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EVALUATION OF BACTERIAL DETACHMENT RATES IN
POROUS MEDIA

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"Evaluation of Bacterial Detachment Rates in Porous Media"

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SUMMARY. The ability of published biomass detachment rate expressions to describe experimental data obtained from porous media reactors using *Pseudomonas aeruginosa* grown aerobically on glucose was evaluated. A first-order rate expression on attached biomass concentration best reflected effluent substrate concentration for combined data sets. Detachment rate coefficient k_d was dependent on initial substrate concentration. Simulation of porous media reactor experiments indicated that responses using higher influent substrate concentrations possessed greater sensitivity to variations in k_d . Simulations of field bioremediation systems suggest the use of accurate biofilm development kinetics is important in the prediction of well bore biofouling.

INTRODUCTION. The success of *in situ* bioremediation of hazardous materials depends on the ability to engineer systems that spatially distribute microbial activity. The main limitation to achieving this goal is the inability to evenly disperse injected fluids throughout the subsurface using recirculation wells. This situation results from the radial flow patterns that emanate from these wells because fluid velocity diminishes rapidly with distance. Hence, injected nutrients tend to react near the well bore, limiting the microbial activity at remote points in the flow field. This phenomena has been observed both in the laboratory (1) and in the field (2).

To extend the biologically active region, researchers have focused on developing nutrient feed strategies that minimize near well growth to allow substrate transport further from the injection point. (2-5). For example, Shouche et al. (3) used simulations of a 1-dimensional flow field to determine a strategy for

pulsed feeding of electron donor (acetate) to minimize biomass growth near the field inlet. This was accomplished by adjusting the acetate concentration, the duration of the feed pulse, and the length of time between feed pulses. The maximum biomass concentration achieved in a 2-m soil column during 50 days of operation could be reduced by more than an order of magnitude as compared to a base case representing the pulsing strategy used in the field work of Semprini et al. (2). Roberts et al. (4), in similar studies, determined that well bore biofouling could be minimized through the use of separate injection pulses of electron donor and electron acceptor. Neither of these investigations accounted for the movement of microorganisms, but considered active biomass as a fixed film. In fact, most reported simulations of *in situ* bioremediation do not incorporate the important processes of bacterial attachment and detachment (6-8).

A limited number of investigations have attempted to describe bacterial transport in porous media through attachment and detachment rate expressions (9-13). For prediction of *in situ* bioremediation activity, this is necessary because contaminant transformation is a direct result of the presence of the subsurface biomass and the spatial distribution of biomass is affected by bacterial attachment and detachment. The goal of this paper is to evaluate the ability of current detachment rate expressions to describe biofilm dynamics in porous media. In addition, the sensitivity of simulated biomass profiles to detachment rate is examined for a porous media column reactor and a field-scale *in situ* remediation system.

METHODS. A pure culture of *Pseudomonas aeruginosa* was used to inoculate porous media reactors. This species is known to form biofilms and has well characterized kinetic parameters (14,15). Before inoculating the reactors, the bacteria were grown aerobically on glucose at 35°C for 2 days in batch culture. This resulted in an inoculum cell concentration of approximately 10^7 cells ml⁻¹.

The 50 mm long by 31 mm in diameter reactor was packed with 1 mm glass beads (Figure 1). Wall effects were minimized by using a bed-to-particle diameter ratio of 30, as recommended by Cohen and Metzner (16). The packing was held in the reactor with #30 brass gauze positioned at each end. Pressure taps, liquid sampling ports, and conductivity ports were provided at each end of the reactor at

the brass gauze-bead interface. Glucose and mineral salts solutions, stored in separate carboys, were diluted with sterile, aerated, distilled water before being continuously fed to the column. Mineral salts media used in these experiments have been described by Siebel and Characklis (15). Glucose solution flow rates were adjusted to give column influent concentrations of either 2.3 mg/L or 19.7 mg/L. Before inoculation, the reactor and feed apparatus were steam sterilized for 1 hour at 18 psig and 125°C. Once sterile and assembled, the reactor was isolated with tubing clamps and inoculated with *Ps. aeruginosa*. After 4 hours, the clamps were removed and the reactor was operated in an upflow mode at a constant flow rate of 38 mL min⁻¹ at a temperature of 20°C. Each experiment was operated for at least 12 days.

