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Article summaries

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Genetically engineered microorganisms

GEMs, genetically engineered microorganisms, are potentially applicable to remediation of contaminated sites; however, the risks that GEMs might pose to the environment are uncertain. This article, which is composed of extracts from a discussion that occurred over the Bioremediation Discussion Group (BioGroup, http://biogroup.gzea.com) Listserver in May 2000, describes some of these risks and GEMs applicability. Responses from BioGroup members have been edited for clarity. However, the complete and original responses can be found at the BioGroup archives at http://gw.cciw.ca/lists/biogroup/.

From Thomas, 2000. Though GEMs have the potential to improve remediation of toxic contaminants, the commercial reality is different. From discussions with property holders in the UK, I find it hard to believe that any property owner will ever allow GEMs on the land in the near future. GEMs have such bad press in the UK and Europe that it would significantly reduce the asset value of the land, however clean it may be. Property holders are risk-averse to bioremediation in Europe and certainly would not apply GEMs to their land. However, GEMs may have a long-term future.

From Lucks, 2000. We have to distinguish between genetically "engineered" microbes and specially "cultured" microbes. The former have foreign DNA, perhaps from pigs or whatever, the latter are organisms that have plasmids from other bacteria, which confer abilities such as the degradation of PCBs for example. These are simply "mixed-blood" organisms. I culture these and do not consider them GEMs.

From Glass, 2000. I would not ordinarily take the time to respond to these [comments], but in a forum such as this, where most members are unfamiliar with GMOs and with the track record of almost 15 years of controlled field testing of GEMs in agriculture, these remarks could well be taken out of context. Please see specific responses below. (Note that GMOs and GEMs were used interchangeably in this discussion).

From Schmid, 2000. I do have some serious concerns/reservations about using GMOs in bioremediating contaminated environments. The impact of such organisms on ecosystems and naturally occurring microbial communities is largely unknown. There are unexpected risks involved, and the release of GMOs in the environment could have devastating consequences. We have to ask ourselves whether we want to release GMOs into the environment. If we do, then we will have to accept the consequences.

From Glass, 2000. Mr. Schmid is entitled to his opinion, but I would strongly dispute that the risks of GMOs are "largely unknown." To the contrary, the potential risks (i.e., the bad consequences that could possibly happen in the worst case scenario) are well known and these are exactly the things that the regulatory agencies look for before approving a field test, and they are exactly the things that have been studied (and largely debunked) in actual field tests. Certain potential negative effects that people have been concerned about since the early 1980s are now known to be very unlikely to occur.

From Schmid, 2000. It is important to remember that GMOs are alive and unpredictable—they can mutate and proliferate, and spread quickly.

From Glass, 2000. Yes, GMOs can mutate and proliferate, but all the available evidence is that they do not spread or multiply quickly in the open environment. In fact, in most cases, laboratory-bred microbes have trouble competing against "real world" microflora and do not survive for very long, in large numbers, in the environment. In the experiments during the late 1980s and early 1990s of which I'm most familiar, most experiments showed low levels of persistence or occasional modest rise in population size in the months following the introduction, followed by decreases to acceptably low, steady-state levels of persistence over the long term. There is no evidence that, in the types of introductions we're talking about, introduced microbes can proliferate out of control and overtake an environment.

From Schmid, 2000. It is known that microorganisms freely associate with one another to form communities. . . . What if a genetically engineered biodegrading microorganism associates with a community and laterally transfers its genes to another microbial species? The community could end up behaving completely differently, perhaps producing toxins instead of biodegrading pollutants. Communities containing genetically engineered organisms could potentially end up destroying crops. GMOs are unpredictable.

From Glass, 2000. This is exactly an issue that regulatory agencies review and address when reviewing proposed uses of GMOs in the environment. The lateral transfer of an introduced gene can only cause harm in the recipient population if the gene or its gene product is itself harmful, or if it encodes a function that could be harmful in some genetic background. Yes, there is some unpredictability here, but it is wrong to say that GMOs are completely unpredictable.

From Schmid, 2000. Once these organisms are released into the environment, they can never be destroyed or stopped.

From Glass, 2000. Although we hear this a lot from opponents, its validity as a scare scenario depends on the ability of an introduced microbe to completely take over its environment and/or spread into other ecological niches. The available evidence is that this just doesn't happen. . . . I am not saying that GMOs should be used indiscriminately in the environment—I was in the ag biotech industry in the 1980s and have long been a proponent of responsible regulation and responsible use of GMOs by academia and industry. I am saying that a regulatory framework has evolved over the

15-plus years to address the concerns Mr. Schmid raises, and a significant scientific track record has also evolved to support this regulatory framework. List members should please not read Mr. Schmid's posting outside the context of the significant track record that exists from 15 years of use and government regulation of GMOs in the environment.

From Russell, 2000. We need to be careful about which organisms we modify, such as *e.coli* and some of the disease organisms; they should never be modified because it is enteric (belonging to or affecting the intestines) in humans. However, we are talking some science and some religion.

From Thomas, 2000. If GEMs are to succeed, a major effort is required to show the benefits of GEMs and doubly prove they are safe, at least if you want to win back the European public. I personally believe that GEMS can deliver a great benefit to society; however, scientific development and public education have to go hand-in-hand.

From Evans, 2000. I am not a microbiologist, but I have worked for more than 20 years with bioremediation, including some of the earliest research for its use with hazardous wastes, and with many commercial applications of biotreatment for process industry wastes as well as remediation. I have a few thoughts to share.

Our inability to eliminate the problem. With respect to spread and control of GEMs, I challenge those who desire to develop and deploy such organisms to first eradicate the imported fire ant from the southern United States. (We cannot even control it.) The population dynamics of microorganisms are much different than ants or other macroorganisms, but fire ants are one example that already demonstrates our inability to anticipate or control the effects of introducing non-native organisms into an ecosystem. One of the most outstanding facts . . . about environmental management over the last 20 years is our common inability and/or unwillingness to characterize and control earth systems to merely contain environmental contaminants, much less eliminate them. This is both a technical limitation and an economic one. Thus have been born such concepts as Superfund's "technical impracticability" (i.e., we can't or won't clean it up) and RBCA, a "dumbed-down" way to institutionalize risk science and place it in the hands of government bureaucrats to justify walking away from environmental contamination. Today, remediation equals containment, thus suggesting similar problems for GEMs in the environment. If they are viable and can propagate and spread, they will.

Economics and the public will. A scientist may be confident that one can eradicate a problem. One can achieve that in a flask or in a batch reactor, but it becomes extremely complex, and costly, in a natural system. At that point, our technical and human limitations and economics take over. Anyone who believes that we have the will to eradicate (not just control) a problem, whether chemical, physical or biological (GEMs), does not understand economics, public policy and politics.

Marginal value of GEMs for remediation*.* The last 20 years of waste treatment and remediation history are littered with "extinct" technologies, great ideas from the simple to the elegant that are now "museum pieces." Some have been quite good and economically viable. So what is the problem? First, remediation does not create a product that can be bought and sold, and it does not create a tangible return on investment. Economies invest in products and services that create wealth. Thus, despite years of enviro-rhetoric from politicians, chemical companies, etc., actions do not necessarily follow words. Environmental professionals have a reluctant consumer group and a weak market for actual cleanup. Second, most of the contaminant classes already have viable appropriate technologies for remediation, are not a big problem (i.e., low risk or not widespread), and/or are not particularly amenable to biological treatment in any form. In this setting, along with regulatory misgivings/ limitations, GEMS have a very limited economic value and would be difficult to apply commercially.

