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FACTORS AFFECTING TRANSPORT OF
PSEUDOMONAS STUTZERI KC

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Patrick D. Radabaugh

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FACTORS AFFECTING TRANSPORT OF *PSEUDOMONAS STUTZERI* KC

By

Patrick D. Radabaugh

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ABSTRACT

FACTORS AFFECTING TRANSPORT OF
PSEUDOMONAS STUTZERI KC

By

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Bioaugmentation is the addition of nonnative microorganisms for bioremediation. In order for this approach to be effective, the injected microorganisms must be transported to specific locations where colonization can occur and contaminants can be degraded. Other researchers have investigated methods of moving bacteria that are easily accomplished in the laboratory, but are difficult to implement in the field. This thesis focused on simulation of options that are practical for field use. Transport properties of *Pseudomonas stutzeri* KC were evaluated in column studies and modeled with clean bed filtration theory. The variables tested include cell physiology, cell concentration, type of electron acceptor, and extent of flocculation. Injection of strain KC as undiluted, non-flocculent, exponentially growing inoculum resulted in the best transport. Diluted and flocculent cultures transported poorly. The results demonstrate that inocula for bioaugmentation purposes can be prepared in a manner that promotes cell transport, without resorting to expensive alternatives.

to Pygmy ruggers everywhere... ...

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V_T	DVLO theory energy of interaction, joules
V_R	DVLO theory repulsive energy, joules
V_A	DVLO theory Van der Waals energy of attraction, joules
D_e	dielectric constant of water, unitless
r_s	radius of sand particles, meters
r_b	radius of bacteria, meters
Ψ_s	zeta potential of sand, volts
Ψ_b	zeta potential of bacteria, volts
δ	inverse double layer thickness, meters^{-1}
x	distance, meters
H_{sb}	Hamaker constant for sand and bacteria, joules
C_o	initial concentration of bacteria, #/l
C	concentration of bacteria, #/l
t	time, minutes
D_a	dispersion term in advection-dispersion equation, m^2/sec
v	groundwater pore velocity, m/hr
u	upstream velocity (Q/A), m/hr

R_a	attachment term in advection-dispersion equation, $time^{-1}$
R_d	Detachment term in advection-dispersion equation, $time^{-1}$
n	column porosity, unitless
α	Collision efficiency (number of collisions that result in attachment compared to total number of collisions)
η	collector mass transfer efficiency (ratio of particles that strike a collector compared to number that flow toward a collector = d_s/d_f)
a_c	accumulation of particles on filter, #/time
d_s	rate at which particles strike a collector, #/time
d_f	rate at which particles flow toward a collector, #/time
N_c	number of collectors in column
A	cross sectional area of column, m^2
α_o	clean bed collision efficiency, unitless
β	Blocking factor (ratio of blocked area of cell to area occupied by the cell), unitless
θ	fraction of surface area covered (ratio of area covered by cells to the total surface area of all the collectors in the column)
A_s	Correction factor in collector mass transfer efficiency equation to correct for neighboring collectors (Happel, 1951), unitless
p	a function of porosity, unitless
N_{Pe}	Peclet number, unitless
N_{Lo}	London/Van der Waals term, unitless
N_R	straining term, unitless

N_G	sedimentation term, unitless
D	diffusion constant of bacteria, m^2/s
ρ_b	density of bacteria, kg/m^3
ρ	density of growth medium, kg/m^3
g	gravitational constant, m/s^2
μ	viscosity of medium, kg/m*s
l	column length, meters
t_{hyd}	column hydraulic retention time, min
λ	filtration constant, $meters^{-1}$
Q	flow rate into column, cm^3/min
A_{col}	surface area available in a column, m^2
d_c	diameter of columns, cm
k	mass transfer coefficient, s

Chapter 1

INTRODUCTION

In recent years, interest in remediation of contaminated soils and aquifers has increased with the increasing number of contaminated drinking water wells. This has led to a rise in the number of treatment technologies. Present technology for the clean up of groundwater includes various pump and treat schemes. However, in situ bioremediation offers a less expensive alternative for sites contaminated with organic compounds. Two processes can be employed for in situ bioremediation: biostimulation and bioaugmentation.

Biostimulation involves "stimulating" the growth of native or indigenous bacteria by providing them with growth-limiting nutrients. A potential problem with use of indigenous bacteria is lack of pathway control and the production of compounds that are more toxic or persistent than the original compound. Biodegradation of carbon tetrachloride is an example. Carbon tetrachloride, a suspected human carcinogen, has been used in various industrial processes and as a fumigant in grain silos and railroad cars. In the laboratory and field, chloroform is often produced during bacterial decomposition of carbon tetrachloride (Criddle et al., 1990; Semprini et al., 1992; Vogel et al., 1987). Chloroform is a "probable human carcinogen of low carcinogenic hazard" (EPA, 1998).

Bioaugmentation involves introduction of non-native microorganisms. This process allows for the use of an organism that has been well studied in the laboratory, whose growth kinetics are known, and whose behavior can be accurately modeled. One potential problem

with this approach is that imported bacteria may be unable to compete with the indigenous flora. Another potential problem is inability to transport the added microorganisms.

A carbon tetrachloride contaminated aquifer, located at Schoolcraft, Michigan, has been the focal point of a combination laboratory/field study for the development of an in situ bioaugmentation remediation process. *Pseudomonas stutzeri* KC (strain KC) degrades carbon tetrachloride to carbon dioxide (CO_2), formate ($HCOO^-$) (Dybas et al., 1994), and an unidentified nonvolatile fraction, without the production of chloroform, under iron limiting, denitrifying conditions (Tatara et al., 1993;, Criddle et al., 1990). Dybas et al. (1994) found that by increasing the pH of the system to 7.9 to 8.2, strain KC had a competitive advantage over microflora in soil and groundwater, including microflora native to Schoolcraft aquifer sediments. When the pH was reduced, strain KC numbers also drastically reduced.

Generally in the field of bioremediation, there is increasing interest in the formation and maintenance of biofilms. In situ bioremediation designs typically involve a biofilm process. A biofilm can be defined as an accumulation of biomass at any solid-water interface, where biomass includes cells and cellular products. In systems with interfaces (soil, streams, etc.) most of the bacteria are attached to surfaces as biofilms (Stotzky, 1972; Wuhrman, 1971; Marshall, 1972). Biofilms occur widely in nature on rocks in flowing rivers or streams, in lakes, on boat hulls, and aquifer and soil systems, on the teeth and in the intestinal tracts of animals, and in systems for the detoxification of hazardous and non-hazardous waste. At Schoolcraft, an in situ biofilm process is being used to degrade carbon tetrachloride.

Prediction of biofilm formation and control of biofilms is of interest because of the many beneficial and harmful roles of biofilms. Biofilms are used in wastewater treatment on

trickling filters, rotating biological contactors, fluidized bed reactors, and various anaerobic processes. Biofilms have also been used in slow sand filters (the "schmutzdecke"), in remediation, in the mining industry to help with ore leaching, and in microbial enhanced oil recovery (MEOL) schemes. Biofilms are also responsible for decreased efficiency of heat exchangers, increased drag on ships, cavities, infections in animals, corrosion, and increased energy requirements for pumping. Despite their widespread use and occurrence, the nature of the formation and maintenance of biofilms is not fully understood.

Because biofilms are ubiquitous, it is clear that attachment is advantageous. Some of the advantages of biofilms include: (Criddle et al., 1991; Loosdrecht, 1990;, Rijarnts, 1994)

- Reduced threat of predation. Attached cells are less prone to predation from viruses, bdellovibrio, and protozoa because they have less exposed surface area.
- Preservation. Some bacteria are known to attach in the presence of substrate, thus securing themselves near a food supply.
- Substrate availability. Numerous organic substrates accumulate at the solid-water interface.
- Benefits of a microenvironment. The biofilm community promotes conditions that are beneficial to the growth and survival of its members. This may include regulation of pH, reduced exposure to toxicants, protection from desiccation, and enhanced capacity for degradation.

Mechanisms of Deposition

Bacterial deposition occurs in a stepwise fashion. The two primary steps are transport and adhesion. Transport is dominated by hydrodynamic phenomena, while adhesion involves of a variety of processes. After the initial deposition, bacteria may secrete exopolymers (fimbrils, adhesins) and multiply. This, in turn, promotes the formation of attached microcolonies and the development of a biofilm.

Transport

The transport step conveys the microorganism to the solid surface where an interaction may occur. The more rapid the transport, the greater the number of microorganisms that come in contact with the solid surface, and the greater the chances of adhesion. This statement holds until the velocity in the medium is so high that shear stresses prevent adhesion. The three major factors affecting cell transport are the diffusion of the organism, convective transport, and motility. Rijnaarts et al. (1993) concluded that convection plays a larger role in attachment than diffusion.

Adhesion

Adhesion refers to the process whereby the colloidal microorganisms attach to solid surfaces. Forces known to affect adhesion include hydrophobicity of the cell, electro-potential of the cell (measured as zeta potential), surface tension, Van der Waals forces, surface protein ionization (pK_a), and physical forces as impaction, straining, and shear stress. Because so many forces contribute to adhesion, there is no single model in the literature that can fully account for the observed interactions between cells and substratum.

Static System Models

Hydrophobicity

Various researchers have developed simple laboratory assays of bacterial hydrophobicity and capacity for attachment to solids (van der Mei, Weerkamp, and Busscher, 1987, Stenström, 1988, Dillon et. al., 1986, Mozes and Rouxhet, 1987, VanHaecke et. al., 1990). Tests used for this purpose include the Bacterial Adherence To Hydrocarbons (BATH) (Rosenberg, 1980), hydrophobic interaction chromatography (van der Mei, 1987, Dillon et. al., 1986), salt aggregation (Lindahl et. al., 1981), adherence to polystyrene (Dillon et. al., 1986), and contact angle measurements (Busscher et. al., 1984). The data collected from these experiments was used as relative measure of hydrophobicity between strains, although there was no correlation between any of the tests (Dillon et. al., 1986).

Surface Free Energy (Interfacial Tension)

This equilibrium model relies on the formation and destruction of interfaces between water-cells, cells-substratum, and substratum-water. Data can be collected in the lab by determining the contact angle of bacteria and substratum for polar and non-polar liquids with known surface tensions and by completing a force balance. This model does not account for electrostatic or Van der Waals forces.

