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Use of a Mathematical Model for Prediction of Optimum Feeding Strategies for *In Situ* Bioremediation†

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ABSTRACT

Liquid wastes containing radioactive, hazardous, and regulated chemicals have been generated throughout the 40+ years of operations at the US Department of Energy (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 ground water. Current DOE policy prohibits the disposal of the contaminated liquids directly to the environment, and remediation of the existing contaminated ground waters may be required. *In situ* bioremediation is one technology currently being developed at the Hanford to meet the need for cost-effective technologies to clean ground water contaminated with CCl_4, nitrate, and other organic and inorganic contaminants. This article focuses on the latest results of an ongoing effort to develop effective *in situ* remediation strategies through the use of predictive simulations. In particular, strategies for nutrient injection are developed that minimize biomass accumulation within the flow field and thus extend the life of injection wells.

Index Entries: *In situ* bioremediation; mathematical model; optimization; carbon tetrachloride.

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INTRODUCTION

The Hanford site, located in southeastern Washington State, is an area of approx 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, plutonium recovery processes discharged carbon tetrachloride (CCl_4) bearing solutions to three liquid waste-disposal facilities: a trench, tile field, and a crib. A minimum of 637 t of CCl_4 was disposed to the subsurface, primarily between 1955 and 1973, along with cocontaminants, 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 ground water contamination of CCl_4 is extensive, covering at least 5 km^2. Concentrations up to 1000 times the Environmental Protection Agency's (EPA) drinking water standard of 5 ppb have been measured in the ground water. 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. At present, *in situ* bioremediation is one technology currently being developed at Hanford to meet the need for cost-effective methods to destroy CCl_4 and nitrate in ground water.

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 (*2*) and denitrifying (*3*) conditions. Carbon tetrachloride biodegradation has also been demonstrated by pure cultures and consortium of denitrifying *Pseudomonas* sp. (*4,5*), the acetogen *Acetobacterium woodii* (*6*), *clostridium* sp. (*7*), and under anaerobic and microaerophilic conditions by *E. coli* (*8*). Sulfate-reducing microorganisms have also demonstrated the ability to biodegrade CCl_4 (*7,9*). In addition, Semprini et al. (*10*) 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 condition is of particular interest at Hanford because of the occurrence of both CCl_4 and nitrates in the unconfined aquifer. Both Hansen (*5*) and Criddle et al. (*8*) identified *Pseudomonas* species capable of degrading CCl_4 with acetate as the electron donor and nitrate as the terminal electron acceptor.

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 pilotscales (*11,12*). For example, a pilot-scale agitated slurry reactor processing a simulated ground-water feed that contained 400 ppm nitrate, 200 ppb CCl_4, and acetate as the primary carbon source demonstrated >99 and 93% destruction of nitrate and CCl_4, respectively. Analysis of all product streams indicated that the concentrations of nitrate and CCl_4 were reduced to levels below the drinking water standards. These promising results with indigenous Hanford microorganisms have 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*.

Although *in situ* bioremediation has many attractive features, implementing the technology will require careful design to circumvent technical constraints. One such constraint is biofouling of nutrient addition wells. Past studies of *in situ* bioremediation have demonstrated that extensive biomass growth near nutrient addition wells can reduce well life (*10*). Since installing wells can comprise a large portion of the capital cost associated with *in situ* bioremediation, it is advantageous to use wells as long as possible. Well clogging occurs because the solutions that are introduced to the subsurface must contain high levels of nutrients, so that after being dispersed throughout the contaminated region, concentrations are still high enough to support microbial metabolism. However, since inhibitory levels of nutrients are typically not used, biological growth is highest where nutrient concentration is highest, that is, next to the injection well. The resulting biomass growth fills pore space and clogs the well. This phenomenon has been observed both in the laboratory (*13*) and in the field (*10*). Well clogging could pose a significant problem for *in situ* destruction of CCl_4, since the process is cometabolic, and requires the addition of large amounts of both electron donor and acceptor to destroy low levels of contaminants. For example, in the field demonstration of CCl_4 destruction reported by Semprini et al. (*10*), the injection well had to be abandoned after only 66 d of operation. In addition to clogging the injection well, the large biomass population near the introduction point may reduce the ability to stimulate destruction throughout a contaminated region. This is because the biomass will consume a significant portion of the added substrate, thus limiting the distribution of nutrients. Hence, contaminant destruction would be limited to a region near the well. It is evident that to lower the cost of *in situ* bioremediation and improve operation, a method to generate more uniform biomass growth throughout the contaminated zone is required. In this article, a mathematical description of 1-D fluid transport and microbial degradation of CCl_4 and nitrate has been used to determine conditions that minimize biomass growth near

