Final report

Field-test of new technology to establish microbial activity in a MTBE/TBA-contaminated drinking water supply

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Abstract

Our Glennville, CA MTBE bioremediation project is designed to build upon existing fluidized-bed bioreactor technology to provide water that is enriched in oxygen and MTBE degraders for re-injection and *in-situ* treatment of the contaminated aquifer. Concurrently, we propose to treat a portion of the bioreactor output to drinking water standards. The community of Glennville was entirely supplied by private well water prior to aquifer contamination and has been without a local water supply since 1998. The project forged 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). If successful, this will be one of the first applications of biological treatment to produce drinking water in the U.S. and will promote changes in policies in local health departments and the water industry. Recognizing the importance of open dialogue with the local community, we have held two information exchange meetings with Glennville residents in mid and late 2008. Further meetings will be held when important milestones of the project are reached. The primary objective in the current phase of the project is to demonstrate the suitability of treated water for re-injection. We are in the process of testing the effluent for concentrations of MTBE and other gasoline components; nutrients added to the bioreactor during the start-up recirculation phase; and potentially pathogenic bacteria. Contaminant, nutrient and pathogen samples collected at the site are analyzed by independent, certified laboratories.
Results of preliminary tests, such as reduced heterotrophic cell counts in effluent of existing ERI bioreactors as well as continuing positive dialogue with the local community and the regulatory authorities, are encouraging for project viability.

Background

Our Multi-project Program entitled, "Biomarkers of Exposure to Hazardous Substances," consists of 8 integrated projects, 3 research support cores, a training core, a research translation core and an administrative core. Dr. Bruce Hammock serves as Project Director for the project team that is determining the "fate and transport of hazardous materials in groundwater, surface water, and air as they move from toxic waste sites using classical and innovative methodologies." The team explores new technologies for thermal and bioremediation of toxic waste and addresses possible health risks associated with these technologies. Project #1, "Transport, Transformation and Bioremediation of Contaminants in the Environment: Exposure Assessment in Heterogeneous Environmental Media," has invented a rapid DNA-based bioassay that can be used to supplement classical technologies for the evaluation of sites contaminated with methyl tertiary butyl ether (MTBE) and tertiary butyl alcohol (TBA). The natural

occurrence of the bacterial strain, *Methylibium petroleiphilum* PM1 (PM1) can be determined as well as validating bioremediation of MTBE and TBA through quantitative spatial and temporal measurements. The purpose of the administrative supplement award that we received was to allow technologies developed through Project #1 to be tested at field sites and transferred to a new partnership of end users, including community members, regulatory agencies and the drinking water industry through efforts of our Research Translation Core.

Figure 1. Glennville, CA,(latitude 35.729, longitude -118.704) is located in Kern County, approximately 36 miles North-East of Bakersfield in the Sierra foothills, at an elevation of 3,176 feet. The MTBE plume extending from a former gas station has contaminated many of the local residents' wells.

Glennville MTBE plume site and Bioremediation

A MTBE plume has contaminated the complex aquifer in Glennville, CA. The point of origin is the Glennville shopping center underground storage tank (UST) at 10675 Highway 155, Glennville, CA (Figure 1). Glenville is located in northern Kern County in the foothills of the Sierra Nevada mountains, in a transition zone to higher elevation bedrock. Gasoline had been released from the former fueling system at the site which consisted of one 6000 gallon UST, fuel dispensers and related piping. The fueling system was removed from the site in August 2002. A groundwater monitoring program consisting of quarterly sampling of up to 44 monitoring wells has been in effect at Glennville since July 1997. Benzene, toluene, ethylbenzene and xylenes (BTEX); total petroleum hydrocarbons reported as gasoline (TPHg); and methyl *tert* butyl ether (MTBE) have typically been detected in certain study area wells. TBA was also suspected to be present at this site and its presence was confirmed by our study.

Our Glennville, CA MTBE bioremediation project is designed to build upon existing fluidized-bed bioreactor technology to provide treated water enriched in oxygen and MTBE degraders for re-injection and *in-situ* treatment of the contaminated aquifer. The project utilizes the naturally-occurring, MTBE/TBA degrading PM1 strain, which was isolated and characterized by Project #1, and a DNA-based bioassay recently developed by Project #1's researchers to track PM1's spatial and temporal occurrence. Concurrently, we aim to treat a portion of the bioreactor output to drinking water standards. The community of Glennville was entirely supplied by private well water prior to aquifer contamination and has been without a local water supply since 1998. The project forged 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 (Water Board); and Environmental Resolution Inc. (ERI). This project builds upon a past small business collaboration with Environmental Resolutions Inc. (ERI), which incorporated UC Davis Superfund research into their fluidized-bed bioreactor for treatment of MTBE and TBA. If successful, this will be one of the first applications of biological treatment to produce drinking water in the U.S. and will promote changes in policies in local health departments and the water industry. Recognizing the importance of open dialogue with the local community, we have held two information exchange meetings with Glennville residents in mid and late 2008.

The primary objective in the current phase of the project is to demonstrate the suitability of treated water for re-injection. We are in the process of testing the effluent for concentrations of MTBE and other gasoline components; nutrients added to the bioreactor during the start-up recirculation phase; and potentially pathogenic bacteria. 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 chief concern for the groundwater environment (10, 14, 28). Groundwater, and by extension the bioreactor environment, are not generally supportive of pathogen growth. For example, enteric bacteria slowly die off in groundwater with a 90% reduction in numbers in 50-100 days (10). Viruses and enteric protozoa such as *Giardia* and *Cryptosporidium* cannot multiply in water in the absence of animal hosts (14). As our objective

was to monitor for potential pathogens that could replicate in the bioreactor, we did not monitor for viruses or protozoa. Contaminant, nutrient and pathogen samples collected at the site were analyzed by independent, certified laboratories.
Results of preliminary tests, such as reduced heterotrophic cell counts in effluent of existing ERI bioreactors as well as continuing positive dialogue with the local community and the regulatory authorities, are encouraging for project viability. Involvement of the California Department of Health Services and Water Quality Control Board and industry review of the outcome serves as a first step and impetus to bring about policy changes with regard to biological treatment of drinking water.

