Designing of an Experiment

Finding a Method for the Dechlorination of TCE at High Toxic Concentrations

Delancy Albers, Mohammad Azizian and Lew Semprini
Department of Environmental Engineering

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

Introduction

Contamination of chlorinated substances such as trichloroehtylene (TCE) is a major issue in United States. TCE is suspected of being a carcinogen(1). Bioremediation is an effective process to remove such contaminants Many organisms are known to be capable of reductive dechlorination of TCE to the non-toxic compound of ethene, including Dehalococcoides spp (2).

In reductive dechlorination, a chlorine atom is replaced with a hydrogen atom and requires two electrons for each step. Hydrogen is the electron donor and the chlorinated compound is the electron acceptor. TCE reducers can be self-inhibited by TCE toxicity (3). In order to determine rates of TCE degradation, its concentration is periodically monitored on a gas chromatograph (GC) as it degrades into cis-1,2-dichloroethylene (cDE), then to vinyl chloride (VC) and to ethylene (ETH). A problem in measuring the effect of high concentrations of TCE arises when TCE cannot be completely purged out of the reactor bottles. Various reasons are possible, from being absorbed into the septa, the glass, or into the microorganisms themselves.

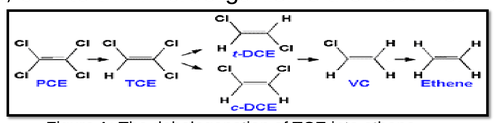

Figure A: The dehalogenation of TCE into ethene. http://www.cpge.utexas.edu/ee/

Objective

The objective of this project was to obtain the best method to determine the effect of high TCE concentration on dechlorination rates using batch experiments. Three methods were utilized:

Method One:
Using batch bottles and adding pure TCE,

Method Two:
Using batch bottles and adding TCE saturated media and

Method Three:
Using a separate reactor to mix the culture with the high concentrations of TCE, then transferring to a batch bottle so that the TCE could be purged out.

Materials and Method

Method One: Only Batch Bottles

- Batch bottles were prepared and sealed anaerobically
- 50 mL of culture is added to the bottle
- 50 uL of pure TCE is added to each bottle
- At various times (3, 6,…, 24 hours) the toxic bottles are purged for 15 minutes with a mix gas of carbon dioxide and argon (80%/20%)
- 2 uL of TCE is added the bottle as well as 2 ml of H2
- The bottles are then monitored TCE transformation using the Gas Chromatography (GC).

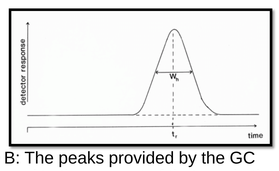

Figure B: The peaks provided by the GC correspond to the amount of that gas in the bottle. *Gas Chromatography and Lipids by William Christie*

Method Two: Batch Bottles with TCE Saturated Media

- Along with the 50 mL of culture, 50 ml of TCE saturated media is added to the bottle
- Bottles are purged at various times
- 1 mL of TCE saturated media is added the bottle Bottles are then TCE transformation products were monitored by GC

Method Three: Large Reactor Bottle Transferred to Batch Bottles

- In a separate 1.16 L bottle put in 500 mL culture and 500 mL media and 600 uL pure TCE
- With time e.g. (3,6,…, 24 hours) siphon anaerobically 50 mL of culture out of the large bottle into batch bottles
- Add 1 mL of TCE sat. media
- Monitor on GC

Figure C: The set-up for method three.

Results

Method One (neat TCE additions):

(D)

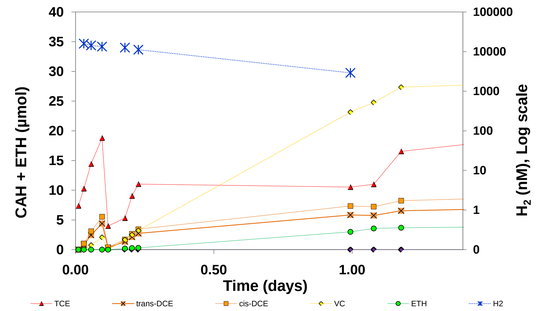

Figure D: Batch bottle purged after 3 hours and monitored on the GC without adding any extra TCE. When the bottles were purged the TCE concentrations were increased. The TCE concentration still rose after purging twice, reaching a maximum of 20 umols.

