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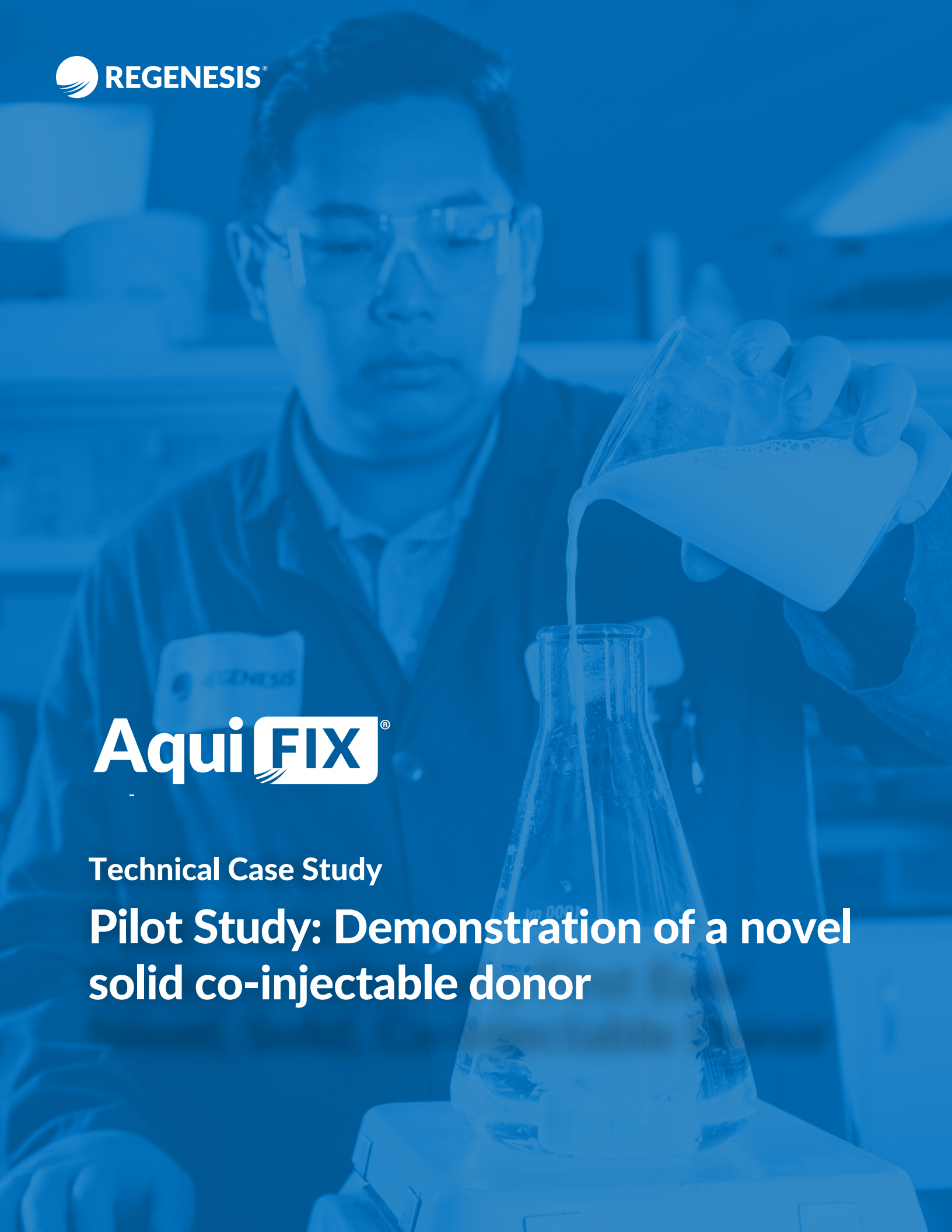


AquiFIX®

Technical Case Study

Pilot Study: Demonstration of a novel solid co-injectable donor

REGENESIS®

Case Study

AquiFix: The first co-injectible, micron-scale, solid electron donor to promote the effective treatment of chlorinated solvents

1.0 Introduction

AquiFix™, a new co-injectable remediation substrate formulated to rapidly establish and sustain anaerobic biodegradation of chlorinated volatile organic compounds (CVOCs) over extended timeframes, was field tested at a CVOC plume site in Southern Michigan. The two-year-long field trial involved installing two short permeable reactive barriers (PRBs) for testing AquiFix. PlumeStop®, a patented colloidal activated carbon amendment, was mixed with AquiFix and injected at one of the PRBs, while only AquiFix was injected at the other PRB.

The new AquiFix technology is chemically compatible, and designed for direct mixing and co-application with colloidal amendments like PlumeStop and S-MicroZVI®. A key pilot test objective was to assess AquiFix in a PlumeStop sorption-enhanced bioremediation system. Bio-Dechlor INOCULUM® (BDI), a bioaugmentation agent, was also injected at each PRB test area.

The PRB area treated with PlumeStop & AquiFix (Treatment Area 1) is in a high to extreme CVOC mass flux area of the plume where the estimated CVOC mass flux exceeds 6,000 mg/m^2/day. In the AquiFix PRB treatment segment where no PlumeStop was added (Treatment Area 2), baseline CVOC concentrations were much lower, representing a more typical CVOC plume.

The results from the *deep, extreme mass-flux zone* at Treatment Area 1 show that total groundwater CVOC concentrations were reduced from baseline by >90% in one month and almost fully eliminated (>99%) through two years of monitoring.

