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In situ Bioremediation of Hanford Groundwater

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ABSTRACT

Liquid wastes containing radioactive, hazardous, and regulated chemicals have been generated throughout the 40+ years of operations at the U.S. Department of Energy's (DOE) Hanford Site. Some of these wastes were discharged to the soil column and many of the waste components, including nitrate, carbon tetrachloride (CCl_4), and several radionuclides, have been detected in the Hanford groundwater. Current DOE policy prohibits the disposal of contaminated liquids directly to the environment, and remediation of existing contaminated groundwaters may be required. In situ bioremediation is one technology currently being developed at Hanford to meet the need for cost effective technologies to clean groundwater contaminated with CCl_4 , nitrate, and other organic and inorganic contaminants. This paper focuses on the latest results of an on going effort to develop effective in situ remediation strategies through the use of predictive simulations.

INTRODUCTION

The Hanford Site, located in southeastern Washington State, is an area of approximately 600 square miles that was selected in 1943 for producing nuclear materials in support of the United States' effort in World War II. Hanford's operations over the last 40+ years have been dedicated to nuclear materials, electrical generation, diverse types of research, and waste management. Some of these operations have produced aqueous and organic wastes that were subsequently discharged to the soil column. In the 200 West area of the Hanford Site (Figure 1), plutonium recovery processes discharged carbon tetrachloride (CCl_4)-bearing solutions to three liquid waste disposal facilities: a trench, tile field, and crib. A minimum of 637 t of CCl_4 was disposed to the subsurface, primarily between 1955 and 1973, along with co-contaminants such as tributyl phosphate; lard oil; cadmium; nitrates; hydroxides; fluorides; sulfates; chloroform; and various radionuclides, including plutonium (1). Near the disposal site CCl_4 vapors have been encountered in the vadose zone during well-drilling operations, and groundwater contamination from CCl_4 is extensive, covering 5 km^2 . Concentrations up to 1000 times Environmental Protection Agency's (EPA) drinking water standard of 5 ppb have been measured in the groundwater. In addition, nitrate concentrations up to 10 times the EPA drinking water standard of 44 ppm have been measured in the same area of the site. Bioremediation is one technology currently being developed at Hanford to meet the need for cost effective technologies to clean groundwater.

The U.S. Department of Energy (DOE) and its operating contractor at Hanford, Westinghouse Hanford Company, are currently pursuing an Expedited Response Action (ERA) to remove volatile CCl_4 from the vadose zone using soil vapor extraction(2). In collaboration with the ERA, DOE's Office of Technology Development has selected the 200 West Area CCl_4 site as the host site for the VOC-Arid Integrated Demonstration. The objective of the Integrated Demonstration is to develop, demonstrate, evaluate, and transfer for deployment new technologies for all phases of cleanup of VOCs and associated contaminants in the subsurface. This program is to bring new technologies forward to provide more effective, cheaper, and safer methods for cleanup. Bioremediation is one technology being developed as part of the VOC-Arid Integrated Demonstration.

The current understanding of microbial degradation of CCl_4 is limited. However, CCl_4 biodegradation has been demonstrated with a number of different bacteria. The conditions that favor biodegradation of CCl_4 are predominantly anaerobic. For example, Bouwer and McCarty observed cultures of sewage treatment bacteria biodegrade CCl_4 to CO_2 and other metabolites under methanogenic (3) and denitrifying (4) conditions. Carbon tetrachloride biodegradation has also been demonstrated by pure cultures and consortium of denitrifying Pseudomonas sp. (5,6), the acetogen Acetobacterium woodii (7), Clostridium sp. (8), and under anaerobic and microaerophilic conditions by E. coli (9). Sulfate-reducing microorganisms have also demonstrated the ability to biodegrade CCl_4 (7,10). In addition, Semprini et al. (11) speculated that sulfate-reducing

bacteria were responsible for the CCl_4 degradation they observed during a field test of in-situ bioremediation. Biodegradation of CCl_4 under denitrifying conditions is of particular interest at Hanford because of the occurrence of both CCl_4 and nitrates in the unconfined aquifer. Both Hansen (6) and Criddle et al. (5) identified Pseudomonas species capable of degrading CCl_4 with acetate as the electron donor and nitrate as the terminal electron acceptor. However, strain KC isolated by Criddle et al. degraded CCl_4 more rapidly and completely than did the P. stutzeri strains studied by Hansen.

