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THE HANFORD SITE

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A DEMONSTRATION OF *IN SITU* BIOREMEDIATION OF CCL<sub>4</sub> AT THE HANFORD SITE

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ABSTRACT

The United States Department of Energy's VOC-Arid Integrated Demonstration Program (VOC/Arid-ID) is developing an *in situ* bioremediation technology to meet the need for a cost-effective method to clean ground water contaminated with chlorinated solvents, nitrates, or other organic and inorganic contaminants. Currently, a field demonstration of the technology is being conducted at the Hanford site in southeastern Washington state. The goal of this demonstration is to stimulate native denitrifying microorganisms to destroy carbon tetrachloride and nitrate. Contaminants are destroyed by mixing an electron donor (acetate) and an electron acceptor (nitrate) into the aquifer, using a matrix of recirculation wells. This work also evaluates the effectiveness of applying scale-up techniques developed in the petrochemical industry to bioremediation. The scale-up process is based on combining fluid mixing and transport predictions with numerical descriptions for biological transport and reaction kinetics. This paper focuses on the necessity of this design approach to select nutrient feeding strategies that limit biofouling while actively destroying contaminants.

## INTRODUCTION

*In situ* bioremediation is one of the technologies currently being developed at Hanford to meet the need for cost-effective methods to remediate ground water contaminated with chlorinated solvents, nitrates, or other organic and inorganic contaminants. This technology has several advantages over current baseline pump-and-treat methods. These methods are limited by the difficulties and costs associated with recovering and handling contaminated materials. For this reason, technology development efforts by the U.S. Department of Energy (DOE) have focused on *in situ* treatment technologies, especially *in situ* bioremediation. Effective *in situ* treatment circumvents the high costs associated with recovering large volumes of contaminated material. In addition, engineering calculations have demonstrated that the time required for remediation can be reduced using *in situ* technologies (Skeen et al., 1993).

One of the primary technical barriers to widespread application of *in situ* bioremediation is the inability to accurately account for geochemical, hydrological, and microbial phenomena during the design process. For the design engineer, the ideal process for biological treatment is an aboveground, continuous-flow reactor that offers relatively simple design and scale-up relationships. Unfortunately, the subsurface environment provides very few of these attributes. Recent bioremediation efforts have focused on this problem by developing and testing simulation tools for scale-up of *in situ* processes (Shouche et al., in press). These tools combine fluid mixing and transport

predictions with numerical descriptions for biological transport and reaction kinetics.

Previous laboratory studies have demonstrated the feasibility of stimulating native Hanford soil organisms to cometabolically destroy carbon tetrachloride (CT) under denitrifying conditions (Petersen et al., in press; Skeen et al., in press; Truex et al., 1994; Hansen, 1990). In these tests, it was shown that CT destruction could be achieved by the addition of an electron donor in the presence of low levels of nitrate. However, higher concentrations of nitrate inhibited the transformation of CT. These experimental results were used as a basis for developing a comprehensive kinetic model of the microbial processes (Hooker et al., 1994). The predictive ability of this model has been confirmed in treatability tests using contaminated Hanford ground water (Hooker et al., in press).

Applying the kinetic model to *in situ* bioremediation is contingent on accurately predicting the transport of microorganisms in the subsurface. This phenomenon has been described for the Hanford denitrifying consortium by estimating attachment and detachment rate coefficients based on experimental measurements of biofilm development in soil column studies. In these experiments, bacterial attachment and detachment rates were related to published kinetic forms (Peyton and Characklis, 1993) using measurements of aqueous and film biomass concentrations as well as nutrient consumption rates for two different feeding arrangements: (1) continuous input of acetate and nitrate and (2) pulse feeding of acetate with continuous addition of nitrate. This information, along with the microbial reaction kinetics, has been

incorporated in a transport simulator that describes temporal and spatial changes in the concentrations of the electron donor, contaminant, electron acceptor, and biomass (Wheeler et al., 1992; Chiang et al., 1989, 1990). The purpose of this paper is to demonstrate the utility of this engineering design tool for estimating operating conditions for *in situ* bioremediation. In addition, we discuss the effects of different nutrient feeding strategies on the predicted biofilm development and CT destruction in the Hanford aquifer.

