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In Situ Bioremediation of TCE-Contaminated Groundwater

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Abstract

This is the final report of a two-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). A barrier to wider use of *in situ* bioremediation technology is that results are often variable and difficult to predict. *In situ* bioremediation has shown some very notable and well publicized successes, but implementation of the technology is complex. An incomplete understanding of the effects of variable site characteristics and the lack of adequate tools to predict and measure success have made the design, control and validation of bioremediation more empirical than desired. The long-term objective of this project is to improve computational tools used to assess and optimize the expected performance of bioremediation at a site. An important component of our approach is the explicit inclusion of uncertainties and their effect on the end result. We have extended our biokinetics model to include microbial competition and predation processes. Predator species can feed on the microbial species that degrade contaminants, and our simulation studies show that species interactions must be considered when designing in situ bioremediation systems. In particular, our results for TCE indicate that protozoan grazing could reduce the amount of biodegradation by about 20%. These studies also indicate that the behavior of barrier systems can become complex due to predator grazing.

Background and Research Objectives

Contamination of groundwater and soils with chlorinated solvents such as trichloroethylene (TCE) is a major national problem. In many cases, these contaminants are present at low concentrations (i.e., parts per million, ppm) over areas as large as several square miles. Cleanup costs are estimated in the billions of dollars, and despite the enormous moneys being spent, present remediation efforts are not effective for widespread, dilute contamination.

Currently, the most common remediation approach is to pump the contaminated groundwater to the surface where the water is treated to remove the contaminants. This

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pump-and-treat approach has been most successful where it is feasible to isolate contamination by forming a hydraulic barrier, and to remove bulk contaminant at heavily contaminated sites. It has been less successful in remediating sites to the near-zero levels (<ppm) of residual contamination required by regulatory agencies. One reason for this is that some of the contaminant is sorbed to aquifer materials, or present as a separate phase. As the contaminant is removed from the aqueous phase, more contaminant leaches from the sorbed or separate phase into the groundwater. The result is that after an initial decrease in contaminant concentrations, low levels of contaminants remain for very long times making pump-and-treat very costly and very slow. Further, for sites at which the contaminant has already widely dispersed, pump-and-treat will be very inefficient simply because of the enormous volumes that would have to be pumped.

Clearly, an alternative strategy is needed. *In situ* treatment has great advantages because it avoids pumping enormous volumes of groundwater in order to remove ppm or lower concentrations of contaminants. A very promising technology is *in situ* bioremediation, the use of microbes to convert hazardous chemicals to environmentally benign products such as water, carbon dioxide, biomass, and salts. Because bioremediation uses autocatalytic processes that occur naturally in the environment, its use has great advantages over other *in situ* destruction techniques that require conditions more difficult to achieve *in situ*. Although applicability *in situ* is perhaps the most significant advantage, bioremediation also has advantages in cost, safety, public acceptance, and effectiveness. A cost effectiveness analysis of an *in situ* bioremediation field demonstration at DOE Savannah River facility concluded that *in situ* bioremediation was ~4 times more cost-effective than traditional pump-and-treat efforts (Saaty & Booth, 1994). Moreover, as shown in Figure 1, field data and simulation results confirm that *in situ* bioremediation results in lower residual contamination levels than alternative treatment technologies.

A barrier to wider use of *in situ* bioremediation technology is that results are often variable and difficult to predict. *In situ* bioremediation has shown some very notable and well publicized successes (e.g., the Exxon Valdez oil spill, Savannah River and Moffet Field), but implementation of the technology is complex. An incomplete understanding of the effects of variable site characteristics (notably site soil heterogeneity, indigenous microbiology, and contaminant speciation) and the lack of adequate tools to predict and measure success, has made the design, control and validation of bioremediation more empirical than desired. In large part, it is the uncertainty associated with bioremediation options that has driven site owners to choose more costly and less effective treatment options. In addition, the performance of bioremediation processes in the field is

challenging to measure because it requires integration of site characterization, laboratory experiments, modeling, and uncertainty analysis during the design of the field process.

The long term objective of this project is to develop accurate computational tools that will position us to work with site owners and providers of remediation services to solve problems in site specific design, implementation, and performance assessment of bioremediation strategies for chlorinated solvents in groundwater. The product will provide an objective way to estimate and optimize the expected performance of bioremediation at a site. An important component of our approach is the explicit inclusion of uncertainties and their effect on the end result.

Importance to LANL's Science and Technology Base and National R&D Needs

This work addresses one of the five major areas identified by the Environmental Management Program Office as best matching primary, near-term customer needs and LANL's capabilities and interests: the removal of solvents from groundwater. Our work brings together LANL's capabilities in modeling and advanced computing, environmental science, chemistry, and microbiology to help develop and implement solutions to a very real and important national problem. Our project helps establish LANL as a leader in solving complex remediation problems requiring multidisciplinary technical teams. This project supports LANL's tactical goals in Great Science, High Performance Computing, and Industry and its missions in Environmental Stewardship and Energy and Environment.

