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Final Report

FUNDAMENTAL QUANTITATIVE ANALYSIS OF
MICROBIAL ACTIVITY IN AQUIFER BIORECLAMATION

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MODELING THE TRANSPORT OF BIOLOGICALLY AND
CHEMICALLY REACTIVE SOLUTES IN A TWO-DIMENSIONAL,
HETEROGENEOUS INTERMEDIATE SCALE SYSTEM

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1. BACKGROUND

In situ bioremediation of hazardous organic chemicals that contaminate aquifer solids and groundwater is a highly promising technique for many sites found at DOE facilities, as well as throughout the United States. Its potential stems from having the agents for destruction of the contaminants, the bacteria, in close proximity to the separate-phase liquid or sorbed contaminants. Instead of relying solely on advective-driven dissolution and desorption -- as in the so-called pump-and-treat approaches -- *in situ* bioremediation uses biodegradation at or very near the source of contamination to replenish the water's ability to accept more dissolving or desorbing contaminant, thereby accelerating contaminant removal.

While *in situ* bioremediation has great promise, its application is far from accepted. More so than for most new technologies, major uncertainties exist. The uncertainties are multiplied by these special aspects of *in situ* bioremediation:

- most of the organic contaminants in the subsurface are not dissolved.
- the subsurface is heterogeneous in terms of physical properties (e.g., porosity and hydraulic conductivity), distribution of contaminants, and distribution of bacteria.
- substrates and nutrients must be supplied to the bacteria to stimulate their growth, but it is impossible to supply these materials uniformly in the subsurface.
- the accumulation of bacteria in a biologically active zone (Odencrantz et al., 1990b) alters the physical, chemical, and biological properties of the subsurface, creating new forms of heterogeneity.
- the subsurface is not easily sampled or monitored.

To address these special issues, research advances are needed in microbiological fundamentals and in mathematical modeling, which provides the only viable tool to connect the many physical, chemical, and biological processes occurring simultaneously during *in situ* bioremediation. This project, Fundamental Quantitative

Analysis of Microbial Activity in Aquifer Bioreclamation, was designed to advance our knowledge in several of the microbiological fundamentals most important to *in situ* bioremediation. Furthermore, it provides significant advances to porous medium modeling that involves microbiological reactions.

This grant was awarded by DOE in March 1989 and renewed for the second and third years in March 1990 and 1991. A supplemental award in 1992 extended part of the work through August 1993. The primary awardee was the Department of Civil Engineering at the University of Illinois at Urbana-Champaign; subcontracts were to the Department of Agronomy at Cornell University and the Department of Civil Engineering at Northwestern University. The principal investigators at the University of Illinois were Bruce E. Rittmann and Albert J. Valocchi. The principal investigator at Cornell University was Philippe Baveye. Dr. Rittmann was at Northwestern University for the final year.

The University of Illinois also had a closely coupled subcontract, entitled "Modeling the Transport of Biologically and Chemically Reactive Solutes in a Two-Dimensional, Heterogeneous Intermediate Scale Experimental System," from Battelle Pacific Northwest Laboratory (PNL). This project was awarded in April 1989 and has been extended through September 1993. Because the two projects were intimately connected, progress of the DOE and PNL projects is reported together.

The project had five primary areas: (1) biodegradation of poorly soluble organic contaminants, (2) dual-limitation kinetics of electron donors and acceptors, (3) kinetics of sequential degradations involving oxygenase reactions, (4) biologically induced clogging in porous media, and (5) two-dimensional modeling of biofilm reactions in nonhomogeneous porous media.

The report summarizes the key findings in each area. Emphasis is placed on identifying the scientific questions, reviewing the experimental and modeling results, and discussing the significance of the results. Detailed presentations of literature review, experimental methods, model development, and experimental results are avoided, as they

can be found in the cited manuscripts, dissertations, and reports.

2. BIODEGRADATION OF POORLY SOLUBLE CONTAMINANTS

The most important organic contaminants that are accessible for *in situ* bioremediation are those that are poorly soluble. Whether in a separate liquid phase or sorbed to aquifer solids, these poorly soluble compounds cannot easily be flushed from the subsurface by water extraction. On the other hand, bacterial activity can destroy contaminants and accelerate dissolution, if the bacteria are located close enough to the interface between the water and the adsorbed or liquid-phase contaminant.

Our research on biodegradation of poorly soluble contaminants focused on nonaqueous-phase liquids (NAPLs) and the factors controlling how close the bacteria are to the water/NAPL interface in a porous medium. The fundamental hypothesis is that biodegradation can significantly speed up NAPL removal by lowering the liquid-phase concentration of the dissolved NAPL near the water/NAPL interface. Bacteria able to locate near the interface will give the greatest effect. Since most bacteria in the subsurface are attached to solid surfaces, their location with respect to the interface can be more-or-less fixed.

Experiments and modeling were performed with toluene, a very commonly encountered hydrocarbon that is characterized by being relatively soluble and inhibitory to bacteria when present at high enough concentrations. Our preliminary experiments with toluene and *Pseudomonas putida* PpG9 demonstrated that toluene was rapidly biodegraded, but only when its aqueous-phase concentration was substantially less than the solubility of pure liquid toluene [i.e., 515 mg/l at 20°C (Verschueren, 1983)]. When a toluene-containing NAPL contacts water, noninhibitory conditions occur when toluene constitutes only a fraction of the NAPL and/or when the dissolved concentration is reduced by advection, dispersion, or degradation. In our preliminary experiments, bacterial growth and toluene biodegradation occurred only when we restricted dissolution

of pure toluene by forcing it to dissolve through a cotton plug. Complete inhibition occurred when pure liquid toluene was in contact with the liquid.

2.1 Quantifying When Bioremediation Significantly Accelerates NAPL Dissolution

Biological reaction is not the only mechanism that reduces the liquid-phase concentration of a NAPL: Advection and dispersion also serve as solute "sinks." In fact, flushing, which is a key component of pump-and-treat technology, relies on advection to remove dissolved contaminant and accelerate NAPL removal. We used a modeling analysis to develop quantitative tools for assessing when biological activity significantly increases NAPL dissolution over that which can occur through flushing. The details of the model development and application are given in Seagren et al. (1992; 1993).

The interactions of NAPL dissolution, biodegradation by attached bacteria, and solute transport are evaluated for two simple domains: homogeneous and immobilized blobs in a saturated, isotropic medium with one-dimensional flow and a NAPL pool adjacent to a saturated, isotropic medium having two dimensions.

2.1.1 Blob Dissolution in a One-Dimensional Domain

The steady-state mass-balance equation for dissolution of a biodegradable solute from uniform blobs in one-dimensional flow is

$$0 = \frac{\partial C}{\partial t} = D_x \frac{\partial^2 C}{\partial x^2} - \frac{q_x}{n S_w} \frac{\partial C}{\partial x} + R_b + R_d \quad (2.1)$$

in which C = NAPL-solute concentration [ML^{-3}]; t = time [T]; D_x = longitudinal dispersion coefficient [L^2T^{-1}]; x = distance in the direction of flow [L]; q_x = specific discharge [LT^{-1}]; n = porosity; S_w = water saturation; R_b = biodegradation sink term [$\text{ML}^{-3}\text{T}^{-1}$]; and R_d = dissolution source term [$\text{ML}^{-3}\text{t}^{-1}$]. The dissolution rate was described by interfacial mass transport:

$$R_d = k_1 (C_s - C) \quad (2.2)$$

in which C_s = the solubility concentration of the pure NAPL [ML^{-3}] and k_1 = a lumped interfacial mass-transfer coefficient [T^{-1}] that depends on hydrodynamics and the NAPL's specific surface area. The biodegradation rate is modeled as pseudo-first order:

$$R_b = -k_{B1} C \quad (2.3)$$

in which k_{B1} = a lumped first-order rate coefficient [$\text{ML}^{-3}\text{T}^{-1}$] that includes the amount of attached biomass (assumed constant in the domain) and the solute's intrinsic biodegradation kinetics.

