

Effectiveness and interspecies competition in colonized porous pellets
by Paul John Sturman

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University
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Abstract:
Bacterial degradation of hazardous compounds has been utilized extensively in the design of pump and treat groundwater remediation schemes. Reactor media can be colonized with either indigenous soil microorganisms or non-native organisms which have been selected to degrade a particular compound. Non-native microbes have historically been quickly outcompeted from reactors exposed to groundwater with a significant native microbial population.

The goal of this research was to evaluate the effectiveness of a particular media (diatomaceous earth pellets) through quantitative analysis of the processes influencing the stability of colonized microorganisms. Experiments were conducted on pellets used in a bench-scale bioreactor study at Tyndall Air Force Base, Florida. These pellets were colonized with a non-native organism capable of degrading chlorobenzene and exposed to groundwater from a contaminated site containing a significant native microbial population. Further experiments sought to determine the effects of organism growth rate, motility) and order of introduction on population stability.

Results indicate that diatomaceous earth pellets may be thoroughly colonized by microorganisms, regardless of their motility. Organism growth rate is a more important factor in bacterial persistence than either motility or order of introduction.

A model for substrate utilization and biomass growth within a pellet was developed. The substrate balance equation was solved using both observed and modified cell density data.

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in

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APPROVAL

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Paul John Sturman

This thesis has been read by each member of the thesis committee and been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency and is ready for submission to the College of Graduate Studies.

August 20, 1991
Date

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Approved for Major Department

21 Aug '91
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ABSTRACT

Bacterial degradation of hazardous compounds has been utilized extensively in the design of pump and treat groundwater remediation schemes. Reactor media can be colonized with either indigenous soil microorganisms or non-native organisms which have been selected to degrade a particular compound. Non-native microbes have historically been quickly outcompeted from reactors exposed to groundwater with a significant native microbial population.

The goal of this research was to evaluate the effectiveness of a particular media (diatomaceous earth pellets) through quantitative analysis of the processes influencing the stability of colonized microorganisms. Experiments were conducted on pellets used in a bench-scale bioreactor study at Tyndall Air Force Base, Florida. These pellets were colonized with a non-native organism capable of degrading chlorobenzene and exposed to groundwater from a contaminated site containing a significant native microbial population. Further experiments sought to determine the effects of organism growth rate, motility, and order of introduction on population stability.

Results indicate that diatomaceous earth pellets may be thoroughly colonized by microorganisms, regardless of their motility. Organism growth rate is a more important factor in bacterial persistence than either motility or order of introduction.

A model for substrate utilization and biomass growth within a pellet was developed. The substrate balance equation was solved using both observed and modified cell density data.

INTRODUCTION

Over 75% of U.S. counties contain wells with some degree of contamination (Lehr, 1985). Although much less than 1% of groundwater in the United States is contaminated with xenobiotic pollutants, the consequences (and concomitant public outcry) from such pollution has made the cleanup of contaminated aquifers a national priority. Engineers and geologists responsible for design and implementation of groundwater remediation schemes can choose from a variety of technologies, such as vapor extraction/soil venting, activated carbon adsorption, groundwater flushing, *in situ* vitrification, and bioremediation. Of these, only bioremediation solves the problem through contaminant mineralization, rather than transfer to a different medium or containment.

Bioremediation of contaminated groundwater involves the use of microorganisms to degrade subsurface contaminant(s). Two forms of bioremediation commonly practiced in field situations are *in situ* and above ground. The latter system involves the use of pump and treat technology, where the contaminant laden groundwater is pumped to the surface and exposed to a microbial population. The microbes may be either indigenous to the soil system or introduced. Introduced species are typically isolated and selected for the ability to degrade the target compound. the origin of these species is generally from a contaminated site or from sewage sludge. Many

refractory contaminants resist degradation by the indigenous soil microbial population, but can be degraded by selected microorganisms. Historically, these compound-specific organisms have been quickly outcompeted by native populations when exposed to groundwater in a pump and treat reactor. Thus stabilization of a microbial population capable of degrading the contaminant(s) is integral to the success of these systems.

