Session 1C.9: Bioremediation of chlorinated solvents in groundwater 1

AEROBIC BIODEGRADATION OF TRICHLOROETHENE WITHOUT AUXILIARY SUBSTRATES

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Keywords: Trichloroethene, aerobic metabolic degradation, carbon isotope fractionation, bioremediation, natural attenuation

Introduction

Trichloroethene (TCE) is a priority pollutant (EU, 2008; ATSDR, 2012; US-EPA, 2012) and among the most frequently detected contaminants in groundwater (Conrad et al., 2010; Mattes et al., 2010). The currently available bioremediation measures have certain drawbacks like e.g. the need for auxiliary substrates (Figure 1). Oxidation of chloroethenes under aerobic conditions represents a promising way to deal with the shortcomings of anaerobic reductive dechlorination (Tiehm and Schmidt, 2011).



Figure 1 Comparison of anaerobic-reductive dechlorination with aerobic cometabolic and metabolic degradation of chloroethenes regarding the need for auxiliary substrates as electron donors and competing reactions. TEA = Terminal electron acceptor. Figure modified after Tiehm and Schmidt, 2011.

Aerobic oxidation is possible via metabolic degradation with the pollutant serving as growth substrate as well as via cometabolic degradation depending on the presence of a suitable auxiliary substrate (Figure 1, Tiehm et al., 2008; Mattes et al., 2010). For field applications metabolic pollutant degradation is superior compared to cometabolic degradation since it does not require the presence of oxidisable auxiliary substrates is avoided (Figure 2). Thus, aerobic metabolic degradation can occur even in aquifers with low organic carbon content, in which cometabolic aerobic degradation as well as anaerobic reductive dechlorination will be hampered due to limited availability of auxiliary substrates.





Figure 2 Consumption of oxygen and auxiliary substrates (ammonia, methane and acetate) during cometabolic and metabolic degradation of TCE.

Material and Methods

Groundwater microcosms were prepared and incubated as previously described (Schmidt et al., 2010). Gas-tight glass bottles (2.3 L volume) were filled with 2 L of groundwater directly from the wells at a chloroethene-contaminated site in the South of Germany (Schmidt et al., 2014). The first sampling was in 2007 from one well. The groundwater contained 1.4 mg/L TCE, 0.15 mg/L cDCE, 0.60 mg/L of dissolved organic carbon (DOC), no oxygen (detection limit: 0.5 mg/L), no ammonium (detection limit: 0.05 mg/L), no methane (detection limit: 0.01 mg/L), no ethene (detection limit: 0.005 mg/L), and no ethane (detection limit: 0.005 mg/L). Four active microcosms were set up: Two microcosms were spiked with cDCE, one microcosm was spiked with VC, and one microcosm was spiked with cDCE plus VC. A second sampling campaign was conducted in 2010 at 8 wells.

Aliquots of the two groundwater microcosms with cDCE were transferred into chloride- and ammoniumfree mineral salts medium (Schmidt et al., 2014). This enriched mixed bacterial culture was maintained active without any auxiliary substrate for five years, with ten transfers into fresh mineral salts medium (10% inoculum). The only carbon substrate was TCE, which was added repeatedly after its degradation.

Abiotic controls were inhibited with 1 g/L sodium azide.

Results

In our study, the aerobic biodegradation of TCE as the sole growth substrate was demonstrated. This new process of metabolic TCE degradation was first detected in laboratory groundwater microcosms (Figure

3). Stable carbon isotope fractionation was observed (Figure 4) providing a reliable analytical tool to assess this new biodegradation process at field sites.



Figure 3 Aerobic degradation of trichloroethene (TCE) in groundwater microcosms sampled in 2007. The error bars represent \pm 6 % reproducibility on standard analyses for the chloroethenes. Figure modified after Schmidt et al., 2014.



