



Underground Tank Technology Update

Vol. 12, No. 3
May/June 1998

Department of Engineering Professional Development The College of Engineering University of Wisconsin–Madison

Underground Tank Technology Update is published bimonthly by 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. For new subscriptions or address corrections, use the form on inside back page.

UTTU is funded by the U.S. EPA under Cooperative Agreement No. L005924-01 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.

If you have a problem locating a reference cited in *UTTU*, please contact Pat Dutt Komor by e-mail at pdkomor@msn.com, or call her at 607/257-6801.

Advisory Board

Gilberto Alvarez, Environmental Engineer
OUST, U.S. EPA, Region 5, Chicago, Illinois

Bruce Bauman, Research Program
Coordinator—Soil and Groundwater, API
Washington D.C.

Robert Hitzig, Geologist
OUST, U.S. EPA, Washington D.C.

George Mickelson, Environmental Engineer
Wisconsin Department of Natural Resources
Madison, Wisconsin

Mark D. Millsop, Hydrogeologist
GME Consultants, Crosby, Minnesota

Phil O'Leary, Professor
Department of Engineering Professional
Development, UW–Madison

Gerald W. Phillips
U.S. EPA, Region 5, Chicago, Illinois

Matt Small, Hydrogeologist
U.S. EPA, Region 9
San Francisco, California

Staff

John T. Quigley Project Director
Pat Dutt Komor Geologist/Writer
Darrell Petska Copy Editor
Debbie Benell Program Assistant
Susan Kummer/Artifax Graphics

Article summaries

Discussion of bioremediation terms 2

This is the first of three articles summarizing bioremediation issues originally debated in November 1997 by members of the BioGroup on the Internet.

Engineered microbe effectiveness, bioaugmentation in lab vs. field, and transgenic microorganisms 5

In this article researchers give practical information on bioaugmentation.

Acceptable hydrocarbon concentrations in soil 7

This article gives technical and philosophical perspectives on “what is the acceptable concentration of hydrocarbons left in soil?”

History of bioremediation references 11

For those interested in the history of bioremediation, a list is provided.

1998 national RNA survey 14

This article summarizes the states' RNA practices.

EPA's internet chat room at www.epa.gov/swerust1/flags.htm consists of EPA, New Mexico, Alaska, Arizona, Montana, Minnesota and California sites. The Minnesota site, for instance, lists documents relating to land treatment, composting and release investigations in karst areas. For a list of state contacts, see the site <http://www.epa.gov/swerust1/states/statcon1.htm>.

UTTU's home page, <http://epdwww.engr.wisc.edu/uttu/>, contains an alphabetical and topical list of every article that has appeared in UTTU since 1987.

For information on “The Environment: The Cleanup and Re-use of Brownfields,” a workshop presented in Seattle, Washington, May 11-12, send an e-mail to dwert@aipt.org or see the web site <http://aipt.org/environment.html>.



Discussion of bioremediation terms

A member of the GZA GeoEnvironmental Inc., BioGroup was approached by a vendor selling treated microbes. The member wanted information on microaerophilic bacteria and degradation/transformation pathways. Here are the responses the member received.

Microaerophilic bacteria, from Whittaker, 1997. Used in bioaugmentation, these guys purportedly need just the minutest amounts of oxygen (no specifications provided) and can biodegrade just about anything. One vendor claims that they are neither aerobic nor anaerobic (rather, microaerophilic) and do not require any nutrient addition. I am not familiar with the term microaerophilic (though I can make the obvious assumptions based on the name itself); in the absence of any technical information provided by the vendor, I wondered if anyone out there could enlighten me.

Degradation/transformation pathways. I have been told from unreliable sources that during bioremediation, one should expect an increase in the abundance of low molecular weight alkanes/aromatic compounds (including BTEX) because they are produced from the transformation of larger molecules, although this will pass as these compounds are themselves degraded. I expressed deep scepticism about this, since from my understanding, aerobic biotransformation proceeds via carboxylic acid/catechol formation, not the discrete alkane/aromatic compounds. Questions: Can biotransformation really result in an increase in lower-molecular-weight compounds (in whatever form) to the extent that BTEX (for example) concentrations would go up? Or more likely, can the actual lower-molecular-weight intermediates be produced in such abundance that they could be mistaken for alkane/aromatic compounds during analysis?

Microaerophilic bacteria, from Schaffner, 1997. It doesn't matter whether they are microaerophilic, or winged pixies, for that matter. The primary rate-limiting factor for biodegradation of contaminants such as petroleum hydrocarbons (PHC) is the electron acceptor. If the "superbugs" are viable using a lower electron acceptor concentration, I believe that means they'll require a longer time to degrade targeted compounds. The bottom line, as always, remains reaction stoichiometry, i.e., you must meet the electron acceptor demand exerted by the PHCs.

Degradation/transformation pathways. I believe you are correct regarding PHC metabolites, i.e., functional groups are not merely stripped from compounds. Regarding increased BTEX concentrations, however, it is possible that stimulation may induce biosurfactant production which may temporarily mobilize sorbed constituents. I have not observed this myself, but I recall that this mechanism has been evoked in some of the early literature to explain increases in contaminant concentrations following biostimulation.

Microaerophilic bacteria, from Barden, 1997. It sounds like another case of overselling things. "Microaerophilic" refers to conditions of very low dissolved oxygen (generally less than 1 mg/L) where dissolved oxygen is not completely absent, hence the description of the condition as not anaerobic, but not fully aerobic. These microbes are capable of various degradation pathways but primarily can utilize organic substrates such as BTEX. They are not "superbugs" and have essentially the same nutrient requirements as other microbes. Very likely they are using nitrate as a terminal electron acceptor (nitrate reduction). If your vendor can't provide you with specific factual technical information supporting their "product," I would tell them to take a hike.

Degradation/transformation pathways. The biodegradation/biotransformation does not produce BTEX compounds (complete aromatic rings). In some cases, degradation of straight- or branched-chain alkanes could potentially produce a slight increase in low-molecular-weight alkanes, but you'd be unlikely ever to see it. It is possible that some degradation intermediates (organic acids) from anaerobic degradation could show up in the same range as BTEX in a GC scan. The lower-molecular-weight hydrocarbons are generally more biodegradable and don't tend to accumulate in the system.

Microaerophilic bacteria, from LaMountain, 1997. By definition, these bacteria are oxygen-requiring organisms that grow only at reduced oxygen concentrations, less than one atmosphere. These organisms are supposedly aerobes that do not have all of the protective enzymes to ward off oxygen toxicity, so they can only grow at low levels of O₂.

Degradation/transformation pathways. I think the vendors are pushing organisms that will use nitrate as an electron acceptor and use O₂ only for the oxygenase enzymes. Ron Olsen has performed some research on these. These organisms would still require nutrient addition, depending on the groundwater characteristics, e.g. nitrate or phosphates. Olsen has said that if you add O₂, then you will select for other organisms that like O₂ better, and these organisms will never get a chance to operate. You are correct to assume that the intermediates would be more polar than the original hydrocarbons. You should see fatty acids and catechols or carboxylic acids as degradation products. I think your source of information was thinking lower molecular weight in general, e.g. big multiple rings chopped into smaller ring units, and not thinking about their substitution, e.g. having hydroxyls or carboxylic acid groups on them.

I have dealt with many vendors of bioremediation techniques or organisms, and many do not understand their products. This is because they are salespeople. I usually ask them to get their company's microbiologist to call me, so I can get the straight story about the product.

If this is a major remediation project or a major expense for your company, I would certainly ask for much more background than they have apparently given to you. Do not buy-in to anything without a good deal of technical information that a trusted microbiologist says is reliable; there are too many people trying to make a quick buck in this business.

Microaerophilic bacteria, from Zang, 1997. I have seen some of these products under different names. They may contain nutrients like nitrate, which can be used as the electron acceptor, for instance.

Degradation/transformation pathways. I really cannot see how a lesser degradable PAH (higher molecular weight) can result in an increase in BTEX. For one thing, when the aromatic rings are broken, the molecule would be destabilized. The thing would essentially "fall apart." More importantly, the resulting BTEX would be more biodegradable than the parents.

Having said all that, I have even seen BTEX "generating" out of thermal desorption processes. I have also seen a graduate of UBC tell me that he saw increased BTEX through slurry phase biotreatment. I think we should go back to the laboratory. If a sample has high hydrocarbons other than BTEX, they will mask the BTEX because in order to perform GC analyses, the laboratory will have to dilute the sample to a certain degree. Thus, the BTEX may become non-detectable in the diluted extract. In the treated sample, when background hydrocarbons are reduced to a lower level, BTEX "stands out."

Microaerophilic bacteria, from Gerlach, 1997. By the way, can't we try to define the terms aerobic, anaerobic, anoxic, microaerophilic . . . so that we can use them consistently throughout our discussions? From a microbiological standpoint, you can always group bacteria into either aerobic or anaerobic species; however, many bacteria have the metabolic capability to grow aerobically or anaerobically, depending on the conditions they discover; they are called facultative aerobes, or facultative anaerobes.

In my opinion (I am an engineer) and the opinion of many microbiologists:

- aerobic means that oxygen (dissolved oxygen) is available
- anaerobic means that no (dissolved) oxygen is available. For metabolism (biodegradation) to occur, other electron acceptors must be available. Electron acceptors in anaerobic environments can be nitrate, sulfate, iron, manganese, carbon dioxide, or organics (fermentation); and there may be a few more that are of little relevance with respect to bioremediation

Saying "no oxygen," however, has an inherent problem: Is "no oxygen" absolutely no oxygen, or is oxygen just below the detection limit of whatever method is being used? People started using the term microaerophilic, or anoxic, for environments where only little oxygen is available, usually less than 0.2 mg/L, which is coincidentally the detection limit of many oxygen tests! In my opinion "anoxic" has mostly been used for environments where no, or little, oxygen is available and nitrate is the dominant electron acceptor. "Anoxic" is most widely being used in wastewater treatment.