Influent and effluent cell samples were collected daily and preserved in buffered formalin prior to analysis. Suspended cells were stained with either Hoechst 33342 or DAPI fluorescent stains, although DAPI was primarily used because it was less susceptible to fading during exposure to ultraviolet light. Cell concentrations were measured using epifluorescent enumeration.

Influent and effluent glucose samples were also collected daily. Once collected, the glucose samples were centrifuged to remove any cells and extracellular polymeric substances (EPS). Glucose samples were analyzed colorimetrically using Sigma Diagnostics® enzymatic glucose determination (procedure 510), modified for measurement at lower concentrations.

MODEL DEVELOPMENT. The equations used to describe nutrient and biomass transport, and biofilm accumulation, in the porous media reactor are presented in Table 1. Biofilm development involves a combination of phenomena including biomass growth in the aqueous phase (r_g), growth in the biofilm (r_g'), and interfacial mass transfer through processes of attachment (r_a) and detachment (r_d). Changes in soil porosity and biofilm thickness are described by the method of Taylor and Jaffe (17). Biomass attachment rate was modeled as a first-order process (Equation 6), similar to that used by Taylor and Jaffe (9). This expression for attachment is based on conservative particle filtration theory (18), assuming that the porosity of the column does not change significantly during the experiment. The effects of a change in porosity on the attachment rate was neglected since calculations indicated that it would account for only 15% of the total attachment rate. The detachment rate expressions evaluated in this study, shown in the

second column of Table 2, are representative of rate expressions found in literature (9-13,19-22).

Numerical simulation of the porous media reactor system used an explicit finite-difference algorithm to reflect one-dimensional spatial variation while time derivatives were estimated using the Runge-Kutta-Feldberg method. Optimum values for detachment rate parameters were determined using Simusolv® version 2.0 optimization software (Dow Chemical Co., Midland, MI). Model parameters were chosen based on the following criteria: 1) standard error between model estimates and experimental data, 2) estimated parameter standard deviations, and 3) percentage variation explained, a parameter similar to the linear coefficient of multiple determination, yet adapted to dynamic, nonlinear systems.

Field-scale numerical simulations in this work used a modification of the multi-dimensional ReActive Flow and Transport code (RAFT) developed by Wheeler et al. (8,23,24). 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 employed 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 fourth-order Runge-Kutta technique. The simulated, two-dimensional flow field was 2.75 m on a side with a single, central injection well. The flow field was assumed to initially contain no glucose and an attached biomass concentration of 1.0 mg-dry biomass per kg of soil (mg-DW/kg-soil).

RESULTS AND DISCUSSION. Figures 1a and 1b show the experimentally measured and simulated effluent substrate concentrations for the porous media reactor. Influent substrate concentrations of 2.3 and 19.7 mg/L are represented in Figures 1a and 1b, respectively. A value of 0.38 min^{-1} was used for the k_a in all simulations represented in Figure 1. The simulated results demonstrate the best fit for the data for each of the detachment equations shown in Table 2. All detachment expressions reasonably reflect experimental data for an inlet substrate concentration of 2.3 mg/L. But, as shown in column 5 of Table 2, Equations 7, 9, 11, and 12 give values for the sum squared errors (SSE) that are significantly lower than those for Equations 8 and 10. For an inlet substrate concentration of 19.7 mg/L, only detachment expressions 7, 11, and 12 accurately represent the experimental data. This trend can be seen

from the SSE data in column 8 of Table 2. For the high substrate loading data, the SSE for Equations 11 and 12 are essentially the same as given by Equation 7 because the second terms in Equations 11 and 12 do not significantly improve the overall fit, representing less than 0.1 and 10% of the overall detachment rate, respectively.

