Self-seeding of a Field-scale MTBE Treatment Bioreactor by Native Aquifer Bacteria

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Environmental Relevance of MTBE

hat is Methyl tertiary butyl ether (MTBE)?

- A fuel oxygenate used in over 85% of reformulated gasoline
- Second most common volatile organic compound (VOC) detected in rban groundwater
- Classified as a potential human arcinogen by the EPA
- More resistant to microbial degradation han other gasoline components
- Exhibits low adsorption to soil surfaces
- Migrates readily with groundwater.

nvironmental Impacts of MTBE

- Principle source of MTBE in groundwater comes from leaking nderground fuel tanks (LUFTs).
- As of 2001, 416,000 MTBE-releasing LUFTs have been identified ationwide (43).

- In California alone, approximately 10,000 LUFT sites are estimated to be releasing MTBE(happel).
- Cleanup of just the remaining, identified releases is estimated to cost $29 billion
- Development of cost-effective field technologies for treating MTBE-impacted water supplies is **critical**

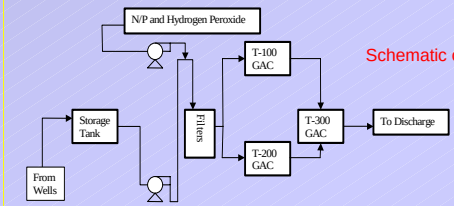
ioremediation as a Treatment Option for MTBE?

- Biological degradation of contaminants (I.e. bioremediation) is a ost-effective, environnmentally-friendly treatment technology used o treat other petroleum hydrocarbons
- Because MTBE was previously believed to be recalcitrant to icrobial degradation, few field-scale demonstrations of MTBE ioremediation have been conducted
- Microbial strains capable of mineralizing MTBE have now been olated and mounting evidence suggests that many aquifers may contain native bacteria with he potential to degrade MTBE
- The first such isolate, *Methylobium etroleophilum* PM1, rapidly mineralizes 20μmol/hr) and has been detected in several TBE-contaminated sites in California

Scientific Approach

Objective: To investigate the potential of native aquifer bacteria to biodegrade MTBE in field-sscale biosimulation test

- In the first phase of this study, colonization of an MTBE-treating bioreactor containing virgin granular activated carbon (GAC) by *in situ* bacteria, including strain PM1, was evaluated.

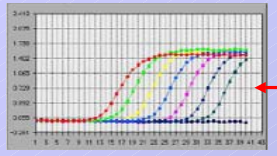


Schematic of Bioreactor Design

- The bioreactor is currently being used to treat MTBE-impacted groundwater (~20 mg/ml) in North Hollywood, California without seeding of the bioreactor by *ex situ* MTBE-degrading organisms.

Phase One Methods

- Total biomass in water and GAC were measured by viable plate counts



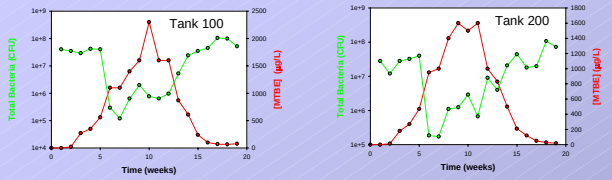
- DNA was extracted from groundwater and GAC samples and quantitative Taqman PCR using 16s universal bacterial primers performed.

Quantitative PCR is a sensitive, fluorescence-based, culture-independent PCR method capable of detecting low concentrations of bacterial DNA in environmental samples.

- Primers specific to the 16s region of strain PM1 were used in Taqman q-PCR to detect and quantify strain PM1 in GAC and groundwater.

Results

- Self-seeding of the bioreactor by native aquifer bacteria occurred rapidly, with colonization reaching 10^5 cells/g GAC within six days of startup and 10^7 cells/g by the fourth week.
- Effluent MTBE concentrations correlated well with bacterial densities. A drop in biomass within both tanks at week four corresponded to a spike in effluent MTBE concentrations. Subsequent recovery in biomass restored bioreactor performance.



MTBE concentrations in bioreactor tank effluents versus biomass within tanks over a 20 week treatment period.

- MTBE-degrading Strain PM1 was detected by q-PCR in all samples and at all dates, including influent, at a concentration of ~2 orders of magnitude below total bacteria and increasing in tandem with total bacteria.

Future/ Ongoing Research

- *Phase Two* → a bench-scale biostimulation study for the North Hollywood aquifer
- Column studies will characterize bacterial densities in the aquifer sediment as a function of MTBE, oxygen and nutrient gradients
- These studies will lay the groundwork for a field-scale biostimulation trial in the MTBE-impacted aquifer

Impacts

- Seeding of biological treatment systems by *ex situ* bacteria along with the associated costs and regulatory approval processes, may not be necessary at sites where native degrading communities exist
- Biodegradation by Indigenous bacteria may offer a rapid and effective alternative to other MTBE treatment strategies
- Increasing our understanding of MTBE biodegradation processes will spur development implementation of MTBE bioremediation technologies in the field