Groundwater contamination by solvents, such as TCE, is one of the most important environmental problems facing industry and government. Recently, one of the nation's largest electronic companies, Motorola, issued a challenge to LANL to work with them and others in the electronics industry to solve the TCE-contaminated groundwater problem. Our work will help to establish LANL as an important R&D partner with industry in addressing this critical problem. Other potential customers include other industries with contamination problems (e.g., chemical, manufacturing, petroleum), environmental remediation service companies, and government (mainly DoD and DOE).

Scientific Approach and Accomplishments

Our approach integrates LANL's capabilities in modeling and high performance computing, hydro-geology, analytical capabilities, and microbiology and biochemistry to address the R&D problems that are currently barriers to the successful implementation of *in situ* bioremediation technologies. The focus of this work is on the modeling aspect, in which we aim to:

- improve our models to include competition and predation in microbial systems, as well as a method of capturing subscale soil heterogeneity, e.g., homogenization theory;
- rank the sensitivity of bioremediation to various site characteristics and microbial metabolic properties; and
- test our improved model against data from a well-characterized site.

For discussion purposes, we divide our effort into three major areas: transport, microbial activity, and performance assessment.

Transport

Transport is one of the most critical issues for *in situ* bioremediation. *In situ* bioremediation will only occur in areas where the appropriate microbes are present in the same location as the TCE and the "food" (electron acceptors such as oxygen, an electron donor carbon source plus nitrogen and phosphate) necessary for microbial activity. This is the goal of any bioremediation technology, whether the technology involves transporting food to the microbes or guiding the TCE contaminated groundwater to a treatment zone or barrier that contains the microbes and their food. Control or accurate prediction of the flow field is obviously important to success of in situ bioremediation. Unfortunately, subsurface geology is very heterogeneous and the various injected substances have different transport characteristics (e.g., diffusivities, sorption coefficients, and solubilities) in different soils. These properties tend to vary on many length scales, from the scale of geologic units to the pore scale and even to finer features within pores. The flow field will accordingly be heterogeneous and multi-scaled. This multi-scale nature of porous flow and reactive transport presents a challenge for computational models.

Another related issue is data uncertainty regarding the heterogeneity of the site. No matter how many wells have been drilled to characterize a site, some uncertainty will remain in geologic and hydrologic properties. Rather than regarding data uncertainty as a negative, we can account for it through statistical and stochastic methods, and in the process, design a more robust system.

Mathematical models of flow and transport provide a mechanism for organizing in a systematic way what we know about a site and the processes involved. They can be used for sensitivity studies, interpretation of experimental data, design of field operations, and optimal management of resources. Because of the complexity of the governing equations, we must resort to numerical solutions solved on computers. Because of the limitations of our computers, we are forced to solve the governing partial differential equations on a coarse scale, missing the details that are finer than the size of computational mesh cells. It is easy to demonstrate however that ignoring the subgrid-scale structure in a field-scale

simulation can lead to serious errors in estimation of flow direction and speed. If flow paths are not correctly captured, then the errors will be compounded in the reactive transport calculations. This could lead to costly policy and operational mistakes. There are several algorithms that approximate subgrid-scale structure, including renormalization group theory, level surfaces tracking, stochastic methods, particle tracking, homogenization and fractal scaling. We have focused on homogenization theory as an attractive alternative to some of the other, more complex multi-scale algorithms.

The relationship that characterizes porous flow is the reduced form of the momentum transport equations known as Darcy's law which relates flow rate U, fluid pressure p, fluid viscosity μ, and a property K of the soil or rock called the permeability:

$$\vec{U} = - \frac{K}{\mu} \nabla p$$

The permeability K(x,y,z) in its most general form is a tensor. Generally, porous flow models ignore this and either use a scalar value or at most a diagonal tensor form. The flow of fluids through subsurface permeable formations can be modeled as a coupled system of nonlinear partial differential equations. In most situations, these must be solved numerically. Even if a million grid cells are used for a three-dimensional (3-D) field-scale simulation (e.g., 100 x 100 x 100 grid cells), the grid cells will be at least several meters in size at best. Small-scale variations in material properties such as permeability will not be accurately represented; they are typically replaced by a scalar obtained through an averaging process. However, a scalar usually is not a good approximation to a tensor (it does not capture the direction information carried in the tensor form), resulting in potentially large errors in the simulated flow field. There have been various attempts to devise a better method of approximating subgrid-scale variability. The recently developed homogenization algorithm has had notable success.

The method of homogenization (Jikov, Kozlov and Oleinik, 1994) essentially involves replacing a fine grid representation of a function with a coarser grid in which function values on the coarse grid are averaged in a particular way. Harmonic and arithmetic averages have been used in the past in an attempt to approximate subgrid-scale variability, but it turns out they are accurate over only a limited range of conditions. The multigrid method of Dendy (1982) provides a much more accurate averaging scheme. Multigrid was developed as a highly efficient algorithm for solving matrix equations. It involves nested grids, with residuals on coarse grids being interpolated to finer grids, and with the solution on the coarser grids being propagated back to the finer grid. The interpolation preserves important properties such as mass conservation. The multigrid concept of averaging from fine grids to coarse grids has been applied to the homogenization

problem in porous media for steady saturated flow (Hyman, Shashkov and Steinberg, 1996). It has shown considerable promise in oil reservoir simulations by simulating 2-D water and oil flow in a heterogeneous reservoir on a coarse mesh to the same accuracy as was obtained with a finer numerical grid; subgrid-scale effects were successfully captured.

