

# Evaluation of Enhanced *In Situ* Bioremediation of Chlorinated Ethenes in Groundwater Using Molecular Tools

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# Background Site Information

## Site in Eastern USA

Major contaminants of concern include chlorinated volatile organic compounds (CVOCs): - **Objective – Treat to achieve a decreasing trend for NJDEP MNA permit approval to close the case.**

- § Tetrachloroethene (96 µg/L)
- § Trichloroethene (2,840 µg/L)
- § *Cis*-1,2-dichloroethene (50 µg/L)
- § 1,1-dichloroethene (1,900 µg/L)
- § Vinyl chloride (10 or less µg/L)
- § 1,1,1-trichloroethane (120 µg/L)

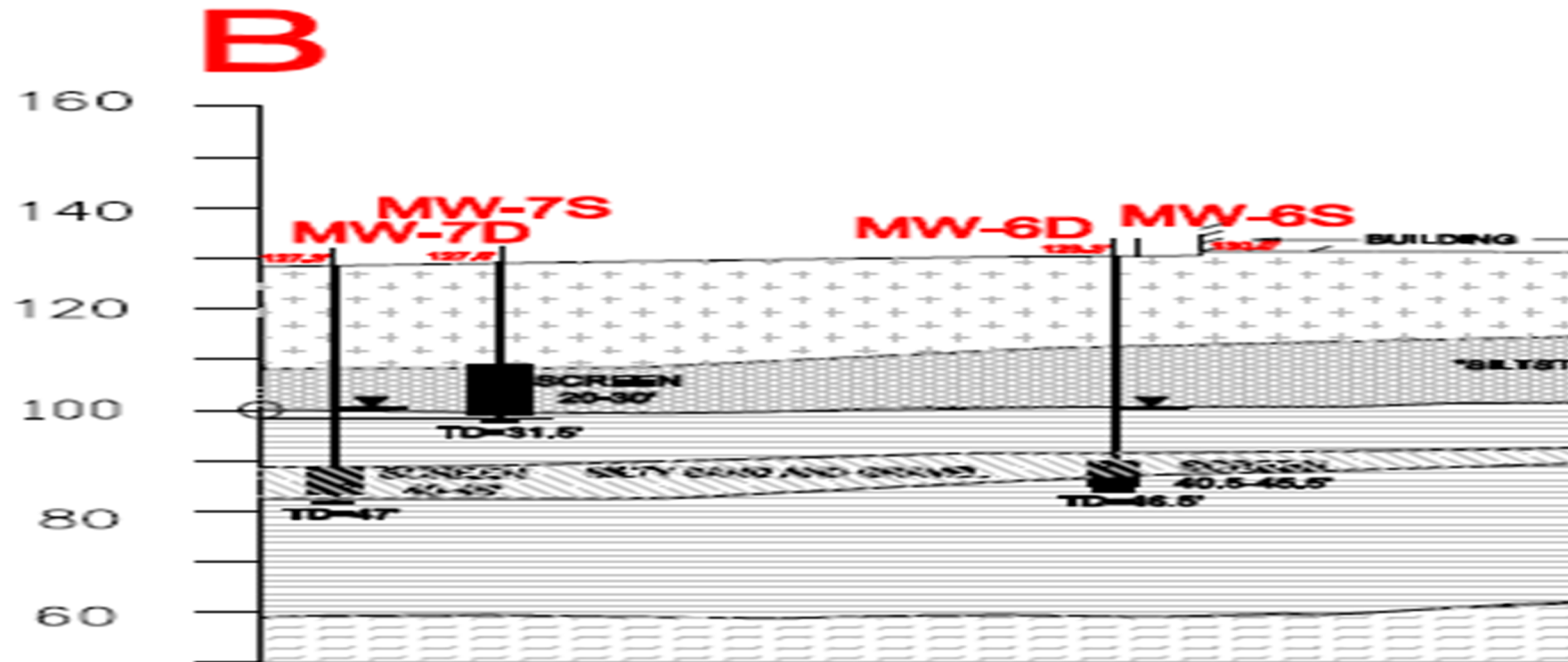
# Background Geochemical and Microbial Data

| Sample Identification | DO   | pH   | ORP   | DHC      | DHG      | Iron  | Total Alkalinity (CaCO3) | Nitrogen (Ammonia) | Nitrogen (Nitrate) | TKN  | Phosphorus | Sulfate | Total Organic Carbon | Ferrous Iron |
|-----------------------|------|------|-------|----------|----------|-------|--------------------------|--------------------|--------------------|------|------------|---------|----------------------|--------------|
|                       | mg/L | SU   | mV    | Cells/mL | Cells/mL | µg/L  | mg/L                     | mg/L               | mg/L               | mg/L | mg/L       | mg/L    | mg/L                 | mg/L         |
| MW-6D                 | 3.09 | 7.48 | 96.00 | 9.70E-02 | <4.6     | 70.60 | 234.0                    | 0.13               | 4.5                | ND   | 0.03       | 28      | 0.780                | ND           |

# Site Geology and Hydrogeology

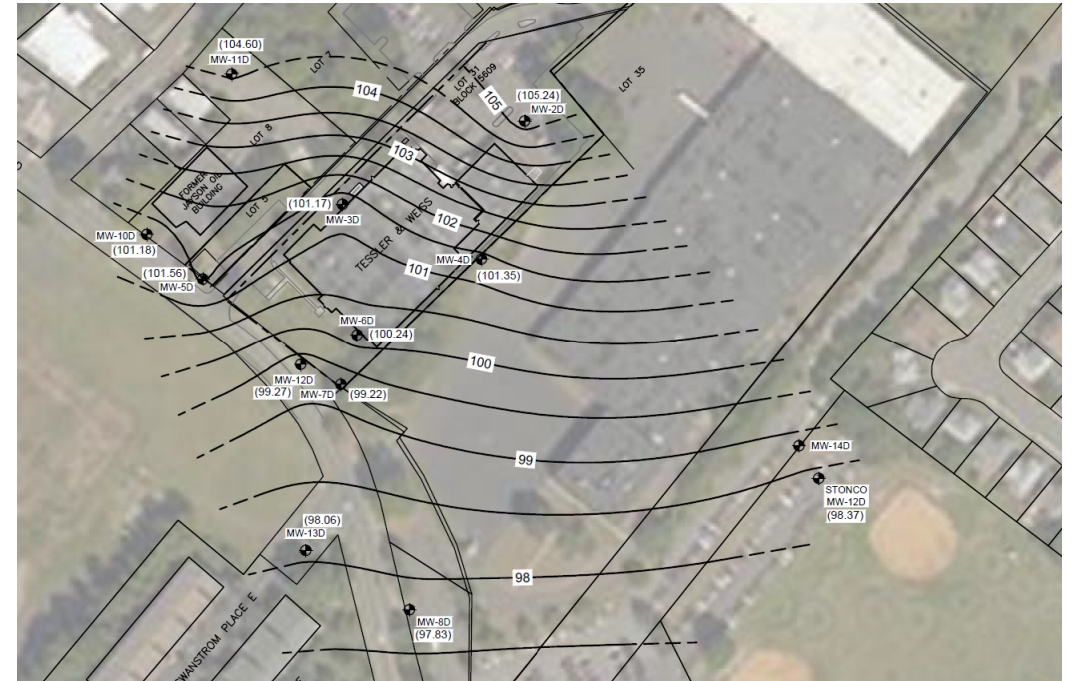
- Basal glacial till composed primarily of silt, with sand, gravel and cobbles
- Lower permeability in basal till and silt zones
- Cobble zone at 10-25 ft below ground surface (bgs). USED SONIC TO GET THROUGH – WENT WITH INJECTION WELLS, CAN'T GET THROUGH WITH DIRECT PUSH
- Key areas of impact identified in preferential thin layers of silty sand and gravel at about 25-35 feet and 40-45 ft below grade
- **WE SEE THE BULK OF IMPACTS HERE – SO THESE PREFERENTIAL ZONES WERE TARGETED**