Additionally, the near-complete conversion of CVOCs to non-toxic daughter products has been observed, with parts-per-million ethene levels sustained for over a year.

In the *shallow, high-mass flux zone* at Treatment Area 1, CVOCs were significantly reduced but less than in the *deep, extreme mass-flux zone*. The less robust CVOC dechlorination in the shallow zone is likely due to the amendments' preferential distribution into the deeper zone. The same direct push injection points were used to deliver amendments into both zones, using a bottom-up approach.

At Treatment Area 2, where no PlumeStop was added, AquiFix reduced the concentrations by more than 90% from baseline with ethene, DHC, and functional gene populations sustained for more than two years thus far.

With the demonstration period now complete, the pilot test has shown that CVOCs can be rapidly removed from groundwater and effectively treated in an extreme mass flux environment using a mixture of PlumeStop and AquiFix. In this system, PlumeStop significantly retards the CVOCs, allowing AquiFix to facilitate their complete reductive dechlorination, regenerating PlumeStop's sorption surface.

Furthermore, AquiFix has demonstrated its utility and effectiveness as a standalone reductive dechlorination amendment. At both pilot test areas, total organic carbon (TOC) continues to be sustained after two years, indicating that AquiFix will persist in promoting effective CVOC bioremediation.

2.0 Technology Description

2.1 AquiFix

AquiFix is an organic remediation amendment developed to promote *in situ* anaerobic biodegradation of CVOCs in groundwater. AquiFix contains an engineered mixture of fast and slow-release organic compounds. The slow-release component comprises fine, solid-phase, plant-based organic particles (<0.5 µm) suspended in water using polymer dispersion chemistry. The rapid-release organic compound dissolves into the aqueous phase.

AquiFix is easily injected into the subsurface employing standard injection equipment and disperses widely within aquifer flux zones. Once in the subsurface, the solid particles bind to the aquifer matrix while the rapidly degradable organic component quickly establishes reducing conditions. The solid particles slowly degrade to produce volatile fatty acids and molecular hydrogen that sustain long-term anaerobic bioremediation. Under most circumstances, AquiFix is expected to stimulate anaerobic biodegradation for 10+ years.

AquiFix is fully compatible with and does not interfere with the performance of co-applied remediation amendments, including PlumeStop and S-MicroZVI.

2.2 PlumeStop

PlumeStop is a micron-scale colloidal activated carbon suspension formulated to achieve wide distribution in the subsurface to treat toxic groundwater contaminants. PlumeStop easily moves through soil pores upon injection, “painting” aquifer solid materials with highly reactive carbon. In sorption-enhanced bioremediation approaches, PlumeStop is used for engineering the retardation of CVOCs and other groundwater contaminants, sorbing the contaminants onto the aquifer matrix and preventing their movement in groundwater for extended timeframes. This process allows bioremediation agents like AquiFix to fully mineralize CVOCs to non-toxic end products like ethene and carbon dioxide within the confined volume of a PRB or other treatment zone.

2.3 Bio-Dechlor INOCULUM (BDI)

BDI is an enriched microbial consortium containing *Dehalococcoides sp.* (DHC) species. Co-applied with controlled release carbon substrates like AquiFix and 3-D Microemulsion, BDI stimulates the rapid and complete dechlorination of CVOCs to non-toxic end products, like ethene, carbon dioxide, and water.