A comparison between a simulation provided by Durlofsky (1991) and a homogenized simulation illustrates the benefit of this subgrid-scale algorithm. In this example, shown in Fig. 2, there are no-flow boundary conditions at the top and bottom of the domain and the pressure is prescribed to be p=1 along the left side and p = 0 along the right side of each square. This sets up a flow through the sand around the shale with a total flux though the system equal to 0.5205. The permeability in the small squares (shale) is $k = 10^{-6}$, elsewhere (sand) it is normalized to $k = 1$.

In Durlofsky's 1991 study (Fig. 2a) on a grid with 1600 unknowns, a mixed finite-element method estimated a total flux of 0.4508 (-13.4% error). Recently Hyman and Dendy found that the same accuracy can be obtained with a coarser grid (Fig. 2b) using a form of homogenization. Efforts are ongoing to fully implement homogenization algorithms into our in situ bioremediation simulator.

Microbial Metabolism/Ecology

Bioremediation performance in the field is usually different from that observed in the laboratory. Two reasons for this, a heterogeneous subsurface environment and issues of transport, were discussed above. Another reason is that, in the laboratory, a species of bacteria is studied in isolation, whereas in the field, these bacteria live in a complex microscopic biosphere. The bacterial species of interest have to compete for resources with other bacterial species, as well as with other microorganisms such as fungi and protozoa. Moreover, these bacterial populations are dynamic. They are capable of rapid adaptation. The rate at which a particular species of bacteria utilizes a substrate, for example, may increase over time. Bacteria can share genetic information, even across species, through transmembrane exchange of plasmids. Competition and predation has been studied in small laboratory batch reactors (see, e.g., Smith and Waltman, 1995), but to our knowledge no previous modeling work has included this type of microbial ecology in a subsurface flow and transport model. We have extended our biokinetics model to include microbial competition and predation processes.

An example of how microbial interactions can affect in situ bioremediation is clearly illustrated in the following example. In this simple 1-D geometry, a hydrocarbon contaminant plume is moving from left to right with groundwater through a region at a fixed velocity of 0.8 ft/day. The groundwater contains dissolved oxygen and nutrients. A bacterial species that will grow on the hydrocarbon substrate is present in the soil. There is

also a common soil protozoan species that will consume the bacterial species. Protozoan grazing of contaminant-eating bacteria has been observed (Sinclair et al, 1993). Protozoan grazing has been found to follow Monod kinetics (Menon et al, 1996). In isolation, the indigenous bacteria would be able to consume the invading contaminant plume rapidly. The presence of a predatory protozoan species complicates this.

Figure 3 shows simulated bacterial and protozoan concentrations and the substrate concentration as a function of position and time. The time window is between 200 and 300 days after first arrival of the plume at X=0. Interesting nonlinear behavior arises due to microbial species interactions that are not seen when only one species is considered. The bacterial and protozoan species experience episodic growth and decay. The oscillations are very regular in the first meter, then undergo a period doubling and become less regular where the substrate is greatly diminished. The contaminant penetrates only about 1 meter into the domain when the microbes are at high levels, but advances 5 meters or more when the microbe levels are low due to protozoan grazing. A barrier design that involved the particular species modeled in this simulation would need to be wide enough to capture all the contaminant, even during protozoan growth episodes.

A range of other behavior is possible for various combinations of species. For example, in the case of a very aggressive predator species and a moderate to slow growing microbial species, the microbial species will experience regular intervals of sustained growth followed by shorter intervals of almost total extinction due to predation. The contaminant is completely removed when the microbes are vigorous, but breaks through completely when the protozoans are at high population levels. This has significant implications for in situ bioremediation, especially for barrier methods. A barrier may function well for a period of time, but then experience almost total failure for a short period of time (a week or so), but then recover. Species interactions must be considered when designing in situ bioremediation systems.

One final task is to consider pore clogging. Data is becoming available (e.g., Jennings et al, 1995) that indicates change in permeability as a function of biomass in pores. Local changes in permeability due to elevated microbial biomass levels will lead to local changes in flow direction and rate, which will strongly affect the rate of bioremediation, another result of the dynamics of soil microbial systems.

Field Application

An important difference between the use of microbes to destroy hydrocarbon contaminants (such as petroleum spills) and their use to destroy highly chlorinated solvents (such as TCE) is that microbes can acquire energy for growth from the metabolism of most hydrocarbons, but metabolism of highly chlorinated solvents (TCE, for example) does not

yield sufficient energy to support microbial growth. Some microbes can, however, transform these contaminants to benign products through a process called cometabolism. Cometabolism results from the fortuitous transformation of non-growth substances (in this case TCE) by enzymes whose function in the microbe is to transform a naturally occurring substrate (such as methane).