Cult science. If one can coin and popularize a new word for the environmental lexicon, one can create a cult around it, generate excitement in the media, and garner government research funding, irrespective of whether it promises much in commercial practice. A good example of this is "phyto-remediation," a concept very well known to and used by soil and crop scientists for many years. . . A quick look at the Internet reveals that, while the number of candidate plant species has increased, we are still limited to the same set of contaminants and treatment mechanisms that were well known over 20 years ago. While practical and effective waste treatment and remediation solutions are ignored (for the political reason that they cost too much today) we reach for marginal methods with ridiculous headlines like "plants guzzle metals." Other than plants' main remediation value in site stabilization and erosion control, or in wetlands systems, phytoremediation has its place only in special circumstances at the margins of environmental management. The same has proven true and likely will remain so in the realm of genetically engineered microorganisms.

From Fisher, 2000. Our incomplete knowledge is always dangerous when the implications are potentially large. I agree that more background information (e.g., long-term micro/macrocosm studies) is necessary before releasing GEMs to the environment.

From Gordon, 2000. We need to have a better background regarding how GEMs can compete once released, although the experience with Rhizobium mutants indicates that laboratory strains are like white rats. They die out. As for higher organisms, they can be released in a form in which they cannot spread, and hence I do not see the need for extreme caution. . . . It is not generally known that there are a number of examples of plants that already contain bacterial DNA. They have been around for about 50 million years, and without doing any harm.

Flint, 2000. Gordon raises a good point about the inability of laboratory strains to compete in a natural environment. A few years ago the University of Maryland conducted a study using microcosms to predict survival rates of GEMs after release into the environment. Dr. Terry McIntyre at Environment Canada put together a team of researchers from across Canada to attempt to validate the usefulness of this methodology. The study was repeated at various sites across the country with laboratory and field trials run in parallel under different environmental and soil conditions. The ultimate goal was to see if it would be possible to use the methodology as a protocol for predicting if GEMs persisting in the environment would be a problem for bioremediation activities. There is some early data from this work available on the web at http://nbiap.biochem.vt.edu/

From Flint, 2000. While the concerns of people such as Dirk Schmid are valid, given the impossible task of trying to predict all possible outcomes of releasing GEMs into the environment, I believe that over the next few years practical application of this technology will show that not only does Mother Nature quickly compensate for the presence of GEMs, but she is almost always ahead of us in the race to create microorganisms for in-situ remediation purposes. In general, we hold too high an opinion of our ability to genetically engineer microorganisms; isolating and adding a single gene can take weeks or months of effort on the part of the researcher. Evolution, on the other hand, is constantly fine-tuning entire genomes of indigenous organisms to adapt to the changing environment with every generation (measured in hours or minutes). Unless you are looking at a recent spill site, odds are good that the indigenous population is already capable of degrading what-ever organic contamination exists and is better at competing for nutrients than any GEM you could add. If the degradation is proceeding slowly, it is usually due to other limiting factors such as lack of contaminant dilution (toxic effects), shortage of electron acceptors or cometabolites, temperature, pH, etc. . . and so far, GEMs don't solve these problems.

From Flint, 2000. I do not wish to come across as completely negative toward the application of GEMs for remediation, because I am not. GEMs may be preferred for some ex-situ applications and there may be good uses for GEMs in remediation of fresh spill sites where the indigenous population has not had time to acclimate to the changing environment, or perhaps some breakthrough could be made in the degradation of some particularly recalcitrant organics. The real question is whether it is economically beneficial to invest the time and money to develop and obtain regulatory approval for a microbial product that Mother Nature will make for you at no cost, given a little time and the right encouragement.

From Taylor, 2000. As a microbiologist I have trouble imagining how a culture added to a contaminated site could ever establish itself enough to actually do anything. With generally 1.5 x 10^9 bacteria/g-soil you would have to be adding a heap of bacteria! *(Reviewer's note: 10^6 bacteria/g-soil is more reasonable.)* I can understand the use of "foreign" cultures if you are using a bioreactor, but I would find it hard to believe that a culture added to a pile of soil would play a real role in contaminant removal. Do we know, for example, that what we see is not just biostimulation of the resident community by addition of extra nutrients along with extra bacteria?

From Muilenburg, 2000. GEMs excepted, thousands of bioaugmentation projects are conducted throughout the world. No one method (natural attenuation, stimulation, augmentation) is correct for all sites at all times. I have seen many many sites and situations where augmentation is conducted more rapidly, at lower cost, has more predictable results with greater control of site and project variables, and with other benefits including less disruption of the local bacterial populations, lesser potential for the stimulation of pathogens, and less nitrification of groundwater. Each of these three approaches has its role. Technical acumen and wisdom lie in recognizing which approach is best for a customer and the environment.

From Glass, 2000. I have always advocated responsible government regulation and public involvement. But more importantly, there has not been a "rush to the market" with GEMs in either agriculture or bioremediation. As other commentators have noted, there are no commercial GEMs used in bioremediation, very few experimental GEMs tested for bioremediation, and frankly very few legitimate targets where use of a GEM offers even a slight advantage over indigenous or other natural bugs for bioremediation. Even in agriculture, 20 years into the biotech era, there are perhaps 20-25 GEMs approved for use as pesticides, many of them variations on the theme of engineered "Bt" organisms, and only one GEM approved for nonpesticidal use, a rhizobium nitrogen-fixing inoculant . . .Finally, I'd also agree that the regulatory framework and agencies themselves are not perfect. But many of the critics would have you believe there's no regulation at all. That couldn't be farther from the truth.

From Schmid, 2000. Microbial communities evolve as do species and ecosystems. Biodegradative communities evolve as well. Changes to the species composition of a community and indeed changes to the spatial positioning of members within a community could change the overall behavior of a community. For example, a biodegrading community could change into a non-biodegrading one. This could seriously impact bioremediation strategies. I fail to see how competition fits into the picture when communities, species, ecosystems and biospheres evolve by means of adaptation and recombination, i.e., genetic recombination and recombination through immigration and emigration of species to/from communities. Species increase their chances of survival if they self-organize into communities. Competition is insignificant and is more likely a product of evolution rather than a mechanism. I am more concerned about recombination.

If you introduce biodegradative GEMs into the environment, you may alter the function of existing communities (and whole ecosystems) in the soils and water through changes in spatial positioning of species within communities and genetic recombination through horizontal transfer of genes between species. Again, we don't know what will happen. Bioremediation strategies that use GEMs could backfire.

From Schaffner, 2000. Mr. Schmid's arguments are persuasive and go right to the issue: the unintended, potentially long-term consequences of releasing non-native genetic material into ecosystems. Actually, though, my issue with GEMs is more basic, i.e., it's the same one I have with bioaugmentation in general. Unless it can be shown that the native population is not up to the task, I can see no good reason for using non-native microbes, genetically engineered or otherwise.

From Radtke, 2000. The release of GEMs for any reason is inherently strapped with limited predictive knowledge. Who knows, maybe a small Tn5-altered sequence will enable an undiscovered soil-borne virus to severely antagonize nitrogenase function. Such detrimental effects wouldn't be all that far out, just unpredicted. It's easy to know the singular desired function provided by the extra molecular baggage a GEM carries, sometimes even several functions, but strictly impossible to predict much beyond that, as many have mentioned in our discussion.

From Carpenter, 2000. Some good points have been raised over the application of GEMs in bioremediation. Even though I do not work in this area, I know that most modifications used in bioremediation aim at improving the efficiency of contaminant degradation in the soil, which in all likelihood has already altered the ecology in that particular site. In the initial case you are hardly "invading" a natural ecosystem.

The movement of the organism out of the contaminated area would be linked to the ability of the microbe to compete effectively against local populations. If you introduce a large number of new species to the site, that could be potentially a problem. However, this is true regardless of the organism's engineered status.

