Effects of High TCE Toxicity On Ability of *Dehalococcoides sp.* to Degrade Chlorinated Solvents

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Research and education focused on life below Earth's surface.

Introduction

Trichloroethene (TCE) was used widely in the United States as a solvent and degreaser; due to inadequate storage and disposal this suspected carcinogen (1) is now a persistent groundwater and soil contaminant. *In situ* bioremediation is an effective and economical process that reductively dechlorinates TCE into ethene primarily via the anaerobic microorganism *Dehalococcoides* sp..(2)

These microbes use an electron donor (H_2) to replace a chorine atom on the hydrocarbon with a hydrogen atom, forming the more toxic daughter products cis-dichloroethene (c-DCE) and vinyl chloride (VC), and finally into benign ethene (ETH). The dechlorination of VC, the most toxic and carcinogenic of the three compounds, is the rate limiting step, so this results in a buildup of it in the groundwater alongside TCE.

Because TCE and its daughter products are dense nonaqueous phase liquids (DNAPL), they collect in high concentration pockets at the bottom of water table, gradually leeching toxins into groundwater. While the microbes are capable of remediating dilute chlorinated aliphatic hydrocarbons (CAHs) *in situ*, their interactions within zones surrounding pockets of DNAPL still needs to be researched.

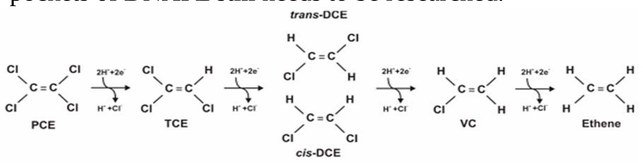

Figure A: The Reductive Dechlorination of TCE to ETH. (4)

Objective

The objective of this project is to simulate microbes encountering areas of groundwater near DNAPL sources of TCE, and their resulting dechlorination rates of TCE and VC after recovering from exposure.

Method

Bottles used were anaerobic 156 ml Weaton batch bottles with septa caps (see figure B) that allowed for samples to be extracted without introducing O_2 to the system.

Figure B: Bottles being purged of CAHs and ETH.

Control:
25 ml culture
0.25 ml sodium formate (NaFR)
1 ml media containing sat TCE
50 ml media from under hood
5 ml H_2 gas

High Toxicity (5mM):
25 ml culture
50 ml media containing sat TCE (5 mM) and 2.25 mmol of NaFR.
5 ml H_2 gas

- Bottles were then placed on shaker table during exposure period. Cultures used were Evanite (EV), and Victoria (VS), and exposure times were 72, 120, 168, and 240 hours.
- 4 controls and 4 exposure bottles for each culture and exposure time were prepared to provide duplicates for TCE and VC dechlorination rates after exposure.
- CAHs have been proven to seep into the glass and septa of bottles as well as the microbes themselves in high toxicity batches (3). Therefore, after exposure, contents of each bottle were transferred to new anaerobic bottles before being purged for 15 minutes with a 75:25 N_2:CO_2 gas mix . 5 ml H_2 was added to each bottle as an electron donor.
- The exposed bottles had nothing else added to them, to provide an overnight recovery period, during which the CAHs absorbed by the microbes would leech out and be converted to ETH. Controls were given 0.5 ml sat TCE to provide continuous dechlorination during recovery.
- After recovery, TCE control and exposed bottles were injected with 1 ml sat TCE and VC control and exposed bottles were injected with 0.25 ml pure VC gas. All bottles were then monitored using gas chromatographs (GC) equipped with flame ionization detectors (FID) to obtain rates of dechlorination.

Figure C: Transferring contents of batches to new bottles.

Results

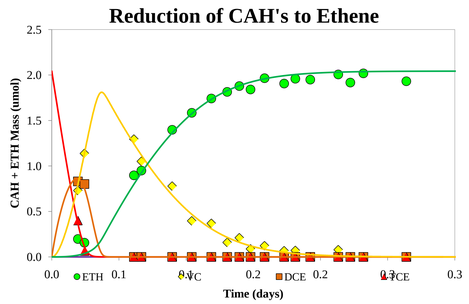

Figure D: Normal dechlorination rate curves for low level CAH concentrations.