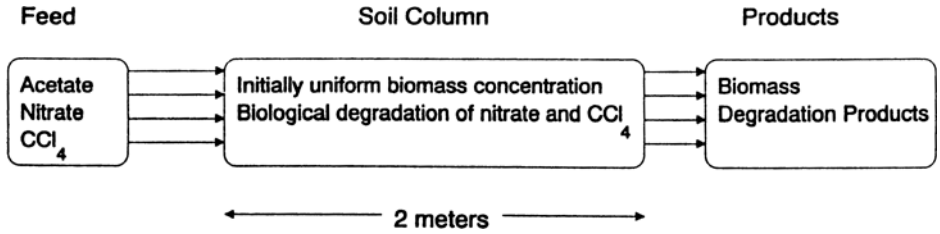

Fig. 1. Schematic diagram of the system being simulated.

the injection point by ascertaining the effects of various nutrient addition strategies. This was accomplished by using a numerical optimizer to determine the feed condition that will minimize the maximum biomass concentration attained in the flow field. In the rest of this article, the mathematical description of the system is first presented, and then the results of a base case and of the best case, as determined by the optimizer, are presented with a discussion of the significance of the results.

MATHEMATICAL DESCRIPTION OF THE SYSTEM

To determine the effects of nutrient addition strategies on biomass growth, a 1-D model of a flow field has been used. The system that is simulated here is shown schematically in Fig. 1. The flow field is assumed to be one dimensional and to be 2 m in length. Nutrients and contaminants are assumed to be introduced to the entrance of the flow field. Within the flow field, biological degradation of the contaminants, consumption of the nutrients, and associated microbial growth are described mathematically. The degradation products are assumed to flow out of the system at the exit of the flow field.

The mathematical description of this flow field is based on that developed by Semprini et al. (*10*). Since the details of this model are provided in (*10*), only the basic elements of the model are described here. In general, this 1-D model takes into account the processes of microbial growth, electron donor and electron acceptor use, and the biotransformation of the chlorinated aliphatics. Transport processes of advection, dispersion, and sorption in the porous medium are included in the model.

In this model, two microbial populations are assumed to be generated. The first population, the concentration of which is designated X_1, is a denitrifying population that uses acetate as the primary substrate (electron donor) and the nitrate as the electron acceptor. The second microbial population, the concentration of which is designated X_2, is assumed to grow on the decay products of the denitrifiers and to be inhibited by the presence of nitrate. Both the denitrifying population and the secondary

microbial population are capable of transforming CCl_4, but at different rates, with the rate of CCl_4 transformation by the second population assumed to be much faster than the transformation rate by the first population. In both reactions, the transformation of CCl_4 is assumed to be governed by Monod kinetics.

Since the CCl_4 is biologically degraded, intermediate compounds, such as $CHCl_3$ and CH_2Cl_2, could be formed. The model accounts for these products as "intermediate" compounds, but does not identify the specific compound(s) that is formed when the CCl_4 is degraded. These intermediate compounds are themselves assumed to be degraded according to Monod kinetics.

Finally, the biomass is assumed to form a thin biofilm on the soil surface that is attached to the soil and is thus immobile. Since this biofilm is assumed to be shallow, it is fully penetrated by the substrates. Carbon tetrachloride and its degradation intermediates are assumed to sorb to the soil matrix, and nonequilibrium sorption kinetics are used to describe this process.

With these assumptions, the governing equations for the transport of the different species is given as:

$$\theta\,(\partial C_i / \partial t) + (1 - \theta)\,\rho_b\,(\partial \overline{C}_i / \partial t) = D_h\theta\,(\partial^2 C_i / \partial^2 x) - \nu\,(\partial C_i / \partial x) - r_i \quad (1)$$

where the C_i represents the concentrations of the electron donor, the electron acceptor, CCl_4, and of the intermediates, C_i represents the concentration of the sorbed species, D_h is the hydrodynamic dispersion coefficient, ν is the superficial velocity of the water through the porous media, θ is the porosity of the aquifer, r_i is the rate of consumption of species i by the biological reactions, and ρ_b is the density of the soil. The values for the kinetic coefficients and the hydrodynamic parameters used in the model were the same as those of Semprini et al. (*10*).

As stated above, nonequilibrium sorption kinetics were used to describe partitioning of the various species to the soil matrix. These phenomena are described using:

$$(d\overline{C}_i / dt) = \alpha(K_d C_i - \overline{C}_i) \quad (2)$$

where K_d is the partition coefficient for the sorption onto the aquifer solids, and α is the mass-transfer coefficient between the aqueous and solid phases. For simplicity, only the CCl_4 and the degradation intermediates were assumed to partition onto the soil matrix.

