



# Underground Tank Technology Update

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## Potential impact of MTBE on community water supply wells

MTBE, which is water soluble and not significantly biodegradable, can persist in subsurface environments for tens to hundreds of years despite natural attenuation processes. Community water supply (CWS) wells, which serve 90 million people in the United States, are especially vulnerable to MTBE contamination. Kilometer-length plumes have been documented.

MTBE has been used widely in the United States as an octane enhancer, a component of RFG (reformulated gasoline) and as a component of oxyfuel. Because of its widespread use, it is likely that "most underground gasoline tanks in use after 1979 in the United States probably contained this compound at some point in time" (*Johnson and others, 2000*). For instance, in Kansas, where neither RFG nor oxyfuel use was required, MTBE was found at 88 percent of 818 LUSTs. MTBE was found at 75 percent of the 9,000 LUFT (leaking underground fuel tank) sites in California. From these data, researchers estimate that of the 385,000 confirmed LUFT gasoline releases nationwide, perhaps some 250,000 involved MTBE. In addition, "recent evidence from California suggests that spills and leaks continue to occur, even at upgraded facilities" (*Johnson and others, 2000*).

In 1996 concern about MTBE contamination of drinking water escalated sharply when seven wells supplying 50 percent of the water for the city of Santa Monica were found to have MTBE concentrations as high as 600 µg/L. "For the city's Charnock well field, an initial review of known and suspected petroleum spill sites identified about 10 potential sources that lay within 1 km of the well field; the sources were above the hydrologic unit accessed by the well field and were created after MTBE use began in the state. At the time that well contamination was discovered, pumping of the Charnock well field was at 5 million gallons/day (mgd). This aggressive pumping was approximately twice the total natural flow of water moving into the aquifer. Despite the presence of a protective aquitard in the system, the pumping had dewatered a significant portion of the upper aquifer, caused water to flow toward the well field from all directions, and had greatly increased the likelihood that the CWS wells in Santa Monica would in fact become contaminated by one or more persistent organic pollutants such as MTBE" (*Johnson and others, 2000*).

### CWS wells: temporal scale

MTBE has the potential to threaten wells for tens to hundreds of years. This period can represent the travel time of MTBE from source areas to a CWS well. MTBE movement from the source to a well is a function of

- source size
- concentration leaving the source
- attenuation mechanisms of dilution, dispersion and degradation

Studies to date suggest that MTBE sources from LUFTs will persist for 10 years with groundwater concentrations of a few hundreds of milligrams per liter (µg/L). To meet maximum allowable concentrations of 20 to 40 micrograms per liter (µg/L) requires an overall reduction factor of 10,000.

"Substantial dilution (by a factor of 250) can occur when a groundwater plume is drawn into a CWS well. If the required overall reduction factor is 10,000, then an additional in-situ reduction factor of about 40 would be required to reduce the concentration in the CWS to an acceptable level." Dispersion will in general not be strong enough to remove the MTBE from a well's capture zone, thus "in most cases, degradation followed by dilution at the well will control the MTBE concentrations found in CWS wells" (*Johnson and others, 2000*).

Although MTBE has been found to degrade in the laboratory, field studies of MTBE biodegradation are inconclusive; the assumption is that MTBE's degradation half-life is at least two years. Thus, to achieve the additional in-situ reduction factor of 40 would require 5 to 6 half-lives, or a time period of 10 years. Thus, "significant numbers of MTBE releases may therefore continue to reveal themselves as problematic sources of contamination for the nation until at least 2010" (*Johnson and others, 2000*).

### Predicting contaminant movement at the local scale

Numerical models using known hydrogeological data and contaminant source locations can help regulators and consultants predict contaminant movement; however, the required data are not always available. Johnson and others (2000) suggest the following:

- determine a 10-year capture zone area using groundwater flow techniques
- determine the areal density of significant sources in the well vicinity
- multiply the density by the 10-year capture zone to obtain the number of sources,  $n_s$ , that will, on average, contaminate the well water at concentrations above set standards

Johnson and others present three examples of this technique in their paper, using a base case, an aquitard case and an infiltration case.

Another factor to consider is the measure of pumping intensity, or pumping stress factor: CWS pumping rate/local aquifer yield. Consider the situation where the MTBE plume is at the water table, with a low pumping stress factor. The MTBE plume can pass over a well without being drawn into it, and the 10-year capture zone will remain zero. Factors other than pumping rate that influence the capture zone area include

- porosity
- aquifer size
- groundwater velocity
- presence of an aquitard
- groundwater recharge

At Santa Monica's Charnock well field, modeling indicated that the pumping rate was about twice the natural groundwater flow, assuring that 100 percent of the water in the aquifer within 1 km of the wells moved toward the well field.

### Predicting risk to nationwide CWS wells

By comparing two databases, the Starview, which has latitude and longitude information on many LUST sites, and the EPA Safe Drinking Water Information System, which has CWS well locations for 31 states, researchers were able to make conclusions about contaminant risk to CWS wells:

- using a cumulative frequency distribution, approximately 35 percent of the CWS wells have, within a 1-km radius, one or more LUST sites; this corresponds to about 9,000 CWS wells
- about 65 percent of all LUSTs are associated with MTBE contamination
- pumping rates, local aquifer yield and other data are not available in one database for all of the nation's CWS wells, or even for a subset of wells
- lack of information prevents determination of the 10-year capture zone, the  $n_s$  (probability of contaminant sources that could impact a CWS), and the cumulative frequency of CWS wells vs.  $n_s$

Johnson and others (2000) argue that "the data to determine the magnitude of that threat (number of MTBE-LUSTs in the immediate vicinity of a particular CWS well) are simply not available at the present time." To address this concern, they suggest the following:

- collect better data on in-situ MTBE biodegradation rates
- understand more completely the stress that pumping puts on local groundwater supplies
- create two new national databases: one for LUSTs and other sources, and one for CWS wells
  - the LUST database, as suggested by EPA's Blue Ribbon Panel on Oxygenates in Gasoline, should focus on sites that actually represent threats to CWS wells; that is, it should include information on the magnitude of each release and contaminant concentrations
  - the CWS databases should contain hydrogeologic and pumping rate data

"These databases will allow improved estimates of the numbers of CWS wells that may be affected by significant concentrations of MTBE over the next 10 years. And, quite independent of the MTBE issue, the databases will help identify aquifer and CWS systems that are being pumped at rates that carry unacceptable risks of contamination by persistent chemicals in general" (Johnson and others, 2000).

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Johnson, R., Pankow, J., Bender, D., Price, C. and J. Zogorski, "MTBE: To What Extent Will Past Releases Contaminate Community Water Supply Wells?" *Environmental Science and Technology*, Vol. 34, May 1, 2000; <http://www.pubs.acs.org>.



## Monitoring biodegradation with carbon isotopes

This article describes basic isotope theory and its potential applicability for monitoring in-situ biodegradation of hydrocarbons.

### Review of isotope basics

"Isotopes, which are almost chemically inseparable from each other, have identical electronic makeups but differ from each other in mass because of subtle differences in the heart of the atom. The word 'isotope' comes from the Greek words 'same' and 'place'; it refers to the fact that isotopes occur in the same place when the chemical elements are arranged in a regular array according to their properties. . . . Any collection of atoms such as encountered in chemical reactions can be characterized by an 'average' atom which has a mass determined by the weighted mean of the various isotopes present. The principal effect of having identical atoms differing only in mass is to change the rate at which chemical transformations occur" (Sienko and Plane, 1974).

