Late Stage Degradation Rates for TCE Daughter Products Correlated with Microbial Community Composition Determined by NGS Analysis

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BACKGROUND/OBJECTIVES

Groundwater at a manufacturing facility in Portland, Oregon was impacted by TCE and manufactured gas plant waste (primarily naphthalene, BTEX, PAHs, and cyanide). In situ chemical reduction enhanced bioremediation for source area treatment was implemented in 2009, with demonstrated success in 2013. Implementation consisted of a 150 foot-long permeable reactive barrier (PRB) consisting of EHC® and bioaugmented with KB-1® installed at 40-112 feet bgs using direct-push technology. Groundwater data were collected from 23 monitoring wells upgradient, within and downgradient of the PRB. Early results confirmed 99.99% TCE mass removal within one year, with most wells below the USEPA MCL. Residual concentrations of cis-dichloroethene (cDCE) and vinyl chloride (TCE degradation products) were observed, with delayed decreases in degradation. To supplement conventional monitoring parameters, next generation sequencing (NGS) was performed to characterize microbial community structure and to provide further insights into remediation performance at specific wells.

APPROACH/ACTIVITIES

The objective of the work was to evaluate a significant time-series data set to identify potential correlations between CVOC degradation rates and NGS data describing the microbial community growth and characteristics under rapid dechlorination phase (RDP). Degradation rates for cDCE and VC (observed from 2010 through 2016) were estimated using first-order slope analysis. The delay (relative to amendment injection and source TCE depletion) was also measured and considered, and half-lives for degradation rates were estimated.

METHODS

Analysis of Degradation Rates

Groundwater concentrations (from wells) of primary (cDCE) and secondary (VC) degradation products of TCE were graphed on log concentration (µg/L) vs. time plots to allow for estimation of pseudo-first order degradation rates. These plots (Figures 1 and 2) demonstrated nearly-simultaneous dechlorination of cDCE and VC, and show the "rapid dechlorination phase" (RDP) that occurred following the non-RDP. The rates are summarized in Table 1; rates for cDCE and VC were well-correlated (Figure 3).

Figure 1

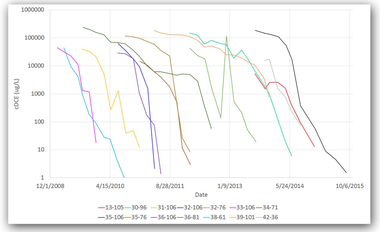


Figure 2

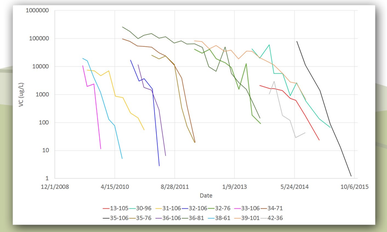
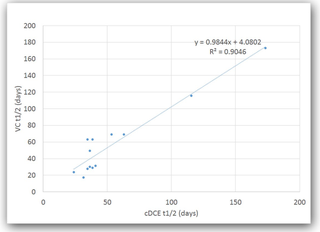


Table 1

	cis-1,2,DCE					Vinyl Chloride				
Well	C at start of RDP (ug/L)	RDP Rate (1/d)	RDP Half-life (d)	Order of Magnitude Reduction		C at start of RDP (ug/L)	RDP Rate (1/d)	RDP Half-life (d)	Order of Magnitude Reduction	
13-105	5,280	0.012	58	3		2,140	0.009	77	3	
30-96	148,000	0.011	63	4		49,700	0.01	69	3	
31-106	39,900	0.018	39	4		7,420	0.011	63	2	
32-106	62,400	0.029	24	5		16,900	0.029	24	4	
32-76	41,500	0.013	53	4		41,500	0.01	69	3	
33-106	44,300	0.022	32	4		10,700	0.04	17	3	
34-71	97,400	0.02	35	4		14,500	0.011	63	4	
35-106	160,000	0.018	39	5		79,700	0.024	29	5	
35-76	118,000	0.017	41	4		25,500	0.022	32	3	
36-106	29,000	0.019	36	5		11,500	0.023	30	3	
36-81	234,000	0.006	116	4		263,000	0.006	116	4	
38-61	43,800	0.02	35	5		19,600	0.025	28	4	
39-101	183,000	0.004	173	3		83,900	0.004	173	2	
42-36	15,600	0.019	36	2		2,960	0.014	50	2	
Mean Data	89,083	0.016	56	4		44,930	0.017	60	3	

Figure 3



Sequencing

NGS data, including a total of 279 DNA samples extracted from monitoring well groundwater (2009 through 2014) had 16S rRNA genes which were amplified using universal primers 515f (5'-GTGCCAGCMGCCGCGGTAA-3') and 806r (5'-GGACTACHVGGGTWTCTAAT 3) (Caporaso, et al., 2011) that target Bacteria and Archaea. Library preparation and sequencing were performed at Genome Quebec and McGill University Innovation Centre (Montreal, PQ) using the MiSeq platform (Illumina Inc., San Diego, CA).