DVLO (Derjaguin and Landau, Vervey and Overbeek)

DVLO Theory describes repulsive and attractive forces between a colloid and a surface. It is applied to bacteria under the assumption that bacteria act as colloids when interacting with surfaces. The energy of attraction, V_T, is described by:

$$V_T = V_R + V_A \tag{1}$$

where V_R = repulsion energy (joules)
V_A = attraction energy (joules)

Electrostatic repulsive forces are a function of the charge on the interacting bacteria and surface (measured as zeta potential), the radius of the interacting species, dielectric constant of the fluid, ionic strength (affects the inverse double layer thickness), and the distance between the bacteria and the surface.

$$V_R = \frac{D_e}{4}\left(\frac{r_s r_b}{r_s + r_b}\right)\left[(\psi_s + \psi_b)^2 \ln\left(1 + e^{-\delta x}\right) + (\psi_s + \psi_b)^2 \ln\left(1 - e^{-\delta x}\right)\right] \tag{2}$$

where r = radius of sand particles ($_s$) and bacteria ($_b$), m
D_e = dielectric constant
ψ = zeta potential of sand ($_s$) or bacteria ($_b$), V
δ = inverse double layer thickness, m^{-1}
x = distance between surfaces, m

Van der Waals forces act to draw the two particles together. The Van der Waals forces, V_A, are a function of distance between the particles, the Hamaker constant, and the radius of the interacting particles.

$$V_A = \frac{-H_{sb}}{6x}\left(\frac{r_s r_b}{r_s + r_b}\right) \tag{3}$$

Numerous studies have shown that increasing ionic strength over several orders of magnitude can decrease the inverse double layer thickness increasing attachment of bacteria to solids (Jewitt et al., 1995; Rijnaarts et al., 1996a). Although sufficient in describing the effects of ionic strength, the DVLO theory cannot account for hydrophobic effects and localized charges on the cell and substratum that could affect attachment.

Isotherm Models

Loosdrecht et. al. (1989) used Langmuir and Volmer isotherms to estimate interaction free energy between the bacteria and the substratum for separation distances greater than 1 nm. In addition to assumptions made when using isotherms, the authors make the assumption that adhesion occurs at the secondary minimum in DVLO theory and that bacteria at this level are free to migrate parallel to the surface.

Modelling Systems

Isotherm models using partition coefficients for the attachment and detachment of bacteria have also been used in the advection-dispersion equation (Witt et. al., 1995, Peterson and Ward, 1989, Corapcioglu and Haridas, 1984, Escher, 1987, and Sarkar et. al., 1994). A typical one dimensional transport equation is as follows:

$$\frac{\partial C}{\partial t} = D_a \frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - R_a + R_d \qquad (4)$$

where C = concentration of a bacteria (#/l)
t = time (s)
D_a = dispersion term (m^2/s)
v = velocity term (m/s)
R_a = attachment term
R_d = detachment term

All of the above-cited authors above use a linear equilibrium partitioning coefficient with the exception of Sarkar et al., (1994) who used the following nonlinear equilibruim coefficient::

$$C_{fD} = \frac{AC_{TD}}{1 + BC_{TD}} \tag{5}$$

where C_{fD} = dimensionless flowing bacterial concentration
C_{TD} = dimensionless total bacterial concentration = $(C_T - C^*)/C_T$
A,B, and C* need to be experimentaly determined

Filtration theory is also derived from the advection dispersion equation. Filtration theory is a mass balance over a control volume similar to equation (4), (see figure 1.1). The sink term, R_s, becomes the filtration equation and R_d is removed. R_d is assumed negligible (Rijnaarts et al., 1996).

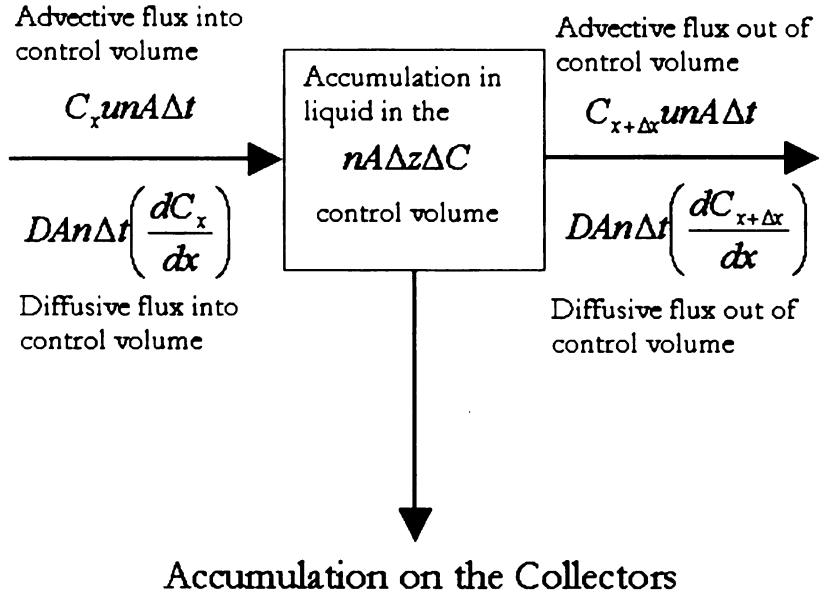

Figure 1.1 Mass balance on particles in a control volume in colloid filtration theory.

where u = upstream groundwater velocity, m/min
n = porosity of column
A = cross sectional area of column, m^2
D = diffusion coefficient of bacteria, m^2/min
x = position, meters

If the accumulation of particles on collectors is defined as

$$a_c = d_s \alpha N_c \Delta t \tag{6}$$

where d_s = the rate at which particles strike a collector = $\eta u n C \pi r_s^2$
α = the collision efficiency
η = collector mass transfer efficiency (unitless)
N_c = the number of collectors in a control volume = $A(1-n)L/(4\pi r_s^3/3)$
r_s = the radius of the sand particles in the column (m)

A mass balance equation can be written over the control volume in the form of equation (7).

$$C_z unA\Delta t - DAn\Delta t\left(\frac{dC_x}{dx}\right) - C_{x+\Delta x} unA\Delta t + DAn\Delta t\left(\frac{dC_{x+\Delta x}}{dx}\right) = nA\Delta z\Delta C + \frac{3}{4r_s}(1-n)\alpha\eta C unA\Delta z\Delta t \tag{7}$$

Dividing equation (7) by $nA\Delta x\Delta t$, taking the limit as Δx and Δt go to zero, substituting in equation (6) for the acculumation on the collector term, and assuming dispersion and accumulation in the liquid are negligible, yields.

$$\frac{dc}{dx} = -\frac{3}{4}\left(\frac{1-n}{r_s}\right)\alpha\eta C \tag{8}$$

Several researchers have used colloid filtration theory to model bacterial deposition in porous media including Martin et al. (1992), Harvey and Garabedian (1991), Logan et al. (1995), Martin et al. (1996), Johnson and Logan (1996), and Rijnaarts et al. (1996b). Assumptions for use of clean bed filtration theory are provided in Table 1.1.

Table 1.1 Assumptions of clean bed filtration model.

Assumptions for clean bed filtration modeling of bacterial deposition in porous media
bacteria are modeled as spherical colloids in the collector mass transfer efficiency equation
collectors are spherical and uniform
ideal plug flow in filter
particles do not interfere with each other
monolayer coverage of bacteria on collectors
steady state conditions

Rijnaarts et al. (1996b) found that bacterial transport in porous media could be modeled using clean bed filtration theory where the collision efficiency is a function of cell-solid interactions and cell-cell interactions as defined in equation (9) for monolayer coverage.

$$\alpha = \alpha_o(1 - \beta\theta) \tag{9}$$

where α_o = the clean bed collision efficiency (dimensionless
β = dimensionless blocking factor (ratio blocked area per cell to cell area)
θ = fraction of collector surface area covered

Cell-cell interactions occur when attached cells prevent the adhesion of more cells by screening or blocking the collector surface (Rijaarnts et al., 1996a). The blocking factor, β, and the fraction of surface covered, θ, are related by $\theta_{max} = 1/B$. Equation (9) states that initially $\alpha=\alpha_o$ and $\theta=0$ and that as surface coverage increases (θ), the collision efficiency (α) decreases.

Unlike isotherm models, which do not separate the contributing forces behind attachment, filtration theory separates and quantifies the contributions of each identified factor that may contribute to attachment. The collector mass transfer efficiency (η) is a "semiempirical" summation of all the forces (equation 10) acting on a cell and substratum and is defined as the probability of a particle colliding with the substratum (the collector) (Rajagopalan and Tien, 1976; Tobiason and O'Melia, 1988; Logan et al., 1995).

$$\eta = 4A_s^{1/3}N_{Pe}^{-2/3} + A_s N_{Lo}^{1/8} N_R^{15/8} + 0.0038 A_s N_G^{1.2} N_R^{-0.4} \tag{10}$$

The first term in equation (8) is for the contribution of advective to diffusive forces. The second term represents the contribution of London/Van der Waals forces and the third term, represents the physical forces of sedimentation and impaction. The variables that compose the terms of η are defined in equations 11 to 15. A_s is a correction factor determined by Happel (1958) to account for the effects of neighboring collectors.

$$A_s = \frac{2(1-p^5)}{(2-3p+3p^5-2p^6)} \tag{11}$$

where $p = (1\text{-}n)^{1/3}$ and n = the column porosity

The Peclet number (N_{Pe}) is a representation of the ratio between plug and diffusive flow.

$$N_{Pe} = \frac{2ur_s}{D_b} \tag{12}$$

N_{Lo} represents the attraction of the bacteria and the sand by Van der Waals/London forces.

$$N_{Lo} = \frac{H_{sb}}{9\pi r_b^2 u\mu} \tag{13}$$

N_R is the ratio between bacterial radius and the radius of the particle with which it is colliding. It represents the effect of straining.

$$N_R = \frac{r_b}{r_s} \tag{14}$$

N_G represents the effects of sedimentation.

$$N_G = \frac{2r_b^2(\rho_b - \rho)g}{9\mu u} \tag{15}$$

The variables used in these equations can be found in Table 1.2.

Table 1.2 List of variables.

Variable	Title	Value	Units
θ	porosity	0.41	unitless
u	velocity	varies	meters/second (m/s)
r_s	radius of sand particle	0.0001	meters (m)
r_b	radius of bacteria	$4.1*10^{-7}$ (aerobic) $3.3*10^{-7}$ (denitrifying)	meters (m)
D_b	Diffusion coefficient of bacteria	$3.65*10^{-13}$ (Rijnaarts, 1995)	meters^2/sec (m/s)
H	Hamaker constant	$2*10^{-22}$ (Rijnaarts, 1995)	joules (J)
ρ	density of media	997.2	kilograms/meters^3(kg/m^3)
ρ_b	density of bacteria	1000 (estimated)	(kg/m^3)
μ	viscosity of media	$9.99*10^{-4}$	Kg/m*s
g	gravitational constant	9.81	m/s^2

If blocking is negligible, equation (9) can be integrated over the length of the column (L) to produce

$$\frac{C}{C_o} = \exp\left(-\frac{3}{4}\frac{(1-n)^{1/3}}{r_s}\alpha\eta L\right) \tag{16}$$

However, when blocking does occur, equation (8) can be solved and the effluent from a soil column becomes a function of time as in equation (17) (Tien, 1989).

$$\frac{c}{c_o} = \frac{\exp\left(\frac{\pi r_b^2 u c_o \eta \alpha_o \beta t^*}{4}\right)}{\exp\left(\frac{3(1-n)_s \eta \alpha_o x}{4 r_s}\right) + \exp\left(\frac{\pi r_b^2 u c_o \eta \alpha_o \beta t^*}{4}\right) - 1} \tag{17}$$

where t^* = actual time minus column hydraulic retention time.

A mass balanced performed on the liquid phase within a column yields.

$$\frac{dc}{dt} = -kC\alpha_o(1 - B\theta) \tag{18}$$

where k is defined as the mass transfer coefficient (equation 19).

$$k = \frac{3u\eta}{4r_s}\left(\frac{1}{n} - 1\right) \tag{19}$$

This theory only accounts for single layer adhesion. Some modification is required for multi-layering attachment ($\alpha > 1$). Further modifications are suggested for this model in this thesis.

