REGENESIS®

Technical Bulletin

Laboratory and Field Results Documenting the Biodegradation of Contaminants from Colloidal Activated Carbon (CAC)



PLUME STOP® Liquid Activated Carbon

PetroFIX® Remediation Fluid

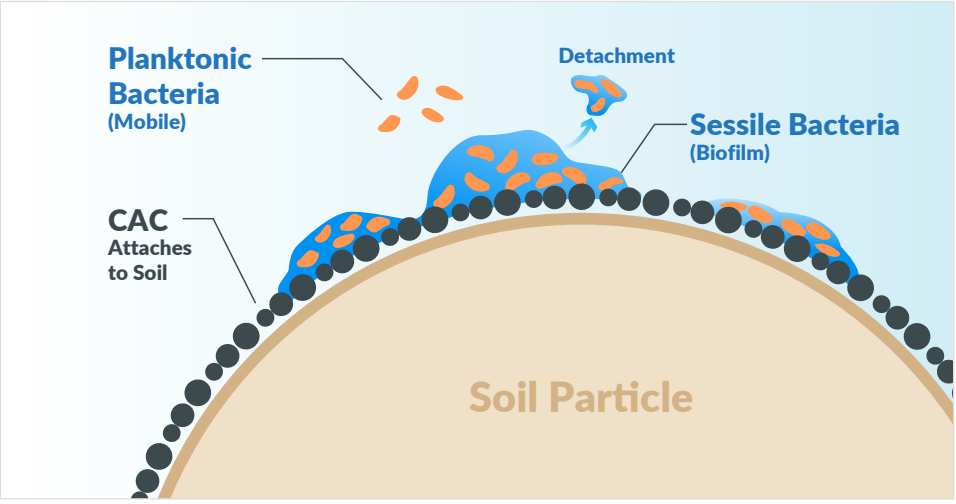
Introduction

The colloidal activated carbon (CAC) technologies developed by REGENESIS, PetroFix® and PlumeStop®, are often paired with biostimulating electron acceptors or donors to degrade adsorbed contaminants. Early laboratory and field research with our CAC-based technologies has shown the ability of bacteria to fully degrade adsorbed contaminants and increase in population in the presence of CAC and electron acceptors or donors added to the system. In the case of PetroFix, soluble electron acceptor salts are included to provide nitrate and sulfate, favorable electron acceptors used for biostimulation of hydrocarbon biodegradation.

We hypothesize that biostimulated bacteria form biofilms Figure 1 on the PetroFix or PlumeStop CAC surfaces coating the soil grains, and this close contact allows for contaminant destruction. As the evidence below shows, including direct microscopy of bacteria colonizing CAC, this mechanism is now well supported. Concentrated contaminants make for a ready food source (hydrocarbons) or electron acceptor (chlorinated solvents) for bacteria to utilize. This adsorb-then-degrade behavior is not unique to REGENESIS technologies; it is increasingly documented across the activated-carbon remediation and biogas literature and reflects a consistent, physically reasonable mechanism.

Figure 1 Biofilm Formation

Conceptual illustration of biofilm forming around CAC particles attached to a soil grain



A growing body of independent, peer-reviewed research supports the central premise of this bulletin: contaminants adsorbed to activated carbon remain biologically available and are actively biodegraded, and that biodegradation renews the carbon's adsorption capacity over time.

Particularly relevant is recent work on micron-scale activated carbon (MAC) — the same 1-micron size class as REGENESIS CAC. Zhang et al. (2022) immobilized indigenous diesel-degrading bacteria on 1-µm MAC and showed by scanning electron microscopy that the bacteria colonized and grew directly on the carbon surfaces, forming biofilm-like extracellular structures. The combined "bio-MAC" removed 86% of diesel in 15 days, versus 71% removed by sorption alone and 24% for free bacteria, a clear synergy of adsorption plus biodegradation. Critically, the authors concluded that the adsorption capacity of the carbon could recover as the adsorbed diesel was biodegraded by the attached bacteria, sustaining long-term removal[1] — the same regeneration behavior REGENESIS field data have long implied.