The transport, reaction and sorption equations are combined to describe the biological processes that occur in the 1-D flow field shown in Fig. 1. This description results in five partial and four simultaneous ordinary differential equations. The partial differential equations describe the concentrations of the acetate, nitrate, second electron donor, CCl_4, and degradation intermediates, which are functions of both time and position within the soil matrix. The ordinary differential equations describe the

Table 1
Initial Concentrations
Used in the Simulation[a]

Species	Conc., mg/L
Nitrate	26
Acetate	0
Biomass 1	1.9
Biomass 2	0.2
Sorbed species	0
Electron donor 2	0
Intermediates	0

[a]Values are assumed to be initially equal throughout the flow fluid.

concentrations of the material that is not transported through the soil matrix, but are functions of time only. These materials are the two biomass populations, the CCl_4 and its degradation intermediates that have partitioned to the solid matrix.

Initial conditions for each species are given in Table 1. In these simulations, the concentration of each component was assumed to be equal to the initial value throughout the flow field. This can be stated mathematically as:

$$C_i(x, t = 0) = C_{o_i} \tag{3}$$

where C_{o_i} is the initial concentration of the ith component.

The boundary conditions at the entrance to the flow field can be stated mathematically as:

$$-D_h(\partial C_i / \partial x) + \nu C_i = \nu g(x = 0, t) \tag{4}$$

In our simulations, the function $g(t)$ was assumed to be either a pulse function or a fixed value. Because the nitrate and CCl_4 were assumed to be fed continuously to the flow field, fixed values were used for these compounds. The nitrate and CCl_4 concentrations in the feed were assumed constant at 26.0 and 0.03696 mg/L, respectively. The acetate, however, was fed in a pulse fashion. The duration, period, and amplitude of these pulses were varied during the optimization to minimize the amount of biomass generated within the flow field.

At the exit of the flow field, the diffusive flux of all components is assumed to be negligible. This condition is stated mathematically as:

$$(\partial C_i / \partial x)\,(x = L, t) = 0 \tag{5}$$

As shown in Fig. 1, in all simulations, it was assumed that the 1-D flow field was 2 m long, since this was the distance between the injection

and sampling wells in the field test used by Semprini et al. For calculational purposes, this 2-m flow field was divided on 40 finite difference grid points. To solve the set of combined partial and ordinary differential equations, the DSS2 system was employed (*14*). In this system, the Runge-Kutta Fehlberg formula was used to integrate, with respect to time, using a time step of 0.001 d. A total simulation time of 50 d was used.

SIMULATION RESULTS AND DISCUSSION

As stated above, the objective of this research was to apply the coupled transport and microbial kinetic model reported by Semprini et al. to determine whether a strategy could be devised for the addition of nutrients to a soil column in such a way that the biofouling near the injection point would be minimized. For *in situ* bioremediation, a reduction in biofouling near the injection point could significantly lower the cost of the treatment, since increased life of the nutrient addition wells would be achieved. By extending the life of these wells, the total number of wells that must be drilled may be reduced, which will, in turn, significantly reduce the cost of the project. To evaluate the potential of such a strategy, a base case was first established.

Base Case Results

In the base case, acetate was fed for 2 h of every 12 h at a concentration of 800 mg/L. The predicted concentrations of CCl_4 (a), intermediates (b), nitrate (c), and acetate (d) as a function of time at the exit of the flow field are plotted in Fig. 2. It is clear that almost complete degradation of the carbon tetrachloride is achieved within this 2-m flow field after 50 d of operation (*see* Fig. 2[a]).

As pointed out above, it was assumed that the CCl_4 was degraded to other, unspecified, intermediate compounds. These intermediates are initially formed and transported beyond the 2-m simulation region (*see* Fig. 2[b]). However, as noted in Fig. 2[b], these intermediates also start to be degraded by the microbial population after a period of 30 d.

In addition to the acetate, nitrate is required for biological growth. Thus, as the microbes grow, the nitrate in the feed stream will be consumed following denitrification pathways. This nitrate degradation is shown in Fig. 2(c), where the calculated nitrate concentration at point 2 m from the inlet is shown as a function of time. In this base case, the nitrate was fed continuously and was consumed by the cells, so that no nitrate is present at the exit of the flow field after only 3 d.

