Report as of FY2006 for 2006CA172B: "Impacts of Ethanol on Anaerobic Production of Tert-Butyl Alcohol (TBA) from Methyl Tertiary Butyl Ether (MTBE) in Groundwater"

Publications

- Articles in Refereed Scientific Journals:
  - Mackay, D., de Sieyes, N., Einarson, M., Feris, K., Pappas, A., Wood, I., Jacobsen, L., Justice, L., Noske, M., Wilson, J., Adair, C., Scow, K., 2007. Impact of ethanol on the natural attenuation of MTBE in a normally sulfate-reducing aquifer. Environmental Science & Technology, 41, 2015-2021.
  - Mackay, D.M., De Sieyes, N.R., Einarson, M.D., Feris, K.P., Pappas, A.A., Wood, I.A., Jacobson, L., Justice, L.G., Noske, M.N., Scow, K.M.., Wilson, J.T., 2006. Impact of ethanol on the natural attenuation of benzene, toluene, and o-xylene in a normally sulfate-reducing aquifer. Environmental Science & Technology, 40, 6123-6130.
  - Nakatsu, CH; K. Hristova, S. Hanada, X.-Y. Meng, J. R. Hanson, K. M. Scow and Y. Kamagata. 2006. Methylibium petroleiphilum gen. nov., sp. nov., a novel methyl tert-butyl ether-degrading methylotroph of the Betaproteobacteria. International Journal of Systematic and Evolutionary Microbiology 56: 983-989
  - Watanabe, N; Schwartz, E; Scow, KM; Young, TM. 2005. Relating desorption and biodegradation of phenanthrene to SOM structure characterized by quantitative pyrolysis GC-MS. Environ. Sci. Technol. 39 (16): 6170-6181.
  - Nozawa-Inoue, M; Scow, KM; Rolston, DE. 2005. Reduction of perchlorate and nitrate by microbial communities in vadose soil. Appl. Environ. Microb. 71 (7): 3928-3934.
  - Scow, KM; Hicks, KA. 2005. Natural attenuation and enhanced bioremediation of organic contaminants in groundwater. Curr. Opin Biotechnol. 16 (3): 246-253.

Report Follows

Introductory text

The widespread use of the gasoline oxygenate MTBE and subsequent spills or leaks of MTBE- laden fuel into the environment has resulted in numerous groundwater supplies becoming contaminated with MTBE. In California alone it is estimated that at least 10,000 groundwater sites currently have MTBE levels above the CA drinking water standard (> 5 ppb). MTBE is a human health concern and relatively recalcitrant under the anaerobic conditions commonly found at gasoline spill sites. Therefore, ethanol is rapidly replacing MTBE as the preferred fuel oxygenate. The increasing use of ethanol amended gasoline (gasohol) is the motivation for this work. The preferential degradation of ethanol in anaerobic environments rapidly consumes predominant electron acceptors (e.g. nitrate, sulfate, iron), and thus spills of gasohol can reduce an aquifer's redox potential to methanogenic conditions. Recent evidence indicates that MTBE may be partially degraded to tert-butyl alcohol (TBA) under methanogenic conditions. Whereas MTBE is a suspected carcinogen and a nuisance chemical in drinking water, TBA is a known toxin, more water soluble than MTBE, and more difficult to remove from water. The biological, geochemical, and environmental factors that influence the formation of TBA from MTBE under methanogenic conditions are not well understood. With an improved understanding of which organisms and environmental factors are involved in anaerobic TBA formation we may be able to design remediation protocols that could prevent TBA from becoming California's next major environmental problem.

This research has the potential to lend insight into the mounting evidence that MTBE partially biodegrades under methanogenic conditions to TBA. Due to the widespread nature of MTBE contamination in California and the current ubiquity of gasohol, the potential for future gasohol releases impacting MTBE contaminated groundwater is high. This research is necessary to understand the potential outcome of gasohol release on MTBE contaminated water supplies.

Problem and Research Objectives

MTBE appears to be biologically converted to TBA under anaerobic conditions. This phenomenon is enhanced by the presence of ethanol, a readily degradable oxygenate that has rapidly replaced MTBE in formulated gasoline. It is important to determine whether TBA formation can be linked to microbial processes in the field and lab, to demonstrate whether ethanol truly enhances its formation and to determine changes in microbial communities during TBA formation.