# Site Geological Cross Section



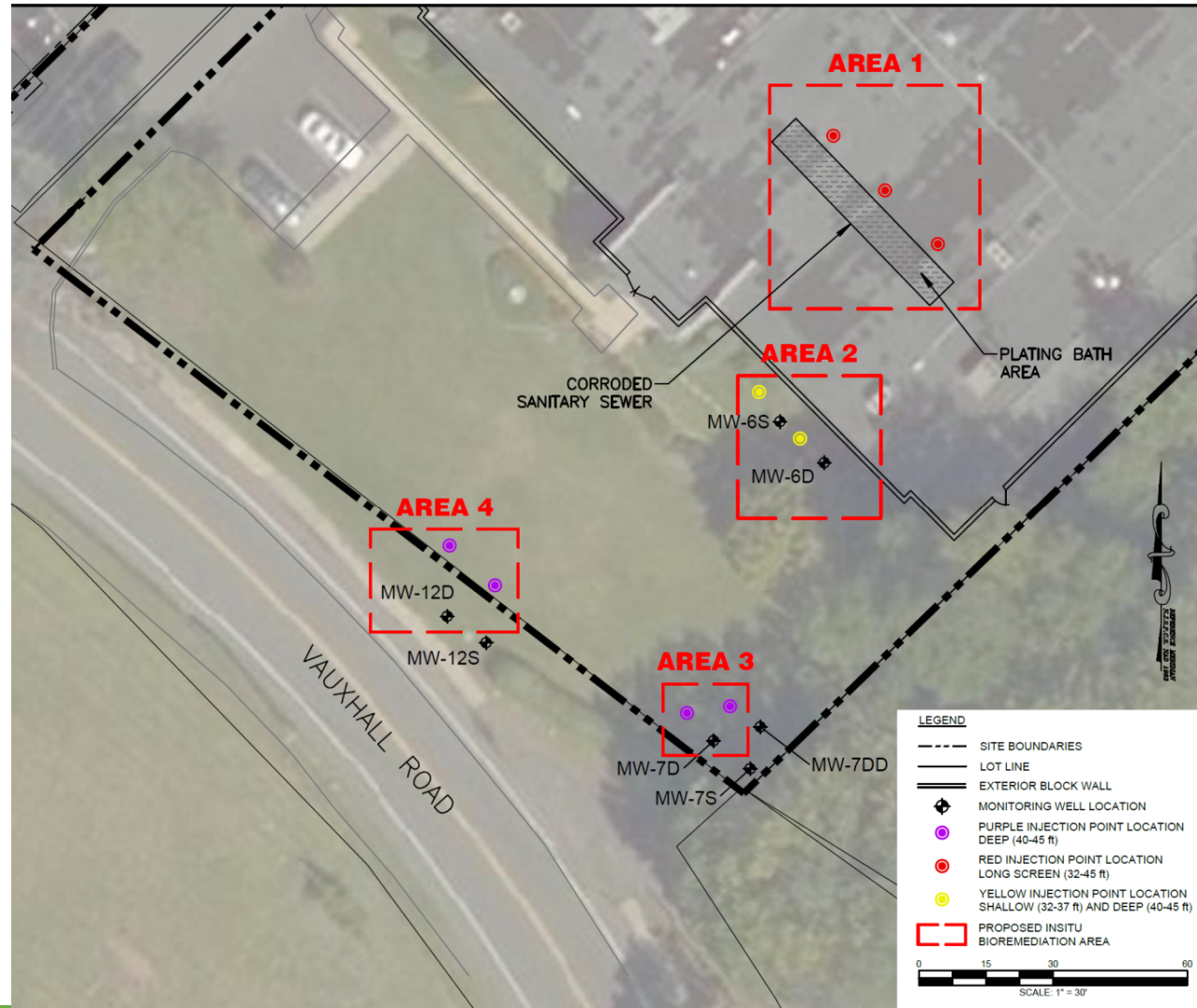
# Site Hydrogeology

- Groundwater flows to the southwest primarily within these more permeable groundwater bearing units, zones and lenses. The treatment focus was within those shallow permeable unconsolidated units which hold the bulk of the impact.