Aerobic MTBE degradation

MTBE and its primary metabolite *tert*-butyl alcohol (TBA) are suspected and known carcinogens, respectively. MTBE biodegradation involves a novel ether cleavage reaction described primarily for cometabolic MTBE-degrading organisms. Previous reports, based on physiology and biochemical studies, suggest that monooxygenase enzymes are involved in the critical steps of biodegradation of MTBE and TBA (5, 24, 26). *Methylibium petroleiphilum* strain PM1 (PM1) is a methylotroph representing a new species within the *Rubrivivax* group (*Comamonadaceae* family) of the beta subclass of Proteobacteria (16). Strain PM1 is one of few pure culture isolates that can completely degrade MTBE (1, 4, 16). Pilot and field studies have demonstrated the efficacy of aerobic bioremediation of MTBE by PM1 (2, 3, 23, 25, 29). Furthermore, PM1-like bacteria (98-99% similar based on 16S rDNA sequences) have been detected in many MTBE-contaminated aquifers in California (8, 11, 12). *In situ* studies correlating total and PM1-like bacterial cell counts with MTBE degradation rates suggest that PM1-like organisms play a significant role in MTBE biodegradation under aerobic conditions in California aquifers (8). We monitored the fate of PM1 in the bioreactor by non-culture based qPCR method. Our lab has extensive experience in developing quantitative real-time PCR assays for detection of the MTBE-degrading strain PM1 in environmental samples (7, 9, 18). Real-time qPCR was used to quantify population densities of total bacteria, and PM1 (27). Recently we identified the MTBE monooxygenase and TBA hydroxylase genes required for MTBE and TBA degradation by strain PM1, respectively, and have developed qPCR primers for these genes.

Existing bioreactor technology

While bioreactor treatment is well accepted as a drinking water purification technology in Europe, the U.S. has lagged behind in implementation of this method. Fast sand filtration was developed to keep pace with the rapidly expanding cities towards the end of the 19th century. Building on existing sand filtration and wastewater treatment technology, fluidized bed bioreactors were developed for nitrate removal from drinking water in Europe in mid 1980s (6, 13). In fluidized bed bioreactors the water flow is from the bottom to the top (Figure 2). This lifts or “fluidizes” the sand particles in the bioreactor, which allows better mixing of water with the bacteria coated sand particles and leads to faster degradation rates. Projects to further explore fluidized bed bioreactor technology for denitrification and trichloroethene and perchlorate biodegradation have taken place (15, 17, 19, 20).

In December 1998, the first biological denitrification plant in the U.S. was put in service to provide drinking water to the town of Coyle, Oklahoma, servicing 290 residents and 400 school children (22). Treated water is passed through a prefilter assembly and a slow sand filter is used to remove organic material (21). A study of capital and operating costs indicated that the total unit cost of water treated in the Coyle denitrification facility was $0.21/cubic meter ($0.79/1,000 gallons) in 1999 dollars (22).

When oxygenate concentrations are above approximately 1,000 ug/l, bioreactor treatment is typically competitive with other available alternatives (i.e., carbon, air stripping with vapor-phase treatment, bioGAC, and chemical oxidation), except for air stripping without vapor-phase treatment.

The total cost of MTBE and TBA cleanup are difficult to estimate, but a recent (May 7, 2008) settlement between state and local water agencies and most major US oil companies illustrates the costs involved. Under the agreement, which started as 59 separate cases that had been filed in 17 different states but were ultimately wrapped into one, the oil companies will pay $422 million up front, and cover 70 percent of the cleanup costs for any of the plaintiffs' wells that become contaminated with MTBE within the next 30 years.