For biodegradation to occur, electron acceptors must be available. Thus, bacteria in microaerophilic environments have to use other electron acceptors to biodegrade contaminants efficiently. I'd be really careful if somebody tells me that

his bugs do not need any nutrient-addition while others do, something the vendor might not explicitly state, but suggest. Nutrients are required for bacterial growth and for efficient bioremediation. If the required nutrients are not available in the system (bioreactor, aquifer), they must be supplied. There is no direct relationship between nutrient supply (usually N, P) and electron acceptor; however, there might be electron acceptors that can also serve as a nutrient, e.g. nitrate. What your sources might be meaning to say is that the relative abundance of BTEX and other aromatics is increasing. That indicates that easily degradable compounds such as straight-chain aliphatic hydrocarbons are being degraded more readily than aromatics or branched aliphatics. Thus, the overall TPH concentration can decrease, but the relative abundance of persistent compounds can increase . . . unless there is a mobilization mechanism, such as biosurfactant production, that increases the concentration of dissolved contaminants. I do not have an idea why low-molecular-weight alkanes should increase. Alkanes are usually degraded via carboxylic acids; these concentrations can increase but are usually of little regulatory concern. However, as mentioned above, the relative abundance of branched alkanes can increase, e.g., pristane and tristane in a diesel contamination.

Terminology consensus

From LaMountain, 1997. I agree that a consensus on terminology would be great. I have found the following to be a general consensus among anaerobic microbiologists, and consequently this is what I teach to my students:

- aerobic—when O₂ is present
- microaerophilic—limiting O₂ (less than 1 mg/L); this describes organisms, rather than environmental conditions; the organisms have specific requirements of low O₂
- anoxic—nitrate is the main electron acceptor
- anaerobic—no O₂, no nitrate, usually sulfate or CO₂ as electron acceptor
- fermentative—no electron acceptor available, characterized by production of low-molecular-weight acids and solvents; this is unusual in engineering reactors or soils but common in industry

I haven't heard of any opinions or new names for iron-reducing conditions; I suppose they would have to go in with anoxic.

Descriptions of aerobic, anoxic-anaerobic and fermentative organisms

Aerobic, from Pelmont, 1997. Aerotolerant organisms should be mentioned, for instance, Lactobacilli. They are anaerobes with no respiratory chain and no use of O₂. However, they still do fermentation in the presence of some O₂, i.e. in "aerobic" conditions. Other anaerobic bugs such as some sulfate-reducing species have been shown to stand low levels of O₂. This is very important in the environment since they can thrive at the limit between aerobic conditions and oxygen-depleted media in water, mats and even biofilms.

Anoxic-anaerobic, from Pelmont, 1997. Nitrate is just one electron acceptor. Here we call denitrification "anaerobic respiration," using nitrate as an acceptor. This is dissimilatory reduction of nitrate, producing typically N_2O and N_2 . Assimilation of nitrate is reduction to ammonia. The distinction between these two modes, however, is not always clear. Nitrate-using respiration shifts easily to other acceptors when nitrate is depleted: dimethylsulfoxide, trimethylamine oxide, fumarate, and others. *E. coli* does so, with a hierarchy of acceptors under control at the transcription level. I do not see the usefulness of this distinction between anoxic and anaerobic. Other respirations involve the reduction of Fe(III), Mn(II). Methanogenesis has been shown to be a respiratory process producing energy with CO_2 or acetate as an acceptor.

Fermentative. We here use a biochemical definition of fermentation. Fermentative processes produce ATP at the substrate level. Respiration is an energetic process building directly a membrane potential, ATP being made with H^+ or Na^+ translocating ATP synthases. This is usually a clear-cut definition, as proposed in the past by Slater . . . According to this definition, acetic acid production from ethanol is not a fermentation, just a respiration going to CO_2 and H_2O if not stopped in time. Fermentation is a word commonly used in industry, whatever the biochemical mechanisms are.

Since fermentation is often a lower energy-yielding process, it is usually characterized by the making of large amounts of organic by-products. Fermentation cannot be carried in oligotrophic conditions. But organic compounds are not the sole by-products: CO_2 and H_2 are also abundant sometimes, as in the case of some obligate anaerobes as *Clostridia* doing efficient fermentation with the production of a lot of gas. A bug like *Clostridium thermoaceticum*, however, is a true acetogenic species, producing acetic acid according to a mechanism that is interpreted as a CO_2 -using respiration, according to the above definition.

Terms applied to bacterial physiology and thermodynamics

The words describing aeration have unfortunately become confusing due to incorrect usage, even among microbiologists. Let's consider these terms as they pertain to bacterial physiology and thermodynamics.

Aerobic, from Focht, 1997. Aerobic refers to life with air, ergo, molecular oxygen. We fall into this category. O_2 is the most efficient electron acceptor known because of the high redox potential (816 mV, pH 7.0) for the O_2/H_2O couple at a 1:1 molar ratio, referred to as the E_h . The E_h for the $H_2/2H^+$ is -414 mV, pH 7.0). Rather than argue about how little oxygen it takes to be anaerobic or anoxic (without molecular oxygen), we should view oxidation/reduction reactions from the perspective of the Nernst equation; that is the basis of electro-chemistry. Energy can be evaluated and compared by use of electro-chemical potentials.

Anoxic, from Focht, 1997. Consider what happens in the environment as a result of respiration. Because diffusion of molecular oxygen in water is about 100,000 times slower

than in the gas phase, an oxic system can become anoxic quite quickly, where there is lots of water. The next most efficient electron acceptor commonly found in the environment is nitrate, which has a redox equilibrium (E_h) of 421 mV. Nitrate, nitrite and nitrous oxide are utilized, in lieu of dioxygen, as oxygen acceptors by many common aerobic soil bacteria, e.g. *Pseudomonas*, *Bacillus*, *Alcaligenes*. Denitrifying bacteria may thus be considered as facultative aerobes. They simply switch over to using the next best electron acceptor when O_2 is not available. Ferric iron ($E_h = 357$, pH 7.0) is the next best electron acceptor. Several books give an erroneous value of 770 mV, which is the value at pH 0. Many, but not all, denitrifying bacteria can use Fe^{+3} as an electron acceptor.

Obligate anaerobes (respiratory), from Focht, 1997. These are the domain of the sulfidogens (sulfate reducers, $E_h = -190$ mV), which may be thought of as having a very truncated respiratory system. This is a tough way to make a living: note the small energy difference, about 200 mV, in oxidizing H_2 this way as opposed to coupling H_2 oxidation with O_2 , 1230mV. But if table scraps are all that remain, then these organisms prevail under these meager conditions. The sulfide producers and the methanogens use molecular H_2 as their energy source. H_2 is generated by the other group of anaerobes immediately below.

Obligate anaerobes (fermentative), from Focht, 1997. So far, we have been talking about inorganic electron acceptors (respiration). Fermentation, by Pasteur's definition, is the use of organic electron acceptors. The production of lactic acid (e.g. yogurt production) from glucose by the lactic acid bacteria (which are microaerophiles) results actually from the reduction of pyruvate to give a stoichiometric balance of protons and electrons in lieu of an inorganic electron acceptor. The bacteria of the genus *Clostridium* actually produce H_2 gas during the production of protons and electrons.

Facultative anaerobes, from Focht, 1997. Microorganisms, including yeasts and other eucaryotes as well as bacteria, that can grow aerobically by respiration or anaerobically by fermentation, fit this term as defined by Pasteur. Unfortunately, too many textbooks have corrupted this term by incorrectly calling denitrifying bacteria facultative anaerobes. Denitrifying bacteria are not fermentative: they are strictly respiratory and are unable to use organic substrates as electron acceptors. Hence, they (and iron and manganese-reducing bacteria) should correctly be referred to as facultative anaerobes, i.e. they are obligate respiratory bacteria.

Microaerophiles, from Focht, 1997. These bacteria are basically fermentative anaerobes. They are different, however, from obligate anaerobes because they are able to tolerate small quantities of oxygen during growth. How small? That depends on the environment. Microaerophiles have an incomplete cytochrome system, such that when protons react with O_2 , hydrogen peroxide is produced. Even more toxic are super oxide radicals (O_2^-) that are generated during respiration. All aerobic organisms have superoxide dismutase (SUD), which converts super oxide radicals to

hydrogen peroxide. We and all other aerobes also have catalase, which converts H_2O_2 to O_2 and H_2O . Microaerophiles have SUD, but not catalase, while obligate anaerobes have neither.

Clinical bacteriologists readily observe that it is quite easy to grow hemolytic streptococci on blood agar plates, but not on other agar plates. So guess where microaerophiles are most commonly found, and what is so unique about blood: the mammalian mouth and the lining of other body cavities, which contain lots of catalase. Not surprisingly, most microaerophiles that reside at low oxygen concentrations are pathogens (hemolytic streptococci, diplococci) while others that reside in anaerobic environments (lactic acid bacteria) are not pathogenic. Based on what we know about the ecology of soil bacteria, the significance of microaerophiles is obscure and not likely to be important vis a vis biodegradation because other bacteria would be better competitors under microaerophilic or anaerobic conditions.

Additional information

For additional information on definitions of anaerobes or associated metabolic processes, consult the issues of *The American Society of Microbiology News* published in the early 1980s. A number of definitions were proposed and debated in the *Letters to the Editor* (Liss, 1997).