## MATERIALS AND METHODS

The numerical design tool used in this work is a modification of the multidimensional Reactive Flow and Transport code (RAFT) developed by Wheeler et al. (1992; Chiang et al., 1989, 1990). This code uses a mixed finite-element method to calculate pressure, velocity, and concentration profiles in the flow field for convection-dominated transport problems. A time-splitting procedure is used to provide a stable solution of the transport and biodegradation equations.

Transport equations are solved by the modified method of characteristics, while microbial reactions are described in a system of ordinary differential equations solved using a 4th-order Runge-Kutta technique. Figure 1 is a two-dimensional plan view of the single injection well. The simulated flow field was 1.75 m on a side, with a 0.25-m node spacing in both the x and y directions. Also in the x and y directions, Dirichlet boundary conditions (equipotential) were employed. The flow field was assumed to initially contain no acetate or nitrate. The initial concentration of biomass in the

flow field was assumed to be  $1.7 \times 10^{-4}$  mg dry biomass per gram of soil, based on representative values of denitrifiers measured from Hanford aquifer field core samples.

Table 1 lists the operating conditions for the five nutrient feeding strategies tested. A flow rate of 5 gpm per meter of well screen and a background CT concentration of 2000  $\mu\text{g/L}$  were used in all cases. In addition, the initial soil porosity and hydraulic conductivity were 0.4 and 0.01 m/day, respectively. In each case, 35 days of injection was simulated. The conditions for Test 1 were chosen as a baseline because they are similar to those that yield rapid CT destruction in batch experiments (Skeen et al., in press). The duration and frequency of pulses for Tests 2 through 5 are based on previous optimization results for a similar denitrifying consortium (Shouche et al., 1993; Semprini et al., 1992). Tests 2 through 4 represent a complete experimental design to test the effects of acetate and nitrate pulsing on the process. The concentrations of the acetate and nitrate pulses were chosen since they result in the addition of the same mass of acetate and nitrate as Test 1. Test 5 uses the same conditions as Test 4; however, the acetate and nitrate are not added simultaneously. Instead, nitrate is added alone for 1 hour, followed by a 1-hr addition of only acetate. This condition was used to demonstrate the utility of separating the addition of electron donor and acceptor (Roberts et al., 1991).

## RESULTS AND DISCUSSION

The five test cases outlined in Table 1 were simulated to select feeding strategies that would sustain CT destruction through the flow field for 35 days. Several of the test cases resulted in a large accumulation of biomass at the well bore, causing complete pore plugging. Figure 2 shows the minimum soil porosity in the flow field as a function of time for the five test cases. Porosity values were calculated based on the attached biomass concentration, assuming a biofilm density of 0.0395 g-dry weight (DW)/ml biofilm (Characklis and Marshall, 1989). For Tests 1, 3 and 4, the minimum porosity always occurred at the well bore (data not shown). This situation is not conducive to *in situ* bioremediation, since biomass is not being distributed throughout the flow field, thus limiting contaminant destruction to a small region near the well. In addition, the phenomenon leads to rapid pore plugging, as demonstrated for cases 1, 3, and 4 in Figure 2. On the other hand, this figure demonstrates that test cases 2 and 5 do not result in pore plugging after 35 days. Figure 3 shows the radial distribution of porosity from the well bore to the edge of the simulated flow field, plotted at different time intervals for (a) Test 2 and (b) Test 5. Also, as displayed in Figure 3, minimum porosity levels observed for test cases 2 and 5 do not occur at the well bore.

From Figure 3A, it is evident that the minimum porosity is shifting in time closer to the well bore for test case 2. This phenomenon is caused by acetate inhibition of cell growth, which also causes the differences in minimum porosity for test cases 2 and 3, as seen in Figure 2. The acetate inhibition constant for these organisms is 413 mg/L (Hooker et al., in press). At a pulse concentration of 1000 mg/L acetate, the inhibition constant results in a



decrease in the specific growth rate of approximately 35%. As substrate concentration is reduced, the inhibition diminishes until a maximum growth rate is reached at an acetate concentration of 270 mg/L. Near the beginning of the simulation, the minimum porosity is located far from the injection well, because the initial low levels of biomass in the flow field result in a slow consumption of acetate. This causes the acetate pulse to move further from the well bore before the concentration is reduced below inhibitory levels. However, since growth near the well is not completely attenuated, biomass accumulates over time, yielding an increase in acetate consumption near the injection well. This activity results in a lower acetate concentration, less substrate inhibition, and an acceleration of biomass growth rates near the well bore.

