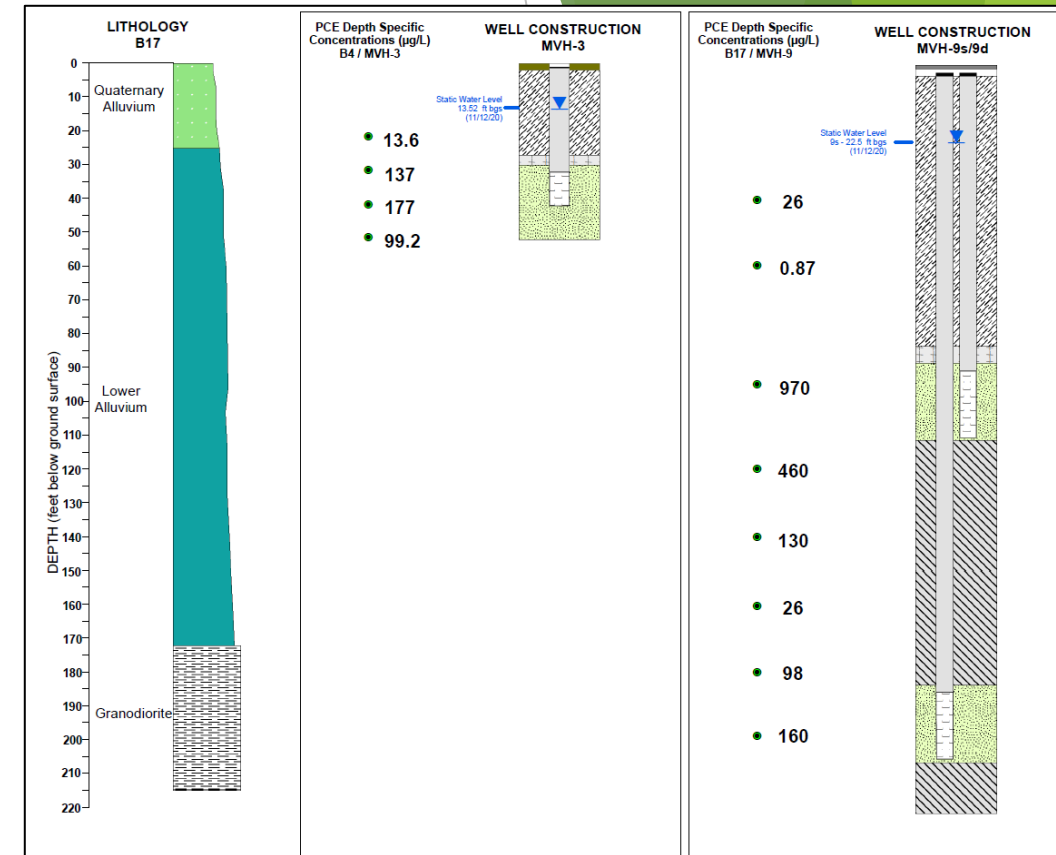
# Lithology & Well Construction of MVH-3 and MVH-9s/9d

## MVH-3

- ▶ Drilled to 52 ft bgs
  - ▶ Screen = 32 to 42 ft bgs
  - ▶ Depth to water = 13.52 ft bgs (Nov 2020)
- ▶ Hydropunch every 10 ft starting at 22 ft bgs
- ▶ Elevated levels of PCE above AWQS below screen

## MVH9s/9d

- ▶ Drilled to 205 ft bgs
  - ▶ MVH-9s screen from 90- 110 ft bgs
  - ▶ MVS-9d screen from 185-205 ft bgs
- ▶ Depth to water - MVH-9s = 22.5 ft bgs (Nov 2020)
- ▶ Hydropunch samples collected every ~ 20 ft
- ▶ Elevated levels of PCE between MVS-9s and MVH-9d screen



# Site Hydrogeology Information

## Shallow Aquifer

- ▶ Flow directions are toward the east
- ▶ Hydraulic gradient of 0.009 ft/ft
- ▶ Hydraulic Conductivity – 0.0061 to 4.1 ft/day
- ▶ Total porosity = 20%; Effective porosity = 10%

*From RI Report (HydroGeochem, 2020)*

## Intermediate Aquifer

- ▶ Flow direction are toward the northeast
- ▶ Hydraulic gradient – 0.014ft/ft
- ▶ Hydraulic Conductivity – 0.12 ft/day
- ▶ Total porosity = 20%; Effective porosity = 10%

*From Groundwater Pilot Test EW-1 (HydroGeochem, 2020)*

# 2020 Chlorinated Volatile Organic Compounds in Site Groundwater

Well ID	Date Sampled	Depth Sampled (ft bgs)	PCE (µg/L)	TCE (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)
MVH-9s	8/26/20	100	<b>670</b>	<4.75	6.53	<5.85
MVH-3	8/26/20	40	<b>273</b>	<b>8.74</b>	5.09	<2.34
AWQS:			5	5	70	2

- ▶ Main CVOC in site groundwater is tetrachloroethene (PCE)
- ▶ Low levels of trichloroethene (TCE) and *cis*-1,2-dichloroethene (*cis*-1,2-DCE) also observed
- ▶ Reductive dechlorination occurring on a limited basis as demonstrated by the presence of TCE and *cis*-1,2-DCE



# 2023 Field Parameters in Site Groundwater

Well ID	Target Installation Depth (Feet bTOC)	Water Parameters					
		Ferrous Iron (mg/L)	pH	Temp (°C)	Specific Cond. (µS/cm)	DO (mg/L)	ORP (mV)
MVH-3	35	0.62	6.90	13.11	167	8.01	191.3
MVH-9s	100	0.48	7.15	15.79	394	6.45	144.1
INJ-1S	37	0.42	7.49	16.16	411	8.19	96.8
INJ-1D	100	0.48	7.50	13.66	199	8.05	155.0
INJ-2S	34.5	0.00	--	--	--	--	--
INJ-2D	100	2.90	12.11	16.83	4626	6.45	74.6

Notes: bTOC= below top of casing  
°C = degrees Celsius

mg/L= milligrams per liter  
µS/cm = microsiemens per centimeter

DO= Dissolved Oxygen      mV= millivolts  
ORP= Oxidation Reduction Potential

# General Groundwater Quality Data

Well ID	Units	MVH-3	MVH-9s	MVH-9d
		10/11/2018	10/11/2018	10/10/2018
<b>Inorganic Chemistry</b>				
Chloride	mg/L	15	46	10
Nitrate	mg/L	2.9	>30	2.7
Sulfate	mg/L	9.3	21	<2.0
Sulfide	mg/L	0.12	0.75	3.15
Ferrous Iron	mg/L	0.02	0.25	0.02
Manganese	mg/L	<0.1	0.1	0.4
<b>General Chemistry</b>				
Alkalinity as CaCO3	mg/L	110	140	130
Bicarbonate	mg/L	134.2	170.8	158.6
Carbonate	mg/L	<6.0	<6.0	<6.0
<b>Dissolved Gases</b>				
Methane	µg/L	<0.00099	<0.00099	0.012
Ethane	µg/L	<0.0020	<0.0020	0.0057
Ethene	µg/L	<0.0028	<0.0028	<0.0028

Source: Hydro Geo Chem Inc. April 2, 2020 Remedial Investigation Report, Miller Valley Rd and Hillside Ave.

**Notes:** \* = WQARF Site

mg/L = Milligrams per liter

µg/L = Micrograms per liter

< = Less than analytical method limit of detection

# Groundwater Microbiology Results

Well ID	MVH-9S	MVH-3	Units
<b>Microbial Parameters Baseline</b>			
Total Biomass	4.28E+04	<1.43E+03	cells/mL
Firmicutes-Terminally Branched Saturated (TerBrSats)	7.48	0	Percent
Proteobacteria-Monoenoic (Monos)	58.44	0	Percent
General-Normal Saturated (Nsats)	22.48	0	Percent
SRB/Actinomycetes- Mid-Chain Branched Saturated (MidBrSats)	4.99	0	Percent
Eukaryotes (Polyenoics)	6.61	0	Percent
Anaerobic Metal Reducers	3.87	0	Percent
<i>Dehalococcoides</i>	<0.5	<0.5	cells/mL
<i>Dehalogenimonas</i>	<4.60	<4.60	cells/mL
<b>Notes:</b>			
mL = Milliliter			
< = Less than the analytical detection limit			

# What's inhibiting biodegradation of CVOCs?

- Dissolved Oxygen (DO): 6 – 8 milligrams per liter (mg/L)
- Nitrate: <2 – > 30 mg/L
- Sulfate: 9 – >20 mg/L
- Total Organic Carbon: 0.6 – 0.9 mg/L
- Total Kjeldahl Nitrogen:  $\leq 0.14$  mg/L
- Ammonia:  $\leq 0.17$  mg/L
- Phosphorus: <0.33 mg/L
- Native microbes need FOOD!!



