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STIMULATION OF BIOLOGICALLY ACTIVE ZONES (BAZ's) IN POROUS MEDIA BY ELECTRON-ACCEPTOR INJECTION

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ABSTRACT

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A methodology involving laboratory-column experiments and computer modeling was utilized to investigate the formation of denitrifying biologically active zones (BAZ's) in a porous medium when a limiting electron acceptor (NO_3^-) is injected along the flow path. Laboratory experiments conducted in a unique one-dimensional porous-medium column demonstrated the relationship between lateral injection of NO_3^- and the location and extent of BAZ's when acetate was present as the sole carbon source. The phenomena of BAZ formation and the utilization of limiting and non-limiting substrates were expresed quantitatively in a computer model that coupled principles of one-dimensional solute transport and steady-state biofilm kinetics. A new, highly efficient solution algorithm was developed to solve directly for the steady-state profiles of the limiting substrate and biofilm mass, as well as for the non-limiting substrate. The predictive ability of the model was verified by successful simulation of particular laboratory experiments using independently determined kinetic parameters for acetate.

INTRODUCTION

In situ bioreclamation is a promising new technique for enhancing the clean-up rate of aquifers contaminated with organic pollutants, such as halogenated solvents, petroleum constituents and pesticides. In situ bioreclamation involves injecting the materials necessary to increase the microbiological activity in the subsurface. The injected material is a component that limits the growth of the desired microorganisms and is usually an electron acceptor, a carbon source, or a macro-nutrient. Injecting the proper amount of the limiting material creates a region of increased microbiological activity, called the biologically active zone (BAZ).

Creation of a BAZ offers major advantages for aquifer clean-up, because microorganisms are in close proximity to all the contaminants, including those dissolved in the water, those sorbed to aquifer materials, and those in a

nonaqueous liquid phase. Thus, the relatively slow mechanism of flushing by water flow is replaced by a degradation reaction very near the source of contaminants. As an example of the ineffectiveness of water flushing, Brown et al. (1987) found in a study of water extraction of various residually contaminated soils that 46 pore volumes of water effectively removed only 1.6% of the adsorbed gasoline fraction. Even after 500 pore volumes of water, soil contamination was still extremely high ($\sim$ 1400 mg gasoline/g soil).

This study investigated fundamental mechanisms that acted when an electron acceptor was injected along the flow path of an electron-donor-rich groundwater to establish a BAZ. The developed BAZ led to the degradation of pollutants that serve as growth-limiting and non-limiting substrates. One-dimensional laboratory column experiments were used to examine the establishment of BAZ's in response to injection of an electron acceptor. A computer model coupling principles of one-dimensional solute-transport and steady-state biofilm kinetics was used to quantitatively describe the laboratory results for the limiting substrate and biofilm mass, as well as for the non-limiting substrate.

EXPERIMENTAL METHODS

Column construction and BAZ *development*

Laboratory-scale, porous-medium columns were constructed of 2.5-cm inside diameter by 22.5-cm-long glass tubes which were filled with 3-mm glass beads. Ports for sampling or injection were placed every 2.5 cm. Special injection assemblies were designed to allow for cross-sectionally uniform injection of electron acceptor into the flow path. The injection assemblies consisted of three injection needles which had, all together, 52 holes of 0.1-mm diameter for discharging injected material.

The injection system is shown schematically in Fig. 1. The orifice spacing along the needle was determined by two factors. The first was the unequal distribution of areas occupied by successive annular segments in the cross-section (see Fig. 1a). Second, the injection pressure at the top of the needle was controlled by the injection pump, but frictional losses caused the fluid pressure to decrease along the needle. Thus, orifice flow rate diminished from the top to the bottom of the needle, because orifice flow rate is a function of the pressure on the inner side of each orifice. An iterative calculation procedure was devised to compute the spacing that guaranteed uniform cross-sectional injection. The Darcy–Weisbach equation (Daugherty and Franzini, 1977) for laminar flow was used to compute the pressure loss along the needle. The calculated orifice spacing used in the experiments is shown in Fig. 1b. Dye tracer tests showed that the injection assembly system gave an excellent approximation of planar, cross-sectionally mixed injection (Rittmann et al., 1988).

