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MTBE and TBA Cleanup-New Research Perspectives

Sponsored by: National Institute of Environmental Health Sciences, Superfund Research Program

Delivered: June 24, 2010, 2:00 PM - 3:30 PM, EDT (18:00-19:30 GMT)

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Moderators: Justin Crane and Monica Ramirez

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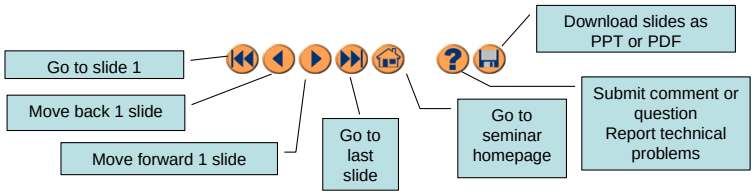

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Although I'm sure that some of you have these rules memorized from previous CLU-IN events, let's run through them quickly for our new participants.

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You should note that throughout the seminar, we will ask for your feedback. You do not need to wait for Q&A breaks to ask questions or provide comments. To submit comments/questions and report technical problems, please use the ? Icon at the top of your screen. You can move forward/backward in the slides by using the single arrow buttons (left moves back 1 slide, right moves advances 1 slide). The double arrowed buttons will take you to 1st and last slides respectively. You may also advance to any slide using the numbered links that appear on the left side of your screen. The button with a house icon will take you back to main seminar page which displays our agenda, speaker information, links to the slides and additional resources. Lastly, the button with a computer disc can be used to download and save today's presentation materials.

With that, please move to slide 3.

MTBE and TBA Cleanup: New Research Perspectives

Krassimira Hristova, Kristin Hicks,
Radomir Schmidt and Kate Scow

Department of Land, Air, and Water Resources,
UC Davis

Superfund Program, Project 1

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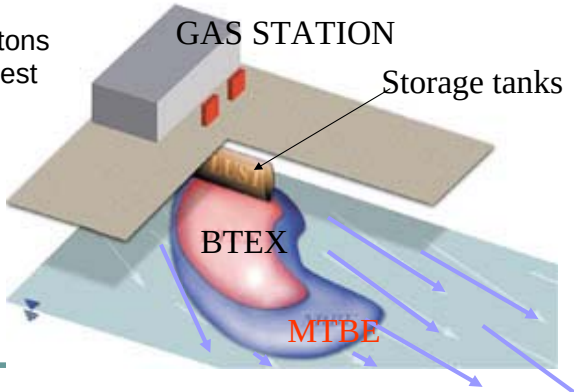
MTBE problem

- MTBE - fuel oxygenate widely used in reformulated gasoline
- *1970s added to replace lead
  *Premium gas amended with MTBE to reach high octane number.
- PRODUCTION: 25 million tons produced in 1999 (2nd highest volume chemical)
- Commonly detected in subsurface water due to
  - large scale production
  - low sorption to soil
  - high solubility in water
  - low biodegradability

SPILLS: Est. 250,000 spills in US- from gasoline storage and distribution systems



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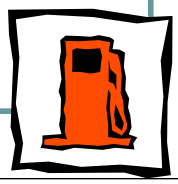
Occurrence of MTBE in the Environment

- Detected in the groundwater of 49 states
- 10,000 sites impacted by MTBE in California

- Frequently detected in water supply wells in US
- 1% of drinking water supplies are above the EPA suggested limit of 20 ppb

- *In situ* degradation apparently slow enough under typical conditions so that significant groundwater migration often occurs
- TBA is a “suspected carcinogen”
- Microbial isolates can degrade MTBE aerobically
- TBA is accumulated in the plumes



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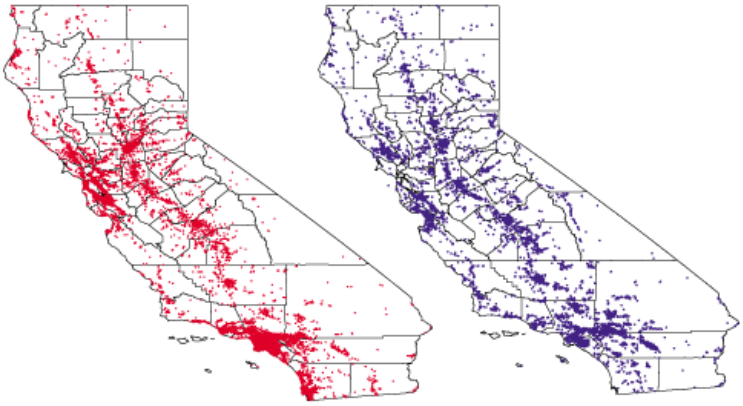
MTBE in the Environment



Leaking Underground Storage Tanks (LUST)

Public Drinking Water Wells

A side-by-side comparison of (a) the locations of leaking underground fuel tanks (LUFTs) in California and (b) the locations of public drinking water wells strongly suggests a high instance of proximity

Wilt (1999) *Lawrence Livermore National Laboratory Science and Technology Review* 21-23

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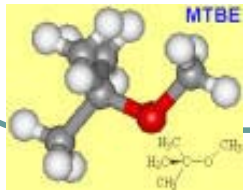
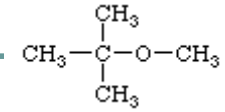
CONTAINS

MTBE

THE STATE OF CALIFORNIA HAS DETERMINED
THAT THE USE OF THIS CHEMICAL PRESENTS
A SIGNIFICANT RISK TO THE ENVIRONMENT



Methyl tertiary butyl ether



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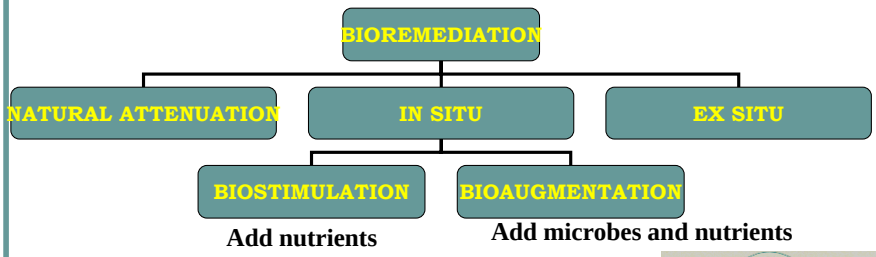
MTBE - today

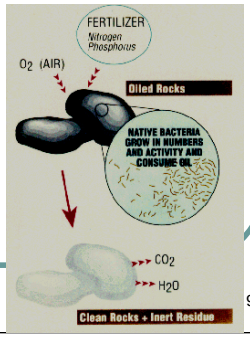
- 7 years after the ban, about 200 public supply wells in California have had to be taken offline
- Undetected migrating plumes in the state
- High cost of clean-up after detection
  - Costs passed on to the consumer
  - TBA, a breakdown product, accumulates in the field