An isotope is "one of two or more atoms of the same element that have the same number of protons in their nucleus but different numbers of neutrons. Protium (1 proton, no neutrons), deuterium (1 proton, 1 neutron) and tritium (1 proton and 2 neutrons) are isotopes of hydrogen. Most elements in nature consist of a mixture of isotopes" (Daintith, 1990).

Stable isotopes of carbon are carbon<sup>12</sup> (<sup>12</sup>C) and carbon<sup>13</sup> (<sup>13</sup>C). These isotopes generally occur in nature at a ratio of approximately 98.89 percent <sup>12</sup>C to 1.11 percent <sup>13</sup>C. Stable carbon isotopes are present in all living organisms and in materials derived from living organisms at a ratio ( $\delta^{13}\text{C}$ ) unique to them. For laboratory quantities, the most suitable

device for separating isotopes is usually the mass spectrometer; however, there are other methods including gaseous diffusion, distillation, electrolysis, thermal diffusion, centrifuging and laser methods (*Daintith, 1990; Reisinger and others, 1999*).

The principal effect of having identical atoms differing only in mass is to change the rate at which chemical transformations occur. For example, in the case of the element hydrogen, which is composed of three isotopes (protium, deuterium and tritium), the lightest isotope generally undergoes chemical reaction most rapidly (*Sienko and Plane, 1974*). The ratio can be changed as a result of natural processes such as hydrocarbon mineralization, a phenomenon known as fractionation. This unique ratio provides for discrimination of carbon sources and renders the approach a valid and valuable bioremediation assessment tool" (*Reisinger and others, 1999*).

"In materials of biological interest, this variation is relatively small, with the most enriched materials (those highest in  $^{13}\text{C}$ ) differing from the least enriched (those lowest in  $^{13}\text{C}$ ) by only 10 percent or 100 parts per thousand (ppt). However, differences in  $^{13}\text{C}$  content between materials in the 1 to 10 ppt range are often significant to biologists, and differences of 0.1 ppt are often significant to paleoclimatologists and geochemists. To make use of these small but significant variations, researchers must measure stable carbon isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) with extremely high precision" (*U.C. Berkeley, 2000*).

"The quantity measured is not the absolute isotope ratio of the sample but the relative difference between the isotope ratios of the sample and standard gases. As a result, a differential notation known as the delta notation has been adopted for expressing relative differences of stable carbon isotope ratios between samples and standards. The  $\delta^{13}\text{C}$  value is calculated from the measured carbon isotope ratios of the sample and standard gases as:

$$\delta^{13}\text{C} (0/00) = [R_{\text{sample}} - R_{\text{standard}} / R_{\text{standard}}]$$

where  $\delta^{13}\text{C}$  is ppt or per mil (0/00), and the difference between the  $^{13}\text{C}$  content of the sample and that of the standard and R is the mass 45/44 ratio of the sample or standard gas" (*U.C. Berkeley, 2000*).

Alternatively, carbon isotope composition can be expressed by

$$\delta^{13}\text{C} = 1000 (R_s / R_{\text{PDB}} - 1)$$

R is the ratio  $^{13}\text{C}/^{12}\text{C}$ . S and PDB refer to sample and standard Pee Dee Belemnite. This standard is based on the internal calcite structure from the fossil *Belemnitella americana* found in the Cretaceous Pee Dee Formation in South Carolina. Units are parts per thousand (per mil) (*Stehmeier and others, 1999; Reisinger and others, 1999*).

### Carbon isotopes and environmental fate

The use of carbon isotopes to characterize the environmental fate of organic molecules was first attempted in 1993

(*Stehmeier and others, 1999*). "It had long been known that biological systems fractionate carbon isotopes. . . [another researcher] suggested that biological fractionation could occur by two mechanisms—enzymatic and mass transfer. Enzymatic fractionation and fractionation by mass transfer both lead to isotopic forms that correspond to the minimum free energy of the system. For biodegradation, the system is assumed to be the boundaries of the microorganism. Kinetic effects of isotopes are produced by differences in the reaction rates of isotopic forms. This means that in an ensemble of interacting atoms, the smaller masses have greater velocities, and molecules containing lighter isotopes are more mobile than those containing heavier isotopes. Second, chemical bonds formed by a heavy isotope are stronger than those formed by the light isotope, resulting in higher activation energy for any reactions in which the heavier isotope participates" (*Stehmeier and others, 1999*).

### Carbon isotopes with respect to in-situ biodegradation

Proof of in-situ biological degradation can be difficult to confirm. According to Stehmeier and others (1999), no single method can unequivocally demonstrate definitive microbial removal of contamination. "Bacterial numbers and activity, metabolic by-products, redox conditions, inorganic carbon isotope ratios and electron acceptor concentration provide circumstantial proof of bioremediation. These types of analyses only indirectly suggest bioremediation because they cannot specifically link contaminant loss to microbial activity. To make matters worse, many bacteria are difficult to isolate, and as much as 75 percent of the targeted organisms may be missed using conventional methods." Stehmeier and others (1999) attempted to define biodegradation extent by measuring the ratio of stable isotopes of benzene, styrene and dicyclopentadiene (DCPD) in laboratory and field studies.

### Laboratory studies

For laboratory studies, researchers

- obtained benzene and styrene degraders from a mixed microbial culture from contaminated groundwater at a petrochemical site; the groundwater sample was amended with nitrogen and phosphorus
- filled vials with 50 to 250  $\mu\text{g/L}$  of hydrocarbon and 5 ml of microbial inoculum
- prepared duplicate vials—one used for optical density measurements (optical density is an indication of microbial growth) and the other for headspace analysis of hydrocarbons
- created an abiotic control to determine if isotope fractionation occurred during shaking and volatilization
- analyzed headspace of vials (hydrocarbon isotope analysis) using a gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS)

### Laboratory study results

Biodegradation experiments using benzene, toluene and styrene as the substrate showed an inverse correlation between hydrocarbon concentration and optical density, indicating the depletion of these hydrocarbons as the microbial culture increased in biomass. The  $\delta^{13}\text{C}$  for residual benzene also increased as concentration decreased and microbial biomass increased. The shift in  $\delta^{13}\text{C}$  was not large but was significantly greater than the analytical reproducibility of carbon isotopic measurements. Additional experiments at different initial concentrations showed similar magnitude of  $\delta^{13}\text{C}$  shift from beginning to end of the experiment, ranging from 80 to 90 percent hydrocarbon consumption (*Stehmeier and others, 1999*).

### Descriptions and results of field studies

Researchers obtained soil and vapor samples from the following four sites:

- site 1: a pyrolysis gas spill (C5+) in very tight clay soil undergoing in-situ bioremediation; samples taken were soil headspace, monitored for 17 weeks
- site 2: a gasoline LUST site, treated by a patented biosparge system; samples were vapors swept from contaminated zones and vapors from discrete soil headspaces, monitored for 6 weeks
- site 3: mixed styrene contaminants in a biopile; samples were vapors swept from the pile, monitored for 6 weeks
- site 4: unknown mixture of hydrocarbons (>C10) undergoing natural attenuation in river sediments; samples were sediments extracted with dichloromethane and sampled for 3 years

Researchers wanted to determine if carbon isotope variations occurred under field conditions, and if these variations occurred in a manner consistent with biodegradation.