Two columns were operated to establish different BAZ configurations. The

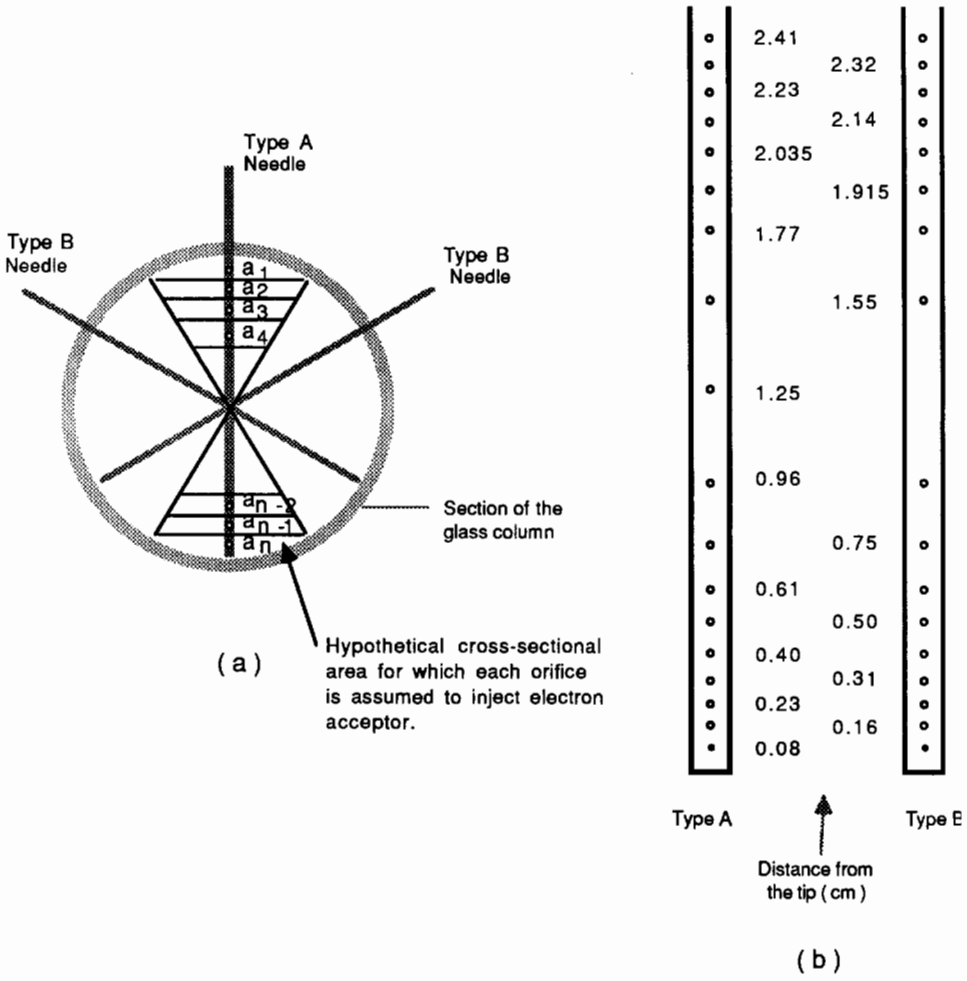
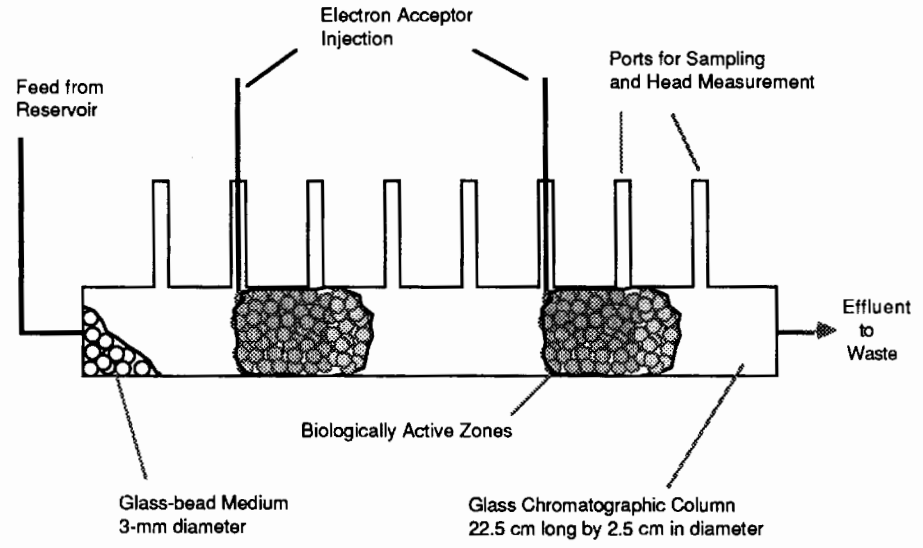
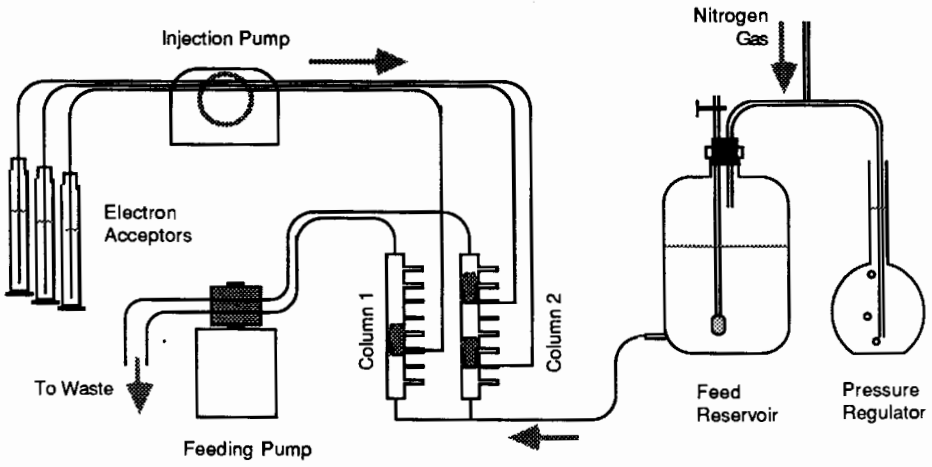