One of the goals of this thesis was to develop a method of conducting cell tranposrt experiments that would realistically simulate field conditions. All experiments were performed and designed so that strategies developed in the laboratory for improved cell transport could be easily implemented in the field. The variables chosen for investigation were those that could be easily modified in the field with mimimal effort. Accordingly, the inocula used in cell transport experiments were not modified following growth (previous researchers have washed and resuspened their bacteria) nor was the sand washed (other esearchers have performed transport experiments on sterile sand).

Hypotheses:

1. A laboratory method can be developed in the laboratory that can mimic field injection conditions without modifying the bacteria.
2. Cell growth stage affects transport. Cells that are exponentially growing are more readily transported than older cells.
3. Flocculating cultures do not transport well. The result is filter ripening and clogging.
4. Lower cell concentrations in the liquid enhances cell transport.
5. Electron acceptors influence cell transport properties.

MATERIALS AND METHODS

Organism

Pseudomonas stutzeri KC (DSM deposit no. 7135, ATCC deposit no. 55595) was routinely maintained on R2A agar (Difco) plates.

Chemicals

All chemicals used were ACS reagent grade (Aldrich or Sigma Chemical Co.). Radioactive acetate with a specific activity of 6.7 mCi/mmol was obtained from Sigma.

Groundwater

Groundwater and the aquifer core samples were obtained from a carbon tetrachloride-and nitrate-contaminated aquifer Schoolcraft, MI. Groundwater samples were obtained from wells in the delivery well gallery constructed for Phase 2 of the Schoolcraft Bioaugmentation Project. These wells are screened from 30 to 80 feet below the ground surface. Groundwater samples were collected in 20 gallon presterilized Nalgene® carboys and stored at 4° C. Aquifer core samples were obtained from the grid for use in the column experiments. Once in the lab, the core material was placed in a presterilized food grade 5 gallon food grade bucket submerged in unpasteurized Schoolcraft groundwater adjusted to pH 8.2. Aquifer material was kept at 4° C until use.

Filters

Radiolabeled cells were removed from their growth medium by filtration through 0.2 μm polycarbonate membranes (Corning Nucleopore Track-Etch Membranes catalog no. 110606).

Columns

Column studies were performed using 2.5 cm diameter chromatography columns (Kontes catalog no. 420400-2510 for columns 10 cm in length and catalog no. 42400-2520 for 20 cm columns) fitted with flow adapters (Kontes catalog no.420415-2500). The flow adaptors allowed the packing of the columns to be different lengths.

Methods

Groundwater Medium

For aerobic growth, Schoolcraft groundwater was supplemented with 1600 ppm acetate (as sodium salt). For growth of denitrifying cells, groundwater was supplemented with 1600 ppm acetate and nitrate (both as sodium salts) prior to pasteurization. Supplemented groundwater was pasteurized by placing it in a 60°C oven for 8 hours (Knoll, 1994). Pasteurized groundwater was stored at 4°C. Just prior to use, 10 ppm of phosphate (PO_4^{-3}) was added and the pH of the groundwater was adjusted to 8-8.2 using sterilized 1 M hydrochloric acid (HCl) or 1 M sodium hydroxide (NaOH). When radiolabeled acetate was used, it was added just prior to inoculation of the groundwater with strain KC.

Analytical Methods

All ions (nitrate, nitrite, phosphate, bromide, acetate, sulfate) were measured by ion chromatography (Dionex model 2000i/SP ion chromatograph with suppressed conductivity detection and a Dionex Ionpak AS4-A anion exchange column). Protein samples were measured using the modified Lowry protein assay (Markwell et al., 1981). pH was measured using a Orion pH probe and meter. Radiation samples were placed in scintillation vials along with 10 ml scintillation cocktail and measured in a Packard 1500 Tri-Carb scintillation counter. Each radiation sample was counted for 5 minutes. Spectrophotometric samples were measured on a Shimadzu UV-160 spectrophotometer.

Growth of ^{14}C-Labeled strain KC

An important criterion for cell transport experiments was that the growth and injection conditions mimic field conditions. Numerous researchers have studied bacterial transport in columns using washed cells harvested in either the late exponential or early stationary phase and resuspended in a buffer to the desired concentration. Such procedures are generally not representative of field methodologies and were therefore avoided in this study.

Because realistic growth conditions were desirable, one goal of this research was to develop a measurement technique that did not alter growth conditions, was easy to measure, and was representative of field conditions. Prior to inoculation of the groundwater medium with strain KC, 20 μCi of ^{14}C-labeled acetate ($^{14}CH_3COONa$) was added to the culture. Acetate served as the electron donor and carbon source in all experiments. Strain KC was labeled as it incorporated acetate into its cell mass. This is illustrated in Figure 2.1.

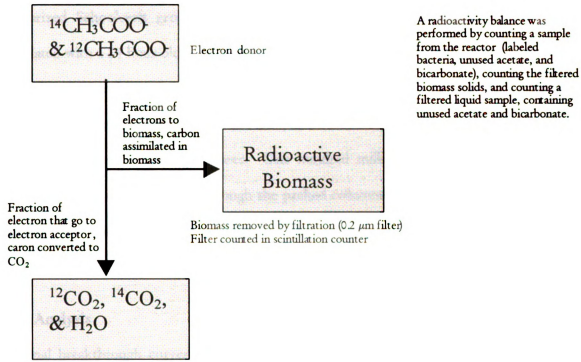

Figure 2.1 Radiolabeling of strain KC.

Preparation of Packed Columns

Columns were wet packed with Schoolcraft aquifer sand to a porosity of 0.41. For each experiment, a set of three columns were packed to approximate lengths of 3, 6, and 9 cm. Because the columns and their retention times were short, growth of the inoculum within the columns is negligible.

Transport Experiments

For transport experiments, *Pseudomonas stutzeri* KC was grown in glass reactors using pasteurized Schoolcraft groundwater medium at pH 8.2. Groundwater medium was inoculated with a 24 hour old culture of strain KC grown in nutrient broth (Difco). A 0.6% (by volume) inoculum was added. Approximately 400 ml of the reactor were removed aseptically for transport experiments at 18, 24, and 30 hours for aerobic cultures and 18, 30, and 48 hours for denitrifying cultures. One hundred milliliter syringes were filled with culture, which was then pushed through the packed columns with syringe pumps. Effluent samples were collected with a fraction collector and then analyzed for either tracer or suspended radioactivity.

Data Analysis

Bacterial breakthrough curves were plotted with time. A filtration coefficient, λ, can be found taking a linear regression of the breakthrough curves and integrating equation (9) to form equation (20).

$$\ln\left(\frac{C}{C_o}\right) = -\lambda L \tag{20}$$

where C_o = influent concentration of bacteria
L = column length
λ = filtration constant (see equation 21)

The collision efficiency, α, can then be calculated from the filtration constant, λ, as in equation (21).

$$\lambda = \frac{3}{4}\left(\frac{1-n}{r_s}\right)\alpha\eta \tag{21}$$

The collision efficiency calculated in equation (21) is substituted into equation (9) and the clean bed collision efficiency, α_o, and the blocking factor, β, can be determined for each column by a simple linear regression procedure. The other variable in equation (9), the fraction of surface covered, θ, can be calculated from equation (22).

$$\theta(t) = \left[\frac{QC_o}{t}\int_0\left(1 - C/C_o\right)dt\right]\frac{\pi a_b^2}{A_{col}} \tag{22}$$

where Q = flow rate into the column, m^3/min
A_{col} = surface area of the porous media, m^2/m^3

The surface area in the column, A_{col}, can be calculated from equation (23).

$$A_{col} = \frac{3(1-n)}{r_s}\cdot\pi\left(\frac{d_c^2}{2}\right)L \tag{23}$$

where d_c = the diameter of the column in meters

INFLUENCE OF CELL PHYSIOLOGY ON TRANSPORT IN POROUS MEDIA

Introduction

For field application, it is desirable to be able to control cell transport properties with minimal effort. If cell transport properties are controlled by the cell physiology, culture growth stage could be a valuable control variable in injection schemes for bioaugmentation cleanups. The goal of this chapter is to investigate the influence of the growth of the culture on its transport properties.

Materials and Methods

Details of the methods used for these experiments can be found in chapter 2. For this experiment, column effluent was monitored by cellular protein as measured in the modified Lowry protein assay (Markwell et al., 1981). The flow rate was set at 2 ml/min for the aerobic culture and 3.5 ml/min for the denitrifying culture, which corresponded to pore velocities of 0.60 and 1.04 m/hr, respectively.

Results and Discussion

The bacterial breakthrough curves for an aerobic strain KC culture in mid-exponential growth (culture grown for 18 hours from a 0.6% inoculum) are provided in Figure 3.1. C/C_o is the ratio of effluent to influent concentration of strain KC.

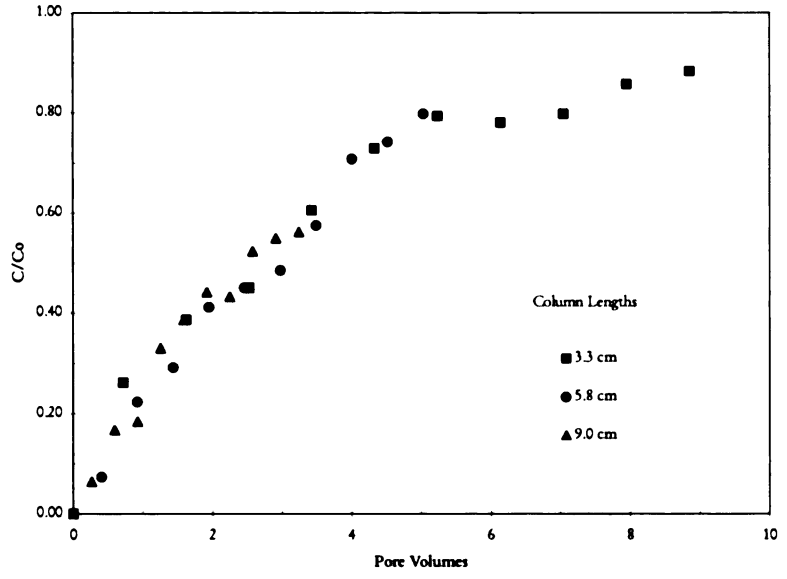

Figure 3.1 Bacterial breakthrough curves for an aerobic strain KC culture during mid-exponential growth.

Figure 3.2 illustrates bacterial breakthrough curves for an aerobic strain KC culture in early stationary phase (24 hours).