# Shallow and Intermediate Groundwater Elevation Maps (January 2019)

## Shallow Aquifer

- ▶ Flow directions are toward the east
- ▶ Hydraulic gradient of 0.009 ft/ft
- ▶ Hydraulic Conductivity – 0.0061 to 4.1 ft/day
- ▶ Total porosity = 20%; Effective porosity = 10%

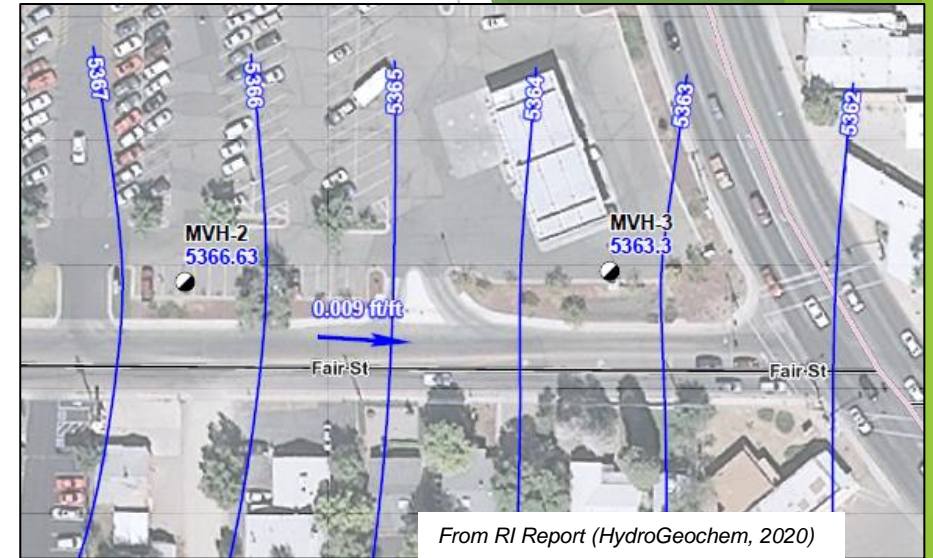
*From RI Report (HydroGeochem, 2020)*

## Intermediate Aquifer

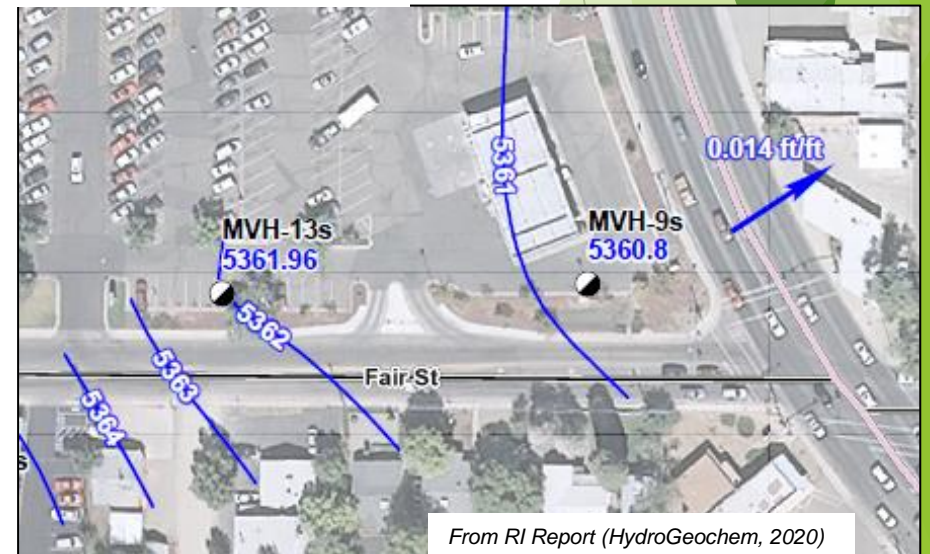
- ▶ Flow direction are toward the northeast
- ▶ Hydraulic gradient – 0.014ft/ft
- ▶ Hydraulic Conductivity – 0.12 ft/day
- ▶ Total porosity = 20%; Effective porosity = 10%