3.0 Pilot Test

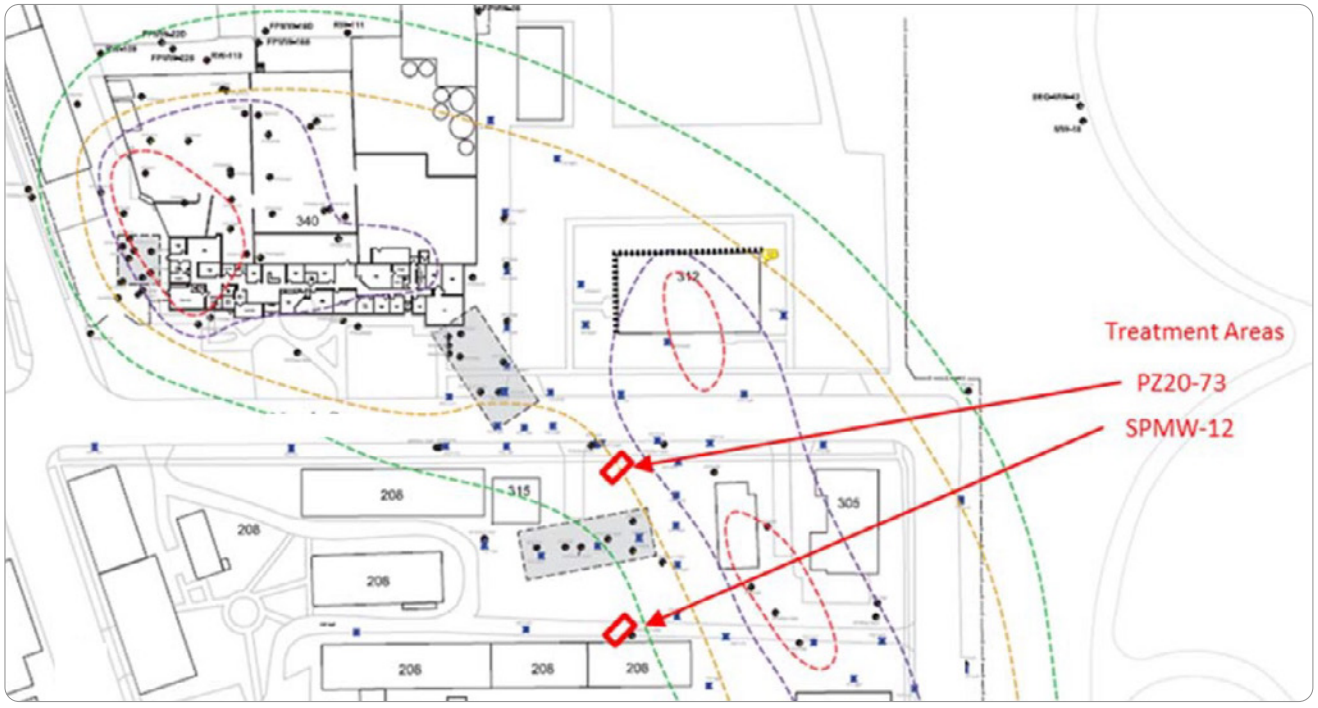
3.1 Site Description

The pilot test site was conducted in a large CVOC plume, originating from a manufacturing and distribution facility in central Michigan. Figure 1 Past site activities resulted in spilled chemical solvents, primarily PCE and TCE, into the subsurface, creating a groundwater CVOC plume extending thousands of feet downgradient beneath a residential neighborhood. High CVOC concentrations, greater than 64,000 micrograms per liter (µg/L), were detected in the pilot study area, based on passive-flux sampling data obtained from a nearby well. The CVOCs are almost entirely comprised of chlorinated ethenes with relatively low concentrations of 1,1,1-trichlorethane and 1,1-dichloroethane reported.

Petroleum hydrocarbons were also spilled at the facility, forming light non-aqueous phase liquids (LNAPL) and creating a commingled plume beneath and near the facility. This commingling resulted in the formation of highly mobile daughter CVOCs, particularly cis-1,2-dichloroethene (cis-DCE) and VC, increasing the overall plume extent and severity.

The hydrogeologic setting is controlled by a prominent esker, a linear, glacially deposited feature comprised of coarse sand, gravel, and cobbles. Groundwater moves through the esker at velocities ranging from 0.3 -3.3m per day. This combination of factors resulted in a persistent, high to extreme contaminant flux environment in the pilot test area. Passive flux monitoring data indicate an average groundwater velocity exceeding 200 feet per year and CVOC mass flux exceeding 6,400 mg/m^2/day in this high flux zone.

Figure 1 **Pilot Test Treatment Areas**



Pilot test treatment areas shown relative to the chlorinated solvent plume.

3.2 Remedial Design Application

Two approximately 6m-long PRB segments were designed for the application, designated as Treatment Area 1 (PZ20-73) and Treatment Area 2 (SPMW-12). The PRBs were oriented approximately perpendicular to groundwater flow.

Seven injection points were advanced to deliver the remedial amendments to the subsurface at each pilot test area. The injection array was positioned approximately 1.3m upgradient of the performance wells at each area. Figure 2A and Figure 2B

PlumeStop and AquiFix were co-applied at Treatment Area 1 (PZ20-73) in a high to extreme mass-flux zone of the CVOC plume. AquiFix (no PlumeStop added) was injected at Treatment Area 2 (SPMW-12), located in a lower mass-flux zone. BDI was injected at both treatment areas.

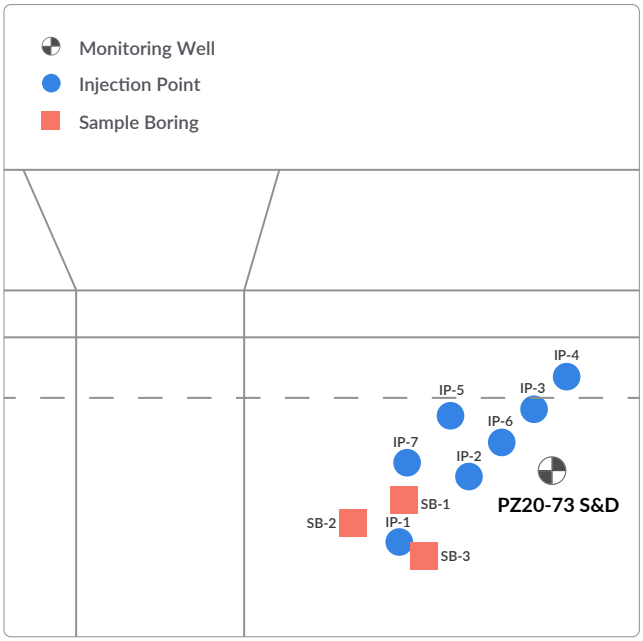
The injection intervals were matched to the performance monitoring well screen depths. At the Treatment Area 1 amendments were injected from 5-8m below ground level (BGL), matching the screen interval of PZ20-73 (S&D). At Treatment Area 2, amendments were injected from 7-9m BGL, encompassing the screen interval of SPMW-12S.

Figure 2A — Treatment Area 1

Treatment Area 1: PZ20-73 AquifFix plus PlumeStop pilot test location.