In contrast to the nitrate feed, the acetate concentration in the feed to the 1-D column was applied in a pulsed fashion, that is, every 12 h, the

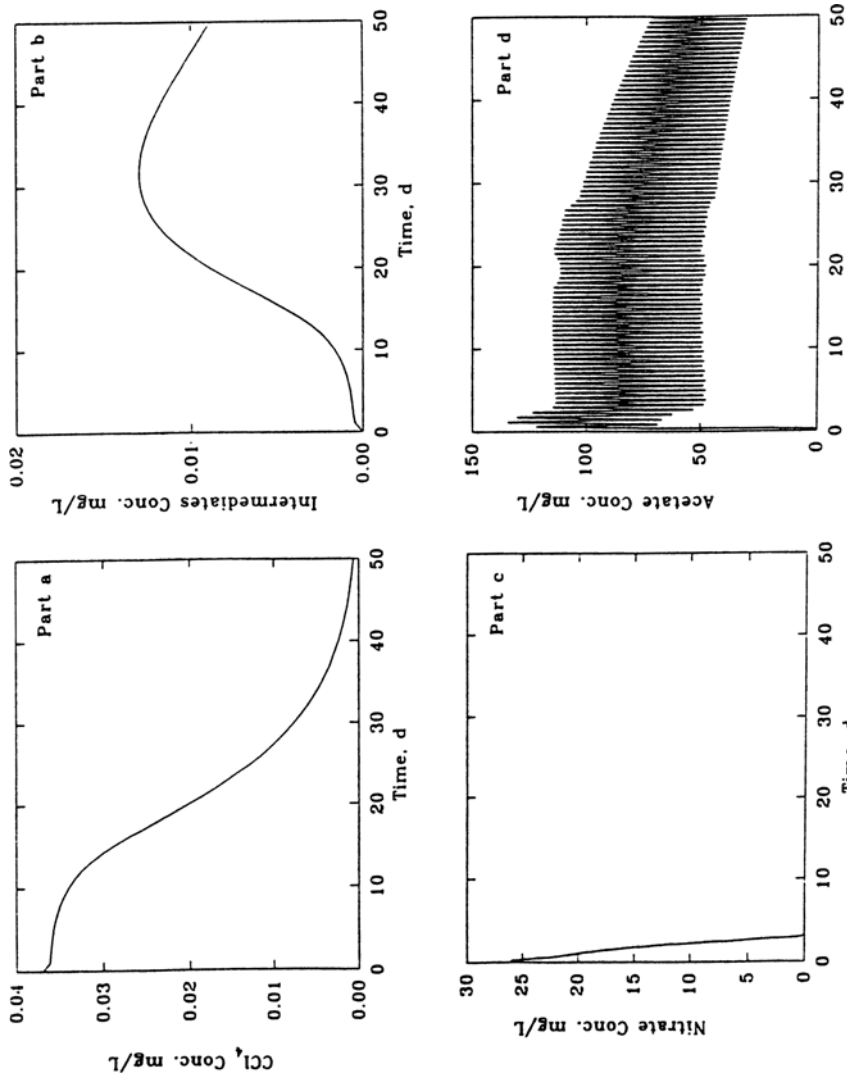

Fig. 2. Predicted concentrations of CCl_4 (a), degradation intermediates (b), nitrate (c), and acetate (d) as a function of time at the exit of a flow field for the base case.

acetate feed (800 mg/L) was turned on for 2 h after which no additional acetate was fed for 10 h. It is evident from Fig. 2(d) that these pulses make their way completely through the column, since pulses of acetate are observed at the exit of the soil column and at no point within the column does the acetate concentration drop to zero. Thus, this large amount of acetate cannot be completely consumed by the cells contained in this 2-m soil column.

As the electron donor and acceptor are fed to the simulated soil column, biomass accumulates within the soil matrix. In Fig. 3(a), the calculated biomass concentration is plotted as a function of time and position within this soil column. Clearly, a significant amount of biomass grows near the inlet of the soil column. Such biomass growth could cause plugging of the injection well and render it inoperative after a relatively short time period. Such behavior was observed by Semprini et al. (*10*) in their field tests in which, after 70 d of bioremediation, the injection well could no longer be used because (presumably) of biofouling.

This effect is evident from estimating the effect of the biomass on the porosity of the soil. The porosity of the matrix was calculated by assuming that the matrix particles were spherical and uniform. The initial porosity of the aquifer was assumed to be 0.23. Based on the concentration of the biomass, the film thickness of the biomass on the solid aquifer particles was calculated. The theoretical porosity of the matrix was then calculated using the equation given by (*15*):

$$\alpha_t = 1 - [\pi(d + 2L_f)^3 / 6\,d^3] \tag{6}$$

where d is the particle diameter, L_f is the biomass film thickness on the aquifer particles, and α_t is the theoretical calculated porosity of the matrix (note that when $L_f=0$, then $\alpha_t=0.476$). To relate this theoretical porosity to the actual porosity of the matrix, the following relation proposed by Cunningham et al. (*15*) is used:

$$(\alpha_a / \alpha_i) = (\alpha_t / 0.476) \tag{7}$$

Where α_a is the actual porosity of the medium and α_i is the initial porosity of the matrix (0.23). In Fig. 3(b), the calculated porosity of the 1-D soil column is shown as a function of time and distance. The large amount of biomass near the inlet of the well significantly reduces the porosity of the matrix and, with time, leads to complete clogging of the well.