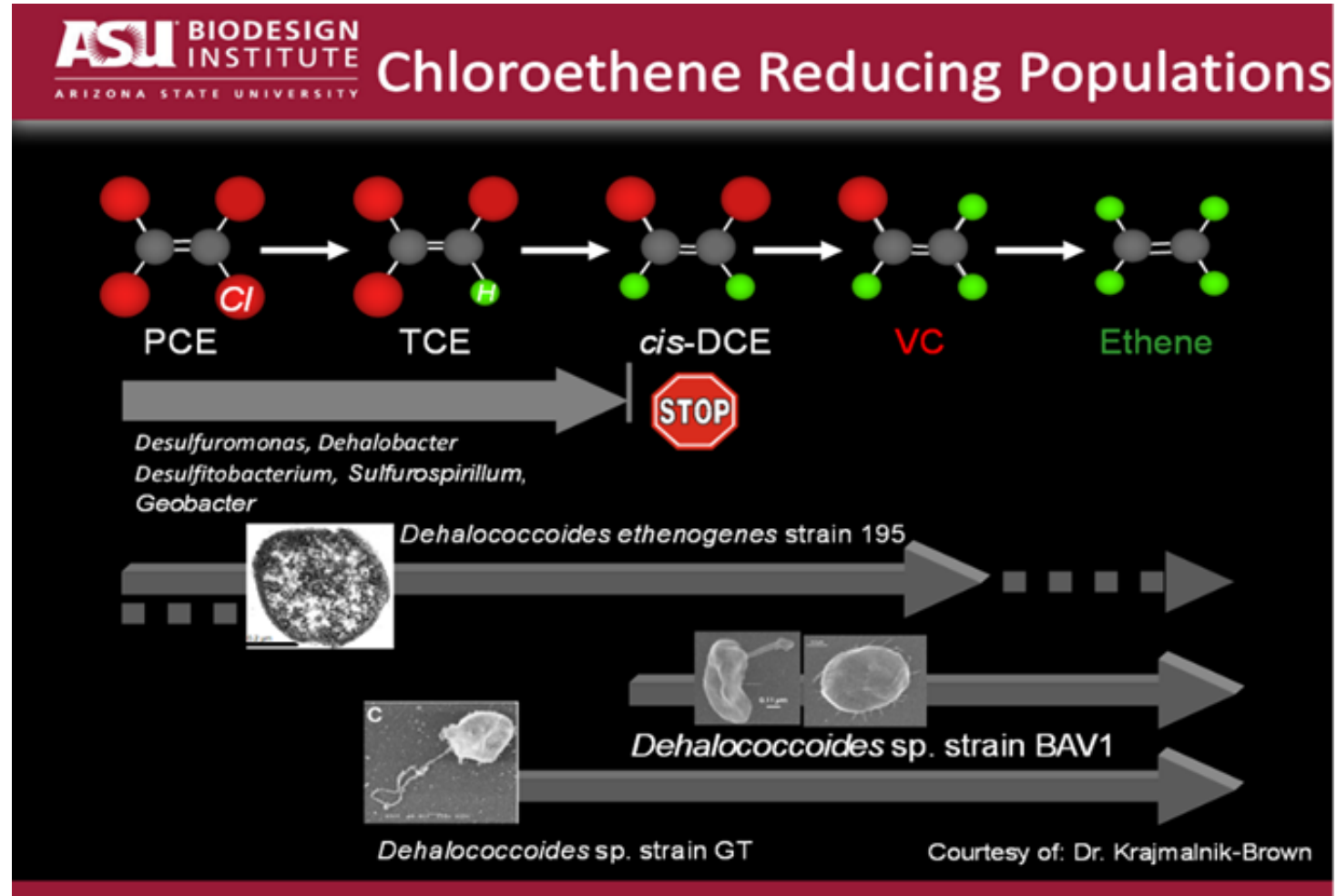




# Well Location Map

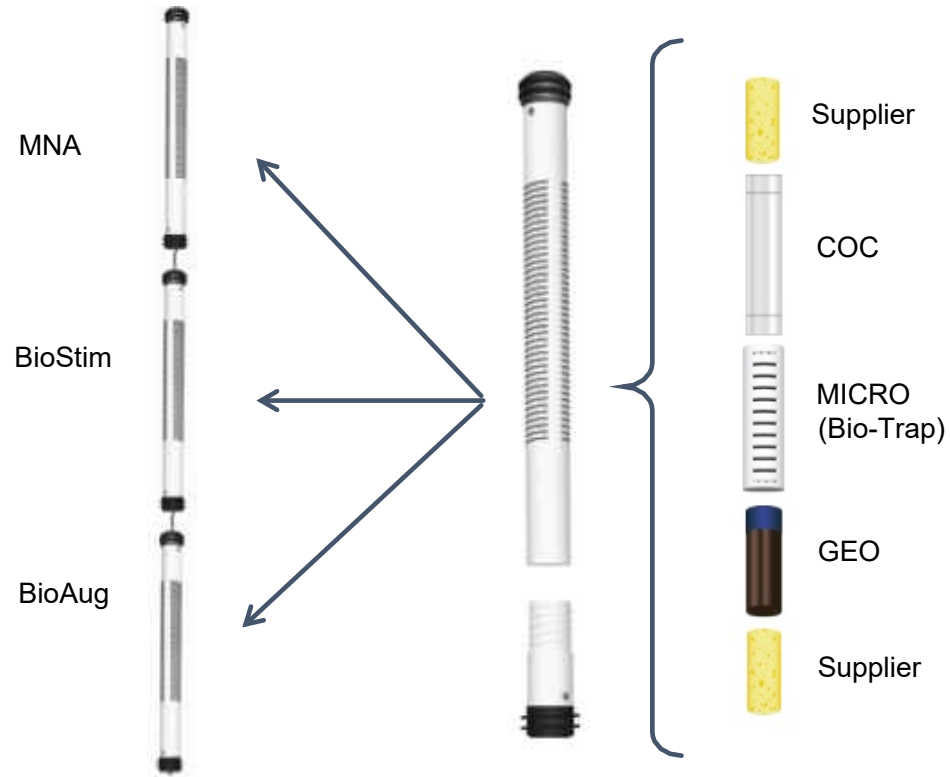


# Anaerobic CVOC Degradation Pathway

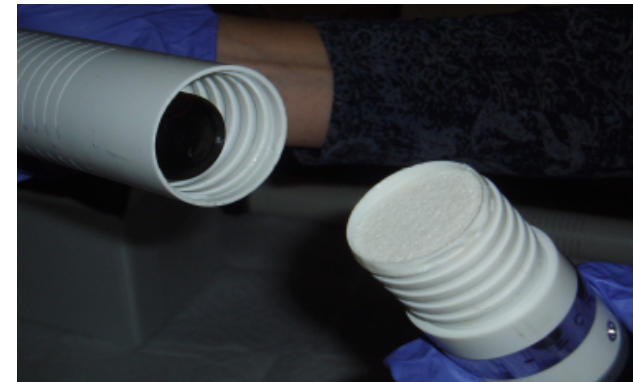
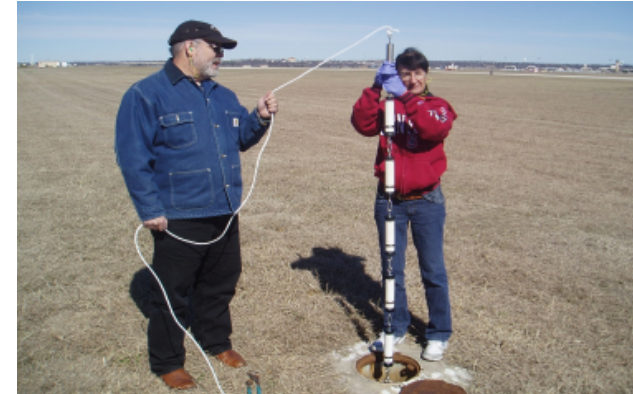




# In Situ Microcosm (ISM) Study Our Molecular Tool Belt



Liang et al., 2011



# In Situ Microcosm (ISM) Study – Results

| Well ID | Date Sampled | PCE (µg/L) | TCE (µg/L) | <i>cis</i> -1,2-DCE (µg/L) | 1,1-DCE (µg/L) | Vinyl Chloride (µg/L) |
|---------|--------------|------------|------------|----------------------------|----------------|-----------------------|
| MW-6D   | 10/23/2019   | 96.40      | 2,840      | 14.20                      | 1,340          | <16.0                 |
| MW-7D   | 10/23/2019   | 18.80      | 862        | 3.60                       | 197            | <3.9                  |

| Well ID | ISM Unit Type                            | Percent Decrease or Increase in Chemical of Concern |        |                     |         | Amount of VC and Ethene Produced (µg/L) |         |
|---------|------------------------------------------|-----------------------------------------------------|--------|---------------------|---------|-----------------------------------------|---------|
|         |                                          | PCE                                                 | TCE    | <i>cis</i> -1,2-DCE | 1,1-DCE | VC                                      | Ethene  |
| MW-6D   | BioStim: EOS PRO+EOS QR                  | -96.4%                                              | -96.7% | +3,216.9%           | -83.2%  | 6.4                                     | 2.3     |
|         | BioStim: EOS PRO + BioAug: SDC-9™        | -85.2%                                              | -85.4% | +1,900%             | -79.6%  | 23.7                                    | 0.8 (J) |
| MW-7D   | BioStim: SRS+10% Glycerol                | -97.3%                                              | -97.5% | +75%                | -97.4%  | 0.8 (J)                                 | 1.4     |
|         | BioStim: SRS+10% Glycerol BioAug: SDC-9™ | -97.3%                                              | -99.5% | +2,333.3%           | -90%    | 0.9 (J)                                 | 0.6 (J) |