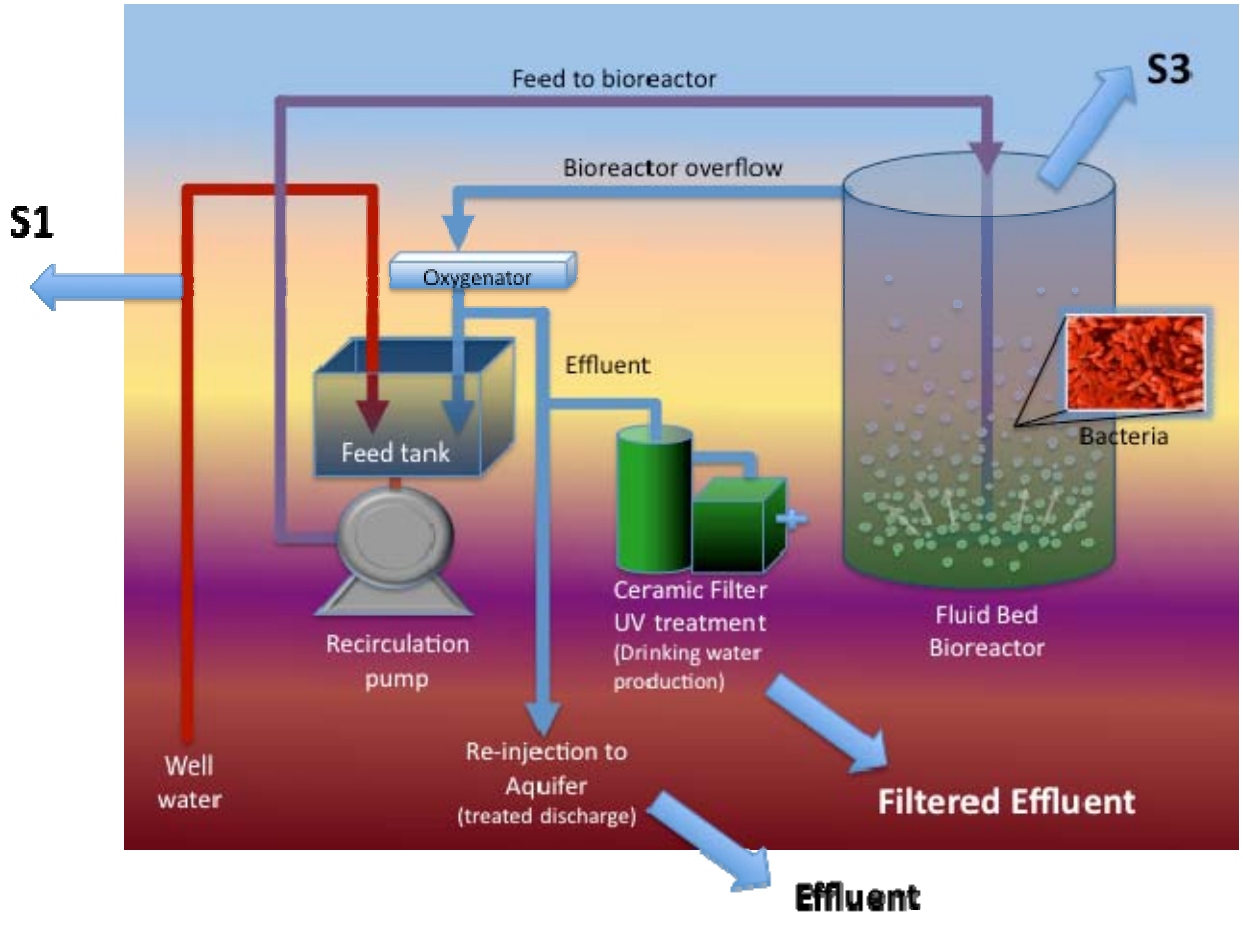

Figure 2. Schematic diagram of ERI-500 fluidized-bed bioreactor. The fluidized-bed medium in this reactor is sand. Sampling points are indicated with arrows.

Materials & Methods

Bioreactor

Environmental Resolutions Inc. installed an ERI-500 Fluidized Bed Bioreactor in a shed behind the old gas station 10675 Highway 155, Glennville, CA (Figures 2 and 3). The entire system includes a bioreactor tank with sand media, feed tank, recirculation pump, oxygenator tower, filter pad, auto dialer, and plastic drum and pump for nutrient solution. The bioreactor can treat 0.2 pounds per day of volatile organic compounds (VOCs). The bioreactor is a two-phase system (solid sand and liquid water – no gas) with a recirculating liquid stream. The biomass is confined to the reaction vessel by its adherence to fine sand. The effectiveness of the fine sand at trapping biomass are demonstrated by higher cell counts in bioreactor influent then in the effluent (see Table 1). The biomass is distributed within the bioreactor by fluidization provided by an upward flow of water maintained by a constant water recirculation rate throughout the system (Figure 2). The water passes upwards through the sand where the biomass degrades VOCs as the water passes by. The reaction on any given pass is limited by the amount of oxygen available in the water. ERI experience to date indicates that the bioreactor operates best between 10 and 35^{o}C (50 and 95^{o}F) at a partial pressure of oxygen above 0.21 atmospheres.

Figure 3. Photograph showing the location of the treatment well (W7), the bioreactor installation and the proposed re-injection well (M33). The photograph is facing northwest. The former gas station building is on the right.

The bioreactor is capable of complete MTBE degradation to below detection limit in one pass through the fluidized bed (the hydraulic residence time being 15 minutes) as long as the dissolved oxygen (DO) is not consumed completely and sufficient nutrients such as nitrogen, phosphorus and potassium (N, P, and K) are available. The required oxygen loading is approximately 3 pounds of oxygen per pound of fuel oxygenates and petroleum hydrocarbons. BTEX and other petroleum hydrocarbons are the preferred carbon substrates for the bacteria. If the total concentration of BTEX and other petroleum hydrocarbons exceeds the total fuel oxygenates concentration, pretreatment using granular activated carbon (GAC) or air stripping may be necessary to remove these more easily treatable compounds to allow the bioreactor to treat the fuel oxygenates.

Benefits of a fluidized bed bioreactor include:
1. The process actually destroys fuel oxygenates and petroleum hydrocarbons, mineralizing them to carbon dioxide and water, rather than merely transferring them to another medium.
2. The process provides one of the very few treatment options for TBA.
3. *Ex-situ* treatment can provide hydraulic control at the site.
4. Bioreactors can be scaled to any mass loading.
5. The process effectively remediates at the source.
6. Bioreactors are good neighbors. They are quiet and do not generate odors.
7. Sand is cheap and readily available.

Water Samples
Samples for waterborne pathogen analysis collected at the site by ERI O&M personnel were sent to Aemtek Inc., Fremont, CA. Aemtek, Inc. is a certified microbiology test laboratory (ELAP certification no. 2607), all samples were processed using EPA standard methods. Alternate VOC samples were analyzed by Kiff Analytical LLC, Davis, CA or processed at UC Davis, LAWR Department. Nutrient samples (nitrate, phosphate, potassium) were analyzed by Kiff Analytical LLC. Kiff Analytical LLC is an environmental testing certified laboratory (ELAP certification no. 2236); all samples were processed using EPA standard methods. Samples were also used at UC Davis for DNA extraction and analysis and plate cell counts.

Molecular methods

Assessment of the suitability of a mixed MTBE degrading culture from an existing ERI bioreactor was performed for its suitability as inoculum for the pilot bioreactor at Glennville. 16S rDNA quantitative PCR amplification and sequencing was used to provide a detailed characterization of the community in the existing ERI bioreactor. DNA was extracted from samples as described previously (9). 16S rDNA was amplified from extracted DNA using universal bacterial 16S rDNA primers and PM1 specific 16S rDNA primers. PM1 is hypothesized to form an essential part of this community based on results from other bioreactors utilizing PM1.

Microbiology
Because of their usefulness as general indicators of fecal contamination of groundwater, coliforms,*E. coli* and several other enteric pathogens were monitored on a regular basis. By sampling upstream and downstream of the bioreactor, we assessed the survival rates of potential enteric organisms within the bioreactor environment. The opportunistic pathogens *Legionella*

pneumophila, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Mycobacterium avium* were used as indicator organisms to assess potential pathogen growth within the bioreactor. The presence of these organisms was determined by growth on appropriate selective media by Aemtek, Inc. Heterotrophic plate counts were used to monitor microbial numbers in the influent and effluent from the bioreactor.

Because they cannot multiply outside of a human/animal host and therefore cannot multiply within the bioreactor environment, viruses and enteric protozoa were not monitored within the water treatment system. A decision on appropriate monitoring schemes for these organisms will be made as part of the overall drinking water quality monitoring downstream of the final microbial treatment.