Bioremediation Approaches



- **Bioaugmentation** - use lab bacterial strain as inoculum
  - inoculate bioreactor (***ex situ*** treatment)
  - inoculate directly in the field (***in situ*** treatment) to create a biobarrier and stop the plume

- **Biostimulation**
  - stimulate native bacteria to degrade the pollutant by providing nutrients and e-acceptors



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TBA problem

- MTBE treatment systems should be tested for TBA treatment efficiency
- Biological remediation of TBA is more effective than traditional adsorption and air-stripping technologies
- Bioreactor effluent is usually discharged as wastewater: biological safety should be tested

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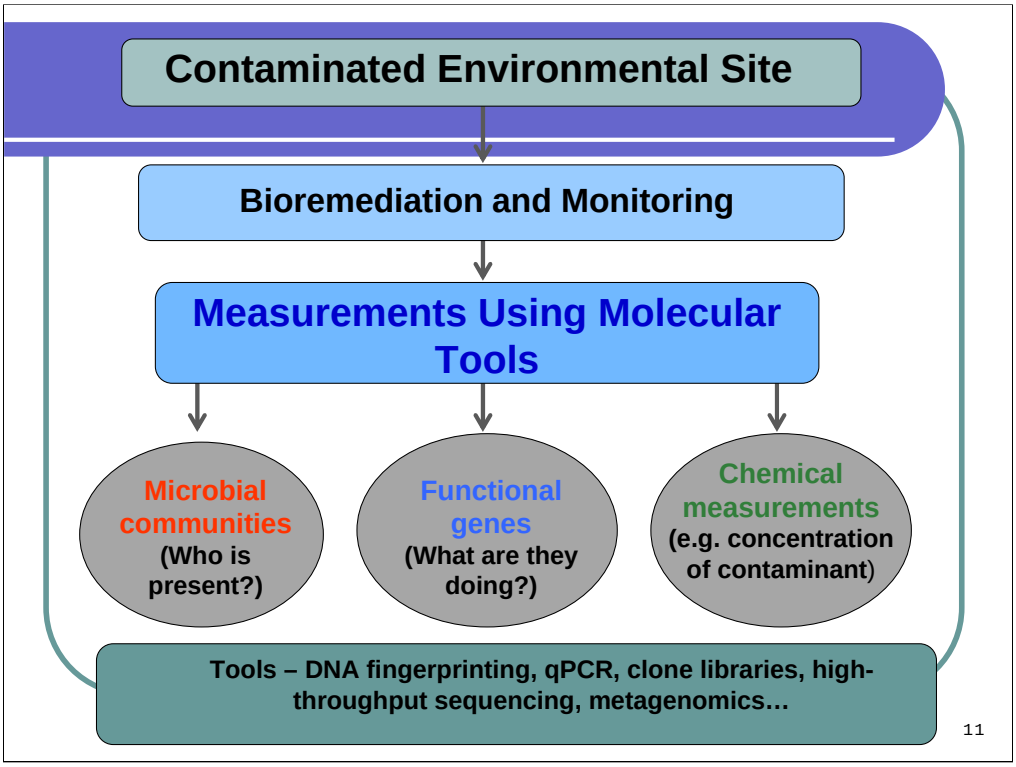
Contaminated Environmental Site
Bioremediation and Monitoring
Measurements Using Molecular Tools
Microbial communities (Who is present?)
Functional genes (What are they doing?)
Chemical measurements (e.g. concentration of contaminant)
Tools – DNA fingerprinting, qPCR, clone libraries, high-throughput sequencing, metagenomics…
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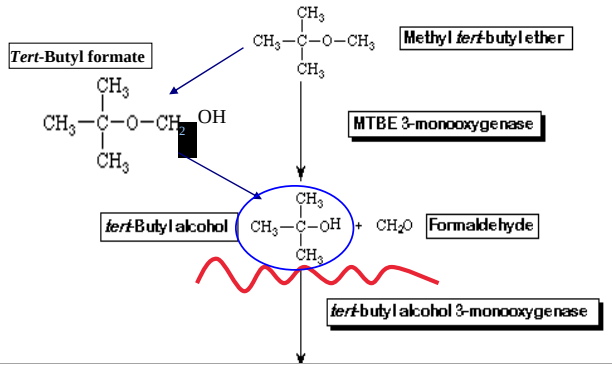
MTBE Biodegradation

Aerobic

Environmental isolates able to degrade MTBE

- *Methylibium petroleiphilum* strain PM1 — 72.6 nmol/min/mg protein
- *Hydrogenophaga flava* ENV735 — 46 nmol/min/mg
- *Mycobacterium austroafricanum* IFP 2012 — 20 nmol/min/mg
- Strain L108 (similar to PM1)



Many MTBE-degraders (cometabolizers and anaerobes) slow down or stop during MTBE biodegradation

TBA undesirable because difficult to treat and more toxic than MTBE

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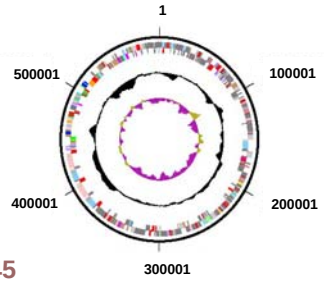
Initially thought to be recalcitrant chemical, difficult to break the ether bond,

But in the last 10 years biodegradation was frequently reported under aerobic conditions in lab and field studies.

MTBE degrading strain *Methylibium petroleiphilum* PM1

- ***Isolated from compost filter***
- ***Strain PM1 readily degrades TBA***
- Aerobic, flagellated, Gram- rod
- Degrades MTBE and TBA completely to CO_2 and cells;
- Beta-proteobacteria (*Aquabacterium, Rubrivivax, Leptothrix)*
- New genus/species
- **The whole genome was sequenced**



Kane at al., 2007, J. Bacteriology, 189:1931-45

Hristova et al., 2007, Appl. Environ. Microbiol., 73:7347-7357

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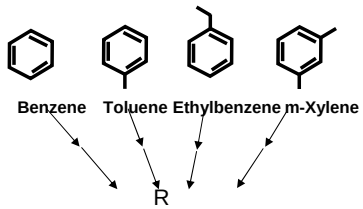
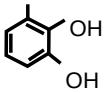
One of the new discoveries based on the whole genome sequence was the megaplasmid

PM1 Degradation abilities

- can grow aerobically on ethanol, methanol, MTBE, alkanes (C4-C12), toluene, benzene, ethybenzene, phenol, and dihydroxybenzoates
- High degradation rates with MTBE, TBA, toluene, phenol, (as single substrates or in mixtures)
- two operons for benzene and/or toluene degradation
- meta-cleavage pathway for catechol and methylcatechols – 2 *dmp* operons





