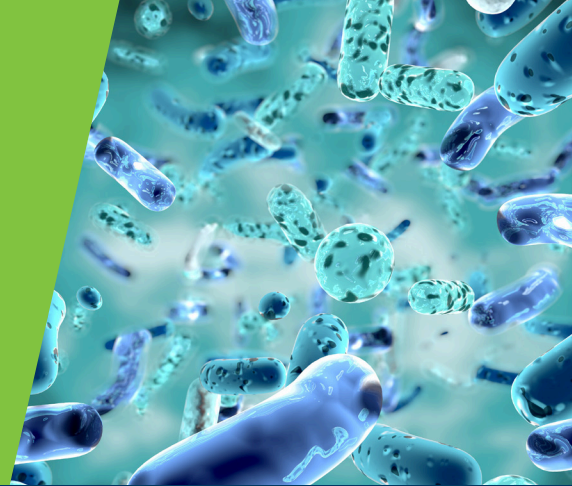


NGS and QuantArray®-Chlor: Characterizing Microbial Communities and Assessing Biodegradation Potential



PROJECT SUMMARY



- QuantArray®-Chlor demonstrated growth of high concentrations of *Dehalobacter* and other halorespiring bacteria in response to electron donor addition.
- Site managers also wanted to know “Who is there?” and used next generation sequencing (NGS) to characterize the microbial community.
- NGS revealed substantial changes after electron donor addition, but fermenting, iron reducing, and sulfate reducing bacteria were consistently among the top genera identified.

PROJECT CHALLENGE



A pilot study was conducted to determine whether addition of an electron donor and other amendments would not only promote biological reductive dechlorination but also biogenic iron sulfide (FeS) for abiotic degradation. QuantArray-Chlor was performed to quantify concentrations of known halorespiring bacteria and assess biological reductive dechlorination. In addition to QuantArray-Chlor, site managers were also interested in answering “Who is there?” to assess impacts of fluctuating subsurface conditions on the overall bacterial community and potential for biogenic FeS and abiotic degradation.

SAMPLING AND ANALYSIS

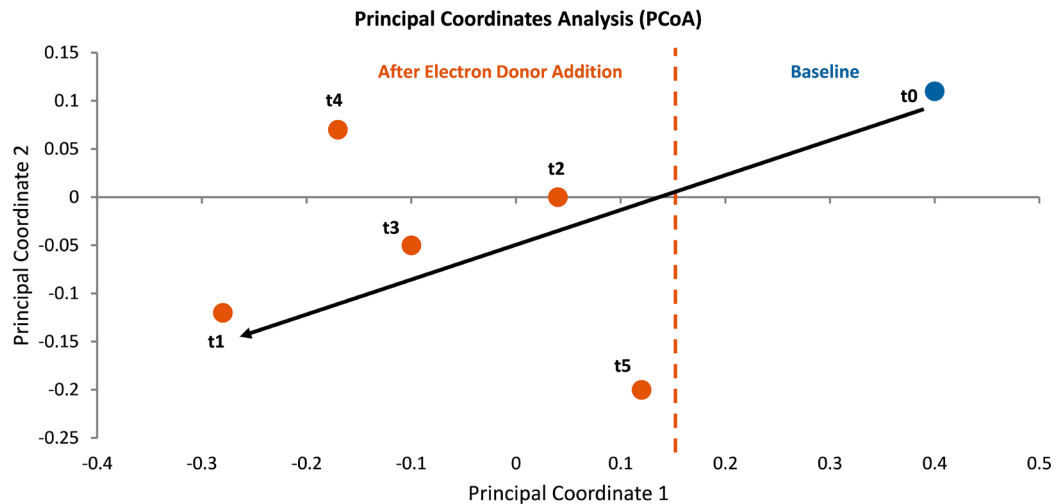


Next generation sequencing (NGS) provides comprehensive identification of microorganisms present in a sample down to the genus and even species level. While not quantitative, the relative proportions of the microorganisms identified can provide insight into potential microbial processes. At complex sites, NGS is performed for an overall profile of the microbial community while QuantArray is used to quantify known contaminant degraders and functional genes. At the site, NGS and QuantArray-Chlor were performed with groundwater samples obtained prior to (t0) and every six months after donor addition (t1-t5).

BASELINE VS AFTER ELECTRON DONOR



Along with tables of top genera and informative descriptions, NGS reports include statistical analyses to aid interpretation. Figure 1 is a Principal Coordinate Analysis (PCoA) of the normalized relative abundances of all genera identified in the samples.



