III. Summary of Current Work

A. Overview

Our current DOE-sponsored project, "Fundamental Quantitative Analysis of Microbial Activity in Aquifer Bioreclamation" was initiated in March 1989 as part of the Biodegradation/Microbial Physiology Subprogram. It is coordinated closely with a second project, "Modeling the Transport of Biologically and Chemically Reactive Solutes in a Two-Dimensional, Heterogeneous Intermediate-Scale System," which is funded by a subcontract from Battelle Pacific Northwest Laboratory as part of the Coupled Processes Subprogram. NAPL biodegradation is one of the key parts of the current work. Before the NAPL work is reviewed in some detail in the next subsection, a brief overview of the rest of the work carried out at the University of Illinois is given here.

Dual Limitation refers to the situation in which the electron donor and electron acceptor limit the cell growth rate together. We carried out extensive experimental and modeling work on the dual limitation kinetics for acetate and O_2 with *Pseudomonas putida* PpFl. The focus for the work was on developing and experimentally evaluating a structured model of dual limitation. The structured model is based upon the realization that the external substrates (acetate and O_2 here) react with internal cosubstrates, such as $NADH_2$, NAD, ATP, and ADP. The bacteria modulate the internal concentrations in response to the external-substrate concentrations.

A unique experimental system was created to be able to independently control the acetate and O_2 concentrations and to gather highly sensitive measurements of all external and internal substrates. Experimental results demonstrated that the major trends predicted by the structural model were correct:

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- The NAD/NADH ratio decreased significantly as O₂ limitation became more severe.
- The NAD/NADH ratio systematically decreased as the acetate loading and concentration increased.
- The ATP/ADP ratio decreased as the acetate utilization rate increased.

Intensive analysis of all the results yielded quantitative expressions for ATP/ADP, NAD/NADH, and the rate of electron-donor utilization, q_d . The most significant is for q_d :

$$q_d = 2.13 \frac{S_d}{184 + S_d} \bullet \frac{S_a}{18.8 + S_a} \quad (2)$$

in which q_d = specific rate of electron donor (acetate) utilization (mg as COD/mg cells-hr), S_d = external electron donor concentration ($\mu\text{gCOD}/\ell$), and S_a = external electron acceptor concentration ($\mu\text{g O}_2/\ell$). Equation 2 is of the form of the so-called multiplicative Monod model; however, its form is derived from a structured model that includes the response of the internal cosubstrate and their reaction with the external substrates.

The work on dual limitation will come to a close by the end of the current project. It has been completely successful in defining the dual limitation phenomenon and providing practical, but fundamentally based, kinetic expressions. The dual-limitation results will be directly applicable in the proposed NAPL research, if O₂ depletion becomes significant in the experiments.

2-dimensional modeling including biodegradation had to be advanced substantially in order to handle the severe nonlinearities of the biological reaction terms, the heterogeneity naturally present or induced by the biological

growth, and the coupling of reaction terms for bacteria and their several substrates. The following achievements have been made in the current project.

- The operator-splitting technique (Wheeler and Dawson, 1988; Odencrantz et al., 1990a) was coded and validated for 2-dimensional domains with stratified layers. The operator-splitting approach makes the code highly flexible so that alternative biodegradation (and other source/sink) submodels can be used. The accuracy of the method was assessed and enhanced by modification of the original method (Valocchi and Malmstead, 1991).
- Systematic evaluation of the strictly macroscopic (Monod) submodel versus the biofilm submodel showed that the added complexity of the biofilm submodel is seldom needed for aquifer applications.
- A comparison of multiplicative versus minimum-rate approaches to describe dual limitation showed significant differences and that the choice of a proper dual-limitation submodel is important.
- Examination of the impact of stratified layering on biodegradation illustrated that transverse dispersion across the layer's interface controlled the mixing between the electron donor and acceptor, thereby leading to enhanced microbiological activity at the interface.
- The code was modified to handle chain-type reactions in which sequential intermediates are produced and consumed, each with its own kinetics for the electron donor and acceptor substrates. This modified code was applied to experiments performed by Battelle Pacific Northwest Laboratory and identified the critical role of O₂ depletion.

- The code was modified to allow equilibrium sorption of substrates. Evaluation with the model of a retarded organic electron donor and an unretarded electron acceptor (O_2) revealed the importance of mixing of the acceptor front into the receding donor front in a region also containing significant biomass.

B. NAPL Biodegradation

The research on NAPL biodegradation in porous media was initiated approximately two years ago. Major efforts have been made in (Seagren, 1991)

- critical review of the literature on NAPL characteristics, transport, and biodegradation.
- definition of the hypotheses upon which the research is based.
- selection and characterization of model NAPL compounds and microorganisms.
- design and characterization of an experimental system to conduct dissolution and biodegradation experiments.
- development of 1- and 2-dimensional models for dissolution and biodegradation
- initial analysis, using the 1-dimensional model, to establish criteria for the successful design of *in situ* bioremediation. The rest of this section summarizes what has been accomplished in the experimental and modeling areas.

