Underground Tank Technology Update

Vol. 17, No. 6 November/December 2003

COLLEGE OF ENGINEERING
UNIVERSITY OF WISCONSIN-MADISON

uttu.engr.wisc.edu | Department of Engineering Professional Development

Underground Tank Technology Update is an electronic bimonthly publication of the University of Wisconsin–Madison, Department of Engineering Professional Development. *UTTU* supplies useful information to federal, state, and local officials working with groundwater technology and to other interested technical specialists.

UTTU is funded by the U.S. EPA under cooperative agreement No. 82933001 to the University of Wisconsin–Madison, which is responsible for its preparation. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Comments and suggestions are welcome and may be directed to John T. Quigley, Project Director, 432 N. Lake St., Madison, WI 53706. Tel 608-265-2083. To comment on an article, or to suggest topics for *UTTU*, please e-mail Pat Dutt or call 607-272-3212.

Staff

John T. Quigley	Project Director
Pat Dutt	Geologist/Writer
Darrell Petska	Copy Editor
Debbie Benell	Program Assistant

Free Subscription To receive this electronic newsletter, e-mail Debbie Benell or call 608-263-7428. There is no cost to subscribers.

Brief article summaries

Notice: Funding Available

Funding is available for creative and innovative projects promoting energy recovery, waste minimization, recycling, environmental protection through land revitalization and homeland security. Those eligible for funding include EPA headquarters, OSWER programs, federal/state/local regulatory agencies, tribes, non-profit groups and academia. Proposals must be submitted by December 5, 2003. For details, go to http://www.epa.gov/oswer/iwg

UST legislation update

By Mike Barolo, U.S. EPA, OUST

Congress recently introduced two pieces of UST legislation: on October 2, Representative Capps introduced H.R. 3231, "Underground Storage Tank Reform and Compliance Act of 2003," and on October 17, Representative Gilmore introduced H.R. 3335, "Underground Storage Tank Compliance Act of 2003." The House Energy & Commerce Committee's Environment and Hazardous Materials Subcommittee has not taken action on either bill, or on H.R. 2733, which was introduced in July.

Simultaneously, Congress has almost completed an omnibus energy bill. Both the Senate and House have passed energy bills, and a conference committee is currently crafting a compromise bill to send to each house for a final vote. While both the Senate and House versions of the energy bill contained a few provisions directly modifying the UST program, the conference committee plans to incorporate a more comprehensive UST bill into the omnibus energy bill. We believe the conference committee intends to include H.R. 3335, or something very close to it, in the final energy bill. Congress is working hard to finalize the energy bill before this session ends (probably in mid-November). A few contentious provisions have held up passage of the energy bill, and we don't know if the issues will be resolved in the near future. Given the strong interest in completing an energy bill, it is likely that Congress will pass an energy bill this session. If Congress does not, they will likely take up the energy bill again when they return after the new year.

Because we believe that Congress plans to include H.R. 3335 (or something very close to it) in the energy bill, I have included a summary of the major provisions. H.R. 3335 is largely based on S. 195, which the Senate passed in May, but contains some important differences.

OUST will continue to monitor the progress of this and other legislation affecting the UST program. We will also continue to provide technical assistance as appropriate.

Major Provisions in H.R. 3335

The 11 major provisions in H.R. 3335 follow.

Authorization for Appropriations: H.R. 3335 authorizes $605 million per year from FY04–FY08.

Expansion of Eligible Uses of LUST Trust Fund: H.R. 3335 expands eligible uses to include various compliance and enforcement activities.

Mandatory Inspections: H.R. 3335 requires on-site inspections within two years for all USTs that have not been inspected since December 22, 1998. After completion of previously uninspected facilities, the bill requires inspection of all USTs every three years. EPA may grant a one-year extension to the first three-year period. The bill also requires EPA to submit a report to Congress on alternative inspection programs within four years.

Use of LUST Funds by State Funds: H.R. 3335 allows state assurance funds to use LUST funds to pay for cleanup in financial hardship cases. Cost recovery is prohibited in these instances. Any state, however, that diverts funds from their state fund for non-UST purposes is no longer eligible to use LUST funds in this way. The bill also grants EPA the authority to withdraw approval of a state fund to be used as a financial responsibility mechanism in SPA states without having to withdraw state program approval.

Operator Training: H.R. 3335 requires all UST operators to be trained. The bill requires EPA to develop training guidance and requires states to develop training requirements based on EPA's guidance. Operators must be trained in accordance with state requirements, initially, and again, if their facility is out of compliance.

Cleanup of Fuel Oxygenates: H.R. 3335 provides specific authority (as well as specific authorization for appropriation) to clean up releases of fuel containing oxygenates.

Compliance Reports/Strategies: H.R. 3335 requires the following:

- states must develop a compliance report on state/local/federal government-owned facilities
- EPA must develop a strategy to ensure compliance and cleanup of USTs in Indian Country and submit a report to Congress summarizing implementation status
- federal agencies must develop a compliance report on federally owned USTs

Public Record: H.R. 3335 requires EPA/states to maintain and make available to the public the following:

- a public record of the number of sources and causes of UST releases
- a record of compliance
- the number of equipment failures

Delivery Prohibition: Within two years of passage, H.R. 3335 makes it illegal to deliver products to a UST at a facility that the EPA or a state has identified as ineligible for delivery. EPA and states must develop both a delivery prohibition roster (made publicly available on the Internet) listing the ineligible facilities, and a process for installing tamper-proof "boots" blocking the fill pipes for these USTs. EPA and states have one year to develop guidance documenting their processes and procedures to implement these provisions.

Waiver of Sovereign Immunity: H.R 3335 clarifies that states may impose penalties against federal facilities for UST violations.

Release Containment: H.R. 3335 requires EPA to submit a report to Congress on the effectiveness of alternative methods for containing UST releases.

Effect of ethanol on remediation performance

This article, excerpted from "Increased Use of Ethanol in Gasoline and Potential Ground Water Impacts," describes how ethanol affects remedial strategies such as source removal, solvent flushing, vapor extraction, pump-and-treat, bioremediation, and natural attenuation (Powers and others, 2001).

Source removal, solvent flushing and soil vapor extraction

Source removal approaches, such as free-phase recovery, may benefit from changes in gasoline distribution when ethanol is present. Solvent flushing techniques using ethanol have been demonstrated to mobilize trapped non-aqueous-phase liquids toward recovery wells. Soil vapor extraction will likely have little value in removing ethanol because the ethanol will be strongly partitioned into any unsaturated zone pore water. On the other hand, the increased flow of air through the unsaturated zone will stimulate aerobic biodegradation of ethanol.

Pump-and-treat

The efficiency of pumping systems designed to contain groundwater contaminant plumes should not be directly affected by ethanol. However, surface treatment of this water by activated carbon may be adversely affected because the sorption capacity of the filter would be exhausted faster due to ethanol adsorption.

Bioremediation

Ethanol is likely to have a negative effect on many current BTEX bioremediation practices. Engineered BTEX bioremediation systems often involve stimulating microbial activities by supplying nutrients and electron acceptors (e.g., O_2 and sometimes NO_3^-), with success often limited by the ability to distribute the stimulating materials throughout the contaminated zone. Ethanol is likely to be present at much higher concentrations than BTEX, which would significantly exacerbate the biochemical oxygen demand (BOD) and nutrient requirements. Thus, maintaining aerobic conditions and precluding nutrient limitation in the contaminated zone could represent a significant increase in treatment costs and a major technical challenge at some sites. In addition, engineered bioremediation works best for high-permeability aquifers ($> 10^{-5}$ m/s), and an ethanol-supported increase in biomass concentration could reduce aquifer permeability and ability to distribute nutrients and electron acceptors throughout the contaminated zone. Clogging problems near injection well screens and infiltration galleries may also be exacerbated due to additional bacterial growth on ethanol.

In sanitary engineering, anaerobic processes are generally used for pre-treatment of high-strength industrial wastewater (e.g., BOD > 1,000 mg/l) because of the high costs and technical difficulties associated with oxygen supply. The effluent from anaerobic reactors is then usually treated aerobically prior to discharge. This suggests that a sequential anaerobic-aerobic approach might be desirable for bioremediation of gasohol-contaminated sites. For example, anaerobic electron acceptors such as nitrate could be injected to accelerate the removal of ethanol and some BTEX contamination. This would alleviate the BOD of the system for more efficient degradation of any residual BTEX in a subsequent aerobic (polishing) stage. Note that anaerobic degradation of ethanol would result in less biomass accumulation (and related clogging problems) than would occur under aerobic conditions, because anaerobic cell yield coefficients are significantly lower. Recent studies have also suggested that anaerobic strategies for in-situ bioremediation of petroleum-contaminated subsurface environments may be as effective as aerobic approaches. This notion is similarly based on the fact that introducing sufficient oxygen can be technically difficult and expensive, whereas anaerobic electron acceptors can be easily added to the subsurface and are chemically more stable. Thus, enhanced anaerobic remediation strategies may become more frequently applied to deal with gasohol releases.