Fig. 1. Design of planar injection system: (a) arrangement of needles; and (b) spacing of orifices along the needles.

superficial flow velocity for each column was 0.10 cm min.$^{-1}$. The overall experimental set-up is shown in Fig. 2. Both columns were inoculated with a mixed population of denitrifying bacteria originally isolated from groundwater and were fed continuously with an anoxic mineral-salts solution containing ^{14}C-labelled acetate at 7.5 mg L^{-1} as soluble organic carbon (SOC). The electron acceptor (NO_3^-) was injected through the injection ports. One column had one injection port (7.5 cm downstream from the inlet), which led to one BAZ and to the designation of the one-BAZ column; the second column had two injection ports (5.0 and 15.0 cm downstream from the inlet), leading to two BAZ's and to the designation of the two-BAZ column.



(a)



(b)

Fig. 2. Schematic diagrams of (a) column reactor; and (b) overall experimental set-up for biologically active zone (BAZ) experiments.

Two major precautions were taken to ensure that only denitrification reactions occurred in the BAZ. First, the feed solution was purged with nitrogen gas for 3 h at boiling temperature to drive off dissolved oxygen. The small DO residual ($\sim 0.1\ mg\ L^{-1}$) present in the feed solution was utilized at the

inlet of the column. During column operation, a slight positive nitrogen gas pressure ($\sim 103\%$ of the ambient pressure) was applied to the feed reservoir to prevent penetration of oxygen from the air and to replace the volume of liquid dispensed by the peristaltic pump. Also, all the sampling ports were capped with serum caps. Second, the mineral medium contained 0.25 m*M* of sodium molybdate (Na_2MoO_4) in order to prevent the growth of sulfate-reducing bacteria (Smith and Klug, 1981).

Samples for SOC and NO_3^- determination were taken from the sampling ports, effluent stream and feed reservoir. The SOC concentration was determined by counting the ^{14}C in a filtered and acidified sample using the following procedures. First, the liquid sample was passed through a 0.45 μm membrane filter to remove the suspended bacteria and particles containing labeled organic carbon. Exactly 1 mL of the filtered sample was pipetted into a scintillation vial. Then, CO_2 was driven off by acidifying the sample to pH $\leqslant 2$ with one drop of 1 *N* HCl and shaking the vial for 10 min. in a shaker. Finally, 9 mL of scintillation cocktail were mixed with the sample, and ^{14}C was counted with a Beckman® liquid scintillation counter (model LS-*100*). NO_3^- was determined using the chromotropic-acid method (A.P.H.A., 1981).

Determination of kinetic parameters

Four kinetic parameters – namely, the maximum specific substrate utilization rate (q_m), the half-maximum rate concentration (K), the cell-yield coefficient (Y) and the cell-decay coefficient (b) — were determined from the results of batch reactor experiments. The units of the kinetic parameters are given in a subsequent section. To consider potential physiological differences of cells grown at different locations along the columns, five batch reactors were run in parallel with five different inocula taken from different points within the columns, i.e. four from the two-BAZ column and one from the one-BAZ column. The values of the kinetic parameters determined from the batch experiments for the two-BAZ column varied only slightly, and the average of each was taken as the representative. However, the kinetic parameters, K and q_m, from the batch experiment for the one-BAZ column were different from the two-BAZ column parameters, particularly the K-value. The variation in the kinetic parameters Y and b within the two-BAZ column and between the two columns was slight. The reader is referred to Rittmann et al. (1988) for details related to the kinetic-parameter determinations.

MODEL DEVELOPMENT

Modeling of the formation of a BAZ was based on application of biofilm kinetics to solute transport in porous media. The steady-state biofilm model, developed originally by Rittmann and McCarty (1980) and improved recently by Sáez and Rittmann (1988), was incorporated into a one-dimensional, steady-state-transport equation. The equation was discretized using finite differences and solved numerically directly for the steady-state profiles of substrate con-

centration and biofilm accumulation. Major modeling advancements were the ability to have lateral injection sources at any point along the column and the use of quasilinearization to give a highly efficient and direct solution for the steady-state substrate profile. The following section describes the model and its solution.

Biofilm phenomena and kinetics

Because of the high specific surface area in an aquifer, almost all of the biological activity is associated with the solids as biofilms or microcolonies. Here, the concept of a biofilm was utilized. A biofilm is generally defined as a layer-like aggregation of microorganisms attached to a solid surface (Rittmann and McCarty, 1980). Modeling of biofilm kinetics has been advanced significantly by considering an ideal biofilm that is locally homogeneous and one-dimensional. The processes affecting substrates and biomass are represented by a set of differential and algebraic equations which must be satisfied simultaneously. Only a very brief summary of biofilm modeling is presented here. The reader interested in further details can consult Rittmann and McCarty (1980) and Sáez and Rittmann (1988).