The first step was to select model NAPL for experimental studies. Two different NAPLs were needed to represent the limiting cases of (a) a low solubility and low toxicity hydrocarbon that allows bacteria to grow very near the NAPL interface, and (b) a relatively high solubility, high toxicity compound

that should prevent bacteria from growing very near the interface. Besides meeting these criteria, the model compounds should be the primary substrate to the bacterium available to utilize it and be available with a C-14 radiolabel.

For the first case, decane and dodecane are good choices. The solubilities are 9 $\mu\text{g}/\ell$ and 3.7 $\mu\text{g}/\ell$ for decane and dodecane, respectively. We have shown that they are biodegraded by strains of *Pseudomonas putida* we have in our laboratory. Dodecane is available as a radiolabel, but the availability for decane is uncertain, although possible.

For the second case, benzene and toluene are acceptable choices, and we have decided on toluene. It has a solubility of 515 mg/ℓ . Although *Pseudomonas putida* PpG9 is completely inhibited by toluene present at its solubility concentration, we have proven that *P. putida* grows well on toluene when it is diffused slowly into the growth medium.

The second step was to design an experimental system for dissolution and biodegradation in a porous medium. Since the purpose of the experiments is to evaluate the phenomena and the interactions, a system with well-defined physical characteristics is paramount. First, a basic system configuration was chosen. Then, the 2-dimensional model, modified to allow NAPL dissolution as a boundary condition, was employed to find a detailed design that assured good plume development when reasonable flow velocities are used.

Figure 1 shows the final design of the reactor. 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 for sampling across the cross-section. Water flows from left to right, and its superficial flow velocity is to be varied from about 0.1 to 1.0 m/day. The NAPL source is a small pool located in a reservoir located

Figure 1. Column design for dissolution and biodegradation experiments in a porous medium. The column is filled with 2-mm glass beads.

2 times the vertical width downstream from the entrance and extending one vertical width downstream. Since the model NAPLs have densities less than water, the NAPL pool is located on the top. Model analysis of this design showed a full plume development with the flow velocities tested.

The reactor must be opened to add the glass bead initially and to remove glass beads from biomass analysis. Therefore, the reactor is fabricated in three parts: the main body and two endplates. The end plates are made of teflon, have a conical section for flow distribution, a square slot to match the reactor body, and holes for tightening rods (Figure 1). A gasket fits in the slot, which also is caulked with silicone after the reactor is assembled.

One reactor system was fabricated, assembled, and tested. Although some trial-and-error adjustments were needed to eliminate leaks and maldistribution of flow, the system has performed well. At this time, a set of experiments is being conducted to characterize the physical phenomena in the reactor. The porosity was measured as $n = 0.364$. Abiotic tracer experiments (using a nonreactive fluorescein dye) are currently underway to determine the longitudinal (α_L) and transverse (α_T) dispersivities for the range of flow velocities proposed. Initial results for the longitudinal dispersion using 1-dimensional flow with a uniform input are quite satisfactory. Table 1 summarizes the values of α_L and D_L for the flow velocities tested so far. Figure 2 illustrates that D_L increased with increasing v_x (as expected) and that the results were well described by the advection-dispersion model. Experiments to measure α_T require a point injection of the tracer and measurement of the tracer concentration along the centerline (Robbins, 1989). These experiments will be conducted during the next several months.

Table 1

Results of 1-Dimensional Tracer Tests to Determine the Longitudinal Dispersion Coefficient (D_L) and Dispersivity (α_L) for a Range of Superficial Flow Velocities (v) and Pore-Water Velocity (v_x)

<u>v, m/day</u>	<u>v_x, m/day</u>	<u>D_L, cm²/hr</u>	<u>α_L, mm</u>
0.37	0.99	0.59	1.43
0.78	2.07	1.87	2.17
1.55	4.14	2.62	1.52

NOTE: Values of D_L were determined by best fit of the experimental (C/C_0 vs t) data with the analytical solution of van Genuchten and Alves (1982) for 1-dimensional advection and dispersion with an initial concentration of $C_i = 0$, a third-type inlet boundary condition, and a second-type (infinite-domain) exit boundary condition. α_L was computed by assuming the hydrodynamic dispersion coefficient was dominated by mechanical dispersion; therefore, $\alpha_L = D_L \div v_x$.

Figure 2. Results of the 1-dimensional tracer tests and model analyses to estimate D_L .

The mass-transfer coefficient for NAPL dissolution, k_t , must be determined in a series of abiotic experiments in which the dissolved NAPL concentration is measured for a grid of locations below and downstream of the pool. These data will be used to construct a 2-dimensional concentration profile for steady-flow conditions. The 2-dimensional transport model having NAPL dissolution as a boundary condition will be used to calibrate the k_t value. Since the flow velocity is fixed and values of a , n , α_L , and α_T will be determined independently, the only calibration parameter is k_t , which will be best fit by minimizing the sum of square of deviations between the experimental and model-predicted results.