The literature also helps explain why carbon enhances biodegradation. Higher carbon dosing increased both bacterial concentration and enzymatic activity up to an optimum, indicating the carbon supplies attachment surface and concentrates substrate for the degraders (Zhang et al., 2022)[1]. Granular activated carbon has been shown to accelerate the anaerobic degradation of organic acids by several-fold, attributed to carbon-mediated interspecies electron transfer (Xu et al., 2018)[2], and biological activated carbon filters achieve prolonged service life specifically because ongoing biodegradation regenerates sorption sites (Piai et al., 2022)[3]. For petroleum hydrocarbons, work at the University of Waterloo indicated that the presence of activated carbon appears to enhance the rate of anaerobic hydrocarbon biodegradation (McLaren et al., 2022)[4].

Taken together, these independent findings — across multiple contaminants and carbon size classes — provide a strong scientific foundation for the REGENESIS laboratory and field results that follow. Because PlumeStop and PetroFix use the same size of activated carbon and are delivered similarly into the subsurface, results are considered interchangeable between the two technologies. The evidence below is organized by contaminant class — **hydrocarbon** and **chlorinated solvent** — with laboratory and field evidence presented within each.

Part A — Hydrocarbon Biodegradation Research

Laboratory Evidence

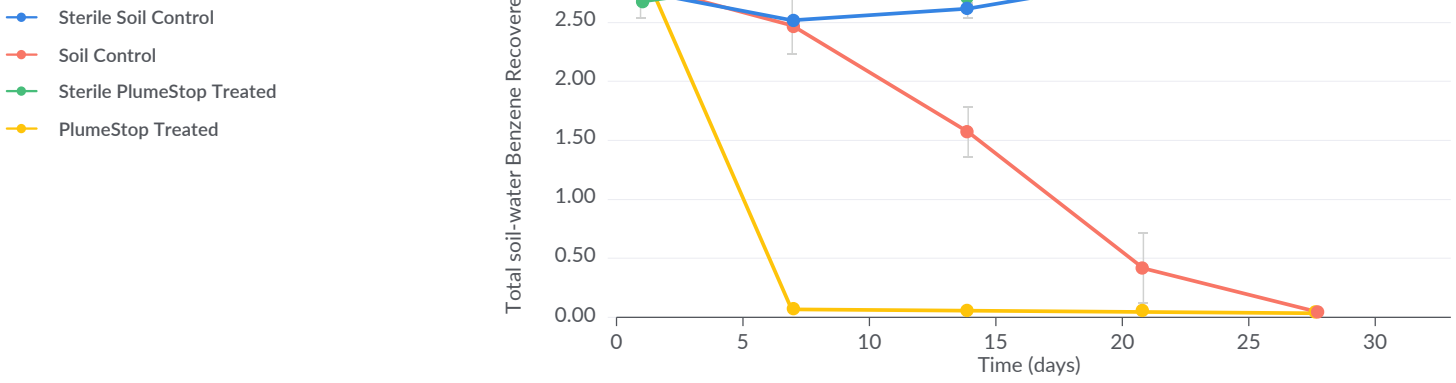
Bench-Scale Study — Full Contaminant Mineralization in the Presence of CAC

Early in the development of CAC, a laboratory batch study evaluated the post-sorption biodegradation of contaminants. Batch-equilibrium samples were prepared in 8 oz amber serum bottles containing 10 g of soil and 70 mL of water spiked with 3 mg of benzene, with oxygen supplied as the electron acceptor from the bottle headspace. Conditions included a sterile control, a live control, a CAC (PlumeStop) treatment, and a sterile CAC treatment. Bottles were sampled destructively in triplicate on days 1, 7, 14, 21, and 28, with benzene quantified in the aqueous phase and by full mass-balance extraction.

In less than seven days, the PlumeStop bottles (yellow line, Figure 2) showed rapid elimination of benzene to non-detect from soil and groundwater, while the soil-only control achieved only a 12.5% reduction by day 7. Sterile controls showed no destruction and no laboratory loss of contaminant.

Figure 2 Impact of PlumeStop on the Degradation of Benzene

Batch-equilibrium total-system extraction (mean of triplicates; error bars ± one standard deviation).



Read PlumeStop Technical Bulletin 3.1

View Recorded Webinar

Key finding: The sorption of contaminants onto PlumeStop does not inhibit their subsequent biodegradation at the studied concentrations.

Further Information: PlumeStop Technical Bulletin 3.1, and a webinar co-presented with Microbial Insights, "Demonstrating Contaminant Biodegradation in Conjunction with PlumeStop Liquid Activated Carbon."

Field Evidence

PetroFix Field Beta Trials — Increased Microbial Activity in the Presence of PetroFix

In the field, multiple lines of evidence are required to show ongoing biodegradation after adsorption, because rapid adsorption onto activated carbon can obscure contaminant destruction. A stronger line of evidence is the utilization of added nitrate and sulfate in the sequence predicted by anaerobic biodegradation (nitrate first, sulfate second).