Our approach to understanding the effect of ethanol on TBA formation at MTBE contaminated sites involves integrating data describing contaminant levels and microbial communities, measured in field and from microcosm studies, using molecular biology techniques and a numerical model of our field site (a contaminated aquifer at the Vandenberg Air Force Base). Controlled release experiments were performed at the site between September 2005 and April 2006. A plume of a mixture of ethanol and MTBE was released alongside a plume of TBA in order to monitor the effect of ethanol on MTBE transformation and to observe degradation of TBA under sulfate reducing conditions, respectively. Specific objectives include:

1. to determine whether TBA concentrations appearing during a controlled ethanol release can be linked to transformation of MTBE concentrations in vicinity by mapping groundwater concentrations of MTBE/TBA in the field and developing a mathematical model to try to understand the mechanisms underlying the patterns of distribution.

2. to measure whether MTBE is converted to TBA under controlled conditions in microcosms, simulating aquifer conditions.

3. to measure changes in microbial communities, both numbers and diversity, when exposed to ethanol and during conversion of MTBE to TBA.

Methodology

Three approaches are used to investigate the potential conversion of MTBE to TBA and the persistence of TBA:

1) Numerical modeling of the aquifer. We are developing a numerical model of the site to quantify the effects of ethanol on MTBE transformation and explore hypotheses regarding TBA formation and persistence. Concentrations of injected contaminants over time are being used to calibrate a finite elements model made with HYDROGEOCHEM 5.0, to determine the most significant factors affecting TBA distribution in the aquifer. Preliminary modeling of diffusion between aquifer layers has been carried out using HydroGeoSphere and validated with field data.

2) Nucleic acid-based microbial analyses. Quantitative polymerase chain reaction (qPCR) and terminal restriction fragment length polymorphisms (TRFLP) are being used to track changes in diversity and abundance of key microbial groups over time.

3) Microcosms. Microbially mediated degradation of MTBE (complete mineralization or partial conversion to TBA) is being confirmed in the lab with anaerobic microcosms of site material. The potential of site microorganisms to degrade TBA is also being tested. Active microcosms and killed controls from site material have been constructed from site material to evaluate the potential of the native microbial communities to carry out the conversion of MTBE to TBA and the complete mineralization of TBA. A second set of microcosms is being used to enrich for these organisms with the goal of identifying the key species or consortia involved.

Principal Findings

Microbial community fingerprints show that the bacterial community experiences a large shift in composition over time (Fig 1a). Additionally, overall diversity appears to have decreased. The extent of change in both parameters was greater over the two months before cessation of contaminant injected than in the first five months of the experiments. At the same time, the selective enrichment of an operational taxonomic unit (OTU) associated with the changing geochemical conditions at the site, is apparent (Fig 1a).

Over the same period, 1) TBA injected into the aquifer did not degrade and 2) MTBE injected into the aquifer together with ethanol resulted in a plume of TBA forming beyond the zone of ethanol depletion, i.e. several feet down gradient of the injection area (Fig. 1b).