These results demonstrate that the first-order detachment equation best represents the combined data sets. In contrast, Equations 8 and 10 do not accurately describe either case, Equation 9 is applicable only to the low substrate loading data, and Equations 11 and 12 are essentially the same as Equation 7 for the high substrate loading case. It is also apparent from estimates of k_{d1} in Equation 7 that a single value of this parameter does not describe both data sets. The value that best represents the low substrate data is 9.79×10^{-4} while a value of 4.4×10^{-3} best represents the high substrate data. Hence, although the first-order model can describe individual experiments, it cannot be used as a predictive expression for variable substrate loadings. This result is consistent with previous observations showing that detachment rate coefficients increase with inlet substrate concentration (25). Because the model is not globally predictive, the sensitivity of simulated results for both the reactor and an *in situ* bioremediation flow-field to the detachment rate was further examined to determine the effects this parameter on *in situ* bioremediation design. Only the detachment rate coefficient was examined because initial calculations revealed that the simulated results were much more sensitive to detachment rate. In fact, a 100% increase in the attachment rate coefficient was offset by only a 4% increase in the detachment rate coefficient.

Predicted behavior of the 2.3 mg/L experiment using values of k_{d1} between 0.5 and 2.0 of the optimum value is shown in Figure 2a. It can be seen that levels of k_{d1} between 0.5 and 1.25 of the optimal level give a reasonable prediction of the data. However, larger k_{d1} values significantly over predict the detachment rate, which results in less attached biomass in the column and higher substrate levels in the effluent. Similar data for the 19.7 mg/L case are shown in Figure 2b. Results of these simulations indicate a greater sensitivity to variations in k_{d1} at higher influent substrate concentrations as only the optimum value of k_{d1} accurately reflects the data. A value for k_{d1} that is one-half of the optimum severely under predicts the detachment rate, causing more substrate consumption than was measured experimentally.

When k_{d1} is raised to 1.25 times the optimum value, complete washout of the attached biofilm ensued.

To estimate the effects of the detachment rate coefficient on a field bioremediation system, the biofilm kinetic equations were applied to a 2-dimensional, radial flow geometry with a central injection well. An influent substrate concentration of 19.7 mg/L at a flow rate of 27.25 m³/day per m of well screen was used for these simulations. In addition, a contaminant was assumed present in the influent at a concentration of 2 mg/L. Contaminant destruction kinetics were first order in biomass and contaminant concentration, based on expressions for carbon tetrachloride destruction reported by Hooker et al. (26). Although these expressions are not applicable to aerobic growth of *Ps. aeruginosa* on glucose, they are typical of contaminant destruction kinetics, and provide insight to the sensitivity of *in situ* bioremediation to bacterial transport. Predicted steady-state biofilm profiles for various k_{d1} values are shown in Figure 3a as a function of distance from the injection well. Values of k_{d1} were chosen to reflect $\pm 25\%$ of the optimum level determined for the high substrate feed rate in porous media reactor studies.

It is apparent from Figure 3a that, near the flow field inlet, biomass increases with decreasing k_{d1} . In addition, lower values of k_{d1} cause a less uniform distribution of biomass. In fact, beyond 1.7 m, the biomass concentration for $k_{d1}=0.0033 \text{ min}^{-1}$ drops below that for $k_{d1}=0.0044 \text{ min}^{-1}$. However, these differences in biomass profiles have little effect on contaminant destruction in the flow field. This result can be seen in Figure 3b which represents field effluent contaminant concentration as a function of time for the three values of k_{d1} . The steady state effluent concentration is XXX, XXX, and XXX for k_{d1} values of XXX, XXX, and XXX, respectively. These data suggest that full field bioremediation is not as sensitive to the detachment rate as the column experiments. This inference is further supported by the fact that all three cases had nearly identical steady-state substrate concentration profiles, and all completely consumed the glucose within the first 0.7 m.