One of the major questions in designing an effective site-specific bioremediation strategy is which of several cometabolic strategies is most promising. At present, five enzymes found in bacteria have been shown to transform TCE cometabolically under aerobic conditions: methane monooxygenase (MMO), toluene dioxygenase, ammonia monooygenase, propane mono-oxygenase (PMO) and $P450_{cam}$. TCE transformations catalyzed by methane monooxygenase (MMO) have been most thoroughly studied in the laboratory and most of the field demonstrations have attempted to stimulate MMO activity to degrade TCE. Methane has a number of advantages: it is cheap, available and a natural constituent of subsurface environments, and it is a gas that is easy to deliver and diffuses readily. Although some notable successes have been shown with MMO, its effectiveness is limited by the irreversible inactivation of the enzyme by the reactive TCE epoxide intermediates generated by the enzyme during TCE transformation. In the analysis that follows, we applied our in situ bioremediation model to a site at which methanotrophs producing MMO were used to remove TCE.

The U.S. Department of Energy (DOE) conducted a field demonstration of bioremediation technology at its Savannah River site in 1992-1993. TCE contamination occurred from the 1950s into the 1980s from a leaking process sewer line. The cleanup technology employed a novel combination of injection of air, methane, N_2O and triethyl phosphate (in an aerosol) below the water table and vacuum extraction in the vadose zone using a pair of subparallel horizontal wells (Fig. 4). The objective was to stimulate aerobic *in situ* bioremediation of TCE contamination in the vadose and saturated zones by certain methanotrophs, methane-oxidizing bacteria that are capable of fortuitously cometabolizing TCE under various conditions.

A generalized description of the hydrogeology at the Savannah River site includes a sand unit, four major clay units, and a water table that lies about 40 m below the surface (Eddy et al, 1991). These sediments are heterogeneous, varying greatly in thickness and continuity across the site. The *in situ* bioremediation demonstration began in late February, 1992, lasted 428 days, and consisted of seven injection and extraction phases.

A plan view schematic of the site is given in Fig. 5, showing the traces of the horizontal wells, the location of monitoring well MHT-4 (data from it was used to test our model), and the orientation of the cross-section (A'-A) used in this study for the model

domain. We use this cross section because this region exhibited the greatest activity during the field demonstration, in terms of methanotroph population changes and TCE mineralization.

A great deal of hydrologic, chemical and microbiological sampling data is available for this site (Eddy et al, 1991; Hazen, 1992, 1993), providing a good test for an in situ bioremediation simulator. Our goals were (1) to model the changes in methanotroph population and TCE concentration observed during the Savannah River field demonstration and (2) to examine the sensitivities of TCE biodegradation to key model biokinetic parameters. Models help us to understand complex subsurface remediation efforts such as those at Savannah River because they provide a mechanism for integrating the many different kinds of data involved (e.g., hydrological, microbiological). Moreover, a calibrated model can provide estimates of the temporal and spatial distribution of concentrations, pressures and saturations everywhere in the subsurface region, and can therefore be used to estimate important quantities that are difficult or impossible to measure in the field, such as the total mass of TCE biodegraded.

This modeling study differs from previous modeling studies in that it includes both the vadose and groundwater zones, unsteady air and water flow, limited nutrients and airborne delivery of nutrients, in addition to toxicity, cometabolic kinetics, predator grazing and kinetic sorption. Previous models (Sturman et al, 1995) have focused almost exclusively on steady saturated flow with waterborne delivery of nutrients or with nutrients in excess. None have considered predator grazing of microbes. Soil protozoan predators include amoebae, various flagellates and fungi, and are observed in many soils at significant numbers (10^3-10^5 /g dry soil weight). A limited sampling at the Savannah River site found enhanced protozoan activity at several wells, and laboratory tests on soil samples from the site indicated that protozoa are present that would in fact feed on methanotrophs (Hazen et al, 1992, 1993).

Only a sampling of results are included here. Details of the simulator, the numerical solution algorithms and simulation results are given in Travis & Rosenberg (1997). Model results for methanotroph cell counts at monitoring well MHT-4 are shown in Fig. 6a, along with field data. When the injection well was turned on at the start of phase two, flooding the subsurface with fresh air, the methanotroph population increased only slightly in response to the higher oxygen content. Population counts rose several orders of magnitude during the methane injection phases (3 and 4), but paradoxically, decreased even though high concentrations of methane were being injected. At late times, during nutrient addition, (phases 6 and 7), population counts oscillated (Fig. 6a). Our model results are shown in Fig. 6b. Two cases were considered. In the first, no protozoan grazing was operating

(dashed curve). This simulation did not show the declines in methanotrophs at 150 and 200 days seen in the data, and the decline at 250-280 days was much shallower than observed. The solid curve is the result of the case with protozoan grazing. Qualitatively, it is a better match to the data; we can not expect a perfect match to data because of the limitations in our model, among other things.

A number of microbial species other than methanotrophs were measured in site soil samples. These included several nitrogen transformers whose population densities also responded to the air, methane and especially the nutrient injection. The population changes in all cases were characterized by oscillatory rises and declines rather than a steady maintained increase. The collective effect of these species on methanotroph dynamics is uncertain. Perhaps the important lesson here is that our modeling indicates that microbial interactions of some kind, predation and/or competition, appear necessary to explain the field data.