Benzene: R = H

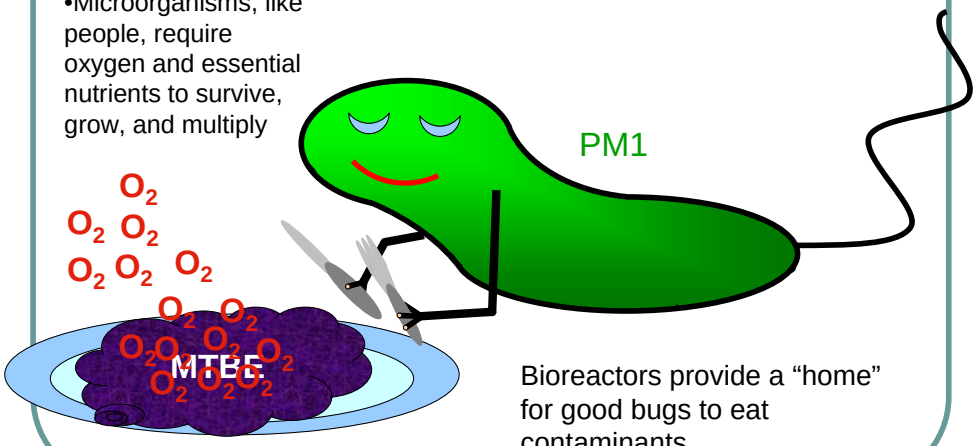
Toluene: R = CH_3

Ethylbenzene: R = CH_2CH_3

m-Xylene: R = CH_3

How it works in the field- good bugs "eat" MTBE and TBA

•Microorganisms, like people, require oxygen and essential nutrients to survive, grow, and multiply



Bioreactors provide a "home" for good bugs to eat contaminants

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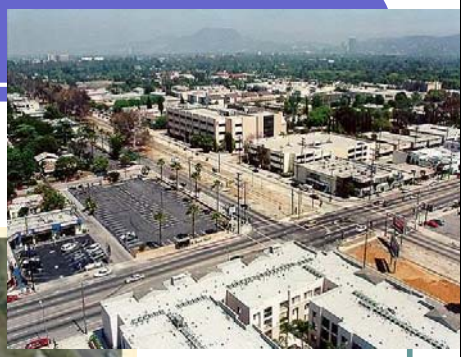
Example 1: Ex situ bioremediation

Treatment of a contaminated drinking water aquifer using native MTBE-degrading bacteria that colonized an *ex situ* bioreactor



North Hollywood
Location of major drinking water aquifer for LA

high concentration MTBE spill from Tesoro gas service station

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Ex situ bioremediation of MTBE-contaminated aquifer at North Hollywood

Can bioreactor successfully treat MTBE and can clean groundwater be returned to aquifer?

UC Davis, Haley & Aldrich, Inc., Tesoro Petroleum Companies, DHS, Miller Brooks Environmental Consultants Inc., Water Resources Control Board

- Groundwater pumped into bioreactor w/oxygen + nutrients
  MTBE biodegradation established within 4 weeks
- MTBE removal was >99%

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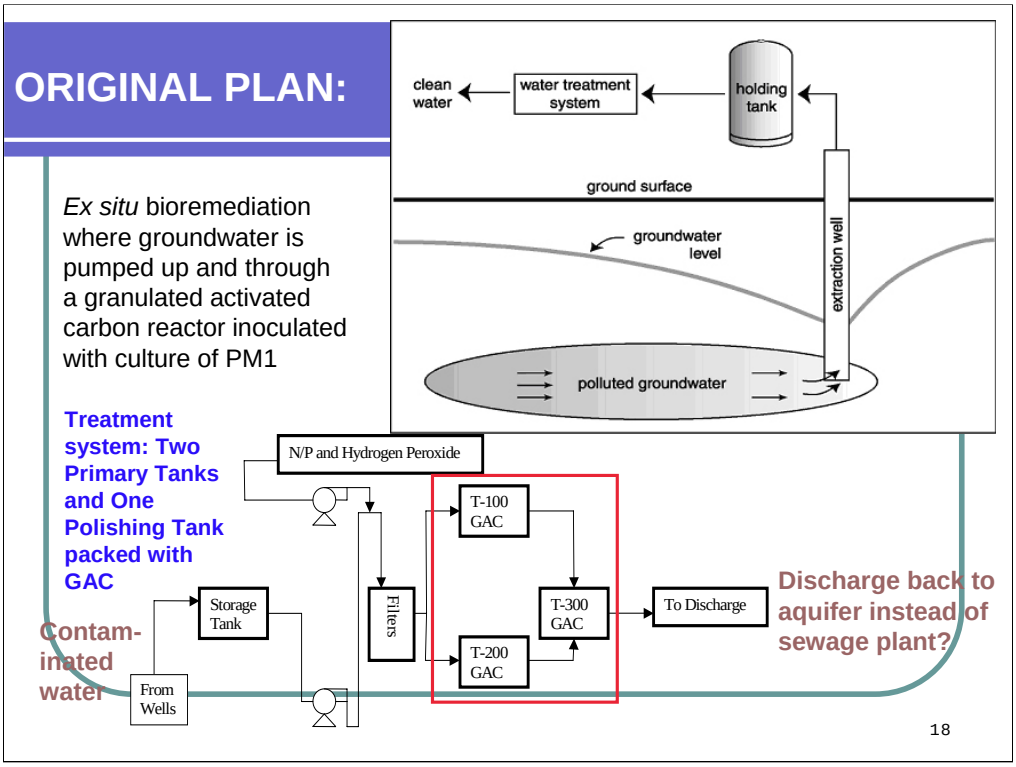


Here you see a simplified schematic of the overall bioreactor design. MTBE-contaminated water is pumped from the wells into a storage tank where it is then aerated using hydrogen peroxide and where nutrients are added to the influent. The influent is split and enters the two primary treatment tanks. Each is packed with 800 kg of virgin GAC and holds about 1600 liters of water? Because of the low affinity of MTBE to GAC the GAC acts as more of a carrier material for the bacteria than an MTBE sorptive

The rationale for exploring self-seeding of the bioreactor was based on preliminary studies done by our lab using sediment and groundwater from the North Hollywood aquifer. No oxygen except for headspace was added. No other amendments like nitrogen and phosphorus were added, in other words very not optimal conditions. No seeding of bacteria was done in this particular experiment although we did a separate one that was seeded with PM1. While the seeded microcosm began degrading faster, both showed MTBE degradation . In the unseeded microcosms, MTBE degradation was down to 1 ppb at the most recent sampling.

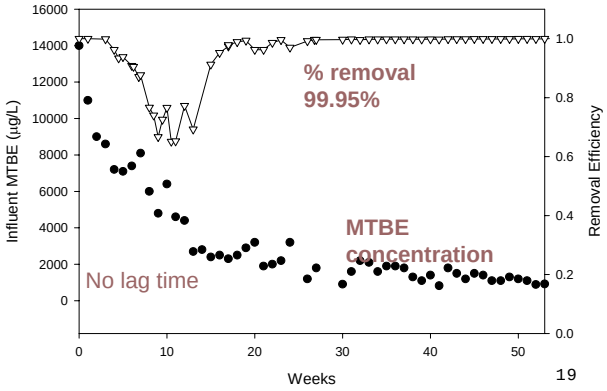
Who will do the job?