Natural attenuation

Natural attenuation is likely to be hindered by ethanol, due to its preferential degradation and the accompanying depletion of oxygen and other electron acceptors that would otherwise be available to support BTEX degradation. One possible outcome is that ethanol would increase the distance that BTEX compounds migrate before attenuating processes decrease their concentrations to acceptable levels. Longer BTEX plumes represent a greater probability of exposure to downgradient receptors and, thus, decreased acceptability of natural attenuation at some sites.

MTBE

If a site has an existing MTBE plume, cleanup of a gasohol release should not significantly affect the existing site cleanup strategy. Since an MTBE plume can be expected to be more mobile and less biodegradable than either ethanol or BTEX, the monitoring and mass extraction approaches used for MTBE will be generally adequate for ethanol and BTEX as well.

Reference

Powers, S., Alvarez, P.J. and D.W. Rice, "Increased Use of Ethanol in Gasoline and Potential Ground Water Impacts," Chapter 1 in *Environmental Assessment of the Use of Ethanol as a Fuel Oxygenate: Subsurface Fate and Transport of Gasoline Containing Ethanol*, Report to the California State Water Resources Control Board, UCRL-AR-145380, October 2001; http://www-erd.llnl.gov/ethanol/pdf_files/Start.htm

Gasohol infiltration and spreading

By Susan E. Powers and Cory J. McDowell

We have very little information on the behavior of ethanol-containing gasoline as it infiltrates the unsaturated zone. Recent studies, however, indicate that adding ethanol to gasoline changes the nature of the capillary phenomena that affect gasoline infiltration and distribution. This occurs at the water table where extensive, rapid partitioning of ethanol into soil moisture and reduced interfacial and surface tensions between phases increase gasohol infiltration.

Fate of ethanol-blended gasoline in the subsurface

The presence of oxygenate chemicals in gasoline can potentially impact their migration and fate in the subsurface following a spill or leak. Numerous processes affect the concentration of such chemicals in aquifers. Generally, these processes include

- infiltration of gasoline through the unsaturated zone
- spreading of the gasoline pool at the water table
- dissolution of slightly soluble species from gasoline into water
- transport of these chemicals with groundwater toward a potential point of contact

Differences in ethanol's biodegradability and hydrophobicity relative to standard gasoline components contribute to the impact of ethanol on the overall fate of BTEX species in groundwater.

Mechanisms that affect gasohol fate during infiltration and spreading

Gasoline from a leak or spill infiltrates the unsaturated zone of the subsurface predominantly under gravitational forces. A fraction of the gasoline is retained in the pore spaces due to interfacial forces, creating a residual saturation of gasoline that generally occupies 1-7 percent of the pore space in the unsaturated zone. Gasoline that migrates to the top of the capillary fringe begins to spread horizontally as it floats on the water. Some gasoline will also continue to migrate vertically and laterally into air-filled voids within the capillary fringe. The net result of the spill is the accumulation of the bulk of the gasoline volume in a pool within the capillary fringe. A decline in the elevation of the water table can result in the spreading of gasoline further into the saturated zone. Droplets or blobs of LNAPL would then be trapped in pore spaces at a lower elevation as the water table elevation increases.

Ethanol changes two primary properties that control the fate of gasoline in the subsurface. The partitioning of ethanol between the gasoline and the aqueous phase is significantly greater than the partitioning of petroleum hydrocarbons, leading to an increased flux of contaminants to the groundwater. The surface and interfacial tensions that cause interfacial forces, which result in entrapment of residual gasoline in the saturated and unsaturated zones, are reduced, leading to less capillary entrapment. Both of these properties change when ethanol is added to gasoline because ethanol is very hydrophilic, whereas petroleum hydrocarbon constituents are hydrophobic. The sections that follow provide a review of the present understanding of these two properties.

Partitioning behavior

Standard-formulation gasoline and water are almost completely immiscible. For example, Polak and Lu (1973) measured the aqueous solubilities of 21 gasoline compounds. The reported values ranged between 0.54 mg/l and 1,800 mg/l for 2,2,5-trimethylhexane and benzene, respectively, at 25°C. Solubilities of water in gasoline compounds range between 84 mg/l and 690 mg/l in 2,3,4-trimethylpentane and benzene, respectively. The reported solubilities do not include the effects of surface-active agents or other gasoline additives that may contain polar components in the molecular structure.

In contrast, ethanol is completely miscible in both gasoline and water at all concentrations. When ethanol is present with both gasoline and water, the preferential partitioning of ethanol into the aqueous phases modifies the overall phase behavior such that water is more soluble in gasoline, and gasoline components are more soluble in water. With a sufficiently large fraction of ethanol in the system, the gasoline and water become completely miscible with each other and merge into a single phase. Conversely, at lower ethanol concentrations, gasoline may separate into two phases if water is added to the blend.

Ethanol partitioning and the effects of ethanol on solubility are illustrated on a ternary phase diagram (Figure 1). Using the three axes, one can determine the overall system mass fractions of gasoline, water and ethanol for any point on the interior. The shaded region indicates the range of water, gasoline and ethanol fractions where the three components exist as two separate phases, while the unshaded region indicates the composition range where these components exist as a single phase. The curve separating the two regions is called the binodal curve. On Figure 1 the binodal curve indicates that gasoline, ethanol and water will exist as a single phase in all relative combinations of water and gasoline, provided that the ethanol present in the entire system exceeds 70 weight percent.

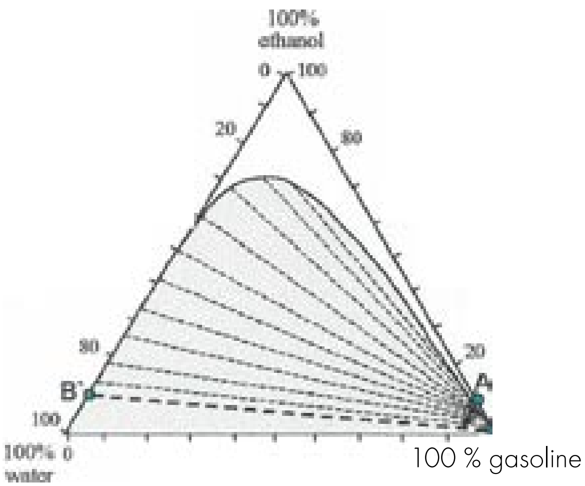
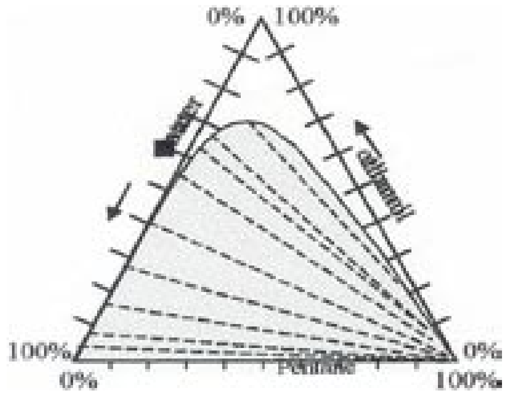
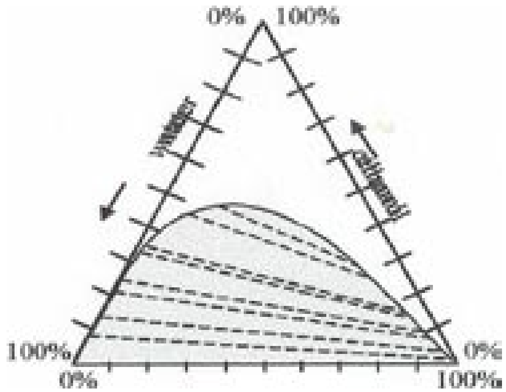


Figure 1. Ternary phase diagram for gasoline-ethanol-water system at 25°C (adapted from de Oliveria, 1997). Axes indicate percent of total mass.



2,2,4-trimethylpentane



toluene

Figure 2. Ternary phase diagram for 2,2,4-trimethyl-pentane-ethanol-water system and toluene-methanol-water system at 25°C. Axes indicate mole percentages (adapted from Peschke and Sandler, 1995).

Please note: Clearer representations of Figures 1 and 2 are available in the Web site of the Powers and McDowell reference at the end of this article.

Tie lines transect the two-phase region and join water-rich and gasoline-rich segments of the bimodal curve. These tie lines indicate the equilibrium compositions of the two phases. The points connected by the lines define the composition of the water-rich phase (on the left side of the two-phase region). The downward slope (left to right) of the tie lines indicates the preferential partitioning of ethanol into water.

