

## CSIA: Actionable Data for Site Assessment and Performance Monitoring

### PROJECT SUMMARY



- During site assessment of a TCE site, compound specific isotope analysis (CSIA) did not demonstrate TCE or cis-DCE degradation, consistent with CENSUS qPCR results indicating low concentrations of *Dehalococcoides* (< 2.0E+01 cells/mL).
- A pilot-scale electron donor injection was performed to promote biological reductive dechlorination and formation of iron sulfides for abiotic degradation.
- Post-injection CSIA results conclusively demonstrated that cis-DCE degradation was stimulated and CENSUS qPCR revealed substantial increases in concentrations of *Dehalococcoides* (> 1.0E+04 cells/mL) and vinyl chloride reductase genes (> 1.0E+04 cells/mL).
- These multiple lines of evidence of enhanced reductive dechlorination gave site managers confidence in this treatment strategy and the treatment area was expanded.

### PROJECT CHALLENGE



At a former manufacturing facility that had operated for 50 years, a fractured bedrock aquifer was impacted by trichloroethene (TCE). Redox conditions were mildly anaerobic and cis-dichloroethene (cis-DCE) was detected. Site managers wanted to (1) conclusively determine whether biodegradation of TCE and cis-DCE was occurring, (2) assess the need for enhanced remediation, and (3) if necessary, evaluate the effectiveness of any enhanced remediation activities.

### SAMPLING AND ANALYSIS



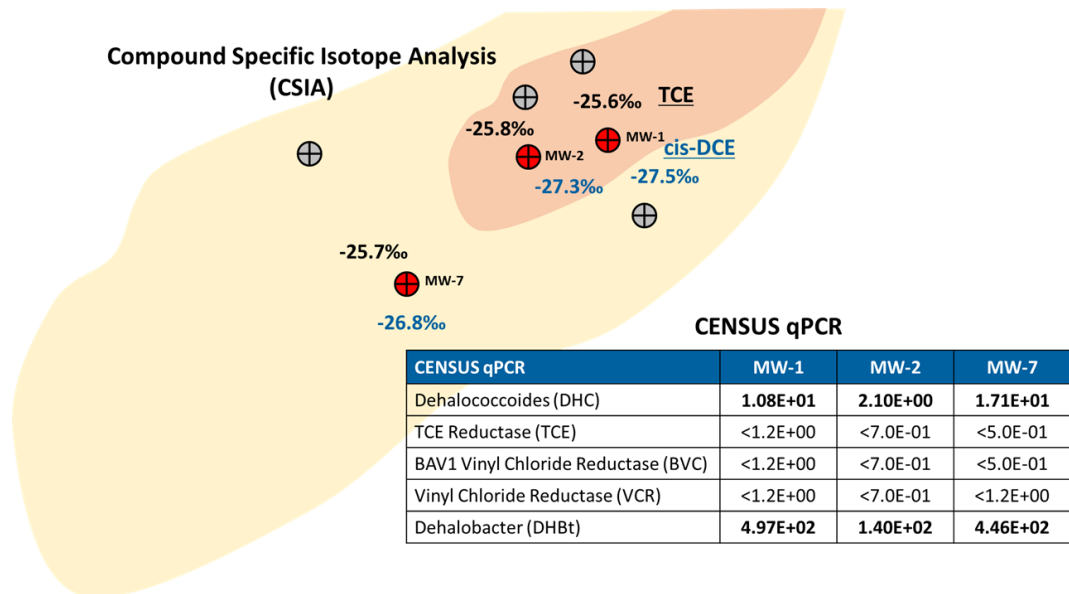
Compound specific isotope analysis (CSIA) was performed to conclusively determine whether TCE and daughter products were biodegrading. CSIA measures the ratio of stable isotopes (e.g.  $^{13}\text{C}/^{12}\text{C}$ ) of a contaminant. During degradation of many contaminants, the ratio of stable isotopes changes in predictable ways whereas physical processes like dilution do not appreciably impact isotope ratios. Therefore, isotopic fractionation (significant increases in  $\delta^{13}\text{C}$  values) conclusively demonstrates degradation of the contaminant. CENSUS qPCR was conducted to quantify *Dehalococcoides* and functional genes for vinyl chloride reductases to evaluate potential for biological reductive dechlorination to ethene.

## SITE ASSESSMENT (BASELINE)



One approach to interpreting CSIA results is to compare the  $\delta^{13}\text{C}$  values of a given contaminant at different monitoring wells along the flow path down the centerline of the dissolved plume.

- If the  $\delta^{13}\text{C}$  values of a contaminant increase by more than 2 ‰ along the flow path, that is significant isotopic fractionation and conclusive evidence of degradation of the contaminant.
- If the  $\delta^{13}\text{C}$  values do not increase by 2 ‰, degradation of that contaminant may or may not be occurring. CSIA cannot prove a negative.



**CSIA:** The  $\delta^{13}\text{C}$  value for TCE at downgradient monitoring well MW-7 (-25.7 ‰) was not significantly greater than observed at upgradient wells MW-1 (-25.6 ‰) and MW-2 (-25.8 ‰). For cis-DCE,  $\delta^{13}\text{C}$  values also did not increase by more than 2 ‰ along the groundwater flow path and all were within the range for manufactured (non-degraded) TCE as compiled in the Microbial Insights CSIA Database.