Unless the new gene supplies an advantage to the introduced microbes above and beyond that of the other organisms in the ecosystem, it will either become part of the community, die or become dormant. Gene flow of the "new" gene to other organisms in the community may confer a fitness advantage in the contaminated site, but it is unlikely to increase the fitness of the organism outside that community. If the modification is such that it relies on the contaminant as a precursor source for a key metabolic pathway, it is possible that once the GEM's job is done, the population will disappear. I agree that this should be examined on a case-by-case basis.

Any native organism from the site that can naturally "bioremediate" also has the above advantage and may be selected by the contaminant. Thus, gene flow from these organisms to others has the same potential impact on surrounding organisms. The advantage of the GEM is that you know the gene and breakdown products. Conversely, with native bioremediators, unless you sample, culture and test, you have no idea of the final breakdown products, including toxicity.

Even the introduction of nutrients to encourage "natural" bioremediation could have adverse effects, including encouraging the wrong species to proliferate, causing a population explosion of the bioremediation organisms, which then upsets the "natural" environment both at the site and in the surrounding area.

My point is that the only way to leave a natural environment in "balance" is to prevent the contamination in the first place. Once contamination has occurred, you no longer have a "natural" environment, and any action, which includes lack of action, is likely to affect both the site and ecosystem.

From Fournier, 2000. Does anyone know how many types of microbes exist in the subsurface? If memory serves me correctly (and it may not), within the last decade, the DOE at Savannah River Labs (Aiken, S.C.) conducted a "Deep Probe" project wherein they installed three vertical wells nearly a mile into the subsurface. During drilling, they took soil and groundwater samples every 3 feet or so . . . As I recall, they found about 30,000 types of microbes, 20,000 of which had never been isolated before. Surprisingly, the microbial population remained fairly constant from about 20 feet below ground surface down to 5,000 feet. In the near subsurface, populations were much higher. Moreover, microbe distribution by type was nearly constant with depth.

Someone can correct me if these numbers are erroneous. However, their conclusion was that the Earth is similar to a giant peach with the fuzz around the outside being equivalent to the "living crust" just below the surface where all manner of microbial and fungal organisms (and others) live in harmony while competing for foodstuff.

Moreover, when it comes to truly understanding the various microbial interactions that occur when contamination is introduced into the subsurface, we have not even scratched the surface. Even the most basic contaminant, such as gasoline, contains hundreds or thousands of individual chemical compounds that change in response to sorption, dissolution, vaporization, precipitation and diffusion. These chemical compounds are also affected by tens of thousands of microorganisms that are evolving, devolving, changing in population, generating enzymes, dying. Even with our best studies concerning microbial community interactions with contaminants, we focus on particular experimental goals and ignore other facts, such as how much contamination is being lost through fungal processes rather than microbial processes.

While it is scientifically interesting to speculate on the potential for positive and negative benefits or interactions of GEMs in the complex environment, it may be even more worthwhile to gain a better understanding of subsurface processes, what tools are available to us naturally, and how to better use them to advantage.

Websites of interest

Websites of interest (*Sayer, 2000*) include the following:

- National biotechnology website gives links to other sites http://www.oecd.org/ehs/biolinks.htm#
- Toxic Substances Control Act (TSCA) Biotechnology Program, U.S. EPA site on GEMs for bioremediation http://www.epa.gov/opptintr/biotech/
- Center for Biotechnology, http://www.ornl.gov/cbt/

Additional references

Additional references of interest include the following (from *Schmid, 2000,* and *Anderson, 2000)*:

"Behavioral Strategies of Surface-Colonizing Bacteria," *Advances in Microbial Ecology,* edited by J. Gwynfryn Jones, 1995, Plenum Press, New York; http://plenum.com.

"Containment of Genetically Engineered Microorganisms During a Field Bioremediation Application," *Applied Microbiology and Biotechnology*, Vol. 51, No. 3, 1999, pgs. 397-400; Ford, C.Z., Sayler, G.S. and R.S. Burlage; http://link.springer.de/link/service/journals/00253/.

"Controlled Field Release of a Bioluminescent Genetically Engineered Microorganism for Bioremediation Process Monitoring and Control," *Environmental Science and Technology*, Vol. 34, No. 5, 2000, pgs. 846-853; Ripp, S.A., Nivens, D.E., Ahn, Y., Werner, C., Jarrell, J., Easter, J.P., Cox, C.D., Burlage, R.S. and G.S. Sayler; http://www.pubs.acs.org.5

"Cultivation and Study of Biofilm Communities," *Bacterial Biofilms,* D.E. Caldwell, edited by H. Lappin-Scott and J.W. Costerton, 1995, Cambridge University Press; http://www.cup.org.

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"Field Application of a Genetically Engineered Microorganism for Polycyclic Aromatic Hydrocarbon Bioremediation Process Monitoring and Control," in *Novel Approaches for Bioremediation of Organic Pollutants*, 42nd OHOLOL Conference, 1999, Plenum Press, New York; Sayler, G.S., Cox, D.E., Burlage, R.S., Ripp, S.A., Nivens, D.E, Werner, C., Ahn, Y. and U. Matrubutham. Book editors are R. Fass, Y. Flashner and S. Reuveny; http://plenum.com.

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"Field-Scale Bioremediation Monitoring Utilizing Bioluminescent Genetically Engineered Microorganisms," Bioremediation Technologies for Polycyclic Aromatic Hydrocarbon Compounds in *In-Situ and On-Site Bioremediation Proceedings, The Fifth International Symposium,* San Diego, CA, Vol. 8, No. 5, 2000, pgs. 277-282, Battelle Press, Ripp, S.A., Nivens, D.E., Burlage, R.S. and G.S. Sayler; http://www.battelle.org/bookstore.

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"Microbial Biofilms," *Annual Reviews of Microbiology*, Vol. 49, pgs. 711-745, Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R. and H.M. Lappin-Scott; http://micro.annualreviews.org

"Microcosms for Evaluating Survival of Pseudomonas Chloroaphis 3732-rn-L11 in Soil," http://nbiap.biochem.vt.edu/brarg/brasym96/angle96.htm.

Planetary Ecology, Caldwell, D.E., Brierley, J.A. and C.L. Brierley, 1985, Van Nostrand Reinhold Co., New York; http://apnet.com.

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GEMs field study

Pseudomonas fluorescens HK44 is the first genetically engineered microorganism approved for field testing in the United States for bioremediation purposes. The microbe bioluminesces as it degrades hydrocarbons by virtue of an introduced lux gene. The gene is fused within a naphthalene degradative pathway. Researchers obtained the original host microorganism and plasmid from manufactured gas plant (MGP) soils contaminated by PAHs (polyaromatic hydrocarbons). Using fiber optic-based biosensors, researchers were able to monitor biodegradation of hydrocarbons by *Pseudomonas fluorescens* HK44 *(Ripp and others, 2000)*.

Researchers began this study with the following goals:

- to acquire permission from the U.S. EPA for a GEM field release
- to develop "a field facility for environmentally controllable and replicated field test of GEMs"
- to release an engineered microorganism into the subsurface to test organism survivability and application
- to understand application of GEM *Pseudomonas fluorescens* HK44 with respect to PAH bioremediation

Researchers conducted the study in the vadose zone of a constructed subsurface soil profile in six replicated soil lysimeters with and without contaminant PAHs. Testing began in October 1996 and continued for approximately two years.

Lysimeter testing facility

To collect samples, researchers used six epoxy-coated, galvanized steel pipes buried 3 meters below ground surface. "A seventh lysimeter was filled with groundwater and used to adjust water levels in the other lysimeters. Each lysimeter was covered with a stainless steel lid. Lysimeters were arranged around a 6-m deep by 6-m diameter central core containing computer equipment, monitoring devices and associated plumbing."