Equations (2.1)-(2.3) were combined and converted to a dimensionless format that includes three dimensionless group parameters:

$$0 = \frac{1}{Pe} \frac{d^2 C^*}{dx^{*2}} - \frac{dC^*}{dx^*} - (Da_1 + Da_2) C^* + Da_1 \quad (2.4)$$

in which C^* = a dimensionless solute concentration = C/C_s , x^* = a dimensionless distance = x/L , L = the length of the domain [L], and the three dimensionless group parameters are defined in Table 2.1. Table 2.1 also defines a fourth group, Da_3 , which equals Da_2 / Da_1 . Boundary conditions are of the third type for the inlet

$$\left(-\frac{1}{Pe} \frac{dC^*}{dx^*} + C^* \right)_{x^*=0} = 0 \quad (2.5)$$

and for a semi-infinite domain for the outlet,

$$\frac{dC^*(\infty)}{dx^*} = 0 \quad (2.6)$$

The analytical solutions for $C^*(x^*)$ and a normalized dissolution rate ($\dot{r}_d^*$) are given as functions of Pe , Da_1 , and Da_2 by Seagren et al. (1992).

Table 2.1 Definitions of Dimensionless Group Parameters

$$Pe = \frac{\text{advection rate}}{\text{dispersion rate}} = \frac{V_x L}{D_x} \quad (2.7)$$

$$Da_1 = \frac{\text{mass-transfer rate}}{\text{advection rate}} = \frac{k_1 L}{V_x} \quad (2.8)$$

$$Da_2 = \frac{\text{biodegradation rate}}{\text{advection rate}} = \frac{k_{B1} L}{V_x} \quad (2.9)$$

$$Da_3 = \frac{\text{biodegradation rate}}{\text{mass-transfer rate}} = \frac{k_{B1}}{k_1} \quad (2.10)$$

Note: v_x = average pore water velocity = $q_x / (n S_w)$

Figures 2.1 and 2.2 summarize the most important findings:

- Maximum dissolution rates (i.e., $r_d^* = 1$ or $C^* = 0$) can be achieved by increasing flushing so that $Da_1 < 0.01$ or by increasing biodegradation so that $Da_3 > 100$.
- Increasing biodegradation effects a significant increase in the dissolution rate (and decrease in C^*) only when Da_3 increases through the range 0.01 to 100.
- If flushing already is decreasing C^* (i.e., $Da_1 < 0.01$), increases to biodegradation have little added benefit for increasing the dissolution rate.

These findings define the ranges of Da_1 , Da_2 , and Da_3 in which flushing and/or enhanced biodegradation can accelerate NAPL dissolution. Further research (here and elsewhere) are required to quantify the values of k_{B1} and k_1 , which are complex functions of hydrodynamics, microbial kinetics, and microbial accumulation.

FIGURE 2.1. Representative curves ($Pe = 1$) showing how $C^*(x^* = 1)$ depends on Da_1 , Da_2 , and Da_3 .

FIGURE 2.2 Representative curves of the normalized dissolution rate, r_d^* , for $Pe = 1.0$.

2.1.2 NAPL-Pool Dissolution in a Two-Dimensional Domain

A similar modeling analysis was carried out for a NAPL pool dissolving into a two-dimensional domain. The governing equation in the flowing liquid is

$$q_x \frac{\partial C}{\partial x} = D_z \frac{\partial}{\partial z} \left(\frac{\partial C}{\partial z} \right) - k_{B1} C \quad (2.11)$$

in which z = the distance dimension normal to the flow direction [L] and D_z = the transverse dispersion coefficient [$L^2 T^{-1}$]. Equation 2.11 is nondimensionalized to

$$\frac{\partial C^*}{\partial x^*} = \frac{1}{Pe_t} \frac{\partial}{\partial z^*} \left(\frac{\partial C^*}{\partial z^*} \right) - Da_2 C^* \quad (2.12)$$

in which Pe_t = a transverse Peclet number = $q_x L_x / D_z$, and $z^* = z / L_x$. Dissolution from the NAPL pool is treated as a boundary condition,

$$C^*(x^*, z^* = 0) = 1 \quad (2.13)$$

for the entire domain, i.e., $0 \leq x^* \leq 1$. Solution of equation 2.13 gives the dimensionless solute concentration at any point in the domain ($C^*(x^*, z^*)$) and the dimensionless dissolution flux j^* , which is converted to the actual flux (J) by multiplying it by $v_x C_s$ (Seagren et al., 1993).

Figure 2.3 summarizes the results very succinctly. The figure plots the enhancement factor E , which equals the dissolution flux with biodegradation divided by the dissolution flux with no biodegradation (i.e., $k_{B1} = 0$). For $Da_2 \leq 0.1$, biodegradation is insignificant and does not increase J . When Da_2 is or can be made greater than 0.1, biodegradation significantly accelerates dissolution. A strategy for increasing Da_2 is to increase k_{B1} by increasing the amount of active biomass and/or by increasing the kinetics of the biomass by alleviating a limitation (e.g., electron acceptor) or improving environmental conditions (e.g., pH).

FIGURE 2.3. $\text{Log } E (= J_{\text{biotic}}/J_{\text{abiotic}})$ for a range of Da_2 .

2.2 Experiments with a Toluene NAPL

A series of experiments was carried out in a laboratory-scale porous-medium reactor in which dissolution from a NAPL pool could be carefully measured. The predicted effects of flushing and biodegradation were evaluated in abiotic and biotic experiments in which the toluene content of the pool and the water flow velocity were systematically varied.

Figure 2.4 shows that experimental system. The reactor is a 37.5-cm-long column having a square (4.5 cm) cross-section. It is packed with 2-mm glass beads. Sampling ports, installed every 4.5 cm, accept syringe needles that can penetrate to different depths. Water flows from left to right, and the flow velocity is variable from 0.1 to 10 m/day. The NAPL source is a small pool located in a reservoir 2 times the vertical width downstream from the inlet and extending one vertical width downstream.

FIGURE 2.4. Schematic of the column design for the NAPL-dissolution experiments

The NAPL pool contains ^{14}C -labelled toluene as the target compound. The bulk of the NAPL is dodecane. Samples in the effluent or from a sampling port are assayed for particulate, soluble organic, and soluble inorganic ^{14}C . The rate at which ^{14}C exits the column is equal to the toluene dissolution rate at steady state.

Initial experimentation characterized the reactors in terms of porosity, longitudinal dispersivity, and transverse dispersivity. Reasonable values were obtained: porosity = 0.37, longitudinal dispersivity = 0.17 cm, and transverse dispersivity = 0.0041 cm.

A series of abiotic experiments evaluated whether or not changes in the advective velocity controlled the dissolution rate as predicted by eqn. 2.11, with $k_{g1} = 0$. Figure 2.5 illustrates that the experimentally determined dissolution flux increased non-linearly with an increase in the advective velocity, as expected for a Pe_l value between 0.1 and 10.

FIGURE 2.5. Experimental data and model predictions for a transverse dispersivity of 0.0041 cm and toluene dissolved in dodecane with a mole fraction of 0.02.

Biotic experiments were carried out after the column had been inoculated with *Pseudomonas putida* PpG9, which oxidizes toluene. Three flow velocities (0.1, 1.0, and 10 m/d) and two toluene contents in the NAPL (giving saturation toluene concentrations of 11.3 and 47.2 mg/l) were used. We expected that biodegradation would have the greatest impacts for the lowest velocity and the lower toluene content. For high flow velocity, advection is a major competing sink, and the relative effects of toluene biodegradation are small. Because the higher toluene content is large enough to cause toxicity, we expected to see a relatively larger impact for the experiments in which the toluene content was lower.

Toluene dissolution rates were determined by the output rate of all forms of ^{14}C . The amount biodegraded was assessed by the loss of soluble organic ^{14}C and by the formation of inorganic ^{14}C via mineralization. A bacterial inhibitor (3mM sodium azide) was added periodically to suspend the biodegradation sink and observe the abiotic dissolution rate for the same physical conditions.