DIDN'T NEED TO INOCULATE WITH ORGANISMS FROM SOMEWHERE ELSE!!

Groundwater pumped into bioreactor w/oxygen + nutrients to condition reactor/GAC

MTBE biodegradation established within 4 wks

NATIVE bacteria rapidly established in bioreactor



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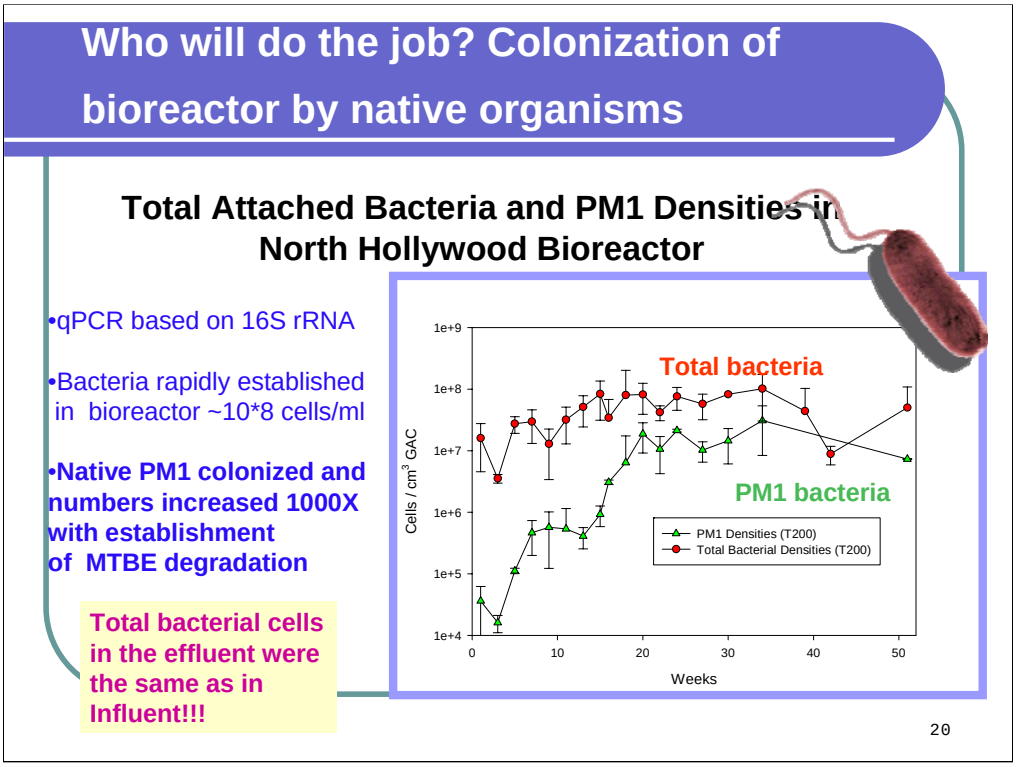


Total Attached Bacteria and Attached PM1 over the course of 52 Weeks in the North Hollywood Bioreactor. MTBE removal was >99%

Cell densities are estimated based on 16S rRNA gene quantification.

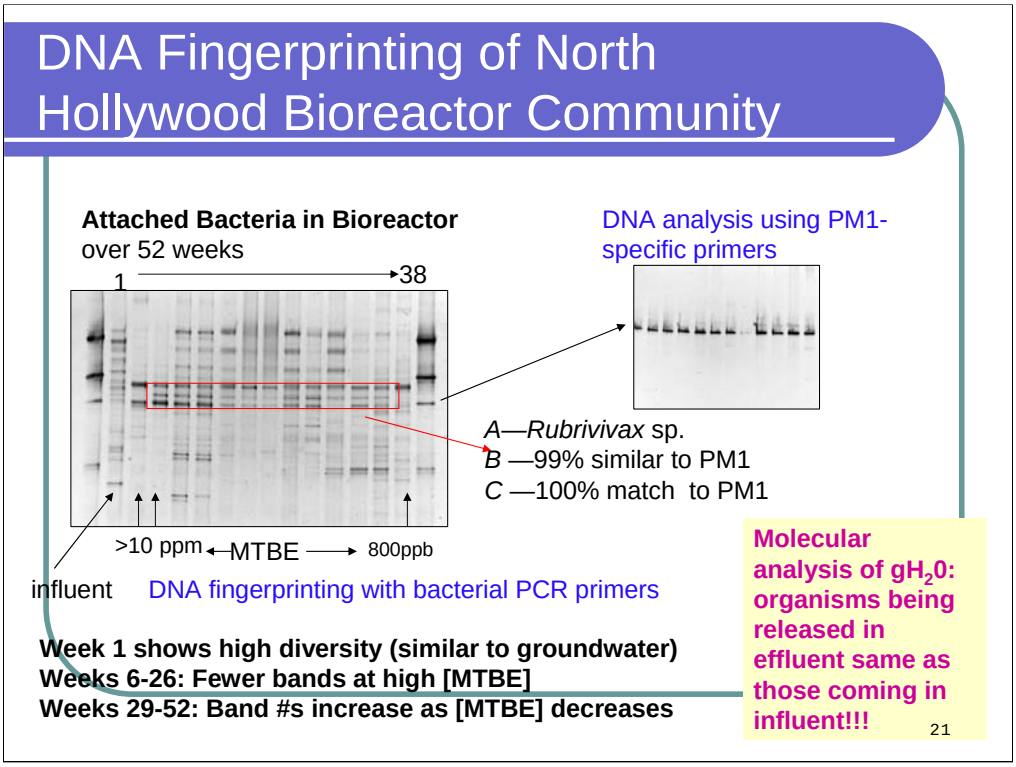
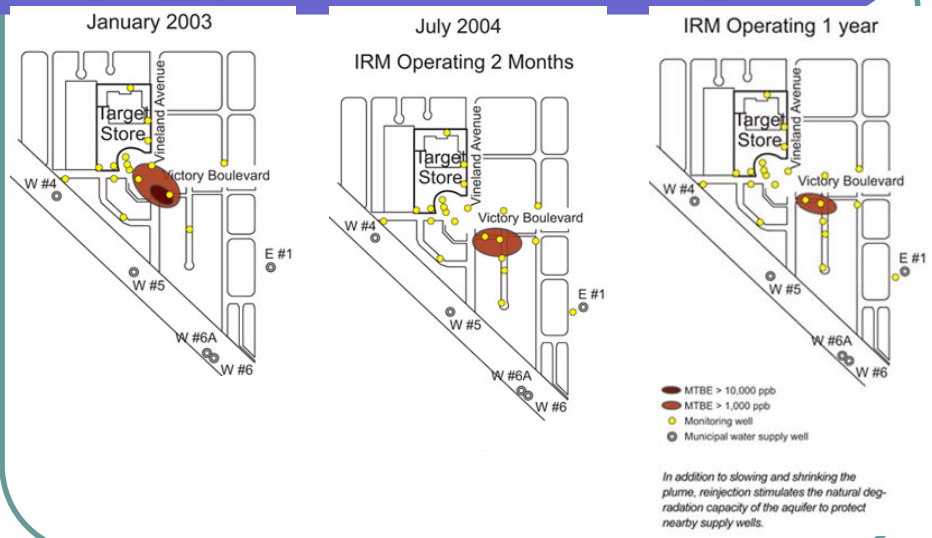