The lysimeters were filled with soil consisting of predetermined amounts of gravel, sand, silt, clay and organic carbon. Lysimeters 3 and 5 received uncontaminated soil inoculated with HK44 while lysimeter 6 received a layer of PAH-contaminated soil not inoculated with HK44. Researchers buried biosensors and fiber optic cables in the soil at various depths within the lysimeters. Lysimeters also contained monitoring devices to measure soil oxygen concentration and temperature. Some took readings every 30 minutes. Ripp and others *(2000)* give additional details in the text concerning the experimental setup. Later, researchers removed soil cores to verify existence of the HK44 genotype. Soil PAHs were analyzed using a gas chromatograph-mass spectrometer.

Biodegradation results

Initial GC/MS analysis of contaminated soils did not detect naphthalene because naphthalene was lost during the extended storage of the soil prior to lysimeter loading." GC/MS analysis of soil cores taken within one month after the supplementary oil addition produced contaminant concentrations that varied widely with position and depth, indicating that contaminants were not homogeneously distributed throughout the treatment zone. . . . A precise assessment of contaminant remediation effectiveness was not derived because of the heterogeneity of contaminants within each lysimeter. However, comparisons between initial contaminant concentrations in lysimeters 1, 2 and 6, following the supplemental oil addition and final contaminant concentrations determined on day 474, indicate that naphthalene levels within the treatment zone decreased, and in most cases rather substantially. Although bioremediation by *P. fluorescens* HK44 accounts for some of this contaminant degradation, natural attenuation by other PAH degraders and air stripping are processes that also affected PAH concentrations, as exemplified in control lysimeter 6 where naphthalene loss occurred in the absence of HK44 microbes. Several indigenous microorganisms capable of utilizing naphthalene as a carbon source were isolated from the PAH-contaminated soils. Although not what we had originally planned or desired, the uneven distribution of pollutants provided a more representative situation of an actual contaminated site, and likely proved to be a better "real world" test of the biosensing capabilities of *P. fluorsecens* HK44."

Bioluminescent detection

The biosensors and multiplexed fiber optic-based light detection system detected volatile PAHs in the HK44-inoculated/PAH-contaminated soils of lysimeters 1 and 2. "Bioluminescence detection from soil was attempted using both buried fiber optic cables and a portable PMT device (photomultiplier tube). The buried fiber optic cables were ineffective at detecting HK44 bioluminescence at population and/or activity levels found in this study. Consequently, a more sensitive portable PMT system (outfitted with Plexiglas windows) that could be lowered into the treatment zone was designed and was ready for use on day 444." Researchers designed experiments that included monitoring bioluminescence of HK44 treatment zones beneath Plexiglas windows to determine if conditions were favorable for growth and biodegradation. Bioluminescence was typically detected within one to two days after inoculation. During the cooler months of winter, researchers observed longer bioluminescence events. However, "No bioluminescence above background was observed in control lysimeter 6 under any of the localized treatment conditions."

Ripp and others *(2000)* contend that "inoculation and maintenance of *P. fluorescens* HK44 within the soil ecosystem was successful. Populations rapidly declined in all inoculated soils during the first 12 days after HK44 introduction but declined more gradually during the remainder of the study.

At no time under any treatments did HK44 populations approach extinction . . . rather cells remained in a state conducive to reactivation and regrowth upon exposure to PAH contaminants and inorganic nutrient amendments."

Modeling predicted a 3-year survival period for a GEM population in soil. GEMs used in agricultural studies have survived four to six years, while in other cases, GEMs were not detected after a year. Modeling of GEM survival rates must consider environmental factors such as soil heterogeneity. "Whereas one area of soil may possess factors conducive to cell growth, an adjacent area may effect growth inhibition. In PAH-contaminated soils, additional parameters are present, such as contaminant concentrations that are too low to adequately support cell population. Successful colonization by a GEM will therefore always rely on a complex balance between the above factors, with controllable factors, such as soil aeration or moisture content, being carefully planned into the overall design of the field release site."

Ripp and others *(2000)* concluded that experiments with *P. fluorescens* HK44

- provided real-time data that reflected naphthalene bioavailability, degradative activity and optimal degradation conditions
- provided a rapid assessment of contaminant bioavailability
- successfully monitored naphthalene vapor using biosensors that utilized alginate-encapsulated HK44 cells
- successfully monitored actively bioluminescing HK44 cells directly from soil using a portable PMT
- suggested that new encapsulation materials, a necessity for increasing cell longevity, will enable development of biosensors capable of long-term, online monitoring of vapor and aqueous phase contaminants
- demonstrated that oxygen was a limiting factor in HK44 survivability in studies performed in the absence of Plexiglas windows
- indicated that decay in population densities does occur, but environmental manipulation can result in regrowth

Reseachers are engineering new strains of bioluminescent bioreporters that will have improved sensitivity, increased levels of bioluminescence and greater genetic stability. At present, successful implementation of HK44 must

- achieve adequate dissemination of the microorganisms in the subsurface
- overcome mass transfer limitations of electron donors and acceptors controlling the degradative response

Related study

Finnish and Swedish researchers have used the luciferase biomarker to monitor bioremediation inocula *(Jansson and others, 2000)*. "Bacteria tagged with the firefly luciferase gene (luc) or bacterial luciferase genes (*luxAB*) can be easily detected and counted as luminescent colonies on agar plates. For example, a biosurfactant producing lux AB-tagged strain of *Pseudomonas aeruginosa* was tracked by counting luminescent colonies in oil-contaminated soil microcosms. . . The main advantage with luminescence markers is the ability to directly monitor light output in a luminometer, without the necessity for cultivation of the cells. The light output is indicative of a metabolically active population of cells, since luciferase enzymes are dependent on cellular activity reserves or reducing equivalents for bioluminescence. . . If the cells are growing, the light output is proportional to the number of cells in the sample. However, after long-term incubation in soil environments, or other 'harsh' environments, microbial cells often become starved or stressed and the light production from luciferase enzymes declines as a response to the change in cellular energy status."

The researchers found that the amount of relative light units (RLU) detected will vary with soil type, indicating a limitation of the in-vitro luciferase assay. For instance, inoculated *Arthrobacter* cells showed a 14 percent RLU in organic soil vs. a 1 percent RLU in clay soil. RLU also varies with bacterial strain—*Pseudomonas* vs. *Arthrobacter*—but to a lesser extent than soil type. These researchers conclude: "The choice of monitoring system depends on the questions to be addressed, the nature of the contaminated environment and the properties of the strain used for bioaugmentation."

Another related study

To test the idea of GEM survival in the world at large, Dr. Brenner of the Molecular Sciences Institute of Berkeley, California, "drank a genetically weakened bacteria." He demonstrated "that such bacteria survived very poorly compared with bacteria that normally came through his intestines and thus would not survive well outside the laboratory" *(Altman, 2000)*.

References

Altman, L.K., "6 Scientists Honored with Prize in Research," *New York Times*, Sunday, September 17, 2000, pg. 34.

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Ripp, W., Nivens, D.E., Ahn, Y., Werner, C., Jarrell, I.L., Easter, J.P., Cox, C.D., Burlage, R.S. and G.S. Sayler, "Controlled Field Release of a Bioluminescent Genetically Engineered Microorganism for Bioremediation Process Monitoring and Control," *Environmental Science & Technology*, Vol. 34, No. 5, 2000; http://www.pubs.acs.org. See also http://www.ornl.gov/cbt /cbt.htm#environmental for articles on GEMs.

See also http://www.ornl.gov/cbt /cbt.htm#environmental for articles on GEMs.

Potential MTBE treatment technologies

This article briefly summarizes research on some of the latest MTBE treatment technologies, including bioremediation, air stripping, advanced oxidation processes (AOP), granular activated carbon (GAC) and synthetic resins, many of which are still at the experimental stage. Table 1 at the article end summarizes these technologies and studies.