Above ground bioreactors utilize a variety of media designed to increase surface area available for microbial colonization per volume of reactor. Attempts to maximize this ratio have lead to the use of porous media for microbial stabilization. One such medium (Manville, Inc., Lompoc, CA) is a diatomaceous earth (D.E.) pellet. An expected benefit of such porous pellets is a degree of protection for interior colonized organisms from both surface shear conditions and microbial competition. A nominal pore size of 20 μm provides a high surface area, and yet does not seriously impede movement and colonization of all pellet interior surfaces (Figure 1).

Microbial colonization of pellet interiors can result in concentration gradients in substrate, electron acceptor, and/or colonizing organisms from pellet exterior to interior sections. Because of the small pore size, diffusive transport is an important consideration in overall mass transport within the pellets.



Figure 1 Scanning electron micrograph of pellet interior. Section shown is at pellet center. Organisms present are *Pseudomonas* sp JS150. Scale bar at lower right is 2 μm.

Goal and Objectives

The goal of this research was to evaluate the effectiveness of colonized D.E. pellets by quantitative analysis of processes influencing the stability of inoculated organisms. The objectives were to 1) determine the rate and extent of bacterial colonization of pellet exterior and interior surfaces, 2) characterize

advective and diffusive transport of cells and substrate into pellet interiors, 3) determine persistence of individual bacterial species in the reactor, and 4) develop a model to predict organism spatial and temporal distribution and substrate utilization with influent flowrate and substrate loading as variables.

To accomplish these objectives, three experimental programs were conducted. In the first, experiments were performed on pellets from a bench-scale bioreactor operated to biodegrade a benzene/chlorobenzene mixture from groundwater samples from Tyndall Air Force Base in Florida. This experiment looked at competition between an inoculated compound-specific organism and native microbes. In a second set of experiments at MSU, columns were used to examine dual species competition and the effect of order of introduction on species persistence. Experiments to determine pellet effectiveness were conducted using a single species, with varying substrate loading.

BACKGROUND

Pump and Treat Bioremediation Techniques

As an option for contaminated groundwater remediation, pump and treat technology is widely employed due to its simplicity and ease of control. Properties of the contaminant and the aquifer impact the success of moving the contaminant from the subsurface strata to an above ground reactor (Quince and Gardner, 1982). For organic contaminants, specific weight, solubility and sorptive properties largely determine whether it will exist as a non-aqueous phase liquid (NAPL) floating atop (or sinking beneath) the groundwater table or dissolved in the groundwater. Aquifer properties such as permeability and organic matter fraction determine the maximum pumping rate and recovery of contaminant.

The success of pump and treat biological systems depends on contaminant dissolution in the aqueous phase, and reactor application rates which allow microbial growth. Reactor configurations include fluidized bed, trickling filter, rotating biological contactor, submerged upflow reactor, and aerated tank. With the exception of the latter, these systems rely on attached microbial cells (biofilms) to degrade the contaminant.

Microbial Survival in Natural and Engineered Systems

Efforts to enhance biodegradation, both *in situ* and above ground, have

led researchers to isolate bacterial strains capable of degrading one or several otherwise recalcitrant organic compounds, frequently as the sole carbon and energy source. Such strains are typically isolated from a contaminated soil site, or from activated sludge (Lee, et al., 1988). The isolation/selection procedure involves exposure of the inoculum to increasing concentrations of the target contaminant, then selection of the most vigorously growing colonies for re-plating and re-exposure to the contaminant (Omenn, 1986).

These efforts have been successful with a diverse range of compounds such as aromatics (naphthalene, styrene, benzene, toluene, xylene), chlorinated aromatics (chlorobenzene), halogenated aliphatics (chloroform, bromodichloromethane, trichloroethylene, tetrachloroethylene) and polychlorinated biphenyls (McCarty, et.al., 1984; Focht and Brunner, 1985).