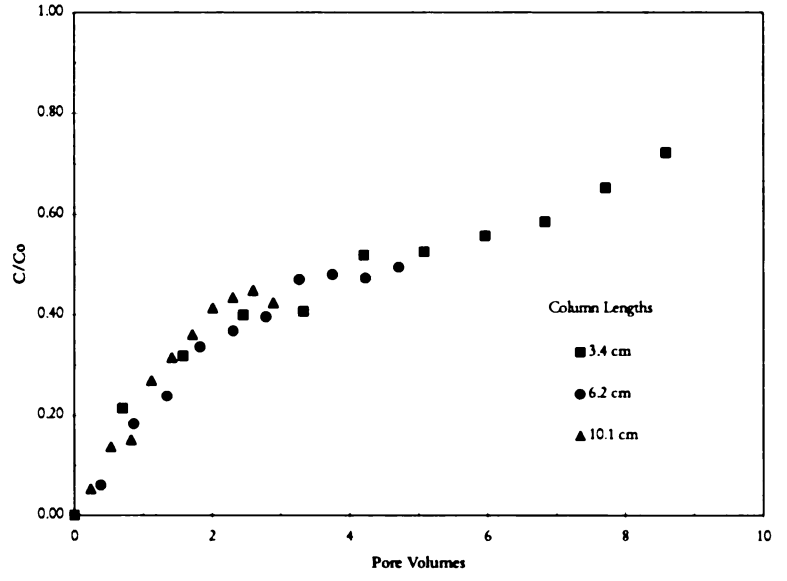

Figure 3.2 Bacterial breakthrough for an aerobic strain KC culture in early stationary phase.

Figure 3.3 illustrates results for a 30 hour aerobic culture in mid-stationary phase.

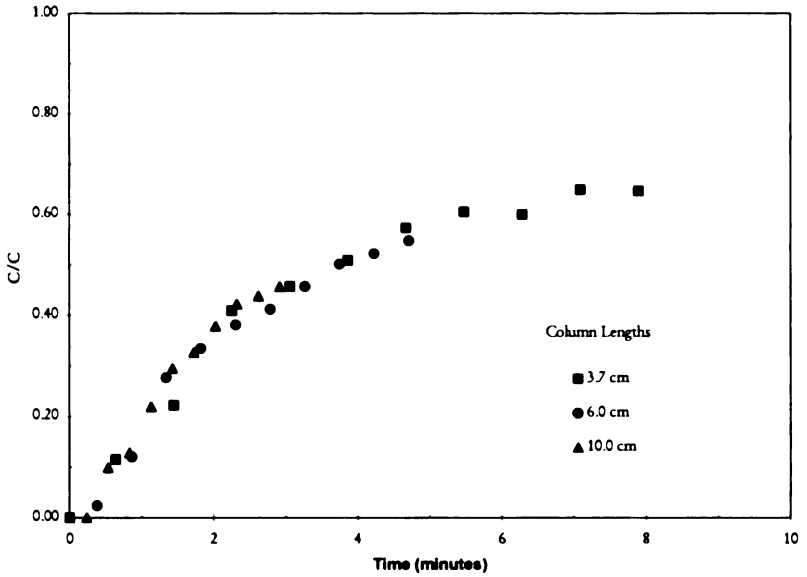

Figure 3.3 Bacterial breakthrough of an aerobic mid-stationary strain KC culture.

Additional transport experiments were performed with nitrate (NO_3^-) as the electron acceptor. While denitrifying, strain KC has two exponential growth phases separated by a short stationary phase (Knoll, 1994). The first exponential growth phase utilizes nitrate as the electron acceptor and the second utilizes nitrite (NO_2^-) (Knoll, 1994). The bacterial breakthrough curves can be found in figures 3.4, 3.5, and 3.6. Figure 3.4 contains the results from a denitrifying culture during its first exponential phase.

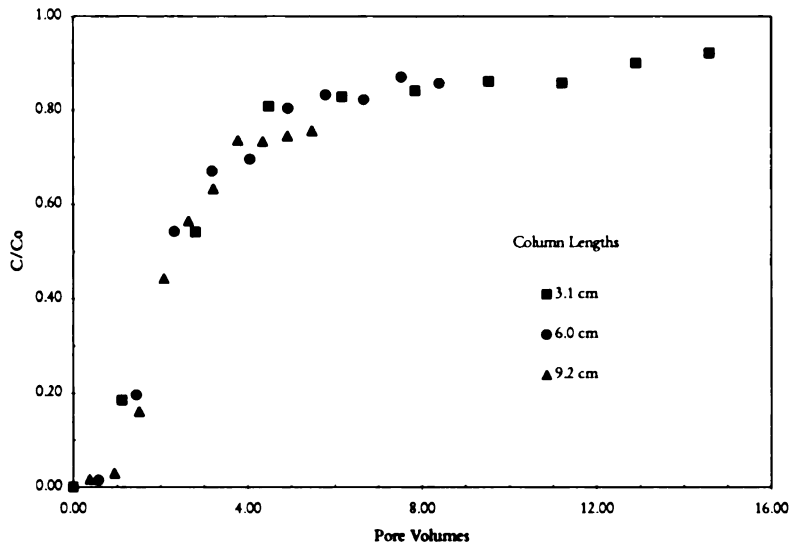

Figure 3.4 Bacterial breakthrough of denitrifying culture during its first exponential phase.

Figure 3.5 illustrates the breakthrough of strain KC during its short stationary phase between its two exponential phases.

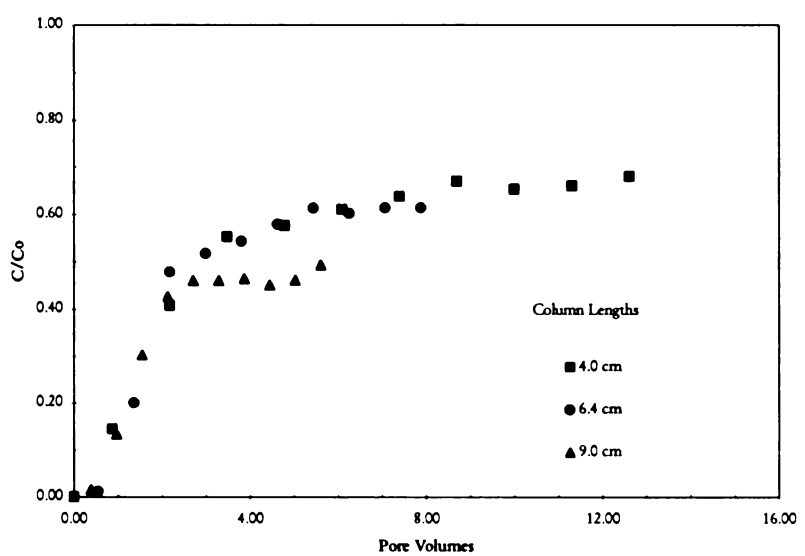

Figure 3.5 Bacterial breakthrough of denitrifying culture during stationary phase.

Figure 3.6 illustrates the breakthrough of a strain KC culture early in its second stationary phase.

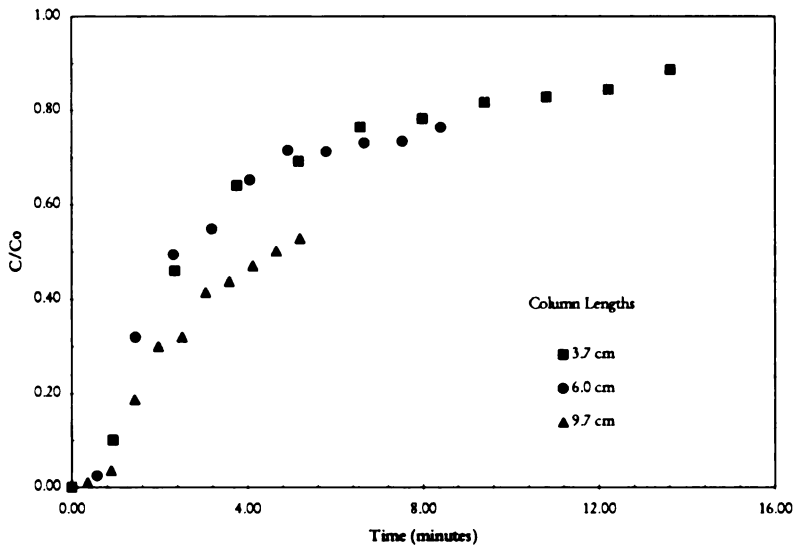

Figure 3.6 Bacterial breakthrough of denitrifying culture during its second stationary phase.

Table 3.1 gives the results of clean bed filtration modeling for figures 3.1-3.6. For α_o, the averages of the three columns are indicated and ,for β, the results are stated for each column. Rijnaarts (1996b) observed a similar trend of increasing blocking factor with an increase in column length and suggests that blocking becomes independent of length with columns of length greater than 7.5 cm (Rijnaarts, 1996b). A likely explanation for the increase in blocking is multilayer adhesion and ripening of the filter (sand column) in the shorter columns. The data of Table 3.1, along with data presented in Chapter 4, suggests that aging cultures of strain KC have altered their cell surface facilitating deposition and attachment to solids.

Table 3.1 Attachment efficiency and blocking factor.

Electron Acceptor	Culture Phase	α_o	β		
			Short	Medium	Long
O_2	mid-exponential	0.44±0.07	2020	2280	2980
O_2	early stationary	0.28±0.00	45.3	82.7	134
O_2	mid-stationary	0.32±0.00	49.6	80.4	134
NO_3^-	exponential	0.64±0.02	1240	2050	2690
NO_3^- & NO_2^-	stationary	0.57±0.00	38.9	62.3	87.6
NO_2^-	stationary	0.78±0.00	59.8	96.9	156

In order to prove that increased attachment in aged cultures was a function of cell age and not cell concentration, another experiment was performed in which cell concentrations were all the adjusted to the same level (approximately $2*10^7$ cfu/ml). Figure 3.4, 3.5, and 3.6 are the bacterial breakthrough curves for the mid-exponential, early stationary, and mid-stationary cells, respectively. A fraction of early stationary and mid-stationary phase culture was removed aseptically from the reactor and diluted using pasteurized Schoolcraft groundwater previously adjusted to the same ionic strength and pH as the medium used to grow the culture.

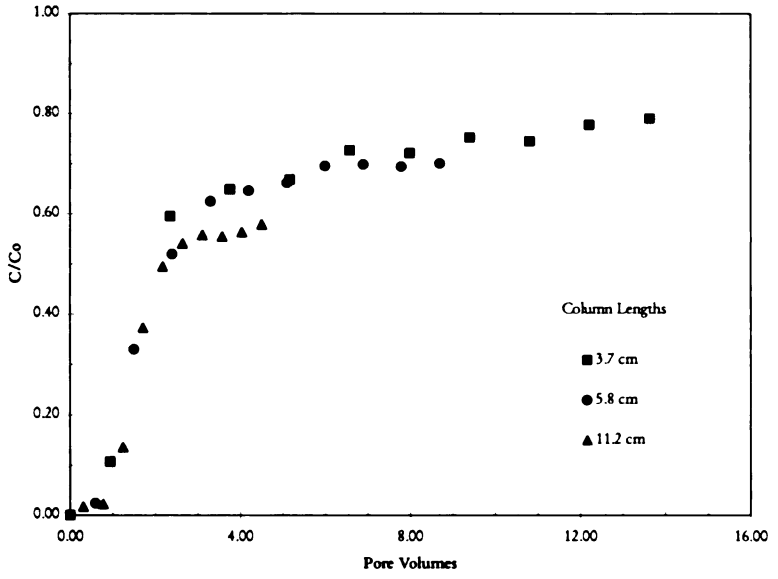

Figure 3.7 Bacterial breakthrough of an aerobic late exponential phase strain KC.