Results

Community involvement in project

To date, we have held three stakeholder meetings in Glennville, which included members of the community as well as regulatory authority representative Greg Issinghoff, ERI representative Dave Klemme, UC Davis researchers Krassimira Hristova and **Error! Reference source not found.**, and superfund Core E representatives Dan Chang and Jim Sanborn. The meetings were held at the Glennville elementary school, which is across the road from the original LUST site. At the first two meetings, ERI and UC Davis gave short presentations explaining the technology to be used to treat the contaminated aquifer and updated the local residents on current state of the project, before fielding questions from all present (Figure 4, see also appendix A1). The third community meeting was used to discuss results at the end of the six month bioreactor trial run. Community members were very active in the meetings, which were also used to coordinate tasks, and were involved in electricity and reactor installation. The Glennville community is situated in Sierra foothills, more than an hour's drive along a narrow, windy road from the nearest major highway, State Route 99. This remoteness has apparently been one of the impediments that has contributed to prior remediation efforts not proceeding past the planning stage. The benefits of the ERI bioreactor technology include robustness and relative simplicity of design. We were able to recruit members of the local community to help with such essential tasks as re-establishing power to the bioreactor in case of a power failure. This hands-on involvement in day-to-day running of the bioreactor was not only essential for the project to get the final go ahead, but it also established a sense of ownership within the community. We also sought residents' opinions on potential drinking water treatment with unanimous support for filtration and UV sterilization in preference to a chlorination step. A third meeting to discuss results at the end of six month trial period was held in Glennville on

Figure 4. Bioremediation101 - Krassimira Hristova giving local residents a glimpse into the inner workings of a fluidized bed bioreactor, Glennville town hall meeting, December 4th, 2008.

Bioreactor establishment

ERI technology typically includes a step of inoculation with biofilm coated sand from established bioreactors. Molecular techniques have demonstrated that the strain PM1 is a significant member of the inoculum community. Two ERI bioreactors located at Laguna Hills, CA (Bioreactor #1) and Healdsburg, CA (Bioreactor #2) are actively degrading MTBE plumes and were analyzed for presence of potential waterborne pathogens to determine if they were suitable as inoculation material for the new Glenville bioreactor (Table 1). Both reactors showed similar trends in total cell counts as enumerated by heterotrophic plate counts, with significantly higher cell counts in the influent than in the effluent of the bioreactor (Table 1). Unfortunately, due to very limited funding, these analyses were based on single sampling events. Total Coliforms were detected in both bioreactors but were either significantly reduced in effluent or

remained the same. Similarly, *Aeromonas hydrophila* was detected in both bioreactors;its numbers were significantly reduced in effluent. Against this trend, *Legionella pneumophila* numbers rose significantly in the effluent of the Laguna Hills bioreactor and very low numbers of this organism were also detected in the Healdsburg bioreactor. For this reason, Healdsburg was chosen as the preferred bioreactor for inoculum. Drinking water production plans at Glennville were altered to include a ceramic bacterial filter followed by a UV sterilization step to ensure that no pathogenic bacteria would be present in the bioreactor effluent.

Table 1. Preliminary waterborne pathogen tests of existing ERI bioreactors in Laguna Hills, CA (#1) and Healdsburg, CA (#2). Water sample dates: Bioreactor #1 – influent (10/29/08), effluent (8/14/08); Bioreactor #2 – influent and effluent (10/30/08). Samples were processed by Aemtek, Inc.

Microbiology test[1]	Reporting units[2]	Detect. Limit	EPA limit[3]	ERI bioreactors			
				# 1		# 2	
				inf.	eff.	inf.	eff.
Total Coliforms	mpn/100 ml	1	0 (MCLG)	1120	4.1	1	2
Legionella pneumophila	cfu/100 ml	1	0 (MCLG)	4	96	ND	2
Aeromonas hydrophila	cfu/100 ml	1	no limit	470	24	2520	55
Pseudomonas aeruginosa	cfu/100 ml	1	no limit	ND	338	ND	ND
heterotrophic plate count	cfu/ml	1	500 (TT)	1470	209	2000	455

[1] No Detects (ND) for *E. coli, Salmonella, Shigella, Camplyobacter jejuni, Yersinia enterocolitica, Vibro cholerae, Mycobacterium avium* complex (MAC).
[2] mpn – most probable number; cfu – colony forming unit
[3] MCLG – maximum contaminant level goal; TT – treatment technology

Glennville bioreactor is treating MTBE and TBA

A strong presence of the MTBE degrading strain PM1 in Glennville treatment well (W7) samples (see Molecular Methods below) led to the proposal to test the startup of the bioreactor with only well water provided to the inoculums. This step was proposed to further reduce the possibility of inadvertent transfer of potentially undesirable bacteria to the newly established bioreactor. The use of only local water as inoculum was also discussed at the second community meeting in Glennville on December 4, 2008. The general consensus showed this to be the preferred method for both local residents and the Water Board. Sand media inoculum was to be used only in the event that an active, MTBE degrading community did not develop sufficiently quickly for the project to continue. The ERI-500 bioreactor was installed at Glennville on December 12, 2008. While plate counts indicated the reactor was populated by bacteria very soon after installation, dissolved oxygen (DO) readings indicated little if any degradation of MTBE took place. Water analysis for MTBE degradation across the bioreactor confirmed MTBE was not being degraded (Table 2). Due to the slow establishment of degrading community, the

bioreactor was inoculated with sand from ERI-Healdsburg on January 15, 2009. The Healdsburg bioreactor is in current use, degrading MTBE, and showed very low *L. pneumophila* counts in previous analysis (Table 1).

Table 2. Oxygenates analysis of ERI-500 Glennville bioreactor in recirculation mode. Sample GC analysis performed at UC Davis or Kiff Analytical LLC as specified. The S2 concentrations vary over time due to feeding of varying concentration of MTBE to bioreactor as carbon source.