The general trends were similar for all flow velocities and toluene concentrations, but important details differ. Figure 2.6 illustrates the general trends for the reactor in contact with the NAPL pool containing the lower toluene fraction ($C_s = 11.3 \text{ mg/l}$) and a flow velocity of 1 m/day. A quasi-steady state was reached between 30 and 50 hours, which correspond to 3 to 5 pore volumes. The steady-state dissolution flux can be computed from the total ^{14}C line and was $8.4 \times 10^7 \text{ dpm/m}^2\text{-day}$. Of the total flux, approximately 50% was CO_2 produced from toluene mineralization. Another approximately 30% was radiolabelled biomass. Thus, only about 20% of the existing ^{14}C could have been from toluene or its breakdown intermediates.

The sodium azide inhibitor was added at 50 hours. Subsequently, the dissolution flux dropped to $4.8 \times 10^7 \text{ dpm/m}^2\text{-days}$, a value only 55% of the uninhibited flux. Furthermore, ^{14}C in CO_2 and biomass declined to close to zero. Clearly, the inhibitor had nearly stopped all biodegradation, which also caused a dramatic reduction in the dissolution rate.

At 64 hours, the inhibitor was removed from the feed. Although the experiment was stopped before a true steady state was re-established, the restoration of the higher dissolution rate with resumed biodegradative activity was clear.

The trends were similar for the higher toluene fraction in the NAPL ($C_s = 47.7 \text{ mg/l}$): The uninhibited dissolution flux increased to $3.8 \times 10^8 \text{ dpm/m}^2\text{-day}$ CO_2 and biomass constituted almost 80% of the total dissolution flux.

Reactor #1; approximately 1 m/d

FIGURE 2.6. Fate of ^{14}C for the reactor in contact with the lower toluene fraction ($C_s = 11.3 \text{ mg/l}$) and a flow velocity of 1 m/day.

Results for pore velocities of 0.1 and 10 m/day showed similar trends as for 1 m/d. As predicted by Seagren et al. (1993), the enhancement effect for the biotic over the inhibited rates were larger for the lower velocities and smaller for the higher velocities. The enhancement from a given biodegradation potential is greatest when the competing sink, advection in this case, is least important.

3. DUAL-LIMITATION KINETICS

Many environments contain two or more solutes that are required for bacterial metabolism, but also are present at concentrations low enough to limit the kinetics of metabolism. Bioremediation frequently creates an environment in which the primary electron donor and acceptor are simultaneously present at limiting concentrations. The electron donor can be an organic contaminant, which must be driven to a low concentration by bacterial utilization. The electron acceptor, such as dissolved oxygen, often has a low concentration, because its supply as part of the bioremediation is limited by solubility and advective transport. This situation of simultaneously limiting concentrations of the primary electron donor and acceptor is called dual limitation.

Although simple mathematical models for dual limitation have been proposed (Bader, 1978), they were either chosen on an *ad hoc* basis because of mathematical convenience or based on an oversimplification of bacterial metabolism. Based on an understanding of how bacteria mediate electron and energy flow, we hypothesized that the kinetics of dual limitation are controlled by intracellular levels of key cofactors that store and transfer energy and electrons. We made a detailed investigation of the responses of the intracellular cofactors to changes in external concentrations of the primary electron donor and acceptor. Finally, we related the kinetics of primary-substrate utilization to the external concentrations of the primary substrates and to the intracellular concentrations of the cofactors.

Details of the experimental program, results, interpretations, and applications are given by Bae (1992), Bae and Rittmann (1990), and Bae and Rittmann (1993a,b,c).

3.1 Experimental Program

The experiments were carried out with *Pseudomonas putida* PpF1, which used acetate as its electron donor and oxygen as its electron acceptor. A consistent source

of the bacteria was cultivated in a mother chemostat operated with a dilution rate of 0.4 hr^{-1} and fed a mineral medium containing $1,000 \text{ mg/l}$ (as COD) of sodium acetate. Cells harvested from the mother chemostat were transferred to a daughter reactor specially designed to allow rapid sampling, independent control of dissolved oxygen (DO) and acetate concentrations, temperature control, pH monitoring, and no contamination. The mineral medium (plus acetate) was fed continuously at different flow rates to give a range of electron-donor loadings. The oxygen concentration was controlled by independent feeding of H_2O , which quickly decomposed to O_2 in the presence of catalase.

Sampling and analytical techniques were developed to be able to obtain instantaneous and highly sensitive assays for bulk-liquid concentrations of acetate and dissolved oxygen, and intracellular concentrations of inorganic phosphate (Pi) and the four key cofactors for energy and electron metabolism: adenosinetriphosphate (ATP), adenosinediphosphate (ADP), oxidized nicotinamide adenine dinucleotide (NAD), and reduced nicotinamide adenine dinucleotide (NADH). The phosphorylation of ADP to ATP creates a high-energy carrier. The reduction of NAD to NADH creates an electron carrier.

Four series of experiments were performed. In Series 1, the electron acceptor (O_2) was in excess, which means that we had only single limitations by the donor. In Series 2, the donor was in excess concentration, giving single limitation by the acceptor. Series 3 and 4 were for dual limitation with DO concentration of 50 and $22 \text{ } \mu\text{g/l}$, respectively.

3.2 Experimental Results

Figure 3.1 summarizes the changes in concentrations of NAD, NADH, and the ratio NAD/NADH for different types of limitation and with different electron-donor utilization rates ($-q_d$). Whereas the NAD concentration was nearly constant at $2.88 \pm 0.28 \text{ } \mu\text{mole/g}$ cells, the NADH concentration depended dramatically on limitation type and $-q_d$. NADH increased systematically as oxygen limitation became more severe. The NADH

concentration also increased gradually with increasing $-q_d$. The range of NADH concentration was large, varying from a low of 0.04 μ mole/g cells for acetate limitation and low $-q_d$ to 1.0 μ mole/g cells for oxygen limitation and high $-q_d$.

The NAD/NADH ratio, which we hypothesize as controlling the overall kinetics, decreased as DO fell or $-q_d$ rose. The range varied 2.4-fold, which suggests that it can have a major impact on overall substrate-utilization kinetics.

The results for the responses of ATP, ADP, and P_i (not presented graphically) showed less of a trend according to type of limitation, but showed a distinct trend with $-q_d$. The trend is shown in Figure 3.2 and expressed mathematically by the correlation equation

$$\log\left(\frac{\text{ATP}}{\text{ADP} \cdot P_i}\right) = -0.423(-q_d) + 2.19 \quad (3.1)$$

in which $-q_d$ has units mg COD/mg cells-hr, and ATP, ADP, and P_i have units mole/g cells.

3.3 Model Development and Results Interpretation

A structured model was developed to describe the rate of electron-donor utilization ($-q_d$) in terms of the external concentration of the donor (S_d) and concentration ratios of the internal cofactors. The form of the model is

$$-q_d = q'_{md} \log\left([NAD/NADH]^{\alpha'} [ATP/ADP \cdot P_i]^{\beta'}\right) \frac{S_d}{K_d + S_d} \quad (3.2)$$

in which q'_{md} is an intrinsic maximum specific rate coefficient [$M_d M_x^{-1} T^{-1}$], K_d is an intrinsic half-maximum rate concentration ($M_d L^{-3}$), S_d is the external concentration of the donor ($M_d L^{-3}$), and α' and β' are internal response parameters.

FIGURE 3.1 Intracellular Responses of (a) NAD, (b) NADH, and (c) their ratio for the different substrate limitations.

FIGURE 3.2 Variation of ATP/ADP·P_i with -q_d for different substrate limitations.

In order to evaluate the model, the experimental data were used to evaluate the kinetic parameters. Because the concentration ratios depended on the dissolved oxygen concentration (S_a), eqn. 3.2 could be rewritten in terms of S_a and S_d . Furthermore, once -q_d was known as a function of S_a and S_d , the internal cofactor ratios also could be expressed in terms of S_a and S_d . The procedures to convert the experimental data to the relationship for -q_d, log(NAD/NADH), and log(ATP/ADP·P_i) were complicated and are reported in Bae (1992). The final results are:

For all limitations:

$$-q_d = 2.13 \frac{S_d}{184 + S_d} \cdot \frac{S_a}{18.8 + S_a} \quad (3.3)$$

$$\log(\text{ATP/ADP} \cdot \text{P}_i) = 2.19 - 0.901 \frac{S_d}{184 + S_d} \cdot \frac{S_a}{18.8 + S_a} \quad (3.4)$$

in which S_d is in $\mu\text{g COD/l}$, S_a is in $\mu\text{g O}_2/\text{l}$, and -q_d is in $\mu\text{g COD/mg cells-hr}$.