Figure 3.8 illustrates transport of stationary phase cells diluted approximately 1:5 to achieve a concentration of cells equivalent to the late exponential phase cells in Figure 3.7 (approximately $2*10^7$ cfu/ml). Note the change in scale on the y-axis.

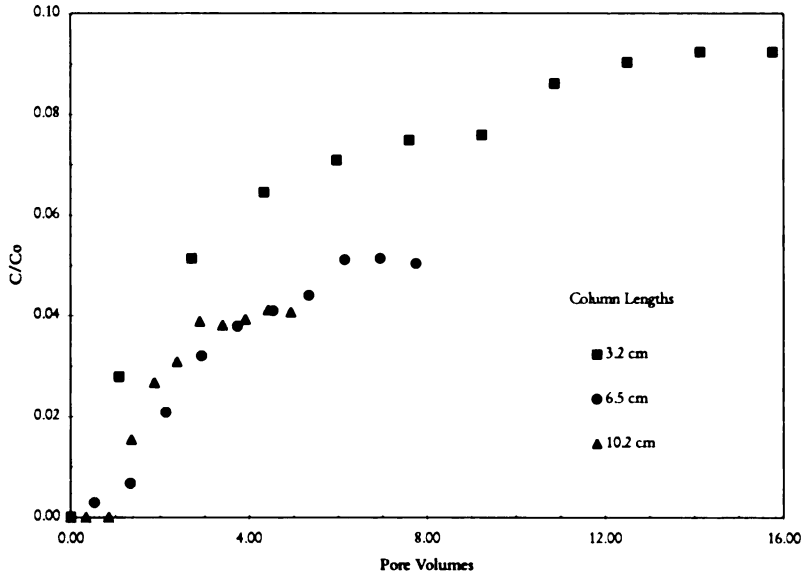

Figure 3.8 Bacterial breakthrough of a diluted early stationary aerobic strain KC culture.

The breakthrough curves in Figure 3.9 are from for a culture removed from the reactor during mid-stationary phase and diluted to about approximately $2*10^7$. Note the scale on the y axis.

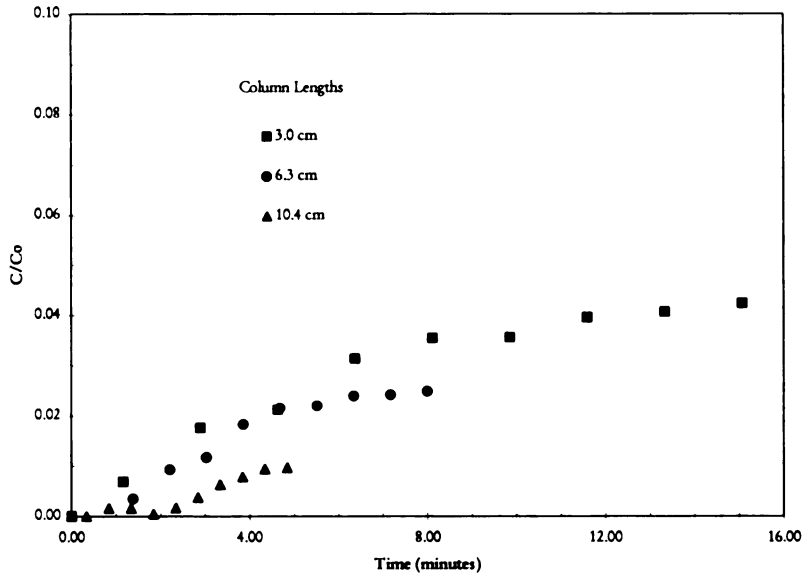

Figure 3.9 Bacterial breakthrough of diluted stationary phase cells.

The experiments with diluted cells clearly demonstrate the dependence of cellular transport on cell physiology. Escher (1986) reported a difference in attachment rate to smooth solids for cells of varying growth rates. In addition, Grasso et al. (1996) reported that that the "partition" coefficient for a *Pseudomonas aeruginosa* on dolomite was a function of physiological state of the cells as was the cell surface free energy. This observation suggests that surface properties of bacterial cells change during growth. A plausible hypothesis is that they exist in a planktonic or dispersed state in rich media permitting more efficient use of nutrients in solution, but when nutrients are limited survival is enhanced by modification of the cell surface to enable deposition and attachment.

The blocking factor listed for strain KC in Table 3.1 is two orders of magnitude higher than those obtained by Rijnaarts (1996b) for *Pseudomonas putida*. There are several possible explanations. One of the more plausible is blocking by native Schoolcraft aquifer flora, which are present on the aquifer solids at a concentration of approximately $3*10^6$ CFU/g. Table 3.2 contains the results of modeling when the native flora are accounted for in the blocking term. The only difference between Table 3.1 and 3.2 is inclusion of native flora in the latter table. The magnitudes of the blocking factor in Table 3.2 are similar to those obtained by Rijnaarts (1996b). The porous media used by Rijnaarts (1995, 1996a, 1996b) and others (Camper, 1993, and Sarkar et al., 1994, Martin et al., 1996, Johnson and Logan, 1996, Jewett et al., 1995) is free of microorganisms prior to the experiment. Another distinctive aspect of this work is that the strain KC cells used in these experiments were not washed as others have done (Loosdrecht et al., 1989;, Johnson and Logan, 1995; Camper, 1993; Sarkar et al., 1994; Gross and Logan, 1995;; Gannon et al., 1991; Fontes et al., 1991; Martin et al., 1996; and Wan et al., 1994). Washing may affect the magnitude of the blocking factor by altering cell surface properties, although this was not proven in the present study. To mimic a field injection, the column experiments in this work were performed with fresh, active, growing strain KC cells, not cells that were resuspended in buffer.

Table 3.2 Attachment efficiency and blocking factor with native flora.

Electron Acceptor	Culture Phase	α_o	β		
			Short	Medium	Long
O_2	mid-exponential	0.44±0.07	399	153	132
O_2	early stationary	0.28±0.00	23.6	30.9	36.1
O_2	mid-stationary	0.32±0.00	27.5	35.0	42.3

These studies suggest that a better understanding of kinetics of bacterial growth could be very beneficial to the success of bioaugmentation activities in subsurface environments. Manipulation of growth conditions may have a profound affect on the extent to which bacteria can be transported away from an injection well. If long travel distances are required, injection of excess nutrients along with the bacteria may promote both growth and transport.

EFFECTS OF CELL CONCENTRATION ON TRANSPORT

Introduction

In this chapter, the effects of cell concentration are investigated to determine the effect of dilution. Intuitively, one might expect that a more dilute culture would bbe more readily transported in as much as less clogging would be expected. On the other hand, dilution may affect the blocking factor. The results presented here were used to design the full scale inoculation scheme for the Schoolcraft Field Bioaugmentation Project.

Methods and Materials

Eight 3 cm long sand columns (hydraulic retention time = 1.7 minutes) were packed with the sand as described in Chapter 2. Four of these columns were used for column transport experiments using pasteurized Schoolcraft groundwater as the dilution medium. The remaining four columns were used for dilution experiments with supernatant from strain KC culture grown as the dilution medium. To obtain supernatant, a culture of strain KC was grown for 24 hours, centrifuged at 5000G for 20 minutes, decanted. The supernatant was then filtered through a 0.2 μm filter and then stored frozen at -20°C until use. Experiments to test the effects of dilution medium on cell transport were conducted with ^{14}C-radiolabeled culture of strain KC in the exponential growth phase. Dilutions were prepared at 10, 25, 50

and 100% of the original concentration. All experiments were performed at a pore velocity of 1.04 m/hr.

Results and Discussion

Figure 4.1 illustrates bacterial breakthrough curves for different concentrations of strain KC with pasteurized Schoolcraft groundwater as the dilution medium. The percentages on the chart represent the percent (by volume) of dilution water. It should be noted that C_o changes for each column depending on the dilution used. For example, the influent to each column can be calculated by subtracting the fraction of dilution medium from one and multiplying that number the reactor concentration ($5.6*10^7$ cfu/ml).

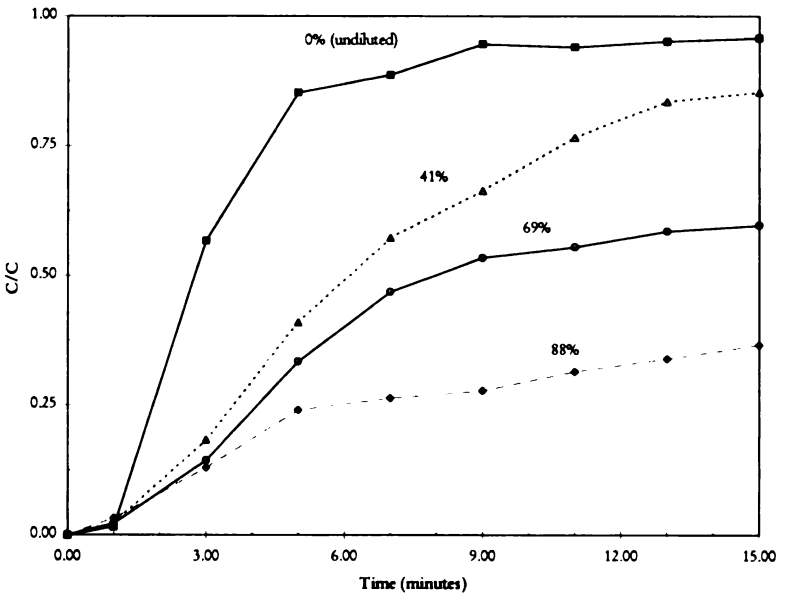

Figure 4.1 Bacterial breakthrough curves of different concentrations of strain KC diluted with pasteurized Schoolcraft groundwater.

From the same reactor, a separate set of columns was operated under identical conditions using supernatant from a culture of strain KC as the dilution medium. Figure 4.2 illustrates the bacterial breakthrough curves obtained.

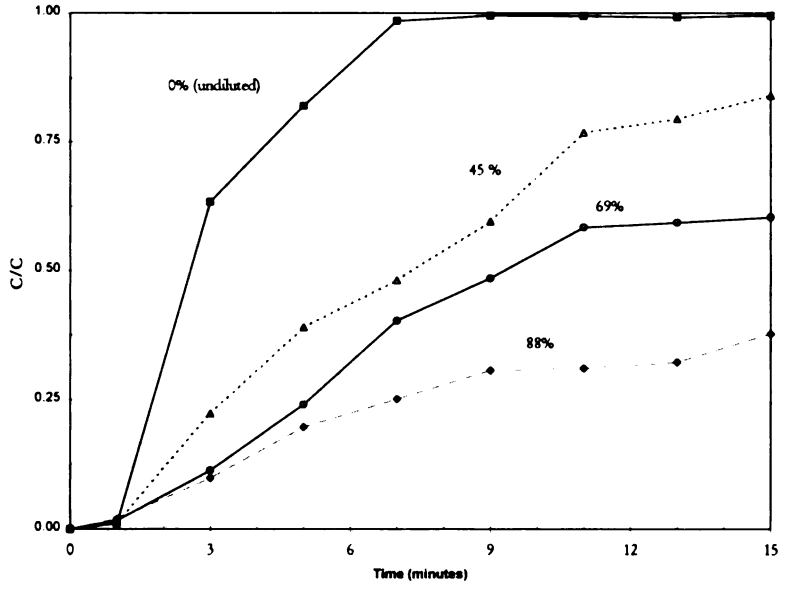

Figure 4.2 Bacterial breakthrough curves for different percentage dilutions of strain KC diluted with filter-sterilized supernatant from a strain KC culture.