Unfortunately, reintroduction of these specific species into natural soil systems has met with limited success. In most cases, introduced organisms are quickly outcompeted by indigenous soil organisms. Goldstein, et al. (1985) cite four reasons for failure: the concentration of the compound is too low, the environment contains some substance or organisms that inhibit growth, the inoculated organism uses a substrate other than the one it was selected to metabolize, or the substrate is not accessible to the organism. In cases where some success with inoculated organisms has been reported, controls often show contaminant removal commensurate with inoculated results (Focht and Brunner, 1985; Westlake, et al., 1978).

Engineered systems offer the advantage of a potentially sterile site for colonization by inoculated bacteria. Since groundwater may contain as many as 10^6 cfu/ml of naturally occurring microorganisms (Bitton and Gerba, 1984), the initial absence of competing microbes allows unhindered colonization by the inoculated organism. The abundance of bacteria in most groundwater insures that a competitive environment will exist in pump and treat reactor systems. Conditions in the reactor will be much different than in the subsurface, however, particularly with regard to electron acceptor in aerated systems.

Inocula survival in engineered systems has shown mixed results. Sojka, et al. (1988) reported limited success in treating landfill leachate containing chlorinated organics and phenol with a sequencing batch reactor system seeded with a consortia isolated from the leachate. During isolation, the consortia exhibited the capability to degrade most of the contaminants present, but did not completely degrade the same mixture in the reactor. Bartha (1986) suggests that repeated inoculations may be necessary to degrade a xenobiotic pollutant, particularly if sufficient, easily degradable organic carbon exists for survival of competing organisms.

Because of their high surface area/volume ratio, D.E. pellets have been used as a substratum for bacterial colonization in both inoculated and indigenous systems. Several researchers have focused directly on quantifying cell adsorption and biodegradation phenomena on manufactured D.E. pellets. Caunt and Chase (1988) developed bacterial adsorption "isotherms" for D.E.

pellets. The pellets compared favorably with sand, anthracite and charcoal in their ability to attach and retain organisms.

Under laboratory conditions, Andrews, et al. (1988) colonized D.E. pellets with organisms isolated from sewage sludge and selected for the capacity to degrade a mixture of benzene, toluene, ethylbenzene and xylene (BTEX). Better than 90% reduction of benzene, toluene and ethylbenzene was reported. No loss of inoculum was reported over time. Column influent was not natural groundwater, however, but was tap water with contaminant added. Similarly, Attaway (1988) successfully degraded a 700 ppm phenol/formaldehyde mixture with microorganisms isolated from sewage sludge and inoculated onto D.E. pellets. Again, influent was tap water.

Friday, et al. (1988) successfully degraded trichloroethylene and dichloroethylene in groundwater using D.E. pellets colonized with an inoculated *Pseudomonas cepacia* strain. Influent cell concentrations and alternate sources of organic carbon are not reported, but the experiment covered only 14 hours, not sufficient time to observe significant competitive losses of the inoculated organism. Pettigrew et al. (1991) found that an inoculated chlorobenzene degrading organism was outcompeted after 2 weeks where influent groundwater contained a wide range of aromatic compounds and approximately 100 ppm total organic carbon as well as a significant native microbial population. In prior experiments the inoculated Pseudomonad survived on chlorobenzene as the sole carbon source for 2 weeks in a chemostat without

competitive pressures (Pettigrew, 1991).

Transport and Effectiveness

Understanding the mechanisms of microbial stabilization and substrate degradation within porous pellets requires some knowledge of intra-pellet transport of these constituents. Cells, substrate and electron acceptor surround the pellet in the bulk fluid. Their movement into the pellet may be caused by advective flow through the pellets (a result of fluid pressure differences across the pellet) or by diffusive transport (a result of concentration gradients within the pellet). Therefore, the reactor configuration, flowrate and pellet pore geometry are important considerations in the transport equations.