DATE	Analysis performed by	MTBE (mg/L)			TBA (mg/L)		
		S2	S3	filtered effluent	S2	S3	filtered effluent
12/12/08	UCD	9.6	9.3	-[1]	ND[2]	ND	-
01/28/09	UCD	6.7	3.9	-	ND	ND	-
02/11/09	Kiff	7.4	6.5	-	1.2	1.0	-
02/26/09	Kiff	0.29	0.11	<0.0005[3]	0.17	0.08	0.04
03/04/09	UCD	0.34	0.07	<0.001[3]	0.20	0.05	0.04
3/11/09	UCD	0.17	<0.001	<0.001	0.01	0.001	<0.001

[1] – not tested
[2] ND – not detected
[3] < - below detection limit

Throughout the Glennville bioreactor operation, pH and temperature stayed close to desired values. The pH remained close to pH 7, fluctuating between pH 6.4 and pH 7.8, while the temperature fluctuated between 65-78^{o}F. Total dissolved solids (TDS) in the reactor inflow rose rapidly from installation date, reaching over 2000 mg/L by the middle of January, and stayed very high while the reactor was in recirculation mode. This high, undesirable value was due to the prolonged stay of the bioreactor in recirculation mode. Due to regulatory concerns and freezing weather that prevented above ground water discharge, the reactor ran in recirculation mode from installation until March 18, 2009. The TDS dropped rapidly to below 1000 mg/L once the reactor was switched to flow-through mode on March 18, 2009. VOC analysis for the bioreactor in run mode indicates it is successfully treating both MTBE and TBA (Figure 5). BTEX compounds were not detected in the bioreactor influent and therefore their effect on bioreactor function was not assessed.

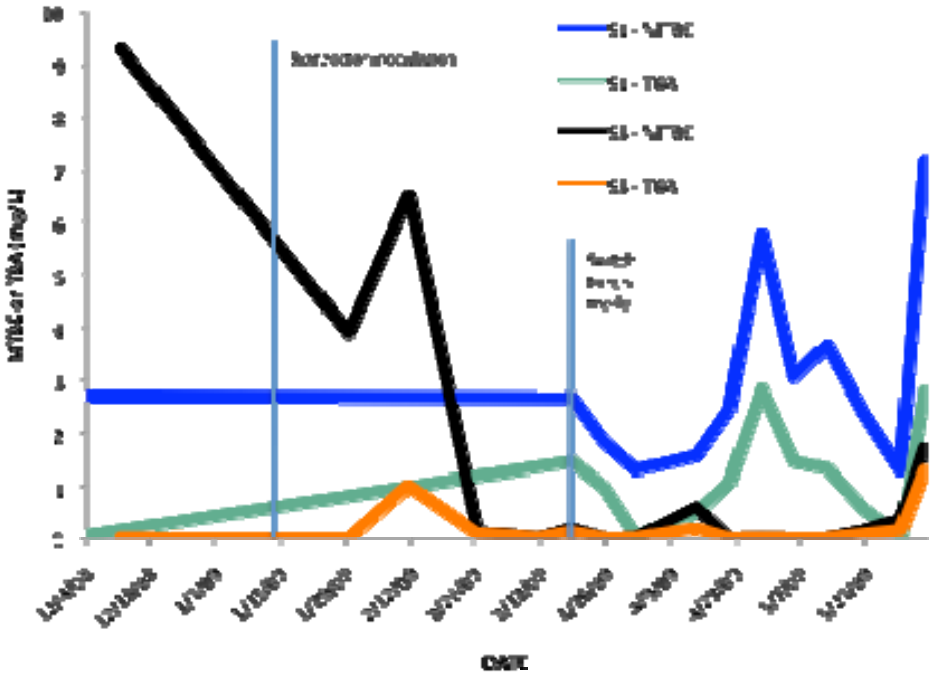

Figure 5. Oxygenates degradation analysis of ERI-500 Glennville bioreactor. Sample GC analysis performed at UC Davis or Kiff Analytical. Both MTBE and TBA were degraded to close to detection limit one week after switch from recirculation to run mode. Effluent MTBE and TBA concentrations remained below detection limit throughout the bioreactor operation.

Microbiology: no pathogens are growing in the bioreactor

Results of waterborne pathogen analysis of well (W7) and ERI-500 bioreactor water indicate that Coliforms were present in the well water but are significantly reduced in the untreated bioreactor effluent and were not detected in the filter/UV sterilizer treated effluent (Table 3). The numbers of the only potential pathogen detected, *A. hydrophila*, were also much lower in the effluent than in the well water. These observations are consistent with previous observations (see Table 1), and suggest that treated water is of the same or possibly better quality than the original ground water from a biological standpoint. No *L. pneumophila* has been detected in the well water or in the bioreactor to date. Heterotrophic plate counts indicate that total cell numbers in the bioreactor were approximately double the numbers in well water while the reactor was in recirculation mode. The switch to run mode significantly reduced the heterotrophic plate counts in the effluent to below drinking water standard levels (Table 3). The bacterial treatment technology consisting of a Doulton Rio 2000 filter and a UV sterilizer were able to keep bacterial numbers in the final product water well below the EPA limit of 500 CFU/ml for treatment.

Table 3. Waterborne pathogens testing in Glennville well-water and ERI-500 bioreactor. **No pathogen from the panel tested was detected.** The heterotrophic plate count (HPC) number in bioreactor effluent is most likely due longer than usual stay in recirculation mode. The removal of coliforms and low HPC number in the filtered effluent samples shows that the combined filtration/UV sterilization water treatment is operating successfully. Samples were processed by Aemtek, Inc.

Microbiology test[1]	Reporting units[2]	Detect. Limit	EPA limit[3]	Glennville					
				10/22/08	2/11/09	2/26/09	5/28/09		
				W7	eff.	Filtered eff.	inf	eff	Filtered Eff.
Total Coliforms	mpn/100 ml	1	0 (MCLG)	178	24.9	ND	2282	ND	ND
Legionella pneumophila	cfu/100 ml	1	0 (MCLG)	ND	ND	ND	ND	ND	ND
Aeromonas hydrophila	cfu/100 ml	1	no limit	153	2	4	832	2	NT[4]
Pseudomonas aeruginosa	cfu/100 ml	1	no limit	ND	1	ND	28	ND	NT
heterotrophic plate count	cfu/ml	1	500 (TT)	3010	6030	34	118	355	89

[1] No Detects (ND) for *E. coli, Salmonella, Shigella, Camplyobacter jejuni, Yersinia enterocolitica, Vibro cholerae, Mycobacterium avium* complex (MAC).
[2] mpn – most probable number; cfu – colony forming unit
[3] MCLG – maximum contaminant level goal; TT – treatment technology
[4] NT – not tested