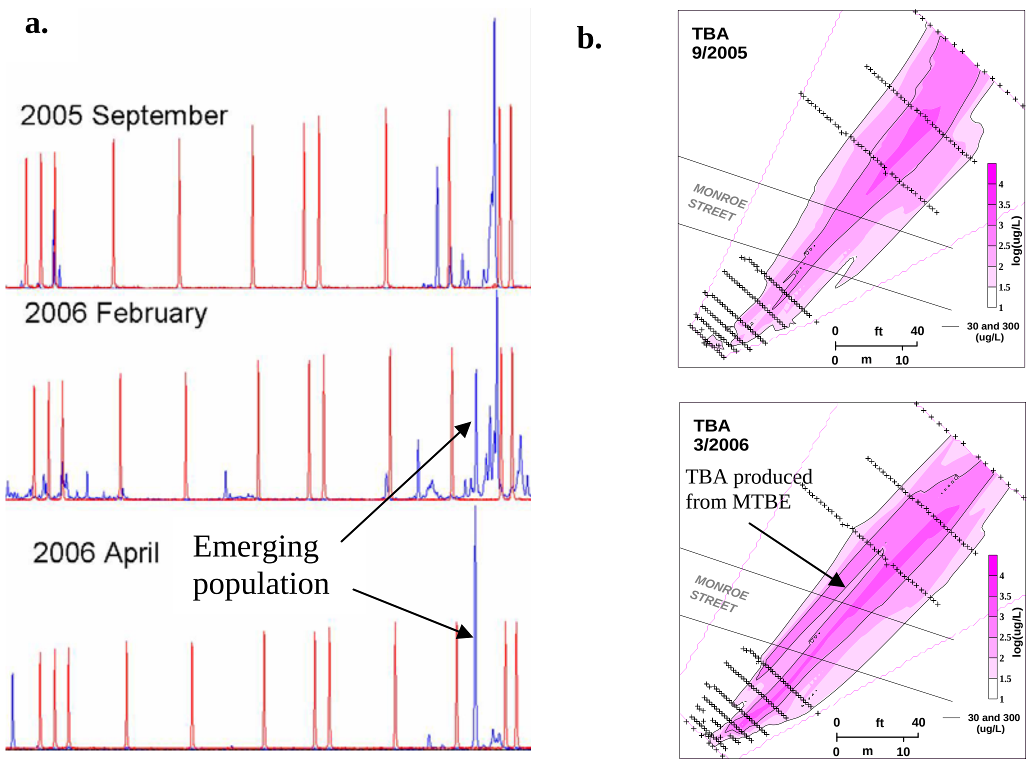

Figure 1. a) Changes in bacterial community structure as determined by TRFLP. Red chromatogram is size standard ROX GS-500. Blue peaks represent bacterial OTUs based on 16S RNA gene. Black arrows highlight emerging population over time (note absence of peak of interest in September 2005). b) Changes in distribution of TBA in the aquifer. Black arrow indicates plume derived from MTBE injection, in a region of the aquifer where TBA was absent at the start of the experiment in September 2005.

Microorganisms of interest, e.g. based on dominant bands and relationships of bands to contaminant concentrations, will be sequenced to determine phylogeny. qPCR methodologies will be used to link changes in their population sizes with macro-scale geochemical data obtained at the site.

Acetogen pure cultures (*Clostridium magnum* and *Sporomusa silvacetica*) were not shown to be capable of carrying out MTBE degradation in pure culture studies, although these results are inconclusive due to possible methodological uncertainties. Primers developed to target the tetrahydrofolate synthetase gene (indicative of acetogenic metabolism) produced a positive result in one out of four site groundwater samples tested. Validation of this result and comparison of acetogenic bacterial abundance to contaminant levels at the site will be carried out next.

An important observation at VAFB is that the persistence of TBA extends well beyond what would be expected, based on the behavior of bromide. Bromide, a conservative tracer similar to TBA in solubility, was co-injected during the experiment. Numerical modeling using HydroGeoSphere was carried out to explore the possibility of diffusion of TBA into the low permeability layer above the layer (S3) containing much of the contamination and the focus of our studies at VAFB. Soil cores were collected from the site (BR04 and BR05 in Fig. 2), divided into sub-sections and concentrations of bromide were measured in each section. Based on these results, it appears that basic assumptions regarding aquifer geology and tracer properties used in conducting the simulation of diffusion of the bromide from the aquifer (S3 sand) into the lower permeability layer above (silt/clay) are valid (Fig 2). In fact, fits to field data could be improved by increasing the extent of mass transfer of TBA from the S3 layer to low permeability layers. However, the high concentrations of TBA persisting in the field cannot be explained with this model alone, due to the more complex history of TBA at the site. The next phase of our research is to develop a more sophisticated numerical model that would improve our understanding of TBA behavior.

