

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

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## Environmental Relevance of MTBE

### What 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 urban groundwater
- Classified as a potential human carcinogen by the EPA
- More resistant to microbial degradation than other gasoline components
- Exhibits low adsorption to soil surfaces
- Migrates readily with groundwater.



### Environmental Impacts of MTBE

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

### Bioremediation as a Treatment Option for MTBE?

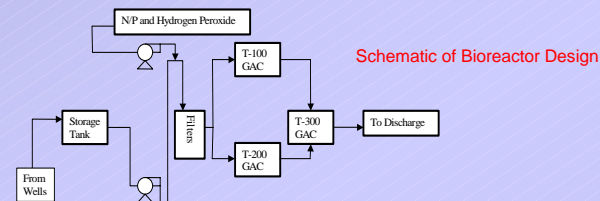
- Biological degradation of contaminants (I.e. bioremediation) is a cost-effective, environmentally-friendly treatment technology used to treat other petroleum hydrocarbons
- Because MTBE was previously believed to be recalcitrant to microbial degradation, few field-scale demonstrations of MTBE bioremediation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



## Scientific Approach

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

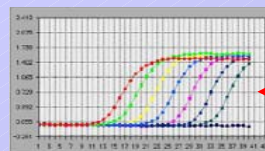
- In the first phase of this study, colonization of an MTBE-treating bioreactor containing virgin granular activated carbon (GAC) by *in situ* bacteria, including strain PM1, was evaluated.



- 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**



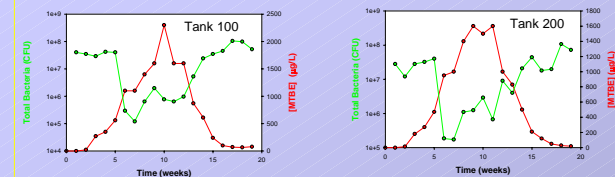
Quantitative PCR is a sensitive, fluorescence-based, culture-independent PCR method capable of detecting low concentrations of bacterial DNA in environmental samples.

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

- Primers specific to the 16s region of strain PM1 were used in **Tagman 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<sup>5</sup> cells/g GAC within six days of startup and 10<sup>7</sup> 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