Church, Pankow and Tratnyek *(2000)* performed column studies using MTBE- contaminated sediments from the Borden aquifer, Lake Erie and New Jersey. They investigated MTBE biodegradation in the presence of cometabolic substrates such as isopropanol, hexane, isopentane, isopentanol, malate and ethanol. The substrates, they discovered, halted MTBE degradation to TBA, confirming that the MTBE-to-TBA conversion occurs only when favorable substrates are present. They determined the MTBE-to-TBA half-life to be about 2 years. Aerobic conditions prevailed and BTEX compounds were absent. Researchers concluded: "Our column results indicate that under typical groundwater conditions in and around plumes from many gasoline spill sites, biodegradation will usually be negligible."

Church, Tratnyek and Scow *(2000)* isolated culture PM1 from a compost biofilter at a water pollution control plant in California. They found that the culture "PM1 rapidly mineralizes MTBE over a range of laboratory conditions, and it grows with MTBE as its sole carbon and energy source at MTBE concentrations as high as 500 mg/L."

Greene and others *(2000)* isolated a gram-negative MTBE-degrading bacterium, *Hydrogenophaga flava* ENV735, that grows rapidly on MTBE and tertiary butyl alcohol (TBA) in a small amount of yeast extract. "MTBE degradation by the strain is not affected by the presence of BTEX or H_2, and the strain does not grow on BTEX as a sole source of carbon. The strain can be grown rapidly to high cell densities on rich media for use in bioaugmentation and bioreactor applications."

Steffan and others *(2000)* used air sparging systems to inject propane and oxygen at two sites. They then added organisms to one site that lacked indigenous propane-oxidizing bacteria. MTBE was mineralized to CO_2; however, researchers ascertained that explosion concerns with propane need to be addressed.

McMillan and Schnoor *(2000)* used hybrid poplar trees to take up ^{14}C-MTBE. First, researchers planted the trees in MTBE concentrations of 0, 10, 100, 1,000 and 10,000 mg/L in an hydroponic experiment. "After three days, the leaves on the cuttings dosed with 10,000 mg/L were droopy and transpiration decreased dramatically to 1 to 2 ml/day. After five days the leaves were completely dry and the cuttings were dead." The trees that received 100 mg/L MTBE stayed healthy. In another experiment, researchers measured MTBE uptake and volatilization from leaf tissue and found that "The amount of ^{14}C-MTBE that was transpired through the leaves constituted the most significant pathway of MTBE movement." McMillan and Schnoor also concluded that

- hybrid poplar trees can tolerate MTBE concentrations greater than 1,000 mg/L
- hybrid poplar trees can effectively remove MTBE f rom aqueous solution
- the primary removal mechanism for MTBE was volatilization through leaf tissue

Macky and others *(2000)* are field-testing a diffusive oxygen-releasing device. By adding oxygen to the subsurface, they hope to encourage native aerobic MTBE-degrading bacteria at the Port Hueneme, California site. The two diffusing methods they developed are the cylindrical and the rectilinear "panel" method.

Barreto and others *(2000)* demonstrated that MTBE, tert-butyl alcohol (TBA), ETBE (ethyl tert-butyl ether) and TAME (tert-amyl methyl ether) were susceptible to photocatalytic degradation in a batch slurry process using TiO_2 as a catalyst. ETBE and TAME degradation produced some intermediates yet to be identified. However, the pH data are consistent with mineralization of ethers to CO_2 and carbonates, then to simple organic acids.

Schreier *(2000)* used Fenton's reagent, an acidified mixture of hydrogen peroxide (H_2O_2) and ferrous iron (Fe(II)), in a batch test to generate free hydroxyl radicals (HO). The hydroxyl radicals react with organic substrates which are mineralized, and then converted to carbon dioxide. Experimental results indicated that greater than 99.7 percent of MTBE in a 1 percent H_2O_2 solution with Fe(II) was destroyed, while up to 71 percent of BTEX in a 5 percent H_2O_2 solution with (Fe(II) was destroyed.

Lien and Zhang *(2000)* used acidified aluminum to oxidize MTBE and reduce carbon tetrachloride. Researchers report that "it is difficult to achieve the simultaneous degradation of both by simply mixing effective oxidants and reductants together. For example, an attempt to achieve the simultaneous transformation of MTBE and CT using Fenton's reagent and zero-valent iron in the same system was unsuccessful. . . . Further research is warranted to better define and understand the underlying mechanisms of acidified aluminum-mediated reactions, and catalytic properties and stability of the acidified aluminum."

Halverson and others *(2000)* tested the ability of a peroxy-acid to oxidize MTBE in the laboratory. The peroxy-acid is formed between an organic acid and hydrogen peroxide. Using 344 moles of acetic acid and hydrogen peroxide each to one mole of MTBE, researchers found that in two hours, MTBE concentration had decreased by 30 percent. By-products formed are being analyzed by researchers for biodegradability via aerobic and anaerobic pathways. Researchers assert that "The acetic acid and hydrogen peroxide solutions should be recyclable, the reagents themselves inexpensive and the process fast. These properties, coupled with the low-energy requirement, make this a promising technology with applications beyond the chemicals being explored at present."

Researchers are currently pretreating MTBE at the wellhead using a combination of ozone (O_3) and hydrogen peroxide (H_2O_2), called HiPOx *(Buartovich-Collins and Bowman, 2000)*. This line-pressure advance oxidation process injects ozone at a line pressure of 30 to 40 psig, resulting in rapid utilization of the ozone. The ozone dissociates and reacts with the peroxide to form hydroxyl radicals, the second most powerful oxidizing agents in nature. "These hydroxyl radicals react very rapidly to oxidize organic contaminants to non-hazardous compounds carbon dioxide and water." Oxidation does not increase water temperature or pressure because the contaminant concentration is very low. The HiPOx system differs from conventional AOP (advanced oxidation process) systems in that HiPOx systems use a series of injector modules in-line to treat the flow of contaminated fluids.

Another possible treatment option is the electron beam process, an advanced oxidation and reduction technology *(Mezyk and others, 2000)*. "The electron beam process can be thought of as a continuous injection of electrons into a flowing stream of water." Scientists endeavored to determine the "biomolecular reaction rate constants between the reactive intermediates and compounds of interest, then to develop a detailed understanding of the reaction mechanism that describes the radiolytic decomposition of the compound(s) in aqueous solution." Although work is at the experimental stage, scientists have concluded that for the oxygenates, the hydroxyl radical is essential for destruction of compounds of interest.

O'Shea and others *(2000)* are conducting experiments to better define MTBE destruction kinetics, in particular with respect to advanced oxidation technologies, TiO_2 photocatalysis, electron beam irradiation and sonolysis. They also found the hydroxyl radical to be the predominant species responsible for compound degradation. "Since these ethers do not appreciably absorb light in the near UV/visible region, direct photo-degradation by solar irradiation is very ineffective. In addition, oxidation in aqueous environments with naturally occurring hydroxyl radicals and other oxidative processes is too slow to yield significant decontamination."

References

Steffan, R.J., Condee, C., Quinnan J., Walsh, M., Abrams, S.H. and J. Flanders, "In-Situ Application of Propane Sparging for MTBE Bioremediation," presented at *The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, May 22-25, 2000; authors can be reached at Envirogen, 4100 Quakerbridge Road, Lawrenceville, New Jersey; 609-936-9300; http://www.Envirogen.com.

References from *Preprints of Extended Abstracts presented at the 219th ACS National Meeting,* San Francisco, California, March 26-30, 2000, Vol. 40, No 1. (publisher Ruth Hathaway, 573-334-3827) include the following:

Barreto, R.D., Barr, P.V. and C. Chavis, "Photocatalytic Destruction of Ethyl T-Butyl Ether (ETBE) and T-amyl Ether (TAME)", pgs. 232-233.