From the data for days 10, 20, and 30 in Figure 3B, it is evident that the shift in minimum porosity toward the well bore is also occurring for skewed pulsing of acetate and nitrate (test case 5). As with test case 2, this phenomenon is caused by substrate inhibition. However, in contrast to the other four test cases, the porosity data for days 20, 30, and 35 of test case 5 suggest that a steady-state minimum will occur 0.7 m away from the well bore. The behavior results from the separate additions of acetate and nitrate, since bioactivity cannot occur until the two reactants mix. In a soil environment, the mixing is accomplished by dispersive flow, which causes the pulses to overlap at a finite distance from the well bore.

Figure 4 shows the predicted CT concentration exiting the flow field as a function of time for test cases 2 and 5. The average exit concentrations are

1942  $\mu\text{g/L}$  and 1690  $\mu\text{g/L}$  for test cases 2 and 5, respectively. It is evident from this figure that significantly more CT is destroyed as a result of skewed pulsing of acetate and nitrate. This behavior results from lower overall nitrate levels afforded by the skewed pulse feeding strategy. The presence of higher nitrate levels is directly inhibitory to CT transformation. In the simulator, this phenomenon is described as:

$$\frac{d[\text{CT}]}{dt} = \frac{\mu_{\text{CT}}[\text{CT}][\text{X}]}{1 + \frac{[\text{NO}_3^-]}{K_{i,\text{CT}}}} \quad (1)$$

where all concentrations are expressed in  $\text{mg/L}$ ,  $\mu_{\text{CT}}$  is  $1.0 \times 10^{-6}$   $\text{L/mg-DW-min}$ , and  $K_{i,\text{CT}}$  is  $6.2$   $\text{mg/L}$  (Hooker et al., in press). The value for the inhibition constant is lower than previously reported (Hooker et al., 1994) but is more consistent with recent unpublished experimental results. As is evident from this equation, a nitrate concentration of just  $6.2$   $\text{mg/L}$  reduces CT destruction by 50%, with greater concentrations yielding higher levels of inhibition. The concentrations of acetate and nitrate leaving the simulated flow field for the first 8 days of test cases 2 and 5 are shown in Figures 5A and 5B, respectively. It is evident from this figure that significantly more nitrate was present in test case 2 than in case 5. This trend continues for the full simulation. The observed response is expected since nitrate is added continuously in test case 2 and is pulsed in test case 5. Hence, for case 2, nitrate is always present in the flow field except when an acetate pulse passes through. Since acetate is pulsed for only 1 hr in every 12, inhibitory levels of nitrate are prevalent. The 12-hr pulse cycles indicated by boxes in

Figures 5A and 5B further demonstrate the greater presence of inhibitory levels of nitrate in test case 2.

It is evident from the five test cases that a wide variation in system response is achieved with the different nutrient feeding strategies. These results demonstrate that the success of *in situ* bioremediation depends on testing operating conditions *a priori* to select appropriate designs that meet the process objectives. The numerical tool used in this paper provides this capability. It should be noted that there will be differences between simulated results and the actual response at a field site because of heterogeneities in properties such as microbial distribution, hydraulic conductivity, and porosity. These differences will require that the actual operating procedures for an *in situ* bioremediation system be modified based on process measurements such as the injection well pressure and concentration profiles for periodic nonreactive tracers. However, the use of numerical tools to evaluate the system provides a basis for making process changes and understanding their results.