Remedial techniques used for each site were as follows:

- site 1:  $\text{NH}_4\text{NO}_3$  and  $\text{PO}_4^{3-}$  simulated in-situ bioremediation; studied for 17 weeks
- site 2: biosparging; studied for 6 weeks
- site 3: biopile; studied for 6 weeks
- site 4: natural attenuation in river sediments; studied for three years

*At site one*, researchers found that at a 90 cm sampling depth, only  $^{13}\text{C}$  fractionation of residual toluene occurred even though substantial quantities of benzene were lost. FDA hydrolysis indicated microbial activity increased by 156 percent. At the 30-cm sampling depth,  $^{13}\text{C}$  fractionation of toluene, benzene and DCPD occurred and FDA hydrolysis increased by 400 percent.

*At site two*, a site actively bioremediated showed a change in  $\delta^{13}\text{C}$  values from 38.5 per mil to 36.65 per mil for gasoline components during the first two months. The  $\delta^{13}\text{C}$  values were generally more enriched where hydrocarbon contamination ranged from 50 to 150 ppm in a uniform sandy clay

soil. Where hydrocarbon contaminations were up to 3,700 ppm, the  $\delta^{13}\text{C}$  values were not enriched.

*At site three*, the styrene-contaminated soil biopile indicated some  $^{13}\text{C}$  enrichment, but the change could not be related simply to the observed hydrocarbon concentration.

*At site four*, residual petroleum-contaminated sediment samples, analyzed at intervals for three years, showed significant increase in  $\delta^{13}\text{C}$  for some components but not for others. Other studies suggested that  $^{13}\text{C}$  hydrocarbon fractionation does not occur with larger molecular weight polycyclic aromatic hydrocarbons at this site.

### Conclusions

Researchers concluded the following from the laboratory and field studies:

#### Laboratory experiments

- with benzene, toluene and styrene, there was an enrichment in  $^{13}\text{C}$  of the residual hydrocarbon with increasing degree of biodegradation; this shift was reproducible and correlated with hydrocarbon fraction degraded; most significant shifts in  $^{13}\text{C}$  occurred when greater than approximately 75 percent of the hydrocarbon component degraded
- enrichment of  $^{13}\text{C}$  in laboratory experiments, while small, ranged between two and seven times greater than the analytical error of 0.3 percent
- while extent of fractionation appeared to be dependent on hydrocarbon amount degraded, the experiments were not sufficient to establish the nature of this dependence; further work has determined fractionation constants for benzene and toluene
- work with C5+ alkanes, alkenes and single ring aromatics as a hydrocarbon mixture indicated that the  $\delta^{13}\text{C}$  of residual hydrocarbons was enriched by as much as 8 per mil after biodegradation

#### In the field

- benzene, toluene, styrene and dicyclopentadiene, as substrates for aerobic growth, showed an enrichment of  $^{13}\text{C}$  in residual hydrocarbon from field sites
- preliminary testing of the isotopic techniques in the field indicated isotopic measurement can be applied to both extracts and vapor samples, although only one sample type should be used for a given site
- the same enrichment was generally observed in several field sites; however, the present study also provides examples of field situations where a single isotopic vector (such as  $\delta^{13}\text{C}$ ) may not be able to resolve complex reaction histories
- at site 1, benzene, toluene and DCPD were monitored for  $^{13}\text{C}/^{12}\text{C}$  fractionation at 2 depths
  - results for the 30-cm depth suggest a shift in  $\delta^{13}\text{C}$  of residual hydrocarbon comparable to that observed in the laboratory

- the shift in  $\delta^{13}\text{C}$  was greater in toluene than in benzene
- data are consistent with aerobic biodegradation (note: fractionation of  $^{13}\text{C}$  by evaporation has been shown to be 0.015 the rate of biodegradation)
- results for the 90-cm sample indicate only a moderate increase of microbial activity (compared to the 30-cm depth), consistent with the dramatic difference in oxidation potential; thus, likely low Eh values (-200mV) dramatically limited activities of aerobic organisms, limiting the resulting  $\delta^{13}\text{C}$ ; if this is correct, then actual biodegradative loss is substantially less than what can be inferred from the total hydrocarbon; or more plausibly, biodegradation may have been anaerobic and may not result in a similar  $\delta^{13}\text{C}$  shift as observed in laboratory experiments
- site 2 results are far from conclusive and may be related to higher hydrocarbon concentrations (in terms of toxicity) and porosity
- site 3, like site 2, used vapor analyses to determine  $\delta^{13}\text{C}$  shifts for styrene; results were not conclusive and may be related to channeling or physical disturbance
- data from site 4, which contained higher molecular weight compounds, indicated that biodegradation may have left only a minimal imprint on the  $\delta^{13}\text{C}$  of residual high-molecular weight recalcitrant compounds

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UTTU thanks Les Stehmeier ([stehmelg@novachem.com](mailto:stehmelg@novachem.com)), for his help on this article.



## Enrichment, isolation and identification of MTBE-degrading bacteria

By Dr. Richard Veeh and Dr. Eric Kern

MTBE research is in its infancy, yet expanded understanding of MTBE fate in environmental systems is essential as approaches to contaminant remediation, such as natural attenuation, are evaluated. Basic research, which this article describes, will benefit all environmental agencies and aid in development of management and remediation strategies. Identifying important MTBE-degrading microorganisms as well as understanding environmental parameters that control MTBE degradation may foster development of engineered systems that accelerate MTBE removal from impacted groundwater.

This study was undertaken with funding from the U.S. Geological Society and the Montana Department of Environmental Quality (DEQ) to assess the potential for MTBE biodegradation at a gasoline release site in Montana and to identify any MTBE-degrading microbial consortia and/or bacterial species present.

### MTBE concerns

MTBE, methyl-tert-butyl ether, is a groundwater concern because MTBE

- is highly soluble and mobile in water
- has an odor threshold detection limit as low as 1.0  $\mu\text{g/L}$  (ppb) in drinking water
- is a suspected carcinogen
- is not amenable to remediation techniques like pump-and-treat or air sparging, because of its chemical properties
- appears to be largely recalcitrant in natural environments
- use is widespread, suggesting a high potential for human exposure

Although addition of hydrogen peroxide can enhance chemical degradation of MTBE through generation of reactive hydroxyl radicals, bioremediation also remains a viable alternative strategy for MTBE removal. General conclusions about MTBE degradation from previous studies include the following:

- MTBE is biodegraded slowly in natural environments
- MTBE biodegradation is primarily aerobic
- MTBE metabolites, tert-butyl alcohol (TBA) and tert-butyl formate (TBF) are of unknown human toxicity and can accumulate in natural systems
- organic co-substrates can enhance MTBE biodegradation

- complete biodegradation may involve a consortium of microbial species
- degradation capabilities of microbial consortia vary for different fuel oxygenates and in the presence of other gasoline constituents

### MTBE site in Montana

The MTBE site we studied is located east of U.S. Highway 93, south of Ronan, Montana. Site characteristics are as follows:

- a 16,000-gallon UST was removed in April 1994
- the UST was estimated to have released 3,000 to 5,000 gallons of gasoline
- a free product plume identified directly west of the tank removal site had migrated beneath a highway
- a dissolved MTBE plume was found to extend west-southwest under a pasture to a south-flowing creek
- both MTBE and BTEX were detected in the spring
- the closest downgradient contaminant receptor was 480 yards away
- dominant lithology identified from boreholes was silt and fine sand with scattered clay lenses, typical lake-bed deposits
- depth to groundwater was 1 to 15 feet

The DEQ initially drilled 20 monitoring wells to identify plume boundaries. DEQ installed another eight wells in September 1997 for free product removal. Site remediation included a combination of passive recovery skimmers, soil vapor extraction/air sparging systems, and an interceptor trench. Well monitoring results for MTBE and BTEX concentrations, from the second quarter of 1998 through the first quarter of 1999, were consistently highest (4-8 µg/L and 27-59 µg/L, respectively) for a monitoring well located about 240 feet downgradient from the point source. Although lower in concentration, MTBE and BTEX were still detectable in monitoring wells adjacent to the creek.