Figure 1 shows attached bacteria in the bioreactor over the course of 52 weeks. The first lane (other than ladder) shows the influent bacterial community. The 2nd and 3rd lanes are from the first month startup period when MTBE concentrations were high (>10PPm). After the first month, the concentrations dropped rapidly in the reactor to ~800 ppb at the last lane.

Because some of the bands were very close together and this interfered with accurate sequencing, DGGE fingerprinting using PM1 specific primers was used. PM1 was detected, cut, and sequenced. Sequencing were 100% identical.

Figure 3 shows prelim results from a column study at the Noho site. MTBE was fed at 10-20 ppm for 8 weeks. Band A is rubrivivax, band B is 99% similar to pM1 and Band C is 100% identical. The banding pattern is similar to that of the Bioreactor at the high concentration period.

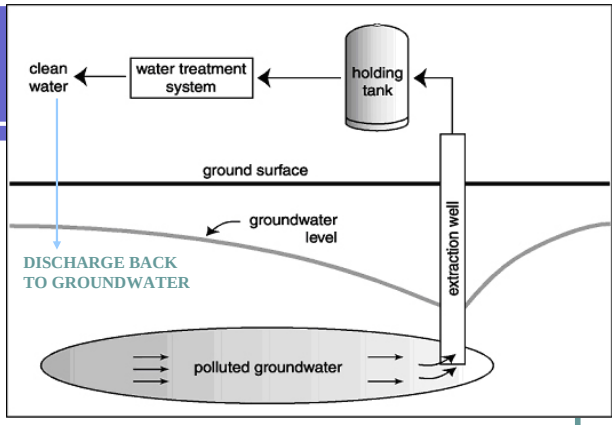
Plume size and concentrations decreased



Can bioreactor successfully treat MTBE /TBA and can clean groundwater be returned to aquifer?

Yes!!!

- Consistent removal of MTBE without formation of toxic products
- SITE BEING SHUT DOWN
- Bioreactor colonized by native microbes (not inoculants)



* Precedent-setting approval by state regulators to treat and re-inject treated water to the aquifer which will save over 10 million gallons of water annually

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Summary I

- Considerable potential for native microbial communities to degrade MTBE and TBA simply with addition of oxygen
- Colonization of bioreactors by native microbial populations supports *ex situ* bioremediation
- Reinjection of clean bioreactor effluent into the aquifer could save $$$$
- Microbial ecology tools help to understand microbial community dynamics, activity, and in the design of remediation strategies

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Example 2: *Ex situ* bioremediation in Glennville, CA

- Supplied by private well water
- MTBE, TBA and BTEX contamination of a drinking water aquifer
- Without a local water supply since 1998
- UC Davis demonstration project started Dec. 2008

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Community Meeting

A *PARTNERSHIP* with members of the community of Glennville; the community water company; the state of California's Department of Health Services; the Central Valley Regional Water Quality Control Board and Environmental Resolution Inc. (ERI)

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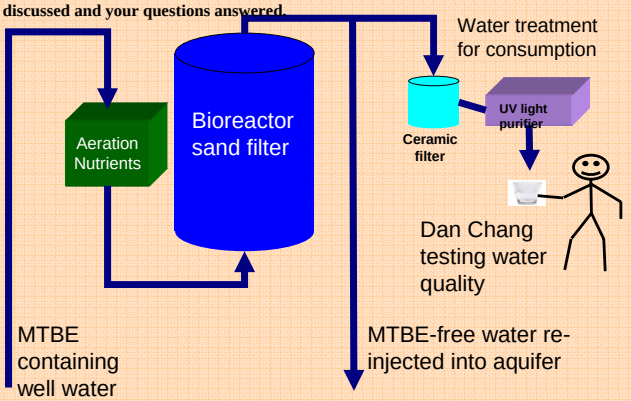
Dear Glennville Residents,

You are invited to a community meeting at 6:00 pm on Thursday, December 4th to be held at the Elementary School Researchers from UC Davis and Environmental Resolutions, Inc. will unveil a bioreactor designed to treat groundwater contaminated by the 1995 MTBE spill. The process has been used to remediate contaminated sites, and this will be the first effort to demonstrate that it can be used to safely produce drinking water. Testing of water from well W7 has shown the presence of the MTBE degrading bacterium *Methylibium petroleiphilum* PM1. W7 groundwater will therefore be used to seed the bioreactor, which will provide the conditions necessary for fast MTBE breakdown. At the meeting the operation of the bioreactor will be discussed and your questions answered.



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Ex situ bioremediation using FBBR

•PM1 mixed culture has been "translated" from UC Davis Superfund researchers to Environmental Resolution Inc. (2000)

•ERI fluidized bed bioreactors have been put into operation at over 30 sites in California, New Hampshire, and Maryland

•The bioreactor includes an aboveground tank containing trillions of microorganisms (*PM1)*, that attach themselves to the surfaces of fine grains of sand.

•The grains are distributed throughout the tank by the upward flow of the water passing through the tank for treatment.