The ternary phase diagram in Figure 1 illustrates the separation of phases following the addition of a small amount of water. The composition of a gasoline is indicated by point A. If water is added to this, the composition gradually changes as indicated by the arrow. Once the composition reaches that indicated by the end of the arrow, the single phase will split into two liquid phases with the compositions indicated by points A` and B`.

For smaller fractions of ethanol, as expected for RFG (reformulated gasoline), much smaller quantities of water are required to cause phase separation. For instance, the American Society for Testing and Materials (ASTM) method D4806 states that if water is added at a concentration of 0.5 percent (by mass) to a gasoline containing 10 percent ethanol (by volume) and 14 percent aromatics (BTEX compounds) at 7°C (45°F), then the gasoline will separate into an alcohol-rich aqueous phase and a hydrocarbon phase.

Because gasoline is a highly complex mixture of alkane, cycloalkane, alkene and aromatic compounds, changes in the gasoline composition may affect the phase behavior. To illustrate this, ternary phase diagrams for two individual components found in gasoline–2,2,4-trimethylpentane (an alkane) and toluene (an aromatic hydrocarbon)–are illustrated on Figure 2. A lower ethanol fraction would be required for phase separation if toluene were the hydrocarbon than if 2,2,4-trimethylpentane were the hydrocarbon. Additionally, the slopes of the tie lines in this figure indicate that ethanol partitions less preferentially into water from toluene than from 2,2,4-trimethylpentane. Assuming that toluene is representative of the general phase behavior of the aromatic hydrocarbons and that the 2,2,4-trimethylpentane represents the properties of alkanes, cycloalkanes and alkenes, then one could generalize that if the fraction of aromatics in the gasoline were increased, the single-phase region would expand. Upon phase separation, the gasoline would contain slightly more ethanol.

Ethanol effect on capillary forces and multiphase flow

The addition of oxygenates to gasoline changes the nature of the capillary phenomena affecting gasoline infiltration and distribution at the water table due to a reduction in the interfacial and surface tensions between the phases. Data were collected for real gasolines equilibrated with water and ethanol. C2 gasoline, for instance, a reformulated gasoline containing 5.8 percent ethanol by volume, was developed by Phillips Chemical Company and certified for testing in California. Ethanol contents were varied by altering the gasoline-water volume ratio or by adding ethanol to the system.

The interfacial tension (IFT) of the C2 system decreased in an almost perfectly linear way from 26.3 dynes/cm at 0 percent ethanol to 7 dynes/cm at 50 percent ethanol – a decrease of about 75 percent. In contrast, neat solvents and a surrogate gasoline comprised of xylene (20 percent by volume) and isooctane had a much more significant decrease in the IFT with the addition of small volumes of ethanol (<10 percent) to the aqueous phase. These differences suggest that some of the other components of the C2 gasoline also strongly affect the interfacial tension and that surrogate mixtures of mono-aromatics and alkanes are not representative surrogates for gasolines in studies of the gasoline migration and distribution at the water table.

The surface tension of water after equilibrating with benzene and ethanol drops substantially with increase in ethanol. In contrast, the organic-air surface tensions are fairly constant with increasing ethanol concentration. The surface tension of the C2 gasoline following equilibration with water and added ethanol was 19.7 ±0.25 over the range of ethanol contents...The independence of the gasoline-air surface tension can be explained by the nearly complete partitioning of the ethanol into the aqueous phase.

Due to the reduction in capillary force associated with the changes in interfacial and surface tensions, the height of the capillary fringe is reduced and depth of the gasoline pool altered. The gasoline can also enter smaller pore spaces, potentially affecting its distribution in the vadose zone and in the gasoline pool. To estimate the depth of a gasoline pool, a model based on hydrodynamics and capillary forces expresses the depth in terms of gasoline and water densities, NAPL surface tension and NAPL-water interfacial tension, maximum saturation of the mobile gasoline phase and a parameter that characterizes the pore-size distribution... This model can be reduced to a direct dependence of gasoline pool depth on the NAPL-water interfacial tension. Thus, a reduction in the interfacial tension is expected to result in a decrease in the thickness of the gasoline pool and a significantly larger areal extent of the gasoline pool. (For more recent data on this phenomenon, see "Another study of ethanol infiltration" in this issue of *UTTU*.)

Recent studies: miscible vs. non-miscible displacement

Researchers at the University of Waterloo, Canada, have conducted the most extensive study of the impact of alcohol as an oxygenate in gasoline on the behavior of the gasoline in the subsurface. They studied the dynamics of M-85 (85 percent methanol and 15 percent gasoline) with respect to infiltration and migration in both saturated and unsaturated systems. The very high alcohol content of this gasoline resulted in a change from the immiscible displacement phenomena (associated with standard gasolines) to a miscible displacement process. Differences in density and viscosity become more important in miscible displacement processes than interfacial tension. Based on the unsaturated zone infiltration experiments, the behavior of the M-85 gasoline relative to the standard gasoline used for comparison can be characterized by:

- less lateral spreading in the unsaturated zone
- a decrease in the height of the capillary fringe, which resulted in the formation of the gasoline pool at a lower elevation (the depth of the capillary fringe returned to its original position after the methanol was flushed from the system)
- the formation of a gasoline pool at the water table with a smooth and regular geometry that was smaller in lateral extent
- the formation of air bubbles in the area where the M-85 displaces water, due to non-ideal mixing of the methanol and water that causes a reduction in the volume of the mixture
- the formation of a "halo" of droplets of gasoline around the periphery of the gasoline pool and infiltration zone due to reduced methanol concentrations and reduction in the effective solubility of the gasoline constituents

The formation of residual droplets of gasoline in the saturated zone substantially increases the NAPL-water contact area for dissolution. In addition to the formation of these droplets by precipitation (when the alcohol concentration drops), droplets can also be formed by multiphase displacement mechanisms as the water table elevation changes. Scientists who studied the changes in hydrocarbon entrapment at the bottom of a pool as the water table fluctuated found that by reducing the interfacial tension from approximately 40 to 10 dynes/cm (with the addition of isopropyl alcohol), the hydrocarbon volume trapped in the saturated zone decreased from 11 to 6 percent of the total pore space. Thus, high alcohol concentrations tend to increase the probability of precipitation but reduce the probability that droplets will form during multiphase displacement.

Other studies

- used ethanol as a flushing agent to enhance trapped NAPL dissolution and recovery
- modeled ethanol-water solutions displacing pure water in a two-dimensional sandbox that represented an unconfined aquifer; displacing fluid was introduced through an injection well, not through the unsaturated zone; when the IFT of the water in the unsaturated zone was reduced, that field capacity was reduced, thereby increasing contaminant transport rate from the vadose to the saturated zone; two observations were made:
  - the ethanol solution preferentially stayed near the top of the sandbox due to its lower density (as opposed to clean water)
  - the capillary fringe was reduced in height by approximately 50 percent, the same percentage as the decrease in the air-water surface tension of the ethanol solution

It is very difficult to predict how the effects described above would compare with the spill of an ethanol gasoline containing 10 percent or less ethanol by volume (see *UTTU* article that follows, "Another study of ethanol infiltration"). If mass transfer of the ethanol to the aqueous phase is rapid relative to the rate of gasoline infiltration, some miscible displacement dynamics could possibly dominate the overall behavior of the infiltrating gasoline. Most of the ethanol in this case could partition into the aqueous phase, causing a change in the relative permeability of fluids in the vadose zone as the aqueous phase swells. Drainage of the ethanol-laden aqueous phase at a later time could dissolve BTEX compounds from the gasoline in a manner different from that anticipated from a gasohol pool. Changes in gasohol retention in the vadose zone and the gasoline pool size and shape at the water table would occur as ethanol partitions into the aqueous phase, resulting in reduced surface and interfacial tensions and the reduced height of the capillary fringe. The extent of these effects is not known.

Other research

We performed other research to explore the impacts of ethanol on gasoline migration and distribution in the unsaturated zone. Research was done in a two-dimensional sand tank. We considered two spill scenarios:

- a spill of ethanol-blended gasoline
- a spill of denatured ethanol after a prior spill of standard formulation gasoline

We found that under conditions tested to date, the retention of ethanol in the unsaturated zone actually creates an initial pool almost identical to that of a gasoline spill. The mass transfer of ethanol to the saturated zone is spread out over a long period of time due to the retention and slow drainage of ethanol from the unsaturated zone. This in effect would cause the biological electron acceptor demand in the vicinity of the source area to also be spread over a longer period of time. With the retention of ethanol in the unsaturated zone, biodegradation of ethanol will occur in this region where sufficient oxygen may be present.

The fraction of ethanol that does partition into the aqueous phase versus that which travels with the gasoline pool depends predominantly on the volume of soil and residual water in the unsaturated zone into which the ethanol can partition. This means that the gasoline spill rate, areal extent of the spill area, residual water saturation and depth to the capillary fringe are important variables that will affect the net impact of ethanol on the distribution of gasoline in the subsurface.