Fig. 7 plots the model domain-wide integrated mass of TCE extracted (via vacuum extraction) and biodegraded as a function of time. This plot indicates that most of the TCE degradation occurred during the 4% methane phase (phase four), with additional degradation occurring during the nutrient addition phase (phase seven). The model result for total extracted TCE is very close to the observed values. These model results indicate that protozoan grazing could reduce the amount of TCE biodegraded by about 20%, a significant factor. This adverse effect on the rate of biodegradation may lead to underestimation of remediation times at other sites if predator grazing is not considered.

Sensitivity Analysis

We determine the sensitivity of the enhanced removal of TCE due to microbial degradation through a series of simulations in which biokinetic parameters of the system were varied one at a time. The parameters varied are: k_{CH4}, K_{CH4}, K_N, k_{TCE}, K_{TCE}, I_{CH4}, T_C, and k_P. Sensitivity is defined as:

$$\left(\frac{\Delta TCE - \Delta TCE_{base}}{\Delta TCE_{base}}\right)\left(\frac{X_{base}}{X - X_{base}}\right)$$

where X is a biokinetic parameter, ΔTCE is the total TCE removed by extraction and biodegradation minus the total TCE extracted when biokinetics is not operating, and the subscript "base" refers to the simulation of the Savannah River site with biokinetics and protozoan grazing. Resulting sensitivities are listed in Table 1. The first row of numbers are the sensitivities as defined above. The second row of numbers are the sensitivities for biodegradation only (vapor extraction differences neglected). These results are specific to the Savannah River site since the amount of TCE removed is a function of the

injection/extraction strategy, the site hydrogeology, and the initial distribution of TCE. Since our model is nonlinear, these sensitivities strictly apply only to a limited part of the parameter space. The relative rankings of the sensitivities, however, are likely to hold more generally and be somewhat site-independent.

Table 1. Sensitivities of TCE removal to key parameters

	k_{CH4}	K_{CH4}	K_N	k_{TCE}	K_{TCE}	I_{CH4}	T_C	k_P
full simulation	1.86	-1.00	-0.42	-1.20	0.06	-0.004	-1.43	-0.24
biokinetics only	1.83	-0.94	-0.45	-1.08	0.07	-0.008	-1.30	-0.27

There are two main conclusions that can be drawn from this sensitivity analysis. First, and the most obvious, is that TCE degradation is strongly sensitive to the factors controlling the rate at which microbes can grow (e.g., k_{CH4}, K_{CH4}, K_N). Second, toxicity is also quite important. There is a strong sensitivity to k_{TCE}, the maximum utilization rate for TCE, but it is a negative correlation because TCE degradation products are toxic to methanotrophs. The faster TCE is degraded, the more toxic products are created and the more microbes are killed. These results imply that using bacteria that are not damaged by TCE, or that can even utilize TCE for energy, will be much more effective than simply using bacteria with higher growth rates. Sensitivity to predators (k_P), although significant, was not as strong as to toxicity.

The simulations support the observations that removal rate of TCE was enhanced by biostimulation of methanotrophs but diminished by protozoan predation. However, no attempt was made to find an optimal field operation. Several modeling results imply that a more efficient operation was likely possible. For example the model shows that much of the TCE between the injection and extraction wells was already removed by the time nutrients were added. Also, the area of high TCE removal rate was limited by the reduced spatial distribution of methane during pulsing. Optimization algorithms, such as developed by Lang (1995), could be used in conjunction with our model to develop a more efficient field operation by determining the best distribution and location of wells, injection-extraction schedules, and pulsing patterns for growth substrates and nutrients. Novel variations may be found. For example, in a very simple optimization model we found that pulsing nutrients as well as methane and systematically changing the length of the time interval between pulses could extend the area of high removal rate. Further use of transport

models coupled with optimization algorithms has considerable potential for reducing remediation time as well as operational costs.

Bioremediation Workshop

The principal investigators of this LDRD-sponsored research were organizers for the first SIAM/CNLS Workshop on "Mathematical Issues in Bioremediation and Porous/Fracture Flow," which took place at Los Alamos June 11-13, 1997, as a satellite meeting to the SIAM Conference on Mathematical and Computational Issues in the Geosciences held the following week in Albuquerque, New Mexico. SIAM and the LANL Center for Nonlinear Studies sponsored the workshop. Fifty scientists attended, with a good balance of representatives from universities, industry and national laboratories, and a few participants from Europe, Canada and South America. The purpose of the workshop was to bring together microbiologists, chemists, hydrologists, engineers and mathematicians to review the state-of-the art in mathematical analysis of in situ bioremediation and to identify major unsolved problems.

Workshop presentations and discussions focused on the complex interactions between bacteria and their chemical environment, the state of the art in coupling biological degradation with complex chemical reactions in mathematical models (coupled nonlinear equations), major differences between microbial degradation of organics and sequestering/ mobilization of metals, bioavailability of contaminants to microbes (sorption/desorption models), optimization of field remediation procedures (neural nets, genetic algorithms), impact of heterogeneity on in situ bioremediation (stochastic differential equations), stability analysis, upscaling, biofilm dynamics, and microbial community dynamics. Major advances have occurred in the last few years in mathematical analysis of bioremediation-related processes and, just as important, much new physical data is available that can be used as a basis for further model development. In particular, the startling recent advances by experimentalists to measure biofilm structure, growth and dynamics will lead to much improved models and predictability. In addition, experimentalists are finding that our Darwinian notions of microbial competition have neglected a very important consideration: microbial community dynamics also rely heavily on cooperation between members.