Data Analysis to Identify increasing or Decreasing Microbes in Rapid Dechlorination Phase

Six wells (13-105 [Dhg but more Dhc increase during RDP, Figure 4], 30-96 (Dhg increase during RDP, Figure 5), 31-106 (Dhc and Dhg decrease during RDP), 32-76 (Dhc increase and decrease during RDP), 33-106, 39-101 (Dhg increase and decrease during RDP, also OTU 10, Figure 6) were selected for further comparison of RDP CVOC data and operational taxonomic units (OTUs) data. The OTUs selected were > 1% of the total OTUs in each of the 6 samples; data were averaged across the rapid dechlorination phase (RDP) and non-rapid dechlorination phase (non-RDP). The fold increase or decrease was plotted (the ratio of the % in RDP: % non-RDP) and expressed on a log scale in Figures 7 and 8. Only changes for OTUs observed in two or more wells and where coefficient of variation values less than 2 were deemed as representing increases or decreases.

Figure 4

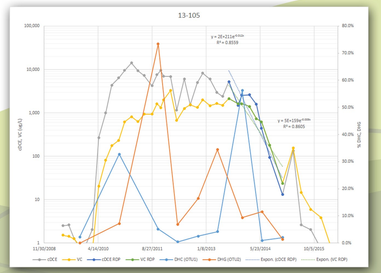


Figure 5

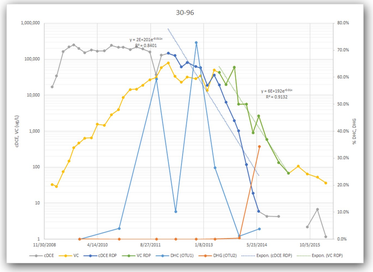


Figure 6

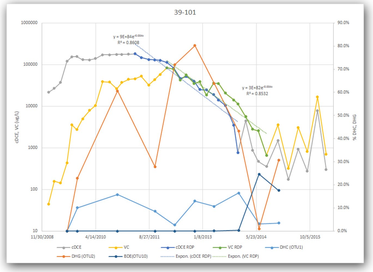


Figure 7

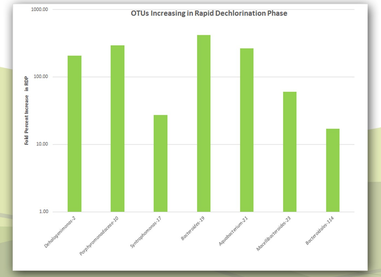


Figure 7: Taxa with observed increases in RDP compared to non-RDP. Dehalogenimonas, a known reductive dechlorinator, increased 205-fold. Several members of the Bacteriodetes (blue bars) increased 17-293 fold. Aquabacterium sp. increased 206- fold and are possible aerobic degraders of VC.

RESULTS/LESSONS LEARNED

Substantial microbial diversity was identified at the site, but in most samples, dechlorinators comprised a high proportion of OTUs, including Dehalococcoides (Dhc) and Dehalogenimonas (Dhg). Other anaerobes including fermenters, sulfate reducers and methanogens, were detected in most samples. Degradation rates, and the delay times for end-stage degradation (depletion of TCE source as indicated by declining/non-detect cis-DCE concentrations) were estimated and correlated to the relative abundance of dechlorinating bacteria.

Taxa Increasing in RDP

A total of 7 OTUs were deemed to have increased in the RDP and therefore represent microorganism abundance strongly correlated with rapid dechlorination. These OTUs are summarized in Figure 7 and Table 2. A total of 8 OTUS were deemed to decrease during the RDP; these are summarized in Figure 8 and Table 3.