# In Situ Microcosm (ISM) Study Results

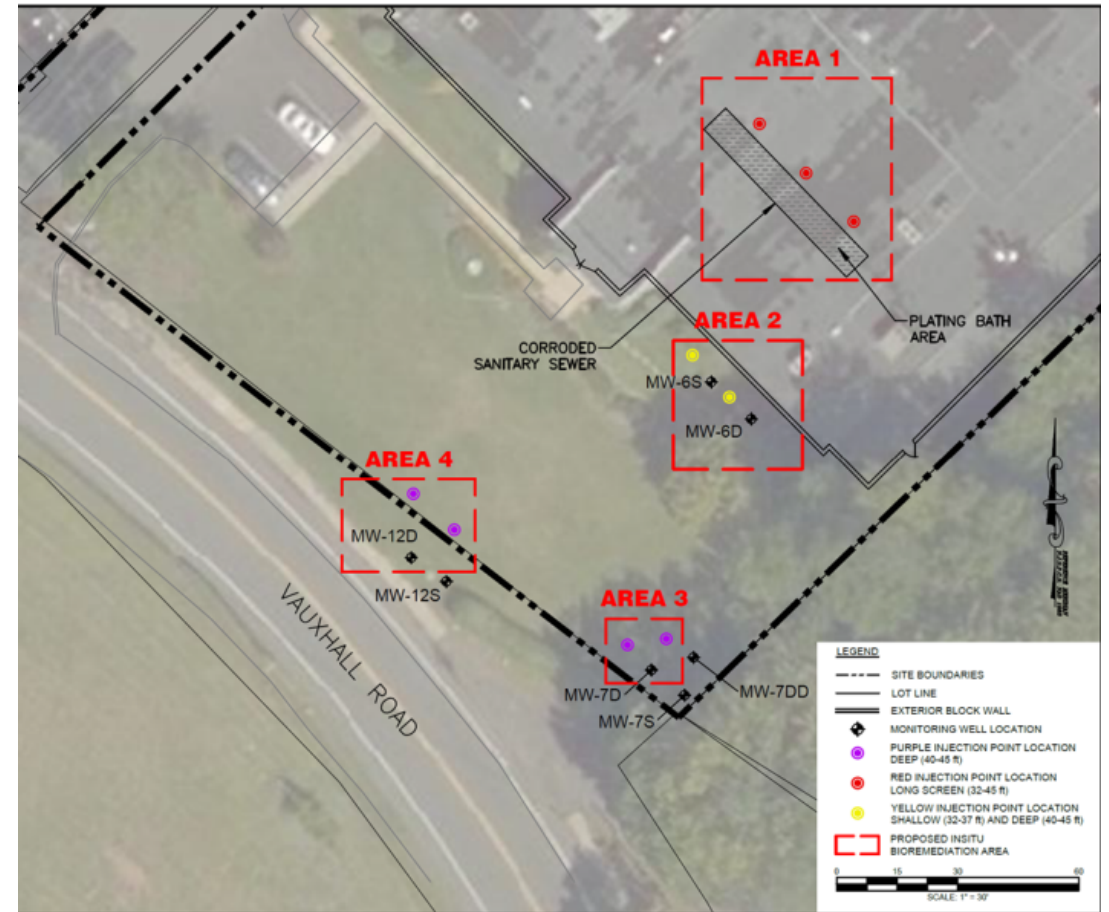
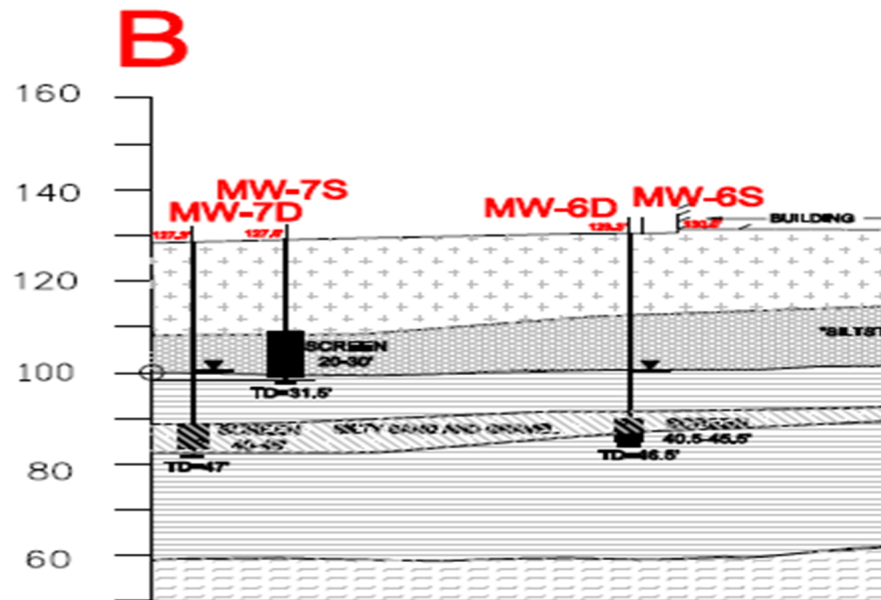
| Well ID | ISM Unit Type                            | Percent Decrease or Increase in Chemical of Concern |        |                     |         | Microbial Results (cells/bead) |                       |                       |
|---------|------------------------------------------|-----------------------------------------------------|--------|---------------------|---------|--------------------------------|-----------------------|-----------------------|
|         |                                          | PCE                                                 | TCE    | <i>cis</i> -1,2-DCE | 1,1-DCE | DHC                            | <i>tceA</i> Reductase | <i>vcrA</i> Reductase |
| MW-6D   | BioStim: EOS PRO+EOS QR                  | -96.4%                                              | -96.7% | +3,216.9%           | -83.2%  | 1.1E+04                        | 1.06E+03              | 8.28E+02              |
|         | BioStim: EOS PRO + BioAug: SDC-9™        | -85.2%                                              | -85.4% | +1,900%             | -79.6%  | 4.44E+06                       | 6.26E+05              | 3.49E+05              |
|         |                                          |                                                     |        |                     |         |                                |                       |                       |
| MW-7D   | BioStim: SRS+10% Glycerol                | -97.3%                                              | -97.5% | +75%                | -97.4%  | 1.44E+04                       | 1.37E+03              | 1.11E+03              |
|         | BioStim: SRS+10% Glycerol BioAug: SDC-9™ | -97.3%                                              | -99.5% | +2,333.3%           | -90%    | 3.84E+06                       | 5.46E+05              | 3.18E+05              |

# Design of In Situ Bioremediation (ISB) System

| Injection Well Areas                | Area 1          | Area 2        | Areas 3 & 4                |
|-------------------------------------|-----------------|---------------|----------------------------|
| Injection Location                  | Inside Building | MW-6S shallow | MW-6D, MW-7D & MW-12D deep |
| ROI (ft)                            | 20              | 20            | 20                         |
| Screen (ft)                         | 10              | 5             | 5                          |
| Porosity ( $n_e$ )                  | 17.5%           | 15%           | 20%                        |
| bulk density (lbs/ft <sup>3</sup> ) | 109             | 99            | 99                         |
| oil retention                       | 0.003           | 0.004         | 0.002                      |
| effective thickness                 | 50%             | 25%           | 75%                        |