For dual and donor limitations:

$$\log(\text{NAD/NADH}) = 0.717 + 1.11 \frac{S_a}{18.8 S_a} - 0.581 \frac{S_a}{18.8 + S_a} \cdot \frac{S_a}{184 + S_a} \quad (3.5)$$

For acceptor limitation alone:

$$\log(\text{NAD/NADH}) = 0.717 - 0.373 \frac{S_a}{18.8 + S_a} \quad (3.6)$$

Equation 3.3 is of the multiplicative form, which means that dual limitation can be described as though two independent Monod expressions are multiplied. This form results from the interacting effects among the external substrates and the internal cofactors. The multiplicative form is quite convenient, since it has been used before for its simplicity.

Equations 3.5 and 3.6, which indicate how $\log(\text{NAD/NADH})$ depends on S_a and S_d , describe how the internal availability of electrons can be controlled. A relatively low value of $\log(\text{NAD/NADH})$ indicates a high electron availability. In general, the internal electron availability is increased whenever $-q_d$ is increased, when the acceptor concentration is reduced, or when the donor concentration is increased.

The potential impact of dual limitation on an electron-requiring reaction, such as reductive dechlorination, is illustrated in Figure 3.3, which was generated by using eqn. 3.5 or 3.6 to predict $\log(\text{NADH/NAD})$, eqn. 3.7 to predict the rate of reductive reaction, and a mass balance (eqn. 3.8) on a completely mixed reactor to compute percent removal. The reaction rate is

$$-r_s = kSX \{ \log(\text{NADH/NAD}) + 1.827 \} \quad (3.7)$$

in which $-r_s$ = rate of reductive reaction (mg/l-day), k = mixed second-order rate constant (l/mg cells-day), S = concentration (mg/l) of substrate to be reduced, X = biomass concentration (mg/l). The reactor mass balance is

$$0 = (S^{\circ} - S) - r_s \theta \quad (3.8)$$

in which θ = hydraulic detention time (days) and S° = influent concentration of the substrate (mg/l). The product $kX\theta$ was set to 2.5 to represent relatively slow kinetics.

The key results in Figure 3.3 are:

- the ratio $\log(\text{NADH}/\text{NAD})$ is most sensitive when S_d is low and when S_a is high.
- the % removal is significantly increased by conditions yielding a high NADH/NAD ratio. The highest removal occurs consistently when the donor is unlimiting. The next best strategy is to minimize the acceptor concentration.
- The % removal is very sensitive to substrate concentration changes for the combination of high acceptor and low donor concentrations.

FIGURE 3.3 Changes in NADH/NAD and % Removal for a combination of donor and acceptor concentrations.

4. KINETICS OF SEQUENTIAL REACTIONS

Quinoline, a frequently found contaminant near fossil-fuel processing facilities, is a nitrogen heterocycle with a benzene ring and a pyridene ring. Researchers at Battelle Pacific Northwest Laboratory (PNL) performed several series of batch and column studies to investigate the biodegradation kinetics of quinoline. The goal of our work was to use mathematical modeling to interpret the experimental results and lead to predictive transport models for quinoline and related compounds.

The fundamental issue for modeling the biodegradation of quinoline is how to describe the several sequential reactions and their intermediates. In particular, quinoline provides an excellent example of a sequence of reactions involving oxygenase steps, which require O_2 as a direct reactant, and hydroxylation reactions, which oxidize the organic compounds without direct action by O_2 . The distinction is important, because evidence suggests that oxygenation reactions are much more sensitive to low oxygen concentrations than are respiration reactions using O_2 as a primary electron acceptor.

The experimental protocols and results, model basis and development, and modeling analyses are presented in Malmstead (1992) and Malmstead et al. (1993).

4.1 Modeling Basis

Figure 4.1 presents the proposed pathway for sequential degradation of quinoline. Only step 1 is proven, but the other steps are firmly based on reaction biochemistry of closely related compounds. The key features of the pathway are:

- Step 1 is a hydroxylation, in which quinoline is oxidized to 2-hydroxyquinoline. As in all hydroxylations, the inserted oxygen comes from H_2O , and the electrons are transferred to an internal electron carrier, the cofactor NAD, which ultimately transfers the electrons to the primary electron acceptor, O_2 , through the cytochromes. Dissolved oxygen concentrations can offset the rate of hydroxylation

reactions through the dual-limitation mechanism described in section 3 of this report. However, oxygen-limitation effects are expected only for dissolved oxygen concentration much less than 1 mg/l.

FIGURE 4.1 Summary of the proposed five key steps for the pathway of quinoline biodegradation.

- Steps 2 and 4 involve oxygenase reactions that require molecular oxygen as a direct reactant. We expect that dissolved oxygen concentrations near 1 mg/l can begin to limit the oxygenase reactions and cause the buildup of their reactants, 2-hydroxyquinoline and 2,6-dihydroxynicotinic acid.
- The reactants in steps 3 and 5, pyruvate and oxaloacetate, are common biochemical intermediates that are rapidly oxidized to CO₂, yielding NADH.
- Cell synthesis requires electrons and carbon from this pathway. Steps 3 and 5 provide the carbon in the form of pyruvate and oxaloacetate. A net of 12 moles of NADH per mole of quinoline is produced, with 10 moles of NADH per mole of quinoline generated in steps 3 and 5.
- Substrate-level oxygen consumption occurs directly via oxygenase reaction (steps 2 and 4) and respiration of NADH generated (net) in steps 1,2,3, and 5. For the overall oxidation of quinoline to CO₂, 4 moles O₂ /mole quinoline are consumed directly by oxygenase reaction, and 6 moles O₂/mole quinoline are consumed through respiration of generated NADH.

The pathway was converted to rate expressions and mass balances for quinoline, each key intermediate, oxygen, and biomass. Mass balances were set up for a batch reactor and for a column biofilm reactor, because each type of system was used experimentally. Although the details of the mass balance equations and their solution can be found in Malmstead (1992) and Malmstead et al. (1993), the rate expressions are summarized here.

Quinoline (Q)

$$r_q = -q_q \times \frac{Q}{K_q + Q} \frac{O}{K_{qo} + O} \quad (4.1)$$

2-Hydroxyquinoline (HQ)

$$r_{HQ} = q_o X \frac{Q}{K_Q + Q} \frac{O}{K_{QO} + O} - q_{HQ} X \frac{HQ}{K_{HQ} + HQ} \frac{O}{K_{HQO} + O} \quad (4.2)$$

2,6-Dihydroxynicotinic Acid (NA)

$$r_{NA} = \frac{6}{9} q_{HQ} X \frac{HQ}{K_{HQ} + HQ} \frac{O}{K_{HQO} + O} - q_{NA} X \frac{NA}{K_{NA} + NA} \frac{O}{K_{NAO} + O} \quad (4.3)$$

Pyruvate (P) and Oxaloacetate (OX)

The compounds were assumed to react instantaneously to form CO₂ and NADH.

Oxygen (O) (needed in column experiments only)

$$r_o = -0.15 q_o X \frac{Q}{K_Q + Q} \frac{O}{K_{QO} + O} - 0.77 q_{HQ} X \frac{HQ}{K_{HQ} + HQ} \frac{O}{K_{HQO} + O} - 1.24 q_{NA} X \frac{NA}{K_{NA} + NA} \frac{O}{K_{NAO} + O} - 2.67 bX \quad (4.4)$$

Biomass (X)

$$r_x = \frac{3}{9} Y_p q_{HQ} X \frac{HQ}{K_{HQ} + HQ} \frac{O}{K_{HQO} + O} + \frac{4}{6} Y_{ox} q_{NA} X \frac{NA}{K_{NA} + NA} \frac{O}{K_{NAO} + O} - bX \quad (4.5)$$

in which the upper case letters refer to the solute concentration in units of mg C/l, O is the dissolved oxygen concentration in mg/l, K_i is the half-maximum rate concentration of substrate i (mg C/l), K_o is the half-maximum rate concentration for oxygen for the degradation of substrate i (mg O₂/l), q_i is the maximum specific rate of utilization of substrate i (mg substrate C/mg biomass C-hour), X is the biomass concentration (mg biomass C/l), b is the endogenous respiration coefficient (hour⁻¹), r_i is the rate of accumulation of material i (mg/l-hour), and the numerical coefficients represent the stoichiometry of the pathway and cell yields for the units used.