Best Case Results

One might assume that the growth of biomass near the well would be a strong function of the acetate concentration and of the manner in which the acetate is fed to the soil column. To find operating characteristics that would extend the life of an injection well, calculations were performed to determine system operating characteristics that would minimize the

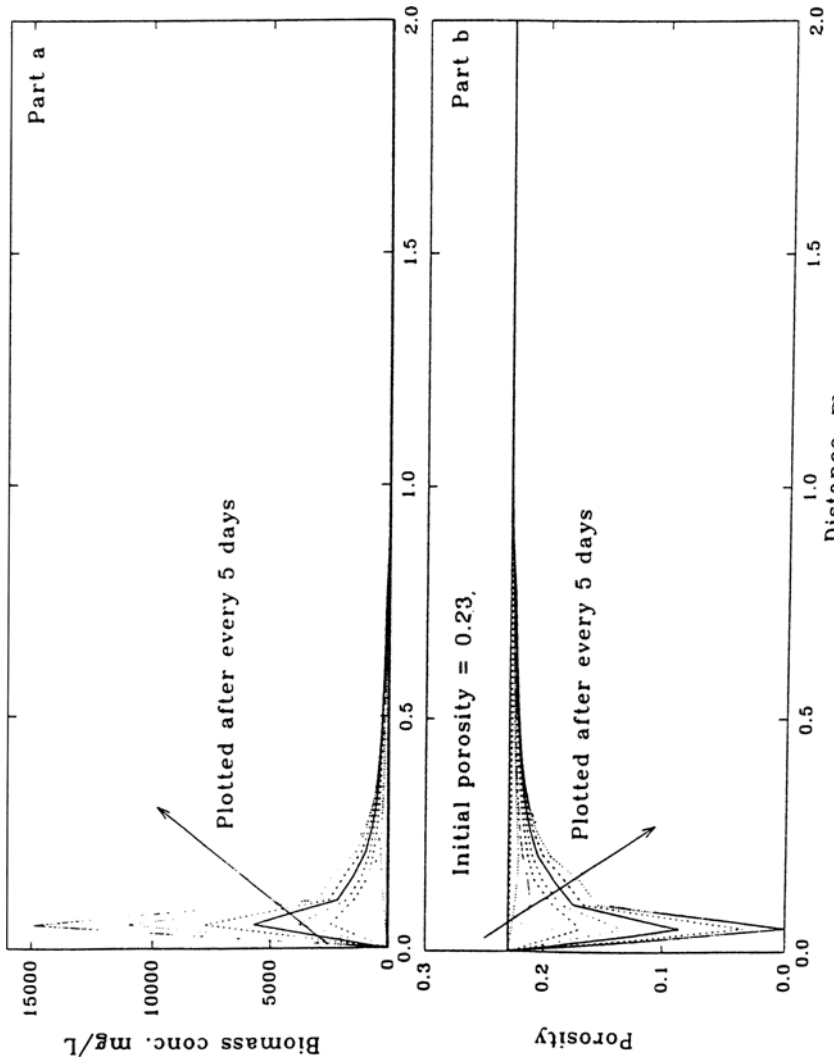

Fig. 3. Predicted concentration of the biomass (a) and porosity (b) as a function of time and position within the flow field for the base case. Profiles are plotted at 5-d intervals.

maximum biomass concentration encountered in the soil column, while ensuring that the CCl_4 concentration at a point 2 m from the column inlet did not exceed 5 ppb, the EPA drinking water standards. To do this, the concentration of acetate fed to the soil column, the amount of time that the acetate was being fed to the column, and the amount of time between the acetage feed pulses were varied.

A Generalized Reduced Gradient optimization algorithm (*16,17*) was used to determine the best strategy for injecting the acetate into the soil column. The optimum values were determined to correspond to a feed concentration of 800 mg/L, a pulse width of 33 min, and a pulse interval (time between pulses) of 12.9 h, respectively. When compared to the base case presented above, the maximum biomass concentration was reduced by about an order of magnitude: from approx 15,000 mg/L to approx 1500 mg/L.

Based on these optimal conditions, the calculated concentrations of the CCl_4 (a), intermediates (b), nitrate (c), and acetate (d) at the exit of the 2-m flow field are plotted as functions of time in Fig. 4. Obviously, the CCl_4 concentration is well below the maximum for drinking water standards, which was one of the primary criteria for this optimal solution. In addition, both the nitrate (part c) and acetate (part d) concentrations at the exit of the soil column are oscillating, and both periodically assume a value of zero. Although the concentration of the intermediates in the exit stream after 50 d is 30% higher than it is in the base case (Fig. 4[b]), it is still in the 10-ppb range.