# Injection Well Construction – Tight Tremie-Grout Seal

## – Injection Well Screens Keyed-In To Geology and Well / Injection Well Locations



# Design of In Situ Bioremediation (ISB) System – Total Injected – A Little Over 60,000 Gallons

|                                                                       | Per Injection Well Type |               |               |
|-----------------------------------------------------------------------|-------------------------|---------------|---------------|
|                                                                       | Long -----              | Shallow ----- | Deep          |
| EOS Pro (gal)                                                         | 429                     | 130           | 195           |
| EOS QR (gal)                                                          | 33                      | 10            | 15            |
| Dilution water (gal)                                                  | 2,792                   | 1,170         | 780           |
| Chase water (gal)                                                     | 5,004                   | 2,225         | 3,725         |
| Total injectant per well of Carbon Substrates (gal)                   | 3,254                   | 1,310         | 990           |
| Total per well type including chase water                             | 8,258                   | 3,535         | 4,715         |
| BAC-9 (L), if needed                                                  | 7                       | 3             | 4             |
| Number of Injection wells per Area                                    | 3                       | 2             | 6             |
| Sodium thiosulfate (gm)                                               | 375                     | 107           | 428           |
| Sodium bicarbonate 0.5 lb per well (lb) if pH decreases significantly | 2                       | 1             | 3             |
| Ascorbic acid to reduce dissolved oxygen (lbs)                        | 6                       | 4             | 12            |
| <b>Total Gallons per injection well</b>                               | <b>8,258</b>            | <b>3,535</b>  | <b>4,715</b>  |
| <b>Total Gallons per injection area</b>                               | <b>24,773</b>           | <b>7,070</b>  | <b>28,289</b> |



# Design of In Situ Bioremediation (ISB) System

- Install injection wells upgradient of the various source and areas of impact
- Obtain baseline data
- Inject ISB amendments (EOS PRO & QR) into injection wells
- Monitor site groundwater
- Inject additional amendments, such as key microbes, additional carbon source (EOS) and/or nutrients, based on groundwater monitoring data

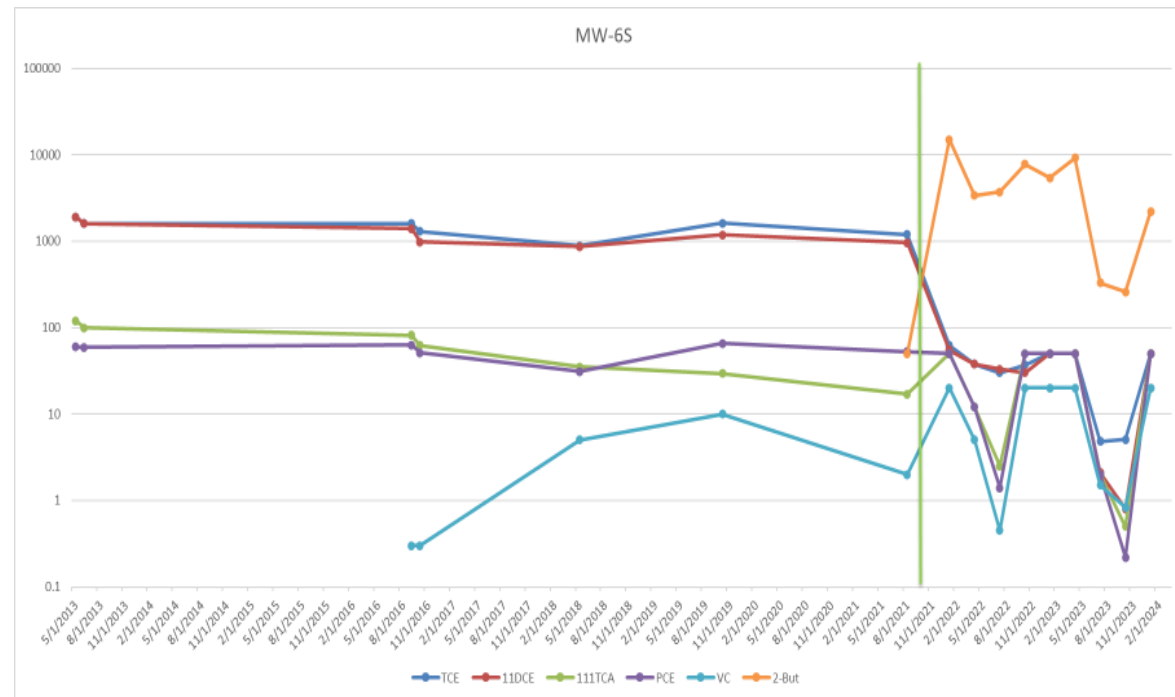
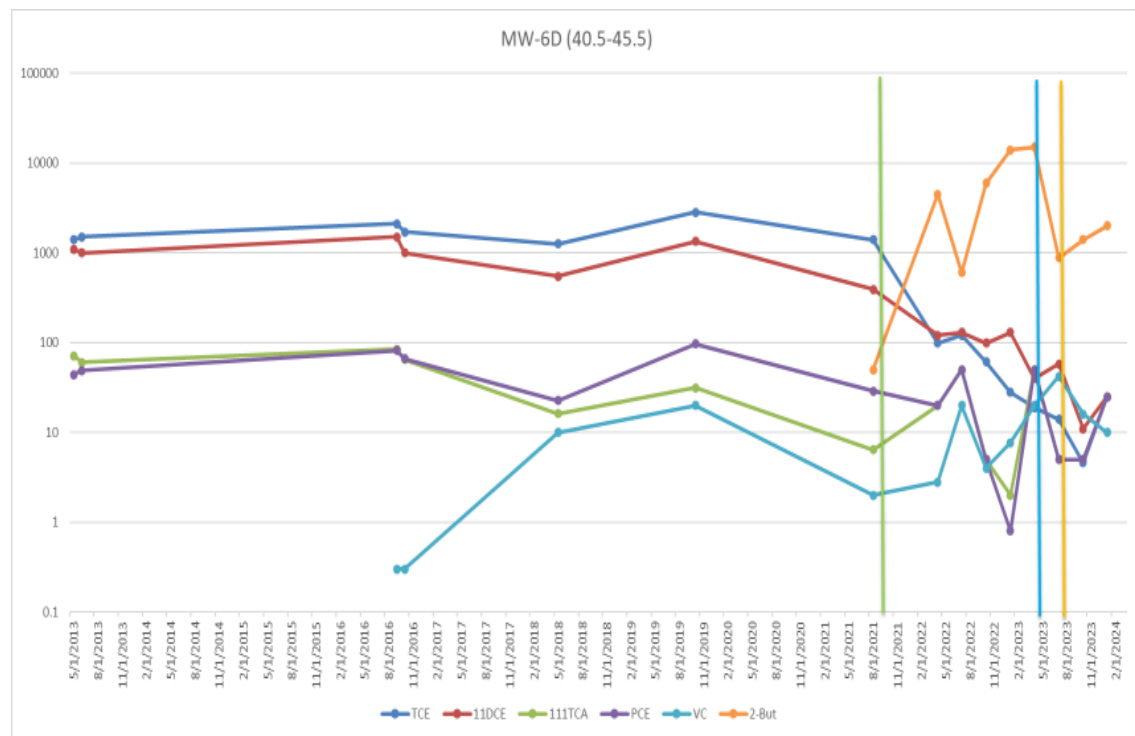
# Total Organic Carbon and Geochemical Parameters Results (micrograms/liter) in MW-6S – GOOD TOC and NITRATE – SHOWS INFLUENCE

| SAMPLE ID:               | MW-6S     | MW-6S     | MW-6S      | MW-6S     | MW-6S      |
|--------------------------|-----------|-----------|------------|-----------|------------|
| COLLECTION DATE:         | 1/12/2022 | 4/27/2022 | 10/26/2022 | 4/27/2023 | 10/30/2023 |
| <b>DISSOLVED METALS</b>  |           |           |            |           |            |
| Iron, Dissolved          | 140000    | -         | -          | -         | -          |
| Manganese, Dissolved     | 119800    | -         | -          | -         | -          |
| <b>GENERAL CHEMISTRY</b> |           |           |            |           |            |
| Chloride                 | 62000     | -         | -          | -         | -          |
| Nitrogen, Ammonia        | 4870      | -         | -          | -         | -          |
| Nitrogen, Nitrate        | 30        | -         | 64.8       | 137       | 34         |
| Nitrogen, Nitrite        | 73.5      | -         | -          | -         | -          |
| Nitrogen, Total Kjeldahl | 6350      | -         | -          | -         | -          |
| Phosphorus, Total        | 7700      | -         | -          | -         | -          |
| Sulfate                  | 10000     | -         | 6700       | 10000     | 1600       |
| Total Organic Carbon     | 3000000   | 1900000   | 2600000    | 1700000   | 1000000    |