Acknowledgments

UTTU thanks the following for their contributions to this article: Dr. Martin Whittaker, Ph.D., Golder Associates Ltd., 905-567-4444, mwhittaker@golder.com; Dr. Debbie Roberts LaMountain, Department of Civil and Environmental Engineering, University of Houston, 713-743-4281, djroberts@uh.edu; Mike Barden, Geoscience Resources LTD, Albuquerque, New Mexico, 505-821-5508, mike-barden@ibm.net; Robin Gerlach, Center for Biofilm Engineering, Montana State University, Bozeman, Montana, 406-994-4770, robing@erc.montana.edu; Dr. Steven Liss, Ryerson Polytechnic University, Toronto, Ontario, 416-979-5000, sliss@acs.ryerson.ca; Dr. Allan Zhang, O'Connor Associates Environmental, Inc., Langely, British Columbia, 604-513-1005, allan-zhang@oconnor-associates.com; Dr. Jean Pelmont, Universite Joseph Fourier, Grenoble, France, 33-0-476-51-48-05, jean.pelmont@ujf-grenoble.fr; Dr. Dennis Focht, University of California, 909-787-3446, focht@citrus.ucr.edu. The views of these individuals are not necessarily those of their organizations.

UTTU also thanks Richard Schaffner, P.G., technical specialist, GZA GeoEnvironmental Inc., moderator of the Bioremediation Discussion Group. For administrative information on the BioGroup, please visit the BioGroup home page (<http://biogroup.gzea.com>); for additional information, send a message to rschaffner@gzea.com.



Engineered microbe effectiveness, bioaugmentation in lab vs. field and transgenic microorganisms

This article is based on a November 1997 discussion within the BioGroup. The exchange began when a member wanted to verify a claim that a "catalyst that contained facultative anaerobes and microaerophiles would draw oxygen down into the substrate" and would complete petroleum hydrocarbon degradation in 60 days." The member wanted to know if there were any methods to demonstrate the "superbugs" effectiveness.

Assessing microbe effectiveness

From Rie, 1997. Several methods are available to check the effectiveness of the additions. First, a word of caution: I have yet to find a refereed, peer-reviewed article in any publication that shows any more than a brief (30-day or less) acceleration of natural biodegradation when microbes are added. This is in comparison to equal treatment of a control plot without microbe addition. Normally, some combination of moisture, nutrient, and/or air addition is enough to stimulate existing microbes. See, for instance, the numerous Air Force studies.

As for assuring the effectiveness of the "native" or "foreign" (indigenous or added) microbes, several methods exist. Probably the best cheap method is a simple respiration test, measuring oxygen and carbon dioxide in test wells that are already in place. Water samples could be checked for dissolved oxygen and pH, which will decrease with increasing carbon dioxide levels.

A second method would be a relatively inexpensive lab count of hydrocarbon-degrading microbes, using core samples, carefully taken during soil sampling for hydrocarbons. Such testing would have to be carefully planned, but it could yield total heterotrophic plate counts as well as hydrocarbon degraders for a cost of less than \$2,000–\$3,000. We recently developed a method for specifically measuring hydrocarbon degraders, which, when combined with TPH data, can give an excellent picture of what is going on. To complete the picture, TPH measurements can be taken for the same series of samples. Several laboratories, including ours, provide the biological testing and planning of sample taking at nominal fees.

Potential biodegradation: the lab vs. the field

From Oppenheimer, 1997. A quantitative jump exists between the ideal lab and the field. Believe me, I've taught geo and marine microbiology for 40 years and for the past 7 years I've been attempting to be a businessman. The bottom line for the multitude of small projects is to get results without an expensive procedure.

We screen all sites by a simple method. Homogenize thoroughly a 500 g soil sample—water is easy—and run a baseline analysis on a composite of at least five grab samples. Separate the soil into two 200 g samples. Place the samples in shallow glass trays to a depth of one inch. One is the control. To the second add your product. Moisten the two samples to a mud consistency. Place on the bench and allow to dry, stirring once a day. If working with high volatiles, use a closed sealed glass pan. There will still be sufficient oxygen because only two atoms of oxygen are required to oxidize one hydrocarbon molecule. The open trays will dry in about seven days. Residual water in the covered trays will require adjustment.

Mix thoroughly all samples and take a composite of at least five small samples from each tray. Mix the composites for analysis. The results will give you information on potential biodegradation rate, and you don't have to bill your customer some outlandish fee. If the test is negative, go back to the lab bench or change microorganisms. Old sites that have no history of contamination are always suspect. The basic problem is to select the right bacteria and understand the environmental conditions to keep the living system viable.

From Wrenn, 1997. Although this method can probably tell you if bioaugmentation will not work at a particular site (assuming an adequate number of replicates are used), it almost certainly will not give you enough information to determine that it will. Two processes are important in determining whether bioaugmentation will be effective: bacterial transport and survival. The extensive and frequent mixing that Dr. Oppenheimer uses simulates landfarming reasonably well, but most other bioremediation processes (especially in-situ processes) are much less well mixed. Transport of bacteria from an injection well into a contaminated formation is difficult, because bacteria are sticky particles that will be filtered out of suspension by the soil particles. Also, if some type of inducer (for a desirable enzyme), or solubility-enhancing compound (to improve the bioavailability of the contaminants) is included in the product being tested, these materials will remain in close proximity to the added bacteria in the laboratory microcosms; however, they probably will not move through the subsurface at the same rate as the bacteria. This will obviously change the way the microbial product performs in the field relative to the laboratory. Furthermore, there is no reason to assume that survival of an introduced microbial population in a well-mixed and aerated laboratory microcosm is an indicator of how well those organisms will survive in the subsurface, where the concentration gradients of oxygen and hydrocarbons can be steep.

Bioaugmentation should always be evaluated in the field. It *should* work in the laboratory. If it doesn't, the microorganisms in the product are poorly matched to the target contaminants or there is a problem with their viability. Success in the lab, however, doesn't indicate that the product will work in the field. The factors that determine success in the field cannot be evaluated in simple laboratory microcosms. Field studies must be carefully designed to distinguish between the effects of bioaugmentation and those due to

pumping water that contains oxygen, nutrients, and/or surfactants.

One minor correction on the subject of laboratory treatability studies: more than two atoms of oxygen are required to oxidize most hydrocarbon molecules. Two moles of O₂ (i.e., four oxygen atoms) are required to completely oxidize one mole of methane, the simplest hydrocarbon. In general, between 1.2 and 1.5 moles of O₂ are required per carbon atom to completely oxidize the hydrocarbons of interest in bioremediation (e.g., BETX and above). Most target hydrocarbons have at least six carbon atoms and will require at least 7.5 molecules of O₂ for complete oxidation of each molecule. The rule of thumb is 3 mg O₂ per mg of hydrocarbon, which is equivalent to 11 ml of air per mg hydrocarbon. Obviously, the volume of air required to completely mineralize the hydrocarbons in 200 g of soil depends on the degree of contamination. If the soil has 5,000 ppm of degradable hydrocarbons, 11 liters of air are required to provide sufficient oxygen for complete biodegradation. Most treatability studies won't be conducted long enough to achieve that degree of mineralization, but it would be foolish to draw conclusions based on less than 10% mineralization, and even that minimal level of treatment requires more than 1 liter of air. Also, the biodegradation rate will become oxygen limited long before the O₂ in the headspace is completely exhausted. So, it isn't valid to assume that enough O₂ will be available, unless you do a few quick calculations on the size of your system relative to the amount of hydrocarbon degradation that you want to observe.

Questions raised concerning transgenic bugs

Another member wanted specific information on a genetically modified microorganism with hydrocarbon-degrading capabilities that was patented in 1981. He also wanted to know if transgenic bacteria were used for ex-situ bioremediation purposes. Finally, he wanted to know what risks transgenic microorganisms presented to the environment.

From Glass, 1997. I have a great deal of experience with U.S. regulation of genetically engineered microbes, including nine years in the agricultural biotech industry when field tests were first conducted in the mid to late 1980s. My company conducted early field tests of genetically engineered rhizobia for nitrogen fixation. I have been involved with the bioremediation industry since 1990, and I have been a proponent of using engineered microbes for bioremediation, when their use made sense technically and economically. Most individuals believe that transgenic microbes pose no additional risk and that the regulatory process to gain approval for their use is manageable and achievable. Many bioremediation professionals, however, are apprehensive about using engineered microbes because of possible adverse public or governmental reaction; the simple fact is, for most currently utilized applications of bioremediation, engineered bugs are just not needed.

With respect to the microorganism that A.M. Chakrabarty patented in 1981, I believe these microbes were never used. There is generally no reason to improve hydrocarbon-degrading bacteria through genetic engineering—naturally

occurring bugs work just fine (particularly indigenous microbes)—and there's no need to incur the expense of any extensive research program to improve them. To the extent that genetic engineering will be useful in bioremediation, it will be for recalcitrant compounds, or for compounds where natural degradation pathways don't exist.

To the best of my knowledge, transgenic bacteria have not been used at all in commercial bioremediation, although some academic groups, notably Gary Sayre's at the University of Tennessee, have obtained government approval for outdoor testing of engineered microbes in bioremediation experiments. Under current EPA regulations, a company could use engineered microbes in a bioreactor without needing a permit for field trials (experiments), but EPA approval would be needed for commercial use. There may have been some experimental, but unlikely commercial, uses of engineered microbes in reactors.