4.2 Batch Results

PNL researchers ran batch experiments using quinoline or 2-hydroxyquinoline as the original substrate and measuring quinoline, 2-hydroxyquinoline, and cell numbers over time. The dissolved oxygen concentration was not measured, and it was assumed to be high enough to preclude significant oxygen limitation. The initial quinoline concentration ranged from 5 to 100 mg/l (3.8 - 75 mg as C/l).

The quinoline experiment was used to find best-fit values of q_Q and K_Q , while the 2-hydroxyquinoline experiments were used to estimate q_{HQ} , K_{HQ} , q_{NA} , and K_{NA} . Fitting was successful for all runs, as substrate and biomass trends could be matched in all cases.

The best-fit parameters are presented in Table 4.1. The maximum rate of substrate utilization declines significantly through the pathway, but the K values are similar. These results indicate the possibility to have intermediate buildup, even without oxygen limitation. This buildup was observed in every batch test and is illustrated by Figure 4.2, which is for quinoline as the initial substrate.

FIGURE 4.2 Experimental and best-fit model results for a quinoline batch-degradation experiment.

4.3 Column Results

Three sets of column experiments were performed with the same quinoline-degrading bacteria used in the batch tests. The input quinoline concentration was 5, 10, or 20 mg as C/l. The flow rates were varied to give different liquid detention times. Because the influent dissolved oxygen was fixed at near-saturation (approximately 8 mg/l) and was not augmented, the column experiments gave considerable oxygen depletion and the opportunity to observe the effects of oxygen limitation, especially on the degradation of 2-hydroxyquinoline. Measurements were of effluent quinoline and hydroxyquinoline. Effluent concentrations of dissolved oxygen and 2,6-dihydroxynicotinic acid were not measured, but were inferred from modeling analysis.

The mass-balance model for the flow-through column was solved for Q, HQ, NA, O, and X. The stoichiometric parameters for O consumption and X production were maintained the same as shown in eqns. 4.4 and 4.5. The kinetic parameters obtained in the batch experiments for degradation of Q and HQ (Table 4.1) were utilized for the column experiments. The oxygen half-maximum-rate concentration (K_{QO} , K_{HQO} , and K_{NAO}) were varied to find best-fit values for all the column runs. Best-fit was judged by the ability to match effluent Q and HQ values.

Table 4.1 shows the K_O values obtained by the best-fit approach. As we anticipated, K_{QO} was very low (0.02 mg/l), reflecting the use of O_2 solely as the primary electron acceptor in respiratory oxidation of NADH. K_{HQO} and K_{NAO} were much higher, 2 mg/l, which supports our hypothesis that the oxygenase reactions are much more severely limited by declining oxygen concentrations.

Table 4.1 Best-Fit Parameters for the Biodegradation Kinetics of Quinoline, 2-Hydroxyquinoline, and 2,6-Dihydroxynicotinic Acid

Substrate	q $\frac{\text{mg substrate C}}{\text{mg biomass C-hour}}$	K $\frac{\text{mg substrate C}}{\ell}$	K_o $\frac{\text{mg O}_2}{\ell}$
Quinoline (Q)	2.1	0.05	0.02
2-Hydroxyquinoline (HQ)	1.0	0.05	2.0
2,6-Dihydroxynicotinic Acid (NA)	0.1	0.05	2.0

Note: q and K values were estimated from batch experiments, while K_o was estimated from column experiments.

FIGURE 4.3 A summary of the 20-mg/l as C column results showing that 2-hydroxyquinoline degradation levelled off as O_2 was depleted. Other refers to the difference between the total input carbon and that measured as Q and HQ.

Figure 4.3 illustrates the O_2 effect for the series of experiments having an influent quinoline concentration of 20 mg/l as C. As the retention time was increased (by lowering the flow rate), oxygen was depleted. Hydroxyquinoline built up to its maximum level at the point where the dissolved oxygen was approaching zero in the effluent. This means that the degradation of HQ was being hindered by low dissolved oxygen, but its formation from Q was still occurring. Figure 4.4 gives a good example, a detention time of 2.2 minutes. The top plot shows that the dissolved oxygen is nearly depleted, which allows HQ to increase substantially over what it would be if no oxygen limitation were occurring (model prediction shown in lower plot) until oxygen was completely eliminated. Only using a multiplicative Monod relation with a relatively high K_{HQO} value could represent the results for the detention times having significant, but incomplete O_2 depletion.

5. BACTERIALLY INDUCED CLOGGING

The growth and metabolic activity of microorganisms are well known for decreasing the permeability of porous media. This "clogging" phenomenon is most likely to be important when a bioremediation scheme is adding substrates and nutrients to stimulate microbial growth and activity. Decreased permeability is most often detrimental, because it can prevent the further introduction of degradation stimulating materials and it can sequester contaminants from all removal mechanisms, including flushing.

Although the phenomenon of clogging is well known, the mechanisms causing the loss of permeability are not resolved. Several mechanisms have been put forward: microbial growth on the solid surfaces decreases pore dimensions, surface growths increase surface irregularities and the friction factor, microorganisms in the liquid are trapped at and plug pore constrictions, metabolism produces materials (e.g., exopolysaccharides) that increase liquid viscosity, and metabolism produces gases (e.g., N_2) or precipitates (e.g., FeS) that reduce liquid porosity and "bind" the medium.

FIGURE 4.4 Double-Monod (upper plot) and simple-Monod (lower plot) column model simulations and experimental data for a 2.2 minute retention time and a 20 mg/l as C influent concentration.

A series of experiments were carried out in sand columns to quantify the degree of permeability loss and to ascertain which mechanisms were most important.

Permeameter columns, shown in Figure 5.1, were inoculated with a range of aerobic strains having different abilities to produce exopolysaccharides (Vandevivere and Baveye, 1992a,b). A nutrient medium containing mineral salts, phosphate buffer, and a range of glucose concentrations was passed through a quartz sand sieved to be in the range 63-125 μm . The permeability was computed at any time from

$$K_s = q_x \left(\frac{\Delta z}{\Delta H} \right) \quad (5.1)$$

where K_s = the hydraulic conductivity [mm s^{-1}], q_x = specific discharge [mm s^{-1}], Δz = the distance along the column [mm], and ΔH = the hydraulic-head difference [mm] across the distance Δz .

FIGURE 5.1 Cross-sectional diagram of the acrylic-plastic permeameter: (1) inlet chamber, (2) support mesh, (3) discontinuous structural support, (4) piezometer connectors, (5) rubber septa, and (6) O-rings.

After a few days feeding, the hydraulic conductivity declined by as much as three orders of magnitude near the inlet of the permeameter. Figure 5.2 illustrates the fact for a set of experiments when the *Arthrobacter* inoculum was injected 20 mm downstream of the inlet screen. Other experiments showed even greater decreases in K_s when the cells grew with heavy growths on the screen (Vandevivere and Baveye, 1992a).

FIGURE 5.2 Evolution of saturated hydraulic conductivity in successive sections of sand. The inoculum (*Arthrobacter* AK19) was injected 20 mm after the screen mesh, and the permeameter was operated with a constant liquid flow rate.

When the cells were induced to take an encapsulated form by severe N limitation, K_s was further decreased, but the effect was not long-lived. Increasing feed concentrations of glucose also greatly accelerated the rate and increased the extent of permeability loss, but the biggest losses occurred at the inlet screen.