Reaction of substrate and electron acceptor to create biomass and products causes concentration gradients to persist in the pellet interior. Cells in the bulk fluid enter the pellets via the interconnected pore structure. Cell attachment, growth, multiplication and detachment occur throughout the pellet, provided nutrient and electron acceptor supply is sufficient. These processes are intimately interdependent, each being both cause and effect of the other.

Modelling efforts in porous media have dealt with both the issue of catalysis, where the media itself catalyses a reaction, and with conventional advection-dispersion models. The porous catalysis model (Satterfield, 1970) begins with the assumption that intra-pellet transport is diffusive only. A flux balance over a differential element of a spherical porous catalyst pellet (Figure

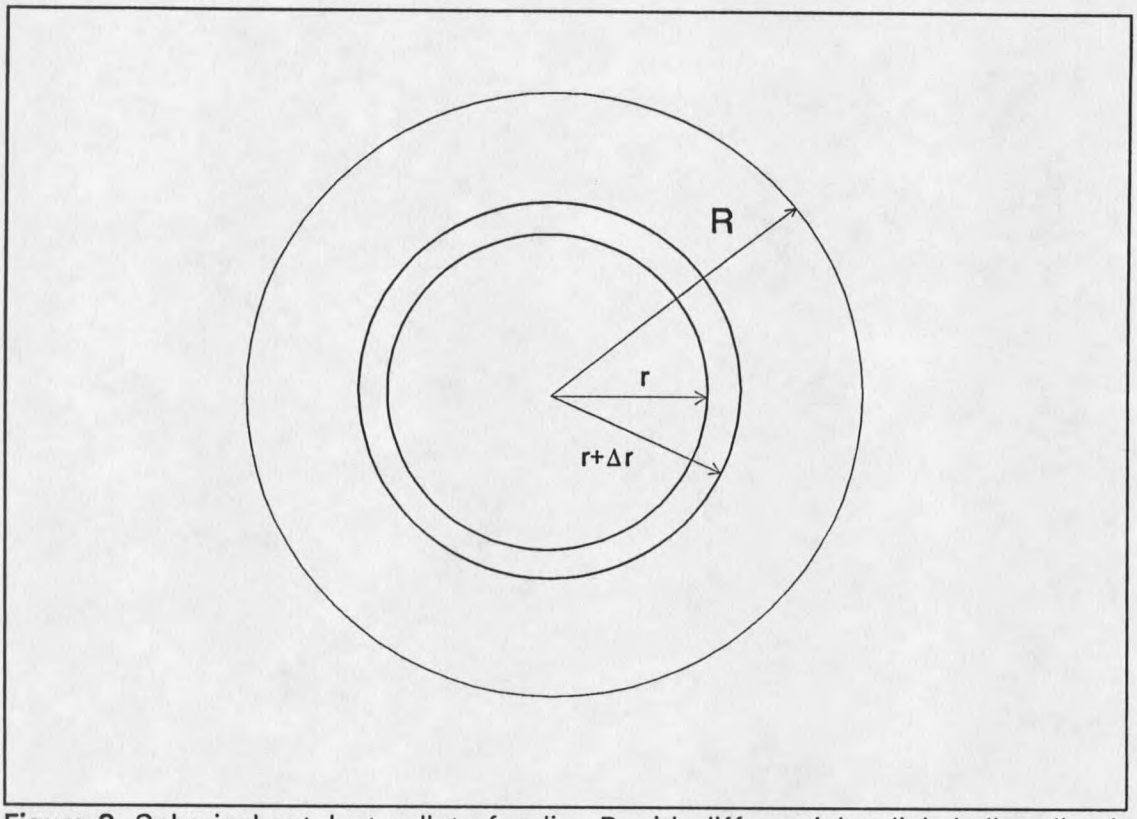


Figure 2 Spherical catalyst pellet of radius R with differential radial shell outlined.