### Soil sample collection and enrichment protocol

We collected soil samples for MTBE-degrading bacterial enrichments from two boreholes drilled to a depth of approximately 1 m below the groundwater surface. One borehole was drilled near the downgradient (leading) edge of the non-aqueous gasoline plume, and the other was drilled approximately 1,200 m farther downgradient adjacent to the creek. We retrieved samples from above, at, and below the groundwater surface.

The three samples from each borehole were mixed and stored at 4°C prior to use in the enrichment experiments. Agricultural soil acquired from a "pristine" (i.e. non-MTBE contaminated) site near Bozeman, Montana, was also used as inoculum for several control enrichments.

We conducted the initial aerobic batch experiment using a 30-reactor matrix consisting of the three inoculum soil

sources, two nutrient concentrations (1x and 10x), MTBE and 100 µg/L 2-propanol added (or omitted) as a co-substrate. Treatments were tested in triplicate and compared to one sterile control for each soils type. Degradation experiments were conducted in 125 ml flasks that contained 25 ml of nutrient medium, simulating a "typical" soil solution. Samples were amended with 0.25 g of composite soil/aquifer material as inoculum. All slurries were spiked with 10 µg/L MTBE and ~100,000 dpm/flask [<sup>14</sup>C]-MTBE. The <sup>14</sup>CO<sub>2</sub> that evolved from the slurries was captured in 0.5 M NaOH (0.3 ml) traps placed in small cups suspended from the stoppers and was used to monitor MTBE degradation.

### Bacterial isolation and MTBE degradation study methods

One flask showed a dramatic increase in <sup>14</sup>CO<sub>2</sub> production in the initial enrichment experiment. We streaked this sampled soil on both R2A and Noble agar plates. We incubated the R2A plates aerobically in the dark, whereas the Noble agar plates were incubated in closed (also aerobic) containers and exposed to MTBE vapors as the sole carbon source. Both sets of plates were incubated at room temperature. Single colonies from both types of plates were transferred three more times for single colony isolation.

Three MTBE degradation experiments were conducted with separate groups of bacterial isolates. In the initial experiment, we evaluated four isolates grown on two types of agar plates. The second degradation experiment involved other isolates:

- a pure culture (from each type of agar plate) added to serum bottles containing 40 ml enrichment medium and 10 µg/L MTBE; 20 µg/L 2-propanol added as a co-substrate
- a pure culture (from each type of agar plate) added to serum bottles containing 40 ml enrichment medium and 10 µg/L MTBE; 2-propanol not added
- a pure culture (from each type of agar plate) added to serum bottles that were sterile

A final degradation experiment was conducted on seven isolates, some of which were re-tested from the second degradation experiment. We used gas chromatography to monitor MTBE degradation and metabolite production.

### MTBE consortium degradation kinetics

MTBE degradation kinetics of the bacterial consortium were studied through several duplicate transfers of the original enrichment culture. Periodically, 3 ml of transfer inoculum was added to fresh nutrient in the serum bottles at MTBE concentrations ranging from 30 to 70 µg/L. Between transfers, 2 ml subsamples were removed weekly and filtered (0.2 µm) for gas chromatographic monitoring of MTBE degradation while fresh air was injected into the headspace. At each sampling point, we removed a subsample and DAPI-stained it for direct microscopic counts of bacteria.

## Molecular analyses

We obtained DNA from MTBE-degrading isolates and consortia. We separated gene fragment mixtures using denaturing gradient gel electrophoresis (DGGE) and thereby obtained "genetic fingerprints" of the consortia and isolates of interest. Bands within the DGGE profiles of the consortia were purified for nucleotide sequence analysis and examined.

## Initial MTBE enrichment results

Analysis of the 30-flask enrichment culture matrix revealed minimal  $^{14}\text{CO}_2$  evolution from microbial degradation of MTBE over a 35-day period, with the exception of one culture (amended with 2-propanol and the 10X nutrient concentration) in which MTBE degradation was significantly greater. This enrichment was used to provide inoculum for subsequent consortium enrichments and for obtaining MTBE-degrading bacterial isolates as described above. The extended "lag phase" and lack of similar activity in replicate flasks for this same treatment indicated that MTBE-degrading bacteria were not a significant fraction of the overall microbial population. Because the one positive flask received 2-propanol as a co-substrate, our work corroborated previous work (Steffan and others, 1997, *Applied Environmental Microbiology*, Vol. 63, pgs. 4216-4222) that identified propane-oxidizing bacteria as the dominant bacteria capable of MTBE degradation.

## MTBE degradation by bacterial isolates

The initial isolate MTBE degradation trial lasted 3 weeks with weekly sample collection. Significant observations include:

- three of the four isolates tested positive for MTBE degradation
- MTBE was degraded by all three MTBE-degrading isolates to a significantly greater extent in the presence of 2-propanol
- both TBA and TBF metabolites accumulated over the three-week experiment

In the subsequent two bacterial isolate studies, similar results were observed:

- significant MTBE degradation occurred only in the presence of 2-propanol
- both metabolites, TBA and TBF, accumulated
- molecular analysis of nine isolates revealed that
  - five were identical and showed 100 percent similarity to a *Pseudomonas* species
  - four were identical, and showed 100 percent similarity to a *Rhodococcus* species

## MTBE degradation by bacterial consortia

MTBE degradation by the consortium was studied through several successive transfers of the original enrichment culture. Although the maximum MTBE degradation rates of  $\sim 0.02 \mu\text{g/day}$  were routinely observed at about 35 days after culture transfer to fresh growth medium, at the highest cell densities

we obtained a degradation rate of  $\sim 0.66 \mu\text{g/day}$ . MTBE concentrations were  $\sim 70 \mu\text{g/L}$ . Interestingly, no metabolite accumulation was observed at any time in any of the bacterial consortium cultures. Thus, we concluded organisms were present in the bacterial consortium that we had not yet isolated. Assuming that these uncultured bacteria would be difficult to isolate using selective solid growth media, we attempted to identify members of the bacterial consortia using the molecular methods previously described. DGGE profiles of four successive cultures obtained by 10 percent inoculum transfer into fresh growth medium clearly showed that the populations of the two MTBE-degrading isolates from the original enrichment decreased over time, whereas two as yet uncultured organisms were becoming more dominant. We also determined that equal numbers of cells removed from a mature consortium culture (i.e., MTBE totally degraded) and introduced into fresh growth medium degrade newly added MTBE at a much lower rate than cells re-suspended in the old growth medium. This suggests that some type of chemical/cell concentration-dependent mechanism may optimize MTBE degradation by this consortium.