Ethanol likely does have some positive impact on remediation efforts. Ethanol in the unsaturated zone will likely stimulate the microbial community, contributing to ethanol and BTEX biodegradation in the unsaturated zone. Pumping the gasoline during recovery efforts will also be improved with the increased LNAPL saturations in the pool area into which the ethanol drains. One should note, however, that a region with a high concentration of ethanol could also have an increased flux of contaminants into the groundwater due to cosolvent effects.

(For a more complete description of this research, access the Web site given at the end of this article.)

Conclusions from research

Our research indicated that adding ethanol to gasoline changes the nature of the capillary phenomena affecting gasoline infiltration and distribution at the water table due to reduced interfacial and surface tensions. These changes result in the following:

- less residual gasoline is trapped in the unsaturated zone following an ethanol-blended gasoline spill compared with a standard gasoline; the reduced surface and interfacial tension in the presence of ethanol results in more complete drainage of fluids from the region
- a significant fraction of ethanol partitions into the residual saturation in the unsaturated zone during gasoline infiltration; depending on the spill volume, this ethanol could then drain slowly into the gasoline pool, creating a central region with high ethanol concentration; the remainder of the pool appears much the same as a spill of gasoline without ethanol

- the fraction of ethanol retained in the unsaturated zone depends greatly on the volume of soil involved, the water content and the rate that gasoline enters the subsurface; the rate that the ethanol-laden water in the unsaturated zone drains to the capillary fringe is limited by the increased viscosity and, therefore, reduced unsaturated hydraulic conductivity of this phase; functional relationships to describe these processes have not yet been developed

Bulk ethanol at bulk distribution terminals can affect the behavior of previously released fuel hydrocarbons in the following ways:

- bulk ethanol dissolves and mobilizes LNAPL trapped in the unsaturated and saturated zones; this process creates vadose regions with high saturation that can drain toward the capillary fringe; the net result is a substantial decrease in the LNAPL trapped in the unsaturated zone
- ethanol creates a depression into which all nearby LNAPL can drain; the reduction in surface tension and consequently in the capillary fringe height in the region that ethanol infiltrates, essentially creates a depression into which all nearby LNAPL can drain; the LNAPL in this region has a reduced interfacial tension, allowing it to fill a greater fraction of the pore spaces; the net result is a region with high LNAPL saturation that would be very mobile
- the ethanol spill could have changed the wetting properties of the soils at the PNW site; it appears that significant surfactant concentrations, which affect capillary properties, exist in the aqueous phase; it is possible that their presence could be related to the stimulation of the microbial community in the presence of ethanol; additional research would be required to confirm these mechanisms

Recommendations

The results of research presented in this study reveal significant uncertainty of the magnitude of the effects examined. For an ethanol-blended gasoline spill, uncertainty in the amount of ethanol that is retained in the unsaturated zone prevents us from adequately predicting gasoline composition that accumulates in a pool at the capillary fringe and therefore the flux of contaminants from the source area into the groundwater. Additional research is needed at several levels to provide sufficient information to predict ethanol and BTEX fluxes to groundwater. Vadose zone studies recommended include:

- laboratory studies to better quantify the functional relationships that control the retention of ethanol in the unsaturated zone; these studies should examine the retention of ethanol as a function of soil type, moisture content and spill rate and include laboratory tests to better define the significance of the observed reversal from water wetting to LNAPL wetting
- numerical modeling; improved modeling efforts are needed to better represent the complex behavior of gasoline LNAPL in the presence of ethanol; a spill of ethanol-blended gasoline into the subsurface should not be modeled as a pool of gasoline with constant composition throughout; this modeling should include the partitioning of ethanol into the aqueous phase of the unsaturated zone as well as the resulting change in interfacial properties, flow characteristics and cosolvency
- a field-scale test that includes the release of ethanol-blended gasoline under unsaturated conditions; a field-scale controlled release is needed to validate in the field the processes observed in the laboratory and to calibrate predictive models of the long-term net flux of ethanol and BTEX into groundwater

Although mostly quantitative, the results of this investigation also provide a basis for recommendations regarding remediation of ethanol/ethanol-blended gasoline contaminated soil:

- increased mobility can enhance LNAPL recovery; because an ethanol spill into soil previously contaminated by an LNAPL consolidates the LNAPL into a pool with high mobility, an opportunity not otherwise available exists for effective free-phase recovery of the LNAPL; efforts to recover this LNAPL should be made in a timely fashion
- enhanced bioremediation can occur within the unsaturated zone; ethanol retention in the unsaturated zone after the spill of ethanol-blended gasoline could provide an opportunity for ethanol biodegradation prior to its entry into the saturated groundwater zone; oxygen, which is likely to become limited, should be added to the unsaturated zone in some form to enhance ethanol degradation rate in this region

References

de Oliveria, E., *Ethanol Flushing of Gasoline Residuals: Micro-scale and Field-scale Experiments*, Dissertation, 1997, University of Waterloo, Waterloo, Ontario, Canada.

Peschke, N. and S.I. Sandler, "Liquid-Liquid Equilbria of Fuel Oxygenate and Water and Hydrocarbon Mixtures," *Journal of Chemical Engineering and Data*, Vol. 40, No. 1, 1995; http://pubs.acs.org/journals/jceaax/index.html

Powers, S.E. and C.J. McDowell, "Infiltration and Distribution of Ethanol and Ethanol-blended Gasoline in the Vadose Zone," Chapter 2 from *Environmental Assessment of the Use of Ethanol as a Fuel Oxygenate: Subsurface Fate and Transport of Gasoline Containing Ethanol*, Report to the California State Water Resources

Control Board, UCRL-AR-145380, Chapter 1, October 2001;
http://www-erd.llnl.gov/ethanol/pdf_files/Start.htm

Ross, S. and R.E. Patterson, "Surface and Interfacial Tensions of Conjugate Solutions in Ternary Systems," *Journal of Chemical Engineering and Data*, Vol. 24, No. 2, 1979; http://pubs.acs.org/journals/jceaax/index.html

UTTU thanks Dr. Susan Powers, SEP@clarkson.edu, for her help on this and other articles in UTTU.

Another study of ethanol infiltration

A more recent study by McDowell and Powers (2003) extends the research of their previous study. The objective of this research endeavor, as before, was to simulate gasoline and gasohol spills in the unsaturated zone and observe the infiltration pattern and gasoline distribution in the vadose zone at the capillary fringe. This time researchers added 10 percent ethanol by volume to the gasoline.

For these studies, McDowell and Powers used dye to enhance visibility of gasoline and ethanol. Researchers spilled gasoline and ethanol into one-dimensional and two-dimensional sand tanks. The pictures taken during the spill experiment were analyzed by image analysis software.

Researchers found the following:

- ethanol at 10 percent in gasoline infiltrated the vadose zone differently from bulk gasoline
- when ethanol entered the unsaturated zone, ethanol quickly partitioned into the residual water saturation in the vadose zone, creating regions of high ethanol concentrations
- gasoline concentrations near the injection site suggest that high ethanol concentrations were sufficient to reduce the gasoline interfacial tension, thereby promoting gasoline drainage and redistribution
- while the gasoline continued to travel through the sand, after reaching the capillary fringe, it pooled and spread laterally; ethanol remained in the unsaturated zone as a component within the residual water
- the gasoline pool size and shape remained "essentially unaffected by the addition of ethanol to the gasoline"
- the "major impact of the ethanol...was an increase in ethanol concentrations and a significant reduction in the residual saturation of gasoline retained in the unsaturated zone; thus, effort for unsaturated zone remediation, such as soil vapor extraction, would have a different efficiency following a spill of gasohol than expected based on experience with gasoline spills"
- 60 ml of ethanol added to "the residual saturation of water in the region near the injection point resulted in a significant increase in the aqueous-phase saturation," which resulted in a lower capacity to retain this phase in the unsaturated zone
- despite a localized increase in LNAPL saturation where the ethanol was added, the overall size and shape of the gasoline pool did not appear affected by the ethanol presence
- "significant decreases in LNAPL saturation in the interfacial tension and increased solubility of the gasoline at these high ethanol concentrations could both have contributed to the drainage of the ethanol-laden water to the capillary fringe and subsequent increase in LNAPL saturation"
- aqueous phase drainage rate is a function of both ethanol density and viscosity
- conducted rainfall events mobilized and caused ethanol in the unsaturated zone to drain; repeated events could flush all ethanol from the column
- BTEX fate and transport models assume that gasoline composition at the capillary fringe has the same ethanol content as that gasoline originally spilled; yet experiments show that "ethanol partitions into and is retained in the residual water in the unsaturated zones, thereby producing an initial condition at the capillary fringe that is significantly different than previously assumed"
- "in larger volume spills, the ethanol retained within the unsaturated zone eventually drains into the existing gasoline pools, acting like a denatured ethanol spill"
- because of a long retention period, ethanol mass transfer occurs over a long time period and "this may cause the electron acceptor and nutrient demands associated with ethanol contamination in the unsaturated zone to also be spread over a longer period of time than previously accepted"; ethanol biodegradation may be shifted and this may reduce the BOD of the contaminant plume and reduce estimated BTEX plume lengths
- as ethanol-laden water begins to drain, increased viscosity would inhibit its velocity, resulting in a greater detention time in the unsaturated zone; this factor should be incorporated into fate and transport models

Reference

McDowell, C.J. and S.E. Powers, "Mechanisms Affecting the Infiltration and Distribution of Ethanol-Blended Gasoline in the Vadose Zone," *Environmental Science & Technology*, Vol. 37, No. 9, 2003;
http://www.pubs.acs.org

Evaluating MTBE attenuation using natural gradient tracers

Researchers used a natural gradient tracer consisting of perdeuterated MTBE to characterize and to distinguish between the contributions of dispersion and degradation of an MTBE plume (Amerson and Johnson, 2003).