2), yields:

$$\begin{matrix}\textit{Rate of diffusion}\\ \textit{inward at } r=r\end{matrix} \; - \; \begin{matrix}\textit{Rate of diffusion}\\ \textit{inward at } r=r+\Delta r\end{matrix} \; = \; \begin{matrix}\textit{Rate of reaction}\\ \textit{within shell}\end{matrix} \qquad (1)$$

Expressed mathematically, the balance becomes:

$$4\pi r^2 N_{A_r}|_r \; - \; 4\pi(r+\Delta r)^2 N_{A_r}|_{r+\Delta r} \; = \; -R_A 4\pi r^2 \Delta r \qquad (2)$$

where: r = radius of pellet to inner shell (L)

$r + \Delta r$ = radius of pellet to outer shell (L)

N_{Ar} = flux of A at r ($ML^{-2}t^{-1}$)

R_A = reaction rate of A ($ML^{-3}t^{-1}$)

The use of a single reaction rate R_A assumes the reaction is homogeneous over the length Δr. Dividing equation 2 by $4\pi\Delta r$, taking the limit as $\Delta r \rightarrow 0$, and applying Fick's first law;

$$N_{A_r} = -D_{eff}\frac{dC_A}{dr} \tag{3}$$

where: D_{eff} = effective diffusivity (L^2t^{-1})

C_A = concentration of A (ML^{-3})

yields:

$$-\frac{d}{dr}(r^2 D_{eff}\frac{dC_A}{dr}) = r^2 R_A \tag{4}$$

Rearranging and expressing the reaction rate term as $R_A = -S_v k C_A^m$;

$$\frac{d^2C_A}{dr^2} + \frac{2}{r}\frac{dC_A}{dr} = \frac{S_v k C_A^m}{D_{eff}} \tag{5}$$

where: S_v = pore surface area per volume ratio (L^2L^{-3})

k = reaction rate constant ($L^{3m-2}M^{1-m}t^{-1}$)

m = order of reaction (-)

Boundary conditions for equation 5 are $C_A = C_{Abulk}$ at $r = R$ and $dC_A/dr = 0$ at $r = 0$. The Thiele diffusion modulus Φ_s is then defined as

$$\phi_s = R\sqrt{\frac{S_v k C_A^{m-1}}{D_{eff}}} \tag{6}$$

This dimensionless number is a ratio of reaction rate to diffusion rate of the reactant at any point in the pellet. With boundary conditions described above and assuming first-order kinetics (m = 1) Φ_s becomes a constant and equation 5 can be solved analytically, yielding;

$$C_A = C_{A_{bulk}} \left[\frac{\sinh\left(\phi_s \frac{r}{R}\right)}{\left(\frac{r}{R}\right) \sinh \phi_s} \right] \tag{7}$$

Equation 7 describes the concentration profile within a porous catalyst pellet.

At steady state conditions, the overall reaction rate of a substance in a pellet will be equal to the flux of that substance into the pellet,

$$\int_0^R R_A 4\pi r^2 dr = 4\pi R^2 D_{eff} \left(-\frac{dC_A}{dr}\right)_{r=R} \tag{8}$$

If all pellet surfaces were exposed to reactant concentrations of C_{Abulk}, the overall substrate flux into the pellet would be

$$\int_0^R 4\pi R_{A_{max}} r^2 dr = -\frac{4}{3}\pi R^3 S_v k C_{A_{bulk}} \tag{9}$$

The effectiveness factor (η) is expressed as the ratio of equations 8 and 9. Substituting the solution to (7) into (8) and simplifying yields:

$$\eta = \frac{3}{\phi_s}\left[\frac{1}{\tanh\phi_s} - \frac{1}{\phi_s}\right] \quad \text{(sphere, first order reaction)} \qquad (10)$$

Effectiveness factor is defined as the ratio of the actual reaction rate to the rate which would occur in the absence of mass transport limitations (Satterfield, 1970), (Grady and Lim, 1980) and (Smith, 1981). The relationship between effectiveness factor and the Thiele modulus is shown in Figure 3.