The substrate is transported from the bulk liquid across an idealized layer, L, through which all the resistance to mass transfer lies. Substrate utilization within the biofilm is assumed to follow a Monod relationship, while molecular diffusion within the biofilm is described by Fick's second law. The coupling and solution of the governing equations for substrate transport to the biofilm surface and utilization with diffusion within the biofilm complete the basics of biofilm modeling.

The growth and loss of the biofilm comprise the next important facets of biofilm modeling, and they are the keys to the steady-state biofilm model (Rittmann and McCarty, 1980; Sáez and Rittmann, 1988). For a steady-state biofilm, the growth and the loss rates are equal. The growth of the biofilm is proportional to the flux of substrate, J, multiplied by Y. The loss of biofilm is described by a first-order loss coefficient, b', multiplied by the amount of biomass per unit surface area, $X_f L_f$, where X_f is the microorganism density and L_f is the biofilm thickness. The overall first-order loss coefficient is comprised of two components, namely the cell decay coefficent and the detachment (shear-loss) coefficient. Since the substrate flux is proportional to the substrate concentration, a key concept of steady-state biofilm modeling is that there exists a minimum substrate concentration, S_{min}, below which no steady-state biofilm can occur because losses are greater than growth.

Repetitive solution of all the governing equations for a steady-state biofilm is not practical when the goal is to model a large system, such as for aquifer bioreclamation. As a result of this, several researchers developed pseudo-analytical techniques which provide algebraic equations that fit the numerical results (Rittmann and McCarty, 1980, 1981; Sáez and Rittmann, 1988). The solution presented by Sáez and Rittman (1988) is the most recent and accurate

method available among the several in existence. More accurate over a large range of substrate concentration than previous methods, the new pseudo-analytical technique is the best option for steady-state biofilm modeling. A short summary of the model's structure is presented here for clarity.

The first premise behind the pseudo-analytical technique is that the actual flux to a steady-state biofilm is a fraction, f, of the flux into a deep biofilm. This is represented mathematically by:

$$J = fJ_{deep} \tag{1}$$

where J_{deep} is the flux into a deep biofilm (Rittmann and McCarty, 1980) exposed to the same bulk concentration, S. A deep biofilm has the maximum flux for a given substrate concentration at its surface. The second premise is that the solution is presented most efficiently with dimensionless parameters, which will be denoted with an asterisk superscript. Sáez and Rittmann (1988) found the value of f could be expressed algebraically as:

$$f = \tanh[\alpha\,(S_s^*/S_{min}^* - 1)^{\beta}] \tag{2}$$

where α and β are functions of $S_{min}^* = b'/(Yq_m - b')$; and $S_s^* = S_s/K$ is the dimensionless substrate concentration at the biofilm surface. Because S^* is not known a priori, it must be computed iteratively, using a Newton's root-finding technique, from:

$$S^* = S_s^* + \frac{\tanh[\alpha(S_s^*/S_{min}^* - 1)^{\beta}]\,[2\{S_s^* - \ln(1 + S_s^*)\}]^{1/2}}{K^*} \tag{3}$$

where S^* is the dimensionless bulk substrate concentration; and K^* is a dimensionless group of kinetic parameters. After the convergence to the appropriate S_s^*-value, the dimensionless flux is calculated by Fick's first law:

$$J^* = (S^* - S_s^*)\,K^* \tag{4}$$

J^* is easily transferred into the dimensional flux using the definition of the non-dimensional flux:

$$J = J^*\,(Kq_m X_f D_f)^{1/2} \tag{5}$$

One-dimensional solute transport model

The governing mass balance for a biodegradable compound for steady-state flow through a homogeneous, one-dimensional column has the form:

$$\varepsilon\,(\partial S/\partial t) = D_H\,(\partial^2 S/\partial x^2) - v\,(\partial S/\partial x) - aJ + Q_s \tag{6}$$

where S is the dissolved bulk-substrate concentration; ε is the porosity; D_H is the hydrodynamic dispersion coefficient; v is the specific discharge (superficial flow velocity); a is the specific surface area of the porous medium; J is the substrate flux into the biofilm; and Q_s is the substrate source term due to lateral

input through the injection ports. For the complicated, nonlinear form of J described above, eq. 6 cannot be solved analytically to give S as a function of t and x. Hence numerical solution is necessary.

For numerical solution of eq. 6, the derivatives are discretized in time or space by well-known and straightforward approximation (Lapidus and Pinder, 1982). The difference equations can be solved at successive time steps until a given stopping point, as defined by a particular problem. Traditional approaches to steady-state biofilm modeling (Rittmann, 1982a) involve solving the transient problem, eq. 6, until steady-state is achieved. Here the time derivative, in addition to the spatial derivatives, is approximated using finite differences. The time dimension adds many more computations than are necessary if the steady-state solution can be obtained directly. Thus, the traditional approaches are computationally inefficient and are not feasible for extension to more complex problems (i.e. multi-dimensional space).