In short, PCoA reveals which samples have similar vs different microbial communities. Samples that are close together or “cluster” on the PCoA plot are more similar. Conversely, samples that are farther apart have notably different microbial communities.

In the current study, the t0 baseline sample prior to electron donor addition (blue) looks like an outlier falling to the far right on Principal Coordinate 1 while the post-injection samples t1 through t5 (orange) are all further to the left. In fact, the samples that are farthest apart on the PCoA are the t0 sample and the t1 first post-injection sample on the far left. Thus, the microbial community at this monitoring well changed substantially following electron donor addition.

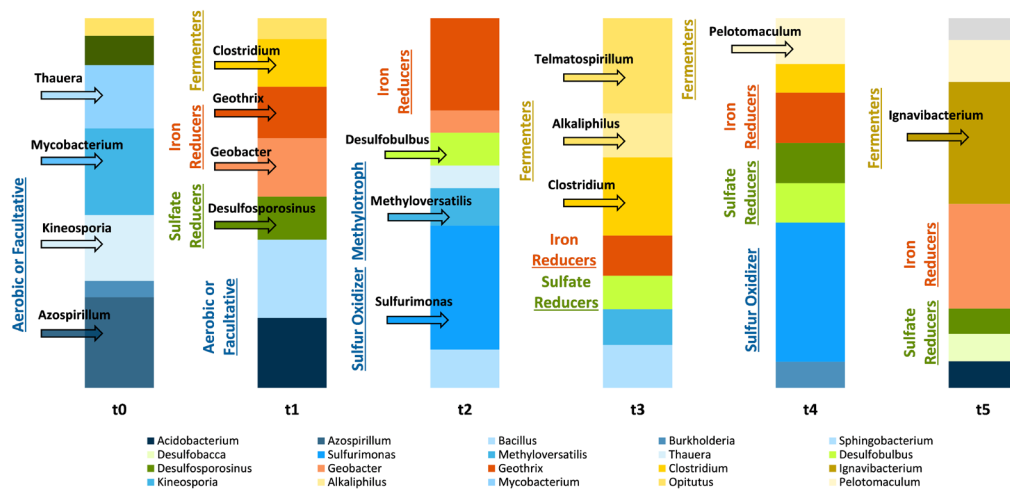
While more similar to each other than to baseline t0, it is interesting to note that the post-injection time points do not form a tight cluster. For example, t1 is separated from t2 along Principal Coordinate 1. Similarly, t5 is separated from t4 and the rest of the post-injection group along Principal Coordinate 2. Thus, the microbial community continued to be dynamic after electron donor addition, changing over time as conditions fluctuated.

In the next section, we dive a little deeper to see which genera were identified at each sampling event.

TOP GENERA OVER TIME



The changes in microbial populations over time are evident in a simplified version of the stacked bar portion of the hierarchical cluster dendrogram (HCD) included in an NGS report.