At an early PetroFix beta site in Indiana, the correct order of electron-acceptor utilization was observed Figure 3. A further line of evidence is the post-application increase in microbial populations and gene targets Figure 4.

Figure 3 Results for Nitrate and Sulfate

Depletion of electron acceptors after PetroFix injection at an Indiana site (May 2018).

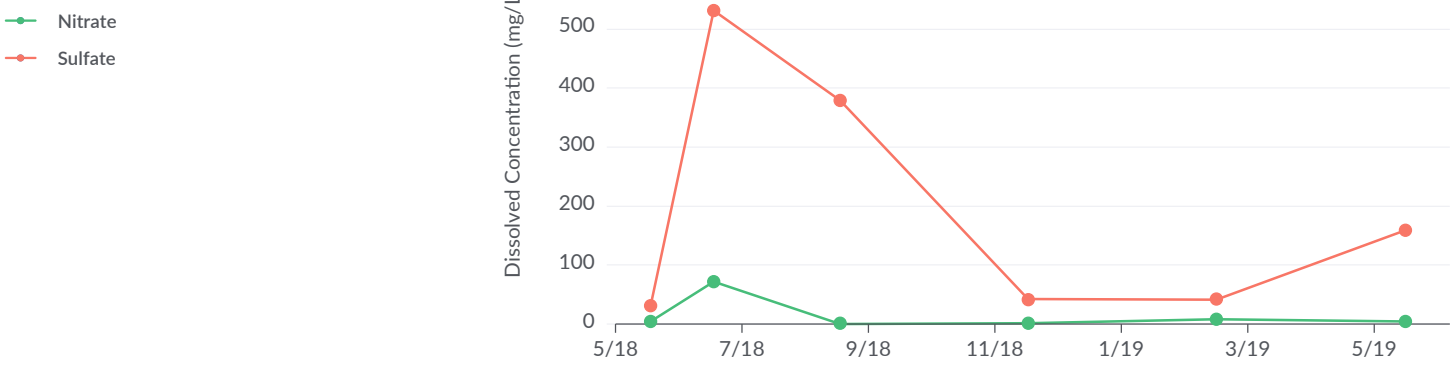
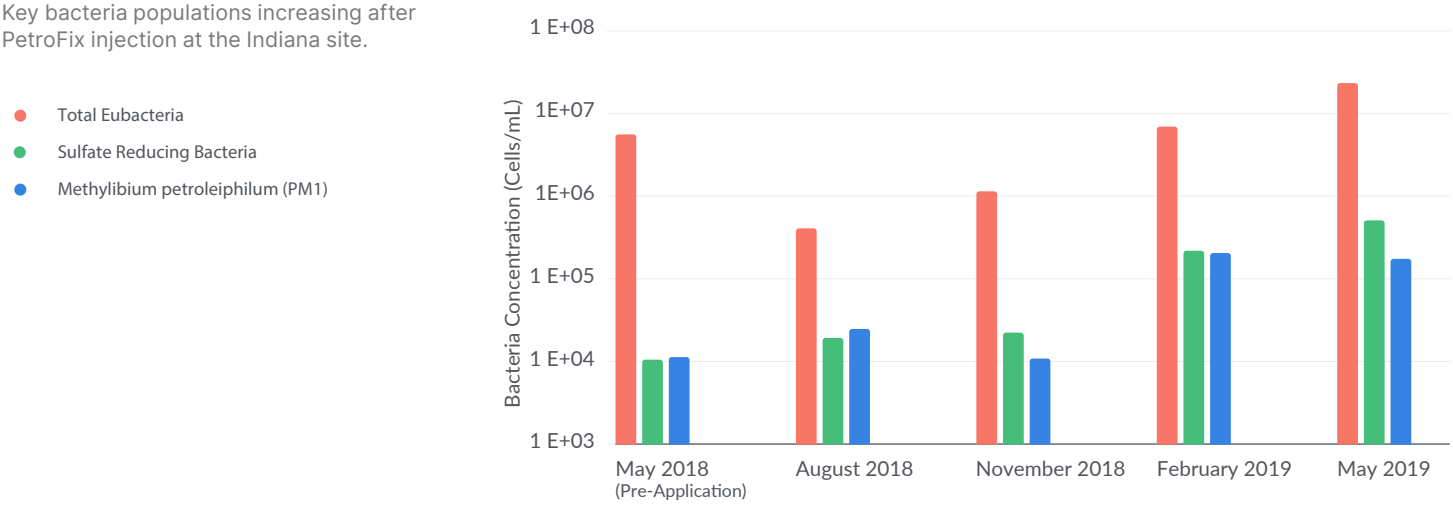


Figure 4 Site Microbial Activity

Key bacteria populations increasing after PetroFix injection at the Indiana site.