Data for the cell density of *Arthrobacter* were consolidated to produce Figure 5.3, which shows that K_s decreases systematically with an increase in cell density. The equation,

$$K_s/K_{s0} = \exp \{-0.355 X^{0.85}\} \quad (5.2)$$

in which K_{s0} = the hydraulic conductivity before bacterial colonization [mm s^{-1}], and X = bacterial density in the porous medium [$\text{mg wet weight cm}^{-3}$]. The correlation coefficient

for eqn. 5.2 is $r = 0.73$. For biomass density less than about $4 \text{ mg wet weight cm}^{-3}$, K_s is not significantly affected. Above that value, reductions in K_s are dramatic. For perspective, a density of $10 \text{ mg wet weight cm}^{-3}$ corresponded to a reduction in pore volume of approximately 2.4%.

FIGURE 5.3 Dependence of the saturated hydraulic conductivity on the bacterial density.

Scanning electron microscopy suggested that the bacteria were not present as continuous biofilms in most of the sand media. Instead, they formed aggregates that appear irregularly on the sand grains. These aggregates appear to act as local plugs when they are trapped at pore lumen.

Experiments comparing mucoid versus nonmucoid strains from the Savannah River collection (Vandevivere and Baveye, 1992b) showed that mucoid strains were very effective at plugging the inlet mesh. Loose slime layers were much more effective at clogging the sand than were cell-bound capsular exopolymers.

Rittmann (1993) analyzed the results of Vandevivere and Baveye (1992a,b), as well as others, with the biofilm-kinetics concept of the normalized surface loading. Rittmann found that the experiments carried out by Vandevivere and Baveye had very low surface loadings, compared to several other experiments on clogging and common practice in

biofilm processes used in wastewater treatment. Having such low surface loadings explain why Vandevivere and Baveye observed discontinuous surface colonization, while others observed continuous biofilms. Even though the influent concentration of glucose was relatively large (up to 100 mg/l), the flow velocity was low and the specific surface area was high in the experiments of Vandevivere and Baveye. This is shown by the equation for the surface loading for the entire column,

$$J_L = \frac{q_x (S^0 - S)}{a L_x} \quad (5.3)$$

in which J_L = surface loading [$\text{ML}^{-2} \text{T}^{-2}$], S^0 = input substrate concentration [M L^{-3}], S = effluent substrate concentration [M L^{-3}], a = specific surface area for the colonization [L^{-1}]. A small J_L value is possible when q_x is small, a is large, or L_x is large, even when $S^0 - S$ is large. The J_L value used by Vandevivere and Baveye -- 0.019-0.038 kg COD (1000 m^3) $^{-1} \text{ day}^{-1}$ -- were less than the minimum flux that gave continuous biofilms (Rittmann, 1993).

On the other hand, the J_L value for the inlet screen was very large, since L_x is no more than a few millimeters. Therefore, the very heavy colonization on the screen is predicted by the concept of the normalized surface loading, even though colonization was sparse throughout the entire column of sand.

6. TWO-DIMENSIONAL MODELING

There will be many physical, chemical, and biological processes occurring simultaneously during *in situ* bioremediation. Realistic groundwater transport problems entail the advection and dispersion of several electron donors and acceptors in a multidimensional, heterogeneous flow domain. The success of any *in situ* bioremediation operation depends in large part upon mixing and transport of limiting substrates in order to enhance subsurface biological activity. Mathematical modeling is the fundamental tool

for synthesizing the basic experimental results described in the other section of this report and extrapolating these results to realistic field scenarios. Moreover, modeling provides a framework for investigating new phenomena arising from coupled physical, chemical, and biological processes.

Our modeling research was conducted under the auspices of a closely coupled subcontract entitled "Modeling the Transport of Biologically and Chemically Reactive Solutes in a Two-Dimensional, Heterogeneous Intermediate-Scale Experiment," from Battelle Pacific Northwest Laboratory. The general goals of our research were: (1) to develop a flexible model that could be used to interpret the results of quinoline degradation experiments conducted in two-dimensional stratified flow cells at PNL; (2) to examine whether different conceptual models of attached biological degradation kinetics lead to significantly different predictions for typical groundwater scenarios; (3) to examine coupling between physical and biological processes, in particular, the effect of velocity field heterogeneity, in the form of ideal stratification, upon biodegradation kinetics; and (4) to examine coupling among chemical and biological processes, in particular, the effect of electron donor adsorption upon biodegradation kinetics.

6.1 Model Development

In order to achieve the modeling goals listed above, our first task was to develop an efficient and flexible computer code capable of accommodating several different types of reaction expressions. We developed a modular computer code based upon using the operator time splitting (OS) technique to solve the coupled transport and reaction equations. OS splits the reactive transport equation into a nonreactive transport partial differential equation and a reaction ordinary differential equation. The two-dimensional nonreactive advective-dispersion equation is solved by the Principal Direction Finite Element Method (PDFEM) described by MacQuarrie et al. (1990), and the reaction equation is solved using a standard second-order Runge-Kutta method. Details of our

implementation of OS are given in Odencrantz et al. (1990) and Odencrantz (1992). Accuracy of our OS code was excellent in comparison to available analytical solutions and other computer codes. The OS technique was up to 10 times faster than conventional solution techniques for the same accuracy. Note that our code is restricted to horizontal flow in homogeneous or stratified domains, since PDFEM is used to solve the transport stage.

OS has become a widely utilized method for solving reactive transport equations. The theory of OS has been advanced by several numerical analysts, including Wheeler and Dawson (1988), who demonstrated that the standard OS technique is first-order accurate in time. Most investigators using OS assume that the overall accuracy of the numerical solution should be guaranteed if each stage of the OS (i.e., the nonreactive and reactive stages) is solved accurately. However, this does not agree with our own theoretical results, which are published in Valocchi and Malmstead (1992). We analyzed a problem for which each stage of the operator-splitting algorithm can be performed exactly (i.e., without discretization error). This analysis revealed that there is an inherent mass balance error present in OS for problems involving continuous mass input boundary conditions. In order for the mass balance error to be less than about five percent, the product of the first-order decay coefficient (k) times the time step size (Δt) must be less than 0.1. We also developed a unique variant of the standard algorithm in which the order of solving the nonreactive and reactive stages is reversed at each time step. This modification significantly reduces the mass balance error for a wide range of $k\Delta t$ values.

Since the model was to be applied to PNL's intermediate scale experiments on quinoline transport and degradation, the code has the capability of handling sequential decay reactions. In fact, a one-dimensional version of the code was used for the column studies reported previously in Section 4. The code, which is named PDPLUS, can handle up to five organic substrates, plus one electron acceptor and biomass. As an example of the code's capability, consider the following sequential biodegradation reaction:

At each step, there is some electron acceptor (A) utilized and some bacterial growth. The mathematical model thus consists of transport equations for S_1 , S_2 , S_3 , S_4 , and A, plus a biomass (M_T) mass balance equation.

$$\frac{\partial S_1}{\partial t} = \nabla \cdot (D \nabla S_1 - v S_1) - R_{S_1} \quad (6.1)$$

$$\frac{\partial S_i}{\partial t} = \nabla \cdot (D S_i - v S_i) + \gamma_{i-1,i} R_{S_{i-1}} - R_{S_i} \quad i = 2, 3, 4 \quad (6.2)$$

$$\frac{\partial A}{\partial t} = \nabla \cdot (D \nabla A - v A) - \sum_{i=1}^4 \gamma_{A_i} R_{S_i} \quad (6.3)$$

$$\frac{\partial M_T}{\partial t} = \sum_{i=1}^4 Y_i R_{S_i} - b M_T \quad (6.4)$$

where D is the hydrodynamic dispersion tensor, v is the pore water velocity vector, R_{S_i} is the biological decay rate of S_i (mass/vol. fluid-t), γ_{ij} is a stoichiometric coefficient equal to mass of S_j produced per mass S_i degraded, γ_{A_i} is a stoichiometric coefficient equal to the mass of A utilized per unit mass of S_i degraded, Y_i is the bacterial yield coefficient equal to the mass of cells produced per mass S_i degraded, and b is the specific decay coefficient. Note that because PDFEM is used, there is only one nonzero component of the velocity field v (i.e., horizontal flow), and the dispersion tensor reduces to a diagonal form.