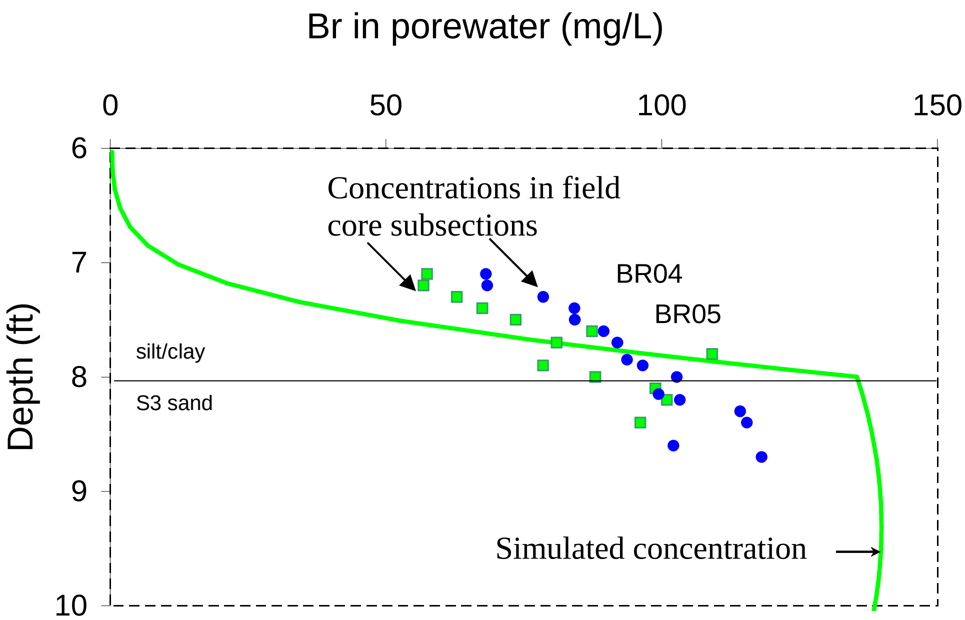

Figure 2. Comparison of simulated results with concentrations measured in field core samples.

Significance

Our results show that TBA behaves unexpectedly at the VAFB field site. We hypothesize that a previously uncharacterized retardation mechanism is impeding the flushing of TBA from the aquifer at a rate that would be expected based on its solubility and sorption properties. TBA is being detected increasingly frequently, throughout the US, at numerous sites previously contaminated with MTBE and thus it is crucial to understand the processes involved in its formation. A better understanding of TBA behavior will lead to improved strategies for its remediation.

INFORMATION TRANSFER PROGRAM

Numerous research presentations have been given based on this work, including a special symposium on MTBE research at VAFB held at the Association of Environmental Health and Sciences (AEHS) meeting in San Diego, CA, this past spring (see notable achievements section). The following are citations of presentations of work specifically related to the UC CWR funded project (all are from the Seventeenth Annual AEHS Meeting and West Coast Conference on Soils, Sediments, and Water: Analysis, Fate, Environmental and Public Health Effects, and Remediation, San Diego, Mar 19-22, 2007).

Feris, K., Mackay, D., Hristova, K., Scow, K., De Sieyes, N.R., Murray, E. **Linking impacts of ethanol on subsurface microbial ecology and anaerobic transformations of BTEX.**

Kaiser, P., Nozawa-Inoue, M., Chakraborty, I., Scow, K., Mackay, D., Murray, E. **Impact of Ethanol on Anaerobic Transformation of MTBE to TBA**

Mackay, D., Chakraborty, I., Nozawa-Inoue, M., Goyal, S., Ginn, T., Scow, K., Einarson, M., Chapman, S. **Flushing of MTBE and TBA from layered media**

STUDENT SUPPORT

	Total Project Funding		Supplemental Awards	Total
	Federal Funding	State Funding		
Undergrad.	1	1	10	12
Masters			1	1
PhD.	1		6	7
Post-Doc.		1	3	4
Total	2	2	20	24

NOTABLE ACHIEVEMENTS AND AWARDS

- Symposium sponsored by the American Petroleum Institute entitled "Review Of Gasoline Oxygenate Research At The Vandenberg AFB Site", at the Association of Environmental Health and Sciences: Seventeenth Annual AEHS Meeting and West Coast Conference on Soils, Sediments, and Water: Analysis, Fate, Environmental and Public Health Effects, and Remediation. The symposium was a three hour session dedicated to highlighting new findings by our collaborative team at Vandenberg, as part of a whole day session on the environmental impact of fuel oxygenates.
- API graduate student award to Irina Chakraborty (2006)
- Superfund Basic Research and Training Program Fellowship to Irina Chakraborty (2005-2007)