*From Groundwater Pilot Test EW-1 (HydroGeochem, 2020)*

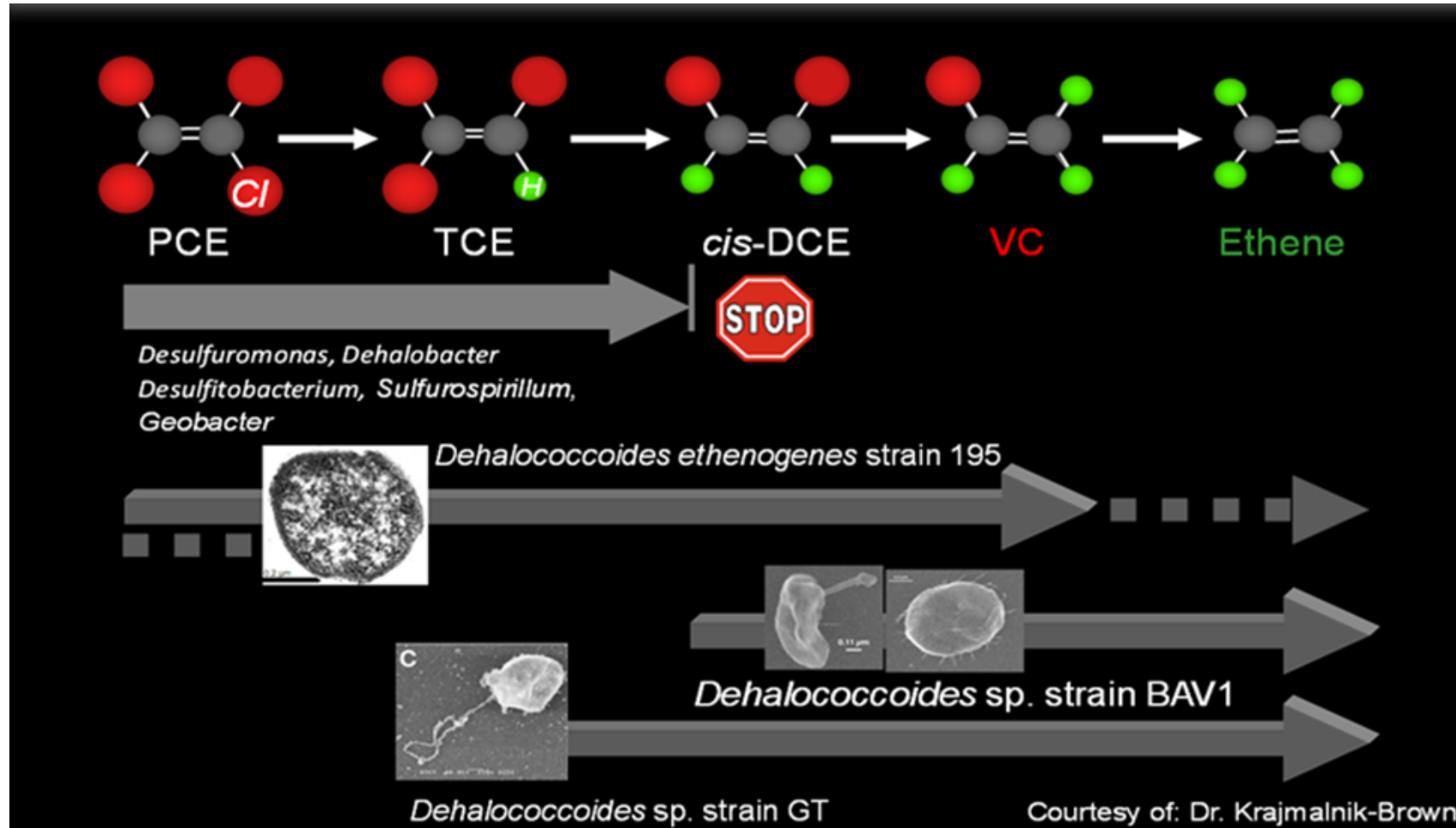
Shallow Aquifer



Intermediate Aquifer



# Why Enhanced Anaerobic Biodegradation?



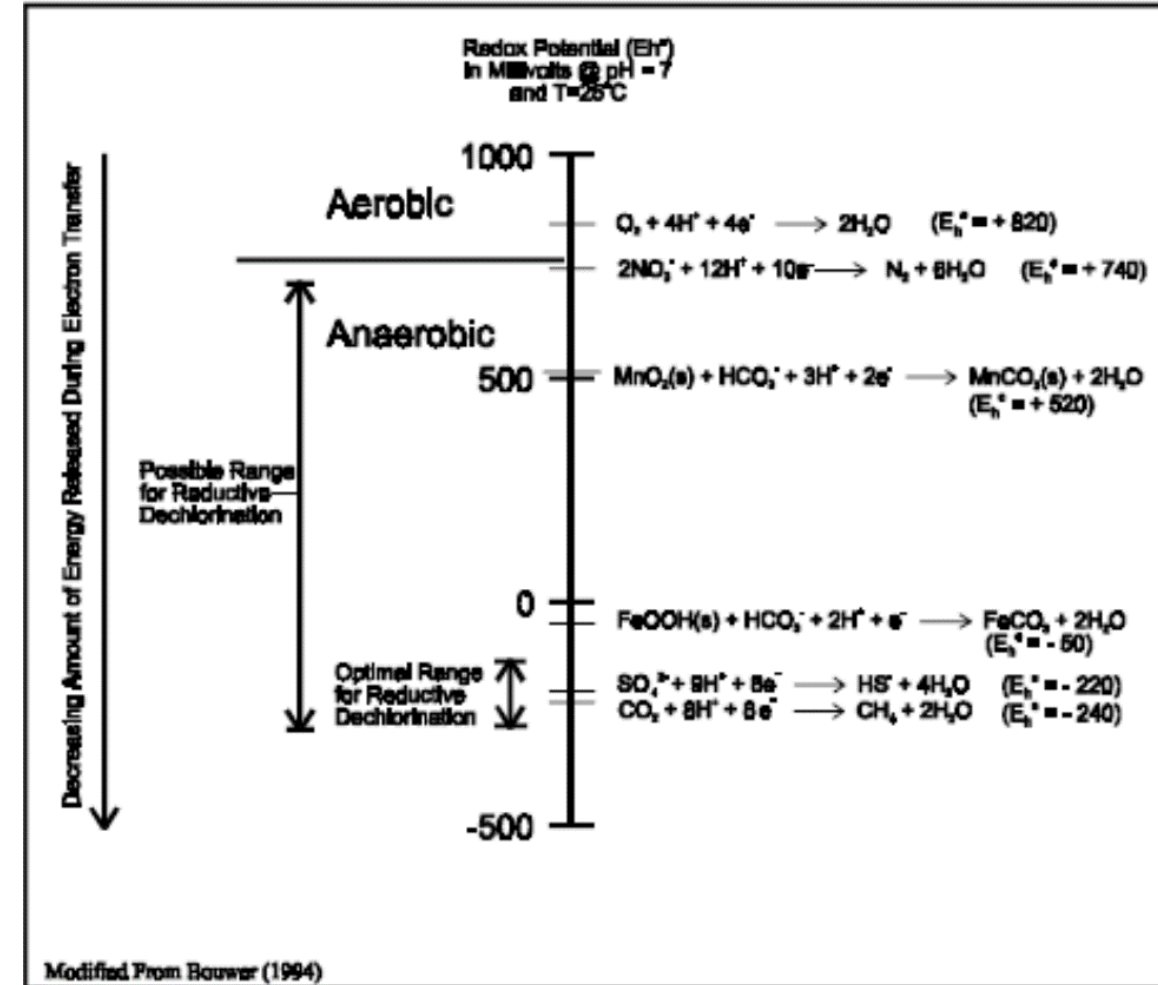
# Significance of Oxidation Reduction Potential

Aerobic Biodegradation: electron acceptor is oxygen

- ▶ Petroleum Hydrocarbons
- ▶ MTBE
- ▶ Less chlorinated, e.g., vinyl chloride

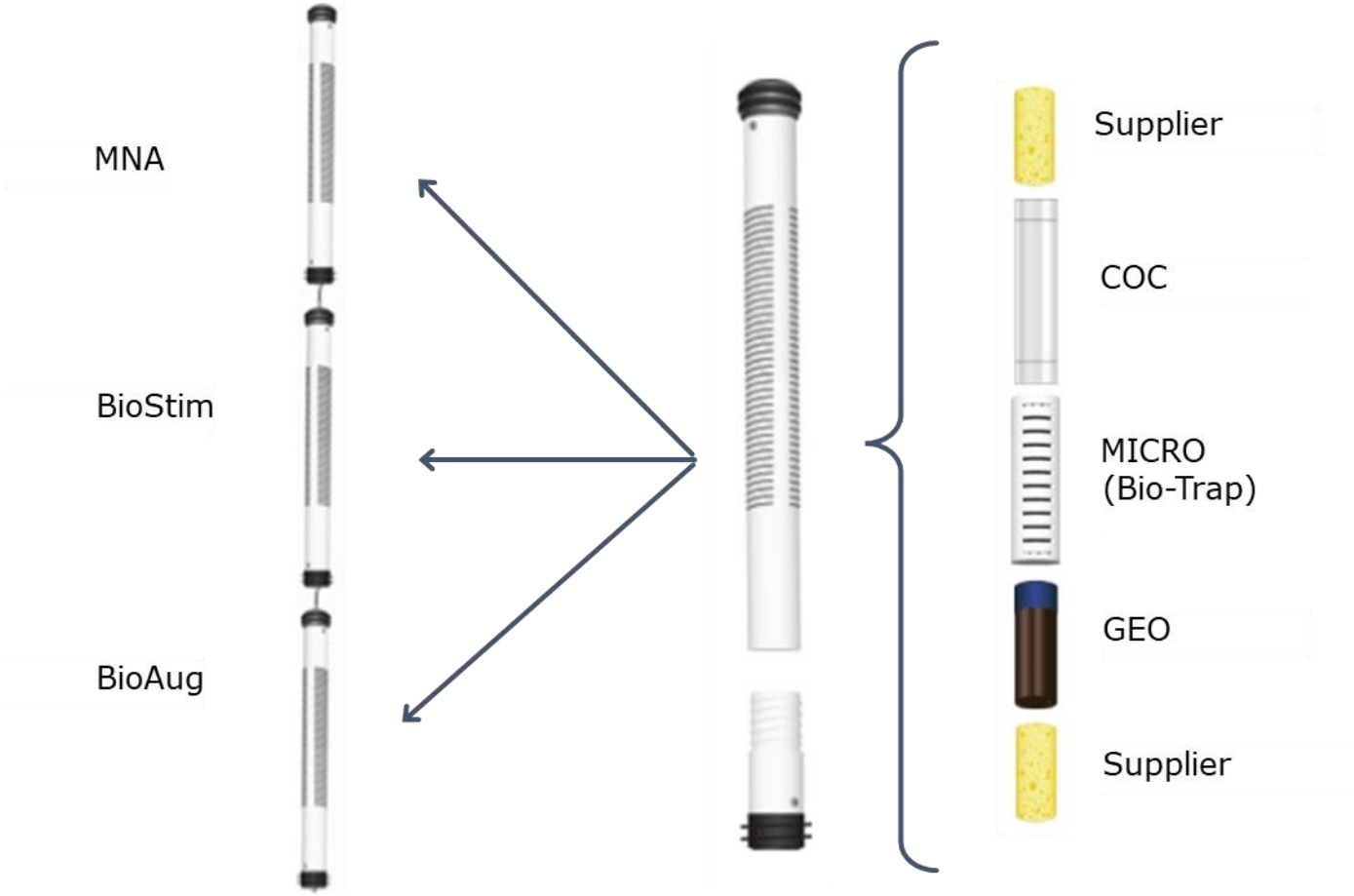
Anaerobic Biodegradation

- ▶ Nitrate reduction (Denitrification); Perchlorate
- ▶ Sulfate reduction (Sulfide formation)
- ▶ Acid formation (-100 to -200 mV)
- ▶ Reductive dechlorination (<-50 mV)
- ▶ Methane formation (-200 mV to -350 mV)



# In Situ Microcosm(ISM) Set Up

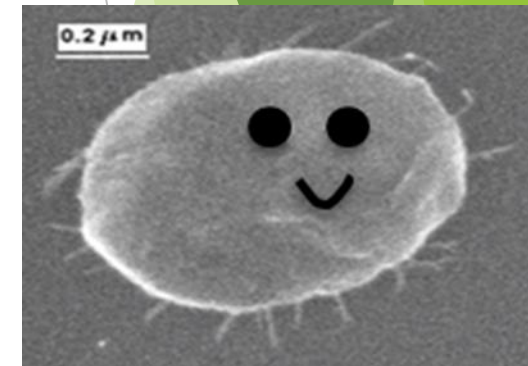
- ▶ In Situ Microcosms (ISM) installed in MVH-3 and MVH-9s