## CONCLUSIONS

A comprehensive, numerical design tool that incorporates microbial metabolic and growth kinetics, bacterial transport, and groundwater flow and transport has been used for testing operating conditions for *in situ* bioremediation of CT in ground water. Specifically, the effects of different nutrient feeding strategies on predicted biofilm development and CT destruction in the Hanford aquifer have been determined. Five operating conditions were tested for their

ability to sustain CT destruction without biofouling the flow field. The results indicate that pulsed additions of acetate and nitrate that are temporally separated provide the greatest CT destruction. In contrast to the other simulated conditions, this injection strategy does not result in the development of high concentrations of biomass near the injection well.

Numerical tools such as that used in this paper will facilitate implementing *in situ* bioremediation since they provide the capability to test operating conditions *a priori* to select appropriate system designs and operating strategies.

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Table 1. Variable Parameters Chosen For Feeding Strategy Simulations



Table 1

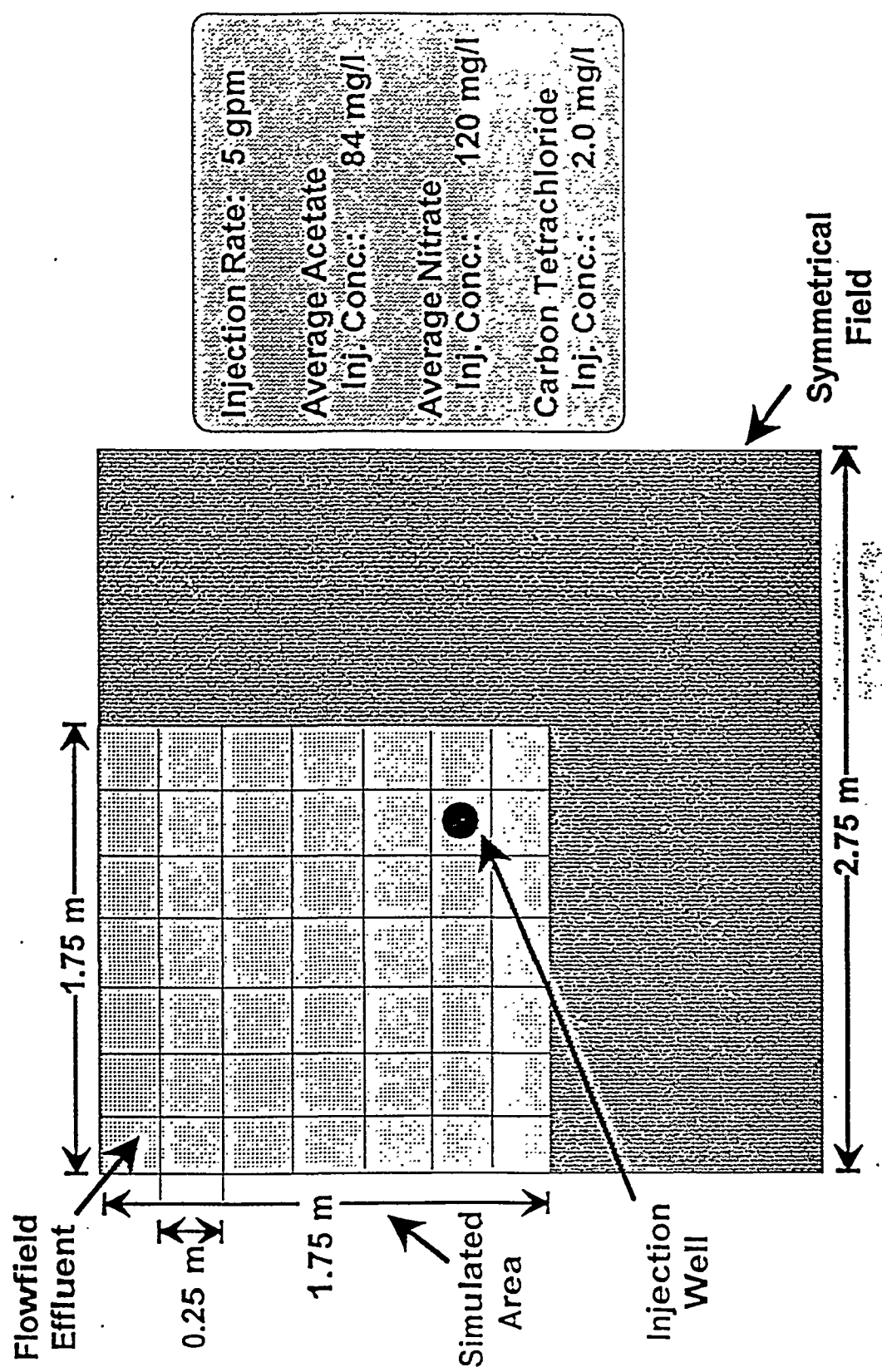
Variable Parameters Chosen For Feeding Strategy Simulations

