

QuantArray®-Chlor

Actionable Data for
Performance Monitoring



PROJECT SUMMARY



- Based on QuantArray®-Chlor results and historical groundwater monitoring, electron donor injection was performed at a former chemical manufacturing facility impacted by a mixture of chlorinated compounds (please see QuantArray®-Chlor: Actionable Data for Remedy Selection).
- Five months after donor injection, concentrations of *Dehalobacter*, *Dehalogenimonas*, and other halorespiring bacteria had increased substantially demonstrating successful biostimulation.
- The exceptions were *Dehalococcoides* and vinyl chloride reductase genes which remained relatively low after five months raising concerns about potential vinyl chloride accumulation.
- Based on QuantArray-Chlor results for competing microorganisms (e.g., sulfate reducing bacteria) however, site managers continued monitoring confident that the impact of competing terminal electron accepting processes was temporary and that *Dehalococcoides* concentrations would increase more substantially during subsequent sampling events.
- By eleven months, concentrations of *Dehalococcoides* and vinyl chloride reductase genes as well as *Dehalogenimonas* and other halorespiring bacteria were greater than 10^4 cells/mL demonstrating the potential for complete reductive dechlorination and the effectiveness of donor injection.

PROJECT CHALLENGE



Groundwater at a former chemical manufacturing facility was impacted by tetrachloroethene (PCE) and trichloroethene (TCE) as well as 1,1,2-trichloroethane (TCA) and 1,2-dichloroethane (DCA). Geochemical conditions were mildly anaerobic but with relatively high dissolved sulfate, a competing terminal electron acceptor. Based on the site assessment including QuantArray-Chlor results for *Dehalococcoides* and functional genes, bioaugmentation was not needed and biostimulation was selected as the remediation strategy. With a mixture of contaminants and complex conditions, site managers incorporated QuantArray-Chlor analysis into performance monitoring to evaluate the effectiveness of electron donor injection.

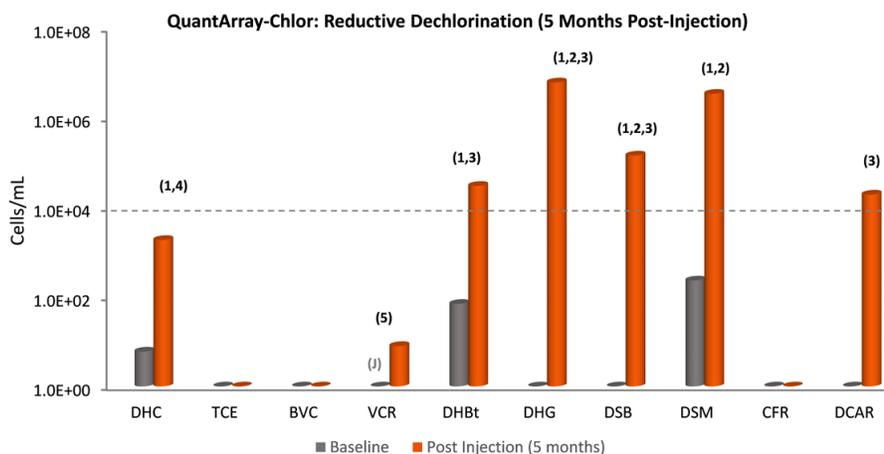
PERFORMANCE MONITORING | 5 MONTHS POST-INJECTION



Along with chemical and geochemical parameters, QuantArray-Chlor was a component of performance monitoring to help answer these crucial site management questions:

1. Was electron donor injection effective?
2. Did concentrations of halorespiring bacteria and functional genes increase?
3. Is reductive dechlorination of all site contaminants likely?
4. Will vinyl chloride accumulate? Is ethene production likely?
5. Are competing microbial processes hindering reductive dechlorination?

Groundwater samples were obtained from selected monitoring wells approximately 5 months after the electron donor injection. A portion of the QuantArray-Chlor results for targets involved in reductive dechlorination are shown below for monitoring well MW-1.