Utility of natural gradient tracer tests

Researchers use natural gradient tracer tests to investigate physical transport processes and chemical fate. Tracer tests can yield field-scale data on specific fate-and-transport processes, given known chemical properties and initial concentrations. Given the importance and challenge of estimating field attenuation rates, natural gradient tracer tests in different geochemical environments can provide critical information on natural attenuation to supplement laboratory measurements and plume studies on accidental releases" (Amerson and Johnson, 2003).

Study area

Amerson and Johnson (2003) studied the aquifer at the U.S Naval Base in Ventura County, Post Hueneme, California. The aquifer has three distinct units:

- 0 to 3 m below ground surface: a fine-grained, clayey silt with the bottom 0.3 m layer water-saturated
- 3 to 6 m below ground surface: fine- to medium-grained sand
- 6 to 7 m below ground surface: a silty-clay unit with intermittent coarse sands

The tracer test took place in the middle layer. Before the test, field workers sampled the plume with a Geoprobe™. Aquifer characteristics included

- a regional hydraulic gradient of about 0.003
- hydraulic conductivity ranging from 0.002 to 0.45 cm/sec
- low DO concentrations, < 1 mg/l
- nitrate ranging from below detection limits to 12.5 mg/l
- Fe (II) present at 0.3 to 12 mg/l
- sulfate concentrations ranging from 10.3 to 1,230 mg/l

Researchers also indicated that sulfate-reducing bacteria were present and active at this site. Characteristics of the contaminant plume include

- two distinct areas: one where the BTEX and MTBE are both present, and one where the MTBE is the sole contaminant
- an MTBE portion that is long and narrow, extending 1.5 km downgradient
- an on-site source zone
- MTBE concentrations that exceed 100 mg/l at 3 m bgs (below ground surface) near the transect midpoint
- total BTEX concentrations ranging from 251 to 577 mg/l
- MTBE and BTEX concentrations that decrease with increasing depth and increasing distance from the transect midpoint
- at its transect ends, MTBE and BTEX concentrations that are both about < 1 mg/l

Tracer injection

The injected solution contained three tracers:

- $^{2}H_{12}$-MTBE, at concentrations of 1 mg/l
- bromide, at concentrations of 500 mg/l
- fluorescein, at concentrations of 250 mg/l

"The deuterated marker provided an analytical mechanism to distinguish the tracer plume from the existing plume. Sodium bromide was chosen as a conservative tracer because it does not volatilize and would not partition into any residual NAPL in the injection zone. The initial bromide mass balance provided confirmation for the initial $^{2}H_{12}$-MTBE mass balance. Naturally occurring bromide, if present at the site, was at concentrations below the method detection limit; therefore, the potential for interference with the tracer was small. Because hydraulic control was critical during the initial plume placement, fluorescein dye was used as a visual method of monitoring the injection process" (Amerson and Johnson, 2003).

Field workers injected a concentrated tracer stock solution over a 1 m interval (3 to 4 m bgs) slightly upgradient from a point about 60 to 65 m along the cross-gradient sampling transect. Workers used a direct push Geoprobe™ to install the five injection wells. Surrounding the five injection wells was a circle of 10 extraction wells. "Each of the five peristaltic pumps used for tracer introduction was connected to two extraction wells and one injection well. This approach was used to maintain hydraulic control during tracer placement and to prevent changing the DO concentration and geochemistry of the ground water" (Amerson and Johnson, 2003). Field workers completed tracer injection in 24 hours.

Plume sampling

Field workers acquired samples from a 9 x 9 m grid that contained 81 fully screened 1-inch PVC wells. They sampled the plume immediately following tracer injection, and at 4, 8 and 12 months after injection. The 3 m to 6 m bgs interval was sampled at a 0.5 vertical interval. After one year, field workers collected samples on a 5 m grid at 0.5 m intervals from 3 to 7 m bgs.

Four-month and one-year plume characterization

Analysis of the four-month sampling data indicated one- to two-orders-of-magnitude reduction from the initial concentrations of $^{2}H_{12}$-MTBE and bromide. Amerson and Johnson (2003) assert that "values were lower than expected and, in retrospect, occurred because the transect sampled only the edge of the tracer plume. A second transect, completed four months later and angled south of the first transect, showed concentrations that were equal to and up to four times higher than the values observed in the first transect, supporting the conclusion that the first transect sampled only the edge of the tracer plume. These data also indicated that the tracer flowpath deviated from the expected direction of groundwater flow, based on the hydraulic gradient at the site and the overall flow direction of the existing MTBE plume. The data acquired from these transects provided the starting location for the detailed tracer plume characterization at the conclusion of one year of transport."

Analysis of one-year data from 81 profiling locations indicated the following:

- plume length grew from 6 m in 1998 to more than 90 m in 1999, while the width increased from 6 m to about 25 m at its widest point
- the plume has several "pockets" of higher concentrations surrounded by low concentrations, indicating influence of a heterogeneous hydraulic conductivity field
- the nonuniform distribution of $^{2}H_{12}$-MTBE indicates portions of the plume have moved at different velocities
- data from a vertical cross section down the plume centerlines show no strong correlation between velocity and depth
- some downward movement was noted, but it was limited by a clay layer
- mass balance suggests that no $^{2}H_{12}$-MTBE was lost during the study

Conclusions

Amerson and Johnson concluded the following from this tracer study of the Port Hueneme, California MTBE plume:

- MTBE biodegradation, based on $^{2}H_{12}$-MTBE mass balance, was negligible at this site
- lack of MTBE biodegradation is consistent with natural sulfate concentrations at the site of approximately 500 to 1,000 mg/l
- dispersion was the dominant mechanism for lowering aqueous $^{2}H_{12}$-MTBE concentrations
- after one year of transport, the tracer plume's mass center moved 120.3 m downgradient; the vertical center was 5.1 m bgs compared to 4.3 m bgs of the original plume
- average velocity of the tracer plume was 0.32 m/day
- average longitudinal and transverse dispersivities were 2.69 and 0.25 m, respectively
- longitudinal dispersion played an important role in tracer plume shape, and the process was also at work at the gasoline plume
- the role of the higher-velocity zones within the MTBE plume is masked by overall plume shape

Amerson and Johnson (2003) found that "the plume's preferential flowpath was not co-located with the apparent centerline of the MTBE plume. The presence of channels in a plume will cause some contaminant mass to move at a different velocity than the overall plume characteristics would suggest. Consequently, the travel time along the apparent centerline may not accurately reflect the travel time of contaminant mass in the aquifer and may lead to errors in the calculated degradation rate. The existence of preferential flowpath may also make locating the actual plume centerline on a mass transport basis very difficult. If the transect and the actual plume centerline diverge with distance from the source, reduced contaminant concentrations caused by dispersion and dilution could support an erroneous conclusion that degradation is occurring" (Amerson and Johnson, 2003).

Finally, authors recommend that anyone using MNA consider the applicability and potential pitfalls of using "'appropriate' monitoring well (or wells) to demonstrate MNA." Furthermore, "Even if microcosm studies indicate that degradation is possible at a site, a defensible field demonstration that MNA is a viable strategy may still be difficult" (Amerson and Johnson, 2003).

Reference

Amerson, I. and R.L. Johnson, "Natural Gradient Tracer Test to Evaluate Natural Attenuation of MTBE Under Anaerobic Conditions," *Ground Water Monitoring & Remediation*, Winter 2003; http://www.ngwa.org

Benzene, toluene and ethanol plume lengths

Ruiz-Aguilar and others (2003) used statistical analyses of plume lengths to identify the impact of ethanol on gasoline plume lengths. In the past, researchers have used mathematical models to predict ethanol's effect on BTEX plume length. These models have predicted increased plume length from 10 percent to 150 percent.