Study/technology	**Results**	**Researchers**
Cometabolic substrates	MTBE-to-TBA conversion occurs only with favorable substrates	Church, Pankow, Tratnyek (2000)
Compost biofilter with degrading culture	Culture PM1 rapidly mineralizes MTBE over wide range of conditions	Church, Tratnyek and Scow (2000)
MTBE biodegradation with a gram-negative bacterium and BTEX	Bacterium grows rapidly on MTBE, not on BTEX	Greene and others (2000)
Air sparging using propane and oxygen	MTBE mineralized to CO_2	Steffan and others (2000)
Phytoremediation	Hybrid poplar trees can tolerate MTBE concentrations to 1,000 mg/L	McMillan and Schnoor (2000)
Diffusive oxygen release device	Still testing	Mackay and others (2000)
Batch slurry process	MTBE and by products susceptible to photocatalytic degradation	Barreto and others (2000)
Fenton's reagent in a batch test	Greater than 99.7 percent of MTBE destroyed	Schreier (2000)
Acidified aluminum	MTBE oxidized	Lien and Zhang (2000)
Peroxy-acid acidification of MTBE	Still testing, but significant MTBE decreases	Halverson and others (2000)
Ozone and hydrogen peroxide treatment at the well head	MTBE successfully oxidized	Buratovich-Collins and Bowman (2000)
Electron beam process	Still experimental	Mezyk and others (2000)
Advanced oxidation technologies, TiO_2 photocatalysis, electron beam irradiation and sonolysis	Still experimental	O'Shea and others (2000)

Buratovich-Collins, J. and R.H. Bowman, "HiPOx™ Advanced Oxidation Technology for the Destructive Removal of MTBE-Contaminated Groundwater," pgs. 253-256.

Church, C.D., Pankow, J.F. and P.G. Tratnyek, "Effects of Environmental Conditions on MTBE Degradation in Model Column Aquifers: II. Kinetics," pgs. 238-239.

Church, C.D., Tratnyek, P.G. and K.M. Scow, "Pathways for the Degradation of MTBE and Other Fuel Oxygenates by Isolate PM1," pgs. 261-263.

Green, M.R., Steffan, R.J., Vainberg, S. and J. Manning, "Treatment of Waters Containing MTBE Using a Membrane Biological Reactor System," pgs. 277-279.

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Lien, H.S. and W.X. Zhang, "Simultaneous Oxidation of MTBE and Reduction of Carbon Tetrachloride Using Acidified Aluminum," pgs. 234-235.

Mackay, D., Wilson, R., Durrant, G., Scow, K., Smith, A., Einarson, M. and B. Fowler, "Field Tests of Enhanced Intrinsic Remediation of an MTBE Plume," pgs. 284-285.

Mezyk, S.P, Cooper, W.J., Bartels, D.M., Thomas, T. and K.E. O'Shea, "Radiation Chemistry of Alternative Fuel Oxygenates—Substituted Ethers," pgs. 250-251.

McMillan, S.K. and J.L. Schnoor, "Phytoremediation of Methyl Tert-Butyl Ether by Hybrid Poplar Trees," pgs. 263-266.

O'Shea, K.E., Larrea, A.A., Kim, D.K. and W.J. Cooper, "Similarities in the Treatment of Gasoline Oxygenates by Advanced Oxidation Processes: Product Studies, Kinetic Evaluations and Mechanistic Conclusions," pgs. 251-253.

Schreier, C.G., "Removal of MTBE and Other Petroleum Hydrocarbons from Water Using Fenton's Reagent," pgs. 242-243.

Other articles of interest in this volume include:

Chen, C. L., Hohnbaum, H., Smith, A., Scow, K., Change, D. and K. Jackson, "Scale-Up of PM1 MTBE-Degrading Culture Production for Field Application," pgs. 282-283.

Deeb, R.A., Nishino, S., Spain, J., Hong-Ying Hu, Scow, K. and L. Alvarez-Cohen, "MTBE and Benzene Biodegradation by a Bacterial Isolate via Two Independent Monooxygenase-Initiated Pathways," pgs. 280-282.

Drogos, D.L. and A.F. Diaz, "Properties and Environmental Behavior of Oxygenates in Use and in Development for Use in Fuel," pgs. 256-259

Flores, A., Stocking, A. and M. Kavanaugh, "Small-Scale Treatment Systems for Removal of Methyl Tertiary-Butyl Ether (MTBE) from Private Drinking Water Wells," pgs. 243-245.

Newman, L.A., Wilson, A., Capron, L., Strand, S.E., Heilman, P. and M.P. Gordon, "Phytoremediation of Methyl-Tertiary-Butyl Ether (MTBE)," pgs. 288-289.

Stringfellow, W.T., Hines, R.D. and S.T. Kilkenny, "Applying Co-Metabolic Biological Reactions for the Ex-Situ Treatment of MTBE-Contaminated Groundwater," pgs. 286-288.

For additional information, see

- the *UTTU* website, http://epd.engr.wisc.edu/uttu/ under the heading MTBE
- the U.S. EPA OUST MTBE site http://www.epa.gov/swerust1/mtbe/index.htm

UST management and operation problems

Improved UST equipment and practices have reduced or eliminated some gasoline releases, although human error and complexity of equipment create opportunities for further releases *(Davidson and Creek, 1999)*. Researchers have identified factors and practices that, if adhered to, are likely to produce fewer releases. The *UTTU* article "UST design/installation factors" *(Vol. 14, No. 5)* described some design and installation parameters of concern. This article lists 13 categories of activities and more than 100 activities/factors that can be sources of problems for the UST owner/operator. These management and operation issues include:

- **product delivery:** predelivery product and water gauging, engaging fill protection and overfill protection, hooking up fuel hose and vapor recovery system, responsibilities of drivers and owner/operators and spill response
- **product dispensers:** customer education, owner/operator staff training, maintenance requirements, visual inspections, filter change out, meter testing/calibration, testing procedure for automatic shutoff systems, testing schedule, response to failed test and tester qualifications
- **pumping systems:** proper usage, inspection and maintenance requirements, inspection and cleanout of check valves and inspector/maintenance personnel qualifications
- **leak detection and prevention systems:** inventory reconciliation/interpretation, evidence of leakage, periodic visual inspection of equipment, tank pit monitoring, automatic tank gauging (ATG) test, tightness testing (tanks and lines), periodic sensor calibration, under dispenser monitoring, pump turbine sump monitoring, inspection and maintenance of overfill sump, interpretation of monitoring/test results, alarm panel testing, response to leak alarms, communication/documentation and system sensitivity

- **vapor recovery systems:** proper usage, fill bucket inspection/drainage, maintenance requirements and inspection/testing criteria
- **spill response:** spill response plan, personnel training and responsibilities, spill response equipment, agency notification and communication, documentation, spill kit maintenance, stopping the spill/leak/release, release reporting, emergency response, spill cause investigation, released product containment and spill response followup
- **tank/line repairs:** industry codes, contractor qualifications, equipment certification, testing requirements, tester qualifications and documentation
- **inventory control:** federal, state, and local requirements, manual tank gauging, manual tank gauging before and after product delivery, proper tank charts, water gauging procedure, accounting system requirements, daily reconciliation, and record and reconciliation forms/instructions, product losses, procedures for reduction of controllable losses, procedure for receipt of product and response to inventory variance
- **tank/line precision testing:** testing requirements/ frequency, environmental conditions recorded/ considered, tester qualifications, criteria for pass/fail, response to failure of test, documentation and sensitivity
- **corrosion protection systems:** maintenance/ monitoring requirements, sacrificial anode replacement, inspection/testing criteria and tester qualifications
- **stormwater control/cleanup:** storm drain inlet inspection/cleaning, catch basin inspection/cleaning, drip/spill spot cleaning and cleanup material disposal
- **training for station personnel and tanker truck drivers:** alarm panel testing, procedures for product receipt, product delivery/tank fillup, inventory reconciliation, station inspection, response to alarms, spill response, site cleanup/waste disposal, on-site record keeping, daily procedures, tank gauging, dispenser inspection/calibration, personnel oversight and communication/documentation
- **record keeping:** history of UST usage prior to current usage, equipment maintenance schedules, equipment certification and testing, equipment operating manuals from manufacturers, station operating permits, maintenance records, calibration, repairs, suspected/confirmed releases, corrective action taken, groundwater monitoring, vapor monitoring, temporary closures, corrosion protection system, analysis of corrosion potential, leak detection performance, inventory variance, repair/upgrade documentation, change-in-service and accidents/incidents/spills

Davidson and Creek *(1999)* believe that "Education is the foundation to improvement. Therefore, if a UST owner/ operator is aware of potential problems in advance, then operation and management practices can be developed to avoid the vast majority of problems."