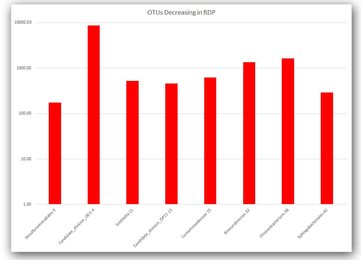
Table 2

Designation	Average Fold Increase Observed in Rapid Dechlorination Phase	Possible Metabolic Role	Comment	Reference
Dehalogenimonas-2	206	Reductive Dechlorination	Dehalogenimonas reported to reductively dechlorinate trans-DCE and VC	Mancher et al., 20 Yang et a 2016
Syntrophomonas -17	27	Fatty acid fermentation specialist produces hydrogen that can be used for dechlorination	Butyrate fermenting *Syntrophomonas wolfe*can form syntrophic relationship with *Dhc*	(Mao et 2017)
Aquabacterium-21	266	Aquabacterium reported to assimilate VC in VC dechlorinating culture	Aerobic VC degradation process	Wilson e al., 2016
Bacteroides-19	419	Fermenter	Bacteroidetes reported to be important symbionts of Dhc in reductive dechlorination/correlated with Efficient conversion of VC to ethene at field site	Pérez-de Mora et 2014; Miura et al., 2015
Porphyromonadaceae-10	293	Fermenter		
Macellibacteroides-23	60	Fermenter		
Bacteroidales-114	17	Fermenter		

Table 3

Designation	Average Fold Decrease Observed in Rapid Dechlorination Phase	Possible Metabolic Role	Comment	Reference
Desulfuromonadales-3	173	Dechlorinators of higher ethenes	This class contains *Geobacter* and *Desulfuromonas* which degrade PCE and TCE	Adrian and Löffler, 201
Candidate_division_OD1-4 (Parcubacteria)	8679	Fermenter of complex carbon and sugars	Generally found in anoxic or aerobic environments	Castelle et 2017
Smithella-11	526	Syntrophs known to ferment propionate	--	a Bok et a 2001
Candidate_division_OP11-13	460	Not well characterized	Widespread but largely uncharacterized organisms sometimes found in hydrocarbon impacted environments	Youssef et 2011
Bacteroidales-14	1.6	Fermenter	Minor fold decrease	
Comamonadaceae-15	616	Respiratory metabolism of organic acids using oxygen or nitrogen	Some members of this family (e.g., *Polaromonas* IS-666 are known aerobic degraders of cis-DCE	Willems et a 1991; Crikman et al., 2002
Brevundimonas-32	1363	--	Some strains reported to degrade aromatic compounds	Parthasarat et al., 2016
Chryseobacterium-38	1620	May use nitrate as	Member of Bacteriodetes Often found in	Wang et 2015

Figure 8



Shifts in Obligate Reductive Dechlorinating Populations

The data indicated that significant shifts in microorganism abundance were correlated with the RDP in multiple wells. Specifically the role of Dhg, a known tDCE dechlorinator, appears to be correlated well with cDCE and VC dechlorination rates in certain wells. The NGS data (for the 6 wells analyzed in Figure 7) clearly indicate, on average, only minor increases in Dhg. More significant and consistent increases in Dhg in some wells may be related to changes in geochemistry and may be responsible for rapid dechlorination of chlorinated ethenes during the RDP. The exact role or reason for changes in numbers of Dhg is not clear, but Yang et al. (2016) recently reported a Dhg population that dechlorinates VC to ethene, raising the possibility that higher rates of dechlorination observed in the RDP may be due to similar shifts in the dechlorinating population.

Role of Bacteroidetes

Some specific taxonomic groups commonly appear in dechlorinating microbial communities including fermenters such as Spirochaetes, Bacteroidetes, Sporomusa (Lorenz and Loeffler, 2016). It is notable that under conditions of rapid dechlorination in this study, 4 of the 7 increasing OTUs were associated with the Bacteroidetes demonstrated high fold increases. Intriguingly, Pérez-de-Mora et al. (2014) observed 3-4 orders of magnitude increases in Bacteroidetes at a fractured bedrock site that was bioaugmented with KB-1®. The authors hypothesized that Bacteroidetes might play role in improved conversion chlorinated of ethenes to ethene either through more beneficial fermentation of by providing trace nutrients to dechlorinating populations.

CONCLUSIONS

- A rapid dechlorination phase was noted in the site shortly after implementation of EISB which significantly and simultaneously reduced the primary degradation products (cDCE and VC) of the source TCE material.
- Degradation rates of cDCE and VC were well-correlated and not sensitive to initial (pre-RDP) concentrations.
- Analysis of average changes in microbial populations associated with the RDP included increases in:
  - Dehalogenimonas - a species known to degrade trans-DCE and recently reported to dechlorinate VC to ethene
  - Several members of Bacteriodetes a class reported to be associated with efficient dechlorination at another KB-1 bioaugmented site
  - Syntrophomonas, which is a known syntrophic partner of Dhc
  - Aquabacterium, which is a known aerobic VC degrader (although the role of aerobic degradation in this data set is unclear)
- The above microbial groups offer intriguing targets for further examination an optimization of reductive dechlorinating systems

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