Figure 4 Compound specific stable carbon isotope fractionation (Rayleigh plot) during aerobic trichloroethene (TCE) degradation in mineral salts medium (three replicate assays denoted by different symbols). The error bars for the isotope analysis are smaller than the plotted symbols (total analytical error: \pm 0.5 ‰). Figure taken from Schmidt et al., 2014.

Further experiments with the enriched mixed bacterial culture in mineral salts medium showed sustained long-term TCE biodegradation down to concentrations below the detection limit. Aerobic TCE degradation resulted in stoichiometric chloride formation (Figure 5) and bacterial growth (Schmidt et al., 2014).



Figure 5 Aerobic trichloroethene (TCE) degradation and chloride formation in mineral salts medium. (A) TCE concentration with repeated spiking, (B) cumulative TCE degraded and cumulative chloride formed. The error bars represent ± 6 % and ± 1 % reproducibility on standard analyses for TCE and chloride, respectively. Figure taken from Schmidt et al., 2014.

Conclusion and Outlook

Further studies are currently conducted to prove field applicability of the aerobic metabolic TCE biodegradation by a field test with in-situ delivery of oxygen into a karstic aquifer.

Aerobic metabolic TCE degradation might represent a new and promising concept for monitored natural attenuation (MNA) approaches or engineered bioremediation of contaminated sites (enhanced natural attenuation, ENA). Those remediation approaches can have advantages compared to reductive dechlorination or cometabolic degradation and can be considered cost-efficient and environmental safe.

Based on these results the assessment of aerobic metabolic TCE degradation at field sites is highly recommended. The observed stable carbon isotope fractionation provides a reliable analytical tool to monitor and quantify aerobic oxidative degradation pathways in the field.

References

ATSDR - Agency for Toxic Substances & Disease Registry, 2012. Priority list of hazardous substances. http://www.atsdr.cdc.gov/SPL/index.html. 21-6-2012.

Conrad, M.E., Brodie, E.L., Radtke, C.W., Bill, M., Delwiche, M.E., Lee, M.H., Swift, D.L., Colwell, F.S., 2010. Field evidence for co-metabolism of trichloroethene stimulated by addition of electron donor to groundwater. Environmental Science & Technology 44 (12), 4697-4704.

EU - European Union, 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. OJ L 348, 24.12.2008, p. 84–97.

Mattes, T.E., Alexander, A.K., Coleman, N.V., 2010. Aerobic biodegradation of the chloroethenes: pathways, enzymes, ecology, and evolution. FEMS Microbiology Reviews 34, 445-475.

Schmidt, K.R., Gaza, S., Voropaev, A., Ertl, S., Tiehm, A., 2014. Aerobic biodegradation of trichloroethene without auxiliary substrates. Water Res. 59, 112-118.

Schmidt, K.R., Augenstein, T., Heidinger, M., Ertl, S., Tiehm, A., 2010. Aerobic degradation of *cis*-1,2dichloroethene as sole carbon source: Stable carbon isotope fractionation and growth characteristics. Chemosphere 78 (5), 527-532.

Tiehm, A., Schmidt, K.R., 2011. Sequential anaerobic/aerobic biodegradation of chloroethenes - aspects of field application. Current Opinion in Biotechnology 22 (3), 415-421.

Tiehm, A., Schmidt, K.R., Pfeifer, B., Heidinger, M., Ertl, S., 2008. Growth kinetics and stable carbon isotope fractioning during aerobic degradation of *cis*-1,2-dichloroethene and vinyl chloride. Water Research 42 (10-11), 2431-2438.

US-EPA - United States Environmental Protection Agency, 2012. Priority pollutants. http://water.epa.gov/scitech/methods/cwa/pollutants.cfm. 21-6-2012.

Acknowledgment

Financial support provided by the German Federal Ministry of Economics and Technology (grant number: 16224 N) is acknowledged. The authors thank Michael Deusch, Siegmund Ertl, Markus Friedrich, Holger Hansel, Michael Heidinger, and Andrey Voropaev for their support.