Here, a technique solves directly for the steady-state is developed. Eq. 6 can be written directly for steady-state by setting the time derivative to zero. The resulting equation is:

$$0 = D_H(d^2S/dx^2) - v(dS/dx) - aJ + Q_s \quad (7)$$

The direct steady-state equation, eq. 7, was chosen for three reasons:

(1) It approximately describes several realistic scenarios of enhanced in situ bioreclamation; for example, the steady-state input of a limiting factor (the electron acceptor here) into an aquifer containing a fairly constant pollutant source.

(2) The numerical solution of eq. 7 provided an opportunity to introduce new, highly efficient solution techniques for strongly non-linear ordinary differential equations into the biofilm modeling literature. The numerical approach, based upon quasilinearization, also can be applied to other groundwater situations involving nonlinear reaction terms.

(3) The laboratory columns were operated under a steady-state biofilm condition.

The quasilinearization technique

The objective of this section is to present a summary of the quasilinearization technique coupled with the finite-difference solution technique. The equation to be solved is eq. 7, the steady-state one-dimensional transport equation with a biological reaction term. Handling the non-linearity of the reaction rate term (J) is the focus of computational strategy, because the biofilm reaction rate term approaches an infinite reaction order at S-values close to S_{min} (Rittmann, 1982a).

The problems of non-linearity can be overcome by quasilinearization (Lee, 1968). The quasilinearization process involves the use of a first-order Taylor's series approximation for the non-linear substrate flux term and, hence, it is closely related to Newton–Raphson linearization. If S^m is assumed to be the

known substrate concentration at an iteration level m, then the substrate flux at the next iteration level can be approximated as:

$$J(S^{m+1}) = J(S^m) + \mathrm{d}J/\mathrm{d}S \cdot (S^{m+1} - S^m) \tag{8}$$

Eq. 8 can be substituted into eq. 7 to yield a linear ordinary differential equation for S^{m+1}. Finite differences are used to approximate the spatial derivatives and yield a system of simultaneous linear algebraic equations which can be solved for S^{m+1}. The spatial domain is discretized into n intervals of size Δx. A three-point finite-difference approximation was used for the dispersion term, and the advective term was approximated by a central difference (Lapidus and Pinder, 1982). Substitution of eq. 8 and the finite-difference approximations for the derivatives into eq. 7, yields the following finite-difference equation for a grid point i with no source term (Q_s):

$$(cS_{i+1} + dS_i + eS_{i-1})^{m+1} = [aJ(S_i) - a(\mathrm{d}J/\mathrm{d}S)S_i]^m \tag{9}$$

where

$$c = D_H/\Delta x^2 - v/2\Delta x$$

$$d = -2D_H/\Delta x^2 - a(\mathrm{d}J/\mathrm{d}S)$$

and

$$e = D_H/\Delta x^2 + v/2\Delta x$$

The discrete equations are subject to two appropriate boundary conditions for the numerical method to be implemented. The influent condition (at $x = 0$) is:

$$vS^{in} = vS - D_H(\mathrm{d}S/\mathrm{d}x) \tag{10}$$

in which S^{in} is the substrate concentration at the inlet of the column. The boundary condition at the effluent end ($x = L_T$) is:

$$\mathrm{d}S/\mathrm{d}x = 0 \tag{11}$$

When the discrete finite-difference equation is written for each grid point and the appropriate boundary conditions are imposed, a tridiagonal system of equations for S^{m+1} at each grid point results.

The key to implementing the numerical technique with quasilinearization is an efficient and accurate evaluation of the $\mathrm{d}J/\mathrm{d}S$ term, i.e. the Jacobian. The new pseudo-analytical equations developed by Sáez and Rittmann (1988) can be differentiated to yield an expression for $\mathrm{d}J/\mathrm{d}S$ for the entire range of concentrations. After convergence to the S_s^*-value (recall eq. 3); eqs. 3 and 4 can be used to calculate the Jacobian:

$$\mathrm{d}J^*/\mathrm{d}S^* = K^*(1 - \mathrm{d}S_s^*/\mathrm{d}S^*) \tag{12}$$

where

$$\frac{\mathrm{d}S^*}{\mathrm{d}S_s^*} = 1 + \frac{1}{K^*}\left[[2\{S_s^* - \ln(1 + S_s^*)\}]^{1/2}\,\mathrm{sech}^2\left(\alpha(S_s^*/S_{\min}^* - 1)^{\beta}\frac{\alpha\beta}{S_{\min}^*} \times (S_s^*/S_{\min}^* - 1)^{\beta-1}\right) + \frac{\tanh(\alpha(S_s^*/S_{\min}^* - 1)^{\beta}][S_s^*/(1 + S_s^*)]}{[2\{S_s^* - \ln(1 + S_s^*)\}]^{1/2}}\right]$$

The method of quasilinearizatin was implemented (Rittmann et al., 1988) and proved to be very accurate and efficient compared to previous methods (Rittmann, 1982a). The new technique was tested with several sets of kinetic and reactor parameters and had very good accuracy and convergence. At each iteration or time step, the relative change in bulk substrate concentration at each grid point was compared to a specified convergence criterion (typically 0.01–0.1%). The algorithm terminated when the convergence criterion was met. Computational efficiency was characterized by the number of iterations and amount of execution time required to converge to the steady-state solution. The new technique was approximately one order of magnitude more efficient, in terms of execution time and number of iterations to convergence (Rittmann et al., 1988).