It is evident from the data in figures 4.1 and 4.2 that higher influent bacterial concentration resulted in improved cell transport. This observation seems counterintuitive given that lower cell concentration would result in fewer collisions and less congestion in the pore spaces. Two possible explanations for the effect of dilution might be: (1) that something secreted by the cells enhances their transport or (2) the solids have some finite capacity for bacterial attachment and once the solids are saturated, additional cells remain are transported further in the bulk solution. Figures 4.1 and 4.2 illustrate that the nature of the dilution medium did not effect cell transport, indicating that a secreted factor is not responsible for the observed dilution effect. The most logical conclusion is that the solids themselves must

have a finite "bacterial demand" and that once the attached cells have achieved a saturating concentration, any additional bacteria entering the system do not attach, but remain in a planktonic state.

Figure 4.3 shows the cumulative number of strain KC cells transported over time from each column in Figure 4.1.

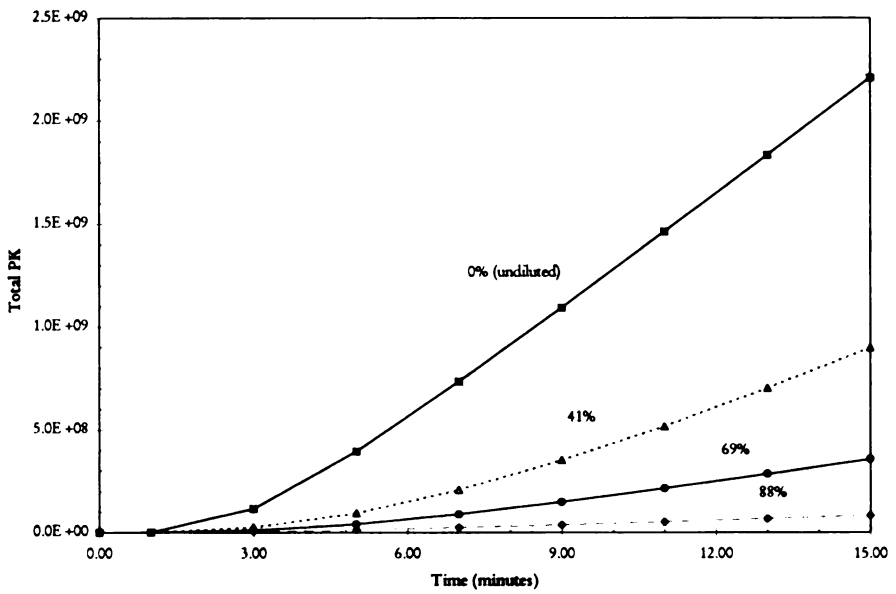

Figure 4.3 Cumulative number of strain KC cells transported for different percentage dilutions prepared with pasteurized Schoolcraft groundwater.

An important aspect of figure 4.3 is that this the results shown are for only 8.7 column retention times. The differences between different dilutions will become greater as time increases. Figure 4.4 shows the total number of strain KC cells deposited over time.

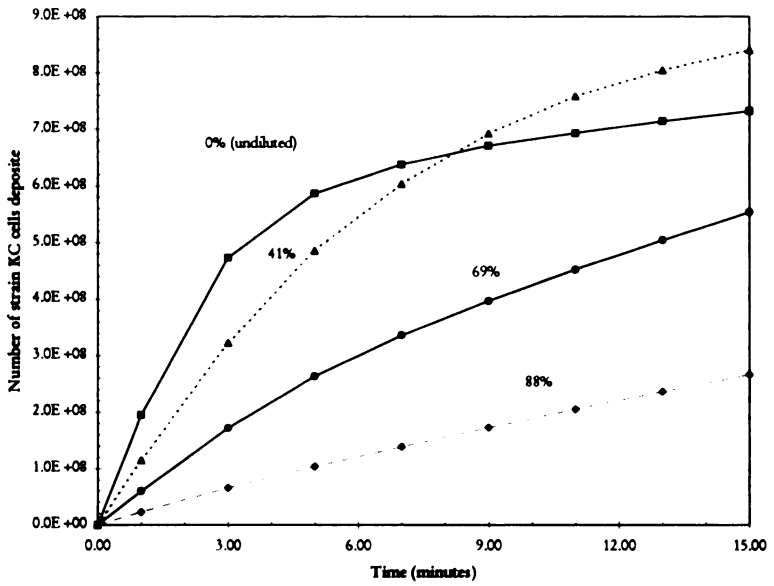

Figure 4.4 Cumulative number of strain KC cells deposited for different percentage dilutions prepared with pasteurized Schoolcraft groundwater.

The decreasing of the slope in figure 4.4 for the culture diluted by 41% dilution and for the undiluted culture suggests that the solids were saturated at a cell concentration of approximately $3*10^7$ strain KC cells/g (referred to as solids loading capacity). This conclusion is also supported by the steepness of the bacterial breakthrough curves in figure 4.1. Breakthrough of strain KC occured after this capacity is achieved. The two major variables influencing the solids loading capacity were growth state and pore velocity of the medium. Although the culture shown here was tested in mid-exponential phase of growth, the same phenomenon was observed for stationary stage cells, as shown in Figure 4.5. The stationary stage culture exhibited even larger differences in transport between dilutions, with considerably less breakthrough of undiluted cells.

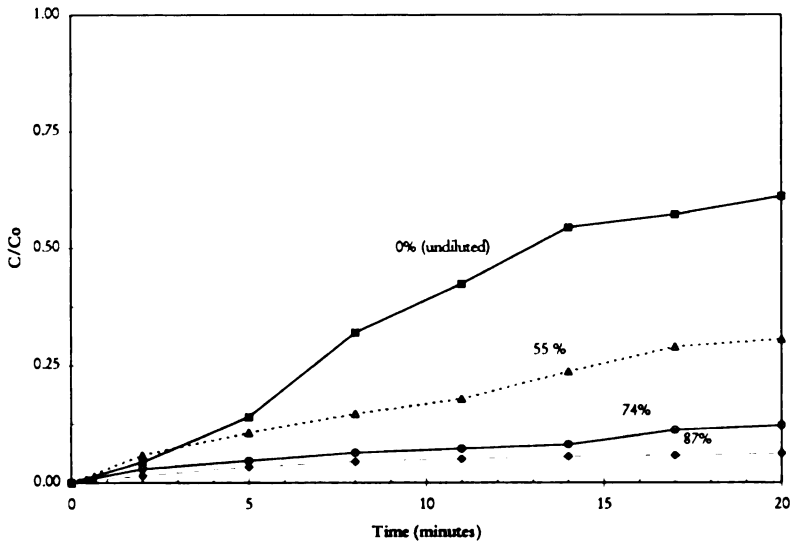

Figure 4.5 Breakthrough curves for a stationary phase culture of strain KC at different percentage dilutions.

No solids loading capacity could be determined from the data for the stationary phase culture because the solids loading capacity was not achieved in the time of the experiment.

The data presented in this chapter establishes that inoculation of cells should be performed using dense cultures without dilution. Use of a younger culture (exponential growth) only partially offsets the effect of cell concentration on cell transport.

Chapter 5

EFFECT OF FLOCCULATION ON TRANSPORT IN POROUS MEDIA

Introduction

Because strain KC is known to occasionally grow as a flocculent or clumpy culture, the effect of this state was examined in a series of cell transport experiments.

Materials and Methods

To evaluate the effects of flocculation on cell transport, flocculent and non-flocculent cultures were evaluated. The flow rate for these flow rates was 4.00 ml/min, corresponding to a pore velocity of 1.19 m/hr.. In flocculent cultures, flocculation, or clumpiness was not visible at 18 hours, barely visible at 24 hours, and obvious at 30 hours old.

Results and Discussion

Figures 5.1-5.3 illustrate bacterial breakthrough curves from a flocculating culture in order from youngest (exponential growth) to oldest (mid-stationary). Note the change in the y-axis on Figures 5.2 and 5.3.

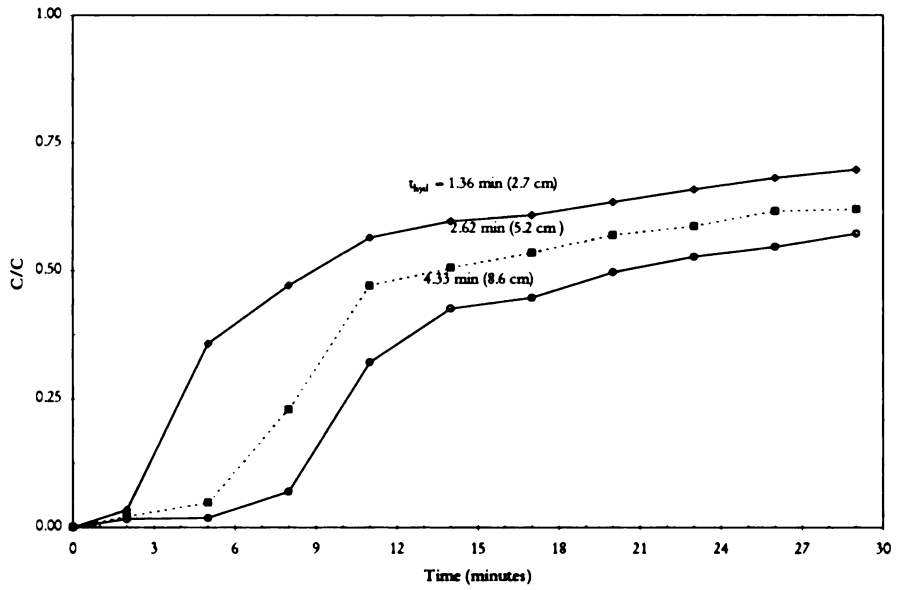

Figure 5.1 Bacterial breakthrough of a flocculating culture during mid-exponential growth.

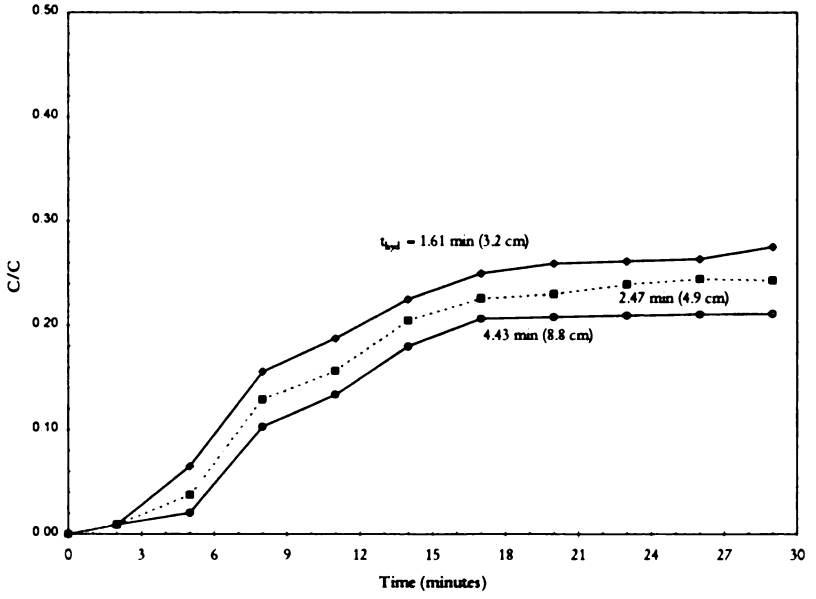

Figure 5.2 Bacterial breakthrough of a flocculating culture at early stationary phase of growth.