## Conclusions

We concluded the following from this study:

- a bacterial consortium capable of biodegrading MTBE does exist at the UST site near Ronan, Montana
- although earlier work with pure culture bacterial isolates obtained from the original enrichment culture degraded MTBE only in the presence of a co-substrate (2-propanol), the enriched bacterial consortium degrades MTBE as the sole carbon source with no metabolite accumulation
- genetic characterization by sequence analysis of two MTBE-degrading isolates (requiring the co-substrate, 2-propanol) identified the isolates as a *Pseudomonas* sp. and a *Rhodococcus* sp., respectively
- molecular analysis of the MTBE-degrading consortia suggests that a diverse group of organisms originally present has become subject to the selection of one to two (not yet cultured) members
- MTBE degradation rate drops dramatically when the bacterial consortium at high cell densities is transferred to a new medium and recovery is slow, suggesting a chemical/cell concentration-dependent mechanism for MTBE degradation
- while several isolates and a consortium capable of relatively high rates of MTBE degradation were enriched from the Ronan site, the lack of significant MTBE degradation in-situ is likely due in part to anoxic conditions in the aquifer, where dissolved oxygen was about  $1 \mu\text{g/L}$ ; observations related to original culture enrichments and generally low MTBE degradation rates under laboratory conditions indicate that microbial biodegradation is probably of minor importance under existing field conditions

### Future research

Future research should focus on the following:

- optimization of growth conditions for important MTBE-degrading bacteria in our consortium
- continuation of efforts to isolate MTBE-degrading bacteria that remain presently difficult to culture
- investigation of possible chemical/cell concentration-dependent co-factors that may be necessary to maintain optimal MTBE degradation rates
- identification of unique RNA sequences peculiar to members of the MTBE-degrading consortium that could be used to develop fluorescent hybridization probes, or PCR amplification products for testing environmental samples
- isolation of possibly unique enzymes that confer complete MTBE-degrading ability

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## Spatial electron acceptor variability in a shallow aquifer

Researchers sought to evaluate the spatial variations of redox patterns in both pristine and BTEX-contaminated portions of a shallow wetland aquifer in Wisconsin. They monitored the aquifer for BTEX, nitrate, dissolved iron and sulfate, dissolved oxygen (DO), dissolved organic carbon (DOC) and methane.

### Site description

The field site, in west-central Wisconsin, includes a military service station contaminated with BTEX from two 12,000-gallon USTs installed in 1943 and removed in 1989.

Characteristics of the plume include the following:

- an upgradient portion, located in a grass-covered upland
- the downgradient portion, which migrates through a forested wetland and ultimately discharges to a shallow stream
- a 110-m length
- a 30-m maximum width
- dissolved BTEX concentrations less than 20 µg/L

Other site characteristics:

- sediment samples near the tank locations showed BTEX concentrations less than 30 µg/kg, suggesting that much of the nonaqueous phase liquid (NAPL) was removed when tanks were excavated
- residual NAPL was discovered in an area of approximately 60 m<sup>2</sup> located downgradient of the former tank location
- the shallow aquifer, in the upgradient area at the water table, contains deposits of well-sorted sand stratified with discontinuous silt lenses with slightly coarser medium sand
- wetland sediments were more heterogeneous than the upland sediments
- below organic-rich deposits, in the wetland area, the aquifer contains fine to medium sand
- hydraulic conductivity of the fine to medium sand, based on slug and tracer tests, is 6 to 12 m/d
- the horizontal hydraulic gradient is approximately 0.02 in the upgradient area and 0.01 in the wetland
- overall direction and magnitude of the horizontal gradient does not vary significantly between dry and wet periods
- vertical gradients varied across the site

### Monitoring

After the tanks were removed in 1989, consultants installed seven monitoring wells. The wells were sampled quarterly for the following:

- BTEX
- nitrate
- dissolved iron
- sulfate

Researchers installed an additional 18 multilevel samplers and five larger bundle-type multilevel samplers. The multilevel samplers were located along two transects parallel to approximate groundwater flow directions, one through a pristine portion of the aquifer, the other through the hydrocarbon plume. The Schreiber and Bahr text (1999) describes in detail the sampling techniques.

Researchers sampled three zones, defined by depth below the water table:

- a shallow zone, 0 to 2 m
- an intermediate zone, 2 to 7 m
- a deep zone, below 7 m

### Pristine portion of the aquifer

For each zone, researchers took samples along the pristine and contaminated transects. For the pristine portion:

*In the upland area, shallow groundwater*

- is aerobic (4 to 5 µg/L DO)
- contains nitrate concentrations (NO<sub>3</sub>) less than 6 µg/L
- has sulfate concentrations averaging 12 µg/L
- has Fe(II) concentrations less than 1 µg/L

*In the wetland area, shallow groundwater*

- DO and nitrate generally decrease along the flowpath (from the upland area), resulting in lower concentrations in the wetland
- researchers attribute electron acceptor reduction to oxidation of dissolved and sediment organic matter in the shallow zone
- concentrations of DOC reach almost 8 µg/L
- oxidation of natural organic matter produces organic acids and a corresponding pH decrease
- low dissolved Fe(II) concentrations and sulfate that do not appear to be significantly depleted indicate DO and nitrate supply are sufficient to prevent iron and sulfate reduction

*In the intermediate zone*

- groundwater DO is low, less than 3 µg/L
- nitrate concentrations are higher
- DOC concentrations reach 14 µg/L and systematically decrease along the flowpath
- Fe(II) (1 µg/L), and sulfate (12 µg/L) concentrations are similar to concentrations in the shallow zone

*In the deep groundwater zone*

- groundwater contains DO less than 1 µg/L
- nitrate is low to undetectable
- Fe(II) concentrations are as high as 18 µg/L
- sulfate ranges from 17 to 38 µg/L
- DOC concentrations are less than 2 µg/L

Here, the low DO and nitrate "suggest the occurrence of organic matter oxidation coupled to the consumption of DO, nitrate and mineral-bound Fe(III). Production of organic acids during oxidation depresses pHs to the 4.9 to 5.8 range. Because the deep portion of the aquifer is not recharged within the area of the field site, DO and nitrate are not replenished along the flowpath. Once DO and nitrate are depleted, the next preferred and available electron acceptor is mineral-bound Fe(III)" . . . then sulfate; sulfate reduction does not appear to occur here.

**BTEX spatial distribution**

Sample analyses indicate that the dissolved BTEX plume is less than 2 m thick and highest concentrations occur at 0.5 to 1 m below the water table. "Near and beneath the stream, strong upward gradients persist and cause the plume to discharge into the stream. Elevated BTEX concentrations extend to the shallow zone of the sampler adjacent to the creek" where

only trace concentrations of benzene were detected. "One sample collected at the creek bank had detectable benzene (0.005 µg/L), suggesting that the plume is also discharging at the seepage face. BTEX has not been detected in the flowing creek water, presumably due to dilution and volatilization." Underflow is unlikely.