# In Situ Microcosm(ISM) Set Up

- ▶ Purpose: to evaluate the potential to promote enhanced reductive dechlorination in site groundwater
- ▶ BioStimulation units included:
  - MVH-9s – EOS PRO + CoBupHMg
  - MVH-3 – Wilclear Plus + CoBupHMg
- ▶ BioAugmentation units included:
  - MVH-9s EOS PRO + CoBupHMg + SDC-9<sup>®</sup>
  - MVH-9s EOS PRO + CoBupHMg + KB-1<sup>®</sup>
  - MVH-9s Wilclear Plus + CoBupHMg + SDC-9<sup>®</sup>
  - MVH-3 Wilclear Plus + CoBupHMg + SDC-9<sup>®</sup>
  - MVH-3 Wilclear Plus + CoBupHMg + KB-1<sup>®</sup>



# Review of ISM CVOC and Gas Results

► BioStim = EOS PRO in MVH-9s

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE* (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride* (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	100	670	<4.75	6.53	<5.85	NA	NA
EOS PRO	11/12/20	92	1.6	1.5	366	0.9	734	<1.3

Change in Concentration (µg/L):                    668.4            0.875            +359.47            2.025

**Percent Reduction/Increase:**                    **-99.76%**            **-36.84%**            **+5,504%**            **-69.23%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

► BioAug = EOS PRO and SDC-9 in MVH-9s

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE* (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride* (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	100	670	< 4.75	6.53	<5.85	NA	NA
EOSPRO and SDC-9	11/12/20	99	2.0	0.6	200	44.3	21,200	12

Change in Concentration (µg/L):                    668            1.775            +193.47            +41.375

**Percent Reduction/Increase:**                    **-99.70%**            **-74.74%**            **+2,962%**            **+1414%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

# Review of In Situ Microcosm Results

## ► BioStim = EOS PRO in MVH-9s

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE* (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride* (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	100	670	<4.75	6.53	<5.85	NA	NA
EOS PRO	11/12/20	92	1.6	1.5	366	0.9	734	<1.3
Change in Concentration (µg/L):			668.4	0.875	+359.47	2.025		
<b>Percent Reduction/Increase:</b>			<b>-99.76%</b>	<b>-36.84%</b>	<b>+5,504%</b>	<b>-69.23%</b>		

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

## ► BioAug = EOS PRO + KB-1 in MVH-9s

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE* (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride* (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	100	670	< 4.75	6.53	<5.85	NA	NA
EOS PRO and KB-1	11/12/20	96	5	2.3	468	2.7	<1.3	<1.3
Change in Concentration (µg/L):			665	0.075	+461.47	0.225		
<b>Percent Reduction/Increase:</b>			<b>-99.25%</b>	<b>-3.16%</b>	<b>+7,067%</b>	<b>-7.69%</b>		

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

# Review of In Situ Microcosm CVOC and Gas Results

In Situ Microcosms (ISMs) installed MVH-3 and MVH-9s

## ► BioStim = EOS PRO in MVH-9s

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE* (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride* (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	100	670	<4.75	6.53	<5.85	NA	NA
EOS PRO	11/12/20	92	1.6	1.5	366	0.9	734	<1.3

Change in Concentration (µg/L):                      668.4                      0.875                      +359.47                      2.025

**Percent Reduction/Increase:**                      **-99.76%**                      **-36.84%**                      **+5,504%**                      **-69.23%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

## ► BioAug = Wilclear Plus + SDC-9 in MVH-9s

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE* (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	100	670	< 4.75	6.53	<5.85	NA	NA
Wilclear Plus and SDC-9	11/12/20	104	39.4	19.7	198	14	263	6.2

Change in Concentration (µg/L):                      630.6                      +17.325                      +191.47                      +11.075

**Percent Reduction/Increase:**                      **-94.11%**                      **+729.5%**                      **+2,932%**                      **+378.6%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

# Inorganics and VFA Results in MVH-9s

Analyte	Units	Well MVH-9s			
		BioStim: EOS PRO, CoBupHMg	BioStim + BioAug: EOS PRO, CoBupHMg, SDC-9	BioStim + BioAug: EOS PRO, CoBupHMg, KB-1	BioStim + BioAug: Wilclea Plus, CoBupHMg, SDC-9
<b>Inorganic Chemistry</b>					
Nitrate	mg/L	<0.2	<1.0	<0.2	<2.0
Sulfate	mg/L	1.1	0.7 J	0.6	<2.0
<b>Volatile Fatty Acids (VFAs)</b>					
Lactic Acid	mg/L	<0.5	1.4	<0.5	1.5
Pyruvic Acid	mg/L	<0.5	1.7	0.5 J	2.4
Acetic Acid	mg/L	20	240	55	100
Propionic Acid	mg/L	3.1	310	9.2	180
Butyric Acid	mg/L	0.2 J	7.4 J	0.5	13

# ISM Results via PLFA & qPCR in MVH-9s

Analyte	Units	Well MVH-9s			
		BioStim: EOS PRO, CoBupHMg	BioStim + BioAug: EOS PRO, CoBupHMg, SDC-9	BioStim + BioAug: EOS PRO, CoBupHMg, KB-1	BioStim + BioAug: Wilclean Plus, CoBupHMg, SDC-9
<b>Microbial Parameters</b>					
Total Biomass	cells/bead	7.67E+06	NA	NA	NA
<i>Dehalococcoides</i>	cells/bead	1.66E+03	7.68E+06	9.92E+05	1.24E+06
<i>Dehalobacter spp</i>	cells/bead	1.45E+06	<2.50E+02	2.20E+05	1.20 x10 <sup>5</sup>
<i>Dehalogenimonas</i>	cells/bead	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Firmicutes - Terminally Branched Saturated (TerBrSats)	Percent	5.05	NA	NA	NA
Proteobacteria-Monoenoic (Monos)	Percent	32.66	NA	NA	NA
General - Normal Saturated (Nsats)	Percent	58.45	NA	NA	NA
SRB/Actinomycetes - Mid-Chain Branched Saturated (MidBrSats)	Percent	0.48	NA	NA	NA
Eukaryotes (Polyenoics)	Percent	2.89	NA	NA	NA
Anaerobic Metal Reducers	Percent	0.47	NA	NA	NA

# ISM Microbial Results via qPCR in MVH-9s

Analyte	Units	Well MVH-9s		
		BioStim + BioAug: EOS PRO, CoBupHMg, SDC-9	BioStim + BioAug: EOS PRO, CoBupHMg, KB-1	BioStim + BioAug: Wilclear, CoBupHMg, SDC-9
<b>Reductive Dechlorination</b>				
<i>Dehalococcoides (DHC)</i>	Cells/bead	7.68E+06	9.92E+05	1.24E+06
tceA Reductase (TCE)	Cells/bead	1.10E+06	5.87E+04	2.00E+05
BAV1 Vinyl Chloride Reductase (BVC)	Cells/bead	<2.50E+01	9.31E+04	<2.50E+01
Vinyl Chloride Reductase (VCR)	Cells/bead	6.92E+05	1.19E+05	1.34E+05
<i>Dehalobacter spp. (DHBt)</i>	Cells/bead	<2.50E+02	2.22E+05	1.20E+05
<i>Dehalobacter DCM (DCM)</i>	Cells/bead	4.82E+04	2.72E+04	4.82E+04
<i>Desulfitobacterium spp. (DSB)</i>	Cells/bead	2.09E+06	1.06E+05	8.22E+05
<i>Dehalobium chlorocoercia (DECO)</i>	Cells/bead	9.89E+05	7.99E+03	4.14E+05
<i>Desulfuromonas spp. (DSM)</i>	Cells/bead	3.19E+06	4.71E+05	1.63E+06
<b>Other</b>				
Total Eubacteria (EBAC)	Cells/bead	1.19E+09	2.44E+08	5.89E+08
Sulfate Reducing Bacteria (APS)	Cells/bead	3.14E+07	1.27E+05	9.06E+06
Methanogens (MGN)	Cells/bead	1.56E+05	3.05E+03	6.54E+04
<b>Notes:</b>	< =			
	J =			

# ISM CVOC and Gas Results

## ► BioStim = Wilclear Plus in MVH-3

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	40	273	8.74	5.09	<2.34	NA	NA
Wilclear Plus	11/12/20	33	13.4	1.3	3.4	<1.0	2,280	<1.3

Change in Concentration (µg/L):            259.6        7.44        1.69        0.67  
**Percent Reduction/Increase:**            **-95.09%**    **-85.12%**    **-33.20%**    **-42.73%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

## ► BioAug = Wilclear Plus + SDC-9 in MVH-3

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	40	273	8.74	5.09	<2.34	NA	NA
Wilclear Plus and SDC-9	11/12/20	40	71.9	16.7	154	<1.0	84	<1.3