Reference

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

For other recent reports on UST systems, see the U.S. EPA OUST site, http://www.epa.gov/swerust1/ustsystm/usteval.htm.

Research notes

Evaluation of the Galega-Rhizobium Galegae System for the Bioremediation of Oil-Contaminated Soil

Suominen, L., Jussila, M.M., Makelainen, K., Romantschuk, M. and K. Lindstrom, *Environmental Pollution*, Vol. 107, 2000, pgs. 239-244; http://www.elsevier.com/locate/envpol.

Researchers experimented with a nitrogen-fixing leguminous plant, *Galega orientalis*, and its microsymbiont with respect to its ability to tolerate m-toluate in the presence of five bacterial strains *(Suominen and others, 2000)*. They monitored seed germination at m-toluate concentration of 0, 500, 1,000, 2,000 and 3,000 ppm. With the aid of bright-field microscopy of roots stained with methylene blue solution, they were able to detect root hair deformation and infection threads. After 30 days, two plants from each m-toluate concentration were transferred into fresh m-toluate-free tubes so researchers could observe their viability over the next 30 days.

Researchers found that the germination of *G. orientalis* seeds decreased with increasing m-toluate concentrations in microcosm studies. "An m-toluate concentration higher than 500 ppm inhibited plant growth and root development, but the presence of the bacterial strain *Pseudomonas putida* inoculant increased the tolerance level to 1,000 ppm. . . . However, in the highest m-toluate concentration tested (3,000 ppm), most of the plants were viable and when transferred into uncontaminated media, half of the plants began to grow normally, and nodules developed on the new lateral roots within 3 weeks." Other strains of bacteria were not successful in degrading the m-toluate."

Results from greenhouse experiments indicated seed germination from 60 percent in uncontaminated soil to 50 and 46 percent in oil-contaminated soil and m-toluate spiked soil, respectively. All plants were able to fix nitrogen but root structure varied with soil type. "After 4 months, the m-toluate concentration in all pots decreased below the detectable level and the roots filled all pots by the end of the experiment."

It is possible "that microbial communities in the rhizosphere are involved in the protection of plants from chemical injury." For instance, a bacterial strain that degrades dibenzothiophene and forms nitrogenous nodules does exist. "This suggests the potential use of rhizobia inoculants for bioremediation connected to a development of strains provided with dual functions: contaminant degradation and nitrogen fixation. At present, no such inoculants are available, and the number of publications concerning the use of legume rhizosphere for soil bioremediation is small. In this work the *G. orientalis* plant and its microsymbiont *R. galegae* showed good potential for the vegetation practice (biodegradation) of oil-polluted soils. The field trials in temperate conditions together with the development of improved inoculant bacteria will show the true efficiency of this bioremedation practice in the future."

Related papers in this volume of *Environmental Pollution* include:

- *Decomposer Animals and Bioremediation of Soils*
- *Bioremediation of Petroleum Hydrocarbon-Contaminated Soil by Composting in Biopiles*
- *Evaluation of Ecological Disturbance and Intrinsic Bioremediation Potential of Pulp Mill-Contaminated Lake Sediment Using Key Enzymes as Probes*
- *Genetic Engineering in the Improvement of Plants for Phytoremediation of Metal-Polluted Soils*
- *On-site Biological Remediation of Contaminated Groundwater: A Review*

Means to Improve the Effect of In-Situ Bioremediation of Contaminated Soil: an Overview of Novel Approaches

Romantschuk, M., Sarand, I., Petanen, T., Peltola, R., Jonsson-Vihanne, Koivula, T., Yrjala, K. and K. Haahtela, *Environmental Pollution*, Vol. 107, 2000, pgs. 179-185; http://www.elsevier. com/locate/envpol.

This article describes bioremediation factors such as limited efficiency, evolution of degradation capacity, use of genetic engineering for strain construction, conjugative plasmids, rhizosphere bioremediation, sufactant bioavailability, electrokinetic extraction, and soil fauna effect. In particular, electrokinesis and electro-osmosis have been shown to mobilize contaminants from clay soils. "Electro-osmosis apparently helps spreading both indigenous bacteria or added inoculum around and into the contaminated site. But even more significant for biological degradation is perhaps that other limiting factors such as low temperature and nutrient limitations can be alleviated by using an electric field. Either way, the results in preliminary application-scale tests have been promising" *(Romantschuk, 2000).*

An Analysis of Low-Flow Ground Water Sampling Methodology

Sevee, J.E., White, C.A. and D.J. Maher, *Ground Water Monitoring and Remediation*, Spring 2000, http://www.ngwa.org.

Researchers recently developed new equations to estimate drawdown equilibrium for sampling water wells. Low-flow sampling can result in excessive water level drawdowns that can cause soil particle and/or aggravated colloidal transport into the well. Limiting water drawdown minimizes the potential for inducing suspended solids into the sample, thereby minimizing sample turbidity.

Field workers can use these equations to "estimate the relative contribution of water entering a sampling device from either the well standpipe or the aquifer. Such equations can be useful in planning a low-flow sampling program and may suggest when to collect a water sample. In low hydraulic conductivity formations, the equations suggest that drawdown may not stabilize for well depths, violating the minimal drawdown requirement of the low-flow technique. In such cases, it may be more appropriate to collect a slug or passive sample from the well screen, under the assumption that the water in the well screen is in equilibrium with the surrounding aquifer" *(Sevee and others, 2000).*

Well-Purging Protocols for Sampling Dissolved Petroleum Hydrocarbons

Miller, H.J., *Practice Periodical of Hazardous, Toxic and Radioactive Waste Management*, Vol. 4, No. 2, 2000; http://www.pubs.asce.org/journals.97hz.html.

Groundwater sampling protocols vary, but they generally recommend that before samples are taken, one of the following conditions should be met:

- upon removal of three well volumes of water, in-situ parameters (pH, temperature, dissolved oxygen, specific conductivity) should have stabilized
- five well volumes of water should have been removed
- the well is pumped dry

Although significant research has been conducted regarding well purging, much of it does not pertain to dissolved hydrocarbon samplings. A major issue is whether to purge or not to purge. Those who promote purging often recommend "low-flow" or "micropurging," which "involves pumping at a very low flow rate (1 L/min or less) from sampling equipment that is either dedicated or placed in the well hours to days prior to use," the objective being to "minimize disturbance to the water column so that stagnant water in the well casing does not mix with water flowing through the well screen." Using bailers, however, disturbs the water column; "many researchers argue that use of bailers introduces excessive variability in measured contaminant concentrations. . . . bailing or pumping at relatively high rates induces mass-averaging effects due to mixing in the well casing and the screened interval. Bailers may induce further effects due to composite averaging."

The no-purge sampling proponents assert that "the inherent variability of the concentration of contaminants in many plumes far outstrips the additional variability potentially induced by incomplete purging."