Treatment Area 1 (PZ20-73)

PRB Length	6 m
Injection Interval	5-8 m BGL
Shallow, high-mass flux zone	5-7 m BGL
Deep, extreme mass-flux zone	7-8 m BGL
Amendments	
AquiFix	097 kg
PlumeStop	650 kg
BDI	9 litres
Total Mix Volume Applied	7,143 L



REGENESIS Remediation Services (RRS) completed the application in December 2020, injecting approximately 7,104 litres of AquiFix and PlumeStop at Treatment Area 1 and 5,583 litres of AquiFix (no PlumeStop added) at Treatment Area 2. These volumes include a nominal volume of BDI culture (9 litres) added to each area.

The amendments were mixed in the RRS injection trailer utilizing two 1,325L tanks equipped with vortex mixers. Mixing water was provided by a nearby hydrant connected to the RRS injection trailer. After mixing, RRS applied the amendments to the subsurface using a positive-pressure, electrically powered pump connected to 38mm diameter Geoprobe rods.

At each injection point, a hydraulic percussion (i.e., direct push) drill rig with a leading three-foot, retractable stainless-steel injection screen was used to advance the borings to the bottom of the target treatment zone. Injection rods were lifted periodically once the desired product volume had been reached. Injection flow rates were maintained between 2 and 5 gallons per minute, with injection pressures maintained between 0.3 and 4 Bar. Overall, higher injection pressures were observed at Treatment Area 1.

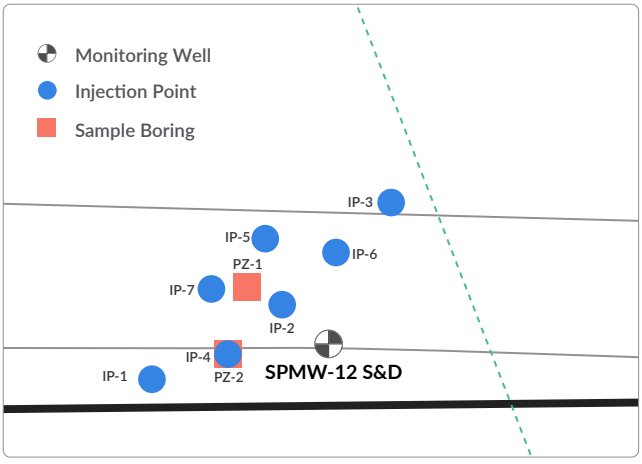
Figure 2B

Treatment Area 2

SPMW-12 AquifFix (no PlumeStop) pilot test location.

Treatment Area 2 (SPMW-12)

PRB Length	6 m
Injection Interval	7-9 m
Amendments	
AquiFix	907 kg
BDI	9 L
Total Mix Volume Applied	5,586 L



4.0 Results

4.1 Treatment Area 1: AquiFix & PlumeStop PRB Segment

4.1.1 Deep Interval - Extreme Mass-Flux Zone (PZ20-73D)

The pilot test results at the Treatment Area 1 - PlumeStop + AquiFix PRB treatment segment in the deep interval - *extreme mass-flux zone (PZ20-73D)* showed the following:

- Total CVOC concentrations in groundwater were reduced by 97% in one month and further reduced by >99% after two years of monitoring Figure 3.
- High ethene concentrations, averaging almost 4,000 micrograms per liter (µg/L), were sustained during the second year of post-injection monitoring. After two years, ethene concentrations are seven times higher than the sum of CVOCs, indicating almost complete mineralization of these contaminants Figure 4.
- DHC and vinyl chloride reductase (VCR) populations, initially augmented by the injection of BDI, have been sustained at greater than 10^4 cells/ml Figure 5.
- TOC concentrations were elevated above 1,000 mg/L immediately following the injection, remaining relatively consistent between 10 and 100 mg/L over the last three monitoring events.

These results suggest extraordinarily effective reductive dechlorination resulting in the almost complete elimination of CVOCs for over two years in this extreme mass flux zone. Further, the residual TOC concentrations indicate that AquiFix will remain effective in facilitating bioremediation of the CVOCs.

Figure 3 **Cl-Ethenes - PZ20-73D - AquiFix & PlumeStop**

Chlorinated ethene concentrations over time at PZ20-73D in AquiFix & PlumeStop PRB segment - deep interval, extreme-mass flux zone.