Read the Case Study

Key finding: Microbial population growth post-application, combined with the decrease of electron acceptors, is strong evidence for biodegradation — the beneficial populations access adsorbed contamination to fuel their growth.

Further information: Northern Indiana PetroFix Case Study.

Multi-Year PetroFix Field Study — Sustained Hydrocarbon Destruction Across Amended Treatment Areas

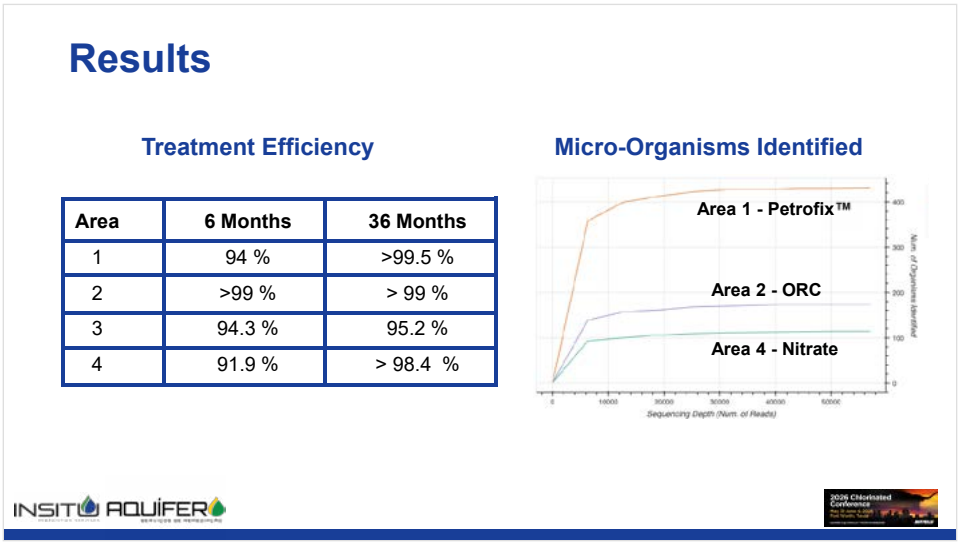
A multi-year field study at a petroleum-impacted site with elevated BTEX (up to ~122,000 µg/L), gasoline-range (up to ~154,000 µg/L) and diesel-range organics, plus residual LNAPL, evaluated whether observed reductions reflected adsorption alone or adsorption coupled with biodegradation[5]. Four 1,000-ft² areas each received PetroFix with a different electron-acceptor package — Area 1 with PetroFix's own recommended supplied amendments (nitrate/sulfate), Area 2 with oxygen-releasing material, Area 3 with sulfate, and Area 4 with nitrate — combining analytical, microbiological, geochemical, and compound-specific isotope monitoring over six to seven years.

Because hydrocarbon biodegradation kinetics generally increase as conditions become more aerobic, oxygen was expected to drive the fastest initial rates — and it did: the oxygen-releasing area showed the fastest early kinetics and reached >99% within six months. Notably, however, **PetroFix's own electron-acceptor blend (Area 1) ultimately outperformed all four amendments, reaching the highest reduction at three years (>99.5%)** — very close to, but ahead of, the oxygen-enhanced area (>99%), with nitrate (>98.4%) and sulfate (~95.2%) also performing well. That the standard PetroFix blend matched and slightly exceeded oxygen at three years is a strong result for the base product.

Equally important is longevity: high hydrocarbon concentrations were driven down and stayed low across the multi-year record. Sustained reduction of this magnitude cannot be explained by finite sorption capacity alone; continued biological turnover is required to regenerate adsorption sites. The microbial data tie performance directly to biology. Every area's community was distinctly modified toward degraders matching its electron acceptor. The PetroFix-blend area (Area 1) developed the most diverse microbial community, with roughly 2.5 times as many organisms identified as the oxygen area and about 4 times as many as the nitrate area. It also achieved the highest three-year reduction Figure 5 and Figure 6. Compound-specific isotope shifts consistent with sulfate reduction provided an additional independent line of evidence.