These oscillations can be seen even more clearly in Figs. 5 and 6 in which the acetate (Fig. 5) and nitrate (Fig. 6) concentrations are plotted as functions of both time and distance during the last 5 d of the simulated bioremediation process. Here, it is obvious that the pulses of acetate that are fed to the column are of the proper magnitude and duration that biological activity exists throughout the entire soil column as both acetate and nitrate continue to be consumed throughout the column. In addition, in Figs. 5 and 6, one can observe that the nitrate concentration is low when the acetate pulse is passing by and increases once the pulse is past. Thus, throughout the flow field, the concentrations of both the electron donor and of the electron acceptor would be always changing and periodically assuming a value of zero.

Semprini et al. hypothesized that two microbial populations were responsible for the degradation of the CCl_4. They assumed that the first population was a denitrifier and that the second population, which accomplished the majority of the CCl_4 degradation, used dead cells from the denitrifying biomass as a carbon source. Thus, CCl_4 degradation would be maximized if the denitrifying biomass were constantly being produced and then immediately dying. The pulses that are observed in Figs. 4–6 would be beneficial for such growth, since the denitrifying

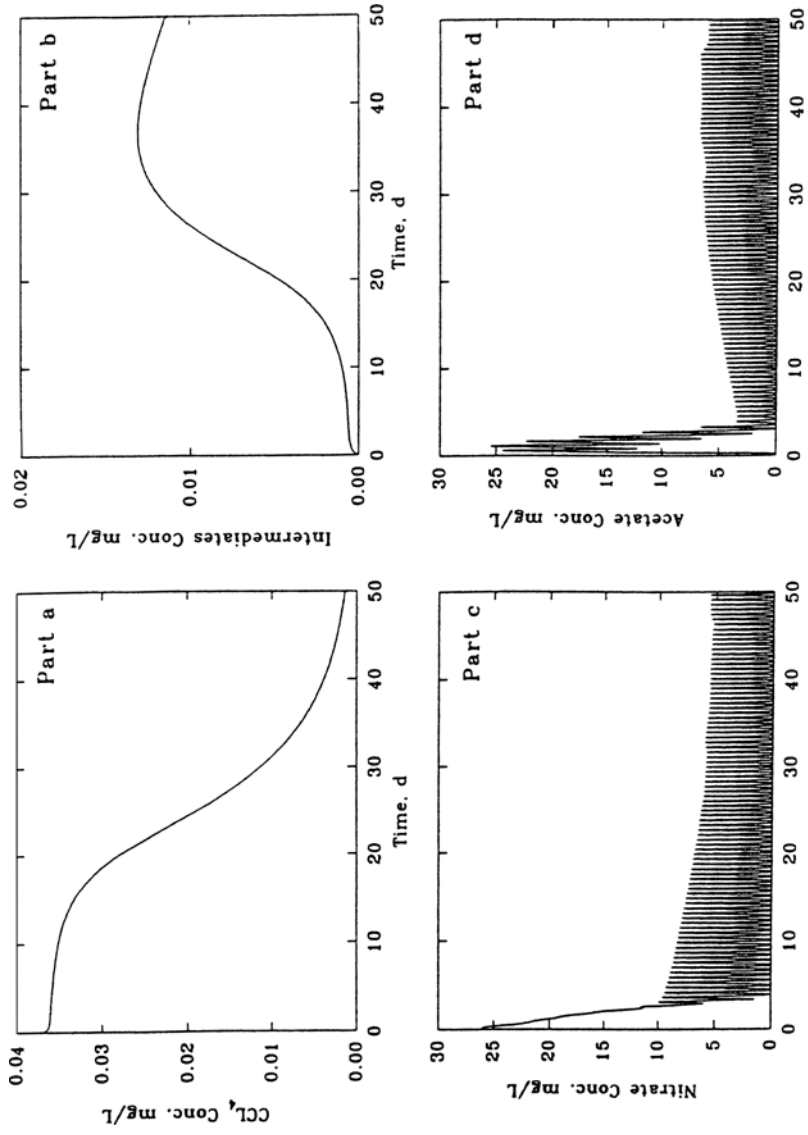

Fig. 4. Predicted concentrations of CCl_4 (a), degradation intermediates (b), nitrate (c), and acetate (d) as a function of time at the exit of the flow field for the best case.