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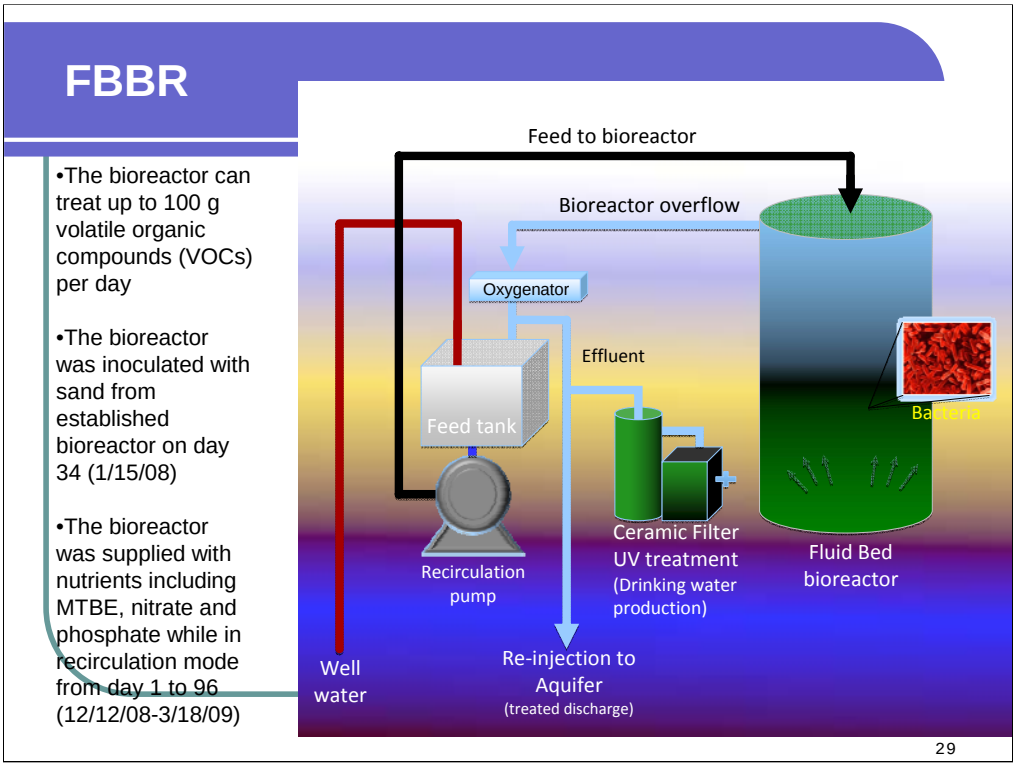
In figure 1, water from the contaminated site wells (1) enters the feed tank, where it is diluted with clean water that has already been processed through the bioreactor. The mixed water stream (2) is pumped to the bottom of the bioreactor, where it flows upward through the sand which is colonized by microorganisms. The microorganisms consume the MTBE, TBA, and other gasoline contaminants, converting them to carbon dioxide and water.
Some of the treated water with depleted oxygen content (3) flows from the bioreactor through an oxygenator where it is reoxygenated and then is sent to the feed tank to dilute incoming contaminated water. The remaining treated water (4) is discharged from the system.



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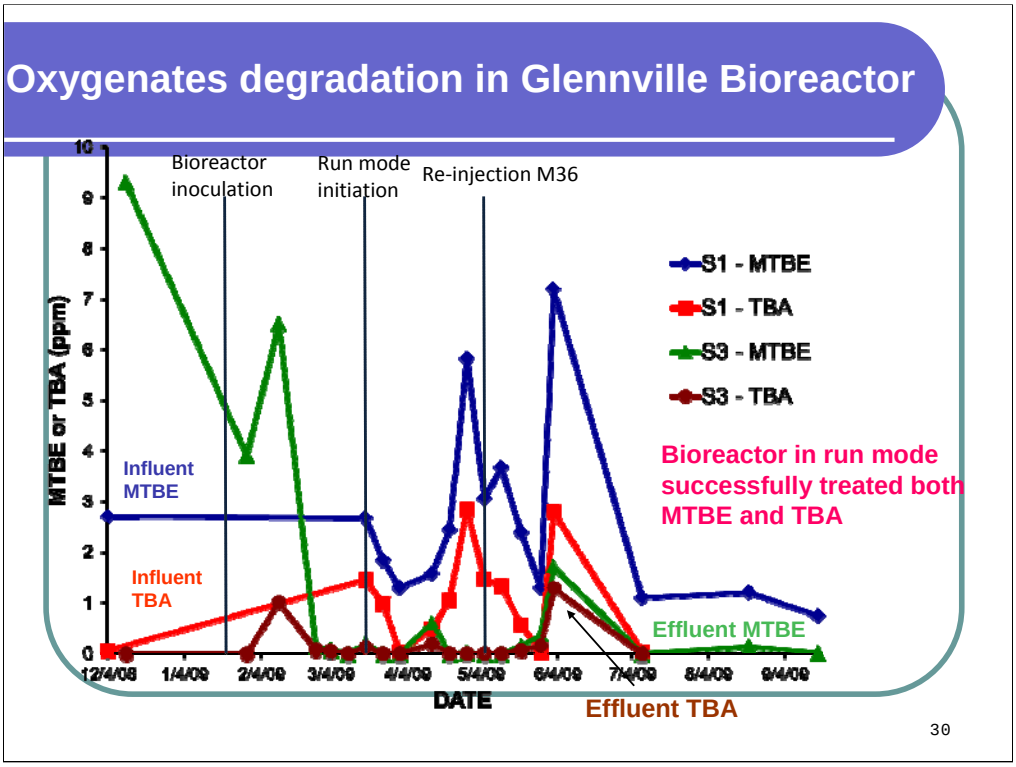


Figure 2. Oxygenates degradation analysis of ERI-500 Glennville bioreactor. Influent (diamond) and bioreactor effluent (square) MTBE (filled symbols) and TBA (empty symbols) concentrations. The bioreactor was supplied with nutrients including MTBE, nitrate and phosphate while in recirculation mode from day 1 to 96 (12/12/08-3/18/09). VOC analysis for the bioreactor in run mode indicates it successfully treated both MTBE and TBA.