Procedure

Researchers collected two sets of groundwater data from about 600 gasoline-contaminated sites. Using telephone surveys and report investigation, researchers excluded sites that

- may have been contaminated by ethanol-amended gasoline

- lacked sufficient data to plot the required plume contours

The first data set consisted of 217 Iowa sites contaminated with regular gasoline, while the second, from Kansas, corresponded to 29 sites contaminated with gasohol (i.e., gasoline with 10 percent ethanol by volume). MTBE contamination did not exist at these sites.

Plume length determination

Researchers used a computer algorithm to contour data and produce plumes. "Plume lengths were then measured as the longest distance between the identified source and the 5 μg/l contour, which corresponds to the drinking water standard for benzene." Using a statistical program (Minitab, version 13.1 State College, Pennsylvania), researchers calculated population statistics such as population mean, standard deviation, median, maximum and minimum and performed distribution analyses at the 95 percent significance level.

The statistical tests determined the following:

- plume length data were log-normally distributed
  - $p = 0.275$ for benzene
  - $p = 0.394$ for toluene
- a cumulative plume length distribution shows that benzene plumes were generally longer for set 2 (with ethanol) than for set 1 (without ethanol)
- 92 percent of the benzene plumes in set 2 were longer than 150 feet, compared to only 74 percent for set 1
- 69 percent of benzene plumes in set 2 were longer than 250 feet compared to 45 percent for set 1
- none of the 29 plumes in set 2 were longer than 500 feet compared to 12 percent of the 217 plumes in set 1—a reflection of set 1's larger data set
- the Turkey method (Turkey, 1977; reference in Ruiz-Aguilar and others, 2003) identified the long plumes as outliers
- data were similar for toluene, although elongations are not as pronounced
- a Kruskal-Wallis test showed the median benzene plume length was longer for set 2 than set 1 (263 vs. 156 feet)
- the same test showed no statistical significance for the toluene plume
- the median length for benzene and toluene plumes without ethanol is within 15 percent of that reported by Newell and Connor (1998), i.e. 132 feet; this number reflects a compilation of other surveys
- the average length of BTEX plumes with ethanol was higher than without ethanol by 36 percent, or 70 feet for benzene, and 17 percent or 26 feet for toluene
- the average benzene plume was 20 percent longer than the average toluene plume for set 2 (with toluene) compared to 4 percent difference for data without ethanol, indicating that the potential elongating effect of ethanol could be more pronounced than that for toluene; benzene is relatively recalcitrant under the anaerobic conditions exacerbated by an ethanol-driven consumption of electron acceptors

The authors contend that while "an increase of 70 feet in the average length of benzene plumes is statistically significant, this does not imply that the corresponding increase in public health risk will also be significant" (Ruiz-Aguilar and others, 2003).

Other factors, besides ethanol presence, that influence plume length include

- hydrogeologic and geochemical factors that affect advection, dilution, sorption, volatilization and biodegradation rates
- site heterogeneity
- release and response scenarios

Researchers found that the type of aquifer material did not have a statistically significant effect on plume length. According to Ruiz-Aguilar and others (2003) "This does not mean that the type of aquifer material (and its associated permeability and sorption capacity) does not affect plume length. Rather it implies that other factors that were not quantified could be more influential." Still researchers assert

- confounding factors were likely randomized by the relatively large data set considered
- Kansas plumes were longer than Iowa plumes even though high temperature (conducive to greater biodegradation) typifies the Kansas plumes
- the statistical analyses indicate consistent experimental and modeling results

Reference

Ruiz-Aguilar, G.M.L., O'Reilly, K. and P.J.J. Alvarez, "A Comparison of Benzene and Toluene Plume Lengths for Sites Contaminated with Regular vs. Ethanol-Amended Gasoline," *Ground Water & Remediation*, Vol. 23, No. 1, Winter 2003; http://www.ngwa.org

Ethanol's effect on hydrocarbon-degrading bacteria

Researchers created a microcosm study to investigate the effects of ethanol on BTEX-degrading bacteria. Their first step involved developing a new DNA-based method to characterize the bacterial community in terms of populations of BTEX-degrading bacteria rather than in terms of species composition. To accomplish this, researchers used molecular biology techniques. Next, researchers created microcosms inoculated with aquifer sediment from four sites with various BTEX and ethanol exposure histories (Beller and others, 2001) and compared the BTEX-degrading activity and bacterial populations in the presence and absence of ethanol.

Beller and others (2002) summarized this work as follows: "We have developed a real-time Polymerase Chain Reaction (PCR) method that can quantify hydrocarbon-degrading bacteria in sediment samples based on a catabolic gene associated with the first step of anaerobic toluene and xylene degradation. The target gene, *bssA*, codes for the alpha subunit of benzylsuccinate synthase... The method proved to be sensitive (detection limit ca. five gene copies) and had a linear range of greater than seven orders of magnitude. We used the method to investigate how gasohol releases from leaking underground storage tanks could affect indigenous toluene-degrading bacteria. Microcosms inoculated with aquifer sediments from four different sites were incubated anaerobically with BTEX and nitrate in the presence and absence of ethanol. Overall, population trends were consistent with observed toluene degradation activity: the microcosms with the most rapid toluene degradation also had the largest numbers of *bssA* copies. In the microcosms with the most rapid toluene degradation, numbers of *bssA* copies increased 100- to 1,000-fold over the first four days of incubation, during which time most of the toluene had been consumed... Use of a companion real-time PCR method for estimating total eubacterial populations (based on 16S rDNA) indicated that, in some cases, ethanol disproportionately supported the growth of bacteria that didn't contain *bssA*. The real-time PCR method for *bssA* could be a powerful tool for monitored natural attenuation of BTEX in fuel-contaminated groundwater."

Culture-independent, molecular method

To detect and quantify bacteria, researchers developed a culture-independent, molecular method, which focused on anaerobic toluene-degrading bacteria because

- anaerobic conditions prevail at LUFT sites due to rapid depletion of dissolved oxygen by indigenous, hydrocarbon-degrading, aerobic bacteria
- oxygen would be even more readily depleted in the presence of ethanol
- toluene is the most readily degradable of the BTEX compounds under anaerobic conditions

Thus the method would detect bacteria with the genetic potential to carry out anaerobic toluene degradation. The analysis method selected was real-time, quantitative polymerase chain reaction (RTQ-PCR), which could detect specific bacterial populations in environmental samples. Specifically, researchers were looking for a gene associated with the enzyme that catalyzes the first step of anaerobic toluene degradation, benzylsuccinate synthase (BSS). This enzyme has been shown to be present in diverse, anaerobic toluene-degrading bacterial cultures that encompass the range of electron-accepting conditions expected to occur at LUFT sites (denitrifying, sulfate-reducing, iron-reducing and methanogenic).

Researchers used the PCR method "to increase the quantity of low abundance genes for detection." In addition, the RTQ-PCR method eliminates problems associated with end-point detection. The technique includes several other advantages:

- quantitative data are collected in real-time during the amplification process
- the linear range often encompasses more than five orders of magnitude

Researchers used RTQ-PCR to investigate factors that would influence populations of toluene-degrading bacteria in microcosms incubated anaerobically with BTEX. Variables studied included

- presence or absence of ethanol
- presence of various electron acceptors
- fuel exposure history of the aquifer sediments used to inoculate the microcosms

Researchers used this method not only to quantify the populations of bacteria containing the *bssA* gene but also to quantify populations of total eubacteria (represented by a universal eubacterial probe based on 16S rDNA). The molecular method was used to investigate how gasohol releases from leaking underground fuel tanks could affect indigenous toluene-degrading bacteria.

Background

Over the past five years, knowledge of the biochemistry and genetics of anaerobic toluene degradation has increased dramatically. "A novel enzyme that catalyzes the first step of anaerobic toluene degradation, benzylsuccinate synthase (BSS), has been reported in cultures that degrade toluene under denitrifying, sulfate-reducing, anoxygenic phototrophic, ferric iron-reducing and methanogenic conditions; all of these studies involved in vitro enzyme assays. BSS has been purified from two denitrifying bacteria, and the gene encoding for the large (alpha) subunit of BSS (*bssA* or *tutD*) has been sequenced from two strains) of the denitrifying bacterium *Thauera aromatica*. Notably, BSS has recently been shown to catalyze the first step of anaerobic xylene degradation. Thus, detection of bacteria harboring genes for BSS are relevant to anaerobic xylene as well as toluene degradation. To date, no enzyme other than BSS has been identified that catalyzes the first (activation) step of anaerobic toluene degradation. Although another activation step was proposed for *Azoarcus tolulyticus* strain *Tol-4*, the proposed enzyme activity has not been demonstrated with in vitro experiments, and in fact, this strain has been shown to contain BSS activity and genes (this study)" (Beller and others, 2002).