Although the integrated microbial reaction characteristics for the flow field are not highly sensitive to detachment rate, it is necessary to include this parameter to account for near well accumulation of biomass and biofouling. If detachment rate is neglected and biomass is considered a fixed film for simulations of 19.7 mg/L glucose injection to the flow field, complete biofouling is predicted after approximately 8 days. In contrast, if the detachment rate applied to this simulation is conservatively

increased to the level observed using the 2 mg/L data from the porous media reactor, a steady-state biofilm develops, possessing a concentration of 1500 mg-DW/kg-soil near the well bore. A biofilm concentration of 15500 mg-DW/kg-soil is required to fully plug the pore space. Finally if k_{d1} is set to 0.0044 min^{-1} , measured for 19.7 mg/L porous media reactor data, a steady-state, near-well biofilm concentration of approximately 360 mg-DW/kg-soil is observed.

These results indicate that it is important to include biofilm development kinetics in the design of *in situ* bioremediation systems. The primary design constraint that is affected by this parameter is biofouling of nutrient injection wells. Because current bacterial transport theory is not predictive and detachment rate coefficient changes with substrate loading, it is important to use conservatively low values of k_{d1} based on experimental measurement. Lower values will under predict detachment, and over predict biofouling. In contrast, other design issues such as volumetric contaminant destruction, and nutrient consumption are relatively insensitive to bacterial transport.

CONCLUSIONS. The ability of published detachment rate expressions to describe experimental results was evaluated. Data was obtained from porous media reactor experiments inoculated with *Ps. aeruginosa* and fed inlet glucose concentrations of 2.3 and 19.7 mg/L. A first order rate expression on attached biomass concentration gave the best prediction for the combined data sets. However, the value of the detachment rate coefficient was dependent on initial substrate concentration, as k_{d1} values of 9.79×10^{-4} and $4.4 \times 10^{-3} \text{ min}^{-1}$ best represented low and high concentration data, respectively. Simulations of the porous media reactor experiments indicate that the response using higher influent substrate concentrations has greater sensitivity to variations in k_{d1} , while responses at both substrate concentrations tested are relatively insensitive to changes in the attachment rate parameter k_a . The effects of detachment rate on a field bioremediation system were also estimated using 2-dimensional radial simulations. These simulations suggest that biomass profiles in the flow field vary with detachment rate. As a result, biofouling of nutrient injection wells is directly dependent on this parameter. In contrast, contaminant destruction, and nutrient consumption in the full flow field are not as sensitive to the detachment rate. These findings suggest that it is important to include biofilm kinetics in the design of *in situ* bioremediation

systems as the primary design constraint that is affected by this parameter is biofouling.

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Nomenclature

- C_s Concentration of a dissolved chemical in the liquid phase ($M L^{-3}$)
- C_{si} Initial concentration of a dissolved chemical in the liquid phase ($M L^{-3}$)
- D_s Dispersivity of a dissolved chemical in the liquid phase ($L^2 t^{-1}$)
- D_x Dispersivity of biomass in the liquid phase ($L^2 t^{-1}$)
- k_a Biomass attachment coefficient ($L t^{-1}$)
- k_{d1} Biomass detachment coefficient (Units are expression dependent)
- k_{d2} Biomass detachment coefficient (Units are expression dependent)
- K_s Monod half-saturation coefficient ($M L^{-3}$)

r_a	Biomass attachment rate ($M L^{-2} t^{-1}$)
r_d	Biomass detachment rate ($M L^{-2} t^{-1}$)
r_g	Rate of liquid phase biomass growth ($M L^{-3} t^{-1}$)
r_g^f	Rate of attached phase biomass growth ($M M^{-1} t^{-1}$)
t	Time (t)
v	Darcy Velocity ($L t^{-1}$)
X	Biomass concentration in the liquid phase ($M_x L^{-3}$)
X_f	Biomass concentration in the attached phase ($M_{xf} M_k^{-1}$)
Y_{xc}	Biomass observed yield ($M_x M_c^{-1}$)
z	Distance from the reactor inlet (L)