**Contaminated transect**

Groundwater along the contaminated transect displays two characteristics:

- geochemical trends that are similar to intermediate and deep zones of pristine groundwater
- significant changes in groundwater chemistry as a result of the BTEX in the shallow zone, due to microbially mediated biodegradation reactions

For instance, in the shallow zone:

- DO and nitrate concentrations are less than 0.5 µg/L (compared to 5 to 6 µg/L in the pristine zone)
- Fe(II) increases because of microbial reduction of mineral-bound Fe(III)
- sulfate is depleted but only in the zones of highest BTEX; in these zones, methane is detected
- only low concentrations of H<sub>2</sub>S (a by-product of sulfate reduction) are detected, likely due to precipitation of Fe(II)-sulfides
- groundwater pH was lower than in the pristine zone because BTEX oxidation by-products include organic acids

*In the intermediate zone, where BTEX is less than 0.05 µg/L*

- DO and nitrate concentrations are depressed
- some iron reduction occurs
- sulfate concentrations are similar to those in the intermediate pristine zone

In the deep zone, where there is very little BTEX:

- electron acceptor profiles are similar to pristine profiles
- Fe(II) concentrations are higher

**Significance of spatial variations of electron acceptors**

Electron acceptor availability is a function of the aquifer's oxidation capacity. Generally, the more oxidative the aquifer, the more readily BTEX will degrade. Oxidative capacity is expressed in terms of assimilative capacity. The assimilative capacity of an aquifer is determined in this way:

- estimate concentrations of electron acceptors available for biodegradation
- calculate difference between background and plume electron acceptors (DO, NO<sub>3</sub> and SO<sub>4</sub>) or metabolic by-products (Fe(II) or CH<sub>4</sub>); metabolic Fe(II), for instance, is a measure of iron reduction, whereas CH<sub>4</sub> is a measure of methanogenesis

- with the difference, multiply by the ratio of the BTEX mass biodegraded to the electron acceptor mass utilized or by-products produced; mass ratios are based on theoretical stoichiometric equations that assume full mineralization of BTEX to carbon dioxide.

"The result is a concentration of BTEX that can potentially be biodegraded in 1 m<sup>3</sup> of saturated soil. Comparison of the assimilative capacity to measured BTEX concentrations provides an initial estimate of the potential for intrinsic biodegradation of a plume." Schreiber and Bahr (1999) mention that analytical biodegradative screening models are available to estimate assimilative capacity as applied to biodegradation potential, but the user should be aware of the models' assumptions and applicability.

### Electron acceptor spatial variation and utilization efficiency

Data from the multilevel sampling indicate that electron acceptor concentrations vary both laterally and vertically. The vertical variability is not accurately represented by the monitoring wells because they are screened over different groundwater zones, and samples often give misleading information. "Errors in assimilative capacity estimates can arise if estimates are based on data from monitoring wells that are screened over multiple groundwater zones, or if the samples are collected from a completely different groundwater zone. . . in addition to yielding inaccurate estimates of assimilative capacity, use of spatially inappropriate data leads to erroneous interpretations of the dominant electron-accepting processes. Based on monitoring well data, calculations of assimilative capacity would suggest that sulfate reduction is the most important electron-accepting process." The multilevel data, however, indicate that iron reduction and methanogenesis are actually more important than sulfate reduction (*Schreiber and Bahr, 1999*).

Assuming that only contaminants use electron acceptors is erroneous: natural organic matter from the wetland, for instance, will consume DO and nitrate. Furthermore, "If the electron acceptor demand due to natural processes observed in the pristine transect is considered in assimilative capacity calculations, results tell a different story. . . The greatest effect of the natural processes on electron acceptor depletion is observed in the shallow zone, where DO and nitrate concentrations decrease along the pristine transect. If these losses are accounted for, the assimilative capacity is lowered. . . This decreased electron acceptor efficiency has implications for remediation systems that seek to enhance biodegradation via electron acceptor addition. Due to non-BTEX losses, the amount of electron acceptor addition required to achieve the optimal biodegradation may significantly exceed that predicted by stoichiometric relationships" (*Schreiber and Bahr, 1999*).

### Conclusions

From their study of electron acceptors in pristine and contaminated zones, researchers conclude the following:

- several distinct geochemical zones exist, and they are controlled primarily by microbially mediated reactions
- although BTEX and other compounds in the plume exert the highest demand for electron acceptors, oxidation of natural organic matter in the wetland also contributes to reduction of dissolved oxygen, nitrate and mineral-bound Fe(III)
- organic matter is likely competing with BTEX for electron acceptors, which is suggested by depletion of electron acceptors in the pristine zone
- samples containing a mixture of contaminated and less contaminated groundwater will underestimate Fe(II) concentrations and overestimate nitrate and sulfate concentrations
- the overall result of waters mixing here is to underestimate the aquifer's assimilative capacity, or the amount of potentially degradable contaminants
- if biases, including electron acceptor inefficiency and groundwater mixing, are not accounted for, assimilative capacity calculations will be inaccurate
- recognizing and quantifying the extent to which electron-accepting processes occur in the absence of contaminants is important
- identifying and using data collected from the spatial zone of interest is essential for accurate data interpretation

### Reference

Schreiber, M.E. and J.M. Bahr, "Spatial Electron Acceptor Variability: Implications for Assessing Bioremediation Potential," *Bioremediation Journal*, Vol. 3, No. 4, 1999.

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## UST design/installation factors

Although improved UST equipment and practices have reduced and eliminated some gasoline releases, equipment complexity combined with equipment failures and human error create conditions for UST releases. Incidents that can result in gasoline and/or MTBE releases include the following:

- failure of gasoline storage, piping, pumping or delivery system
- spillage during fuel delivery
- spillage during fuel dispensing
- vapor-phase losses

According to Davidson and Creek (1999) "Significant strides have been made as each successive generation of leak prevention and detection equipment was implemented. It seems to be industry consensus now that small, slow or subtle gasoline losses from USTs remain the primary challenge. Preventing these small/subtle spill sources becomes even more important when MTBE (or any other generally recalcitrant compound) is present in the gasoline."

After reviewing numerous documents, Davidson and Creek compiled several tables of information. The list in this article consists of 13 UST design and installation parameters/activities that parties responsible for UST management/operation should be aware of. Following are these UST design and installation parameters/activities of concern:

- preinstallation site analysis: includes factors such as site conditions, subsurface structures such as utilities and wells, site drainage and topography and corrosion protection requirements
- site design/layout: includes factors such as local requirements and permits, stormwater drainage system, shoring evaluation, customer entry/exit pathways and nearby structures/utilities
- materials/equipment selection: includes factors such as local requirements and permits, material selections and specifications, preinstallation inspection and testing
- excavation: includes factors such as OSHA/safety considerations and inspection/oversight requirements
- tank placement: includes factors such as tank bedding material and its placement/compaction, piping connections, drop tube for gauging, installer qualifications and inspection/oversight

- overfill protection: includes factors such as automatic shutoff devices and flow restrictors/ball float valves
- fillport spill containment: includes factors such as catchment basins/containment sumps, sump manway and inspection/oversight
- vapor recovery systems: includes factors such as system design/layout, Stage I and Stage II systems, vent lines and testing requirements
- product dispensers: includes factors such as dispenser pans, sensors, under dispenser piping and meter calibration
- pumping systems: includes factors such as suction pumping systems and sump sensors
- other equipment: includes waste disposal equipment and station spill kit
- tankpit and trench backfilling: includes factors such as initial UST system tightness test and soil placement/compaction
- documentation: includes factors such as facility plot plan, equipment warranties and O & M plan

In addition, many of these activities require that workers have proper qualifications, that work is inspected and that testing requirements are met. A companion article in the next issue of *UTTU* will address potential solutions.