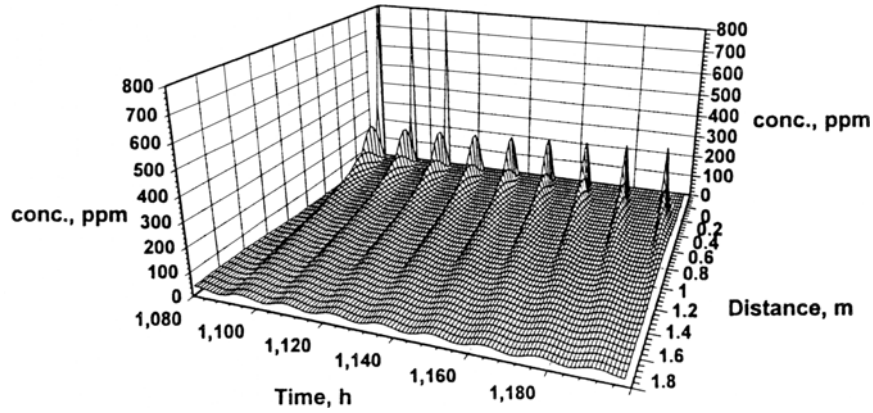

Fig. 5. Predicted acetate concentration as a function of time and position for the best case during days 45–50. Calculated values are plotted every hour.

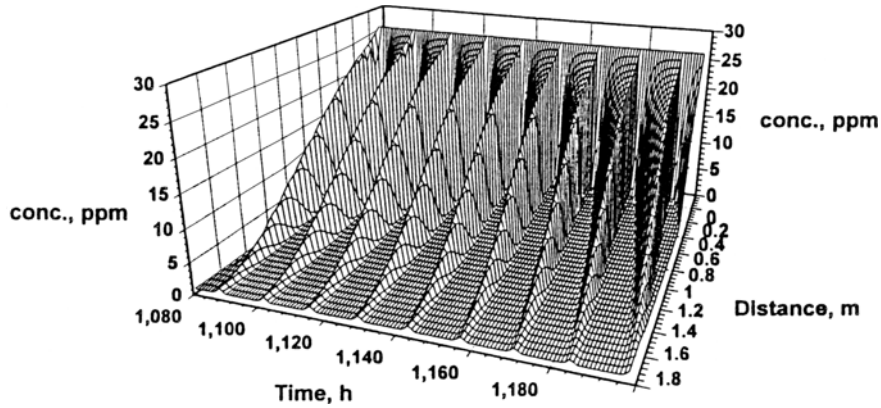

Fig. 6. Predicted nitrate concentration as a function of time and position for the best case during days 45–50. Calculated values are plotted every hour.

biomass would start to grow as a pulse of nutrients came past a point in the column, but then after the pulse had passed, this biomass would tend to die and allow CCl_4 degradation to occur with the second biomass population. In addition, this strategy would tend to minimize the buildup of any microbial population within the column.

The acetate feed pulses are sufficiently close together that the CCl_4 concentration does not show any effect owing to these pulses, however. Rather, the profile shown in Fig. 7 is representative of the pseudo-steady state that is developed within the column at this time. In this figure, the CCl_4 concentration is plotted as a function of distance through the soil column at the end of the 50 d of simulation. Degradation is observed throughout the entire soil column and not just in a limited zone near the entrance of the column.

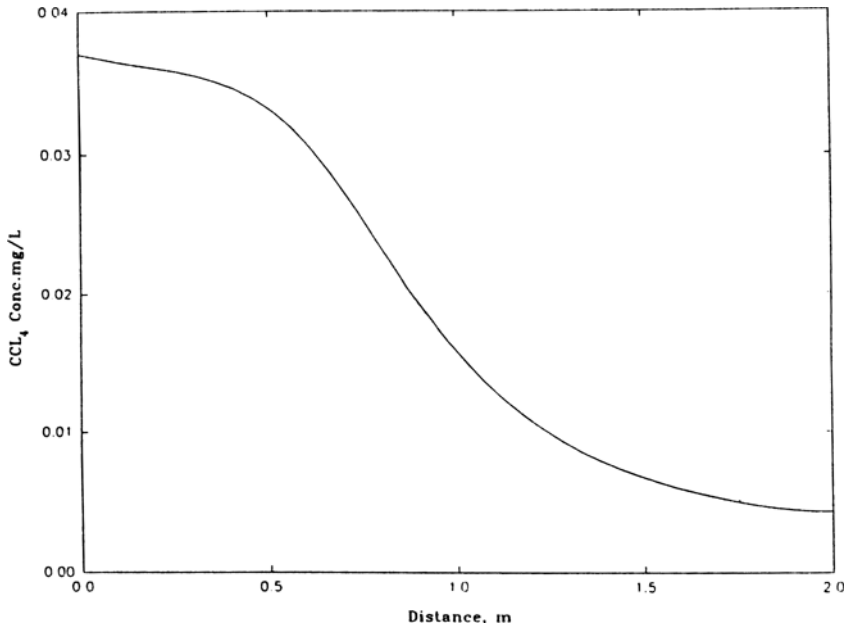

Fig. 7. CCl_4 concentration as a function of distance through the soil column at the end of the 50-d simulation.