Comparison of the QuantArray-Chlor results for baseline (gray) versus 5 months post-injection (orange) samples clearly demonstrated successful biostimulation – significant growth of halorespiring bacteria in response to electron donor injection.

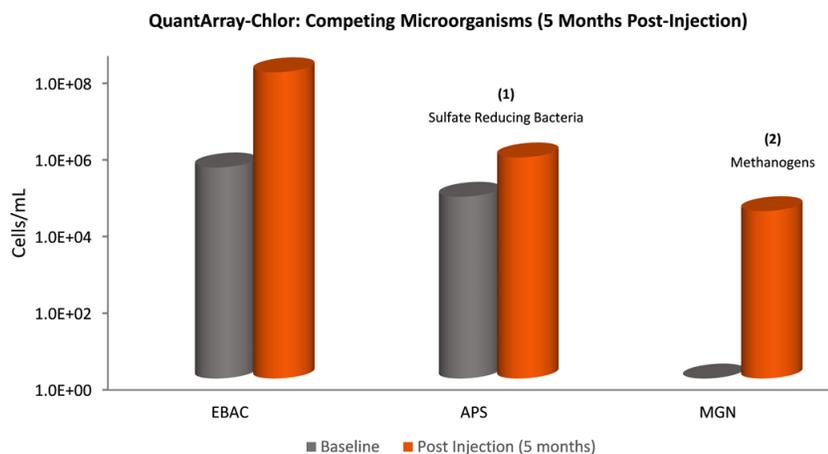
- (1) Concentrations of key groups of halorespiring bacteria increased by two to as many as six orders of magnitude following electron donor injection.
- (2) In fact, site managers accessed the Microbial Insights Database to reveal that post-injection concentrations of *Dehalogenimonas* (DHG), *Desulfitobacterium* (DSB), and *Desulfuromonas* (DSM) at MW-1 were greater than detected in 90% of groundwater samples submitted from sites worldwide.
- (3) *Dehalobacter* (DHbt), DHG, and DSB concentrations increased to between 10⁴ and 10⁶ cells/mL following electron donor injection, demonstrating substantial growth of bacterial populations capable of anaerobic biodegradation of chlorinated ethanes including 1,1,2-TCA. In addition, 1,2-DCA reductase genes (DCAR), which were below detection limits prior to injection, were detected on the order of 10⁴ cells/mL after biostimulation.

PERFORMANCE MONITORING | 5 MONTHS POST-INJECTION (CONT.)



- (4) *Dehalococcoides* (DHC) concentrations (10^3 cells/mL) also increased substantially following electron donor injection. However, DHC concentrations were still less than the 10^4 cells/mL recommended by Lu et al for generally effective rates of reductive dechlorination.
- (5) Moreover, vinyl chloride reductase genes (VCR) were detected post-injection but only on the order of about 10^1 cells/mL.

While demonstrating substantial growth of halo-respiring bacteria, post-injection *Dehalococcoides* concentrations less than 10^4 cells/mL could have been cause for concern. As shown below however, QuantArray-Chlor includes not only quantification of halo-respiring bacteria but also sulfate reducing bacteria and methanogens which can compete with *Dehalococcoides* for available hydrogen.



- (1) Populations of sulfate reducing bacteria (APS) were already high prior to injection and increased by more than an order of magnitude after electron donor addition.
- (2) Methanogens, which were below detection limits in the baseline samples, increased to more than 10^4 cells/mL following injection.