Molecular Methods

Quantitative PCR was used for assessment of the suitability of a mixed MTBE degrading culture from an existing ERI bioreactor for use as inoculum for the pilot bioreactor at Glennville. The qPCR results of existing bioreactors and Glennville well water (W7) are summarized in Figure 6 and 7. In all cases, PM1 was detected. The highest PM1 numbers and PM1 proportion of total bacteria were in the ERI Breeder bioreactor, which has been running in recirculation mode with constant supply of MTBE for several years. No influent data was collected for this bioreactor because it is in recirculation mode with no fresh influent. The well water in Glennville also showed very high PM1 numbers (1.2×10^8 cells/ml) and a value of 6.8% for PM1 as percentage of total bacteria (Figure 6 and 7). This is likely due to the selection for PM1 in the MTBE contaminated aerobic zone near the surface of W7 and probably does not reflect PM1 concentrations elsewhere in the aquifer.

Due to logistical, regulatory and weather considerations, the Glennville bioreactor could only be switched over to run mode on March 18, 2009. The molecular analysis of the microbial community in the bioreactor in relation to MTBE concentration is shown in Figure 8. Very high PM1 numbers correlate with high MTBE concentrations at S3 of the bioreactor. Low PM1 % in the influent (S1) (Figure 8) support the above observation that the high numbers of PM1 bacteria in W7 (Figure 6 and7) were due to high concentrations near the surface of well water and do not reflect high PM1 numbers in the aquifer.

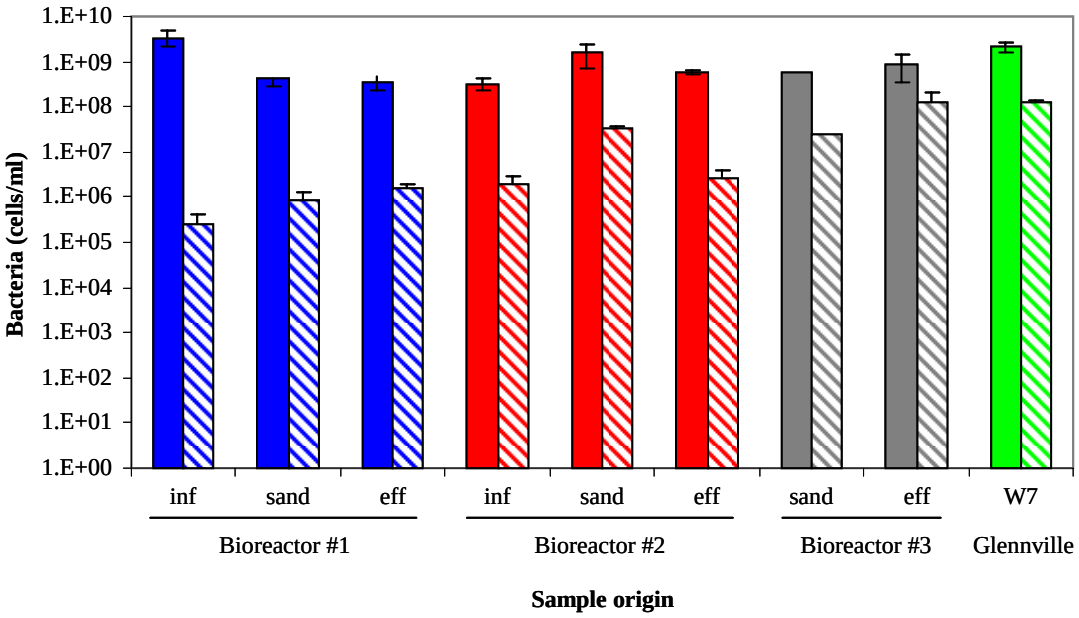

Figure 6. Bacterial enumeration by qPCR. Samples from Glennville well water (W7) and three ERI bioreactors located at: Healdsburg, CA (#1); Laguna Hills, CA (#2); and Forest Lake, CA (#3). DNA extractions from samples were performed in triplicate. Error bars represent one standard deviation. Solids – total bacterial cells/ml; stripes – PM1 cells/ml.

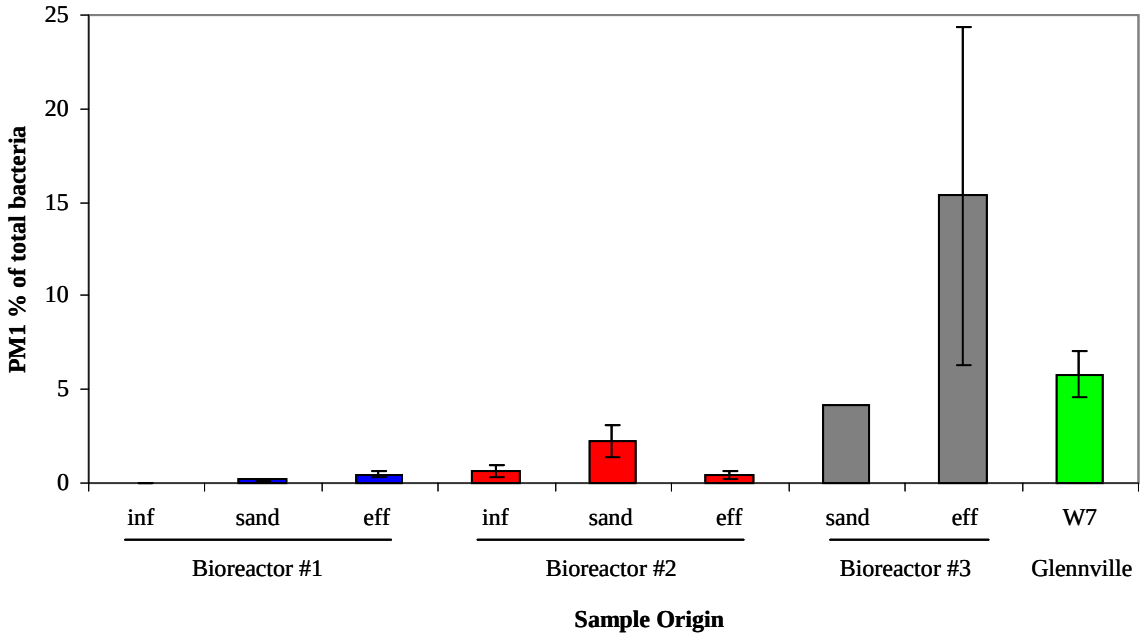

Figure 7. Ratio of PM1 to total bacteria in samples from Glennville well water (W7) and three ERI bioreactors located at: Healdsburg, CA (#1); Laguna Hills, CA (#2); and Forest Lake, CA (#3). PM1 formed a significant subset of the microbial population in all three bioreactors and well water. DNA extractions from samples were performed in triplicate. Error bars represent one standard deviation.