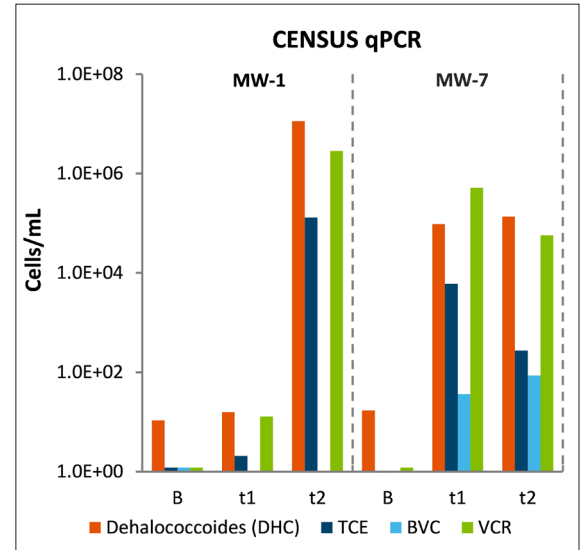
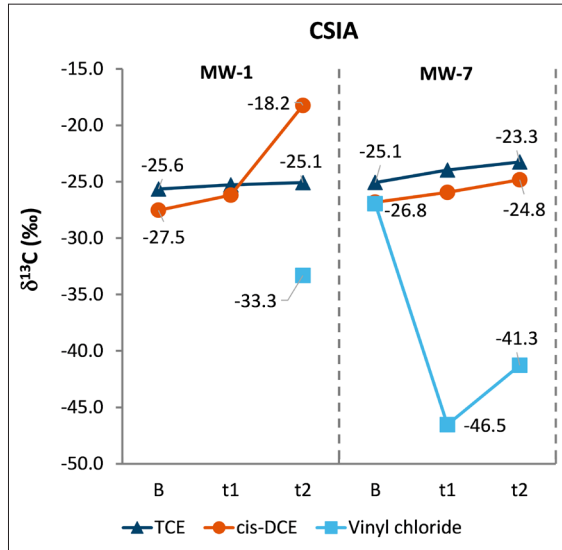
**CENSUS qPCR:** The CSIA results were consistent with CENSUS qPCR results indicating low concentrations of *Dehalococcoides* (DHC) and *Dehalobacter* (DHBt). With DHC below the recommended concentration of  $10^4$  cells/mL, effective rates of reductive dechlorination were unlikely under existing conditions.

**Decision:** While degradation may be occurring, without conclusive proof of cis-DCE degradation and with low concentrations of DHC and other halo-respiring bacteria, enhanced remediation was clearly warranted. CSIA and CENSUS qPCR results from the site assessment were used as the baseline to evaluate the effectiveness of enhanced bioremediation.

## PERFORMANCE EVALUATION



A pilot scale electron donor injection was conducted near MW-1. CSIA and CENSUS qPCR results for the baseline (B) and two post-injection sampling events (t1, t2) are shown below for MW-1 and downgradient well MW-7.



MW-1: The  $\delta^{13}\text{C}$  value for cis-DCE (orange line) increased significantly from the baseline event (-27.5 ‰) to time t2 (-18.2 ‰) demonstrating cis-DCE degradation had occurred following electron donor injection. In addition, CENSUS qPCR demonstrated a substantial, concurrent increase in concentrations of *Dehalococcoides* (DHC; orange bar) and vinyl chloride reductase genes (VCR; green bar) in response to the electron donor injection. The  $\delta^{13}\text{C}$  value for TCE (dark blue line) did not increase likely due to back diffusion of non-degraded TCE from an impacted less permeable zone present at this site (“masking”).

MW-7: For cis-DCE, significant isotopic fractionation was observed from baseline (-26.8 ‰) to the time t2 (-24.8 ‰) sampling event demonstrating degradation had occurred. While not confirming vinyl chloride degradation, the decrease in vinyl chloride  $\delta^{13}\text{C}$  values was consistent with enhanced reductive dechlorination of cis-DCE. Moreover, high concentrations of DHC and vinyl chloride reductase genes at both monitoring wells at time t2 demonstrated the potential for reductive dechlorination of vinyl chloride in subsequent sampling events.

Decision: Multiple lines of evidence indicate success - expand the treatment area. CSIA results conclusively demonstrated cis-DCE degradation had occurred while CENSUS qPCR revealed substantial increases in concentrations of *Dehalococcoides* and vinyl chloride reductase genes in response to the amendment addition.

## KEY BENEFITS



- **Actionable:** During site assessment, CSIA and CENSUS qPCR data demonstrated that enhanced remediation was warranted.
- **Conclusive:** CSIA results conclusively demonstrated that cis-DCE degradation occurred while CENSUS qPCR demonstrated substantial growth of *Dehalococcoides* and increases in concentrations of vinyl chloride reductase genes (VCR) following donor injection.
- **Confidence:** Increased stakeholder confidence in the treatment approach led site managers to expand the treatment area.

## LAB LOCATIONS



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