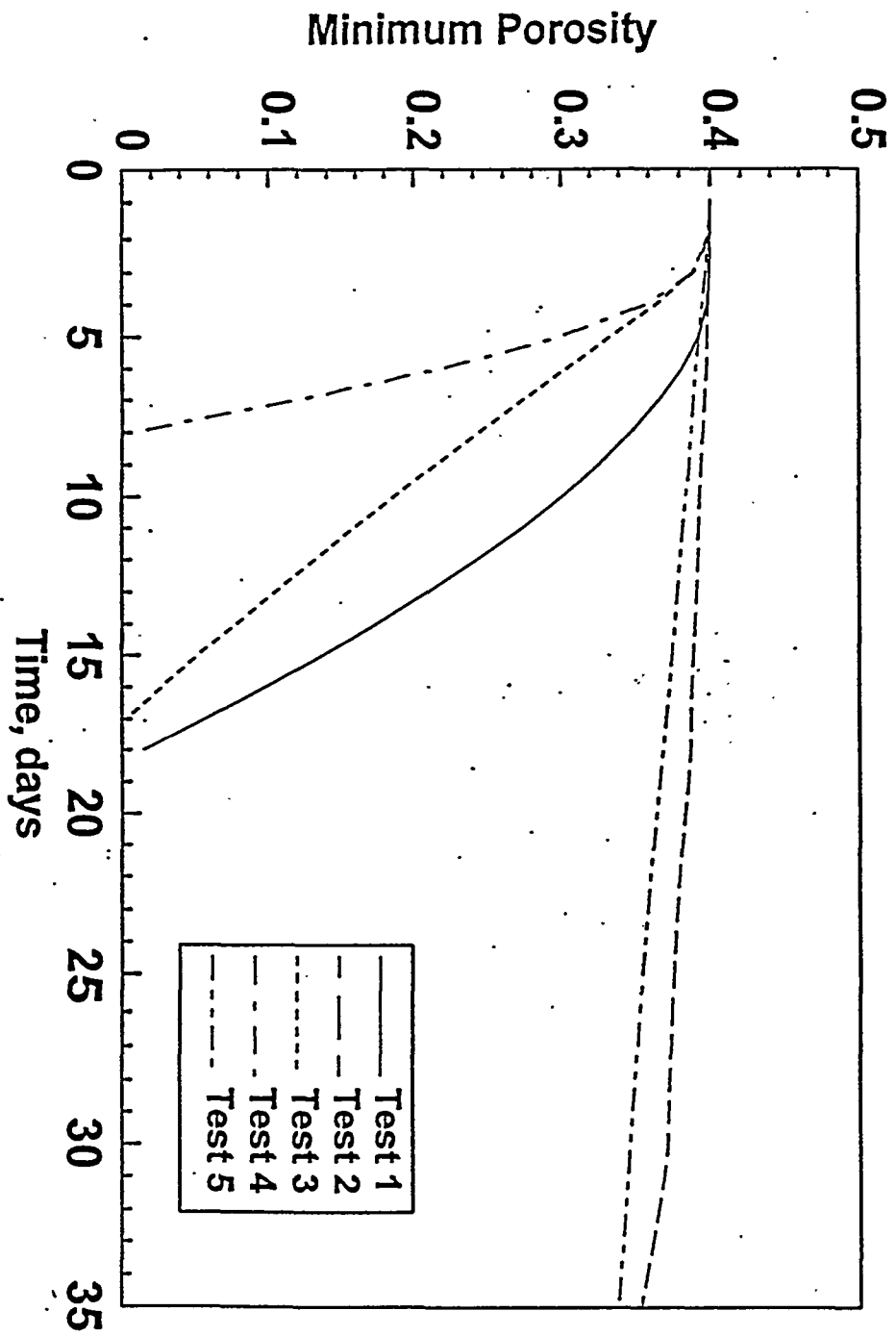
Parameter	Test 1	Test 2	Test 3	Test 4	Test 5
Injected Acetate, mg/L	84	1000	84	1000	1000
Injected Nitrate, mg/L	120	120	1400	1400	1400
Pulsed Components	none	acetate	nitrate	acetate, nitrate	acetate, nitrate
Continuously Feed Components	CT <sup>(a)</sup> , acetate, nitrate	CT, nitrate	CT, acetate	CT	CT
Pulse Duration	continuous	1 hr	1 hr	1 hr	1 hr
Pulse Frequency	continuous	12 hr	12 hr	12 hr	12 hr
Pulse Skew	none	none	none	none	1 hr