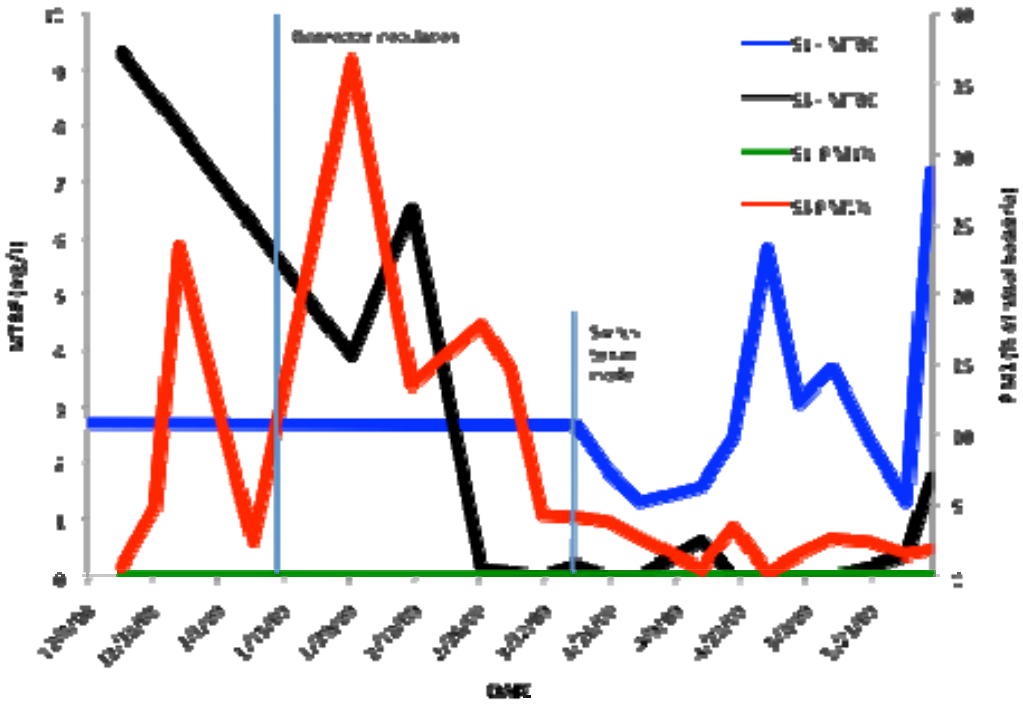

Figure 8. Correlation of MTBE concentration in ERI-500 Glennville bioreactor with PM1 prevalence. High concentrations of PM1 as a fraction of total bacteria were detected in the at S3 in the bioreactor during the initial stage of bioreactor function when MTBE was supplied as carbon source and concentration of MTBE at S3 remained high. The start of degradation and removal of MTBE at S3 was followed by a similar reduction in the percentage of PM1. The percentage of influent PM1 remained low throughout bioreactor operation.

Water samples from the well nearest to the injection well (M36), well M33, and downstream well M51 were tested for MTBE, TBA, and BTEX concentrations, and PM1 prevalence. Trace amounts of BTEX (< 0.05 mg/L) were detected in well M33, but not in M51. MTBE and TBA concentrations in correlation to PM1 prevalence are shown in Figures C and D. Well M51 had significantly higher MTBE concentration than M33, and somewhat higher proportion of PM1 as a fraction of total bacteria. No clear change in PM1, MTBE or TBA concentrations took place during the period tested. Further molecular analyses of filtered well water are pending.

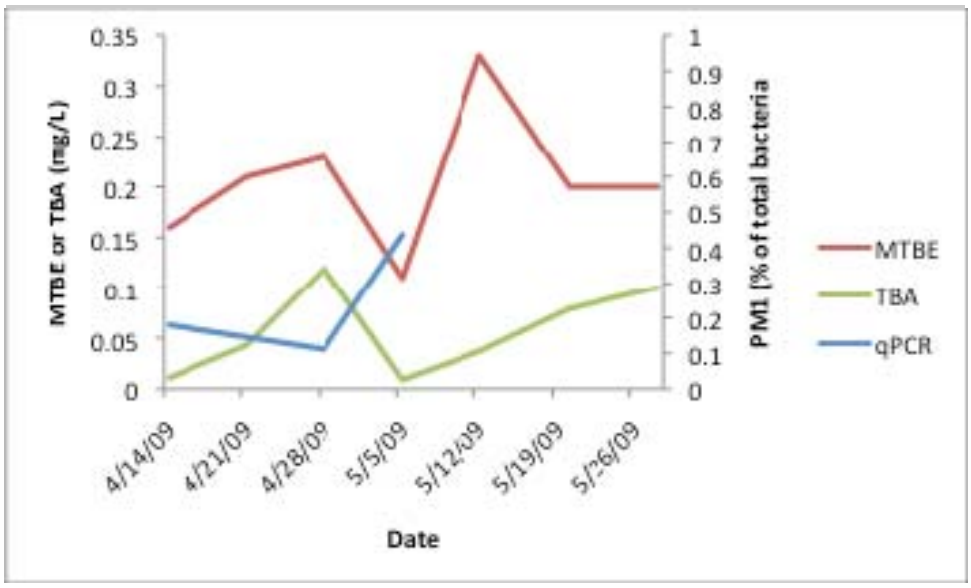

Figure 9. MTBE and TBA concentrations, and PM1 prevalence in well M33. Well M33 is located adjacent to injection well M36.