### Reference

Davidson, J.M. and D.N. Creek, *Survey of Current UST Management and Operation Practices*, December 1999; published by the Center for Groundwater Restoration and Protection, National Water Research Institute, Fountain Valley, California 92728-0865; 741-378-3278; <http://www.ocwd.com/NWRI>.

For other recent reports on UST systems, see the U.S. EPA OUST site <http://www.epa.gov/swrust1/ustsystem/usteval.htm>.



## Information sources

### Recent publications

*Assessment of Phytoremediation as an In-Situ Technique for Cleaning Oil-Contaminated Sites*, published by the University of Saskatchewan, can be viewed or downloaded at <http://www.rtdf.org>.

*Biofiltration Project Report: Scale-up and Design Guide*, from the Center for Waste Reduction Technologies, American Institute of Chemical Engineers, New York, New York; <http://www.aiche.org/>.

*Bioremediation: Principles and Practices, Vol. I: Fundamentals and Applications* and *Vol. III: Bioremediation Technologies*, 1998, is available from Technomic Publishing Company, Inc., Lancaster, Pennsylvania; <http://techpub.com/tech/>.

*Biotreatment of Industrial and Hazardous Waste* is available from McGraw Hill Publishing Co.; <http://www.books.mcgraw-hill.com/>.

Environmental forensics books and videos, from amazon.com (<http://www.amazon.com>), include:

- *Environmental Forensics: Principles & Applications*
- *Environmental Law*
- *Glossary of Terms to Environmental Forensics*
- *Dictionary of Environmental Science for Lawyers*
- *Handbook of Environmental Data*
- *Hydrocarbon Chemistry*
- *Organic Chemistry on Video II*
- *Chemometrics in Environmental Analysis*
- *Chemometrics: A Practical Guide*
- *Chemometrics: Statistics and Computer Application in Analytical Chemistry*
- *Chemometrics: A Textbook*
- *International Environmental Law*
- *Environmental Law in a Nutshell*
- *How to Lie with Statistics*
- *Stable Isotope Geochemistry*
- *Statistics on Video*

*European Community Deskbook*, published by Environmental Law Institute, is available for \$89.95 by calling 800-433-5120 or 202-939-3844.

*Field Inspection Manual for Leak Detectors*, by Ken Wilcox Associates, Inc., gives descriptions, certification information, vendor contact information and operational procedures for many common leak detection systems. For a table of contents see [http://www.kwaleak.com/fields\\_manual.htm](http://www.kwaleak.com/fields_manual.htm), or call 816-443-2494.

*Guidelines for Successful Phytoremediation* is available from the American Institute of Chemical Engineers, <http://www.aiche.org/cwrt>, or call 800-242-4363.

*Handbook of Storage Tank Systems: Codes, Regulations and Designs* is available from Marcel Dekker, Inc., New York, New York; call 212-696-9000 or <http://www.dekker.com>.

*Innovative Remediation and Site Characterization Technology Resources* (EPA 542-C-99-001) is available from the U.S. EPA Innovation Office. The CD ROM can be ordered by calling 800-490-9198 or 513-489-8190.

*Natural Attenuation for Groundwater Remediation*, published by the National Research Council, is posted at <http://books.nap.edu/books/0309069327/html/index.html>.

*OnSite InSights*, a periodic newsletter produced by the Northeast Hazardous Substance Research Center, can be viewed or downloaded at <http://www.hsrg.org/hsrg/html/pub.html>. For hard copies, contact Andrea Kinney at 508-358-3532.

*Steel Tank Technology Guide* is available at no charge from STI at 847-438-8265.

*Strategies for Characterizing Subsurface Releases of Gasoline Containing MTBE* is available from the American Petroleum Institute; e-mail [ehs-api@api.org](mailto:ehs-api@api.org) or download from <http://www.api.org/mtbe>.

### U.S. EPA publications and software

*An In-Situ Permeable Reactive Barrier for the Treatment of Hexavalent Chromium and Trichloroethylene in Ground Water* provides detailed design, installation and performance monitoring data on full-scale PRB applications. The three volumes can be obtained from <http://www.epa.gov/ada/reports.html>. Contact Kay Cooper at [cooper.kay@epa.gov](mailto:cooper.kay@epa.gov) or 580-436-8651 for hard copies.

*Assessing Contractors' Capabilities for Streamlined Site Investigations* (EPA 542-R-00-001) and other Brownfields technical documents are available at <http://www.brownfieldstsc.org> or <http://clu-in.org/techpubs.htm>. For hard copies call 800-490-9198 or 513-489-8190 or fax 513-489-8695.

Department of Energy information is available on research efforts and applications of permeable reactive barriers. View or download at <http://clu-in.org/techpubs.htm>.

*ETV Verification Report: Environmental Decision Support Software, Environmental Software SitePro™ Version 3* (EPA 600-R-99-093) is software that integrates a geographic information system (GIS), computer-aided design, mapping, contouring, boring logs, cross-sections, graphing, imaging and reporting. See <http://clu-in.org/techpubs.htm> or <http://www.epa.gov/etv/verifprt.htm#02>.

*Hydraulic Optimization Demonstration for Groundwater Pump-and-Treat Systems, Vol. 1: Pre-Optimization Screening (Method and Demonstration)* (EPA 542-R-99-011A) and *Vol. 2: Application of Hydraulic Optimization* (EPA 542-R-

99-011B) provide spreadsheets on how to optimize existing or planned pump-and-treat designs. View or download at <http://clu-in.org/techpubs.htm>; for a hard copy, contact 800-490-9198 or 513-489-8190 or fax 513-489-8695.

*Hydrocarbon Spill Screening Model v. 1.50* simulates petroleum emplacement during a defined release scenario, formation of fuel lens in the subsurface, dissolution in groundwater and fate and transport in a shallow aquifer. For information, contact [weaver.jim@epa.gov](mailto:weaver.jim@epa.gov).

*Innovative Treatment Technology Developer's Guide to Support Services, Fourth Edition* (EPA 540-B-99-008) lists more than 100 organizations, government programs and on-line databases that can provide business support to developers. View or download at <http://clu-in.org/techpubs.htm>; for hard copies, contact 800-490-9198 or 513-489-8190 or fax 513-489-8695.

*Introduction to Phytoremediation* (EPA 600-R-99-107) defines terms and provides a framework to understand phytoremediation applications. View or download at <http://clu-in.org/techpubs.htm>.

*LUSTRisk*, a spreadsheet program for estimating risk of migrating benzene and MTBE, is at the peer-review stage. Those willing to test the software and provide comments to the authors should contact Matt Small at 415-744-2078 or e-mail [small.matthew@epa.gov](mailto:small.matthew@epa.gov).

*Natural Attenuation of MTBE in the Subsurface under Methanogenic Conditions*, EPA 600-R-00-006, can be viewed or downloaded at <http://clu-in.org/techpubs.htm>, or call Kay Cooper at 580-436-8651 or fax 580-436-8503.