The potential of stimulating microorganisms indigenous to the Hanford Site to degrade both nitrate and CCl_4 has been demonstrated at the laboratory-, bench-, and pilot-scales. (12,13). For example, a pilot-scale agitated slurry reactor processing a simulated groundwater feed containing 400-ppm nitrate, 200-ppb CCl_4 , and acetate as the primary carbon source demonstrated greater than 99% and 93% destruction of nitrate and CCl_4 , respectively. Analysis of all product streams indicated that the concentration of nitrate and CCl_4 were reduced to levels below the drinking water standards. These promising results with indigenous Hanford microorganisms has led to the speculation that it may be possible to introduce the appropriate nutrients to the subsurface to induce the native bacteria to biodegrade both the nitrate and CCl_4 contamination in-situ. This paper outlines the latest results in an effort to design and implement an in-situ bioremediation test site. At present, work is proceeding to measure hydrodynamic and pertinent chemical properties of the proposed bioremediation test site, and to rigorously

study the kinetics of contaminant degradation and the growth of the indigenous microorganisms (14). This information is being incorporated into 1- and 3-dimensional simulations of in situ bioremediation to help design proper remediation conditions, as depicted in Figure 2.

EXPERIMENTAL DESIGN

Batch Experiments

Batch experiments are being performed to obtain reliable data that will allow credible prediction of the results of using in situ bioremediation technologies. These experiments will provide data for verifying mathematical expressions that describe the biological reactions involved in the degradation of CCl_4 and nitrate and the simultaneous production of biomass. They are designed to allow the investigation of the effects of various experimental conditions upon the proposed expressions. A balanced 2^{6-2} fractional factorial design for these experiments is being used. Correct reaction kinetics will result in none of the experimental conditions having a statistically significant effect upon the parameters in the kinetic expression. The experimental parameters that are being considered are the pH and the concentration of CCl_4 , acetate, nitrate, nitrite and biomass that is present initially in the reactor. The data being collected as a function of time include the biomass, CCl_4 , acetate, nitrate, and nitrite concentrations, and the pH and redox potential of the media. Ion chromatography is used to measure the ion concentrations, while CCl_4 is determined using a gas chromatograph equipped with an ECD detector. Redox

potential and pH are measured continuously in the reactor with standard electrodes. Biomass concentration is determined as dry weight in the solution.

The reactions are assumed to convert nitrate and acetate to biomass, N_2 , CO_2 , and water. In addition, to maintain a charge balance, H^+ is consumed in the reaction, and intermediate nitrite ions may be produced if all of the nitrate is not converted completely to N_2 . Carbon tetrachloride is assumed to be converted to biomass, CO_2 and Cl^- via a co-metabolic pathway. The microbial reactions are assumed to be inhibited by high acetate and CCl_4 concentrations. Thus, substrate-inhibited Monod kinetics are assumed for acetate and CCl_4 , while the reactions are assumed to be first order with nitrate, nitrite, and cell concentrations.

Flow Cell Experiments

A flow cell has been constructed and packed with soil obtained from a borehole drilled during FY 1991. This borehole was drilled to a total depth of approximately 85 m. The depth to groundwater at this site is 74 m, and the soil packed in the flow cell was removed at 81 m. The soil was obtained from the borehole in a sterile manner, and packed aseptically into the flow cell with minimal disruption of the soil's characteristics. The cylindrical flow cell is 7.62 cm in diameter and 33 cm in length. This flow cell is shown schematically in Figure 3.

The flow cell experiments are designed to allow the confirmation of denitrification and CCl_4 degradation by the indigenous bacteria, to confirm the reaction kinetics obtained in the batch

experiments, to ascertain the degree to which the biomass will plug the column, and to allow the confirmation of predictive computer simulations at selected experimental conditions. Here, the data collected include the acetate, nitrate, nitrite, sulfate, and CCl_4 concentrations and the column pressure as a function of time and position, and the biomass concentration in the column effluent and the dispersion in the column and porosity of the column as functions of time. Ion concentrations are again determined using an ion chromatograph, and CCl_4 concentration is determined using an ECD-equipped gas chromatograph.

To date, this column has been fed with simulated groundwater spiked with acetate and nitrate for three months. In Figure 4, the acetate and nitrate concentrations which result from continuous feeding of these compounds to the soil are shown as a function of position through the column. Clearly, the nitrate is being completely consumed in this system, while the acetate is metabolized. There was not any nitrite in the column feed stream, and the concentration initially increased to approximately 350 ppm, and then was relatively constant throughout the remainder of the column. This indicates that nitrite was initially produced from the nitrate, and then both were being utilized as the microbes metabolized the nutrients.