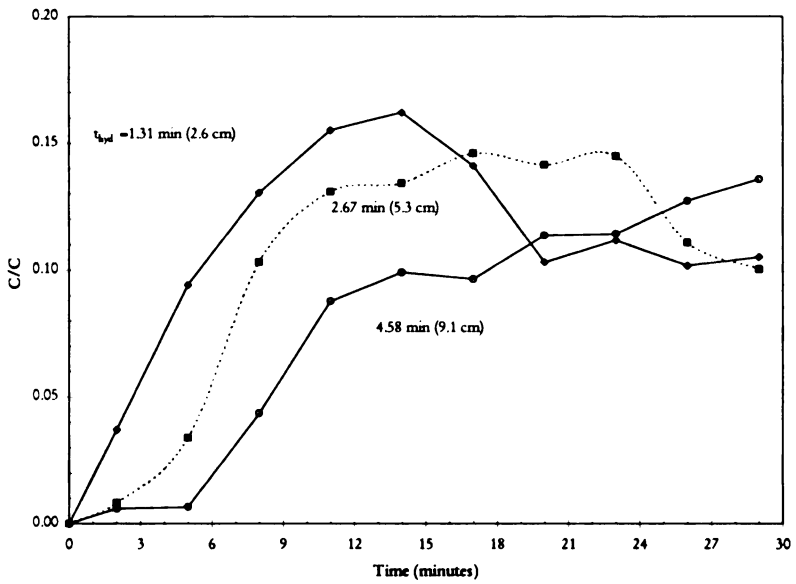

Figure 5.3 Bacterial breakthrough of a flocculating culture at mid-stationary phase.

Figures 5.1-5.3 illustrate breakthrough patterns suggestive of filter ripening. Filter ripening is a state in which, multilayer bacterial adhesion causes increased removal rates. This results in large removal efficiencies because of decreaseing pore size over time. In addition, there is a significant increase in head loss.

To better understand the nature of the flocculent state, both flocculating and non-flocculating cultures of strain KC were observed under a microscope. The visual observations were:

1. Flocculant cultures were not as motile as non-flocculent cultures. Studies have shown motility to be a factor in transport (Jewitt et al., 1995; Camper, 1993).

2. Clumps of cells within the flocculent culture increase in size with cell age. Mid-stationary phase clumps are visible to the naked human eye and settle by gravity in the absence of mixing.

The flocculation of cells is apparently triggered by nutrient stress. An experiment was performed in which 6 Erlenmeyer flasks were inoculated with a culture of strain KC. To 3 of these vials, 10 ppm phosphate was added. No phosphate was added to the other three cultures. The three cultures with no phosphate added flocculated, while the three containing phosphate did not. Dybas (1998) performed a similar experiment with filter-sterilized Schoolcraft groundwater and established that, for this medium, flocculation was triggered by a limitation in trace metals.

The results of clean bed filtration theory modeling of figures 5.1-5.3 can be found in table 5.1.

Table 5.1 Attachment efficiency and Blocking factor for flocculating strain KC.

Physiological State	α_o	β		
		Short	Medium	Long
mid-exponential	0.52±0.01	231.08	399.98	605.44
early stationary	1.08±0.00	17.49	26.78	48.09
mid-stationary	1.26±0.00	14.83	30.23	51.90

The trends for the blocking factor are similar to those reported in Chapter 3 for a well-dispersed culture of strain KC Most of the deposition occurs in the first few centimeters, as evidenced by the breakthrough curve of figure 5.3. All three columns had almost the

identical breakthrough profiles despite their different lengths. This suggests that sorting by cell age is occurring within the columns. A batch culture, like the one used in this experiment, is composed of cells with a myriad of cell ages, distributed around some average Cells with the highest α and the lowest β are the first to deposit. The young cells are not as likely to attach and are smaller in clump size. As a result, they are more likely to pass through the column, while the older, larger cells more likely to attach and be filtered out at the column entrance.

When flocculent culture breakthrough curves are compared with breakthrough curves for non-flocculent cultures lower breakthrough levels are observed for flocculent cells and transport is nearly twice as retarded. This is probably related to the solids loading capacity and cell sorting. In a flocculent culture, most of the cells are associated with clumps. Planktonic cells are in the minority. Because the clumps are removed in porous media near the well head, the distal end of the column "sees" fewer cells and it takes longer for the bacterial demand of the solids to be achieved. Breakthrough of cells is therefore retarded when compared to the breakthrough of a well dispersed culture.

It is critical that flocculent cultures be avoided in field applications where effective cell distribution is needed. Flocculating cells have significantly impaired properties for transport. If a flocculent culture is injected, the expected outcome is deposition of a large number of bacteria near the well head, which will result in an increase in head loss. This could cause problems for the injection pumps or the pipes carrying the substrates.

Chapter 6

ENGINEERING APPLICATION

In most field applications, biostimulation is the preferred method of bioremediation. However, there is now increasing interest in bioaugmentation. Two obstacles to the use of bioaugmentation are competition with indigenous microflora and transport of the added microorganism. Competition can be facilitated by modifying the environment to favor the added microorganisms, but cleanup can only occur if the added organisms are transported to the desired location.

Past researchers have proposed many methods to increase transport of microorganisms in porous media. Suggested methods include: use of low ionic strength cell suspensions (Fontes, 1991; Rijnaarts, 1996; Loosdrecht et al., 1989; Jewitt, et al., 1995; Martin et al., 1996; Sarkar et al., 1994; Martin et al., 1992; Gross and Logan, 1995), use of ultra micro bacteria (Jang, 1983), injection of non-attaching mutants (Camper et al., 1993), injection of a surfactant to decrease attachment (Johnson and Logan, 1996; Gross and Logan, 1995; Sarkar et al., 1994), pH modification of inoculum (Rijnaarts et al., 1995; Jewitt et al., 1995), decreasing the concentration of cells (Sarkar et al., 1994; Escher, 1986), and increasing velocity of the cell suspension (Sarkar et al., 1994; Rijnaarts, 1996b; Escher, 1986). Some of the studies provide basic mechanistic understanding of bacterial transport. However, the proposed strategies are often difficult to implement at the field scale. In this research, laboratory studies were designed to simulate field operations, so that the strategies developed could be readily implemented.

Data presented in this thesis indicates that some common practices in previous studies, such as washing and resuspending bacteria, could be detrimental to cell transport. The results also indicate that transport is dependent upon growth state and medium. The data also suggest that native microorganisms already present on the collectors probably play a role in attachment of the injected microorganism.

Results presented in this thesis demonstrate that simple steps may be taken to increase cell transport. Transport of strain KC can be controlled by cell physiology under both aerobic and denitrifying conditions. These observations are probably also true for other cell types. Dilution of the inoculum resulted in a decrease in transport and that flocculent cultures were transported poorly.

This thesis demonstrates that there are cost-effective variables that can be manipulated to promote cell transport. For application of bioaugmentation, the feasible transport distance of the organism may be an essential design parameter. The feasible transport distance depends on the concentration of the microorganisms required for colonization, inoculum volume, injection time, and cell physiology. At the Schoolcraft Bioaugmentation Site, closely spaced wells (15 wells 1 meter apart) and a relatively small inoculum volume (approximately 6.7 gallons inoculum per linear foot of well screen) were used to inoculate the biocurtain currently in use at the site. The inoculum was injected undiluted when the culture was in exponential growth. Early evidence indicates that this injection strategy allowed strain KC to colonize the space between the wells to form a continuous biocurtain capable of efficient degradation of carbon tetrachloride.

CONCLUSIONS AND FUTURE WORK RECOMMENDATIONS

Conclusions

1. Radiolabeling of strain KC with ^{14}C-acetate allows strain KC to be grown and used in transport experiments in the laboratory as it would be grown and injected into the subsurface in the field.

2. Clean bed filtration theory can accurately model transport of strain KC in repacked Schoolcraft sediments.

3. Cell physiology is a variable in attachment. Cells that are exponentially growing display high blocking factors and are easily transported, while cells in stationary phase have low blocking factors and are not easily transported.

4. Transport properties of cells grown under aerobic and denitrifying conditions were similar.

5. Dilution of a strain KC culture resulted in decreased transport of the cells in Schoolcraft aquifer sediments.

6. Schoolcraft aquifer sediments display a capacity for cells that are exponentially growing. Strain KC is readily transported, once saturation of the solids is achieved,.

7. Flocculent strain KC cells are non-motile. Dispersed cultures of strain KC are composed of very motile cells.

8. Flocculent cultures of strain KC are poorly transported and promote filter ripening.

9. Clump size in flocculent strain KC cultures increases with age of the culture.

Future Work Recommendations

1. Determine the effect on transport of the native Schoolcraft flora present on the aquifer sediments used for the work in this thesis.

2. Evaluate how the different mineral components of Schoolcraft aquifer sediments influence cell attachment.

3. Determine the effect of velocity on attachment. In addition, attempt to quantify the effect of a radial flow field.

4. Examine effects of growth medium. This includes metals, pH, and surfactants.

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Appendix A

CHAPTER 3 RAW DATA

Table A.1., Table A.2., and Table A.3 contains the results from the column experiments shown in figures 3.1, 3.2, and 3.3, respectively.

Table A.1: Effluent protein concentrations (μg/l) for transport experiments in figure 3.1

Time (minutes)	3.3 cm column	5.8 cm column	9.0 cm column
0.00	0.00	0.00	0.00
2.40	20.31	5.66	4.99
5.40	29.97	17.31	12.99
8.40	34.96	22.64	14.32
11.40	46.95	31.97	25.64
14.40	56.61	34.96	29.97
17.40	61.60	37.63	34.30
20.40	60.60	44.62	33.63
23.40	61.93	54.94	40.62
26.40	66.60	57.61	42.62
29.40	68.59	61.93	43.62

Table A.2: Effluent protein concentrations (μg/l) for transport experiments in figure 3.2

Time (minutes)	3.4 cm column	6.2 cm column	10.1 cm column
0.00	0.00	0.00	0.00
2.40	21.62	6.03	5.32
5.40	32.25	18.43	13.82
8.40	40.40	24.10	15.24
11.40	41.11	34.02	27.29
14.40	52.45	37.21	31.90
17.40	53.16	40.05	36.51
20.40	56.35	47.49	41.82
23.40	59.19	48.56	43.95
26.40	65.92	47.85	45.37
29.40	73.01	49.97	42.88

Table A.3: Effluent protein concentrations (μg/l) for transport experiments in figure 3.3

Time (minutes)	3.7 cm column	6.0 cm column	10.0 cm column
0.00	0.00	0.00	0.00
2.40	15.59	3.19	0.00
5.40	30.13	16.30	13.47
8.40	55.64	37.57	17.37
11.40	62.02	45.37	29.77
14.40	69.11	51.75	40.05
17.40	77.97	56.00	44.30
20.40	82.23	62.02	51.39
23.40	81.52	68.05	57.42
26.40	88.25	70.88	59.54
29.40	87.90	74.43	62.02

The concentration of strain KC cells in these three experiments represented in tables A.1., A.2., and A.3. can be found in table A.4.. Strain KC concentrations were determined by serial dilution and plating on R2A agar.