For microbially catalyzed reactions within a porous pellet, the above equations must be modified to reflect more complex Monod kinetics, and spatial variations in reaction rate, i.e., microbial colonization on interior pellet surfaces may vary with both position and time.

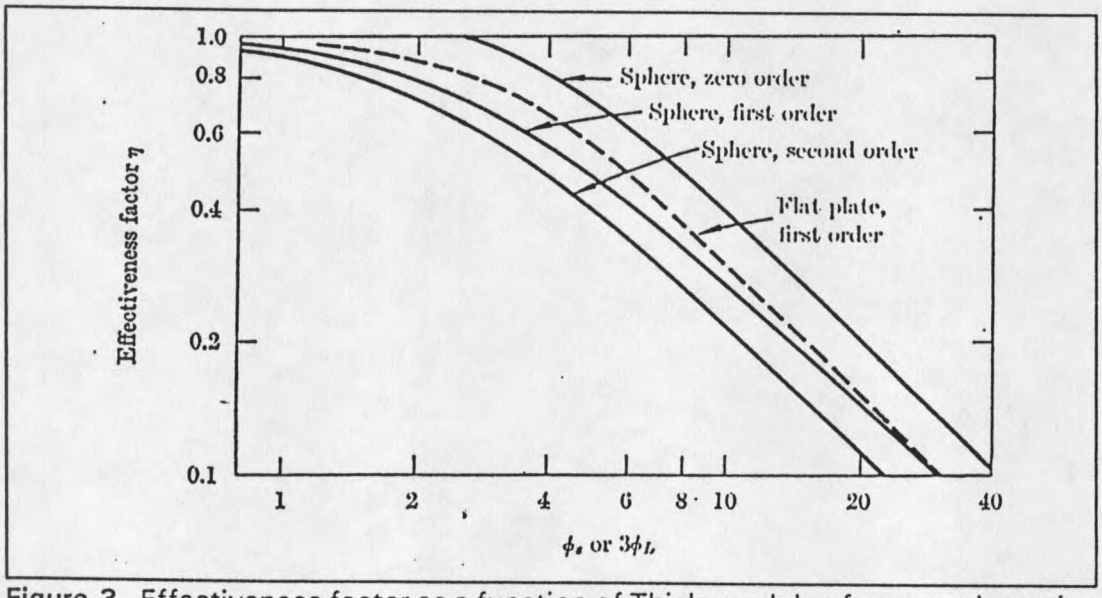


Figure 3. Effectiveness factor as a function of Thiele modulus for several reaction orders and shapes (Satterfield, 1970).

Monod or saturation kinetics are described by:

$$\mu = \frac{\mu_{max} C_A}{K_A + C_A} \tag{11}$$

where: μ = specific growth rate of organisms (t^{-1})

μ_{max} = maximum specific growth rate (t^{-1})

K_A = half-saturation coefficient (ML^{-3})

Reaction rate within the pellet becomes

$$R_A = -\frac{X \mu_{max} C_A}{Y(K_A + C_A)} \tag{12}$$

where: X = cell mass per unit pellet volume (ML^{-3})

Y = substrate yield ($M_{cells}/M_{substrate}$)

Equation 5 becomes;

$$\frac{d^2 C_A}{dr^2} + \frac{2}{r}\frac{dC_A}{dr} = \frac{X \mu_{max} C_A}{D_{eff} Y(K_A + C_A)} \tag{13}$$

The addition of saturation kinetics makes equation 13 first-order with respect to C_A when $K_A >> C_A$, or zero-order with respect to C_A when $C_A >> K_A$. Either of these conditions makes the equation analytically solvable. If $K_A \sim C_A$, a numeric solution is required.