# Total Organic Carbon and Geochemical Parameters Results (micrograms/liter) in MW-6D – AGAIN INCREASED NUTRIENTS AND TOC

| Sampling Event:          | Post-Injection Q2 | Post-Injection Q4 | Post-Injection Q6 | Post-Injection Q8 |
|--------------------------|-------------------|-------------------|-------------------|-------------------|
| <b>SAMPLE ID:</b>        | <b>MW-6D</b>      | <b>MW-6D</b>      | <b>MW-6D</b>      | <b>MW-6D</b>      |
| <b>COLLECTION DATE:</b>  | <b>4/27/2022</b>  | <b>10/27/2022</b> | <b>4/27/2023</b>  | <b>10/30/2023</b> |
| <b>DISSOLVED GASSES</b>  |                   |                   |                   |                   |
| Ethane                   | 2.48              | 3.08              | -                 | -                 |
| Ethene                   | 3.46              | 6.24              | -                 | -                 |
| Methane                  | 1350              | 2560              | -                 | -                 |
| <b>DISSOLVED METALS</b>  |                   |                   |                   |                   |
| Iron, Dissolved          | <b>61400</b>      | <b>93800</b>      | -                 | -                 |
| Manganese, Dissolved     | <b>75040</b>      | <b>108400</b>     | -                 | -                 |
| <b>GENERAL CHEMISTRY</b> |                   |                   |                   |                   |
| Chloride                 | 66000             | -                 | -                 | -                 |
| Nitrogen, Ammonia        | 40.7              | 62.6              | -                 | -                 |
| Nitrogen, Nitrate        | 48.5              | 60.3              | 80.8              | 100               |
| Nitrogen, Nitrite        | 61.9              | -                 | -                 | -                 |
| Nitrogen, Total Kjeldahl | 4520              | 482               | -                 | -                 |
| Phosphorus, Total        | 1290              | 1110              | -                 | -                 |
| Sulfate                  | 10000             | 3800              | 2700              | 10000             |
| Total Organic Carbon     | 1900000           | 2000000           | 2500000           | 1200000           |

# CVOC Results in MW-6D & MW-6S



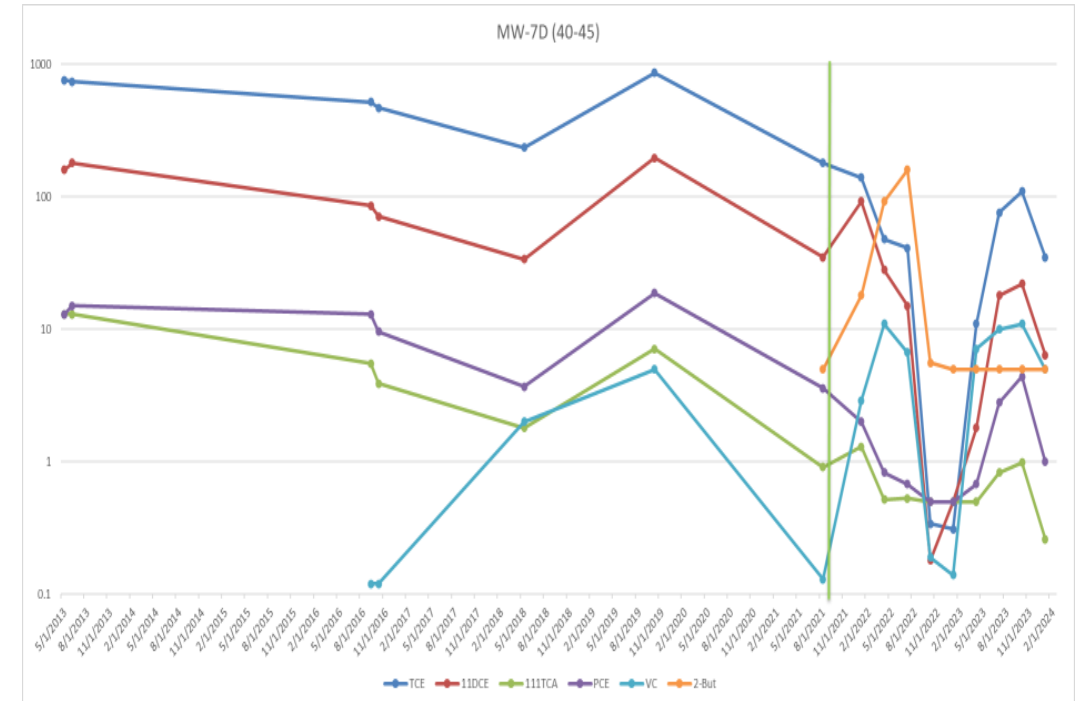
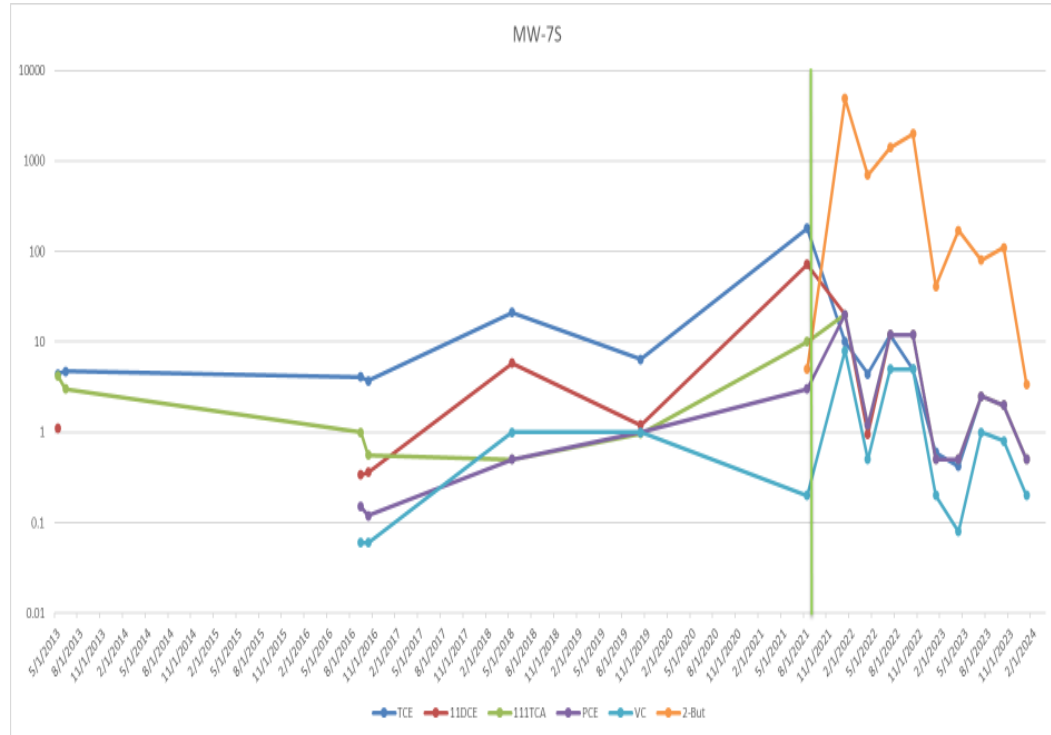
Within less than two years of injection (green vertical line), there was more than two orders of magnitude reduction in chlorinated ethenes (TCE and DCE) in the source area - MW-6S & MW-6D. Due to slight accumulation of DCE and VC in the MW-6D area, Dehalococcoides (DHC) Consortium (gold vertical line) was injected into the groundwater in that area. Solid decreases thereafter, with DCE going from about 80 ug/L (over NJDEP GWQS of 70) to less than 40 (now below NJDEP) and VC going from about 70 ug/L to 10. NJDEP closure via MNA appropriate. Quick note: added some buffer (blue vertical line), as pH dropped a little lower than we wanted after a year – further TCE reductions after.