The central problems yet to be solved center on the following issues:

- heterogeneity/multiscale processes/upscaling. Bioremediation involves complex biogeochemical interactions operating on many time scales and taking place in a stochastic medium that itself has structure over many space scales. How can upscaling to the field scale be accomplished efficiently under these conditions?
- predictability and sensitivity. What level of predictability can we achieve, given the uncertainty in data, in characterization of the subsurface environment and even in

knowledge of processes? Apart from this, how inherently predictable or unpredictable is bioremediation? How can measurements at certain scales be used to predict dynamics at other scales?

- communication . How can we create more interaction between the applied mathematics community and the microbiologists, chemists and engineers who measure what happens in the laboratory and in the field? Mathematicians build models; engineers want reliable predictions. Models presently come in a great variety of types but their usefulness is doubted, or not understood, by many laboratory and field scientists. How can greater acceptance of models be promoted? How do we bridge the 'terminology gap' between different disciplines? Is it time to create a bioremediation community model, or at least a community library, much like the community climate model?

Areas for Future Work

We have identified several areas to investigate with our extended model. The first is the transport limitations associated with different proposed microbial co-metabolite strategies (e.g., methane + methanotrophs vs. propane + propane oxidizing bacteria) and different environments, beginning with a very simple system of well injection and extraction. The second is optimization of well injection/extraction, such as changing well location and pumping schedules (i.e., time-dependent rather than steady), taking into consideration data and hydrogeological uncertainties. The third area for investigation concerns ways to increase transport into lower permeability areas, such as using other forces (e.g., electro-osmotic), using surfactants and using the microbes themselves (through pore-clogging) to help channel flow into low-permeability zones. The higher permeability areas will be relatively easy to remediate because the microbes will grow more readily there (better access to the injected co-substrate). We may want to consider stimulating their growth sufficiently to clog pores deliberately in regions of high permeability to force flow into regions of low permeability. Finally our model can be used to evaluate the essential role of microbial competition, cooperation, predation, and gene transfer on in situ bioremediation efficiency. This is an area that is only just beginning to be investigated experimentally. We have developed a computational tool that makes possible all of these lines of study.

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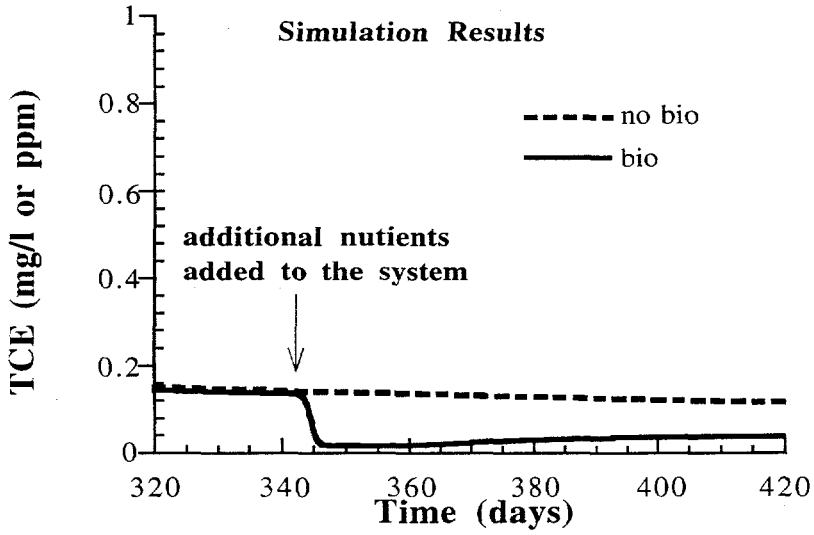


Figure 1. Typical reduction in TCE concentration over time between in situ bioremediation and pump-and-treat technologies.

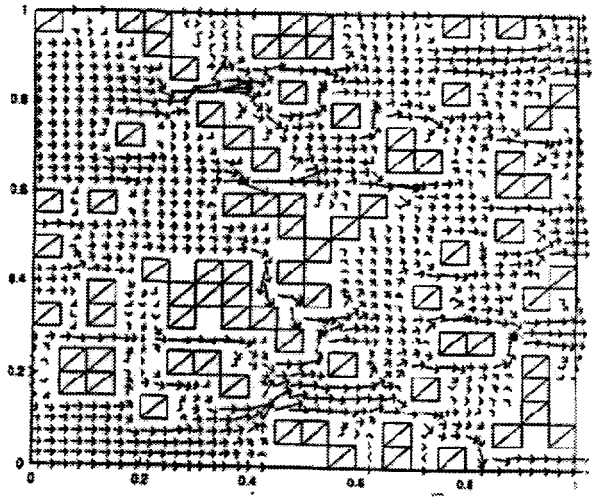


Fig. 2a. The nodal control volume method with 1600 unknowns has a 12% error in the flux.