If a pellet control volume is confined to a thin cross-sectional slice

through the pellet center, termed the x-direction, the advection-dispersion model (Freeze and Cherry, 1979) for a non-reacting, non-sorbing compound yields the following equation in one dimension;

$$D_l \frac{\partial^2 C_A}{\partial x^2} - v \frac{\partial C_A}{\partial x} = \frac{\partial C_A}{\partial t} \qquad (14)$$

where: D_l = coefficient of dispersion (L^2t^{-1})

v = average linear pore velocity along a flowline (Lt^{-1})

The dispersion coefficient can be further expressed as;

$$D_l = \alpha v + D_{eff} \qquad (15)$$

where: α = dispersivity (L)

The relative importance of diffusion and dispersion (advection) within pellets can be determined through comparison of the dimensionless Peclet number (Pe) and D_l/D_{eff} (Freeze and Cherry, 1979). The Peclet number is defined as

$$Pe = \frac{vd}{D_{eff}} \qquad (16)$$

where: d = average pore diameter in pellet (L)

Each system component (biotic and abiotic) has its own diffusion coefficient (Table 1), therefore the Pe vs. D_l/D_{eff} will differ for each. The general form of this relationship is shown in Figure 4 (Freeze and Cherry, 1979).

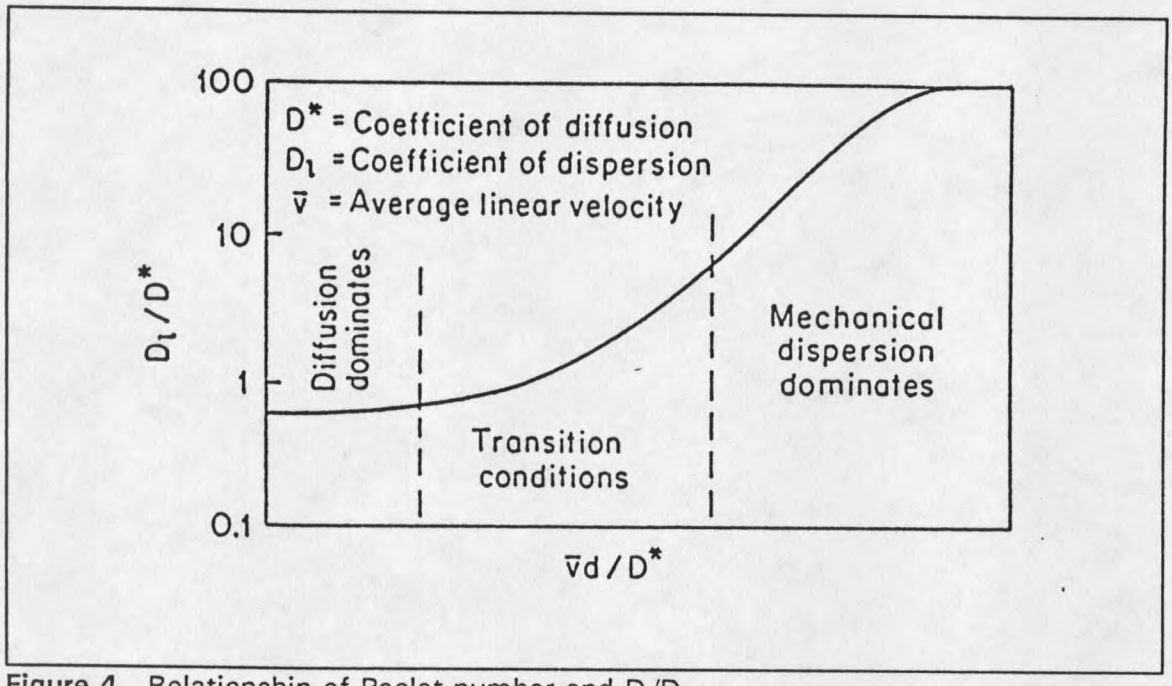


Figure 4. Relationship of Peclet number and D_l/D_{eff}.

Dispersivity (α) must be determined experimentally for a particular porous medium (see Experimental Approach). Once D_{eff} and α are known, D_l becomes a function of v. For a given medium and component, Peclet number is also a function of v. The dominant transport mechanism, as depicted by Figure 4, is then a function of v only (for a given constituent and media).