Figure 5 Treatment Efficiency and Microbial Response by Area

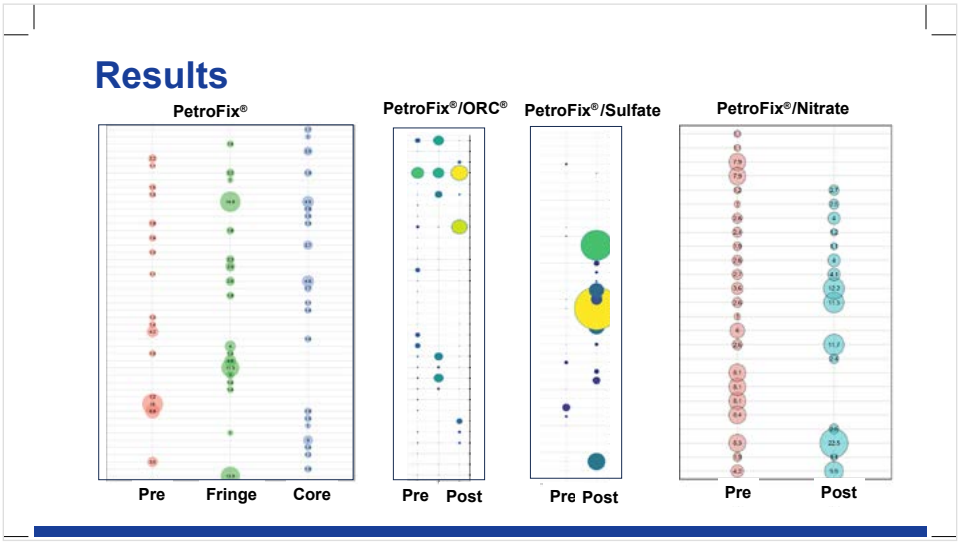
BTEX treatment efficiency at 6 and 36 months, alongside the number of microorganisms identified by sequencing. The PetroFix-blend area (Area 1) supported the most diverse microbial community and achieved the highest three-year reduction[5].



Area	6 Months	36 Months
1	94 %	>99.5 %
2	>99 %	> 99 %
3	94.3 %	95.2 %
4	91.9 %	> 98.4 %

Figure 6 Pre- & Post-Injection Microbial Communities by Treatment Area

Community shifts after application, reflecting the supplemental electron acceptor used in each area[5].



Reference: R. McGregor, "The Effect of Various Amendments on the Performance of Colloidal Activated Carbon for the Treatment of a High-Mass Petroleum Hydrocarbon Plume," presented at the 2026 Battelle Chlorinated Conference. Publication of data planned.



Image of a flow-through tank in operation. Dark areas show PlumeStop in the more conductive area and diffusing into the lower k zones (lighter color bands).

Read the Technical Bulletin

Part B — Chlorinated Solvent Biodegradation Research

Laboratory Evidence

Flow-Through Tank Study — Enhanced Microbial Activity

Professor Tom Sale of Colorado State University led a SERDP-funded tank study using alternating high- and low-conductivity soil layers to simulate storage and release of TCE from low-permeability zones. REGENESIS worked with Tom Sale and Kevin Saller, Ph.D. to build a similar flow-through system to evaluate PlumeStop distribution and biological response. Four glass-paned tanks were packed with nine alternating silt and sand layers, flushed with TCE-saturated then clean anaerobic water, and dosed with PlumeStop, lactate, and other amendments after day 103. Water and soil were analyzed by qPCR at Microbial Insights.

PlumeStop distributed through high-K zones and penetrated low-k zones. *Dehalococcoides sp.* (DHC) populations and functional genes (TCE and vinyl chloride reductases, VCR and BVC) increased at least 1 to 2 orders of magnitude versus the treatment without PlumeStop (Figure 7), and effluent showed 90–99.9% TCE mineralization in the PlumeStop tanks.