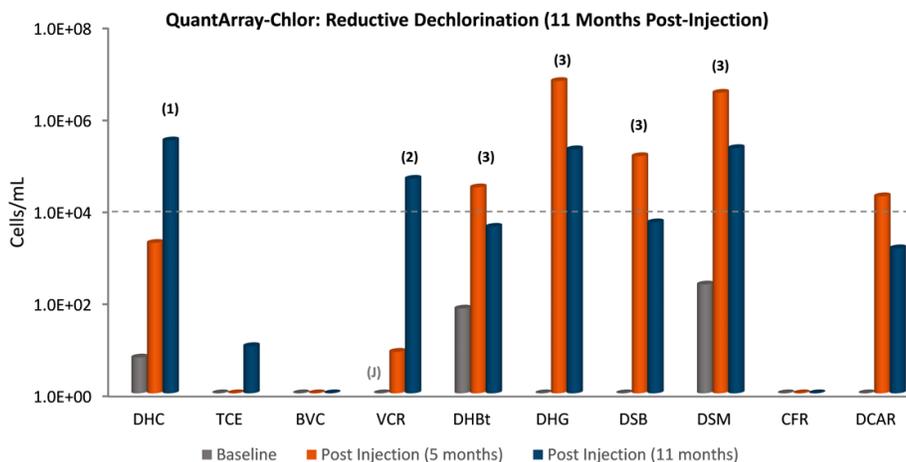
Thus, competition with sulfate reducing bacteria and methanogens likely hindered the initial growth of *Dehalococcoides* during the first 5 months after electron donor addition.

Decision: Continue monitoring. Increases in sulfate reducing bacteria and methanogens suggested that competing electron accepting processes may have temporarily limited growth of *Dehalococcoides*. Site managers continued monitoring confident that *Dehalococcoides* concentrations would ultimately increase.

PERFORMANCE MONITORING | 11 MONTHS POST-INJECTION



By the eleven month sampling event, QuantArray-Chlor demonstrated continued growth of *Dehalococcoides*, increases in vinyl chloride reductase genes, and a large and diverse population of halorespiring bacteria almost a year following electron donor injection.



- (1) *Dehalococcoides* (DHC) concentrations had increased to a high concentration (10⁵ cells/mL) by the subsequent groundwater sampling event (blue bar, 11 months post-injection).
- (2) Moreover, vinyl chloride reductase (VCR) genes increased to 10⁴ copies/mL, confirming the potential for continued reductive dechlorination of vinyl chloride to ethene.
- (3) Concentrations of other halorespiring bacteria including *Dehalogenimonas* (DHG) and *Desulfuromonas* (DSM) continued to be detected at concentrations near or greater than 10⁴ cells/mL, indicating the continued presence of a large and diverse population of halorespiring bacteria almost a year following electron donor injection.

Decision: Successful biostimulation. High concentrations of *Dehalococcoides*, vinyl chloride reductase genes, and other halorespiring bacteria like *Dehalogenimonas* were detected following electron donor injection. Moreover, QuantArray-Chlor results for competing microorganisms increased stakeholder confidence in biostimulation as the selected remedy and confirmed that no additional actions such as bioaugmentation or re-injection were needed.

KEY BENEFITS



As a component of remedy selection and performance evaluation, QuantArray-Chlor was a critical line of evidence that directly impacted site management decisions.

- **Actionable:** Increases in concentrations of *Dehalococcoides*, *Dehalobacter*, *Dehalogenimonas*, and other halorespiring bacteria demonstrated the effectiveness of electron donor addition as the treatment strategy.
- **Comprehensive & Cost-Effective:** QuantArray-Chlor includes quantification of a broad spectrum of key halorespiring bacteria, functional genes and competing microorganisms like sulfate reducing bacteria in a single analysis.
- **Increased Confidence:** With QuantArray-Chlor showing a temporary increase in competing microorganisms (e.g., sulfate reducers), stakeholders were confident that *Dehalococcoides* concentrations would eventually increase and that complete reductive dechlorination to ethene was likely.
- **Saved Money:** With confidence in the effectiveness of biostimulation, site managers continued monitoring knowing that bioaugmentation and additional electron donor injection were not necessary.
- **Conclusive:** QuantArray-Chlor results for the eleven month sampling event conclusively demonstrated successful biostimulation by documenting growth of high concentrations of *Dehalococcoides* as well as other key halorespiring bacteria.