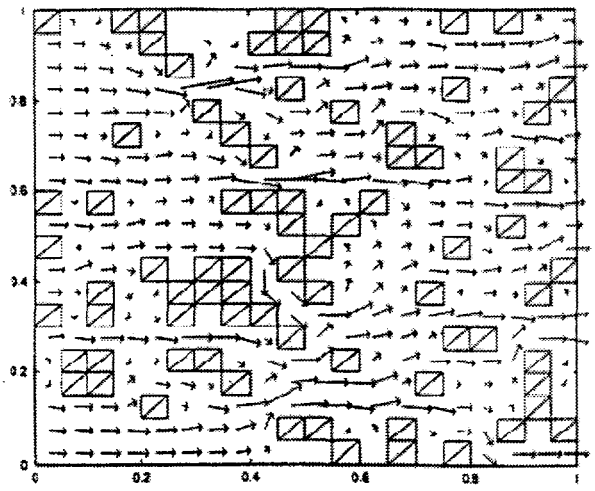


Fig. 2b. The homogenized solution with 400 unknowns has the same accuracy.

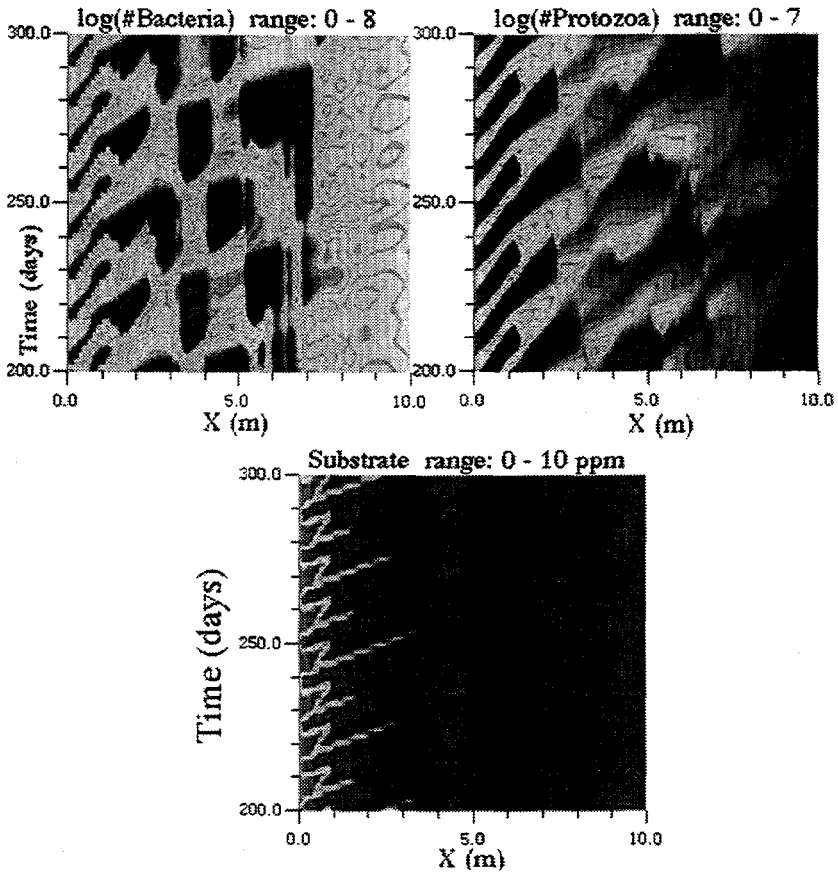


Figure 3. Banded gray scale shading of bacterial and protozoan dynamics. Flow is from left to right at 1 cm/hr, and substrate concentration at X=0 is a constant 10 ppm.

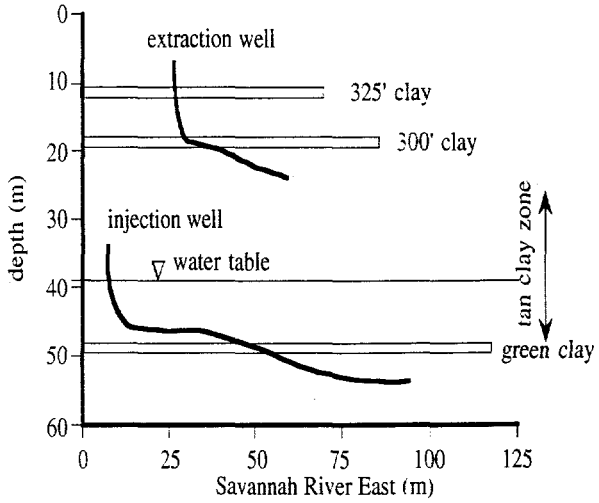


Fig. 4. Vertical section at the Savannah River site contaminated with TCE showing the depths of geologic units, the water table and traces of the subhorizontal injection and extraction wells. TCE is present between depths of 10 to 60 meters.