The pH of the column feed stream was near 7.0, and as the media flowed throughout the column and was reacted by the microbes, the pH increased to a value in excess of 9. Prior studies have shown that once the pH exceeds values of about 8, the microbial

reaction rate is severely diminished. These results indicate that some sort of pH control is necessary in the in situ case.

The results shown above demonstrate that, consistent with previous studies (13,6), the microbes in the Hanford aquifer are capable of denitrification. Ongoing research will demonstrate that concurrent CCl_4 degradation also takes place in this soil sample.

To better understand these results, and to be able to predict the results of in situ remediation, predictive mathematical simulations of the column and of the aquifer are also being performed.

PREDICTIVE PARAMETER DATA ANALYSIS

Development of a successful in situ bioremediation site requires a good understanding of the microbial growth and degradation kinetics, as well as the hydrodynamics and chemical properties of the aquifer. The capability to accurately predict these parameters is essential to the proper design of bioremediation application strategies. For these reasons, predictive mathematical simulations of the system are being undertaken. A 1-dimensional model has been developed to allow the investigation of various operational strategies, while a full 3-dimensional model, which requires significant quantities of computer time to solve, is used to ascertain the effects of the application of these technologies to the aquifer.

1-Dimensional Simulation

The utility of modeling the in situ degradation process was demonstrated by Semprini et al. (11), who used a 1-dimensional transport model to simulate their field in situ bioremediation experiments. In the mathematical description of this system, 1-dimensional transport is assumed. In addition, Semprini assumed that two biomass populations are responsible for the degradation of CCl_4 . The first of these is a denitrifying bacteria that uses acetate as the carbon source, while the second, which degrades CCl_4 much faster than the first, used dead cells from population 1 as the carbon source and is inhibited by nitrate. For both the cellular growth and consumption of the nutrients, Monod kinetics are assumed. Non-linear dynamic sorption of CCl_4 and intermediates to the soil is also assumed. With this predictive tool Semprini et al. accurately predicted the concentrations of substrate, electron acceptor, and contaminant that were observed during operation of a field in situ remediation system. In addition, these authors determined a priori that feeding a pulse of substrate would reduce microbial growth around the injection well. Reducing the microbial growth in this region will reduce the biofouling of the well. To take advantage of this effect, a 1-hour pulse of acetate was introduced during their experiments on a 12-hour cycle.

Based upon their description of the problem, we have developed a mathematical simulation of a 1-dimensional system. The problem is formulated as five partial differential equations, and four ordinary differential equations. The partial differential equations are used to describe the concentrations of acetate,

nitrate, CCl_4 , electron donor 2 (decay products of microbial population 1), and chemical intermediates such as chloroform, etc. The ordinary differential equations describe the microbial population concentrations, and the concentrations of the adsorbed CCl_4 and intermediates. The reaction kinetic expressions used in the results reported here were the same as those reported by Semprini, et al. (11). The resulting set of equations was solved using the numerical method of lines with 40 grid points in a 2.0-m simulation length. The Runge Kutta Fehlberg formula was used to integrate with respect to time, using a step of 0.001 days. Figure 5 shows the predicted CCl_4 concentration as a function of time and distance using the conditions reported by Semprini et al. Clearly, most of the CCl_4 degradation takes place near the inlet. This behavior is caused by the fact that most of the microbial growth is concentrated in this region, as shown by Figure 6, which illustrates the predicted biomass concentration as a function of position and time.

As can be seen, the model predicts that a significant quantity of biomass is accumulated within 0.2 m of the feeding well, even with a 1-hour feed pulse. This was observed experimentally since fouling of the injection well was reported after 66 days. If, however, the same total amount of acetate had been added to the system using a 10-minute pulse width, the model predicts that the biomass concentration could have been reduced significantly. In Figure 7, the biomass concentration is again shown as a function of time and position. Here, however, a 10-minute pulse width was used. Clearly, significantly less biomass would be produced near

the feed well if this feed strategy were employed. However, this strategy would not be useful if the CCl_4 were not degraded. On Figure 8, the CCl_4 concentration at the exit of the column is shown for both a 10-minute and 1-hour feeding pulse. The predicted CCl_4 concentration is essentially the same regardless of the feeding strategy. Thus, by using a 10-minute pulse, bioremediation could be carried out for a longer period of time before the well would be plugged and made inoperable.