You asked about the special environmental risks of using transgenic microorganisms tailored for bioremediation: how do strains derived from a contaminated site differ from those derived from a lab culture (with adapted degradation capability)? This question is probably too complicated to answer in a brief e-mail. There are likely no unique risks from transgenic microbes, but there are several risks one should assess before the large-scale introduction of ANY non-indigenous microbe, including the ability to survive and disseminate beyond the plot, and any adverse effects on nontarget organisms. With respect to the use of "bioaugmentation" with natural bugs vs. engineered bugs: lab cultures often cannot compete with indigenous populations, and most studies do not show any lasting effect of introduced populations (e.g., in increasing rates of degradation). Thus, introduced populations don't persist long enough to do any good, and they probably don't last long enough to do any harm either.

From LaMountain, 1997. Another BioGroup member expressed similar views in that she'd heard that the new organism could neither survive nor compete in the world outside the lab. I have never heard that transgenic bacteria have actually been used to successfully treat anything. In most cases, if you talk to the authors who published the wonderful paper about the creation of a strain that could degrade new compounds, they will tell you that it did not survive the competitive environment of a treatment process.

Although Gary Sayre has shown that some genetically altered organisms can survive in the environment for at least short periods of time, in most cases the organisms released do not survive long. The major changes in their genome are in degradative pathways, so it is unlikely that this would affect their pathogenicity. The largest risk involves wasting a lot of money.

Organisms that derive their degradative properties "naturally" through exposure to the compound or a similar compound are much more stable and usually the best competitors for that substrate. If they are soil organisms, and not kept in the lab too long, they will thrive back in the

soil where they came from. If you keep the organisms happy in the lab too long, they will not be as effective when put back into the soil.

Acknowledgments

UTTU thanks the following for their comments: Dr. John Rie, CBRS, Inc., Meriden, Connecticut, 203-237-1382, cbrs@snet.net; Dr. Carl Oppenheimer, www.obio.com, carlo@mail.utexas.edu; Dr. David J. Glass, D. Glass Associates, Inc., Needham, Massachusetts, 617-726-5474, DGlassAssc@aol.com; Dr. Debbie Roberts LaMountain, Department of Civil and Environmental Engineering, University of Houston, 713-743-4281, djroberts@uh.edu; and Brian Wrenn, Rochester, New York, 715-787-0502, bawrenn@rpa.net. The comments expressed by these individuals are not necessarily those of their organizations.

UTTU graciously thanks Richard Schaffner, P.G., technical specialist, GZA GeoEnvironmental Inc., moderator of the Bioremediation Discussion Group. Please visit the BioGroup home page (<http://biogroup.gzea.com>) for information or send a message to rschaffner@gzea.com.



Acceptable hydrocarbon concentrations in soils

A member of the BioGroup posed the following question: "For bioremediation of hydrocarbons in soils, what final concentration is considered as acceptable?" The comments received from the BioGroup follow.

From Willits, 1997. To accurately respond to the question of acceptable limits for soils, you need to know the potential for impact to groundwater and subsequent food chain consumption. In general, levels in the United States range between 100 and 1,000 ppm for soils and virtually non-detect for groundwater. Different states have different levels for standards.

From Cooley, 1997. I must take issue with this response, or at least part of it. Soil standards for total petroleum hydrocarbons (TPH) have, in the past, been typically set to arbitrary base-10 numbers (e.g. 100, 1,000 or 10,000 ppm). Some states are moving toward using risk-based standards for TPH. These standards usually are based on both protection of groundwater from cross-media contamination and consideration of dermal exposure, soil ingestion, inhalation of volatiles or particulates, or a combination of these exposure routes. For example, the Texas risk-based standards take soil ingestion and inhalation of particulates into account in establishing near-surface soil standards and potential for leaching to groundwater for all impacted soil.

Because TPH is not a chemical but a mixture, risk analysis is conducted using surrogate compounds. The TPH is speciated through GC/MS methods and a surrogate is used for each carbon range. For example, C9-C12 aromatics are treated as naphthalene. Alternatively, the TPH Criteria Working Group has developed transport and fate properties (e.g. K_{oc}) for these carbon ranges through laboratory testing. Surrogates are also used for toxicological data.

In our experience, the resulting risk-based standards for soil vary from approximately 500 mg/kg to concentrations that exceed soil saturation. Typically they fall in the 1,000-5,000 mg/kg range. The composition of TPH varies significantly from site to site, depending primarily upon release origin and TPH degradation. Furthermore, soil properties, such as fraction of organic carbon used in calculating risk-based standards, vary from site to site.

Regarding TPH in groundwater, there is no human health-based justification for requiring "non-detect" as a cleanup standard. Even if you treat the TPH mass as consisting entirely of pyrene (a very conservative assumption), the resulting risk-based standard for human ingestion in a residential setting is approximately 1 mg/L. However, ecological risk issues, such as endangerment of critical habitats, or aesthetic concerns, such as taste or odor, may result in more stringent cleanup standards.

From Focht, 1997. I note that scientists have found concentrations that are quite variable. We have found that diesel fuel-contaminated soil gives an excellent fit to a fractile log-normal distribution plot (27 samples). My question therefore has less to do with science, but rather with law.

In California the legal limit for TPH-contaminated soil is 100 ppm. But what does this mean? A log-normal mean, or all samples being less than 100 ppm? I have found no one to give an answer. The few attorneys who do understand statistics have little appreciation for the meaning of a log-normal distribution. The general rule of thumb among soil scientists is that contaminants—including natural ones such as nitrate—are log-normally distributed, while most other intrinsic soil properties (organic matter, texture, mineral constituents) are normally distributed. Even this is a questionable generality if one samples soil located on terminal moraines.

In lieu of recognizing spatial variability of contaminants, I maintain that we are chasing a rainbow, with no pot of gold nearby—except to snake oil peddlers and their remedies.

From Miller, 1997. Concentrations are quite variable. But many books, articles, and guidance documents are available on the subject of environmental statistics. Perhaps most important are the guidance documents published by the USEPA. In the absence of any specific state guidelines on the subject, these documents will provide the recommended rules. The following are some of the most applicable:

- U.S. EPA, Office of Policy, Planning and Evaluation, "Methods for Evaluating the Attainment of Cleanup Standards, Volume 1: Soils and Solid Media," Statistical Policy Branch, Washington D.C., 1989
- U.S. EPA, Office of Solid Waste, "Statistical Analysis of Ground-Water Monitoring Data at RCRA Facilities, Interim Final Guidance," Washington D.C., 1989
- U.S. EPA, Office of Solid Waste, "Statistical Analysis of Ground-Water Monitoring Data at RCRA Facilities, Addendum to Interim Final Guidance," Washington D.C., 1992
- U.S. EPA, Office of Emergency and Remedial Response, "Guidance for Data Usability in Risk Assessment (Part A)," Washington D.C., 1992
- U.S. EPA, Office of Policy, Planning and Evaluation, "Methods for Evaluating the Attainment of Cleanup Standards. Volume 3: Reference-Based Standards for Soils and Solid Media," Environmental Statistics and Information Division, Washington D.C., 1994

A very useful textbook on the subject is R.O. Gilbert's *Statistical Methods for Environmental Pollution Monitoring*, Van Nostrand Reinhold, New York, 1987. The concentrations of foreign chemicals (xenobiotics) in the soil and groundwater frequently do follow a log-normal distribution. Many exceptions exist, however, so it is crucial to determine the distribution of your data for each measured parameter. Such tools as probability plotting, Lilliefors normality tests, and Shapiro-Wilk normality tests can be used here. Once the distribution has been determined for each chemical, the appropriate measure of the data's central tendency (e.g., mean, geometric mean, median) can be calculated. You will find that the usual regulator-accepted value is an upper confidence limit for that calculated central tendency. Note that in your calculations you must use only data from the contaminated area in your calculations—no averaging in values from any clean background areas.

On the subject of environmental law: many state regulations discuss very elementary statistical methods that are acceptable for evaluating environmental data. (I am not familiar with California laws.) In the absence of any such guidance, it is usually acceptable to use the EPA procedures (see references above), but get approval from your regulators. This may be heresy, but you can read and interpret the state environmental laws yourself—you don't necessarily need a lawyer. I am an environmental chemist, and I have been interpreting for years the relevant environmental laws of the federal government and of Massachusetts, New Jersey, New York, Pennsylvania, Ohio, and Illinois. A little patience is required to plow through the verbiage to find the applicable portions of the law.

How to classify pyrene and its relationship to RBCA

From Willits, 1997. Maybe pyrene is lumped in with TPH in Texas, but here in New Jersey, pyrene is grouped with PAHs with an entirely different standard. In fact, I think that one of the few things we use TPH analysis for is diesel contamination. On another note, RBCA certainly seems to have enabled a lot less actual action and cleanup.

From Cooley, 1997. Pyrene, when detected, is treated as a separate PAH constituent in Texas and other states that I

have worked in. From a risk standpoint, pyrene can be used as a conservative surrogate for an entire TPH mass. In other words, when calculating risk, treating 1,757 mg/kg TPA as 1,757 mg/kg pyrene is conservative. Therefore, a RBCA where TPH is treated as pyrene is protective of human health and the environment.

I wish there were enough money in the world to remediate every spill to background concentrations, but there isn't. Therefore, we have to make intelligent choices on where and how to spend remediation dollars. The best available tool is RBCA, which is designed to achieve cleanups protective of human health and the environment.

From Rothstein, 1997. Concerning "what final hydrocarbon concentration is considered as acceptable?": this is a loaded question! It depends what compounds are in the hydrocarbon. Benzo(a)pyrene is only allowed to be in residential soil to 2.5 ppm while naphthalene is allowed to 3,000 ppm in Pennsylvania. If the oil is fairly innocuous, such as mineral oil or food grade oil, then any concentration that does not permit free product to cause a sheen when it rains would be acceptable. This concentration (when no sheening will occur during rain) would be the residual concentration, which for mineral oil ranges from 12,400 ppm in sand to approximately 50,000 ppm.