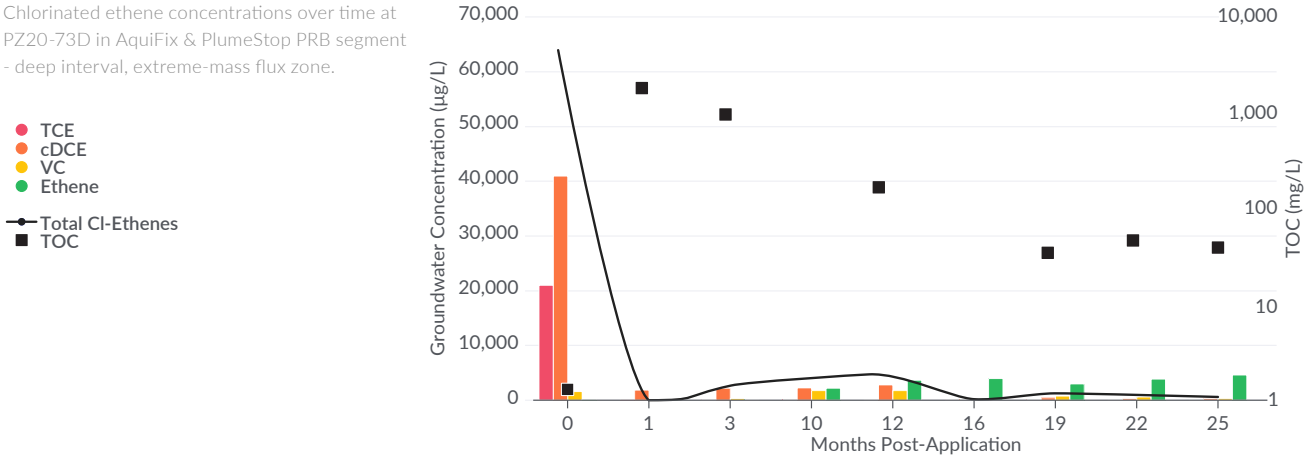


Figure 4 Change in Molar Fraction PZ20-73D - AquiFix & PlumeStop

Chlorinated ethene molar fraction changes at PZ20-73D in AquiFix & PlumeStop PRB segment – deep interval, extreme mass-flux zone. Molar fractions are plotted as stacked columns on the primary axis and the total molar concentration of chlorinated ethenes plus ethene is shown by the solid line on the secondary axis.

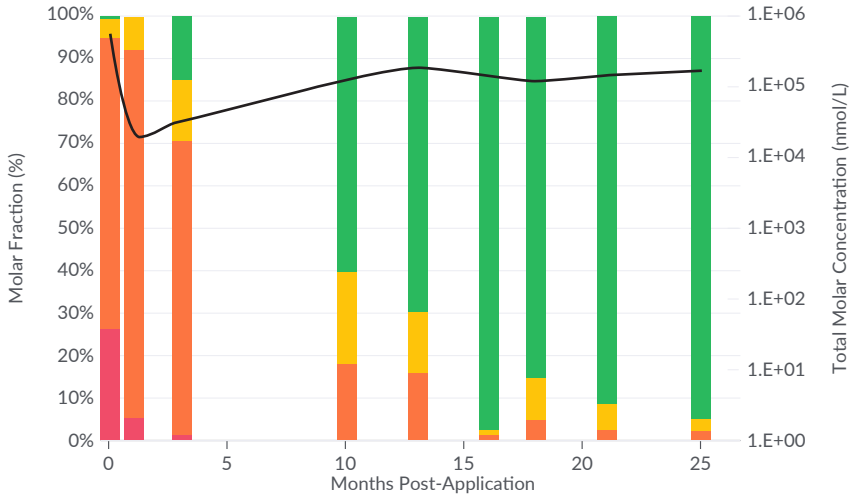
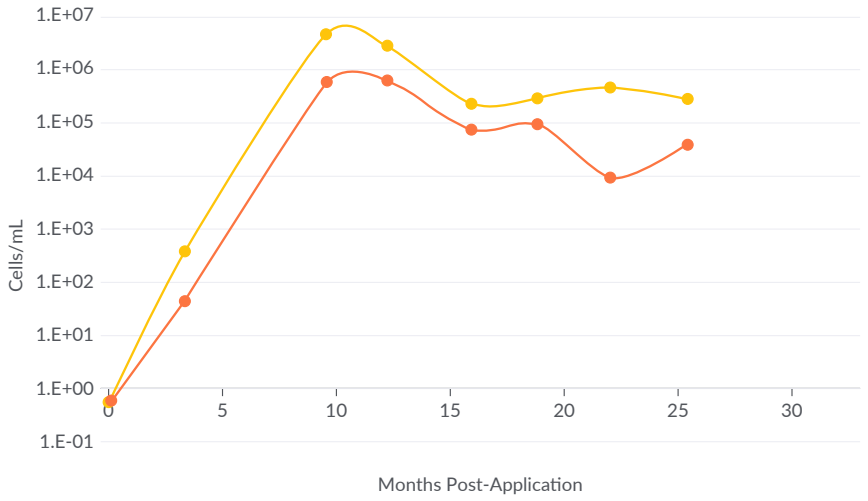


Figure 5 DHC and VCR Populations - PZ20-73D - AquiFix & PlumeStop

DHC and VCR populations at PZ20-73D in AquiFix & PlumeStop PRB segment – deep interval, extreme mass-flux zone.



4.1.2 Shallow Interval - High Mass-Flux Zone (PZ20-73S)

The pilot test results at the Treatment Area 1 - PlumeStop & AquiFix PRB treatment segment in the shallow interval - *high mass-flux zone (PZ20-73S)* showed the following:

- Total CVOC concentrations in groundwater were reduced by 92% in one month, rebounding to some extent. After two years, total VOCs remained reduced by 53%, including an 87% decrease in TCE. Figure 6
- VC and ethene were detected at their highest concentrations during the 25-month monitoring event, while DHC and VCR populations remained elevated Figure 7 and Figure 8.
- TOC is present but at lower concentrations than in the deeper interval.

These results indicate that robust dechlorination occurs in the *shallow, high mass-flux zone* but at slower biotransformation rates than in the *deep, extreme mass flux zone*. In comparing the shallow and deep intervals, likely, the AquiFix and PlumeStop amendments were preferentially distributed into the deep interval - extreme-mass flux zone using the bottom-up injection approach.

Figure 6 Cl-Ethenes - PZ20-73S-AquiFix & PlumeStop

Chlorinated ethene concentrations over time at PZ20-73S in AquiFix & PlumeStop PRB segment - shallow interval, extreme mass-flux zone.