3-Dimensional Simulation

Recently, Rice University and Pacific Northwest Laboratory have begun a collaborative research effort that involves laboratory, field, and simulation work directed toward validating remediation strategies, including both natural and in situ bioremediation at DOE sites such as Hanford. Because of chemical, biological, geologic, and physical complexities of modeling these DOE sites, one of the major simulation goals of the project is to formulate and implement accurate and efficient (parallel) algorithms for modeling multiphase/multicomponent flow and reactive transport. For in situ bioremediation in heterogeneous media, modeling is critical because the underlying processes scale nonlinearly. Also, important reactions take place at small scales that are difficult to average over a "control volume" since, even in the field, fronts often remain sharp and the reactions are usually very sensitive to concentrations.

To date, a 3-dimensional simulator has been developed which incorporates fluid transport, contaminant sorption, and microbial

degradation kinetics to describe the temporal and spatial changes in the concentration of substrate, contaminant, electron acceptor, and biomass. Currently, Monod and Michaelis-Menten-type kinetics are used to describe the microbe reactions, but as more information on Hanford and other arid-site microorganisms is obtained the site-specific information can easily be incorporated. In the simulations, the biodegradation terms are treated as generalized sources and sinks in the transport equations, while sorption is treated as a linear equilibrium isotherm.

The 3-dimensional simulation uses an accurate mixed finite-element method to calculate the flow field. A time-splitting procedure is used to solve the transport/biodegradation equations. Transport is solved by the modified method of characteristics (MMOC), and the reactions are treated as a system of ordinary differential equations and are solved by integration in time. The MMOC is important to keep transport fronts sharp (15,16).

An example 3-dimensional simulation is shown in Figures 9 and 10. Monod kinetics were assumed for the microbial reactions. In this case there is injection of a single "nutrient," which stimulates indigenous microorganisms to degrade a single contaminant. The contaminant is a co-metabolite and is initially distributed nonuniformly. The complex interplay of flow, transport and biodegradation is suggested by the twists of these "isosurfaces." The light gray is the highest concentration, and dark gray is the lowest. There is an intermediate gray for intermediate concentrations, and the closeness of these isosurfaces indicates the sharpness of the front. The soil conductivities in

this model vary by two orders of magnitude. These were originally synthesized randomly and subsequent processing with filters introduced a characteristic length scale of approximately three grid lengths. Thus, the results shown represent a complex flow field where regions of high conductivity weave through regions having very low hydrodynamic conductivity. Biological activity in the model and in some laboratory experiments is highest at interfaces between high and low permeability zones (17). One explanation for this behavior that is suggested by our model is that the low permeability areas act as a reservoir of contaminant, and the higher permeability zones advect nutrients and carry away inhibiting metabolic products. The densest microbial populations develop, therefore, at the interface where both co-metabolites are readily available. The target reactions for degrading CCl_4 are most likely more complex than the reaction in our 3-dimensional example, and more realistic expressions for the microbial process are currently being developed.

CONCLUSIONS

This paper outlines the latest results in an effort to design and implement an in-situ bioremediation test site to degrade CCl_4 and nitrate at DOE's Hanford Site in southeastern Washington State. Recent results indicate that indigenous soil microorganisms are capable of denitrification, and based on previous work this indicates a good potential for CCl_4 degradation. At present, work is proceeding to measure hydrodynamic and pertinent chemical properties of the proposed bioremediation test site, and to

rigorously study the kinetics of contaminant degradation and the growth of the indigenous microorganisms (14). This information is being incorporated into 1- and 3-dimensional simulations of in situ bioremediation to help design proper remediation conditions.

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FIGURE LEGENDS

Figure 1. Location of the 200 West Area on the Hanford Site in Washington State.

Figure 2. Schematic of the approach to the design of the in situ remediation technology.

Figure 3. Schematic of the flow cell.

Figure 4. Nitrate and acetate concentration through the flow cell.

Figure 5. Predicted CCl_4 concentration as a function of time and distance using the conditions reported by Semprini et al. (11).

Figure 6. Predicted biomass concentrations as a function of time and distance using the conditions reported by Semprini et al. (11).

Figure 7. Predicted biomass concentration with 10 minute feeding pulses as a function of time and position.

Figure 8. Predicted CCl_4 concentration at 2 m as a function of time. Results for both 10 minute and 1 hour feeding pulses are shown.

Figure 9. Contaminant concentration profile predicted by the 3-dimensional simulation after 0.3 pore volumes of fluid have been introduced.

Figure 10. Biomass concentration profile predicted by the 3-dimensional simulation after 0.3 pore volumes of fluid have been introduced.

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Contaminant Concentration

Microbe Population

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