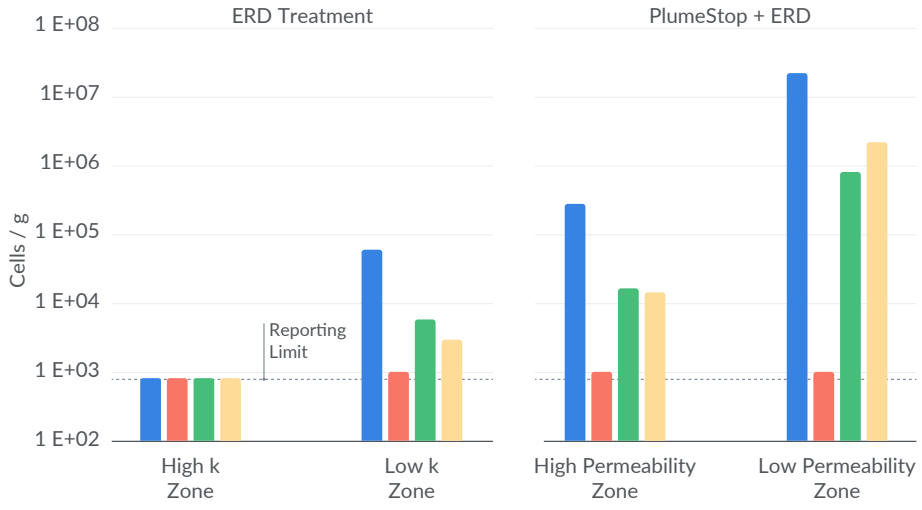
Key finding: Dechlorinating bacteria and functional genes increased 1–2 orders of magnitude in the presence of PlumeStop, and TCE was degraded.

Further information: A Solution to the Problem of Back Diffusion (PlumeStop Technical Bulletin 6.1).

Figure 7

PlumeStop Effects on DHC Populations

DHC and functional genes increase in the presence of PlumeStop versus without, in both high- and low-permeability (K) zones.



Bench-Scale Column Study — Chlorinated-Ethene Biodegradation Associated with CAC

A SERDP-funded bench-scale column and modeling study (Project ER21-1224) led by Dr. Dimin Fan of Geosyntec Consultants, with SiREM providing the KB-1® dechlorinating culture, directly examined the interaction between sorption on CAC and biological reductive dechlorination[6]. Five flow-through columns were operated with a continuous ~10 mg/L TCE influent at two CAC loadings (200 and 1,000 mg/kg), with and without bioaugmentation, and a 10-cm CAC reactive zone so that behavior within and downgradient of the barrier could be observed independently.

CAC first sorbed TCE, delaying breakthrough and lowering dissolved concentrations, but contaminant fate did not stop at adsorption. In the non-sterilized sorption-only columns, TCE was biologically converted to cis-DCE within the CAC zone with no culture added, indicating indigenous organisms used the CAC product's polymer stabilizer as an electron donor. In the bioaugmented CAC columns, sequential dechlorination proceeded through cis-DCE and vinyl chloride to ethene. Ethene production increased with increasing CAC loading: at the higher CAC dose, downgradient cis-DCE + VC was lowest and ethene highest, while a large sorbed cis-DCE mass remained on the carbon — evidence that CAC acts as a reservoir that slowly releases daughter products back into solution where they remain available for continued dechlorination.

The presence of activated carbon did not suppress biological activity — it enhanced it. *Dehalococcoides sp.* biomarkers expanded significantly within the CAC zone during dechlorination, and Dr. Fan concluded that the presence of activated carbon enhanced the dechlorination process. A HYDRUS-1D reactive-transport model reproduced the sorption-then-dechlorination sequence and is being refined to add competitive sorption and an explicit carbon-enhancement term — work that remains under study.

Highlighted finding — CAC enhanced biodegradation: Ethene production increased with increasing CAC loading, *Dehalococcoides sp.* grew significantly within the CAC zone, and TCE was biologically converted even in columns with no culture added. Dr. Fan concluded that the presence of activated carbon enhanced reductive dechlorination — direct, independent evidence that biodegradation occurs on and in the presence of CAC rather than being inhibited by it.

Reference: D. Fan et al. (Geosyntec Consultants; SiREM), "Modeling Laboratory Experimental Data: The Effects of Colloidal Activated Carbon on Biological Dechlorination," SERDP ER21-1224, presented at the 2026 Battelle Chlorinated Conference[6]. Publication of data planned.

Field Evidence

Multiple Lines of Evidence for Biodegradation Following PlumeStop Application

A 2026 webinar co-presented with Microbial Insights — Evidence-Based Rapid CVOC Remediation with Proof: Linking Microbial, Geochemical, and Analytical Data, by Paul Erickson (Vice President of Research & Development, REGENESIS) and Jack Sheldon (Senior Consultant, Antea Group), introduced by Dr. Dora Taggart — assembled microbial, geochemical, analytical, and imaging evidence that chlorinated solvents adsorbed to CAC remain biologically available[7]. PlumeStop was paired with a non-interfering electron donor (AquiFix) to drive enhanced reductive dechlorination.