# Total Organic Carbon and Geochemical Parameters Results (micrograms/liter) in MW-7S and MW-7D – AGAIN GOOD INFLUENCE – ELEVATED TOC AND NUTRIENTS

| Sampling Event:          | Post-Injection Q2 | Post-Injection Q4 | Post-Injection Q6 | Post-Injection Q8 |
|--------------------------|-------------------|-------------------|-------------------|-------------------|
| SAMPLE ID:               | MW-7S             | MW-7S             | MW-7S             | MW-7S             |
| COLLECTION DATE:         | 4/28/2022         | 10/26/2022        | 4/27/2023         | 10/30/2023        |
| <b>GENERAL CHEMISTRY</b> |                   |                   |                   |                   |
| Chloride                 | 32000             | -                 | -                 | -                 |
| Nitrogen, Ammonia        | -                 | -                 | -                 | -                 |
| Nitrogen, Nitrate        | 47                | -                 | -                 | -                 |
| Nitrogen, Nitrite        | 62.9              | -                 | -                 | -                 |
| Nitrogen, Total Kjeldahl | -                 | -                 | -                 | -                 |
| Phosphorus, Total        | -                 | -                 | -                 | -                 |
| Sulfate                  | 10000             | -                 | -                 | -                 |
| Total Organic Carbon     | 670000            | 1300000           | 180000            | 140000            |

| Sampling Event:          | Post-Injection Q1 | Post-Injection Q2 | Post-Injection Q4 | Post-Injection Q6 | Post-Injection Q8 |
|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| SAMPLE ID:               | MW-7D             | MW-7D             | MW-7D             | MW-7D             | MW-7D             |
| COLLECTION DATE:         | 1/12/2022         | 4/27/2022         | 10/27/2022        | 4/27/2023         | 10/30/2023        |
| <b>DISSOLVED GASSES</b>  |                   |                   |                   |                   |                   |
| Ethane                   | -                 | -                 | 18.6              | -                 | -                 |
| Ethene                   | -                 | -                 | 8.46              | -                 | -                 |
| Methane                  | -                 | -                 | 15400             | -                 | -                 |
| <b>DISSOLVED METALS</b>  |                   |                   |                   |                   |                   |
| Iron, Dissolved          | 50                | -                 | 19.3              | -                 | -                 |
| Manganese, Dissolved     | 3038              | -                 | 3467              | -                 | -                 |
| <b>GENERAL CHEMISTRY</b> |                   |                   |                   |                   |                   |
| Chloride                 | 59000             | -                 | -                 | -                 | -                 |
| Nitrogen, Ammonia        | 80.6              | -                 | 124               | -                 | -                 |
| Nitrogen, Nitrate        | 450               | -                 | 100               | 100               | 173               |
| Nitrogen, Nitrite        | 50.4              | -                 | -                 | -                 | -                 |
| Nitrogen, Total Kjeldahl | 300               | -                 | 260               | -                 | -                 |
| Phosphorus, Total        | 67                | -                 | 93                | -                 | -                 |
| Sulfate                  | 10000             | -                 | 1900              | 4800              | 6000              |
| Total Organic Carbon     | 12000             | 40000             | 5800              | 2100              | 1500              |

# CVOC Results in MW-7S and MW-7D



In MW-7D, TCE went from about 1,000 ug/L to a low of 0.31 ug/L (below 1 ug/L NJDEP GWQS) after 5Q; but rebounded to 110 ug/L after 8Q. In the 9Q, TCE decreased to 35 ug/L. Other CVOCS decreased to less than 8 ug/L. TOC at background concentration. MW-7S Total CVOCs have dropped to less than 0.1 mg/L. Decreasing trends noted in both 7S and 7D – ought to be able to close with NJDEP permit.

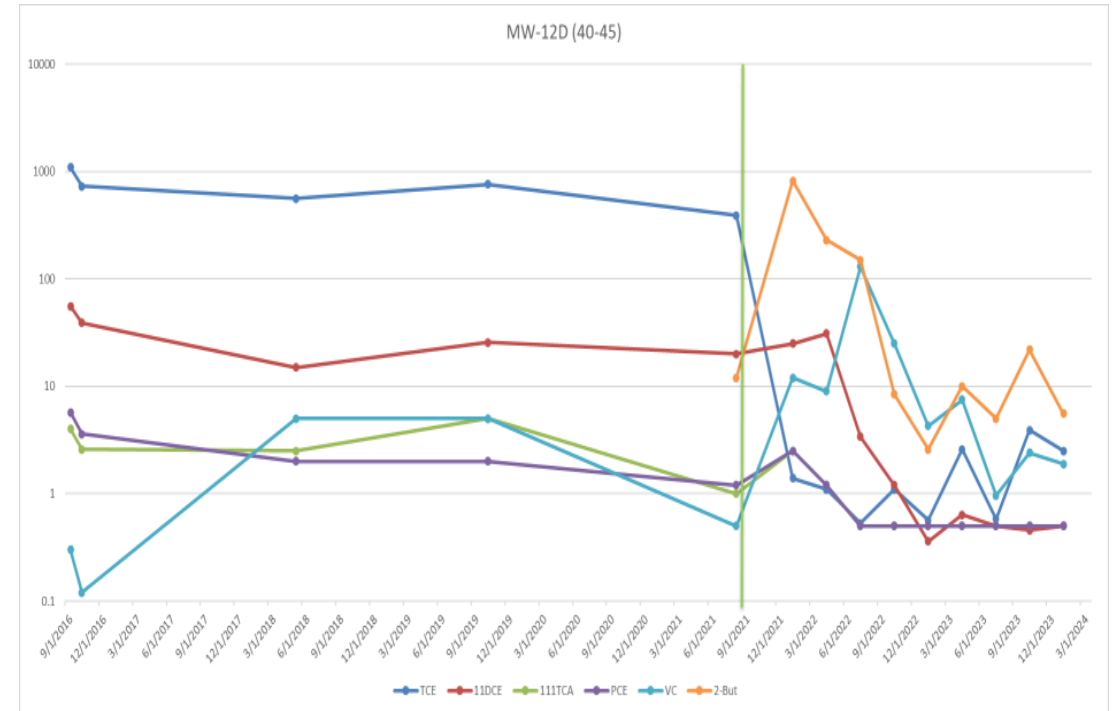
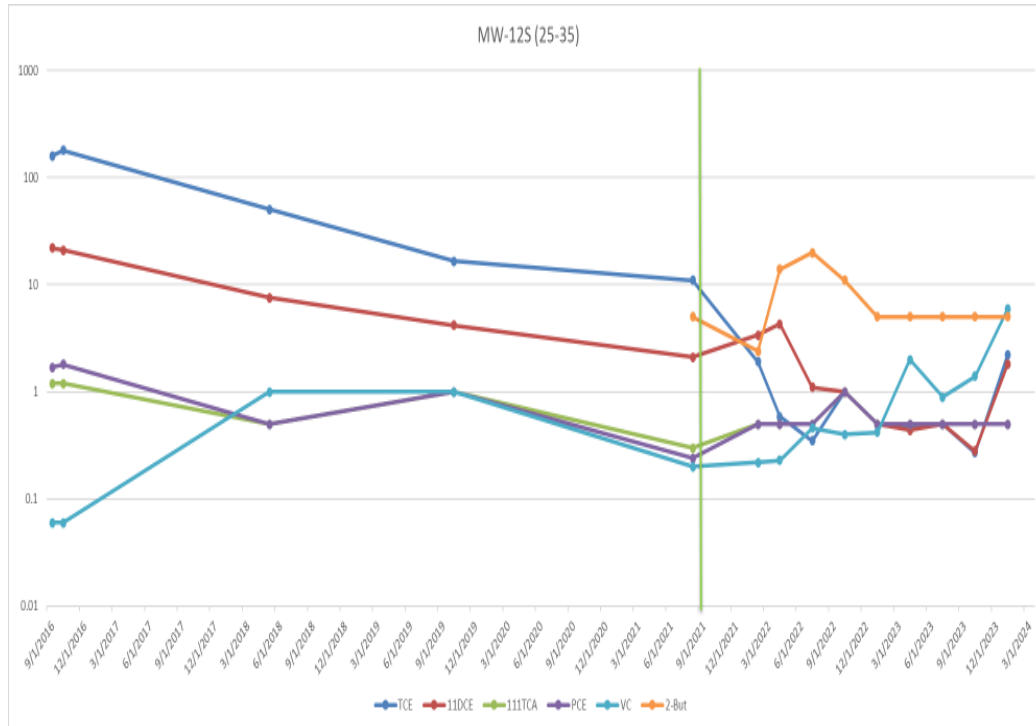
# Total Organic Carbon and Geochemical Parameters Results (micrograms/liter) in MW-12S and MW-12D – SOME TOC / NUTRIENTS / INFLUENCE