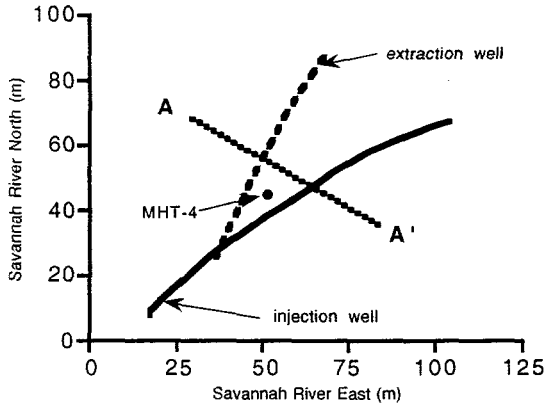
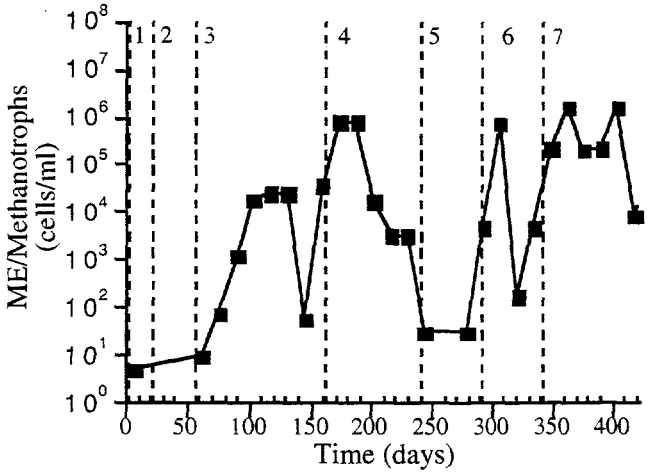
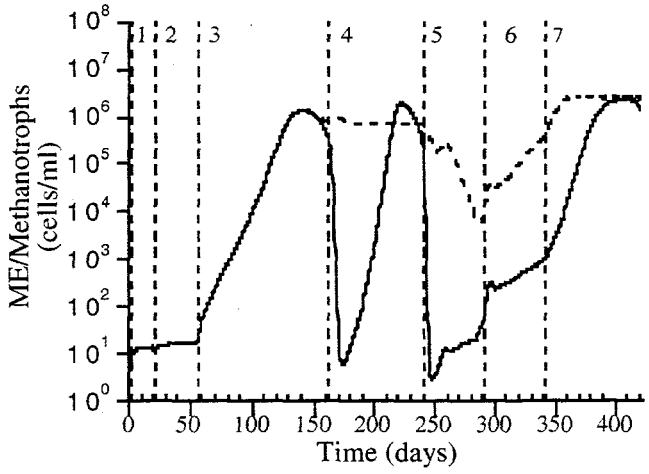


Figure 5. Plan view schematic of the Savannah River treatment site, showing the traces of the horizontal wells, monitoring well MHT-4, and the orientation of the cross-section A-A', used for the modeling study.



(a)



(b)

Figure 6. Measured (a) and computed (b) microbial concentrations versus time at the principal monitoring well. Vertical dashed lines mark the beginning of injection phases. In (b), the solid curve is the computed result with-predators, the dashed line without-predators.

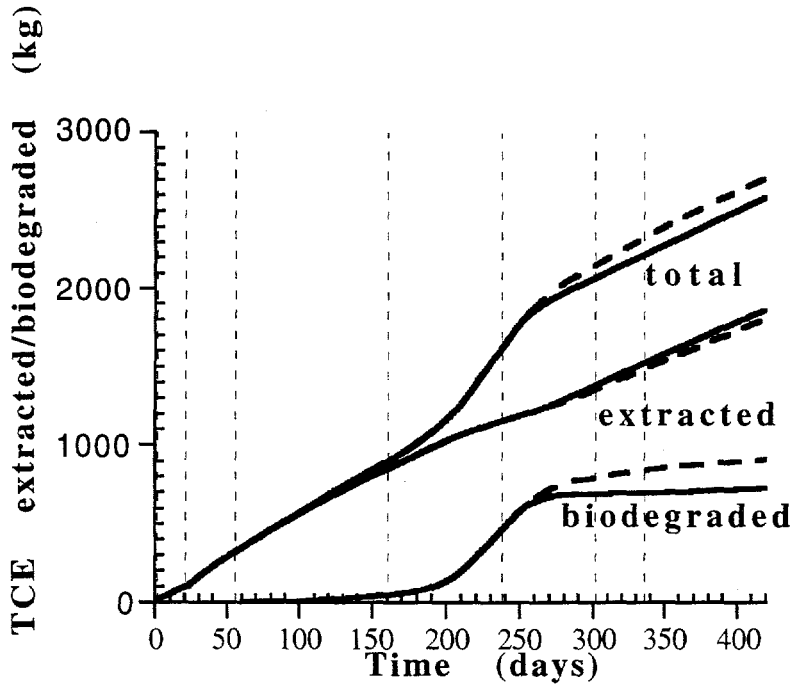


Figure 7. Cumulative TCE removed, through vacuum extraction and biodegradation versus time. Vertical dashed lines mark the injection phases. Solid curves include predation; dashed curves do not.