In Fig. 8, the calculated biomass concentration and the porosity of the soil column are plotted as functions of distance at various time intervals. Here, the maximum biomass concentration is an order of magnitude less than it was in the base case presented in Fig. 3. In addition, the biomass is more uniformly dispersed though the soil column than it was in the base case, where a large fraction of the total biomass was within 0.2 m of the column inlet. Correspondingly, the porosity is not impacted to the degree that it was in the base case. In the base case simulation, the porosity dropped to nearly zero after the 50-d simulation time, but in this "best" case, the porosity only decreased 16% from the original value. Thus, in theory, the life of the well could be extended before plugging would occur.

Note that the assumption of two biomass populations developing within the soil column may not be the best explanation for the observed phenomena. Skeen et al. (*18*) present data that show that denitifying bacteria may degrade CCl_4 only when in transition from an environment in which the electron acceptor is limited to an environment in which excess electron acceptor is present. This explanation, as offered by Skeen et al., also explains the phenomena observed by Semprini et al. in their field case. In any case, the kinetic expressions offered by Semprini et al. describe the observed phenomena and can be used to obtain a better understanding of the *in situ* biological remediation of CCl_4-contaminated sites by denitrifying bacteria.

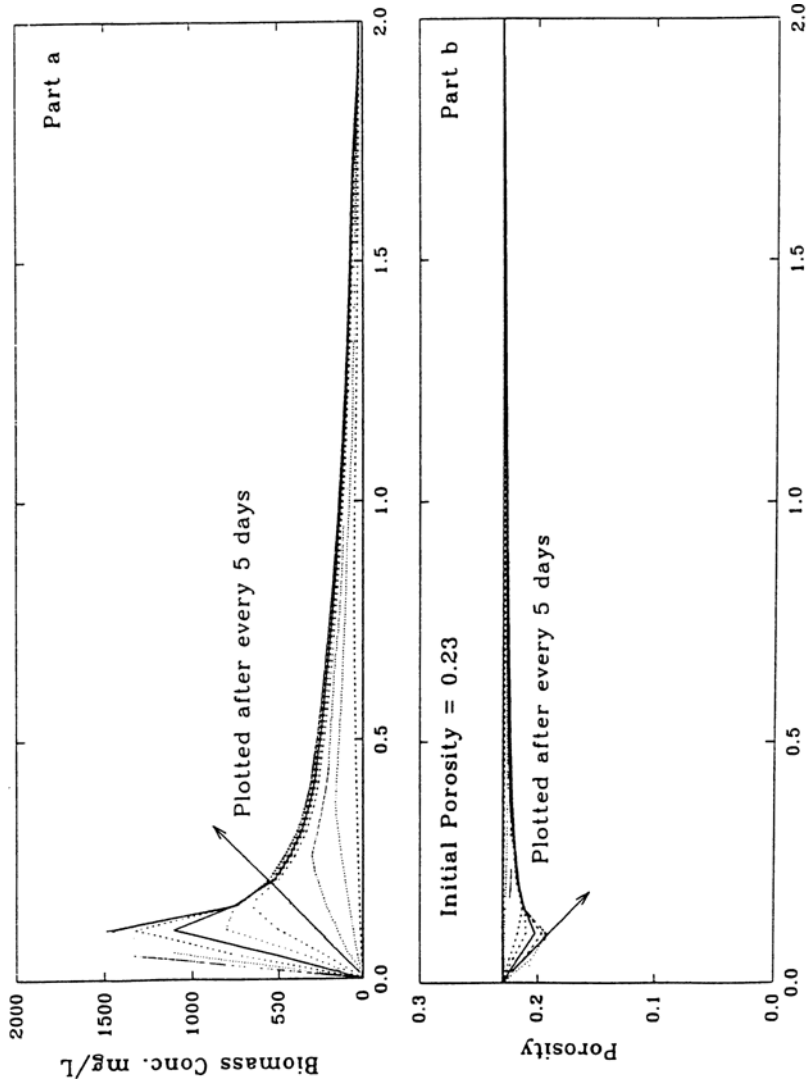

Fig. 8. Predicted concentration of the biomass (a) and porosity (b) as a function of time and position within the flow field for the best case. Profiles are plotted at 5-d intervals.

CONCLUSIONS

A simulation of a 1-D soil column was completed using previously published expressions for the biological reaction kinetics. This simulation was used to determine a strategy that would allow the life of an injection well to be extended. This was accomplished by adjusting the concentration of the acetate feed, the duration of the feed pulse, and the amount of time between feed pulses. It was found that the maximum biomass concentration that was achieved in a 2-m soil column during 50 d of operation could be reduced by more than an order of magnitude as compared to a base case. Thus, for long-term bioremediation, it is imperative that optimal strategies be devised for the injection of nutrients to the subsurface since such strategies have a profound impact on the life and performance of bioremediation projects.

ACKNOWLEDGMENTS

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