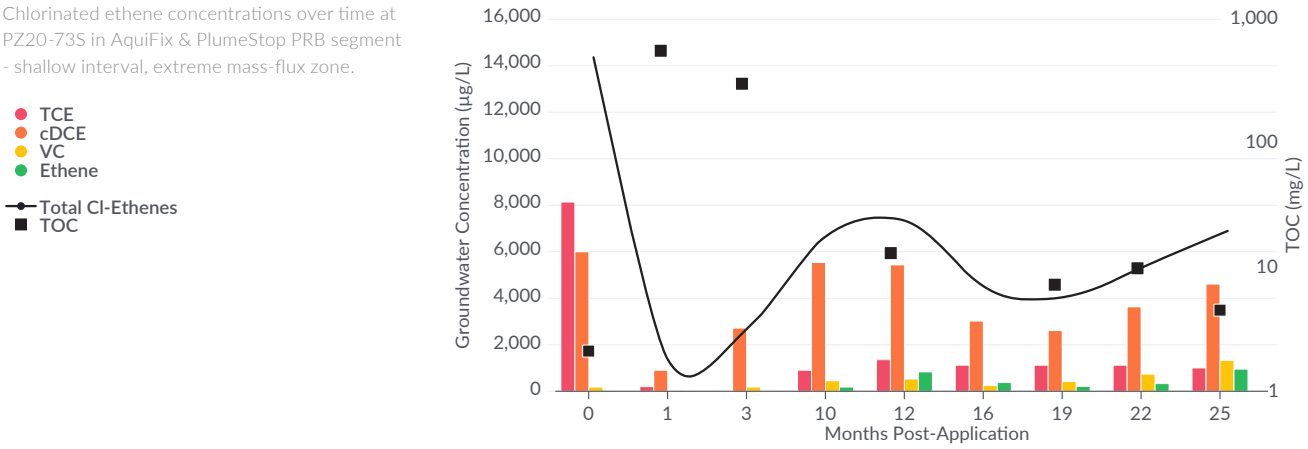


Figure 7 Change in Molar Fraction PZ20-73S - AquiFix & PlumeStop

Chlorinated ethene molar fraction changes at PZ20-73S in AquiFix & PlumeStop PRB segment – shallow interval, high mass-flux zone. Molar fractions are plotted as stacked columns on the primary axis and the total molar concentration of chlorinated ethenes plus ethene is shown by the solid line on the secondary axis.

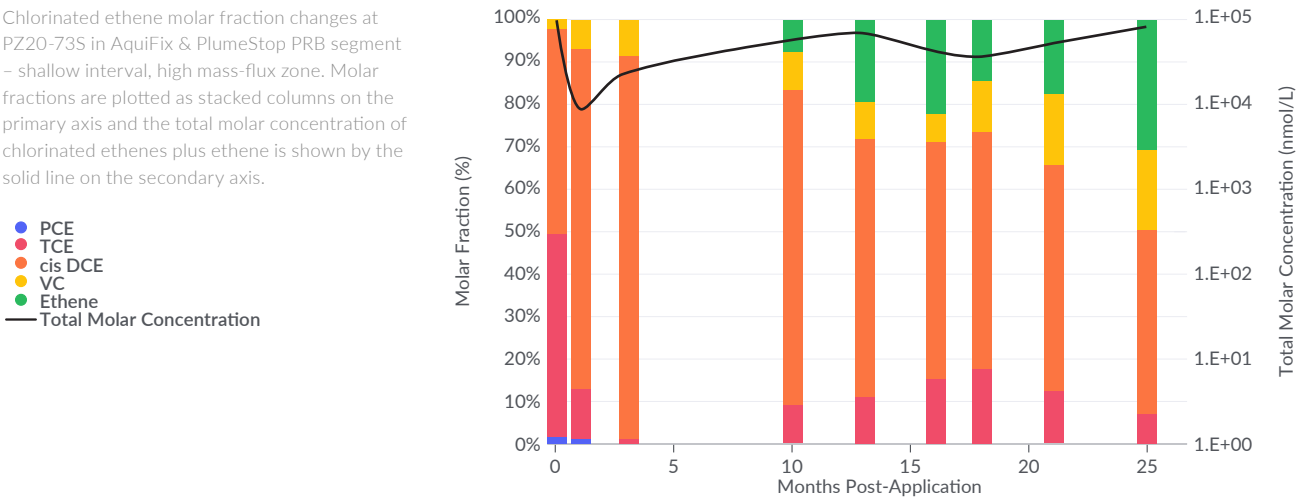
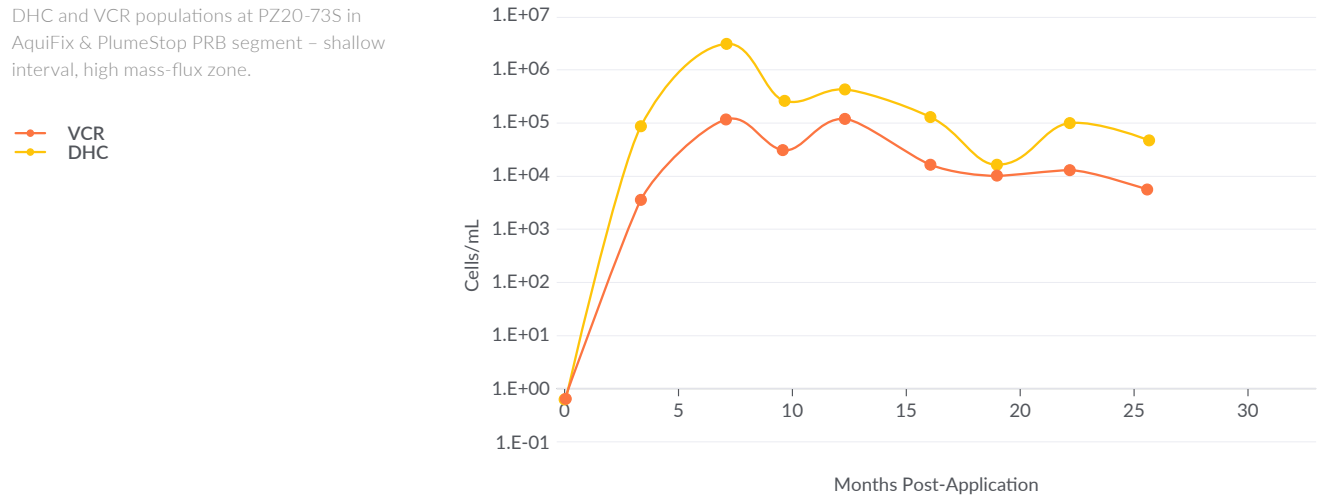