We have implemented a simple network-type representation of the reacting substrates. This enables PDPLUS to handle any arbitrary number and form of sequential decay reactions among the five donor substrates.

The mathematical form of the reaction terms R_{S_i} in equations (6.1)-(6.4) is determined by the particular biodegradation kinetic submodel adopted. In the early versions of the model, we implemented both conventional Monod kinetics and more

sophisticated biofilm kinetics. However, based upon results to be summarized in the next subsection, we decided that the additional sophistication (and computational burden) of the biofilm model was not warranted. Therefore, the final PDPLUS code implements either single-Monod or multiplicative-Monod kinetic submodels. In the latter case, for example, the reaction term takes the form

$$R_{Si} = q_{mi} M_T \left(\frac{S_i}{K_{Si} + S_i} \right) \left(\frac{A}{K_{Ai} + A} \right) \quad (6.5)$$

where q_{mi} is the maximum specific rate of substrate utilization, K_{Si} is the half-maximum-rate concentration of S_i , and K_{Ai} is the half-maximum-rate concentration of A for reaction i . The need for possibly using different K_A values was described in Section 4. Also, the final version of the code accounts for electron acceptor utilization due to endogenous decay of biomass.

The PDPLUS code was partially verified by comparison with analytical solutions for sequential first-order decay. The code is fully documented in a user's guide.

6.2 Effect of the Type of Biodegradation Kinetic Model upon Groundwater Transport

Although it is widely accepted that most of the active biomass in the subsurface is attached to soil grains, there are several possible alternative conceptual models of biodegradation kinetics (Baveye and Valocchi, 1989; Odencrantz, 1992). The simplest model, here termed Monod, assumes that all the attached biomass is exposed to the macroscopic solute concentration, as given in equation (6.5). More sophisticated approaches, such as the biofilm and microcolony models, assume that the macroscopic solute concentration first diffuses from the bulk pore fluid across an external boundary layer to the bacteria/liquid interface, and then can subsequently diffuse and react within the biofilm or microcolony. In order to address whether the added sophistication of the biofilm model is warranted in typical groundwater problems, we used dimensional analysis and numerical simulations to conduct a detailed comparison of the Monod and biofilm

models.

When both internal and external mass-transfer resistances are small, the biofilm and Monod submodels are identical. The relative importance of internal and external mass-transfer phenomena can be quantified using dimensionless groups, as shown by Suidan et al. (1987) and Odencrantz (1992). A dimensionless biofilm thickness can be defined by

$$L_r^* = \frac{L_r}{\sqrt{\frac{2K_s D_r}{q_m X_r}}} \quad (6.6)$$

and a dimensionless external film thickness by

$$L^* = \frac{L}{\sqrt{\frac{2K_s D_r}{q_m X_r}}} \quad (6.7)$$

where L_r is the biofilm thickness, L is the external film thickness, q_m is the maximum rate of substrate utilization, K_s is the half-maximum-rate concentration, D_r is the molecular diffusion in the biofilm, and X_r is the biofilm density. L_r^* indicates the importance of internal diffusion relative to biological reaction. Suidan et al. (1987) showed that biofilms are fully penetrated for $L_r^* \leq 0.2$ (i.e., no internal gradient within the biomass). Thus, the Monod model is possible when $L_r^* < 0.2$, and biofilm effects will be more evident when L_r^* is increased, such as by increasing substrate inputs to increase L_r , decreasing K_s , or increasing q_m or X_r . We conducted simulations using the biofilm submodel for a wide range of groundwater conditions. Details are reported in Odencrantz (1992). In every case, L_r^* was very small (L_r^* never exceeded 0.011 at steady-state). This is due to the relative high surface area and low substrate loading typical of groundwater systems.

Odencrantz (1992) developed a new dimensionless parameter quantifying the relative importance of external mass transport. The parameter, termed the mass transport Damkohler number, is defined by

$$Da_{(mt)} = 2L \cdot L_f^* D_f^* K_s / S \quad (6.8)$$

where D_f^* is the ratio of the molecular diffusion coefficient in the biofilm to that in the bulk liquid. $Da_{(mt)}$ represents the ratio of the maximum possible reaction rate to the maximum possible rate of external mass transport; hence, a large value of $Da_{(mt)}$ means that external mass transport is limiting the overall rate of biodegradation. We found through numerical simulations that external mass transport limitations became significant when $Da_{(mt)}$ is greater than about 10.

Although the dimensionless parameters L_f^* , L^* , and $Da_{(mt)}$ give insight into the interaction of processes occurring at the "REV" scale (or the scale of a single discrete element in a numerical model), the total amount of biological growth will be governed by the flux of substrate due to advection and dispersion into the REV. Odencrantz (1992) developed another new dimensionless parameter, Da_{MAC} , the macroscopic Damkohler number. Da_{MAC} , which equals the maximum amount of mass degraded in a single finite element over one time step divided by the mass advected into the element over one time step, is given by

$$Da_{MAC} = \frac{\Delta x q_m M_T}{\theta v S_{in}} \quad (6.9)$$

where Δx is the element size, θ is the porosity, and S_{in} is the substrate concentration incoming to the element. Very large values of Da_{MAC} imply that the system is "reaction-controlled", and, hence, most of the substrate mass is degraded over the first grid block adjacent to the source. This situation could correspond to a high amount of biomass growth so that L_f^* becomes large and a biofilm model is needed. However, in this case, the issue of choosing among the biological kinetic submodels becomes moot because the

numerical discretization is too coarse to resolve the small-scale biological processes. In other words, any kinetic model can be chosen when Da_{MAC} is very large, because the grid spacing is too coarse to resolve profiles of biomass and substrate. In this case, the biological reaction appears to be nearly instantaneous for the given resolution.

The results of our dimensionless analysis and numerical simulations indicate that both the internal and external diffusion components of the biofilm model can usually be neglected when modeling biodegradation processes in natural groundwater systems. Hence, we conclude that the Monod model is sufficient for practical applications. Internal diffusion can be neglected because biofilm thicknesses are very small due to the high surface area and low substrate loading typical of groundwater cases. Although modeling of external mass transfer limitation is difficult for naturally occurring porous media, the most widely utilized correlations from the chemical engineering literature indicated that this process was not significant for typical groundwater scenarios.

6.3 Effect of Porous Medium Stratification upon Biodegradation Kinetics

By controlling mixing between water having different amounts of electron donor and electron acceptor, field scale heterogeneity may have a major influence upon biodegradation. Stratification is a simple type of heterogeneity that is commonly encountered in the field.

We conducted extensive simulations in stratified porous media in order to examine the influence of heterogeneity on coupled transport and biodegradation processes. Figure 6.1 shows the problem definition we used in some of the work; the system consists of two layers with mass input along the entire influent boundary. At time zero there is a uniform background concentration of electron donor (acetate) present but no electron acceptor (nitrate). The acceptor (but no donor) is continually input into the domain as shown in Figure 6.1. We also studied the case with input of both electron acceptor and donor; these results are given in Odencrantz (1992).

FIGURE 6.1 Domain and parameters used for the simulation experiments in stratified porous media.

Since the multiplicative Monod model was employed (eqn. 6.5), both donor and acceptor must be present simultaneously for degradation to occur. Hence, transverse dispersion of electron acceptor from the fast into the slow layer plays the key role in controlling the overall degradation of the electron donor. We therefore conducted a series of numerical simulations for varying values of dispersivity.

Some results of the simulations are illustrated in Figure 6.2, which shows the total mass of electron donor in the system as a function of time. The general shape of these "mass-loss" curves can be understood by examining the nonreactive case, which shows two distinct limbs corresponding to the washout of the donor from the fast and slow layers. For the simple case of plug flow, the first limb corresponds to washout from both layers; after $t = 2$ days, all the donor is washed out of the fast layer, which means that

the second limb corresponds to washout from the slow layer. Comparison of each case with the nonreactive curve indicates the amount of biodegradation. It is apparent that very little degradation occurs in the fast layer because of the short detention time available. Enhanced transverse dispersion leads to enhanced amounts of biodegradation. This is corroborated by Figure 6.3, which shows the total amount of biomass in the system as a function of time. Clearly, there is enhanced biological activity as α_T increases. Detailed examination of the spatial distributions of substrates and biomass reveals that the layer interface is a region of enhanced biological activity. This is because there is a "reservoir" of acceptor in the high velocity top layer and a "reservoir" of donor in the low velocity bottom layer. Increasing the longitudinal dispersivity also has a very significant influence due to the resultant large increase in length of the mixing zone between the advancing acceptor front and the displaced donor front.