Pennsylvania has risk-based standards for a large number of compounds. The list is contained in the "Land Recycling Act," also known as Act 2. This act has soil- and water-based numbers. The soil numbers are different for residential and commercial sites; they also differ with proximity to the aquifer. The big problem with Act 2 is that it doesn't address oils, such as transformer mineral oil, that contain some of the compounds listed but all of which are below PQLs in the oil itself. In this case we had the state of Pennsylvania agree that we would simply reduce the oil concentration to below residual saturation so that there would be no sheen. Another problem with Act 2 is that virtually all of the methods require EPA GC/MS techniques such as 8270B, which are very expensive.

From Willits, 1997. Mr. Cooley appears to believe that certain cleanup criteria are unrealistic when balanced against costs and risks. I believe that the main reason we are at this juncture in accepting lowered standards for environmental quality is that we have propounded a massive failure in accomplishing cleanups over the past 20 years. It seems that both greed on the part of the environmental industry as a whole and failure to exercise proper caution in evaluating and applying technologies has brought us to this sorry point. Our clients are exhausted and fed up with spending millions of dollars for systems to clean up soils and groundwater that are still not cleaned to acceptable standards. No amount of statistical analysis is adequate to prevent accidental contact or seepage to some higher risk location by an unidentified preferential pathway. Yet what we are doing has had such a high percentage of failure: pump-and-treat systems, vapor extraction systems, peroxide treatments, etc.

From Oppenheimer, 1997. We are a company who has pushed inexpensive applied bioremediation with near 100%

success in cleanup. We are continuously in competition with large funded companies that have the capability of selling inefficient systems that can never reach criteria. Now they are pushing for criteria reduction through risk assessment or just plain politics.

Our governments are responsible for the health of the population. It is inexcusable for any government unit to reduce criteria when potential health hazards are present, especially in groundwater. Bioremediation, involving proper use of selected natural microbial populations, is a natural, sensible way of removing pollutants from soil, air, and water. We have proven it cost-effective for all natural hydrocarbons and many chlorinated hydrocarbons in-situ. It is currently estimated that in the U.S. we use approximately 35% of the GNP for pollution abatement.

A large company in Japan has spent \$5-10 million on research to produce microorganisms that degrade TCE, and they will spend more on developing mass production. I have been producing TCE organisms in mass amounts for two years and have used them successfully in groundwater cleanup. If the Japanese company (who had been contacted by our Japanese partner) had spent their millions on the application of our proven microorganisms, they could have cleaned up a great amount of TCE pollution generated by industry in Japan. Sometimes I think our scientists are more interested in salaries than cleaning up our polluted environment. This also applies to certain government agencies. Bioremediation using applied microorganisms and appropriate application technology can and will turn a pollutant into a resource.

From Cooley, 1997. Certainly using a default-conservative pyrene standard for evaluating risks due to TPH needs to be done within the framework of a risk-based corrective action (RBCA). For example, if you are following the ASTM RBCA standard, generic non-site-specific tier 1 risk-based screening levels (RBSLs) would be established for pyrene for the various media and exposure pathways. If the TPH values are less than the tier 1 RBSLs, no further action would be required for TPH. If the TPH values exceeded the tier 1 pyrene RBSLs, then you would have several options:

1. Speciate the TPH and develop tier 1 RBSLs for the TPH, then compare the TPH concentrations to the TPH RBSLs
2. Develop tier 2 site-specific target levels (SSTLs) for pyrene; compare TPH concentrations to pyrene SSTLs
3. Speciate TPH and develop tier 2 SSTLs for TPH; compare TPH concentrations to TPH SSTLs
4. Perform a tier 3 evaluation using numerical methods (i.e. modeling); develop tier 3 SSTLs and compare TPH concentrations to tier 3 SSTLs for TPH; tier 3 SSTLs could be based on pyrene or speciated TPH

The concept behind RBCA is to do sufficient analysis on which to base decisions. For example, if you have a small TPH release and you can live with cleaning up to tier 1 RBSLs for pyrene, then why spend the money performing a detailed risk assessment? On the other hand, if you have a very large release, it is worthwhile to develop tier 3 SSTLs based on TPH speciation.

Please note that even a tier 3 based on speciated TPH is protective of human health and the environment. All RBCA programs that I am familiar with, including the ASTM RBCA, are based on the EPA Risk Assessment Guidance for Superfund (RAGS). Toxicity factors are typically taken from the integrated risk information system. Yes, there is conservatism built into the toxicological data, and yes, there is some redundancy in conservatism with pathway analysis. In the end, however, we have a system that is protective of human health and the environment without burdening the private and public sector with unreasonable remediation costs.

From Ketcheson, 1997. I'm from Canada, and I thought the majority of cleanup guidelines were based on toxicological evaluation using a generic, yet conservative set of exposure conditions. The established value actually represents a no-effects limit for the most sensitive receptor, which has then been reduced by some factor of safety to establish the guideline value. I understand that the value represents an upper concentration limit that will be tolerated in the environment. Thus any decision above the limit represents an unsatisfactory condition in the environment that conceptually needs to be mitigated.

I would be concerned if contaminant levels were close to this value because the sampling is suggesting levels close to an unsatisfactory limit. Given the heterogeneity at most sites, I have no confidence that there would not be TPH levels above the sampled value in close proximity to the sampling location. This is where geostatistics may prove useful.

Testing contaminated soil

From Smith, 1997. Regarding TPH cleanup standards, in many places where large quantities of fuel have spilled into the subsurface, there are large subsurface fuel pockets that are difficult to detect with soil sampling and conventional soil gas sampling. I've been on several sites where soil and soil gas have tested clean, but high methane levels and low oxygen levels indicated TPH pockets somewhere. At one Air Force base, these pockets supported a soil vapor extraction system for several years **without TPH ever being detected in soil samples**, and TPH being detected in soil gas only after several hours of vapor extraction. Yet methane concentrations were high, and low oxygen concentrations were found in the original samples of undisturbed soil gas.

Most investigations don't look for methane or measure oxygen concentrations in the soil gas. In the San Francisco Bay area some environmentalists and citizens are beginning to insist that these measurements be made before they will agree to "no action." The methane at Naval Air Station in Alameda, California, constitutes 60 percent of the soil gas under parking lots. This constitutes a very real explosive hazard if it leaks into utility trenches or buildings.

Yet methane is not regulated by federal or state toxics laws, nor accounted for in quantitative risk assessments. Quantitative risk assessments have a very narrow scope, and qualitative risks such as transformation into more toxic products and explosive hazards should be taken into account. In general, if oxygen is present in the subsurface where hydrocarbons are found, some Bay Area citizens are more comfortable with higher TPH cleanup levels.

Original inquiry?

From Morgan, 1997. Some time ago an individual asked a question regarding "permissible" or "legal" limits of hydrocarbon contamination in soil. Our discussion group has proceeded to give that individual all sorts of information about what is wrong (or at least inconsistent) with "permissible" levels of contamination, but little to answer his or her basic question. The original posting seemed to be asking a general question and hoping, I think, to receive responses from various geographic regions regarding cleanup criteria used in their area. If my premise is correct, I suggest that the people scan the ASTM document, *DS64-Cleanup Criteria for Contaminated Soil and Groundwater*, edited by A. Buonicore. The document is a summary of criteria used by various states as well as other countries; it should not, however, be viewed as the definitive or final document on this subject. I offer it here only to assist the original poster in getting an overview of the topic.

Acknowledgments

UTTU thanks the following for their comments: Austin Cooley, Brown and Caldwell, Houston, Texas, acooley@brwncald.com; Dr. Dennis Focht, 909-787-3446, focht@citrus.ucr.edu; University of California, Riverside; Jim Willits, BioActive Remediations Technologies, Inc., New Jersey, willits@bioactive.com, <http://www.bioactive.com>; Mike Miller, Camp Dresser & McKee Inc., Cambridge, Massachusetts, millerme@cdm.com; Dr. Carl Oppenheimer, carlo@mail.utexas.edu; Tony Morgan, hydrogeologist, LGI, Inc., 909-390-2833, quatinvest@earthlink.net; William Smith, chair of the Sierra Club's East Bay Military Conversion Task Force, Fremont, California, 510-490-3008, WJASmith@AOL.com; David Ketcheson, dketches@niagara.com; Mark Rothstein, Peco Energy Co., 215-841-4868, mrothstein@legal.peco.com. Comments expressed by these individuals are not necessarily those of their affiliated organizations.

UTTU also graciously thanks Richard Schaffner, P.G., technical specialist, GZA GeoEnvironmental Inc., moderator of the Bioremediation Discussion Group. For information on the BioGroup, please visit the BioGroup home page, <http://biogroup.gzea.com>) or send an electronic message to rschaffner@gzea.com.



History of bioremediation references

The following references offer a glimpse of bioremediation history.

