

Summary of Information About Lawrence Livermore National Laboratory Studies on the Applicability of Bioremediation for Removing Fuel Oxygenate Compounds from LUFT Sites

Site Name: Not provided

Site Location: Not provided

Contaminant: MTBE

Media: Soil and Groundwater

Technology: Bioremediation

Technology Scale: Bench

Period of Operation: Not Provided

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Description:

Researchers at Lawrence Livermore National Laboratory (LLNL) conducted several studies to evaluate the bioremediation potential of soil and groundwater containing MTBE [1-3]. The results of their investigations on MTBE/TBA degraders and a screening study of Palo Alto leaking underground fuel tank (LUFT) sites are presented below.

Research on MTBE/TBA Degraders

To identify microorganisms capable of degrading MTBE and/or TBA, studies were conducted to evaluate alkyl-ether degradation by methanotrophs, long-term in situ enrichment for TBA-degrading microorganisms, and pure and mixed cultures reported to use MTBE as a growth substrate. Results of these studies are summarized below.

Methanotrophs Study

Research was performed to determine whether two methanotrophic microorganisms, *Methylosinus trichosporium OB3b* and *Methylosinus sporium*, were capable of transforming TBA and various alkyl ether compounds. The bacteria were grown with methane as the single carbon source. Cells were harvested and washed, and the presence of methane monooxygenases was confirmed by measuring TCE degradation activity. In resting cell assays, both organisms failed to degrade detectable amounts of TBA, MTBE, ethyl tert butyl ether (ETBE), diisopropyl ether (DIPE), and tert amyl methyl ether (TAME).

Long-term In Situ Enrichment of TBA Degraders

This study attempted to isolate TBA degraders from a chemical manufacturing site in New Jersey that had been contaminated with pure and dissolved TBA for more than 10 years. A range of 67 to 460,000 TBA degraders per gram of sediment had been reported for this site. However, these results could not be duplicated. During the study, a few colonies appeared on selected plates containing solidified minimal medium plus TBA. No growth was observed, however, when these colonies were transferred into liquid media, suggesting that the microorganisms were growing on agar constituents and/or impurities rather than on the test compound (TBA). Thus, after 10 years of potential microbial enrichment at the site, no microorganisms were detected that could grow on minimal medium using TBA as the sole carbon and energy source.

Cultures Using MTBE as Growth Substrate

Two microbial cultures were tested that had been reported to use MTBE as a growth substrate - the Pelorus Environmental and Biotechnology Corp. (PEL) presumed pure culture (PEL-Pg) and the PEL consortium culture (PEL-CON). Both cultures were derived from leaking underground fuel tank (LUFT)-sediment samples and reportedly used MTBE as the sole carbon and energy source. During testing, the PEL-Pg culture was found to be a consortium of two different strains; neither of these two isolates nor the PEL-CON culture grew on MTBE/TBA or degraded these compounds. In discussions with PEL representatives, it was concluded that the apparent microbial growth observed on MTBE by PEL most likely was due to the presence of ppm-levels of triacetic acid in the test media.

Culture Derived from Biofilter

Biomass from an experimental biofilter was obtained from the Joint Water Pollution Control plant in Whittier, California, and tested to confirm its ability to degrade MTBE. The biofilter material had been observed to rapidly degrade >90% MTBE after a one year adaptation period. Tests at LLNL confirmed the ability of the mixed culture to degrade MTBE and TBA. However, microbial growth associated with MTBE depletion was extremely slow and resulted in undesirable cell clumping. Both properties potentially will limit the applicability of this culture to the restoration of subsurface environments.

Palo Alto LUFT Sites Screening Study

A study was conducted at LLNL to assess the potential use of intrinsic and/or engineered in situ bioremediation for the restoration of MTBE-impacted soils and groundwater at leaking underground fuel tank (LUFT) sites [1-3]. The study locations, all situated in Northern California, were identified by the Santa Clara Valley Water District and the San Francisco California Regional Water Quality Control Board as high risk sites, based on their proximity to public drinking water wells and/or the presence of extremely high concentrations of MTBE in shallow groundwater. The overall goal of the study was to provide general conclusions concerning the fate of MTBE at LUFT sites.

Soil (sediment) and groundwater samples from one particular site in Palo Alto were collected at a depth of 18-26 feet where aqueous MTBE concentrations were in the low ppm range. Microcosms constructed from these materials either mimicked the anaerobic conditions prevailing at the site, or contained various amendments ranging from nutrients, to oxygen, to microbial biomass. The groundwater used to construct the microcosms was first sparged to drive off volatile contaminants and then respiked to yield an initial aqueous MTBE concentration in the microcosm of about 420 µg/L. Microcosms were incubated for 3 months at 20°C and 6 rpm. Table 1 presents a summary of experimental conditions and results obtained during the initial screening study.