Table A.4. Strain KC concentration in transport experiments represented in tables A.1-A.3

Table	Reactor Age (hours)	Growth Phase	Protein concentration (μg/l)	Strain KC concentration (cfu/ml)
A.1.	18	mid exponential	77.7	9.6E+06
A.2.	24	early stationary	101.2	8.5E+07
A.3.	30	mid stationary	136.1	1.1E+08

Tables A.5, A.6, and A.7 contain the solid ^{14}C scintillation readings from the column effluents shown graphically in figures 3.4, 3.5, and 3.6.

Table A.5: Cell associated ^{14}C readings for transport experiments in figure 3.4 in CPM (counts per minute)

Time (minutes)	3.1 cm column	6.0 cm column	9.2 cm column
0	0.00	0.00	0.00
2	457.73	35.95	40.79
5	1339.54	485.69	74.01
8	2002.27	1343.75	399.07
11	2052.94	1660.18	1097.12
14	2084.27	1722.42	1399.89
17	2133.47	1990.91	1568.51
20	2125.90	2063.66	1823.13
23	2230.61	2038.01	1818.72
26	2284.01	2155.55	1847.10
29	2280.86	2124.85	1874.22

Table A.6: Cell associated ^{14}C readings for transport experiments in figure 3.5 in CPM

Time (minutes)	4.0 cm column	6.4 cm column	9.0 cm column
0	0.00	0.00	0.00
2	451.14	36.19	49.03
5	1265.67	623.47	416.42
8	1719.75	1487.03	941.83
11	1791.71	1608.44	1327.11
14	1897.76	1689.03	1431.69
17	1984.67	1799.92	1432.11
20	2084.41	1907.02	1444.10
23	2030.12	1873.99	1401.39
26	2054.95	1909.13	1434.00
29	2117.02	1909.55	1535.42

Table A.7: Cell associated ^{4}C readings for transport experiments in figure 3.6 in CPM

Time (minutes)	3.7 cm column	6.0 cm column	9.7 cm column
0	0.00	0.00	0.00
2	371.21	90.08	37.29
5	1693.27	1173.75	131.56
8	2358.39	1820.43	688.58
11	2545.04	2019.23	1104.41
14	2813.18	2401.54	1176.47
17	2879.59	2632.40	1524.22
20	3005.28	2622.55	1610.74
23	3048.02	2689.80	1733.70
26	3106.25	2702.37	1848.92
29	3264.21	2811.09	1946.54

Table A.8 contains the reactor concentrations of the initial cell ^{14}C and strain KC.

Table A.8: Strain KC concentration in transport experiments represented in tables A.5-A.7

Table	Reactor Age (hours)	Initial Cell ^{14}C concentration (CPM/ml)	Strain KC concentration (cfu/ml)
A.5	20	2478.3	1.0E+07
A.6	30	3108.9	6.5E+07
A.7	48	3682.5	9.7E+07

Tables A.9., A.10, and A.11 contain the solid ^{14}C scintillation readings from the column effluents represented in figures 3.7, 3.8, and 3.9, respectively.

Table A.9: Cell ^{14}C readings for transport experiments in figure 3.7 in CPM

Time (minutes)	3.7 cm column	5.8 cm column	11.2 cm column
0	0.00	0.00	0.00
2	210.48	46.96	34.17
5	1172.93	649.88	44.44
8	1280.05	1025.34	267.71
11	1317.79	1231.84	735.41
14	1434.98	1274.18	976.58
17	1422.61	1305.84	1067.90
20	1485.08	1372.29	1101.44
23	1470.62	1377.95	1094.52
26	1535.18	1369.78	1112.13
29	1560.55	1381.73	1141.69

Table A.10: Cell ^{14}C readings for transport experiments in figure 3.8 in CPM

Time (minutes)	3.2 cm column	6.5 cm column	10.2 cm column
0	0.00	0.00	0.00
2	19.60	2.00	0.00
5	36.10	4.70	0.00
8	45.30	14.60	10.80
11	49.80	22.50	18.80
14	52.60	26.60	21.70
17	53.30	28.80	27.30
20	60.50	30.90	26.80
23	63.50	35.90	27.60
26	64.90	36.10	28.90
29	64.90	35.40	28.60

Table A.11: Cell ^{14}C readings for transport experiments in figure 3.9 in CPM

Time (minutes)	3.0 cm column	6.3 cm column	10.4 cm column
0	0.00	0.00	0.00
2	5.97	0.00	0.00
5	15.27	2.97	1.37
8	18.47	8.07	1.37
11	27.37	10.17	0.37
14	30.97	15.87	1.47
17	31.07	18.77	3.27
20	34.47	19.17	5.47
23	35.47	20.87	6.77
26	36.97	21.07	8.17
29	37.37	21.67	8.37

Table A.12 contains the reactor concentrations of the initial cell ^{14}C and strain KC.

Table A.12: Strain KC concentration in transport experiments (prior to dilution) represented in tables A.9-A.11

Table	Reactor Age (hours)	Dilution	Initial Cell ^{14}C concentration (CPM/ml)	Strain KC concentration (cfu/ml)
A.9	18	N/A	2478.3	4.E+07
A.10	24	1:4.54	3108.9	8.E+07
A.11	30	1:5.00	3682.5	1.E+08

Appendix B

DATA FROM CHAPTER 4 EXPERIMENTS

Table B.1 contains the cell associated ^{14}C column effluent CPM for exponentially growing strain KC inoculum diluted with Schoolcraft groundwater represented in figure 4.1.

Table B.1: Column effluent ^{14}C readings for transport experiments in figure 4.1 in CPM

Time (minutes)	0.12 Dilution	0.31 Dilution	0.59 Dilution	1.00 Dilution
0.00	0.0	0.0	0.0	0.0
1.00	9.4	17.8	35.7	46.1
3.00	38.3	119.6	293.7	1771.4
5.00	71.3	278.5	656.5	2666.0
7.00	78.1	390.1	918.7	2771.9
9.00	82.3	445.2	1064.5	2958.6
11.00	93.3	462.5	1230.2	2940.8
13.00	100.7	488.2	1340.3	2974.4
15.00	108.5	498.2	1369.7	2995.3

Table B.2 contains the cell associated ^{14}C column effluent CPM for a exponentially growing strain KC inoculum diluted with supernatant from a strain KC culture represented in figure 4.2.

Table B.2: Column effluent ^{14}C readings for transport experiments in figure 4.2 in CPM

Time (minutes)	0.12 Dilution	0.31 Dilution	0.55 Dilution	1.00 Dilution
0.00	0.0	0.0	0.0	0.0
1.00	5.2	14.2	22.0	29.4
3.00	26.2	93.3	373.4	1703.2
5.00	52.4	199.3	652.3	2203.5
7.00	67.1	333.9	805.2	2647.1
9.00	81.8	402.1	997.2	2674.4
11.00	82.9	484.4	1285.5	2672.3
13.00	86.0	492.2	1329.9	2665.0
15.00	100.7	500.8	1406.4	2674.4

Table B.3 contains the information about growth of the inoculum.

Table B.3: Reactor growth for tables B.1 and B.2

Reactor Age (hours)	Strain KC (cfu/ml)	Cell assimilated ^{14}C (CPM/ml)
0	4.7E+06	0.00
18	1.0E+07	
24	5.6E+07	3127.5
30	2.8E+08	

Note: The experiments were performed at a reactor age of 24 hours.

Table B.4 contains the cell associated ^{14}C column effluent CPM for a stationary phase strain KC inoculum diluted with Schoolcraft groundwater represented in figure 4.5.

Table B.4: Column effluent ^{14}C readings for transport experiments in figure 4.5 in CPM

Time (minutes)	0.13 Dilution	0.26 Dilution	0.45 Dilution	1.00 Dilution
0.0	0.0	0.0	0.0	0.0
2.0	4.0	19.5	78.6	118.1
5.0	9.1	31.4	141.7	373.0
8.0	12.1	43.1	195.0	855.4
11.0	13.9	48.6	236.8	1132.8
14.0	15.3	54.4	314.4	1455.2
17.0	15.9	75.5	384.6	1529.6
20.0	17.0	81.7	406.5	1636.6

Table B.5 contains the information about growth of the inoculum.

Table B.5: Reactor growth for table B.4

Reactor Age (hours)	Strain KC (cfu/ml)	Cell assimilated ^{14}C (CPM/ml)
0	6.2E+05	0.00
18	8.6E+06	
24	8.8E+07	2678.9
30	9.6E+07	

Note: The experiment was performed at a reactor age of 24 hours.

Appendix C

DATA FROM CHAPTER 5

Tables C.1, C.2, and C.3 contain the solid ^{14}C scintillation readings from the column effluents shown graphically in figures 5.1, 5.2, and 5.3.

Table C.1: Column effluent ^{14}C readings for transport experiments in figure 5.1 in CPM

Time (minutes)	2.7 cm column	5.2 cm column	8.6 cm column
0	0.00	0.00	0.00
2	166.09	37.68	29.05
5	687.54	86.73	32.42
8	956.15	414.71	126.10
11	1020.99	850.89	348.61
14	1079.09	913.63	559.33
17	1131.93	966.47	807.53
20	1147.51	1029.62	898.05
23	1191.71	1062.25	952.36
26	1233.61	1114.88	956.36
29	1263.08	1121.19	961.41

Table C.2: Column effluent ^{14}C readings for transport experiments in figure 5.2 in CPM

Time (minutes)	3.2 cm column	4.9 cm column	8.8 cm column
0	0.00	0.00	0.00
2	92.52	39.89	39.89
5	494.76	165.20	89.80
8	791.74	569.32	224.72
11	932.93	690.03	380.94
14	994.12	904.31	445.06
17	1103.55	997.88	536.53
20	1145.32	1016.26	614.01
23	1154.93	1056.35	696.09
26	1164.75	1080.37	723.24
29	1215.91	1074.52	734.31

Table C.3: Column effluent ^{14}C readings for transport experiments in figure 5.3 in CPM

Time (minutes)	2.6 cm column	5.3 cm column	9.1 cm column
0	0.00	0.00	0.00
2	223.91	49.66	35.99
5	569.24	204.76	39.98
8	789.36	623.53	263.26
11	938.98	791.46	530.94
14	981.07	812.72	599.12
17	853.54	883.43	583.55
20	623.53	856.07	687.72
23	675.51	877.11	690.87
26	615.12	670.04	769.79
29	635.53	606.70	821.56

Table C.4 contains the reactor concentrations of the initial cell ^{14}C and strain KC.

Table C.4: Strain KC concentration in transport experiments represented in tables A.5-A.7

Table	Reactor Age (hours)	Initial Cell ^{14}C concentration (CPM/ml)	Strain KC concentration (cfu/ml)
C.1	18	1809.8	7.7E+06
C.2	24	4418.8	5.6E+07
C.3	30	6050.4	6.7E+07