- Alexander, M., *Introduction to Soil Microbiology*, Wiley, N.Y., 472 p., 1961.
- American Petroleum Institute, Federal Water Pollution Control Administration, *Proceedings to the Joint Conference on Prevention and Control of Oil Spills*, 345 p., 1969.
- American Petroleum Institute, *Annual Oil Spill Conference*, Washington D.C., 1969 to present.
- Atlas, R.M., "Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective," *Microbiological Reviews*, No. 45, p. 180-209, 1981.
- Azoulay, E. and J.C. Senez, "Degradation bacterienne des hydrocarbures paraffiniques II. Determination des produits intermediares par la methode des adaptations simultanees," *Ann. d. Inst. Pasteur*, No. 8, p.868-879, 1960.
- Baas Beeking, L. G. M., Kaplan, I. R. and D. Moore, "Limits of the Natural Environment in Terms of pH and Oxidation Reduction Potentials," *Journal of Geology*, No. 68, p. 243-284, 1960.
- Baas Beeking, L. G. M., "Geology and Microbiology," *Contr. Marine Microbiology, New Zealand, New Zealand Oceanographic Institute*, Memoir 3, p. 48-64, 1959.
- Beerstecher, E., *Petroleum Microbiology*, Elsevier Press, 375 p., 1954.
- Blank, M., Ed., "Chemistry of Biological Systems," *Advanced Experimental Medicine and Biology*, Vol. 7, Plenum Press, New York, 1970.
- Blumer, M. and J. Sass, "Oil Pollution Persistence and Degradation of Spilled Fuel Oil," *Science*, No. 176, p. 1120-1122, 1972.
- Bowan, H.J.M., *Trace Elements in Biochemistry*, Academic Press, New York, 241 p., 1966.
- Boylan, D.B. and B.W. Tripp, 1971, "Determination of Hydrocarbons in Sea Water: Extracts of Crude Oil and Crude Oil Fractions," *Nature*, No. 230, p. 44-47, 1971.
- Brock, T.D., Smith, D.W. and M.T. Madigan, *Biology of Microorganisms*, Prentice Hall, Englewood Cliffs, New Jersey, 847 p., 1984.
- Brown, S.O., "Microbial Oxidation of Crude Oils and Water Soluble Fraction of Crude Oils as Shown by the B.O.D. Method," *Texas A&M Research Foundation*, Project 9, p. 2, 1950.
- Bushnell, L.D. and H. F. Haas, "The Utilization of Certain Hydrocarbons by Microorganisms," *Journal of Bacteriology*, No. 41, p. 653-673, 1941.
- Calvin, M., *Chemical Evolution*, Oxford University Press, 278 p., 1969.
- Campbell, R., *Microbial Ecology*, Blackwell Science Publication, Oxford, 191 p., 1983.
- Carey, F.A., *Organic Chemistry*, McGraw-Hill, New York, 1219 p., 1989.
- Clerk, R.B., *Marine Pollution*, Oxford, Clarendon Press, 168 p., 1992.
- Colwell, R.R. and J.D. Walker, "Ecological Aspects of Microbial Degradation of Petroleum in the Marine Environment," *Critical Reviews in Microbiology*, No. 5, p. 423-445, 1977.
- Conservation Foundation, "State of the Environment, An Assessment Mid-Decade," *Conservation Foundation*, Washington D.C., 586 p., 1984.
- Corredor, J.E., Morell, J.M. and C.D. Castillo, "Persistence of Spilled Crude Oil in a Tropical Intertidal Environment," *Marine Pollution Bulletin*, No. 21, p. 358-388, 1990.
- Council on Environmental Quality, *Environmental Quality 9th Annual Report*, Washington D.C., 599 p., 1978.
- Davis, J.B., *Petroleum Microbiology*, Elsevier Publications, Amsterdam, 604 p., 1967.
- DeRosa, M., Gambacorta A. and A. Gliozzi, "Structure, Biosynthesis and Physicochemical Properties of Archaeobacterial Lipids," *Microbiology Review*, No. 50, p. 70-80, 1986.
- Dobell, C., *Antonie van Leeuwenhoek and his "Little Animals,"* London Staples Press, 1932.
- Driver, J.I., *The Geochemistry of Natural Waters*, Prentice Hall, Englewood Cliffs, New Jersey, 437 p., 1988.
- Drucker, H. and R.E. Wildung, "Biological Implications of Metals in the Environment," *Proceedings Symposium, Technical Information Center, Energy Research and Development Administration*, 682 p., 1975.
- Ehrlich, H.L., *Geomicrobiology*, Marcell Dekker, New York, 393 p., 1981.
- Englemann, T.W., "Bacterium photometricum, Ein Beitrag zurvergleichenden Physiologie des Licht und Farbensinnes," *Pflugers Arch.*, No. 30, p. 95, 1883.
- Fenchel, T. and T.H. Blackburn, *Bacteria and Mineral Cycling*, Academic Press, London, 225 p., 1979.
- Ford, B.J., *Microbe Power*, Stein and Day, New York, 181 p., 1976.
- Garrels, R.M., *Mineral Equilibria*, New York, Harper Brothers, 254 p., 1960.
- Gabler, R.E., Sager, R.E., Brazier, S.M. and D.L. Wise, *Essentials of Physical Geography*, Saunders College Publication, Philadelphia, 550 p., 1987.
- Gibson, D.T., Cardini, G.E., Maseles, F.C. and R.E. Kallio, "Incorporation of Oxygen-18 into Benzene by Pseudomonas Putida," *Biochemistry*, Vol. 9, p. 1631, 1970.
- Gliozzi, A., Rolandi, R., DeRosa, M., Gambacorta, A. and B. Nicolaus, "Membrane Models of Archaeobacteria," in *Transport in Biomembranes*, Edited by R. Anolini, Raven Press, New York, 1982.
- Gortner, R.A. and W. Gortner, *Outlines of Biochemistry*, John Wiley, New York, 1078 p., 1949.
- Gundlach, E.R., Boehm, P.D., Atlas, R.M., Ward, D.M. and D.A. Wolfe, "The fate of AMOCO CADIZ Oil," *Science*, No. 221, p. 122-129, 1980.
- Gunkel, W., Gassmann, G., Oppenheimer, C.H. and I. Dundas, "Preliminary Results of Baseline Studies of Hydrocarbons and Bacteria in the North Sea," 1975, 1976 and 1977, *Spanish Conference on Hydrocarbon Pollution, Santiago de Compostela*, 38 p., April 1977.

- Haas, H.F., Yantzi, M.F. and L.D. Bushnell, "Microbial Utilization of Hydrocarbons," *Trans. Kansas, Academic Science*, No. 44, p. 39-45, 1941.
- Hildebrand, H.H. and G. Gunter, "Deposition of Petroleum Tars and Asphalts, Beaches of the Northern Gulf of Mexico," *UTMSI Report*, University of Texas, Port Aransas, Texas, 88 p., 1955.
- Hitzman, D.O., "Petroleum Microbiology and the History of its Role in Enhanced Oil Recovery," in E.C. Donaldson and J.B. Clark (Eds.), *Proceedings, 1st International Conference on Microbial Enhanced Oil Recovery*, May 16-21, 1982, Afton, Oklahoma, p.162-218, 1983.
- Horvath, R.S., "Microbial co-Metabolism and the Degradation of Organic Compounds in Nature," *Bacteriological Reviews*, No. 36, p. 146-155, 1972.
- Hunt, John M., *Petroleum Geochemistry and Geology*, W.H. Freeman and Company, San Francisco, 617 p., 1979.
- Hutner, S.H., "Nutrition of Protists," in *This is Life*, Johnson and Steere, Editors, Holt Reinhart and Winston, New York, 1962.
- James, A.M., "The Electrochemistry of the Bacterial Surface," *Proceedings of Biophysics and Biophysical Chemicals*, No. 8, p. 98-144, 1957.
- Johnson, F.H., Goodale, W.T. and J. Turkevich, "The Bacterial Oxidation of Hydrocarbons," *Journal Cell. Comp. Physiol.*, Vol. 19, No. 163-172, 1942.
- Kator, H.I., *Utilization of Crude Oil Hydrocarbons by Mixed Cultures of Marine Bacteria*, Unpublished PhD Thesis, the Florida State University, Department of Oceanography, 1972.
- Kator, H.I., Oppenheimer, C.H. and R.J. Miget, 1971, "Microbial Degradation of a Louisiana Crude Oil in Closed Flasks and Under Simulated Field Conditions," *Proceedings of the Joint Conference on Prevention and Control of Oil Spills*, American Petroleum Institute, EPA, and U.S.C.G., p. 287-296, 1971.
- King, J.W. and D.A. Stevens, *Proceedings of the First International MEOR Workshop*, Department of Energy, April 1-3, 1986, Bartlesville, Oklahoma, NTIS DOE/BC/10852-1, (DE87001216), 373 p., 1987.
- Kinghorn, R.R.F., *An Introduction to the Physics and Chemistry of Petroleum*, Wiley and Sons, New York, 420 p., 1983.
- Krumbein, W.E., Editor, *Microbial Geochemistry*, Blackwell Science Publications, 330 p., 1983.
- Kuznetsov, S.I., Ivanov, M.V. and N.N. Lyalikova, *Introduction to Geological Microbiology*, Trans. 1963, McGraw-Hill, New York, 1962.
- La Riviere, J.W.M., "The Production of Surface Active Compounds by Microorganisms and its Possible Significance in Oil Recovery II," *Antonie v. Leeuwenh, Journal of Microbiology Serol.*, No. 21, p. 9-27, 1955.
- Lederberg, J. and E.M. Lederberg, *Journal Bacteriology*, 63:399, 1952.
- Lee, C.C. and W.K. Craig, "Water Soluble Hydrocarbons from Crude Oil," *Bulletin Environmental Contaminated Toxicology*, Vol. 11, p. 212-217, 1974.
- Leeuwenhoek, A. van, 1694, "Ondervindingen en Beschouwingen der Onsigthar Geschapene Waarheden," 2nd Ed., p 45., Delft, in Dobell, 1932.
- McKenna, E.J. and R.E. Kallio, "The Biology of Hydrocarbons," *Annual Reviews of Microbiology*, Vol. 19, p.183, 1965.
- Marr, E.K., *The Bacterial Oxidation of Benzene*, Dissertation, Pennsylvania State College, 1959.
- Margulis, L., *Early Life*, Jones and Bartlett, Boston. 260 p., 1984.
- Mason, S.F., *Chemical Evolution, Origin of the Elements, Molecules and Living Systems*, Clarendon Press, Oxford, 317 p., 1992.
- Master, M. and C.H. Oppenheimer, "On the Solution of Quartz and Precipitation of Dolomite in Sea Water During Photosynthesis and Respiration," *Zeit. F. Allgemeine Mikrob.*, Vol. 5, p.48-51, 1965.
- Meinschein, W.G., "Origin of Petroleum," *Bulletin of the American Association of Petroleum Geologists*, Vol. 43, p. 925, 1959.
- Meinschein, W.G., "Biological Markers and n-Alkanes as Geological Agents," in *Organic Geochemistry of Contemporaneous and Ancient Sediments*, Ed. Meinschein, Great Lakes Section Society of Economic Paleontologists and Mineralogists, Bloomington, Indiana, Chapter 2, p. 29, 1983.
- Miget, R.J., Oppenheimer, C.H., Kator, H.I. and P.A. LaRock, "Microbial Degradation of Normal Paraffin Hydrocarbons in Crude Oil," *Proceedings for the Joint Conference on Prevention and Control of Oil Spills API-FWPCA*, December, p. 327-331, 1969.
- Montgomery, C.W., *Fundamentals of Geology*, W.C. Brown, 357 p., 1989.
- National Academy of Sciences, *Productivity of World Ecosystems*, NAS, Washington D.C., 166 p., 1975.
- Oppenheimer, C.H., Thesis, University of California at Los Angeles, *Effect of High Pressure on Marine Microorganisms*, 1951.
- Oppenheimer, C.H. and L. Kornicker, "Effect of Microbial Production of Hydrogen Sulfide and Carbon Dioxide on the pH of Recent Sediments," *Publication of the Institute of Marine Science*, The University of Texas, Vol. 5, p. 5-15, 1958.
- Oppenheimer, C.H., "Bacterial Production of Hydrocarbon-like Materials," *Zeitschrift fur Allgemeine Mikrobiologie*, Vol. 5, No. 4, p. 284-307, 1965.
- Oppenheimer C.H., "Eh and pH of Marine Sediments," in *Encyclopedia of Earth Sciences*, Reinhold Publications Company, New York, 1966.
- Oppenheimer, C.H., *Testimony Presented to the Oversight Hearings on the Effect to the United States on the Blowout of the Pemex IXTOC Oil Well*, September 11, 1979.
- Oppenheimer, C.H., Personal Paper, Collection of Papers Prepared for the Bureau of Land Management, Offshore Oil Lease Hearings, Louisiana, Mississippi, Florida, New York, 1980.
- Oppenheimer, C.H., *Oil Ecology*, Chapter 1, "Marine Environmental Pollution, 1, Hydrocarbons," R. Geyer, Editor, p. 21-35, Elsevier Oceanography Series, Amsterdam, 1980.
- Oppenheimer, C.H. and W. Drost-Hansen, "A Relationship Between Multiple Temperature Optima for Biological Systems and the Properties of Water," *Journal of Bacteriology*, Vol. 80, p.21-24, 1960.