OUST publications, available from <http://www.epa.gov/oust/pubs>, include

- *Catalog of EPA Materials on Underground Storage Tanks* (EPA 510-B-00-001)
- *List of Known Insurance Providers for Underground Storage Tanks* (EPA 510-B-00-004)
- *Underground Storage Tank Program Directory* (EPA 510-B-00-005)

*Profiles of Innovative Technology Providers* (EPA 542-R-99-012) provides technical, financial and stock analysis of six years of data from EPA's former Vendor Innovation Office. View or download at <http://clu-in.org/techpubs.htm>. For hard copies, call 800-490-9198 or 513-489-8190 or fax to 513-489-8695.

*OnSite*, a collection of on-line calculators for solving basic contaminant fate and transport equations, simple models and unit conversion factors, can be accessed at <http://www.epa.gov/athens/software/training/WebCourse/part-two/onsite/index.htm> or contact [weaver.jim@epa.gov](mailto:weaver.jim@epa.gov).

*Risk-based Decision Making Performance Assessment Study*, Bulletin No. 2, summarizes a study to develop practical, quantitative measures for evaluating the impact of RBDM on

achieving state goals. The bulletin also contains an application of these measures to five states and provides guidelines for others interested in tracking RBDM performance programs. This bulletin is available at <http://www.epa.gov/OUST/rbdrm>. Also see ASTM, <http://www.astm.org>, and the RBCA State Policy Issues Database, <http://www.gsi-net.com/RBCAPOL>.

*The National Contingency Plan Product Schedule* from the U.S. EPA Oil Program Center contains information on dispersants, surface washing agents, surface collecting agents, bioremediation agents and miscellaneous oil spill control agents. Data can be viewed or downloaded at <http://www.epa.gov/oilspill/ncp.htm>.

*Understanding Oil Spill Response* (EPA 540-K-99-007) can be obtained by calling 800-490-9198 or 513-489-8190 or faxing 513-489-8695.

*Understanding Variation in Partition Coefficient,  $K_d$ , Values* (EPA 402-R-99-004A&B) can be obtained from <http://www.epa.gov/radiation/technology/partition.htm> or call 800-490-9198 or 513-489-8190 or fax request to 513-489-8695.

U.S. Department of Energy Subsurface Contaminants Focus Area reports:

- Chemical oxidation: <http://www.envnet.org/scfa/tech/dnapl/factsheets/tms167.pdf>
- EPA Chemical Oxidation report: <http://www.epa.gov/swertiol/download/remed/chemox.pdf>
- Fenton's reagent: <http://www.envnet.org/scfa/tech/dnapl/factsheets/tms2161.pdf>

*Wellhead AnalyticElement Model (WhAEM)* provides an interactive computer environment for design of protection areas based on radius methods, uniform flow solutions and geohydrologic modeling methods. Contact [kraemer.steve@epa.gov](mailto:kraemer.steve@epa.gov) for more information.

## Websites and web publications

Advisory to UST Owners/Operators Regarding Ethanol-Blend Fuel Compatibility, <http://www.swrcb.ca.gov/cwphome/ust/ethanol.htm>

Brownfields Technology Support Center  
<http://brownfieldstsc.org/>

Centerpoint, a periodic newsletter from the Southeast Hazardous Substance Research Center  
<http://hsrc.org/hsrc/rbriefs/RB1/rbrief1.html>

Center for the Evaluation of Risks to Human Reproduction  
<http://www.cerhr.niehs.nih.gov/>

ChemicalDesk, exchange of water treatment chemicals and services, <http://www.chemicaldesk.com>

Compatibility of remediation, well construction and sampling materials with nonaqueous phase liquids  
<http://www.epa.gov/swertiol/tsp/download/napl.pdf>

Cost and Performance Reports, [http://www.estcp.org/technical\\_documents.htm](http://www.estcp.org/technical_documents.htm)

Environment Canada Bioremediation Archive, archived message links from the Bioremediation Newsgroup <http://gw.cciw.ca/lists/bioremediation/>

Environmental Expert.com, includes environmental news, resources, software guide and links, <http://www.environmental-expert.com>

Environmental Working Group, <http://www.foodnews.org>

Field Analytic Technologies Encyclopedia (FATE), an online encyclopedia of field analytic technologies <http://fate.clu-in.org>

Gehm Environmental, for a paper on subsurface hydrocarbon mapping, <http://www.gehm.com>

GeoPotential, <http://members.aol.com/rpsiii/geomain.htm>

GeoTracker, a geographic information system that contains water quality, water use and infrastructure data for California, <http://www.swrcb.ca.gov/cwphome/UST/ethanol.htm>

Groundwater Circulating Well Technology Assessment (NRL/PU/6115-99-384), [http://www.estcp.org/technical\\_documents.htm](http://www.estcp.org/technical_documents.htm)

Ground Water Currents (EPA 542-N-99-008), <http://clu-in.org/techpubs.htm>

Human Factors Assessments of Environmental Technologies program, <http://hazmat.vv.net/>

Improving the Cost-Effectiveness of Hazardous Waste Site Characterization and Monitoring, <http://clu-in.org/techpubs.htm>

*Journal of Industrial Ecology*, <http://www.yale.edu/jie/dissert1.htm>

Management of Contaminated Sediments—Research Supporting a Risk-Based Approach, a slide presentation <http://maven.gtri.gatech.edu/ws/title.html>

Phytoremediation Decision Tree, produced by Interstate Technology Regulatory Cooperation Workgroup <http://clu-in.org/techpubs.htm>

Remedial Systems Evaluation Checklists, <http://www.environmental.usace.army.mil/library/guide/guide.html>

Science Applications International Corp. <http://www.greenonline.com/brownfields>

Sensor Technology Information Exchange <http://www.wpi.org/sensors/>

Site Assessment Quick Reference Guidance Series, <http://www.epa.gov/superfund/programs/siteasmt/index.htm#site>

Tank Scope, <http://www.TankTech.com>

Technology Status Review: In-Situ Oxidation, published by DoD Environmental Security Technology Certification Program, [http://www.estcp.org/technical\\_documents.htm](http://www.estcp.org/technical_documents.htm)

**U.S. Geological Survey sites**

- Introduction to the Quality of Our Nation's Waters, <http://www.water.usgs.gov/pubs/circ/circ1225/html/intro.html>
- Table of Contents, The Quality of Our Nation's Waters: <http://www.water.usgs.gov/pubs/circ/circ1225/>
- National findings and their implications for water policies and strategies: water-quality patterns in agricultural areas, [http://www.water.usgs.gov/pubs/circ/circ1225/html/wq\\_agric.html](http://www.water.usgs.gov/pubs/circ/circ1225/html/wq_agric.html)
- National findings and their implications for water policies and strategies: water-quality patterns in urban areas, [http://www.water.usgs.gov/pubs/circ/circ1225/html/wq\\_urban.html](http://www.water.usgs.gov/pubs/circ/circ1225/html/wq_urban.html)

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*UTTU obtained many of these sites and other information from the Groundwater Mailing List (<http://groundwater.com>), the Bioremediation Discussion Group (<http://biogroup.gzea.com>) and TechDirect (<http://clu-in.com/techdrct.htm>). UTTU thanks the moderators/editors from these groups—Ken Bannister of Groundwater, Richard Schaffner of Biogroup and Jeff Heimerman from U.S. EPA's TechDirect.*

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