Table 1. Summary of Results Obtained in a Screening Study using Environmental Samples from a Palo Alto LUFT Site [1].

| Experimental Condition | Results (% loss) |
|---|---------------------|
| Anaerobic – 125 mL serum bottles, 3.4 g of sediment, 15.5 mL of groundwater (MTBE air-stripped), spiked with MTBE to ~420 µg/L | |
| Sterile control | <10 % |
| No amendments | <10 % |
| Oxygen release compound (ORC [®]) | <10 % |
| Lactate (1 g/kg) | 18% (349 ± 31 µg/L) |
| BioPetro commercial bioaugmentation product [4] | <10 % |
| BioPetro control (buffer and sucrose without BioPetro) | <10 % |
| Aerobic – VOA vials, 5 g of sediment, 23 mL of groundwater (MTBE stripped), spiked with MTBE to ~420 µg/L | |
| Sterile control | <10 % |
| No amendments | 100 % (<5 µg/L) |
| Isopropanol, 5 ppm | <10 % |
| <i>M. vaccae</i> JOB5 (10 ⁸ CFU/g) + isopropanol | 100 % (5 µg/L) |
| Pelorus strain | <10 % |
| Pelorus consortium | <10 % |
| Biofilter consortium | 100 % |
| BioPetro commercial bioaugmentation product | <10 % |
| BioPetro control (buffer and sucrose without BioPetro) | <10 % |

The results of the screening study indicated that MTBE generally persists under anaerobic conditions. In addition, ORC[®] (an oxygen release compound) and BioPetro (sawdust coated with 5×10^9 CFU of more than 22 species of different facultative anaerobic microorganisms per gram [4]) did not stimulate MTBE degradation under anaerobic conditions; minor losses of MTBE observed in lactate-amended microcosms could not be reproduced in later studies. Although not expected by the researchers, MTBE was degraded completely in live, aerobic microcosms. As a result, it was difficult for the researchers to judge the effectiveness of some of the bioaugmentation formulations. However, the presence of additional carbon sources (sucrose contained in the BioPetro microcosms, isopropanol, and pasteurized cells) inhibited intrinsic MTBE biodegradation.

The kinetics of intrinsic aerobic MTBE degradation and the respective microorganisms currently are being investigated at LLNL. Initial attempts to reproduce the above results failed when old sediment cores were used that had been stored at 4°C for a period of about one year. However, rapid aerobic biodegradation of MTBE was observed when microcosms were constructed from fresh sample material. Follow-up experiments also showed that indigenous microorganisms in aerobic microcosms could consume higher concentrations of MTBE of up to 5 ppm over a period of only 15 days. During these experiments, TBA was detectable as a metabolite of MTBE biodegradation.

Conclusions

The LLNL researchers provided the following conclusions from their work:

- Two methanotrophic microorganisms did not transform MTBE, TBA, ETBE, DIPE, and TAME
- Long-term enrichment under site-specific conditions may fail to produce strains that effectively degrade TBA/MTBE
- Careful selection of experimental media is important to identify “real” MTBE degraders
- Fast-growing MTBE degraders that are needed for bioaugmentation are currently not available
- MTBE may biodegrade naturally at LUFT sites in some instances when conditions are favorable (e.g., aerobic conditions, no BTEX compounds present).

References:

1. Rolf Halden et. al. Environmental Protection Department, Lawrence Livermore National Laboratory. “Evidence for Intrinsic Bioremediation of MTBE at a LUFT Site”. Presented at: In-Situ Remediation Alternatives for MTBE Impacted Aquifers, Oxnard, CA, August 10, 1999.
2. Anne Happel and Rolf Halden. Environmental Protection Department, Lawrence Livermore National Laboratory. “Examining in Situ Biodegradation of MTBE at Commercial LUFT Sites.” *In: An Evaluation of MTBE Impacts to California Groundwater Resources*, pp. 65-68. 1998. UCRL-AR-130897. <http://geotracker.llnl.gov/LiverReports.htm>.
3. Record of Telephone Conversation. Steve Michener, Tetra Tech EM Inc., and Rolf Halden, Lawrence Livermore National Laboratory. “Obtain Information on Comparative Case Study of Biodegradation at Lawrence Livermore.” October 5, 1999.
4. BioPetro Vendor Information. <http://www.infohwy.com/business/betusa/hycar.html>