Treatment of lateral injection ports

The limiting material added via an injection well to enhance in situ bioreclamation is often consumed very rapidly near the injection well. Such localized biological activity prevents adequate microorganism–contaminant contact throughout most of the aquifer and can also lead to clogging problems. The problem of localized biological activity can be solved, at least in principle, by providing multiple injection wells along the groundwater flow path. Although several experimental investigations have examined the degradation kinetics of specific compounds in porous-medium reactors (Bouwer and McCarty, 1983; Stratton et al., 1983; Bouwer and Wright, 1988), there have been no studies that considered multiple-input locations.

The object of having multiple injections of the electron acceptor is to spread out the BAZ, thus reducing the potential for clogging and ensuring better microorganism–contaminant contact. The Q_s terms in eq. 7 represents injections along the flow path. Accurate and efficient solution of the finite-difference equations becomes a more difficult problem when lateral injections are allowed, because the inputs create local numerical instabilities. Therefore, special treatment is necessary to incorporate the multiple lateral injections. The approach used here was to implement local upstream weighting of the advection term at grid points where any lateral injection ports are located. This technique is a commonly used method to smooth out numerical oscillations (Lapidus and Pinder, 1982). Further numerical details are presented by Rittmann et al. (1988).

Non-limiting substrates

The modeling also was advanced by explicit coupling of the steady-state biofilm model solution, which solves for the concentration profile of the limiting substrate and the amount of biofilm, to a model for the non-limiting substrate. An example of a non-limiting substrate is NO_3^- when SOC is limiting; the flux of NO_3^- into the biofilm was set equal to the flux of SOC multiplied by

a stoichiometric coefficient. Although the flux of the non-limiting substrate was determined by the flux of limiting substrate, it had its own rates of advection, dispersion and injection. Therefore, a solute-transport equation for the non-limiting substrate was solved after obtaining the solution for the limiting substrate. The appropriate equation is similar to eq. 7, but with J stoichiometrically proportional to the flux of limiting substrate.

EXPERIMENTAL RESULTS AND MODEL APPLICATION

One-BAZ *column results*

An amount of nitrate (7.32 mg NO_3^--N L^{-1}) stoichiometrically sufficient for full acetate oxidation was injected through a single injection port in the one-BAZ column. The SOC concentration in the effluent gradually decreased for ~120 days, after which it maintained a very low, steady-state concentration, except for a few cases of fluctuations which were caused by occasional system disturbances, e.g. gas removal from the column (Rittmann et al., 1988). The average exit SOC after day *120* was ~0.2 mg L^{-1}, which corresponds to 97% removal of the input SOC. The ratio of nitrate consumption to acetate removal across the column was typically 0.67 mg NO_3^--N/mg SOC. This compares well to the theoretical stoichiometry (0.64–0.72 NO_3^--N/mg SOC, depending upon the degree of cell decay) predicted by mass and energy balances of bacterial growth (McCarty, 1971).

Fig. 3a shows that the majority of the SOC removal took place in the 2.5-cm region immediately downstream from the nitrate injection port: the rate of

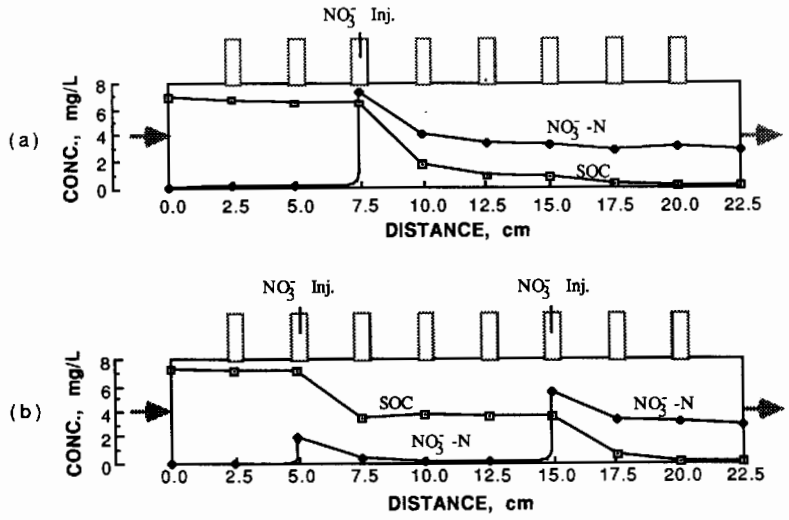


Fig. 3. Typical one- and two-BAZ steady-state profiles: (a) one-BAZ column; and (b) two-BAZ column.

removal diminished toward the column outlet, in most cases showing a plateau of concentration starting at 7.5–10.0 cm downstream after injection. Physical examination of the column also showed that most of the BAZ was contained within ~7.5 cm of the injection. The removal of SOC immediately upstream of the nitrate injection was assumed neglibible, because the calculated back-diffusion of nitrate was insignificant. Thus, the SOC concentration at the injection port, which could not be determined experimentally, was assumed to be the same as the SOC of the immediate upstream port.