One of the goals of this research is to develop practical tools for analysis and design of *in situ* bioremediation. We accomplished an important initial step towards this goal by completing a systematic analysis of a simple model scenario. This simple analysis provides immediate insight into conditions necessary for successful *in situ* bioremediation, and it also demonstrates the approach we can take to derive practical tools for more complex scenarios.

The domain studied is the simplest one possible: a saturated, homogeneous, isotropic medium with 1-dimensional flow in the x direction and length L . The NAPL is a single component present uniformly as residual saturation of immobilized blobs. This situation conforms exactly to the experimental system used in Dr. Abriola's research on NAPL dissolution rates. We are exchanging information to compare and complement each other's results. For steady flow and steady-state concentrations (a reasonable assumption as long as the NAPL surface area does not change), the advection-dispersion-reaction equation is

$$0 = D_L \frac{d^2 C}{dx^2} - v_x \frac{dC}{dx} - K_t (C_s - C) - K_B C \quad (3)$$

in which D_L = longitudinal dispersion coefficient [L^2/T], v_x = pore liquid velocity [L/T], K_d = lumped dissolution mass-transport coefficient [$1/T$] = $k_d a/n$, K_B = a lumped first-order biodegradation rate coefficient [$1/T$].

The critical step is nondimensionalization of Eqn. 3 by introducing dimensionless variables $x^* = x/L$ and $C^* = C/C_s$. Substitution of these variables on Eqn. 3 and rearrangement give

$$0 = \left(\frac{D_L}{v_x L} \right) \frac{d^2 C^*}{dx^{*2}} - \frac{dC^*}{dx^*} - \left(\frac{L}{v_x} (K_d + K_B) \right) C^* + \left(\frac{K_d L}{v_x} \right) \quad (4)$$

The parameters in parentheses are characteristic dimensionless groups that relate the different phenomena. Table 2 summarizes the dimensionless groups, and Eqn. 5 shows how Eqn. 4 is written in terms of the dimensionless groups,

$$0 = \frac{1}{Pe} \frac{d^2 C^*}{dx^{*2}} - \frac{dC^*}{dx^*} - (Da_1 + Da_2) C^* + Da_1 \quad (5)$$

Equation 5 was solved analytically for wide ranges of Pe , Da_1 , and Da_2 . The dimensionless concentration at the end of the domain ($C^* (x^* = 1)$) and the normalized dissolution rate over the domain,

$$r_d^* = \frac{\int_0^1 Da_1 (1 - C^*) dx^*}{Da_1} \quad (6)$$

were computed. Figure 3, which is for $Pe = 1$, summarizes the key findings. For very low Da_2 , the dissolution rate is at a minimum determined by Da_1 . For high rates of advection ($Da_1 < 1$), the dissolution rate is kept high by flushing. But, for relatively low advection ($Da_1 > 1$), the dissolution rate becomes very

Table 2

Definitions of the Dimensionless Groups

Pe - Peclet No. - advection/dispersion - $v_x L / D_L$

Da_1 - Damkohler No. 1 - dissolution/advection - $K_d L / v_x$

Da_2 - Damkohler No. 2 - biodegradation/advection - $K_B L / v_x$

Da_3 - Damkohler No. 3 - biodegradation/dissolution - K_B / K_d

Figure 3. Summary of how the normalized dissolution rate depends on Da_1 , Da_2 , and Da_3 ($= Da_2/Da_1$) for $Pe = 1$.

small. When biodegradation becomes important ($Da_2 > 0.01$), the dissolution rate can be high even when advection is small ($Da_1 > 1$), because biodegradation is an effective sink that keeps C^* well below 1.

The practical impact of this analysis can be stated succinctly.

- Flushing can increase the dissolution rate only as Da_1 decreases from about 100 to about 0.1.
- Biodegradation can replace or enhance flushing when Da_2 increases in the range 0.01 to 100.
- Increasing biodegradation will not further enhance the dissolution rate when $Da_3 > 100$.
- Decreasing Pe (e.g., by increasing dispersion) broadens the ranges of the Damkohler numbers given above, but the trends are the same.

C. Publication and Training

The publication of the current project are listed in Section IX. Publications from the Current Project. The following students were educated through participation in the project at the University of Illinois:

- Mr. Joseph Odenchantz -- Ph.D. expected in December 1991
- Mr. Wookeun Bae -- Ph.D. expected in early spring 1992
- Mr. Eric Seagren -- currently a Ph.D. candidate at the post-preliminary-exam stage. Degree expected in 1993
- Mr. Michael Malmstead -- M.S. degree expected in May 1992
- Mr. Zach Hall -- M.S. degree expected in December 1991
- Mr. Robert Brunswick -- M.S. degree awarded in May 1991
- Mr. Robert Bowen -- beginning Ph.D. student
- Mr. Daniel Pastor -- beginning M.S. student

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