Figure 8 DHC and VCR Populations - PZ20-73S - AquiFix & PlumeStop

DHC and VCR populations at PZ20-73S in AquiFix & PlumeStop PRB segment – shallow interval, high mass-flux zone.



4.2 Treatment Area 2: AquiFix (no PlumeStop added) PRB Segment (SPMW-12S)

The pilot test results at Treatment Area 2 - AquiFix (no PlumeStop added) PRB treatment segment showed the following:

- Total CVOC concentrations in groundwater have been reduced by 93% after two years of monitoring Figure 9.
- Molar fraction percentage analysis shows increasing ethene relative to the chlorinated ethenes beginning at 10 months and peaking at 22 months when it was detected at its highest concentration of 760 µg/L Figure 10.
- DHC and VCR populations have remained elevated, ranging from 10^3 to 10^5 cells/ml Figure 11 .
- TOC concentrations were elevated above 1,000 mg/L immediately following the injection, remaining relatively steady over the last three monitoring events.

These results show effective AquiFix treatment in a low to moderate flux regime. A sustained source of TOC remains available to facilitate bioremediation of the CVOCs fluxing into the barrier in this relatively low-mass flux zone.

Figure 9 Cl-Ethenes - SPMW-12S - AquiFix (no PlumeStop)

Chlorinated ethene concentrations over time at SPMW-12S in AquiFix (no PlumeStop) PRB segment.

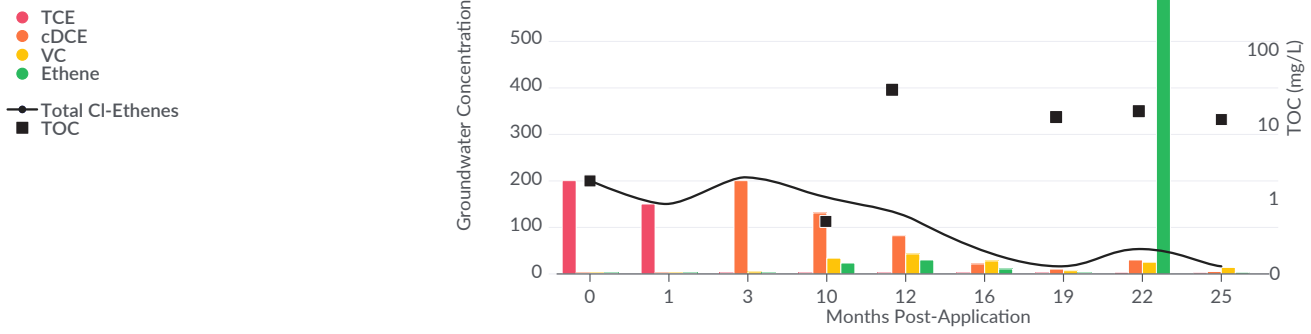


Figure 10 Change in Molar Fraction SPMW-12S - AquiFix (no PlumeStop)

Chlorinated ethene molar fraction changes at SPMW-12S in AquiFix (no PlumeStop) PRB segment. Molar fractions are plotted as stacked columns on the primary axis and the total molar concentration of chlorinated ethenes plus ethene is shown by the solid line on the secondary axis.