Model application to the one-BAZ column

The steady-state, solute-transport model for the limiting substrate (acetate) and the coupled transport model for the non-limiting substrate (NO_3^-) were used to evaluate the one-BAZ column experiment. Kinetic parameters for the utilization of the SOC (q_m and K) were determined, as explained previously, independently in batch experiments with bacteria taken from the column (Rittmann et al., 1988); thus, model results were true predictions. The kinetic

TABLE 1

Reactor and biofilm parameters used in modeling

Parameter	Units	One-BAZ column	Two-BAZ column
Acetate:			
S^0	mg SOC L^{-1}	6.5	7.09
S_{min}	mg SOC L^{-1}	0.0131	0.0497
q_m	mg SOC (mg cells)$^{-1}$ day^{-1}	2.22	2.00
K	mg SOC L^{-1}	0.218	0.80
X_f	mg cells cm^{-3}	15	15
Y	mg cells/mg SOC	0.678	0.678
b	day^{-1}	0.07	0.07
D_{SOC}	cm^2 day^{-1}	1.07	1.07
Nitrate:			
S^0	mg NO_3^--N L^{-1}	7.32	1.92, 5.52
S_{min}	mg NO_3^--N L^{-1}	–	0.0090
q_m	mg NO_3-N (mg cells)$^{-1}$ day^{-1}	–	1.45
K	mg NO_3^--N L^{-1}	–	0.146
X_f	mg cells cm^{-3}	–	15
Y_N	mg cells/mg NO_3^--N	1.02	1.02
b	day^{-1}	–	0.07
D_N	cm^2 day^{-1}	1.40	1.40
Reactor:			
v	cm day^{-1}	144	144
a	cm^{-1}	20.0	20.0
ε	cm^3 cm^{-3}	0.30	0.30

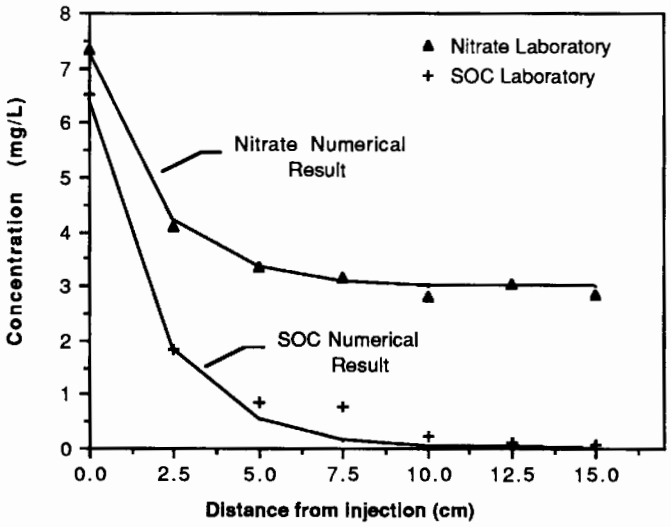


Fig. 4. Comparison of laboratory and numerical results for the one-BAZ column. Zero distance indicates the injection port.

and reactor parameters used in the numerical simulation are shown in Table 1. The hydrodynamic dispersion coefficient, D_H, was determined using the Hiby relationship (Rittmann, 1982a) and the diffusion-layer thickness, L, by the mass transfer correlation reported by Namkung et al. (1983).

Fig. 4 shows the model prediction compared to the experimental results for the one-BAZ column. Model predictions and experimental results agreed quantitatively that removals of SOC and NO_3^- and accumulation of biofilm were greatest in the first 2.5 cm beyond the injection port. Removal rates and biofilm accumulation declined gradually in the next 5.0 cm, and substrate concentrations attained a steady plateau value thereafter. The model predictions correctly described all trends, and absolute deviations between predicted and experimental results were small in all cases.

Two-BAZ *column results*

The two-BAZ column was operated by injecting nitrate in such a manner that about one half of the SOC fed was removed in the first BAZ, and the other half was removed in the second BAZ. The total nitrate injection was the stoichiometrically sufficient amount required to completely oxidize the fed acetate. The ratio was such that the upstream port injected 25% of the total NO_3^- and the downstream injected 75%; this corresponds to 1.92 and 5.52 mg NO_3^-–N L^{-1}, respectively.

Fig. 3b shows typical profiles of SOC and nitrate concentrations. Although nitrate was the rate-limiting substrate after the first injection, it was in surplus after the second injection, making SOC the rate-limiting substrate.

Model application to the two-BAZ column

The two-BAZ column was modeled using the solute transport model and the same reactor parameters as for the one-BAZ column. The kinetic parameters for acetate (as SOC) utilization, K and q_m, were slightly different from those determined for the one-BAZ column (Rittmann et al., 1988). Similar influent SOC concentration was used as in the one-BAZ experiment; however, the electron acceptor was injected in two locations. The two-injection strategy caused NO_3^- to be the rate-limiting substrate in the first BAZ, where it was depleted to close to its S_{min} just before the second injection. At this point, there was ~50% removal of acetate. After the second injection, NO_3^- was in ample supply, and SOC (acetate) became the rate-limiting substrate.