- Oppenheimer, C.H., Miget, R.J. and H.I. Kator, "Ecological Relationships Between Marine Microorganisms and Hydrocarbons in the O.E.I. Study Area, Louisiana," *Rice University Studies*, Vol. 65, p. 287-325, 1979.
- Oppenheimer, C.H. and F.K. Hiebert, "Microbially Enhanced Oil Production Field Tests in Texas," *Proceedings of the Symposium on Applications of Microorganisms to Petroleum Technology, USDOE/NIPPER*, Bartlesville, Oklahoma, 1988.
- Oppenheimer, C.H. and F.K. Hiebert, *Microbiological Techniques for Paraffin Reduction in Producing Oil Wells*, Department of Energy Final Report, DOE/BC/14014-9 (DE89000741), 67 p., 1989.
- Pasteur, L., *Ann.Chim.Physic.*, Vol. 58, 323 p., 1860.
- Pashley, R.M., McGuigan, P.M., Ninham, B.W. and D.F. Evans, "Attractive Forces Between Uncharged Hydrophobic Surfaces: Direct Measurements in Aqueous Solution," *Science*, Vol. 229, p.1088-1089, 1985.
- Perfiliev, B.V. and D.R. Gabe, *Capillary Methods of Investigating Microorganisms*, Translated from Russian, University of Toronto Press, J.M. Shewan, Editor, 1969.
- Perry, J.J., "Microbial Cooxidations Involving Hydrocarbons," *Microbiological Reviews*, Vol. 43, p. 59-72, 1979.
- Pinta, M., *Detection and Determination of Trace Elements*, Translated from French, Ann Arbor-Humphrey Science Publisher, Ann Arbor MI, 588 p., 1970.
- Science News Letter*, Nov. p. 297, 1942.
- Shabtai, Y. and D.L. Gutnik, "Exocellular Esterase and Emulsan Release from the Cell Surface of *Acinetobacter Calcoaceticus*," *Journal of Bacteriology*, Vol. 161, p.1176-1181, 1985.
- Sharpley, J.M., *Elementary Petroleum Microbiology*, Gulf Publications, 256 p., 1966.
- Shennan, J.L. and I. Vance, "Microbial Enhanced Oil Recovery Techniques and Offshore Oil Production," in *Microbial Problems in the Offshore Oil Industry*, p. 73, Ed. E.C. Hill, et.al., Wiley and Sons, Chinchester, 1987.
- Sieburth, J.Mc.N., *Microbial Seascapes*, University Park Press, Baltimore, 248 p., 1975.
- Sieburth, J.Mc.N., *Sea Microbes*, Oxford University Press, 491 p., 1979.
- Simanov, A.I., Nazarov, M.I., Gruzinov, N.V., Afanas-Eva, M.A. and V.P. Andryukov, *Meteorologiya i Gidrologiya*, Vol. 3, p. 64-72, 1984.
- Smith, P.V., "Preliminary Note of Origin of Petroleum," *Bulletin of American Association of Petroleum Geologists*, Vol. 36, p. 411, 1952.
- Sondheimer, E. and J.B. Simeone, *Chemical Ecology*, Academic Press, N.Y., 336 p., 1970.
- Stephenson, M., *Bacterial Metabolism*, Longmans, Green and Co., London, 398 p., 1949.
- Stone, R.W., White, A.G.C. and M.R. Frenske, *Journal of Bacteriology*, Vol. 39, p. 91, 1940.
- Stotzky, G., "Influence of Clay Minerals on Microorganisms: III Effect of Particle Size, Cation Exchange Capacity and Surface Area on Bacteria," *Canadian Journal of Microvbiology*, Vol. 12, p. 1235-1246, 1966.
- Tausson, W.O., "Bacterial Oxidation of Crude Oils," *Neftyanoe Khoz*, Vol. 14, p. 220-230, 1928.
- Teal, J.M. and R.W. Howarth, "Oil Spill Studies: A Review of Ecological Effects," *Environmental Management*, Vol. 8, p. 27-44, 1984.
- Thimann, K.V., *The Life of Bacteria*, Macmillan, N.Y., 909 p., 1964.
- Twenhofel, W.H., *Treatise on Sedimentation*, Dover Publications, New York, Two Volumes, 926 p., 1961.
- Vandermeulen, J.H., "Some Conclusions Regarding Long-Term Biological Effects of Some Major Oil Spills," *Phil. Trans. R. Society of London*, Vol. 297, p. 335-351, 1982.
- Van der Linden, A.C. and G.J.E. Thijssse, "The Mechanisms of Microbial Oxidations of Petroleum Hydrocarbons," *Adv. Enz.*, Vol. 27, 1965.
- Ward, C.H., Bender M.E. and D.J. Reish, "The Offshore Ecology Investigation," *Rice University Studies*, Vol. 65, Nos. 3&4, 589 p., 1979.
- Warner, J.S., "Determination of Aliphatic and Aromatic Hydrocarbons in Marine Organisms," *Analytical Chemistry*, Vol. 48, p. 576-583, 1976.
- Wentworth, C.K., "A Scale of Grade and Class Terms for Classifying Sediments," *Journal of Geology*, Vol. 30, p. 377-391, 1922.
- Yen, T.F., University of Southern California, *A State of the Art Review on MEOR*, NSF Grant OIR-8405134, 1988.
- Young, L.Y. and C.E. Cerniglia, Eds., *Microbial Transformation and Degradation of Toxic Organic Chemicals*, Wiley-Liss Inc., New York, 1995.
- ZoBell, C.E., "The Effect of Solid Surfaces Upon Bacterial Activity," *Journal of Bacteriology*, Vol. 46, p.39-56, 1943.
- ZoBell, C.E., *Marine Microbiology*, Chronica Botanica, Waltham, MA., 240 p., 1946.
- ZoBell, C.E., U.S. Patent #2,413,278, Described a process by which bacteria release oil from geological formations, 1946.
- ZoBell, C.E., "Studies on Redox Potential of Marine Sediments," *Bulletin of American Association of Petroleum Geologists*, Vol. 30, p. 477-513, 1946.
- ZoBell, C.E., "Assimilation of Hydrocarbons by Microorganisms," *Advance Enzym.*, Vol. 10, p. 443-486, 1950.
- ZoBell, C.E., "Bacterial Activities and the Origin of Oil," *World Oil*, Vol. 130, p. 128, 1950.
- ZoBell, C.E., U.S. Patent # 2,742,398, described a process to reduce paraffin in oil wells, 1956.

UTTU graciously thanks Dr. Carl Oppenheimer, carlo@mail.utexas.edu., for sending us his reference list.