Method Two (TCE-saturated media additions):

(E) (F)

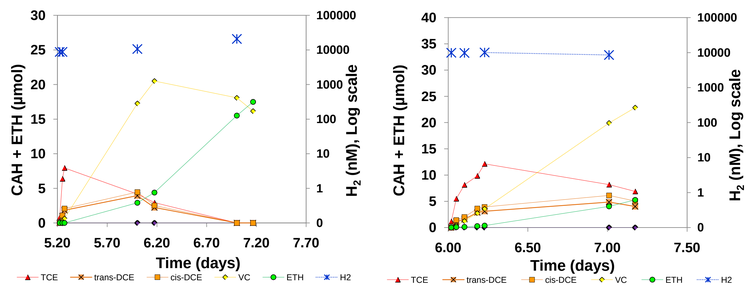

Figure E: Increase in TCE concentration following purging after 5 hours of exposure. With no additions, 9 umols of TCE was still detected.

Figure F: Increase in TCE concentration following purging after 24 hours of exposure and no further TCE additions. Reached a maximum of 13 umols of TCE.

Method Three (neat TCE additions and bottle transfer):

(G) (H)

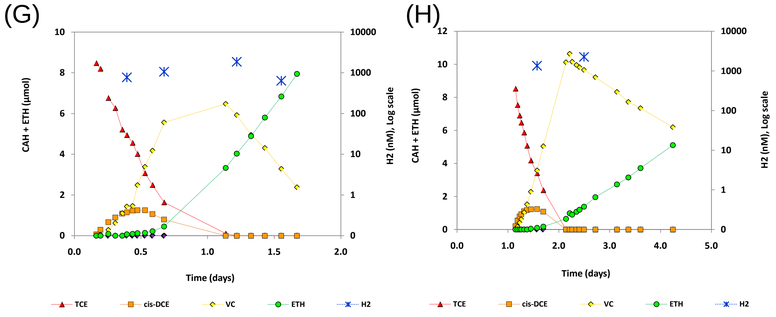

Figure G: Culture transferred to batch bottles after 3 hours of exposure and purged. Then 1 mL of TCE sat. media added. TCE concentration never rose.

Figure H: After 24 hours of exposure, the culture was transferred to a batch bottle and purged. Then 1 mL of TCE sat. media added. TCE concentration never rose.

Conclusion

- TCE can be absorbed into septa, glass and microorganisms. The biggest problem is TCE being absorbed into glass and which is not able to be purged out.
- Using TCE saturated media helps prevent excessive TCE adsorption since the TCE is soluble already.
- Using a separate bottle to hold the high TCE concentrated mix, then transferring to a different bottle to purge and perform rate tests, eliminated the TCE-adsorption effects observed previously.

Acknowledgements

I would like to thank Mohammad Azizian for his help in the lab as well as mentoring my project. Also, I'd like to thank Lew Semprini and the Subsurface Biosphere Initiative for this opportunity.

Literary References

(1) Vogel, T. M., and P. M. McCarty. "Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions. -- Vogel and McCarty 49 (5): 1080." *Applied and Environmental Microbiology*. Web. 31 Aug. 2010. <http://aem.asm.org/cgi/content/abstract/49/5/1080>.

(2) Maymo-Gatell, X., Y. Chien, J. M. Gossett, and S. H. Zinder. 1997. Isolation of bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 276:1568–1571.

(3) Haest, P.J., D. Springael, and E. Smolders. "Dechlorination Kinetics of TCE at Toxic TCE Concentrations: Assessment of Different Models." *Water Research* (2009). Print.