The change of rate-limiting substrate after the second injection of NO_3^- presented an interesting modeling situation. If the electron acceptor had limited the growth throughout the length of the column, the model with lateral injection ports could have been used without modification. In the case of a change of limitation, however, two coupled solute-transport equations had to be used. In the section of the column before the second injection, quasilinearization and finite differences were used to solve the solute transport equation for NO_3^- as the rate-limiting substrate. Then, the profile for SOC (the non-limiting substrate) was calculated using a flux into the biofilm determined by the NO_3^- fluxes and stoichiometry, as reported in the previous section. At the point of the second injection of nitrate, a new solute-transport equation had to be solved. For the points downstream of the second injection, this new solute-transport equation was solved using SOC as the limiting substrate; it was coupled to the upstream segment of the column by requiring the continuity of SOC flux at the injection port. For NO_3^-, the upstream flux of NO_3^- was added to the flux through the injection port, as the upstream flux represented only ~0.18% of the flux through the port. Since NO_3^- was the non-limiting substrate, the NO_3^- profile after the second injection was obtained from the SOC fluxes and stoichiometry.

The kinetic parameters for NO_3^- utilization, which are shown in Table 1, were not independently measured in the laboratory and had to be estimated. The maximum specific rate of substrate utilization, q_m, was taken from the q_m of SOC, adjusted by stoichiometry. The K-value was varied until proper fit of the laboratory column data was obtained. The low value for K for NO_3^- is within the common range for denitrification processes (Rittmann and Langeland, 1985).

Fig. 5 shows the numerical results compared to the laboratory data. The numerical results are in extremely good agreement with the laboratory data. The stoichiometric values used in the numerical modeling, 1.5 mg SOC/mg NO_3^--N and 0.67 mg NO_3^--N/mg SOC for the first and second BAZ, respectively, allowed proper representation of both substrate profiles in both BAZ's. Thus, the choice of which substrate was rate-limiting is justified.

Comparison of Figs. 4 and 5 demonstrates that having two NO_3^- injections

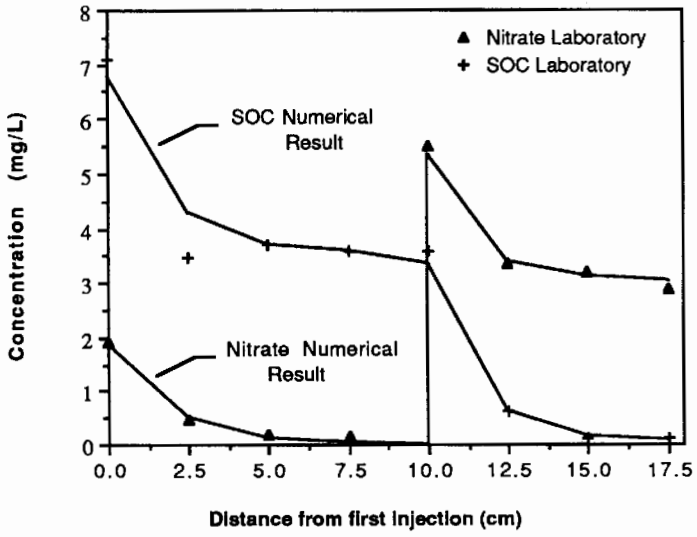


Fig. 5. Comparison of laboratory and numerical results for the two-BAZ column. Zero distance indicates the injection port.

spread out the distance over which a BAZ was present, even though the total amount of injected NO_3^- and the overall removal of SOC were similar in both columns. The importance of spreading the distance of the BAZ is to reduce the biofilm thickness which lowers the clogging potential.

SUMMARY AND CONCLUSIONS

This study investigated fundamental mechanisms that can act when an electron acceptor is injected along the flow path of an electron-donor-rich groundwater to establish a biologically active zone (BAZ) for degradation of pollutants that serve as growth-limiting and non-limiting substrates. The research methodology consisted of laboratory column experiments that were coupled with computer modeling. The laboratory experiments demonstrated that lateral injection of NO_3^- could be successfully utilized to control the location and extent of BAZ's in systems where acetate was the carbon source.

A highly efficient numerical model that couples solute-transport mechanisms and biofilm kinetics was developed by employing a quasilinearization technique for the biofilm reaction term. The model was capable of solving directly for the steady-state profiles of limiting substrate, biofilm thickness and non-limiting substrates. The predictive ability of the model was successfully verified by modeling the results of the laboratory experiments using independently determined kinetic parameters for acetate utilization, cell growth and cell decay. The model predictions correctly described all trends.

The results of this research demonstrate that injection of limiting substrate along the groundwater flow path is a viable means of establishing spatially

distributed BAZ's for enhanced in situ bioreclamation. The phenomena of BAZ formation and substrate utilization within BAZ's can be quantitatively interpreted and predicted at the laboratory scale by rigorous mathematical models that couple principles of solute transport and biofilm kinetics.

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