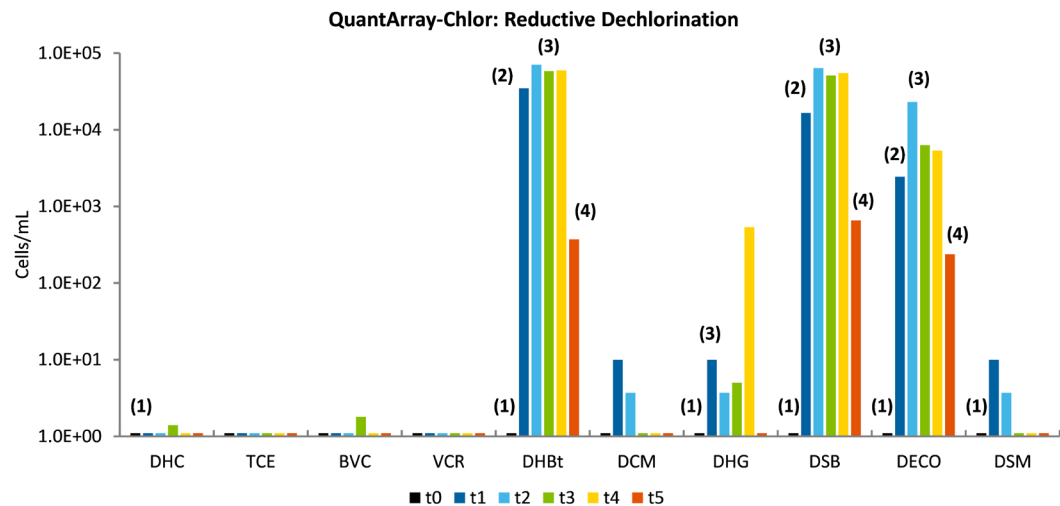
- **Baseline t0:** The top genera identified were aerobic, microaerobic, and facultative bacteria (shades of blue) including *Kineosporia*, *Azospirillum*, and *Thauera*.
- **Time t1:** NGS analysis revealed substantial changes in response to electron donor addition. While aerobic populations were still present, several genera of anaerobes became among the top detected. More specifically, the percentages of reads from fermenters (shades of yellow; *Clostridium*), iron reducing bacteria (shades of orange; *Geobacter* and *Geothrix*), and sulfate reducing bacteria (shades of green; *Desulfosporosinus*) increased considerably.
- **Time t2:** Genera of iron and sulfate reducing bacteria remained among the top detected, but the aerobes *Sulfuromonas* and *Methyloversatilis* were also among the most frequently identified. *Sulfuromonas* utilize reduced sulfur compounds and *Methyloversatilis* are methylootrophs. While possibly signaling more aerobic conditions, both thrive at the fringes of anaerobic zones and during temporary fluctuations when elemental sulfur, methane, and oxygen could all be available.
- **Time t3:** Dominated by fermenters, iron reducing bacteria, and sulfate reducing bacteria consistent with continued anaerobic conditions.
- **Time t4:** As with t2, sulfur oxidizing *Sulfuromonas* was the top genus identified, but genera of iron reducing and sulfate reducing bacteria were still among the most frequently detected reads.
- **Time t5:** Reads from fermenters, iron reducers, and sulfate reducers were again the most frequently detected.

Overall, NGS revealed a dynamic microbial community but consistently high proportions of anaerobic bacteria involved in fermentation, iron reduction, and sulfate reduction.

QUANTARRAY®-CHLOR



QuantArray-Chlor analysis was performed to quantify concentrations of known halo-respiring bacteria and functional genes and evaluate the potential for biological reductive dechlorination.



- (1) Prior to electron donor addition (t0), concentrations of *Dehalococcoides* (DHC), *Dehalobacter* (DHBT), and a broad spectrum of other halo-respiring bacteria and functional genes included in QuantArray-Chlor analysis were below detection limits.
- (2) Following electron donor addition (t1), concentrations of *Dehalobacter* (DHBT), *Desulfitobacterium* (DSB), and *Dehalobium* (DECO) increased by three to four orders of magnitude demonstrating that electron donor addition effectively promoted growth of known halo-respiring bacteria. *Dehalogenomina*s (DHG) spp. were also detected but at relatively low concentrations. Concentrations of *Dehalococcoides* (DHC) and vinyl chloride reductase genes (BVC, VCR) however, remained below or near detection limits through the study.
- (3) For sampling events t2 through t4, concentrations of *Dehalobacter* (DHBT) and *Desulfitobacterium* (DSB) remained on the order of 10^4 cells/mL and DECO continued to be detected at over 10^3 cells/mL.
- (4) By time t5 however, concentrations of *Dehalobacter* (DHBT), *Desulfitobacterium* (DSB), and *Dehalobium* (DECO) had all decreased substantially.

Decision: Expand to Full Scale. Although *Dehalococcoides* (DHC) concentrations were low, QuantArray-Chlor demonstrated growth of substantial concentrations of *Dehalobacter* (DHBT) and *Desulfitobacterium* (DSB) in response to electron donor. Moreover, high concentrations of these halo-respiring bacteria were maintained for over two years before decreasing at time t5.

NGS and QuantArray®-Chlor: Characterizing Microbial Communities



KEY BENEFITS



Multiple lines of evidence always provide a more complete picture. NGS gave an overall profile of the microbial community while QuantArray-Chlor quantified known halo-respiring bacteria and functional genes to assess biological reductive dechlorination.

- **Conclusive:** QuantArray-Chlor demonstrated growth and maintenance of high concentrations of *Dehalobacter* and other halo-respiring bacteria for two years after electron donor injection.
- **Actionable:** After two years, concentrations of *Dehalobacter* and other halo-respiring bacteria decreased substantially suggesting that additional electron donor may be needed to continue to support biological reductive dechlorination.
- **Revealing:** NGS analysis to characterize the bacterial community clearly highlighted the change in the microbial community after electron donor addition and provided insight into likely microbial processes such as fermentation, iron reduction, and sulfate reduction and potential FeS production.

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