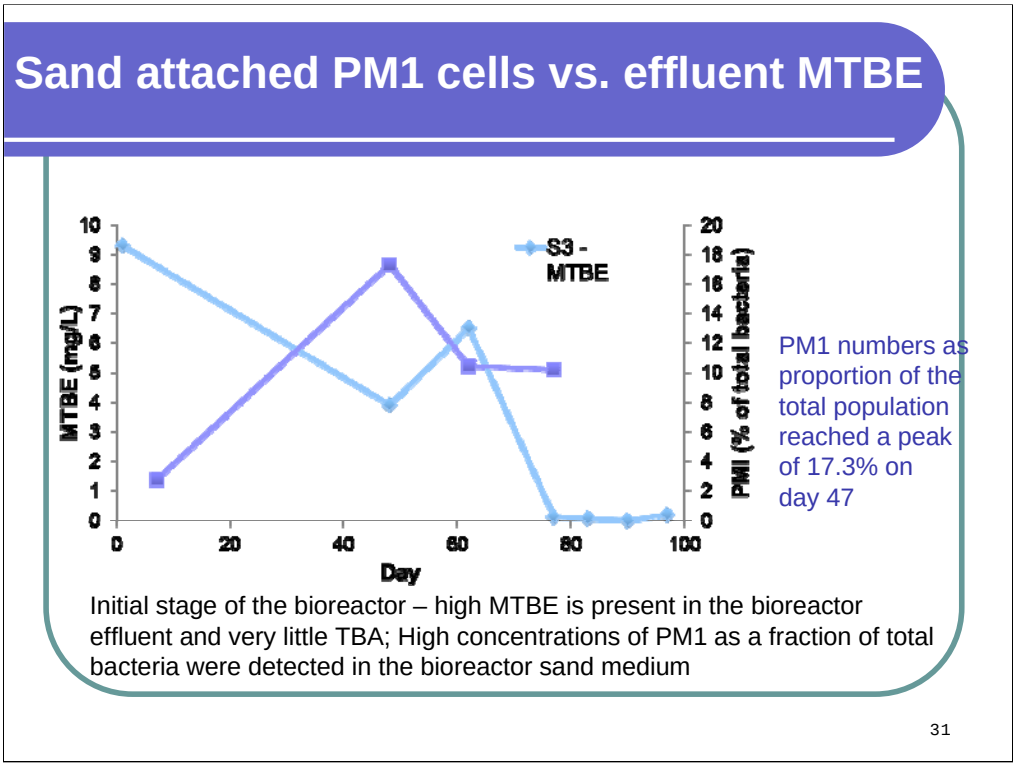


Figure 3. Correlation of MTBE concentrations in ERI-500 Glennville bioreactor with PM1 prevalence. High concentrations of PM1 as a fraction of total bacteria were detected in the bioreactor sand medium during the initial stage of bioreactor function when MTBE was supplied as carbon source and concentration of MTBE in the bioreactor effluent remained high. Little TBA was detected in the bioreactor at this time. Total bacterial numbers in the bioreactor biofilm reached a relatively steady state after day 50.

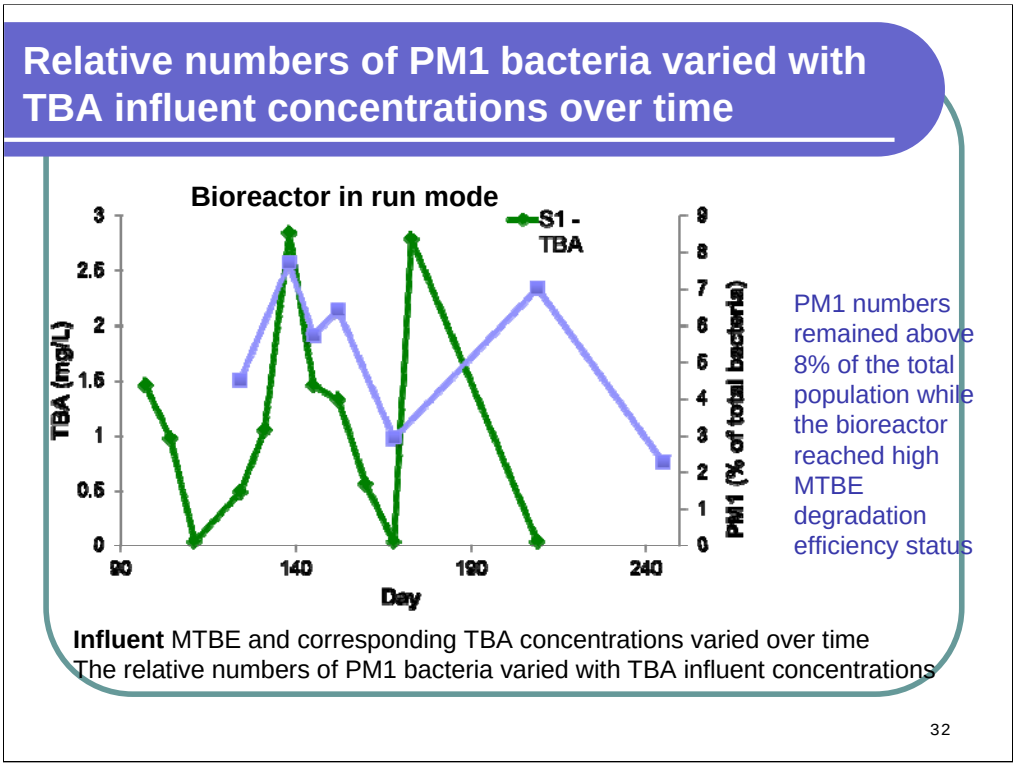
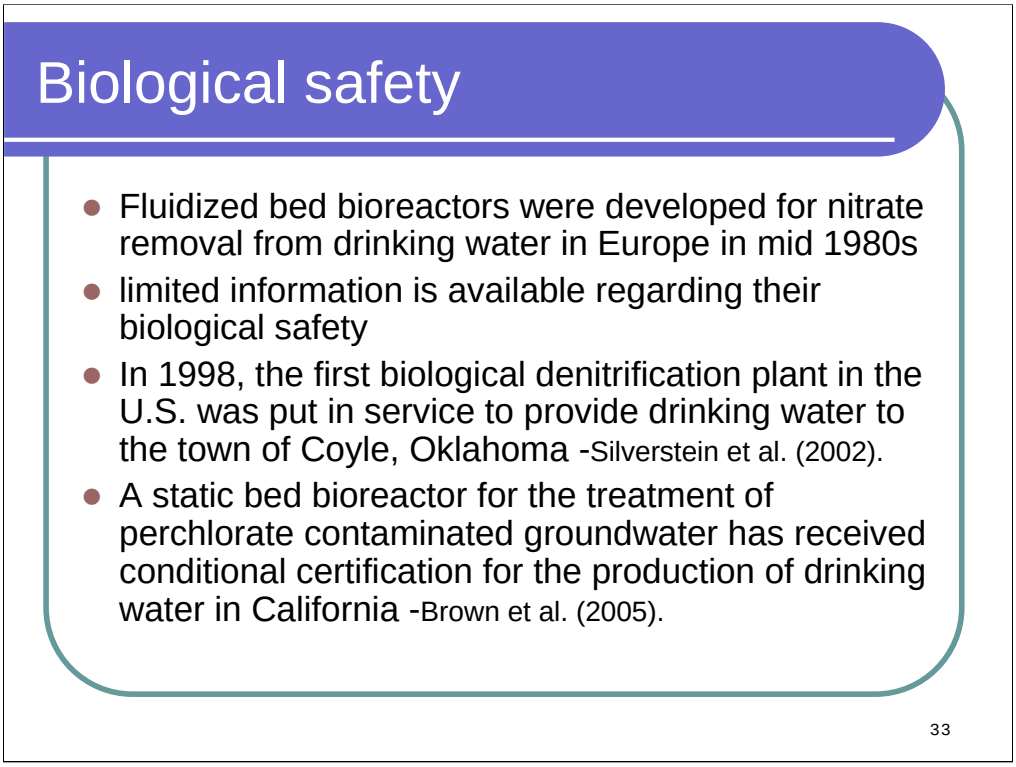


Figure 4. Correlation of TBA concentrations in ERI-500 Glennville bioreactor with PM1 prevalence. During run mode, influent MTBE and corresponding TBA concentrations varied over time. The relative numbers of PM1 bacteria varied with TBA influent concentrations.



Treated water passed through a prefilter assembly and a slow sand filter removed organic material Silverstein (1997). Due to the lack of availability of comprehensive pathogen monitoring methods, regulators have required the installation of downstream membrane technology for microbe removal from such systems Sanders et al. (2004).

A static bed bioreactor for the treatment of perchlorate contaminated groundwater has received conditional certification for the production of drinking water in California -Brown et al. (2005).