Studies of purging vs. no purging often give contradictory results. Using results from an extensive study (4,808 prepurge/postpurge data points), Miller (*2000)* concluded that there was a "systematic tendency toward slightly higher petroleum hydrocarbon concentrations in the prepurge samples than in the postpurge samples." Variables in the data could be attributed to well construction, site hydrogeology, purging methods and sampling devices. Miller (*2000)* contends that "The growing problem of groundwater contamination is driving researchers, consultants and regulatory agencies to consider, when appropriate, more cost-effective approaches for sampling and analyzing groundwater. The no-purge methods of sampling may present one such approach and may be particularly appropriate during long-terms monitoring of UST sites with wells screened above the water table."

Miller tested a ChemSensor, which is a 25 mm-diameter probe that can be lowered into a well to a depth of 200 feet. "A cable links the probe to a hand-held meter that provides the user with a response in terms of p-xylene equivalents measured in parts per million (ppm). The device has a temperature correction coefficient that is established and factory preset. One advantage of a ChemSensor over traditional laboratory analysis is its ability to obtain a statistically significant quantity of data at lower cost and reduced time. "Additionally, since ChemSensor measurements are made in-situ, many of the QA/QC issues typically associated with storage and handling of samples between the field and lab are eliminated, and the potential exposure of the operator to harmful contaminants is greatly reduced."

A test of the ChemSensor indicated that prepurge BTEX concentrations tended to be slightly higher than postpurge; in addition, there was no significant difference between these samples and those analyzed at a laboratory. Another advantage of using such a probe is sampling of a discrete interval. Bailing usually involves collecting samples over an interval; furthermore, during purging, water mixes within the well screen. Advantages of no-purge also include

- reduction of cross-contamination between wells
- elimination of storage and disposal of large volumes of purge water
- substantial cost savings

Miller *(2000)* recommends the no-purge and sensor sampling method for long-term dissolved hydrocarbon monitoring where

- the monitoring well is screened in an unconfined aquifer of coarse-grained sediments
- the top of the well screen is above the water table
- the screened interval is located predominantly within a single soil type
- no measurable free product is observed in the well

The no-purge method should not be used when opening or closing a site, or when contamination may affect residential or municipal water supply wells. Miller *(2000)* also recommends that practitioners consider a "confirmatory purged sample protocol."

See also M.J. Galloway's *(Ground Water Monitoring and Remediation, Summer, 2000, pg. 194)* "Discussion of Active Monitoring and Thoughtful Remediation." Galloway compares contaminant concentration data taken from a well that was purged vs. a well that was not purged. Results differ by an order of magnitude.

Flow Dynamics and Potential for Biodegradation of Organic Contaminants in Fractured Rock Vadose Zones

Geller, J.T., Holman, H.Y., Su, G., Conrad, M.E., Pruess, K. and J.C. Hunter-Cevera, *Journal of Contaminant Hydrology, Vol. 43, 2*000, pgs 63-90; http://www.elsevier.com/locate/jconhyd

This article describes "an experimental approach for investigating the potential for bioremediation of volatile organic compounds (VOCs) in fractured rock vadose zones," and in particular, in arid regions that are considered to be biologically inactive by virtue of dry conditions and minimal organic matter. Researchers achieved the following:

- established a conceptual model for fluid and contaminant distribution in a geologic matrix
- created seepage experiments to identify features of liquid distribution in the fracture plane
- identified microorganisms by non-destructive monitoring of biotransformations on rock surfaces at the micron-scale
- integrated flow and biological activity in natural rock geocosms

Geller and others *(2000)* define geocosms "as core-scale flow cells that incorporate some aspects of natural conditions, such as seepage in the fracture plane."

Researchers found the following:

- bacteria are ubiquitous, but few, in fractured rock vadose zones of arid regions
- water seepage in a fractured rock vadose zone is localized and intermittent; however, adequate water to maintain bacteria viability will likely exist
- in layered basalt flows, perched water can occur at sedimentary interbeds with water flowing through fractures and rubble zones and moving into the high-storativity vesicular basalt
- a mixed culture of viable bacteria does exist on the vesicle surfaces of defensible samples, thus supporting existence of bacteria on fracture surfaces
- possibly, addition of water and organic contaminants could stimulate bacterial activity
- NAPL will pond at vertical discontinuities and the presence of residual water may redirect the NAPL to areas not contacted by the flowing water
- high-resolution spectroscopic techniques can map bacteria, mineral and contaminant distributions, and this provides potential for non-destructive monitoring of biological activity at the microscale

Researchers are applying these mapping techniques to "test the effect of various environmental factors on biotransformation rates and to quantify potential isotopic shifts caused by biodegradation, by measuring the abundance and stable isotopic composition of residual contaminants and of potential metabolic byproducts (e.g., CO_2)."

Information sources

U.S. EPA publications and information

The following publications can be viewed or downloaded at http://clu-in.org/techpubs.htm or obtained by calling 800-490-9198 or 513-489-8190 or faxing 513-489-8695.

- *Engineered Approaches to In-Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications* (EPA 542-R-00-008)
- *Innovations in Site Characterization: Geophysical Investigation at Hazardous Waste Sites* (EPA 542-R-00-003)
- *Proceedings of the Ground-Water/Surface-Water Interactions Workshop* (EPA 542-R-00-007)

Other EPA/government documents/software:

- *A Guide to Developing and Documenting Cost Estimates During the Feasibility Study* (EPA-540-R-00-002) http://www.epa.gov/superfund/resources/remedy/costest.htm
- *A User's Guide to Environmental Immunochemical Analysis,* a general troubleshooting guide http://www.epa.gov/crdlvweb/chemistry/immochem/user-guide.htm#TABLE
- *Evaluating the Hanby Test Kits for Screening Soil and Groundwater for Total Petroleum Hydrocarbons: Field Demonstration* (ERDC/CRREL TR-00-007), http://www.crrel.usace.army.mil/techpub/CRREL_Reports/reports/TR00-7.pdf.

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Website: http://epd.engr.wisc.edu/uttu/

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- *ETV* (Environmental *Technology Verification) Reports for Environmental Decision Support Software:*
  - Tech Development Corporation, Environmental Visualization System Pro http://www.epa.gov/etv/02/evs_vr.pdf
  - Decision FX- SamplingFX http://www.epa.gov/etv/02/samplingfx_vr.pdf
  - Environmental Software – SitePro Version 3.0 http://www.epa.gov/etv/02/siteprovr.pdf
  - Environmental Systems Research Institute – ArcView GIS Version http://www.epa.gov/etv/02/esrivr.pdf
  - University of Tennessee Research Corporation – Spatial Analysis and Decision Assistance (SADA) http://www.epa.gov/etv/02/sada_vr.pdf
- *Innovative Technology Summary Report: Electrical Resistance Tomography for Subsurface Imaging* (DOE/EM-0538) http://ost.em.doe.gov/ifd/scfa/itsrs/itsr17/itsr17.pdf
- *The OnSite On-line Tools for Site Assessment* Helps regulators review modeling reports and enhance contaminant transport knowledge http://www.epa.gov/athens/software/training/WebCourse/part-two/onsite
- *Site Remediation Technology InfoBase: A Guide to Federal Programs, Information Resources and Publications on Contaminated Site Cleanup Technologies* (EPA 542-B-00-005), http://www.frtr.gov.

Websites

Environmental Fate Data Bases (EFDB), which contains data on environmental fate, microbial toxicity and biodegradation, http://esc.syrres.com

"Water Quality: Key EPA and State Decisions Limited by Inconsistent and Incomplete Data," (RCED-00-54) which contends that statistics from the National Water Quality Inventory "are unreliable and subject to misinterpretation." Available at http://www.gao.gov. Go to Search GAO Archives and type in the title for a pdf version of the document.

U.S.G.S. site on arsenic http://www.epa.gov/safewater/arsenic.html.

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

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