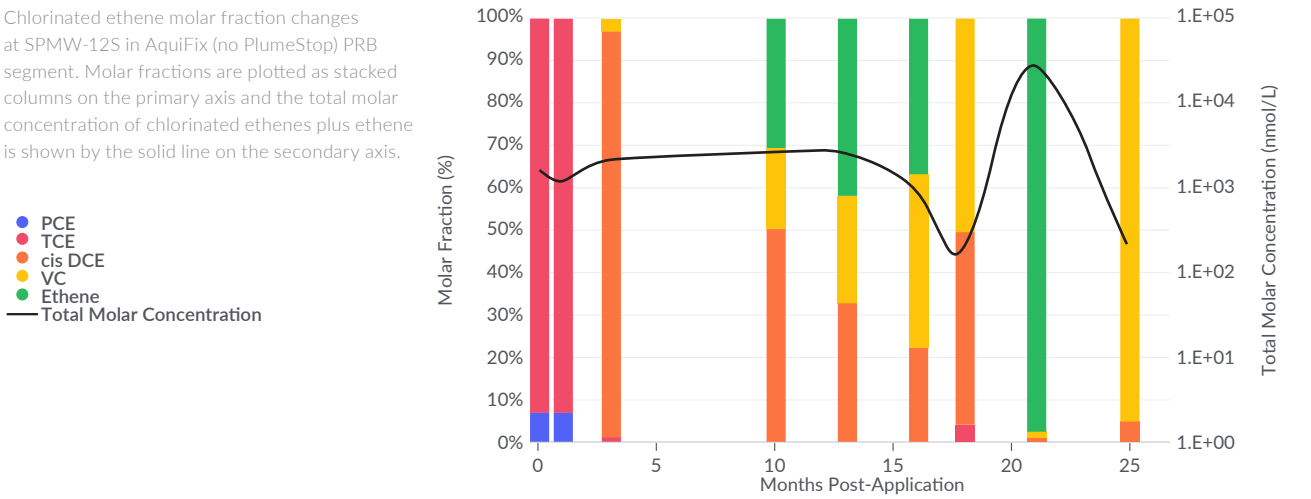
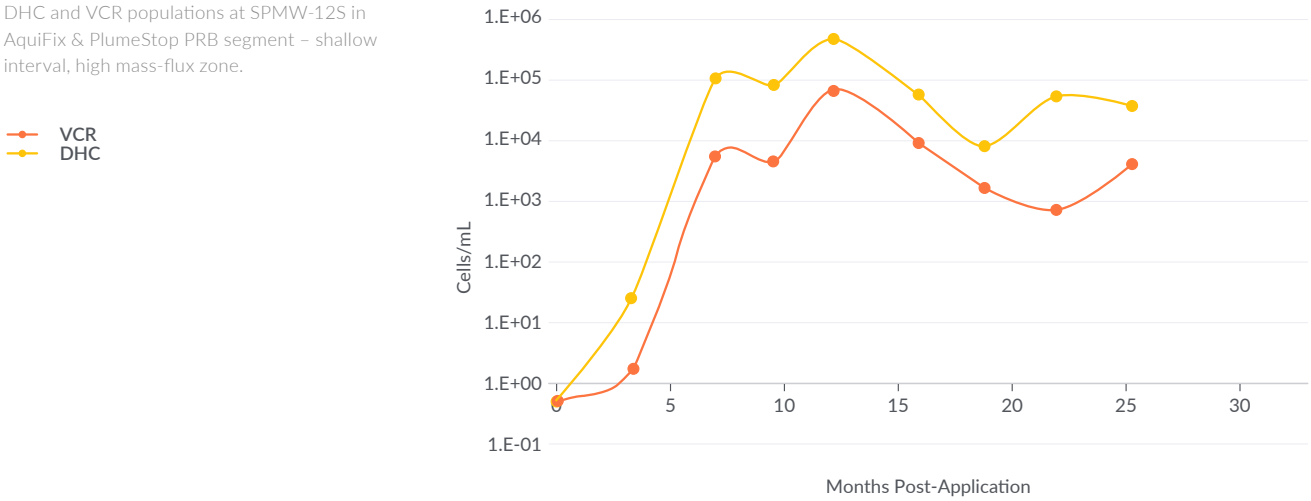


Figure 11 DHC and VCR Populations SPMW-12S - AquiFix (no PlumeStop)

DHC and VCR populations at SPMW-12S in AquiFix & PlumeStop PRB segment – shallow interval, high mass-flux zone.



5.0 Summary and Conclusions

AquiFix has shown a high level of efficacy in providing a staged release of organic carbon to rapidly promote and then sustain anaerobic biodegradation of CVOCs.

The results from the *deep, extreme mass-flux zone* treated with AquiFix & PlumeStop (Treatment Area 1) show almost total elimination (>99%) of over 60,000 µg/L CVOCs sustained through 25 months of performance monitoring. Ethene concentrations have averaged approximately 4,000 µg/L in this area for over a year. The change in molar fraction shows the near-complete transformation of CVOCs moving into the barrier to non-toxic species.

In the *shallow, high-mass flux zone* at Treatment Area 1, CVOCs were less reduced, as it appears the amendments preferentially distributed into the deeper zone. This may have resulted from using the same direct push injection points for both shallow and deep intervals while employing a bottom-up injection approach in highly conductive aquifer materials.

In the Treatment Area 2 - AquiFix PRB treatment segment (no PlumeStop added), modest concentrations of CVOCs have been reduced from baseline by 93%, with ethene, DHC, and functional gene populations sustained for more than two years, thus far.

With the demonstration period now complete, the pilot test demonstrates that sorption-enhanced bioremediation of CVOCs using a PlumeStop/AquiFix mixture is a highly effective treatment method, even as a PRB in an extreme mass-flux environment. In this system, PlumeStop substantially slows the CVOC mass flux allowing AquiFix the contact time necessary to fully degrade the CVOCs trapped in the PRB.

Ethene is non-sorptive, so as sorbed parent compounds like PCE, TCE and cis-1,2-DCE are transformed, PlumeStop's sorption surface is regenerated. This regeneration is important to prevent PlumeStop from becoming overwhelmed by the incoming CVOC mass flux.

Furthermore, AquiFix has demonstrated its utility and effectiveness as a standalone reductive dechlorination amendment. At both pilot test areas, total organic carbon (TOC) continues to be sustained after two years, indicating that AquiFix will likely remain effective in promoting bioremediation of the CVOCs for the foreseeable future.

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