Change in Concentration (µg/L):            201.1        +7.96        +148.91        0.67  
**Percent Reduction/Increase:**            **-73.66%**    **+91.08%**    **+2,926%**    **-42.73%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed



# ISM CVOC and Gas Results

## ► BioStim = Wilclear Plus in MVH-3

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	40	273	8.74	5.09	<2.34	NA	NA
Wilclear Plus	11/12/20	33	13.4	1.3	3.4	<1.0	2,280	<1.3
Change in Concentration (µg/L):			259.6	7.44	1.69	0.67		
<b>Percent Reduction/Increase:</b>			<b>-95.09%</b>	<b>-85.12%</b>	<b>-33.20%</b>	<b>-42.73%</b>		

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

## ► BioAug = Wilclear Plus + SDC-9 in MVH-3

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE (µg/L)	<i>cis</i> -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	40	273	8.74	5.09	<2.34	NA	NA
Wilclear Plus and SDC-9	11/12/20	40	71.9	16.7	154	<1.0	84	<1.3
Change in Concentration (µg/L):			201.1	+7.96	+148.91	0.67		
<b>Percent Reduction/Increase:</b>			<b>-73.66%</b>	<b>+91.08%</b>	<b>+2,926%</b>	<b>-42.73%</b>		

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

# ISM CVOC and Gas Results

## ► BioAug = Wilclear Plus + KB-1 in MVH-3

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE (µg/L)	cis-1,2-DCE (µg/L)	Vinyl Chloride* (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	40	273	8.74	5.09	<2.34	NA	NA
Wilclear Plus and KB-1	11/12/20	37	<1.0	0.6	193	7.8	580	2.4

Change in Concentration (µg/L):                      273                      8.14                      +187.91                      +6.63

**Percent Reduction/Increase:**                      **-99.99%**                      **-93.14%**                      **+3,693%**                      **+566.7%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

## ► BioAug = Wilclear Plus + SDC-9 in MVH-3

Amendment	Date Sampled	Depth (ft bgs)	PCE (µg/L)	TCE (µg/L)	cis-1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Methane (µg/L)	Ethene (µg/L)
None	08/26/20	40	273	8.74	5.09	<2.34	NA	NA
Wilclear Plus and SDC-9	11/12/20	40	71.9	16.7	154	<1.0	84	<1.3

Change in Concentration (µg/L):                      201.1                      +7.96                      +148.91                      0.67

**Percent Reduction/Increase:**                      **-73.66%**                      **+91.08%**                      **+2,926%**                      **-42.73%**

**Notes:** \* Percent reduction/increase and change in concentration determined using ½ the method detection limit. NA- Not analyzed

# ISM Inorganics and VFA Results in MVH-3

Analyte	Units	Well MVH-3		
		BioStim: Wilclear Plus, CoBupHMg	BioStim + BioAug: Wilclear Plus, CoBupHMg, SDC- 9	BioStim + BioAug: Wilclear Plus, CoBupHMg, KB-1
<b>Inorganic Chemistry</b>				
Nitrate	mg/L	<4.0	<1.0	<1.0
Sulfate	mg/L	<4.0	1.2	<1.0
<b>Volatile Fatty Acids (VFAs)</b>				
Lactic Acid	mg/L	<5.0	<0.5	<0.5
Pyruvic Acid	mg/L	4 J	0.2 J	1.6
Acetic Acid	mg/L	330	0.4 J	20
Propionic Acid	mg/L	670	18	70
Butyric Acid	mg/L	98	0.5	0.9

# ISM Microbial Results in MVH-3

Analyte	Units	Well MVH-3		
		BioStim: Wilclear Plus, CoBupHMg	BioStim + BioAug: Wilclear Plus, CoBupHMg, SDC- 9	BioStim + BioAug: Wilclear Plus, CoBupHMg, KB-1
<b>Microbial Parameters</b>				
Total Biomass	cells/bead	NA	1.03E+07	2.88E+06
<i>Dehalococcoides</i>	cells/bead	6.48E+03	1.36E+06	6.22E+05
<i>Dehalobacter spp</i>	cells/bead	4.10E+05	4.10E+05	<2.50E+02
<i>Dehalogenimonas</i>	cells/bead	<2.50E+02	<2.50E+02	<2.50E+02
Firmicutes - Terminally Branched Saturated (TerBrSats)	Percent	NA	23.56	19.71
Proteobacteria-Monoenoic (Monos)	Percent	NA	36.77	47.98
General - Normal Saturated (Nsats)	Percent	NA	29.35	26.35
SRB/Actinomycetes - Mid-Chain Branched Saturated (MidBrSats)	Percent	NA	2.47	2.37
Eukaryotes (Polyenoics)	Percent	NA	0.29	0.39
Anaerobic Metal Reducers	Percent	NA	7.53	3.19

# ISM Microbial Results in MVH-3

Analyte	Units	Well MVH-3		
		BioStim: Wilclear Plus, CoBupHMg	BioStim + BioAug: Wilclear Plus, CoBupHMg, SDC-9	BioStim + BioAug: Wilclear Plus, CoBupHMg, KB-1
<b>Reductive Dechlorination</b>				
<i>Dehalococcoides (DHC)</i>	Cells/bead	6.48E+03	1.36E+06	6.22E+05
tceA Reductase (TCE)	Cells/bead	9.10E+02	2.49E+05	3.77E+04
BAV1 Vinyl Chloride Reductase (BVC)	Cells/bead	2.86E+01	<2.50E+01	3.54E+04
Vinyl Chloride Reductase (VCR)	Cells/bead	7.02E+02	1.64E+05	6.10E+04
<i>Dehalobacter spp. (DHBt)</i>	Cells/bead	4.10E+05	4.10E+05	<2.50E+02
<i>Dehalobacter DCM (DCM)</i>	Cells/bead	5.85E+04	4.29E+04	5.11E+04
<i>Desulfitobacterium spp. (DSB)</i>	Cells/bead	4.66E+03	2.32E+05	1.08E+05
<i>Dehalobium chlorocoercia (DECO)</i>	Cells/bead	1.45E+05	3.17E+05	3.74E+05
<i>Desulfuromonas spp. (DSM)</i>	Cells/bead	3.31E+04	1.75E+06	1.13E+06
<b>Other</b>				
Total Eubacteria (EBAC)	Cells/bead	2.58E+08	4.12E+08	1.84E+08
Sulfate Reducing Bacteria (APS)	Cells/bead	3.45E+05	8.22E+06	1.29E+06
Methanogens (MGN)	Cells/bead	4.68E+04	1.41E+04	3.13E+05
<b>Notes:</b>	< =	Not detected above laboratory reporting limits		
	J =	Estimated value		

# Review of In Situ Microcosm Results

## ► Comparison of ISM units in MVH-3 and MVH-9s

Well ID	ISM Unit Type	Percent Reduction/Increase in Chemical of Concern			Relative Amount of VC and Ethene Produced (µg/L)	
		PCE	TCE	<i>cis</i> -1,2-DCE	VC	Ethene
MVH-9s	BioStim: EOS PRO	-99.76	-36.84*	+5,504	0.9J	< 1.3
	BioStim: EOS PRO + BioAug: SDC-9	-99.70	-74.74*	+2,962	44.3	12
	BioStim: EOS PRO + BioAug: KB-1	-99.25	-3.16*	+7,067	2.7	< 1.3
	BioStim: Wilclear Plus + BioAug SDC-9	-94.11	+729.47*	+2,932	14.1	6.2
MVH-3	BioStim: Wilclear Plus	-95.09	-85.12	-33.20	< 1.0	< 1.3
	BioStim: Wilclear Plus + BioAug: SDC-9	-73.66	+91.08	+2,926	< 1.0	< 1.3
	BioStim: Wilclear Plus + BioAug: KB-1	-99.99	-93.14	+3,693	7.80	2.4

**Notes:** J- The reported value is an estimate  
 \* Percent reduction/increase and change in concentration determined using ½ the method detection limit.

# Review of In Situ Microcosm Results

- ▶ Purpose of ISMs to evaluate the potential for enhanced reductive dechlorination of CVOCs in Site groundwater.
- ▶ In Situ Microcosms (ISMs) incubated for three months in MVH-3 and MVH-9s
- ▶ In MVH-3, Wilclear Plus demonstrated the highest level of overall reductive dechlorination of CVOCs; PCE initial concentration 273 µg/L.
- ▶ In MVH-9s the combinations EOS PRO SDC-9 demonstrated the highest level of overall reductive dechlorination of CVOCs; PCE initial concentration 670 µg/L.