Researchers chose the quantitative polymerase chain reaction (PCR) analysis method to identify specific bacterial populations in studies of gasohol releases.

Microcosm setup and analysis

Beller and others (2001) inoculated microcosms with aquifer sediments from four sites with different histories of exposure to fuel hydrocarbons, then incubated them anaerobically with BTEX and nitrate in the presence and absence of ethanol. Microcosms (constructed in duplicate) consisted of

- 10 g of homogenized aquifer sediment
- artificial groundwater, rigorously deoxygenated before use
- BTEX concentrations of 0.4–3 mg/l
- ethanol, if added, in concentrations of 50 to 100 mg/l
- bicarbonate-buffered medium

For the molecular studies, researchers focused on microcosms that showed strong evidence of toluene removal. Thus, they analyzed "eight sets of microcosms (a 'set' is a group of microcosms inoculated with aquifer sediment from a given site under given electron-accepting conditions, in both the presence and absence of ethanol). In general, microcosm sampling was designed to investigate the effects of ethanol on the populations of bacteria carrying the *bssA* gene and of total eubacteria" (Beller and others, 2001). For each set of microcosms, researchers sampled four times:

- sampling 1, control: microcosms were sacrificed without incubation (initial conditions)
- sampling 2: sampled when ethanol was depleted; samples provided direct evidence of the effect of ethanol degradation on bacterial populations incubated under given conditions
- sampling 3: sampled when first BTEX compound (usually toluene) was depleted (in either ethanol-amended or unamended microcosms, whichever degraded BTEX more quickly)
- sampling 4: sampled when the first BTEX compound (usually toluene) was depleted (in either ethanol-amended or unamended microcosms, whichever degraded BTEX more slowly)

Samplings 3 and 4 "were intended as a means of 'calibrating' the *bssA* results because they indicated the amount of increase in numbers of *bssA* copies after a known amount of toluene had been completely depleted" (Beller and others, 2001).

Microcosms created from four sites

Researchers procured samples from four sites:

- a LUFT site at Travis Air Force Base
- a LUFT site at Sacramento, California
- an ethanol- and fuel-contaminated terminal in the Pacific Northwest (Northwest terminal)
- a background uncontaminated site in Tracy, California

Researchers regularly monitored BTEX, ethanol and nitrate, using a gas chromatograph equipped with an autosampler, and flame ionization and photoionization detectors. Researchers used ion chromatography to analyze nitrate and added additional nitrate when it became depleted. Beller and others (2002) describe in detail the method of DNA extraction and real-time PCR analysis.

Microcosm results

Travis AFB microcosm, LUFT site. Researchers found that under denitrifying conditions, ethanol did not appear to affect toluene degradation. "Ethanol and toluene were largely depleted within the first 5 days of incubation; the two compounds were degraded concurrently, and toluene degradation rate was similar in the presence and absence of ethanol. A very steep increase in the number of *bssA* copies occurred between day 0 (i.e., the controls) and days 4-5; copies of *bssA* increased from ca. 10^5 initially to between 10^7 and 10^8. Analyses of two additional replicates confirmed the control *bssA* data. The presence of ethanol had no clear effect on numbers of *bssA* copies, which is consistent with its lack of effect on toluene degradation activity... In contrast to its apparent lack of effect on toluene degradation, ethanol had an effect on total eubacterial populations (represented by the number of copies of 16S rDNA). In the absence of ethanol, the number of copies of 16S rDNA did not change notably from the initial value of ca. 2 x 10^8. Ethanol-amended microcosms, however, showed a four- to five-fold increase relative to initial conditions. The apparent number of additional eubacterial cells resulting from growth on ethanol is generally consistent with the estimate of 6 x 10^9 cells based on thermodynamic calculations, the known mass of ethanol consumed under denitrifying conditions, and the assumption that the number of 16S rDNA copies/genomes was the same in the samples and the real-time PCR standards" (Beller and others, 2002).

Under sulfate-reducing conditions:

- toluene degradation was slower, and possibly, "ethanol slightly enhanced the toluene degradation rate"
- degradation of toluene and ethanol occurred concurrently
- benzylsuccinate synthase may have been involved in anaerobic xylene degradation in addition to toluene degradation; thus degradation of *m,p*-xylenes could have impacted on *bssA* abundance
- ethanol may have enhanced abundance of total bacteria

Under methanogenic conditions toluene showed a rapid initial phase of degradation (50-75 percent degraded) followed by a slower phase of degradation.

With ferric iron-amended conditions, trends of toluene and ethanol degradation were similar (Beller and others, 2001).

Sacramento microcosm, LUFT site. Toluene degradation was slower for these sediments than for those from Travis AFB. Rates for ethanol-amended and unamended microcosms were similar until day 30, at which time the rate appeared to increase somewhat in ethanol-amended microcosms. Beller and others (2002) assert that "Ethanol degradation may have slightly increased the number of *bssA* copies; at day 3 (after ethanol was degraded), the numbers of *bssA* copies in ethanol-amended microcosms were approximately 170 percent in ethanol-amended microcosms. As was observed for Travis AFB microcosms, growth on ethanol appreciably increased the populations of total eubacteria. Numbers of 16S rDNA copies in ethanol-amended microcosms were, on average, approximately 50 times those of the controls. Smaller increases in total eubacteria were also observed for unamended microcosms, presumably as a result of growth on indigenous organic matter in aquifer sediment."

Tracy, California, non-LUFT site. Researchers documented slower toluene degradation for this sediment under denitrifying conditions. Ethanol did not affect degradation rate. "Numbers of *bssA* copies in all incubated samples were roughly equal to numbers in the control (initial) samples... The detection of *bssA* in aquifer material that has not been exposed to LUFT contamination is not particularly surprising, as toluene-degrading, denitrifying bacteria have been found in a range of pristine environments" (Beller and others, 2002).

"It appears that ethanol enhanced the numbers of eubacterial 16S rDNA copies, as the average abundance in ethanol-amended microcosms was ca. 5.5 times the average in the unamended microcosms. However, total eubacteria also increased in unamended microcosms" (Beller and others, 2002).

Northwest Terminal, fuel- and ethanol-contaminated site. Researchers studied anaerobic toluene degradation under denitrifying and sulfate-reducing conditions.

Under denitrifying conditions, ethanol had a "dramatic and positive effect on anaerobic toluene degradation... In the presence of ethanol, toluene was mostly degraded by day 15, whereas in the absence of ethanol, there was no clear degradation in more than 50 days. The differences in toluene concentrations between ethanol-amended and unamended conditions cannot be explained by analytical error, as the median RSD for all toluene data was 2.5 percent."

"If ethanol promoted toluene degradation by fortuitously supporting the growth of anaerobic, toluene-degrading bacteria, this is not reflected in the data for *bssA*. Indeed, the numbers of *bssA* copies at day 5 (when ethanol was depleted) were 2-3 times lower in ethanol-amended microcosms than in unamended ones."

Researchers suggest several explanations for "the lack of an apparent increase in *bssA* copies in ethanol-amended microcosms despite their enhanced toluene degradation activity:

- a minor increase actually occurred but was within the range of experimental error for these samples
- ethanol stimulated toluene degradation by some means unrelated to growth (e.g., by induction of genes associated with toluene degradation, although this seems unlikely based on current knowledge of the anaerobic toluene degradation pathway)
- the primer-probe set used for real-time PCR did not efficiently match the actual *bssA* sequences of the toluene-degrading bacteria in these particular microcosms (although the primer-probe set was based on a consensus sequence from four different denitrifying strains)
- anaerobic toluene degradation proceeded by a metabolic pathway that did not involve BSS (although there is currently no in vitro or genetic evidence for such a pathway)"

Under sulfate-reducing conditions, researchers found "no marked differences in the numbers of *bssA* copies as a function of the presence of ethanol...there were also no clear effects of ethanol on the abundance of 16S rDNA copies" (Beller and others, 2001).

Environmental applicability

"Ideally, a molecular method for in-situ monitoring would target a functional gene associated with pollutant degradation, such that a strong relationship between the number of target gene copies and pollutant-degrading activity could be confidently assumed. Our real-time PCR method for *bssA* follows this principle by targeting a key gene associated with anaerobic toluene and xylene degradation. The application of real-time PCR in this study is atypical in the sense that the primer-probe set was designed to be specific to *bssA* yet still allow for the natural genetic diversity of *bssA* among denitrifrying bacteria that might be encountered in the environment...As *bssA* sequences from a wider range of bacteria become available and the diversity of this gene is better understood, it will become possible to assess whether the primer-probe set described here will need to be altered to encompass natural diversity or whether multiple sets will be required. Regardless, real-time PCR targeted at *bssA* appears to be a promising monitoring tool for assessing natural attenuation of BTEX in fuel-contaminated groundwater" (Beller and others, 2002).