FIGURE 6.2 Effect of transverse dispersion on total mass of electron donor in the system.

FIGURE 6.3 Effect of transverse dispersion on the total biomass in the system.

6.4 Effect of Electron Donor Adsorption upon Biodegradation

Our analysis of coupled transport and biodegradation processes in stratified systems revealed that the key phenomena are those that control the mixing between the donor and acceptor. Most organic electron donors will adsorb to some degree, thus retarding these advective velocity. However, since most electron acceptors are not retarded, the advective velocity of the donor can be less than that of the acceptors. Because many field *in situ* bioremediation schemes will entail injection of electron acceptors, donor adsorption can lead to enhanced mixing with the acceptor and thus be an extremely significant process controlling overall biodegradation phenomena. Accordingly, we conducted a major study of the interaction between sorption and biodegradation. We assumed linear equilibrium adsorption of the electron donor only. Details are given in Odencrantz (1992).

In order to eliminate any complicating effects caused by heterogeneity, we focused our numerical simulations upon a one-dimensional homogeneous column in which a constant flux of electron acceptor is input and displaces a background electron donor at uniform concentration. In the absence of any adsorption, mixing between the donor and acceptor occurs across the moving dispersed boundary that forms as the acceptor displaces the donor. However, if the background donor is adsorbed, then it will migrate at a retarded velocity, whereas the input electron acceptor is nonadsorbing and will migrate at the pore-water velocity. Because of the difference in velocity, the input acceptor front will "overtake" the displaced background donor front leading to increased overlap and mixing between the acceptor and donor. Therefore, in general, we find that an increased retardation factor of the donor leads to an increased amount of biodegradation.

More detailed examination of the simulation results revealed some very intriguing behavior. Figure 6.4 shows one example for the case with pore-water velocity equal to 0.1 m/day and donor retardation factor equal to 3. At very early times ($t \leq 5$ days), little biomass has accumulated, and the acceptor front has migrated further than the donor front. But, it is in this region of overlap that there is simultaneously high donor and acceptor concentrations; the resulting high biomass growth leads to increased utilization of the donor and acceptor. Since a greater portion of the influent acceptor is being consumed by the increased biomass, the downstream acceptor flux diminishes, and the profile develops the peculiar shape shown at $t = 15$ days. It is interesting that eventually the donor and acceptor fronts travel at the same speed and maintain approximately the same shape. Therefore, the degradation kinetics decreases the velocity of the acceptor front and increases the velocity of the donor front.

what appears to be a cyclic "steady state." The biomass development has a direct effect upon the overall donor mass that is biodegraded. The rate of donor mass utilization is initially high during the early growth phase of the biomass and then diminishes to a roughly constant value during the cyclic steady-state phase. After this steady-state phase is reached, the donor and acceptor fronts travel at approximately the same speed, as shown in Figure 6.4.

7. CONCLUSIONS

This project made important progress in several different, but related areas of *in situ* bioremediation. The following summary statements highlight the most significant contributions.

- Bacteria must be located very close to the NAPL/interface if *in situ* biodegradation is to accelerate NAPL dissolution and aquifer cleanup.
- For bioremediation of blobs, the impact of *in situ* biodegradation is greatest only when the dimensionless group Da_3 increases in the range 10^{-2} to 10^2 , and as long as the dimensionless group Da_1 is greater than about 10^{-3} , which means that dissolution is not already mass-transport limited by flushing from advection.
- For bioremediation of NAPL pools, biodegradation is effective in accelerating the dissolution rate when Da_2 is increased when it is greater than 0.1. This can be effected by increasing the amount of biomass and/or its specific reaction rate.
- Experiments carried out with NAPL pools containing toluene confirmed the *in situ* biodegradation can accelerate NAPL-dissolution rates when the biodegradation rate near the NAPL-water interface is large enough, compared to the flushing (advection) rate.

FIGURE 6.4 Profiles of the donor (top), acceptor (middle), and biomass (bottom) for the one-dimensional simulation with adsorption.

- The two critical ratios of intracellular cofactors -- NAD/NADH and ATP/ADP·P_i -- vary systematically as the degrees of primary electron-donor and electron-acceptor limitation change. The NAD/NADH ratio increases as the acceptor concentration increases or as the specific rate of electron-donor utilization (and donor concentration) increases. The ATP/ADP·P_i ratio increases as the specific rate of donor utilization (and the donor concentration) increases.
- The observed cofactor responses can be incorporated into a structured kinetic model that predicts the donor utilization rate and both cofactor ratios as a function of the donor and acceptor concentrations. Through linkage of the cofactor responses to donor and acceptor concentrations, the structured model is transformed into the multiplicative (or double Monod) model for dual limitation. This result resolves the long-standing debate concerning the correct form for dual-limitation modeling.
- The systematic changes in cofactor responses are large (more than 30-fold). Thus, proper manipulation of the primary substrate concentrations can greatly increase the removal of contaminants that require an intracellular source of electrons.
- The sequential biodegradation of quinoline, a nitrogen-containing heterocycle, can be modeled with a 5-step pathway that contains a mixture of hydroxylation and oxygenation reactions.
- The oxygenase steps, which require O₂ as a direct reactant, are much more sensitive to oxygen limitation than are the hydroxylation reactions, which generate the cofactor NADH. The half maximum rate concentration is 100 times larger for the oxygenase reactions (2 mg/ℓ) than for the hydroxylation reactions (0.02 mg/ℓ).
- Substrates for oxygenase reactions (e.g., 2-hydroxyquinoline) build up when dissolved oxygen concentrations are relatively low, such as less than about 2 mg/ℓ.
- Permeameter experiments with fine sand demonstrated rapid and extensive clogging, as measured by the decrease in saturated hydraulic conductivity.

- The decrease in permeability was strongly correlated to the accumulation of biomass for biomass densities greater than about 4 mg wet weight per cm³ of porous medium.
- Within the sand columns, biomass colonization was not as continuous biofilms, but as irregular aggregates. Straining of these aggregates at pore throats was implicated as the primary clogging mechanism.
- Clogging at the inlet screen was severe and accompanied by accumulation of a dense mat.
- Analysis of the permeability results with the biofilm-kinetics concept of the normalized surface loading explained why the colonization was not continuous: The high specific surface area of the sand produced a surface loading less than needed to support a continuous biofilm, such as is observed in other clogging experiments and in wastewater-treatment processes.
- A flexible and efficient two-dimensional numerical model for multi-substrate transport in stratified aquifers was developed. The code is based upon using the operator splitting (OS) technique to separate the overall problem into purely nonreactive and reactive stages. The code implements either single or multiplicative Monod kinetics and can handle up to 5 substrates plus one acceptor and biomass. The code has been tested and is fully documented.
- We performed a rigorous error analysis of OS and found that there is an inherent mass balance error for problems involving continuous mass input boundary conditions. Minimization of this error introduces a time step size restriction which depends inversely upon the magnitude of an approximate first-order rate coefficient.
- We used dimensional analysis and numerical simulations to assess differences between transport calculations based upon biofilm and Monod kinetic submodels. This analysis revealed that there are no substantial differences between the two submodels for typical groundwater conditions.

- We examined the impact of stratification upon biodegradation for a simple two-layer system. We found that transverse dispersion across the layer interface controlled mixing between the donor and acceptor, thereby leading to enhanced biomass growth at the interface.
- We found that electron donor adsorption has a major impact upon biodegradation in a system with electron acceptor injection. In general, increasing the retardation factor of the donor led to increased biodegradation rates. For large domains and continuous input, the traveling profiles of donor, acceptor, and biomass reach a cyclic steady-state condition.

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