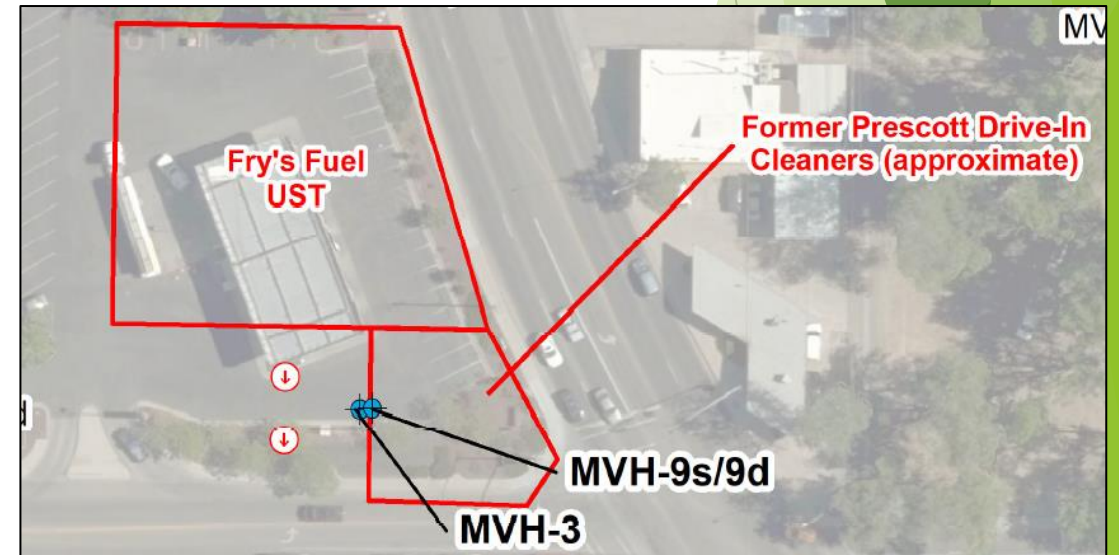
Well-ID <sup>α</sup>	ISM-Unit-Type <sup>α</sup>	Percent-Reduction/Increase-in-Chemical-of-Concern <sup>α</sup>			Relative-Amount-of-VC-and-Ethene-Produced-(µg/L) <sup>α</sup>	
		PCE <sup>α</sup>	TCE <sup>α</sup>	<i>cis</i> -1,2-DCE <sup>α</sup>	VC <sup>α</sup>	Ethene <sup>α</sup>
MVH-9s <sup>¶</sup>	BioStim: EOS-PRO <sup>α</sup>	-99.76 <sup>α</sup>	-36.84* <sup>α</sup>	+5,504 <sup>α</sup>	0.9J <sup>α</sup>	<1.3 <sup>α</sup>
	BioStim: EOS-PRO+ BioAug: SDC-9 <sup>α</sup>	-99.70 <sup>α</sup>	-74.74* <sup>α</sup>	+2,962 <sup>α</sup>	44.3 <sup>α</sup>	12 <sup>α</sup>
	BioStim: EOS-PRO+ BioAug: KB-1 <sup>α</sup>	-99.25 <sup>α</sup>	-3.16* <sup>α</sup>	+7,067 <sup>α</sup>	2.7 <sup>α</sup>	<1.3 <sup>α</sup>
	BioStim: Wilclear-Plus+ BioAug: SDC-9 <sup>α</sup>	-94.11 <sup>α</sup>	+729.47* <sup>α</sup>	+2,932 <sup>α</sup>	14.1 <sup>α</sup>	6.2 <sup>α</sup>
MVH-3 <sup>¶</sup>	BioStim: Wilclear-Plus <sup>α</sup>	-95.09 <sup>α</sup>	-85.12 <sup>α</sup>	-33.20 <sup>α</sup>	<1.0 <sup>α</sup>	<1.3 <sup>α</sup>
	BioStim: Wilclear-Plus+ BioAug: SDC-9 <sup>α</sup>	-73.66 <sup>α</sup>	+91.08 <sup>α</sup>	+2,926 <sup>α</sup>	<1.0 <sup>α</sup>	<1.3 <sup>α</sup>
	BioStim: Wilclear-Plus+ BioAug: KB-1 <sup>α</sup>	-99.99 <sup>α</sup>	-93.14 <sup>α</sup>	+3,693 <sup>α</sup>	7.80 <sup>α</sup>	2.4 <sup>α</sup>

**Notes:** J- The reported value is an estimate<sup>¶</sup>  
 \* - Percent reduction/increase and change in concentration determined using ½ the method detection limit<sup>α</sup>

# In Situ Bioremediation (ISB) Early Implementation

## In Situ Bioremediation Injection Well Design

- ▶ Two nested injection wells (shallow and intermediate aquifers)
- ▶ Drilled using Sonic methodologies
- ▶ Depth specific hydropunch samples to determine vertical contaminant profiles
- ▶ Target screen intervals within shallow and intermediate aquifers where contamination is present
- ▶ 2-inch diameter wells
- ▶ SCH 40 PVC well casing and screen
- ▶ 0.020"ml slots with 12-20 filter pack

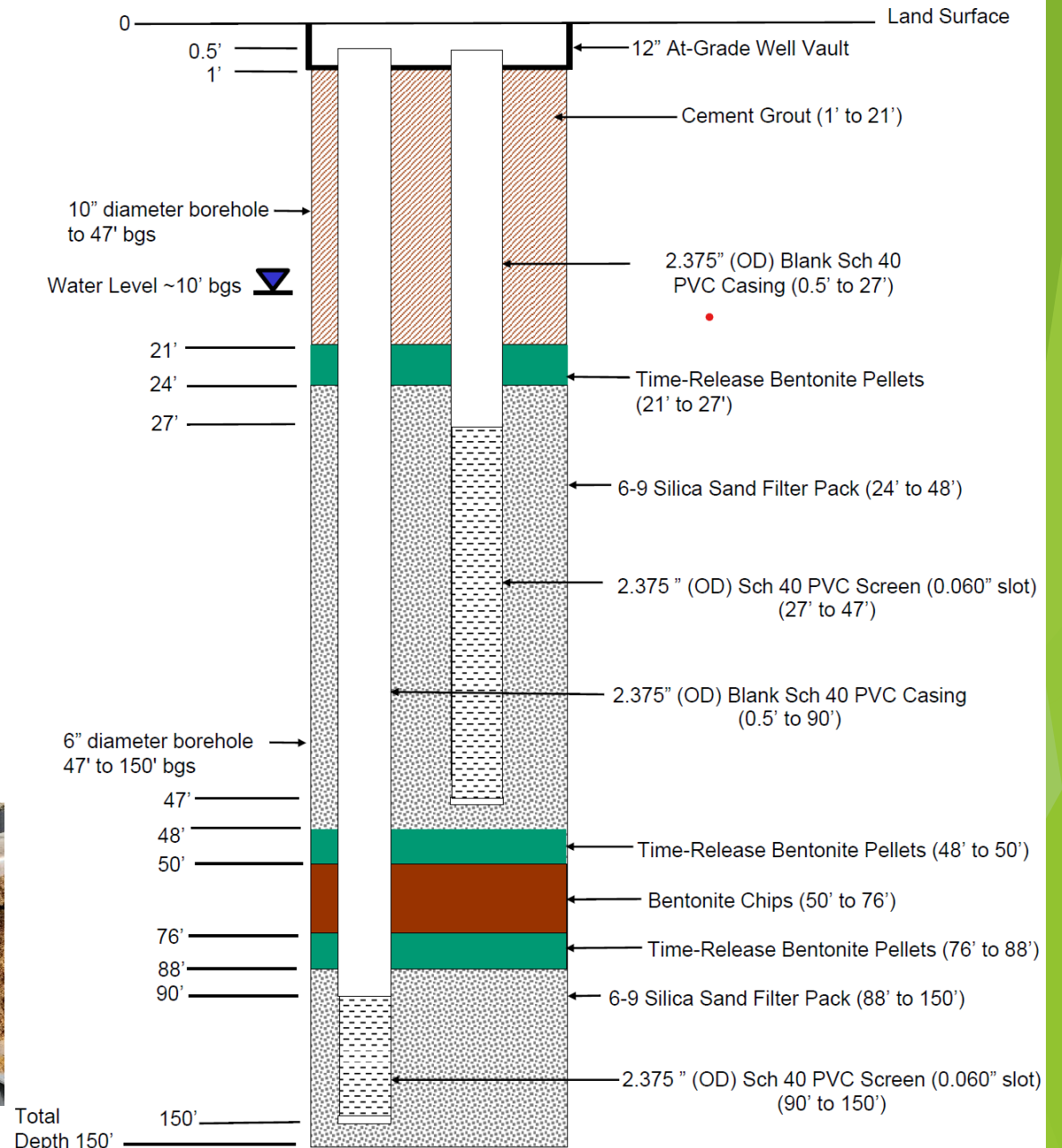




# ISM Early Implementation

## In Situ Bioremediation Injection Well Construction

- ▶ Injection well INJ-1 screened in shallow aquifer
- ▶ Injection well INJ-1D screened in intermediate aquifer
- ▶ 2-inch diameter wells
- ▶ SCH 40 PVC well casing and screen
- ▶ 0.020" slot with 12-20 filter pack