Biological safety

- Determine the biological safety of final waters produced by a sand-based FBBR
  - Enteropathogenic *E. coli, Salmonella* and *Shigella spp., Campylobacter jejuni, Aeromonas hydrophila, Legionella pneumophila, Vibrio cholerae, Yersinia enterocolytica* and the *Mycobacterium avium* complex (MAC) have been identified as **pathogens of concern** for the groundwater environment
  - **Coliforms and Heterotrophic plate counts**
  - Viruses and enteric protozoa such as *Giardia* and *Cryptosporidium* cannot multiply in water in the absence of animal hosts

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Pathogens detection in Glennville Bioreactor

Microbiology test[1]	EPA limit[2] (cfu/ml)	Glennville Bioreactor (#3)			
		Initial (day 61)		Established (day 167)	
		inf.	eff.	inf.	eff.
Legionella pneumophila	0 (MCLG)	BDL	BDL	BDL	BDL
Aeromonas hydrophila	no limit	153	2	832	2
Pseudomonas aeruginosa	no limit	BDL	1	28	BDL
Total coliforms	0 (MCLG)	178	24.9	2282	BDL
Escherichia coli	0 (MCLG)	BDL	BDL	BDL	BDL
Heterotrophic plate count	500 (TT)	3010	6030	118	355

- Potentially pathogenic microorganisms were either not detected or their numbers decreased across the bioreactor
- The drinking water production system consisted of a bacterial grade filter followed by UV sterilization

BDL – below detection limit
NT – not tested
MCLG – maximum contaminant level goal
TT – treatment technology

Not detected:
E. coli, *Salmonella*, *Shigella*, *Camplyobacter jejuni*, *Yersinia enterocolitica*, *Vibro cholerae*, or *Mycobacterium avium* complex (MAC)

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Pathogens detection in ERI Bioreactors

Microbiology test[1]	EPA limit[2] (cfu/ml)	ERI bioreactor – Lake Forest				Eri bioreactor - Healdsburg			
		(Oct 2008)		(Feb 2010)		(Oct 2008)		(Feb 2010)	
		inf.	eff.	inf.	eff.	inf.	eff.	inf.	eff.
Legionella pneumophila	0 (MCLG)	4	96	5	BDL	BDL	2	BDL	BDL
Aeromonas hydrophila	no limit	470	24	$1.5x\ 10^5$	BDL	2520	55	BDL	BDL
Pseudomonas aeruginosa	no limit	BDL	338	NT	NT	BDL	BDL	NT	NT
Total coliforms	0 (MCLG)	1120	4.1	488	57.6	1	2	1	3
Escherichia coli	0 (MCLG)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Heterotrophic plate count	500 (TT)	1470	209	271	38	2000	455	470	375

Total bacteria enumerated by HPC also decreased across the bioreactors

Ongoing pathogen monitoring would be prudent for any aerated, degradative bacteria-rich waters injected back into the aquifer

Summary II

- Bioreactors efficiently treats MTBE, TBA, BTEX (unique degradation abilities of PM1)
- Inoculation with MTBE-degrading organisms has to be evaluated on case by case basis
- Highest proportions of PM1 as percentage of total bacteria occuring in samplings following a peak of MTBE/TBA in the influent
- Reductions in oxygenate concentrations lead to reductions in PM1 ratios
- Potentially pathogenic microorganisms were either not detected or their numbers decreased across the bioreactor

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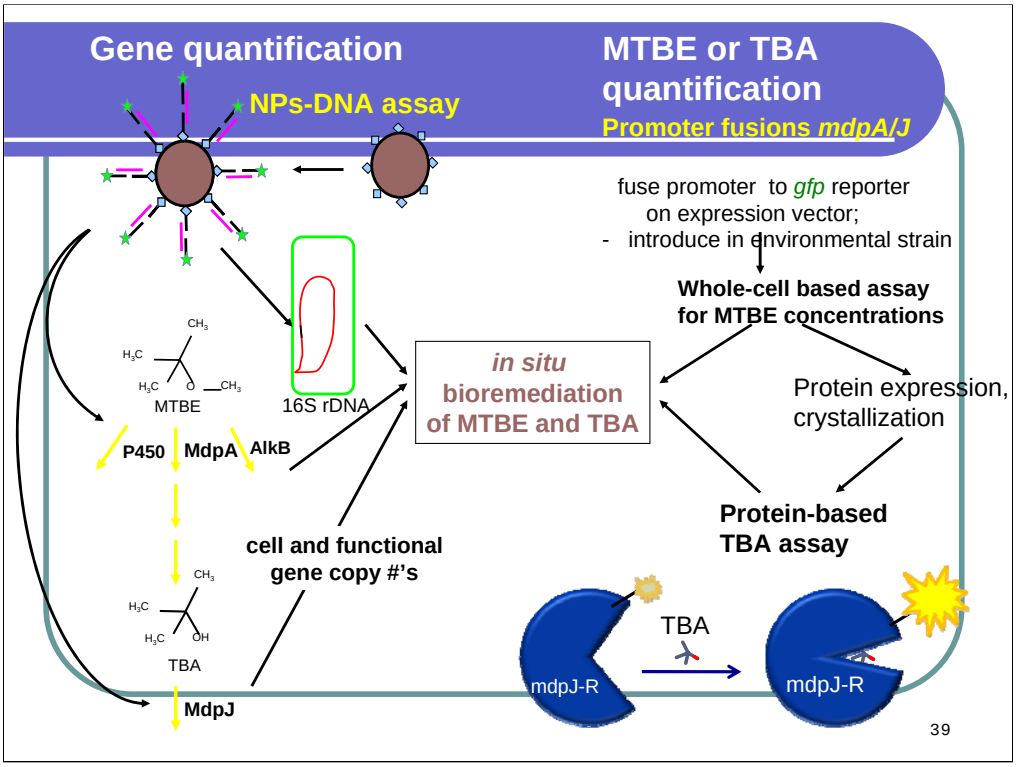
Summary II - continue

- Based on results of bacterial loads, contaminant analysis and physical characterization of treated effluent waters, sand-based FBBR's can produce water of similar quality to uncontaminated source groundwater
- Comprehensive testing for pathogens by high throughput methods is not available yet. In the meantime, conventional drinking water treatment and testing is recommended following MTBE and TBA removal


Gene quantification
NPs-DNA assay
MTBE or TBA quantification
Promoter fusions mdpA/J
fuse promoter to gfp reporter on expression vector;
- introduce in environmental strain
Whole-cell based assay for MTBE concentrations
MTBE
16S rDNA
in situ bioremediation of MTBE and TBA
Protein expression, crystallization
P450
MdpA
AlkB
Protein-based TBA assay
cell and functional gene copy #'s
TBA
MdpJ
TBA
mdpJ-R
mdpJ-R
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- ***Haley & Aldrich***

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Resources & Feedback

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