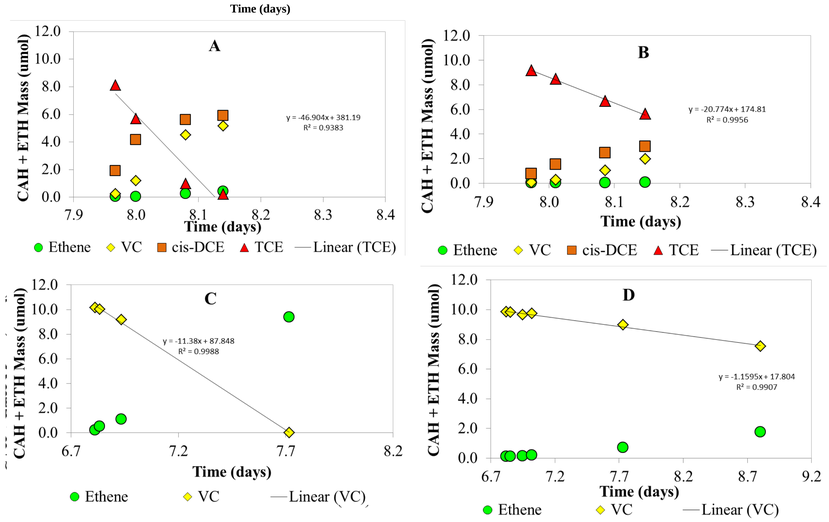

Figure E: Graphs of transformation rate after high tox exposure and in controls. Graph A is from 168 EV TCE-C-2 (Control), graph B is from 168 EV TCE-1 (high tox), graph C is from 120 EV VC-C-1 data (Control), and graph D is from 120 EV VC-1 (high tox).

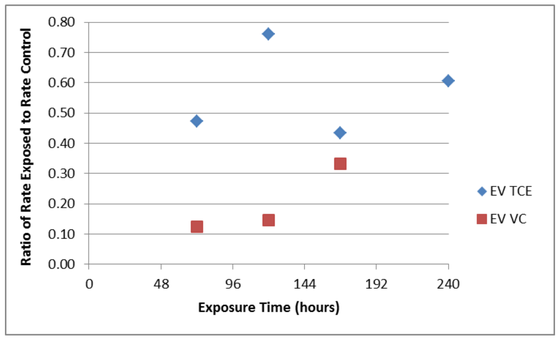

Figure F: Graph shows the ratio of the dechlorination rate of exposed microbes to the dechlorination rate of the control microbes for both TCE and VC for the **Evanite** culture at various exposure times.

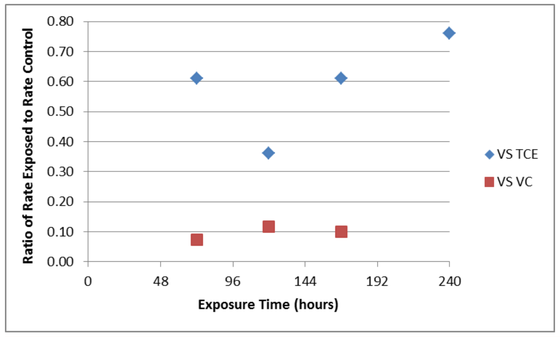

Figure G: Graph shows the ratio of the dechlorination rate of exposed microbes to the dechlorination rate of the control microbes for both TCE and VC for the **Victoria** culture at various exposure times.

Conclusions

- For both cultures, the ratio of rates pattern was almost identical. For all exposure times and cultures, the TCE ratio of rates was higher than the VC ratio of rates, which correlates the fact that the microbes have the more difficulty dechlorinating VC than TCE. The last step of VC transformation to ETH are much more sensitive to high concentration TCE exposure.
- Looking at the rates of dechlorination (figure E) it is clear that the control microbes were much quicker than the exposed microbes for dechlorinating TCE and VC. However, the exposed microbes, given a recovery period, were still able to dechlorinate the CAHs, indicating that *in situ* microbes that encounter zones near NAPL sources can survive and still perform its function even if they are exposed for up to ten days.

Acknowledgements

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References

(1) "Trichloroethylene MSDS." . Mallinckrodt Baker, Inc., n.d. Web. 30 Aug. 2011. <http://hazard.com/msds/mf/baker/baker/files/t4940.htm>.

(2) He, J., Sung, Y., Krajmalnik-Brown, R., Ritalahti, K. M. and Löffler, F. E. (2005), Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. Environmental Microbiology, 7: 1442–1450. doi: 10.1111/j.1462-2920.2005.00830.x

(3) http://sbi.oregonstate.edu/education/su10interns/Albers_Delancy.pdf

(4) http://www.afcee.af.mil/resources/technologytransfer/programsandinitiatives/enhancedinsituanaerobicbioremediation/basics/index.asp