1998 national RNA survey

by Mike Martinson

The May/June 1997 *UTTU* gave results from a national survey of states on the use of remediation by natural attenuation (RNA). The survey, using written questionnaires or phone calls, gathered information from UST programs. By late January 1997, all 50 states, the District of Columbia and Guam had responded to the survey.

In January 1998, in cooperation with *UTTU*, Mike Martinson of Delta Environmental Consultants, Inc. completed a similar telephone survey. The original respondents from the *UTTU* survey (if available) were asked basically the same questions to facilitate comparison with the *UTTU* 1997 survey. In addition, questions pertaining to upcoming RNA and methyl tert-butyl ether (MTBE) issues were posed.

Both surveys contained questions that could be construed as somewhat ambiguous. The 1998 Delta survey, however, was undertaken completely over the phone so as to assist in clarifying questions. Authors of each survey acknowledge that responses were subject to human bias: that is, even regulators from the same state might not give the same answer to the same question. In addition, the 1998 survey queried other staff experts as suggested by the initial regulatory contacts.

The telephone poller began the survey by specifying that the topic was "RNA's use in state UST programs, specifically for groundwater petroleum hydrocarbon cleanup"; thus, responses were focused on the major chemical constituents (i.e., BTEX, PAHs, and MTBE) of gasoline and diesel, rather than on the rarer UST chlorinated compounds. In addition, the focus of the 1998 survey was on groundwater contamination; very little information was obtained concerning soil contamination.

The respondents were familiar with the definition of natural attenuation: biological, physical and chemical transformations that can make contaminants less mobile and toxic, as well as reduce their mass, volume and concentration. RNA is regarded as a passive remediation technique whereby natural processes are used to clean up a site; in contrast, active remediation involves an engineered intervention that expedites or enhances natural processes.

Comparison of results from both surveys indicate the following:

- all states continued in their previous assessment that RNA was acceptable in combination with other remedies to clean up petroleum-contaminated sites
- the use of RNA as a sole groundwater cleanup remedy increased from 1997 results (to about 75%), with only 7 states (<14%) in 1998 specifying that RNA was **not allowed** as a sole remedy

- the 1997 survey indicated that most states had or planned to establish statutes, regulations or guidance on RNA; the 1998 survey further clarified this data:
 - 4 states had statutes specific for RNA
 - 1 state would add statutes by 1999
 - 11 states had RNA-specific regulations
 - 1 state planned to have new regulations for RNA
 - 17 states currently had RNA-specific guidance
 - 14 states planned to develop or consider guidance
- the 1998 requirements for implementing RNA on a groundwater remediation project were even more consistent than those reported in 1997:
 - site characterization was mandatory for all states
 - all states favored free-phase removal to either minimal measurement levels or until technical infeasibility was demonstrated
 - the majority of the states required or preferred groundwater monitoring (49 states) and source control (45 states)
- as stated in the 1997 survey, a stable or shrinking groundwater plume extent (47 states) was considered the most important (or primary) line of evidence to demonstrate RNA effectiveness; decreasing concentration trends, according to 44 states, were also a primary line of evidence

Additional RNA trends emerged from the 1998 survey:

- groundwater RNA (including monitoring-only policies) was considered an acceptable or site-specific remedy alternative for most states' gasoline/BTEX-impacted sites (50 states) and diesel fuel/PAH-impacted sites (49 states)
- only 13 states required a contingency plan prior to RNA implementation; 34 states did not require such a plan
- risk assessment requirements to implement RNA were approximately the same in 1997 (24 states) compared to 1998 (25 states); in addition to the states requiring risk assessment, 9 states required site-specific consideration for risk assessment in 1998, compared to 4 states in 1997
- groundwater cleanup goals for petroleum hydrocarbon contamination were set at generic levels for 44 states and as site-specific levels for 46 states
- states increased their use of required and site-specific use of geochemical indicators (i.e., secondary lines of evidence) to support plume extent and concentration trend data; geochemical indicators were required by 19 states in 1997; 20 states in 1998 had requirements while 11 additional states were evaluating site-specific cleanup requirements for secondary lines of evidence

- states did not widely accept using RNA for MTBE contamination:
 - only 5 states permit an RNA remedy for MTBE
 - 25 states consider RNA of MTBE to be a site-specific decision
 - 24 states have MTBE regulations
 - 3 states require that MTBE cleanup requirements be site-specific
 - 24 states currently have no MTBE regulations
 - 9 states expect to adopt MTBE regulations in 1998-99
 - 6 states anticipate adopting regulations soon after the U.S. EPA issues its final health risk advisory and/or establishes an MTBE maximum contaminant level (MCL)

The following section lists the questions posed to each of the 51 state contacts and their responses.

1. Does your state allow the use of natural attenuation (often termed remediation by natural attenuation, or RNA) as a groundwater cleanup remedy for UST sites with petroleum hydrocarbon contamination:
 - (a) in combination with other treatment technologies or methods?
 - 49 Yes
 - 1 Yes — *site-specific*
 - 1 Yes — *post-remediation*
 - (b) as a sole remedy?
 - 38 Yes
 - 3 Yes — *site-specific*
 - 1 Yes — *not always*
 - 1 Yes — *as monitoring only*
 - 1 No — *site-specific possible*
 - 7 No

- (c) Is a contingency plan required prior to RNA implementation?
 - 34 No
 - 2 Yes — *afterwards*
 - 13 Yes
 - 2 *Site-specific*

2. Pertaining to the specific use of natural attenuation or RNA, has your state established:

- (a) statutes
 - 46 No
 - 1 *Planning to*
 - 4 Yes
- (b) regulations
 - 39 No
 - 1 *Planning to*
 - 11 Yes
- (c) guidance
 - 17 Yes
 - 14 *Considering/Developing*
 - 20 No

3. What cleanup goals are available for closure of groundwater contamination in your state?

- (a) Generic levels
 - 44 Yes
 - 1 *Planning to*
 - 6 No
- (b) Site-specific levels
 - 46 Yes
 - 5 No

4. To implement natural attenuation (or RNA, or monitoring only), does your state require:

- (a) site characterization
 - 51 Yes
- (b) source/hot spot control
 - 43 Yes
 - 2 *Preferred*
 - 1 No
 - 3 *Site-specific*
 - 2 *Yes — not always*
- (c) free-phase/free-product removal
 - 51 Yes — *to either minimal measurable levels or to extent of technical feasibility*



Subscriptions and address corrections

Any person or organization wanting a subscription to *Underground Tank Technology Update (UTTU)* should send requests and subscription fee (free to state government employees) to

Debbie Benell
432 North Lake St.
Madison, WI 53706
tel. 608/263-7428

Subscriptions begin with the first issue of each year; those who subscribe during the year will receive all issues in the volume.

Please send address corrections to the above address. Back issues (bimonthly from April 1987) are available. Please check the form.

- YES, put me on your *UTTU* mailing list.
 - I'm enclosing the \$30 (1-yr) subscription fee.
 - Free. See my state government employer below.
- YES, send me *UTTU*'s previous issues.
 - I am enclosing \$30.
 - Back issues free. See state government employer below.

NAME _____

TITLE _____ PHONE _____

COMPANY/ STATE GOV. AGENCY _____

ADDRESS _____

CITY _____ STATE _____ ZIP _____

Make checks payable to University of Wisconsin—Madison

- (d) risk assessment
 25 Yes
 9 Site-specific
 1 Optional
 1 No response
- (e) monitoring
 48 Yes
 1 No response
5. Is RNA an acceptable cleanup remedy for the following contaminants?
- (a) gasoline/BTEX compounds
 46 Yes
 1 No
- (b) diesel fuels/PAH compounds
 40 Yes
 2 No
- (c) MTBE
 22 Site-specific
 13 Not regulated
 5 Yes
 3 Yes — possible w/acceptable risk
 3 Probably no
 3 No NA policy
 2 No acceptable risk data available
6. For RNA, does your state:
- (a) specify a minimum number of monitoring points?
 28 No
 8 Site-specific
 15 Yes
- (b) specify the number of points?
 32 Site-specific
 19 Other
- (c) require a minimum duration of monitoring?
 17 1-2 yrs
 17 Site-specific
 15 to <GW standards/goals
 2 ≥2 yrs
- (d) specify a monitoring frequency (typical)?
 38 1 to 4 events per year
 13 Site-specific
7. What evidence does your state require to demonstrate RNA's effectiveness?
- (a) shrinking or stable plume
 47 Yes
 1 Yes — not always
 2 No formal method/policy
 1 Preferred — models OK
- (b) decreasing contaminant concentrations
 44 Yes
 2 No formal method/policy
 1 Yes — not always
 3 No
 1 Preferred — models OK
- (c) geochemical indicators
 20 Yes
 11 Site-specific
 20 No
8. Does your state have groundwater regulations for MTBE as of January 1998?
 24 Yes
 24 No
 3 Site-specific
9. Of the 24 states without current MTBE regulations, does your state anticipate adopting MTBE groundwater regulations in the future?
 9 No change
 8 Adopt regulations in 1998
 1 Adopt regulations in 1999
 6 Adopt regulation pending EPA issuing final health advisory/MCL

UTTU thanks Mike Martinson, Delta Environmental Consultants, Inc., for contributing this article. Mike can be reached at 612-697-5165 or mikema@deltaenv.com.

The next issue of UTTU will contain the specific data that Mike Martinson obtained during the course of this survey.

Underground Tank Technology Update

 **The College of Engineering**
University of Wisconsin-Madison

Engineering Professional Development
 432 North Lake Street
 Madison, Wisconsin 53706

Nonprofit
 Organization
 U.S. Postage
PAID
 Madison, WI
 Permit No. 658