Greek Symbols

ϵ	Volume fraction of the media filled with liquid (Unitless)
μ	Specific growth rate of biomass (t^{-1})
μ_m	Maximum specific growth rate of biomass (t^{-1})
ρ_k	Substratum bulk density ($M_k L^{-3}$)

Table 1. Model of Nutrient and Biomass Transport and Biofilm Accumulation in Porous Media.

$$(1) \text{ Substrate} \quad \frac{\partial C_a}{\partial t} = D_a \frac{\partial^2 C_a}{\partial z^2} + \frac{D_a}{\epsilon} \frac{\partial \epsilon}{\partial z} \frac{\partial C_a}{\partial z} - \frac{v}{\epsilon} \frac{\partial C_a}{\partial z} - \frac{C_a}{\epsilon} \frac{\partial \epsilon}{\partial t} - \frac{r_g}{Y_X/C} - \frac{r_g' \rho_k}{\epsilon Y_X/C}$$

$$(2) \text{ Suspended Biomass} \quad \frac{\partial X}{\partial t} = D_x \frac{\partial^2 X}{\partial z^2} + \frac{D_x}{\epsilon} \frac{\partial \epsilon}{\partial z} \frac{\partial X}{\partial z} - \frac{v}{\epsilon} \frac{\partial X}{\partial z} - \frac{X}{\epsilon} \frac{\partial \epsilon}{\partial t} - \frac{r_a}{\epsilon} + \frac{r_d \rho_k}{\epsilon} + r_g$$

$$(3) \text{ Attached Biomass} \quad \frac{d X_f}{d t} = \frac{r_a}{\rho_k} - r_d + r_g'$$

where

(4) Suspended Growth Rate

$$r_g = \left(\frac{\mu_m C_a}{K_s + C_a} \right) X$$

(5) Attached Growth Rate

$$r_g' = \left(\frac{\mu_m C_a}{K_s + C_a} \right) X_f$$

(6) Attachment Rate

$$r_a = k_a X$$

Detachment Rate Expressions are Given in Table 2

Table 2. Detachment Rate Expressions and Optimum Coefficient Values Obtained.

Eq. #	Expression	Low Substrate Loading			High Substrate Loading			Reference
		k_{d1}	k_{d2}	SSE	k_{d1}	k_{d2}	SSE	
(7)	$k_{d1} X_{af}$	0.00098	-	0.43	0.0044	-	18	(11) Chang and Rittmann 1988 (19) Kreikenbohm and Stephan 1985 (20) Rittmann 1989
(8)	$k_{d1} X_{af}^2$	2.3	-	3.4	6.2	-	480	(12) Bryers 1987 (21) Trulear and Characklis 1982 (22) Wanner and Gujer 1986*
(9)	$k_{d1} \mu \eta X_{af}$	0.55	-	0.27	0.84	-	120	(10) Speitel and DiGiano 1987*†‡
(10)	$k_{d1} \mu \eta X_{af}^2$	2600	-	2.8	1400	-	470	(13) Stewart 1993*†‡
(11)	$k_{d1} X_{af} + k_{d2} \mu \eta X_{af}$	0.00064	0.21	0.21	0.0044	.001	18	(10) Speitel and DiGiano 1987*†
(12)	$k_{d1} X_{af} + k_{d2} \mu \eta X_{af}^2$	0.00098	0.026	0.43	0.0043	110	14	(13) Stewart 1993*†

*Assuming biofilm density is constant.

†Assuming $\eta = 1$

‡If $k_{d2} = 0$

Figure Captions

- Figure 1. Comparison of various detachment rate expressions optimized to give the best possible fit of experimental data obtained for influent substrate concentrations of (a) 2.3 mg/L and (b) 19.7 mg/L.
- Figure 2. Sensitivity of predicted effluent substrate concentration to the detachment rate coefficient used in Equation 7 for influent substrate concentrations of (a) 2.3 mg/L and (b) 19.7 mg/L.
- Figure 3. Sensitivity of predicted (a) biomass distribution and (b) contaminant concentration to the detachment rate coefficient used in Equation 7, under radial flow conditions for an influent substrate concentration of 19.7 mg/L.

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