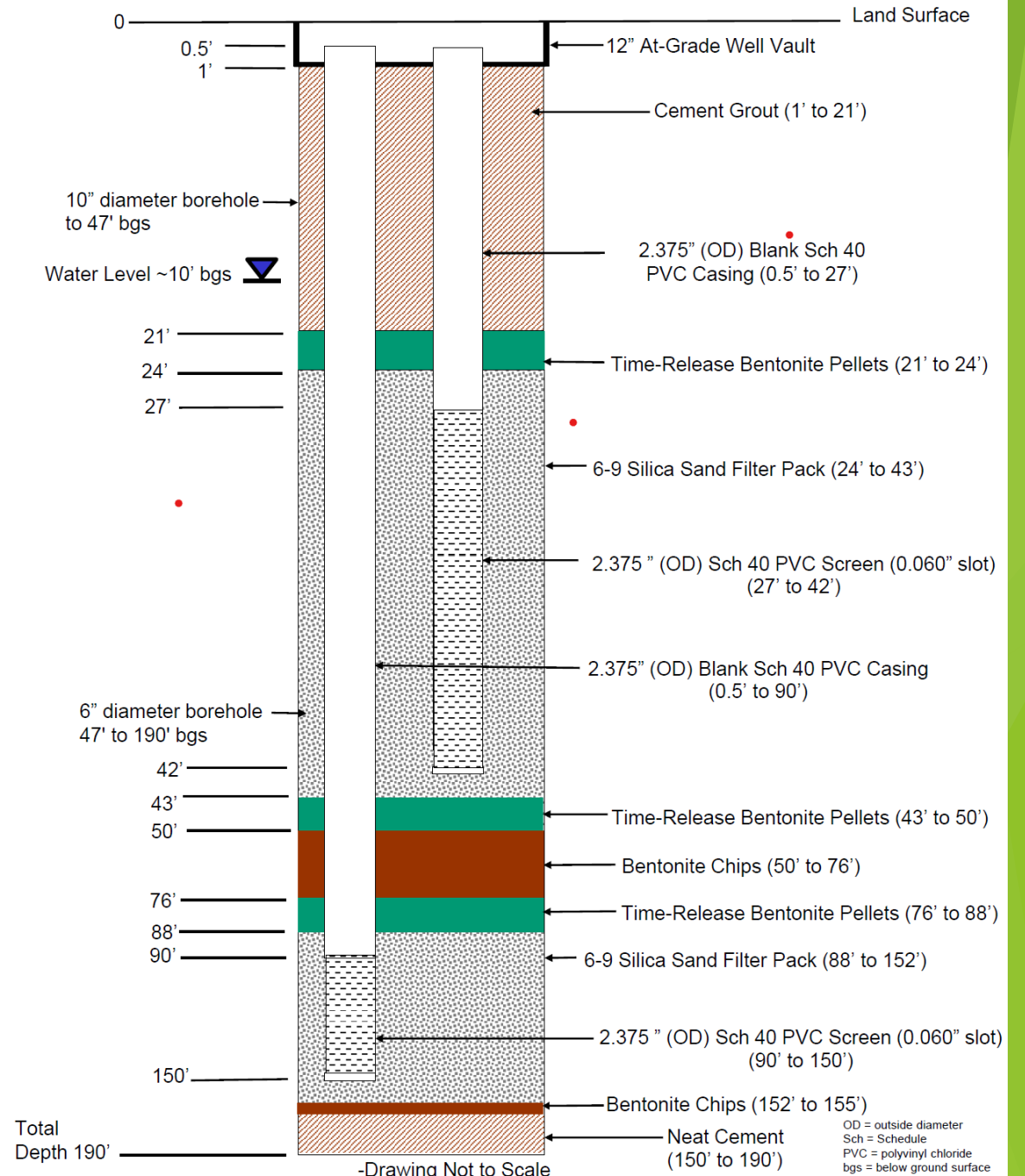
OD = outside diameter  
Sch = Schedule  
PVC = polyvinyl chloride  
bgs = below ground surface

-Drawing Not to Scale

# ISB Early Implementation

## In Situ Bioremediation Injection Well Construction

- ▶ Injection well INJ-2 screened in shallow aquifer
- ▶ Injection well INJ-2D screened in intermediate aquifer
- ▶ 2-inch diameter wells
- ▶ SCH 40 PVC well casing and screen
- ▶ 0.020" slot with 12-20 filter pack



# Recent Groundwater Results

## ➤ CVOC Results in Injection Wells and Site Monitoring Wells

Sample ID	Sample Date	Depth to Groundwater (ft bTOC)	Sample Depth (ft bTOC)	PCE	TCE	<i>cis</i> -1,2-DCE	<i>trans</i> -1,2-DCE	Vinyl Chloride
<b>ADEQ Aquifer Water Quality Standards (AWQS)</b>				<b>5.0</b>	<b>5.0</b>	<b>70</b>	<b>100</b>	<b>2.0</b>
INJ-1S	2/21/2023	3.08	37	<b>7,660</b>	<b>97.0</b>	<b>63.5</b>	<b>1.14</b>	<0.234
INJ-1D	2/21/2023	10.09	100	<b>684</b>	<b>6.80</b>	<b>6.72</b>	<0.149	<0.234
INJ-2S	2/21/2023	6.43	34.5	<b>220</b>	<b>4.93</b>	<b>4.84</b>	<0.149	<b>0.481 J</b>
INJ-2D	2/21/2023	10.11	100	<b>1,750</b>	<b>16.2</b>	<b>14.3</b>	<b>0.367 J</b>	<0.234
MVH-3	2/21/2023	3.09	37	<b>812</b>	<b>23.5</b>	<b>14.0</b>	<b>0.221 J</b>	<0.234
DUP				<b>798</b>	<b>23.9</b>	<b>13.6</b>	<b>0.260 J</b>	<0.234
MVH-9s	2/21/2023	9.08	100	<b>987</b>	<b>15.0</b>	<b>17.6</b>	<b>0.171 J</b>	<b>0.598 J</b>

**Notes:** Groundwater samples analyzed by United States Environmental Protection Agency (USEPA) Method 8260B. All results reported in micrograms per liter (µg/L). Orange box indicates result above AWQS

# Recent Groundwater Results

## ➤ Field Parameter Results in Injection Wells & Monitoring Wells

Sample ID	Sample Date	Depth to Water (ft btoc)	Ferrous Iron (mg/L)	Temp. (°C)	ORP (mV)	DO (mg/L)	pH (SU)	Specific Conductance (µS/cm)
			Measured with Hach DR900 Colorimeter	Measured with field instrumentation (YSI-556 Meter)				
MW-3	2/21/2023	3.09	0.62	13.11	191.3	8.01	6.90	167
MVH-9S	2/21/2023	9.08	0.48	15.79	144.1	6.45	7.15	394
INJ-1S	2/21/2023	3.08	0.42	16.16	96.8	8.19	7.49	411
INJ-1D	2/21/2023	10.09	0.48	13.66	155.0	8.05	7.50	199
INJ-2S	2/21/2023	6.43	0.00	--	--	--	--	--
INJ-2D	2/21/2023	10.11	2.90	16.83	74.6	6.45	12.11	4,626
<b>Notes:</b>								
ft btoc = Feet below top of casing.			ORP = Oxidation-reduction potential.					
mg/L = Milligrams per liter.			µS/cm = Microsiemens per centimeter					
mV = Millivolts			°C = degrees Celsius					
DO = Dissolved oxygen.			SU = Standard units.					