<sup>(a)</sup>carbon tetrachloride

- Figure 1. Two-dimensional plan view of simulated system consisting of a single injection well and the adjacent aquifer.
- Figure 2. Minimum porosity in the injection well field (due to biofouling) over the simulated duration of tests.
- Figure 3. Spatial distribution of porosity, measured radially from the well bore at 10, 20, 30, and 35 days, for A, test case 2 and B, test case 5.
- Figure 4. Effluent concentrations of carbon tetrachloride, mg/L (leaving the simulated flow field shown in Figure 1) over the simulated duration of test cases 2 and 5.
- Figure 5. Effluent concentrations of acetate and nitrate, mg/L (leaving the simulated flow field shown in Figure 1) over the first 8 days of simulated duration for test cases 2 and 5.

Figure 1





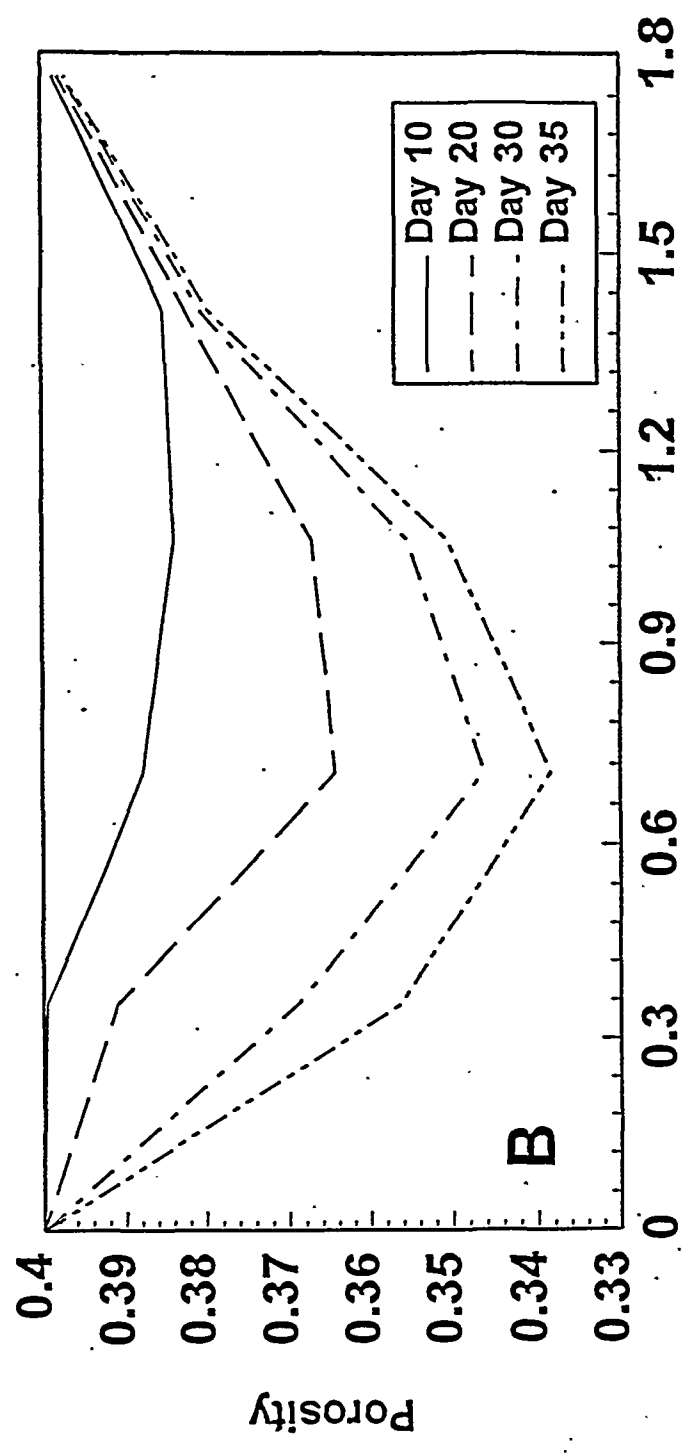
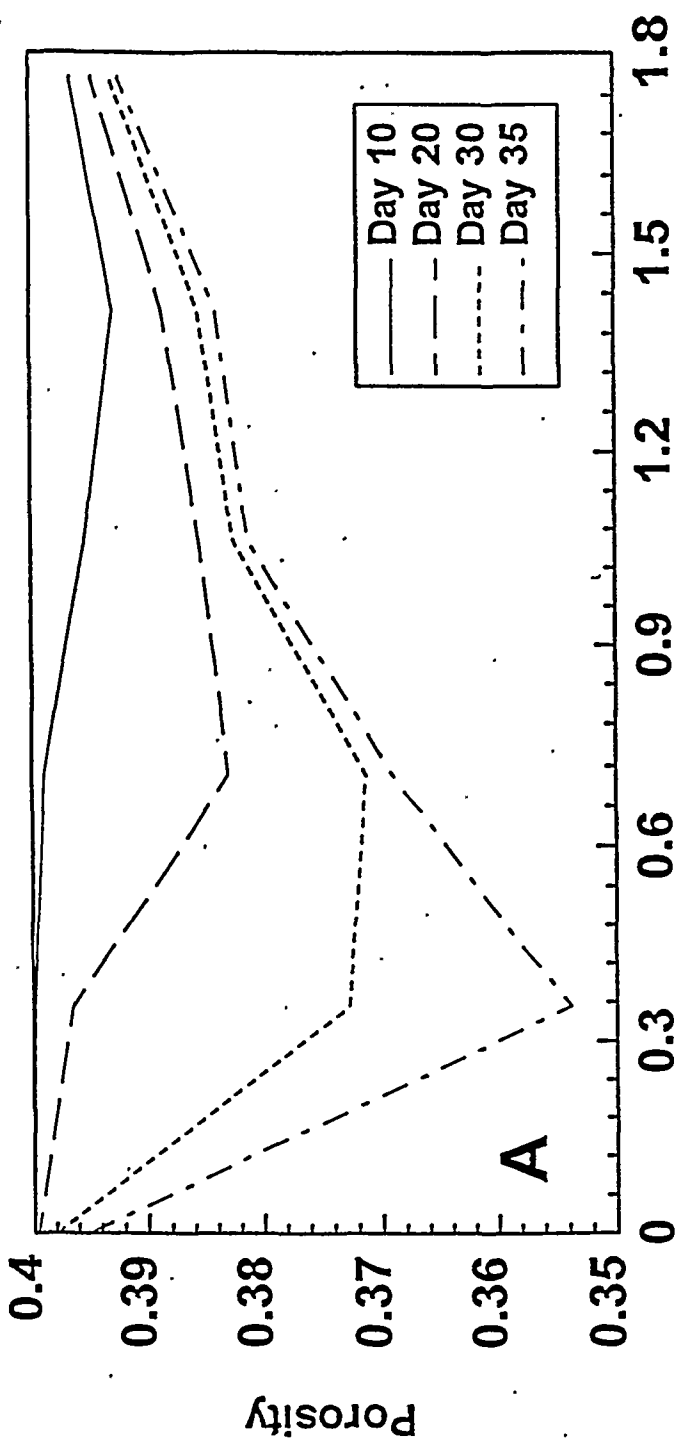


Figure 4