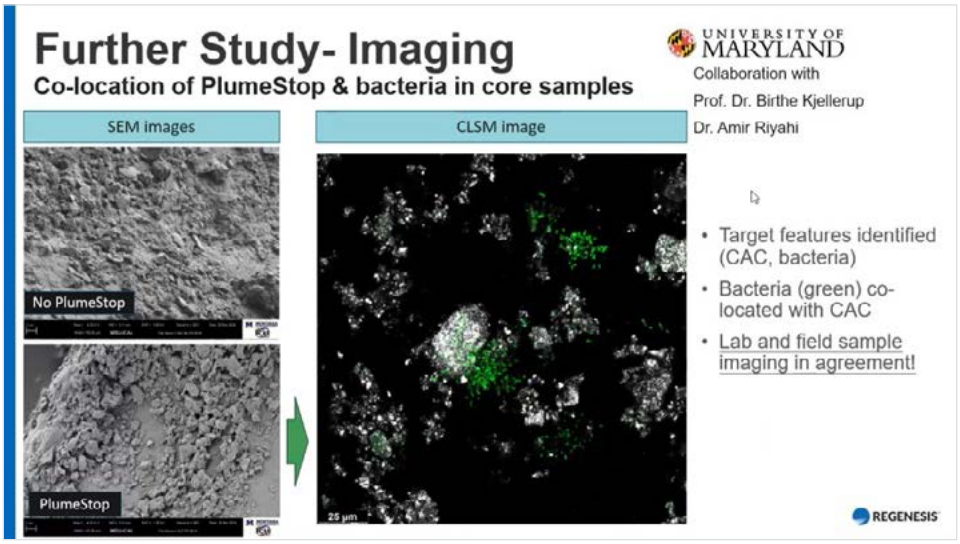
Watch the Webinar

Following application, an initial rapid drop in dissolved solvents reflected adsorption; subsequent monitoring showed continued destruction, with ethene — the terminal product of complete reductive dechlorination — accumulating while solvent concentrations stayed suppressed and did not rebound. Molecular tools confirmed active biology: *Dehalococcoides sp.* and vinyl-chloride-reductase (VCR) gene counts rose from near non-detect to sustained levels, and in a larger multi-mechanism application dechlorinators increased by at least two orders of magnitude, accompanied by strongly reducing geochemistry. Direct imaging of field cores by scanning electron and confocal microscopy showed bacteria co-located on and around the CAC particles, with laboratory and field images in agreement — visual confirmation that degrading organisms establish directly on the carbon.

> **Key finding:** Independent microbial, geochemical, analytical, and imaging lines of evidence converge on the same conclusion — CAC functions as a biologically active treatment matrix on which adsorbed solvents are dechlorinated to ethene, not merely stored.

Figure 8 Co-Location of PlumeStop and Bacteria in Core Samples

SEM and confocal laser scanning microscopy of core samples showing bacteria co-located with CAC particles. Recorded webinar featuring Paul Erickson (REGENESIS) and Jack Sheldon (Antea Group). (2026 Microbial Insights webinar; publication of data planned.)



Journal References

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2. Xu, S.; Han, R.; Zhang, Y.; He, C.; Liu, H. Differentiated stimulating effects of activated carbon on methanogenic degradation of acetate, propionate and butyrate. *Waste Management* 2018, 76, 394–403. https://doi.org/10.1016/j.wasman.2018.03.037

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Conference and Webinar References

4. McLaren, B., et al. *Does Activated Carbon Enhance the Biodegradation of Petroleum Hydrocarbons?* Poster presented at the 2022 Battelle Chlorinated Conference, Palm Springs, CA, May 22–26, 2022.