| Sampling Event:          | Post-Injection Q2 | Post-Injection Q3 | Post-Injection Q4 | Post-Injection Q6 | Post-Injection Q8 |
|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| SAMPLE ID:               | MW-12S            | MW-12S            | MW-12S            | MW-12S            | MW-12S            |
| COLLECTION DATE:         | 4/27/2022         | 7/27/2022         | 10/26/2022        | 4/27/2023         | 10/30/2023        |
| <b>DISSOLVED METALS</b>  |                   |                   |                   |                   |                   |
| Iron, Dissolved          | -                 | 3040              | -                 | -                 | -                 |
| Manganese, Dissolved     | -                 | 49980             | -                 | -                 | -                 |
| <b>GENERAL CHEMISTRY</b> |                   |                   |                   |                   |                   |
| Chloride                 | -                 | -                 | -                 | -                 | -                 |
| Nitrogen, Ammonia        | -                 | -                 | -                 | -                 | -                 |
| Nitrogen, Nitrate        | -                 | -                 | -                 | -                 | -                 |
| Nitrogen, Nitrite        | -                 | -                 | -                 | -                 | -                 |
| Nitrogen, Total Kjeldahl | -                 | -                 | -                 | -                 | -                 |
| Phosphorus, Total        | -                 | -                 | -                 | -                 | -                 |
| Sulfate                  | -                 | -                 | -                 | -                 | -                 |
| Total Organic Carbon     | 140000            | 84000             | 33000             | 9200              | 4600              |

| Sampling Event:          | Post-Injection Q1 | Post-Injection Q2 | Post-Injection Q4 | Post-Injection Q6 | Post-Injection Q8 |
|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| SAMPLE ID:               | MW-12D            | MW-12D            | MW-12D            | MW-12D            | MW-12D            |
| COLLECTION DATE:         | 1/12/2022         | 4/27/2022         | 10/27/2022        | 4/27/2023         | 10/30/2023        |
| SAMPLE MATRIX:           | WATER             | WATER             | WATER             | WATER             | WATER             |
| <b>DISSOLVED GASSES</b>  |                   |                   |                   |                   |                   |
| Ethane                   | -                 | -                 | 0.5               | -                 | -                 |
| Ethene                   | -                 | -                 | 25.8              | -                 | -                 |
| Methane                  | -                 | -                 | 13300             | -                 | -                 |
| <b>DISSOLVED METALS</b>  |                   |                   |                   |                   |                   |
| Iron, Dissolved          | 10700             | -                 | 107               | -                 | -                 |
| Manganese, Dissolved     | 26120             | -                 | 5397              | -                 | -                 |
| <b>GENERAL CHEMISTRY</b> |                   |                   |                   |                   |                   |
| Chloride                 | 42000             | -                 | -                 | -                 | -                 |
| Nitrogen, Ammonia        | 208               | -                 | 229               | -                 | -                 |
| Nitrogen, Nitrate        | 100               | -                 | 100               | 26.6              | 100               |
| Nitrogen, Nitrite        | 29.6              | -                 | -                 | -                 | -                 |
| Nitrogen, Total Kjeldahl | 470               | -                 | 690               | -                 | -                 |
| Phosphorus, Total        | 226               | -                 | 128               | -                 | -                 |
| Sulfate                  | 10000             | -                 | 1500              | 1400              | 10000             |
| Total Organic Carbon     | 360000            | 160000            | 13000             | 5100              | 11000             |



# CVOC Results in MW-12S and MW-12D



In MW-12S, VC rose from 1.4 to 5.9 ug/L, but TCE has gone from an average of over 50 ug/L to 3. TOC now at background concentration.

In MW-12D, TCE remains low at 2.5 but has dropped by more than two orders of magnitude, as DCE has as well from baseline.

VC went from 2.4 to 1.9 mg/L (close to NJDEP GWQS). TOC at background concentration – MNA appropriate – apply for permit.

# Summary of ISB Program

- The carbon source amendments led to a rapid increase in TOC in the groundwater, which led to highly anaerobic conditions (beer garden). Background conditions and CVOC levels were conducive for this simple carbon substrate approach (EOS).
- We saw major drops (more than 1-2 orders of magnitude) in our key CVOC of concern, TCE.
- Interesting note - Significant increase in 2-butanone, which is a breakdown product from the VFA butyrate – we are seeing and have seen good degradation. There is a NJDEP standard for 2-butanone (300 ug/L), we were over that but see it attenuating to background over time.
- In process of transitioning to monitored natural attenuation – NJDEP MNA permit application – closes case upon approval.
- Success attributed to good distribution of amendments within the specially designed and installed injection wells (screened / grouted tightly into those thin impacted geological units), can't stress the tremie-grout seal enough, and,
- Our strong but simple molecular tool belt with the results from the ISM study that showed proof of concept (both carbon / EVO / nutrient substrates showed +/- >90% reduction in ISM study; and, favorable ongoing monitoring data collected on a molecular level (DHC counts, geochemical / nutrients and VOC data continue to be favorable).

# Questions?

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