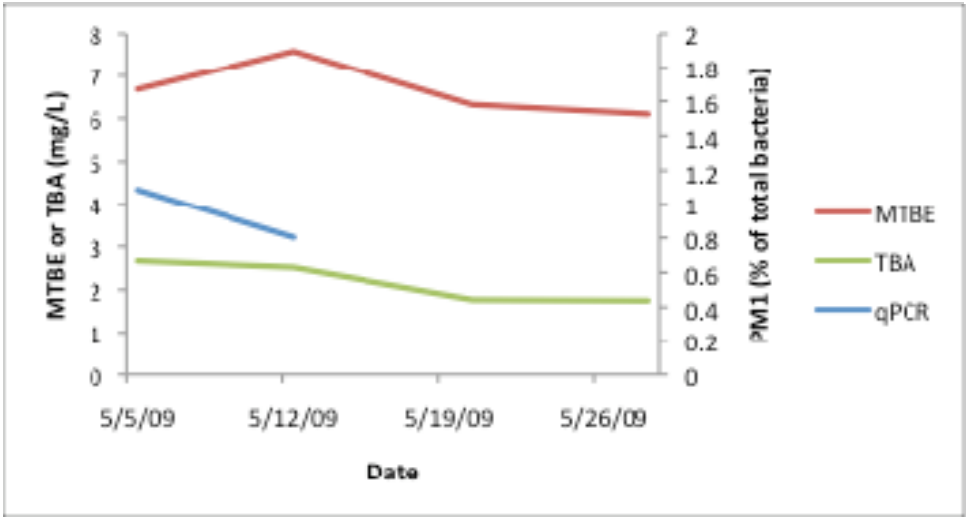

Figure 10. MTBE and TBA concentrations, and PM1 prevalence in well M51. Well M51 is located at the opposite end of the parking lot and approximately 50 m from injection well M36.

Nutrient analysis

During recirculation mode, the microbial community is fed on a mixture of carbon source (MTBE) and nutrients (N, P, K). In order to obtain a discharge permit to begin re-injecting treated water back into the aquifer, the nutritional status of the bioreactor effluent had to be established. Samples taken on March 4, 2009, while the bioreactor was still in recirculation mode,showed high levels of nitrate, phoshpate and potassium (Table 4). To wash out the nutrients out of the system, we sought and obtained permission from the Regional Water Board to run the bioreactor at 0.5 gal/min and discard processed water on a field at the back of the property at 10675 Highway 155, Glennville, CA. After one week in run mode at 0.5 gal/min (i.e. with fresh water as influent and treated water discharged), the bioreactor effluent nutrient levels were significantly reduced, with N only slightly above the drinking water MCL (Table 4). Although no nutrients were added to the bioreactor in run mode, the N concentration in the effluent fluctuated between 15 and 41 mg/L, before decreasing to below detection limit in the first week of June 2009.

Table 4. Nutrient analysis of end product effluent water produced by the Glennville ERI-500 bioreactor while in recirculation mode and after switching to run mode. High concentrations of N, P, and K were detected in recirculation mode, necessitating flushing of bioreactor before re-injection into aquifer. After one week in run mode, nutrient levels were close to acceptable levels. Water samples were analyzed by Kiff Analytical LLC.

Analyte	Reporting units	Reporting Limit	EPA limit (MCL)[1]	Recirc. mode (3/4/09)	Run Mode				
					3/25/09	4/1/09	4/21/09	5/5/09	6/2/09
Nitrate (as N)	mg/L	5	10	300	15[2]	25	17	41	BDL[3]
Total Phosphate (P)	mg/L	1.5	no limit	67	3.8	3.2	NT[4]	NT	NT
Potassium (K)	mg/L	0.5	no limit	96	2.6	3.2	NT	NT	NT

[1] MCL – maximum contaminant level, drinking water standard
[2] sample processed outside recommended hold time
[3] BDL – below detection limit
[4] NT – not tested

Conclusions

ERI bioreactors have been used successfully at approximately 30 leaking underground storage tank (LUST) sites to date, mainly in California. The technology destroys the contaminants, mineralizing them to carbon dioxide and water, rather than merely transferring contaminants to another medium. No air emissions or noise are generated. Energy requirements are low. The equipment is relatively compact and can be reused at other sites. *This is one of the few technologies that can effectively treat TBA.*

Quantitative PCR showed strong presence of MTBE degrading bacterium PM1 at W7 in Glennville (Figure 6, 7), and strong correlation between MTBE concentration and PM1

prevalence (Figure 8). A MTBE and TBA degrading microbial community was established in the bioreactor, with both contaminants degraded to below instrument detection limit (Figure 5).

To our knowledge, no comprehensive study assessing health risks of fluidized-bed bioreactor systems due to growth of waterborne pathogens has been published to date. *Our study therefore represents one of the first steps towards an objective assessment of these systems for the production of drinking water*. The results of our study are promising. In the specific cases we were able to test, we usually observed reduction of total bacteria from groundwater across the bioreactor and only rarely detected low levels of the potential pathogens included in our list (Table 1 and 3). More extensive sampling is urgently needed to determine if these observations translate into broader trends. Our approach of using DNA technology in conjunction with plate counts to monitor establishment of the microbial community in the bioreactor is also novel and could lead to better monitoring of the degradation capacity of each bioreactor as well monitoring the biological safety of these systems.

Because nutrients had to be added to the bioreactor during recirculation mode, water samples from this period showed elevated levels of N, P and K. Once the bioreactor switched to run mode and supplementary feeding stopped, water quality of the final effluent improved rapidly (Table 4), with water close to the drinking water MCL quality being produced within one week of run mode start and decreasing to below detection limit by the final analysis in this report.

There were significant delays caused by budgetary concerns, technical and regulatory concerns – the discovery of the presence of *L. pneumophila* in at least one ERI bioreactor, nutrients in discharge waters, slow establishment of MTBE degradation community within the bioreactor, as well as weather concerns – above ground discharge of effluent in a field behind property could not take place due to freezing temperatures. Despite these difficulties, the bioreactor is still operating and degrading MTBE and TBA, and we are proceeding with treated water re-injection into the aquifer and analysis of the effectiveness of this water on aquifer remediation. The unique opportunities of this project, especially the favorable attitude of the local community and the regulatory authorities towards this university-industry cooperative effort, warrant its continuation.

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Appendix A.
Flyer for the town hall meeting on 12/4/08.

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.