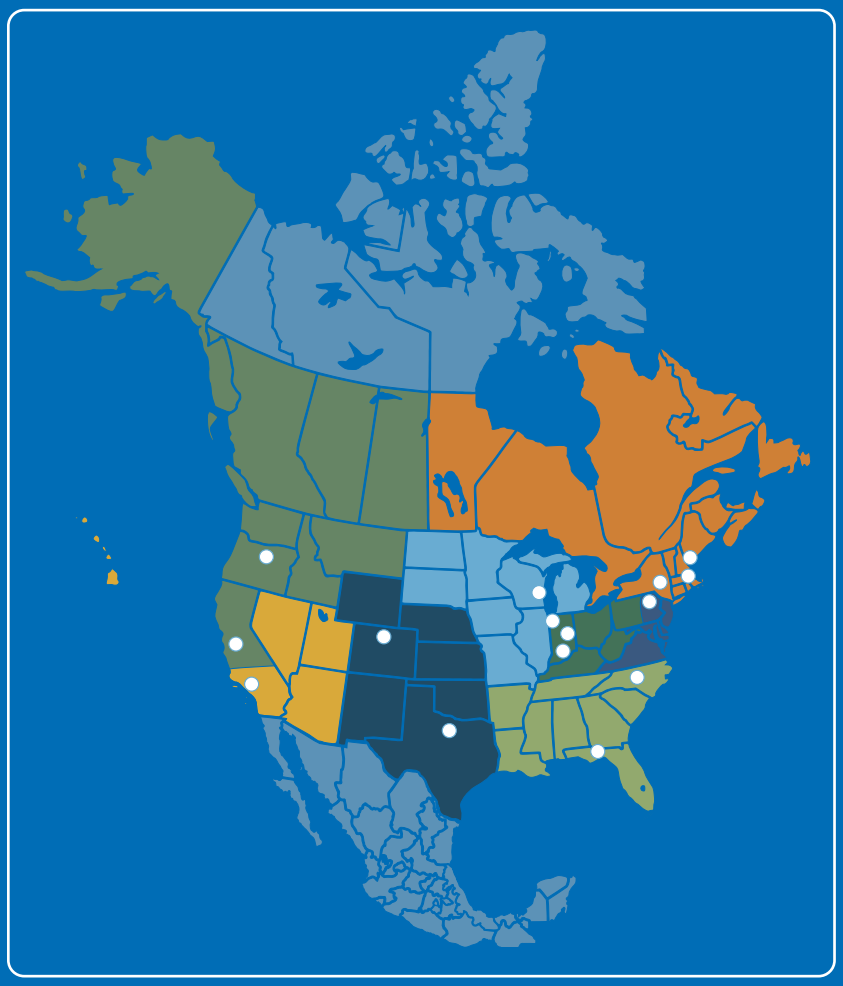
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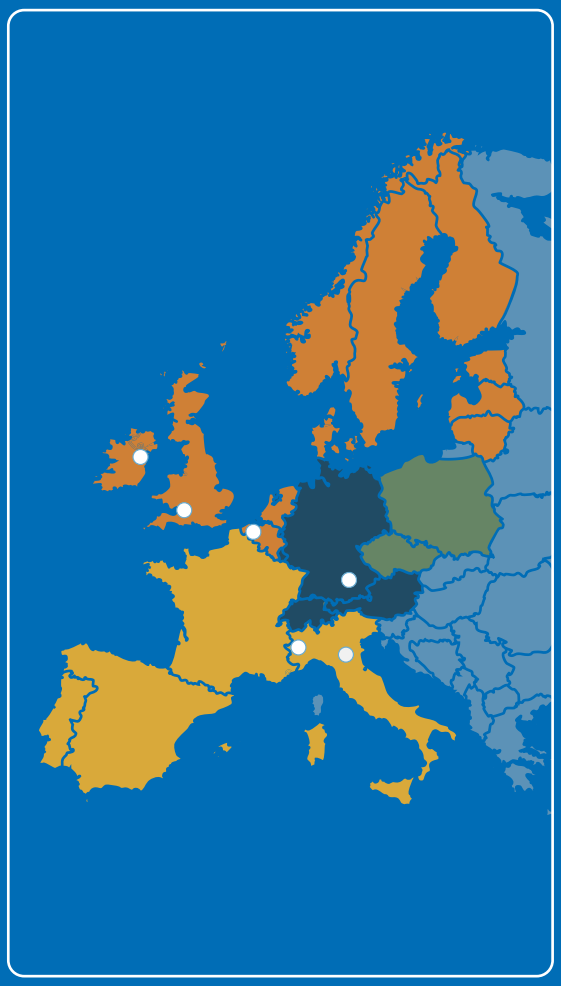
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7. Erickson, P.; Sheldon, J. *Evidence Based Rapid CVOC Remediation with Proof: Linking Microbial, Geochemical, and Analytical Data. Microbial Insights Insights in Practice* webinar, June 18, 2026. https://microbe.com/webinar/evidence-based-rapid-cvoc-remediation/.

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