Reference

Beller, H.R., Kane, S.R. and T.C. Legler, "Effect of Ethanol on Hydrocarbon-Degrading Bacteria in the Saturated Zone: Microbial Ecology Studies," Chapter 4 in *Environmental Assessment of the Use of Ethanol*

as a *Fuel Oxygenate: Subsurface Fate and Transport of Gasoline Containing Ethanol*, Report to the California State Water Resources Control Board, UCRL-AR-145380, October 2001; http://www-erd.llnl.gov/ethanol/pdf_files/Start.htm

Beller, H.R., Kane, S.R., Legler, T.C. and P.J.J. Alvarez, "A Real-Time Polymerase Chain Reaction Method for Monitoring Anaerobic, Hydrocarbon-Degrading Bacteria Based on a Catabolic Gene," *Environmental Science & Technology*, Vol. 36, No. 18, 2002; http://www.pubs.acs.org

UTTU thanks Dr. Harry Beller, beller2@llnl.gov, for his help on this article.

Research notes

A Lost Generation

Nyer, E.K., Fierro, P. and B. Guillette, *Ground Water Monitoring & Remediation*, Vol. 22, No. 4, Fall 2002; http://www.ngwa.org

In this article, Dr. Nyer describes some of the skills that will be lost to hydrogeologists unless such skills are properly passed down to the next generation. Skills with the potential of being lost include

- installing a pumping well in a silty aquifer
- proper use of drilling with a hollow-stem auger so that clay-rich cuttings are not smeared upward (skin effect)
- proper evaluation of pilot tests so as not to rule out remedial alternatives, such as vapor extraction
- awareness of data accuracy crunched through computer programs
- awareness that maps contoured by software programs do not necessarily depict the true state of nature

A Pilot Study of Passive Diffusion Bag (PDB) Sampling in a Fractured Bedrock Environment

Bilosi, T.W., Feldmann, S.G., O'Grady, B.A. and J.M. Dale, *Contaminated Soil Sediment and Water*, January/February 2003; http://www.aehsmag.com

The U.S. Geological Survey, the Navy and an engineering firm cooperated on a study of passive diffusion bag (PDB) sampling. They found that "The use of PDB samplers substantially reduces equipment and labor costs and produces virtually no investigation-derived waste, as compared to conventional purging and sampling techniques. However, unlike typical PDB samplers that are usually constructed of polyethylene-based material and used to monitor for VOCs only, a regenerated cellulose-based material was used in this PDB pilot study to also monitor inorganic parameters... The majority of the organic data indicate that the PDB samplers yielded similar results to conventional purging and sampling techniques."

Enhanced In-Situ Chemical Oxidation Using the PRP System

Scrudato, R.J. and J.R. Chiarenzelli, *Contaminated Soil Sediment and Water*, January/February 2003; http://www.aehsmag.com

Remediators used a programmable release process (PRP), a three-phase remedial process that

- oxidizes contaminant of concern
- modifies groundwater redox chemistry
- maintains the modified geochemistry of the impacted groundwater

This application can be used for smear and vadose zones. The procedure, performed at a BTEX/MTBE spill site in New York, reduced BTEX concentrations from about 15 mg/l to 20 µg/l.

Other papers and abstracts of interest

From *Contaminated Soil Sediment and Water*, January/February 2003; http://www.aehsmag.com unless indicated otherwise

A Bioremediation Case Study: Butane Biostimulation for the Remediation of Petroleum Contaminants, Begley, J.F., Perriello, F.A., DiCesare, G. and J. Longo

Advances in Methods to Stage Injection of ORC, HRC and Other Materials, A. Livadas

Analysis of Volatile Organics in Produce Using Solid-Phase Microextraction and GC/MS, Tan, C.K., Shannon, K., Acosta, R. and J. Rodriguez

Biodegradation Kinetics of MTBE in Laboratory Batch and Continuous Flow Reactors, Wilson, G.J., Pruden, A., Suidan, M.T. and A.D. Venosa, *Journal of Environmental Engineering*, September 2002; http://ojps.aip.org/eeo

Bioremediation of Polyaromatic Hydrocarbons Using a Groundwater Recirculating Treatment System, Cagnetta, P.J. and D.B. Lewis

Calibration of a Model for Volatile Organic Compound Mass Removal by Multiphase Extraction, Edwards, D.A., Little, J.W., Lanik, W. and P.A. Hajali, *Journal of Environmental Engineering*, May 2002; http://ojps.aip.org/eeo

Chelated Native Iron in Fenton-Like Oxidation of BTEX and PAHs in Soils, Jazdanian, A.D. and K.R. Reddy

Correlation of Field Analytical Detectors in the Analysis of Soil Contaminated with Diesel Fuel, Clark II, C.J. and J.J. Delfino

Cost and Performance of Vegetable Oil Injection for Enhanced In-Situ Bioremediation at Two Air Force Sites, Henry, B.M., Griffiths, D.R., Love, A.M., Guest, P.R. and J. Gonzales

Degradation of MTBE and Related Gasoline Oxygenates in Aqueous Media by Ultrasound Irradiation, Kim, D.K., O'Shea, K.E. and W.J. Cooper, *Journal of Environmental Engineering*, September 2002; http://ojps.aip.org/eeo

Detailed Chemical Fingerprinting of Gasoline for Environmental Forensic Investigations: Part 2. Analytical Method Performance, Uhler, R.S., Healey, E.M., McCarthy, K.J., Uhler, A.D. and S.A. Scout

Diffusive Partitioning Tracer Test for Nonaqueous Phase Liquid (NAPL) Detection in the Vadose Zone, Werner, D. and P. Hohener, *Environmental Science & Technology*, Vol. 36, No. 7, 2002; http://www.pubs.acs.org

Effects of Biodiesel Blends and Arco EC-Diesel on Emissions from Light Heavy-Duty Diesel Vehicles, Durbin, T.D. and J.M. Norbeck, *Environmental Science & Technology*, Vol. 36, No. 8, 2002; http://www.pubs.acs.org

Electroremediation of Contaminated Soils, Page, M.M. and C.L. Page, *Journal of Environmental Engineering*, March 2002; http://ojps.aip.org/eeo

EPA Performance Study for Field Measurement of Total Petroleum Hydrocarbons Using Ultraviolet Fluorescence Technology, S. Greason

Facilitated Desorbtion and Incomplete Dechlorination: Observations from 350 Applications of HRC, Koenigsberg, S.K., Lapus, K. and G. Pasrich

Groundwater Reaeration and Hydrocarbon Plume Length: A Modeling Analysis, Neale, C.N., Holder, A.W., Ward, C.H. and J.B. Hughes, *Journal of Environmental Engineering*, January 2002; http://ojps.aip.org/eeo

Implementation of an In-Situ Chemical Oxidation Program for Remediation of Saturated Zone Petroleum Hydrocarbons at the Navy Exchange Service Station, Naval Air Station, Easterday, A.C. and C.J. Varner

In-Situ Biodegradation Determined by Carbon Isotope Fractionation of Aromatic Hydrocarbons in an Anaerobic Landfill Leachate Plume (Vejen, Denmark), Richnow, H.H., Meckenstock, R.U., Reitzel, L.A., Baun, A., Ledin, A. and T.H. Christensen, *Journal of Contaminant Hydrology*; http://www.elsevier.com/

In-Situ Chemical Oxidation Misfires and Recent Innovative Improvements, Adams, R., Mahaffey, B., Slack, B. and M. Vigneri

Molecular Changes During Petroleum Combustion, S. Emsbo-Mattingly

MTBE and its Degradation Products: Molecular Modeling Study, Bahr, K.L., Hayik, S., Bock, C.W. and W.H. Brendley

Multiple Methods for Determining Stability of Attenuating MTBE Groundwater Plumes, Gibbs, J.T., Williamson, T.K.J., Naber, S.J. and T.L. Macchiarella, *Journal of Environmental Engineering*, September 2002; http://ojps.aip.org/eeo

Stimulation of Environment-Polluting Oil Residues Degradation by Microbial Associations, Voicu, A., Dobrota, S., Stefanescu, M., Lazaroaie, M., Lazar, I.I., Petrisor, I.G. and J.M. Kuperberg

Sourcing Hydrocarbons at Fire Training Areas: A Molecular Characterization of the Combusted and Evaporated Residues of Distillate Fuels, Emsbo-Mattingly, S., Stout, S., Uhler, A. and K. McCarthy

TBA Biodegradation in Surface-Water Sediments Under Aerobic and Anaerobic Conditions, Bradley, P.M., Landmeyer, J.E. and F.H. Chapelle, *Environmental Science & Technology*, Vol. 36, No. 19, 2002; http://www.pubs.acs.org