# Recent Groundwater Results

## ► Injection Well and Monitoring Well Groundwater Geochemical Parameter Data

Sample ID	Depth to Groundwater (ft bTOC)	Bromide	Chloride	Nitrate	Sulfate	Total Organic Carbon	Methane	Ethane	Ethene
		EPA Method 9056A				EPA Method 9060A	EPA Method RSK 175		
		Results in mg/L					Results in µg/L		
INJ-1S	3.08	<b>0.881 J</b>	<b>25.7</b>	<b>0.538</b>	<b>39.9</b>	<b>2.02</b>	<b>59.4</b>	<b>21.4</b>	<4.26
INJ-1D	10.09	<b>0.705 J</b>	<b>31.4</b>	<b>2.84</b>	<b>15.9</b>	<b>0.737 J</b>	<2.91	<4.07	<4.26
INJ-2S	6.43	<b>60.6 J</b>	<b>40.0 J</b>	<b>8.06 J</b>	<59.4	<b>7.52</b>	<b>46.8</b>	<4.07	<4.26
INJ-2D	10.11	<b>614 J</b>	<379	<b>72.7 J</b>	<594	<b>2.63</b>	<2.91	<4.07	<4.26
MVH-3	3.09	<b>0.839 J</b>	<b>20.4</b>	<b>2.83</b>	<b>18.7</b>	<b>0.554 J</b>	<2.91	<4.07	<4.26
MVH-9s	9.08	<b>0.764 J</b>	<b>38.6</b>	<b>6.81</b>	<b>15.9</b>	<b>0.473 J</b>	<b>47.4</b>	<4.07	<4.26

Notes: EPA =Environmental Protection Agency

mg/L=Milligrams per liter

µg/L=Micrograms per liter

Estimated concentration above minimum laboratory MDL and minimum laboratory Method Reporting  
J =Limit.

# Recent Groundwater Results

## ► Monitoring Well Microbial Data

Sample ID	Sample Date	Depth to Ground-water (ft bTOC)	<i>Dehalococcoides</i> (DHC)	<i>tceA</i> Reductase (TCE)	BAV1 Vinyl Chloride Reductase (BVC)	Vinyl Chloride Reductase (VCR)
MVH-3	2/21/2023	3.09	<b>3.20</b>	<b>0.90 J</b>	<1.00	<b>0.80 J</b>
MVH-9s	2/21/2023	9.08	<b>4.70</b>	<b>0.30 J</b>	<b>0.20 J</b>	<b>0.80 J</b>
All results reported in cells per milliliter (cells/mL).						

# In Situ Bioremediation Approach

- ▶ Prepare dilution water: extract & treat groundwater with GAC; promote anaerobic conditions with ascorbic acid.
- ▶ Inject about 800 gallons of diluted EOS QR into each well along with sodium bicarbonate, if appropriate
  - Anticipate injection flow rate will be  $< 0.3$  gallons per minute based on clean water injection test results
  - Monitor dissolved oxygen (DO) and oxidation reduction potential (ORP)
  - After DO is less than  $0.5$  mg/L, perform bioaugmentation injection with *Dehalococcoides* (SDC-9).
  - Inject additional diluted EOS QR followed by anaerobic chase water.
  - Evaluate contaminants, geochemical and microbial parameters in downgradient wells.



# In Situ Bioremediation Approach

## Injection Well Design Parameters

Aquifer / Well Parameter	Value
Radius of Influence	~7.5 feet
Screen Length Impact	20 feet
Porosity ( $n_e$ )	10%
Pore Volume per Injection Well	2,644 gallons
Target Pore Volume 30% of Total Pore Volume per Injection Well*	793 gallons
Amendments Per Injection Well	Quantity
EOS QR	280 pounds (or 27 gallons)
Estimated Total Organic Carbon (TOC) Post-Injection**	4,950 milligrams per liter
BAC-9 Dose	2 liters
Sodium Bicarbonate	14 pounds
Sodium Ascorbate	6 pounds
Sodium or Potassium Bromide	5 pounds
Dilution Water and Chase Water per Injection Well	Quantity
EOS QR (27 gallons) and Dilution Water (270 gallons)	297 gallons
Anaerobic Chase Water	497 gallons
Anaerobic Chase Water post BAC-9 Injection	25 gallons
<b>Total Volume of Injected Materials per Well:</b>	<b>819 gallons</b>





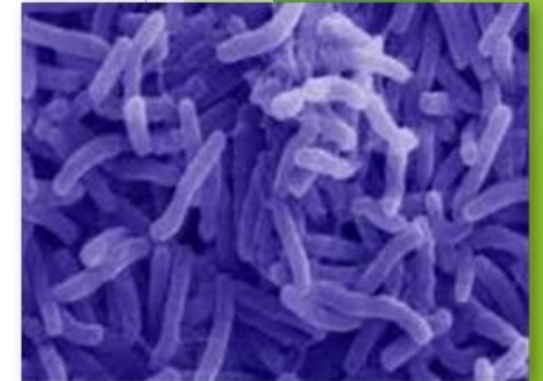
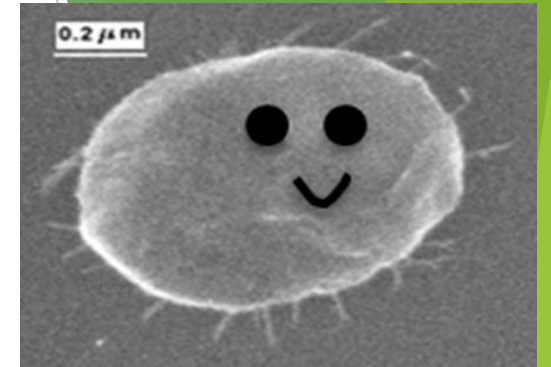
# In Situ Bioremediation Approach

- Baseline groundwater sampling 1<sup>st</sup> week of February 2024
- Commenced ISB program during 2<sup>nd</sup> week of February
- Extracting groundwater from EW-1
- Injection of EOS-QR, sodium bicarbonate & sodium bromide
- Injection rate into INJ-1S and INJ-2S was approximately 0.14 – 0.2 gallons per minute (gpm) and 0.05 – 0.19 gpm, respectively
- Injection rate into INJ-1D and INJ-2D was 0.14 – 0.54 and 0.24 – 0.5 gpm, respectively
- Inject *Dehalococcoides* into injection wells under anaerobic conditions after 50% EOS QR injected



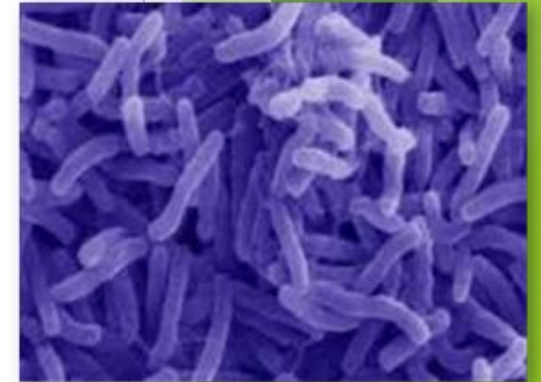
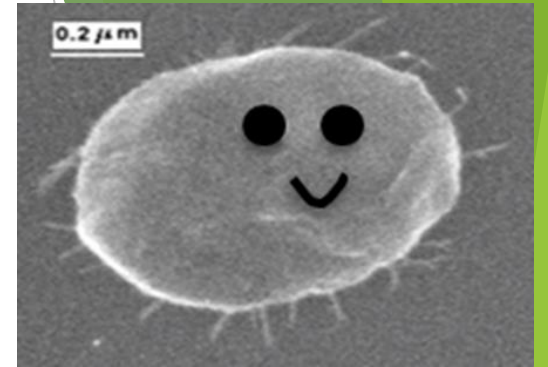
# In Situ Bioremediation Monitoring Program

- Monitor quarterly groundwater
  - Field parameters: pH, DO, ORP, conductivity, temperature
- Monitor analytical parameters:
  - cVOCs
  - TOC
  - Terminal electron acceptors (O<sub>2</sub>, nitrate, Fe, sulfate)
  - Volatile fatty acids (acetate, propionate, etc.)
  - Total Kjeldahl nitrogen, ammonia, nitrate, nitrite
  - Total Phosphorus
  - Dissolved gases: methane, ethane, ethene,
  - Microbial biomass: key microbe - *Dehalococcoides*

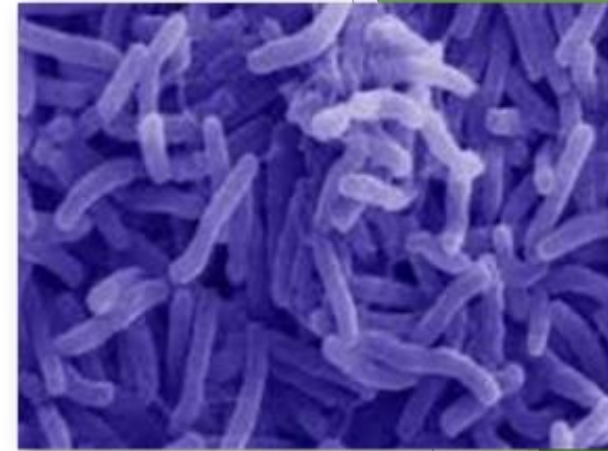


# In Situ Bioremediation Monitoring Program

**Stay Tuned for Groundwater  
Results in 2024**



# QUESTIONS?



THANK YOU!  
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