Table 1. Diffusion Coefficients of System Components.

Component	Diffusivity	Source
Motile cells	1×10^{-5} cm^2s^{-1}	Characklis and Marshall, 1990
Non-motile cells	5.5×10^{-9} cm^2s^{-1}	Characklis and Marshall, 1990
Glucose	7×10^{-6} cm^2s^{-1}	Goldberg and Tewari, 1989
Oxygen	2×10^{-5} cm^2s^{-1}	Himmelblau, 1964

EXPERIMENTAL APPROACH

The experimental work can be divided into 3 parts 1) determinations of pellet physical properties, 2) experiments performed on pellets sent from Tyndall Air Force Base, and 3) laboratory experiments with colonized pellets.

Diatomaceous Earth Pellet Physical Properties

The diatomaceous earth pellets used herein (Manville, R-635) are irregular cylinders approximately 6mm in diameter and varying in length from approximately 2 to 10 mm. Pellet chemical composition (Table 2) and physical properties (Table 3) were provided by the manufacturer.

Table 2. Diatomaceous earth pellet: chemical analysis.

Compound	% by Weight
SiO	82.3
Al_2O	7.2
CaO	2.6
MgO	1.2
Fe_2O_3	1.9
Na_2O	3.3
K_2O	0.9
P_2O_5	0.4
TiO_2	0.2

Table 3. Diatomaceous earth pellets: physical properties.

Property	
Average Size	0.64 cm D x 0.5-1.25 cm L
Mean pore diameter	20 μm
Surface area	0.27 m^2/g
Total pore volume	0.61 cm^3/g
volume fraction 1-10μm	12.5%
" 10-20 "	35.8%
" 20-30 "	39.0%
" 30-40	" 8.1%
Compacted bed density	513 kg m^{-3}

Hydraulic Conductivity

Reactors. Fluid flow in a packed bed reactor using D.E. pellets as a substratum material will follow a tortuous flow path around the individual pellets. Such a system (Figure 5) has a dual pore size distribution, where spaces between pellet contribute to macroporosity and intrapellet voids account for microporosity. A packed-bed reactor system with inlet and outlet manometers (Figure 6) was used to measure headloss (dh/dl) across the reactor. The reactor consisted of a distilled water feed reservoir, a Cole Parmer (Chicago, Il.) peristaltic pump, a Gelman Sciences (Ann

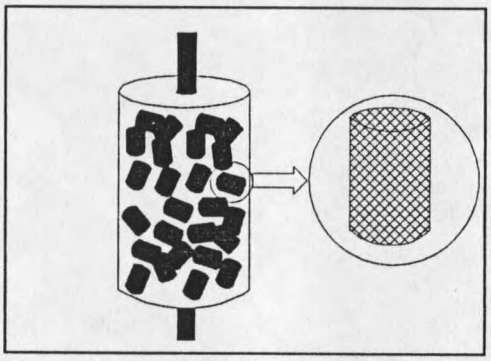


Figure 5. Detail of D.E. pellet reactor.

Arbor, Mi.) flow filter, and water manometers at the inlet and outlet of the 3.4 cm diameter, 9 cm long packed-bed reactor.

Measurement of hydraulic conductivity of a single pellet utilized a similar setup, with the exception that a single pellet was tightly packed into Masterflex (Cole-Parmer, Chicago, Il.) tubing and substituted for the packed-bed reactor (Figure 7). Short circuiting of flow is prevented by the tight fit between tube and pellet.

Measurements. Hydraulic conductivity (K) of the packed-bed reactor is then calculated via Darcy's Law:

$$Q = KA\frac{dh}{dl} \tag{17}$$

where: Q = volumetric flow rate (L^3t^{-1})

K = hydraulic conductivity (Lt^{-1})

A = total reactor cross sectional area (L^2)

dh/dl = head loss (-)

Advective flow velocity into a pellet can then be calculated for any flow situation as follows:

1) Total reactor headloss $(dh/dl)_r$ is observed for a particular flow volume.

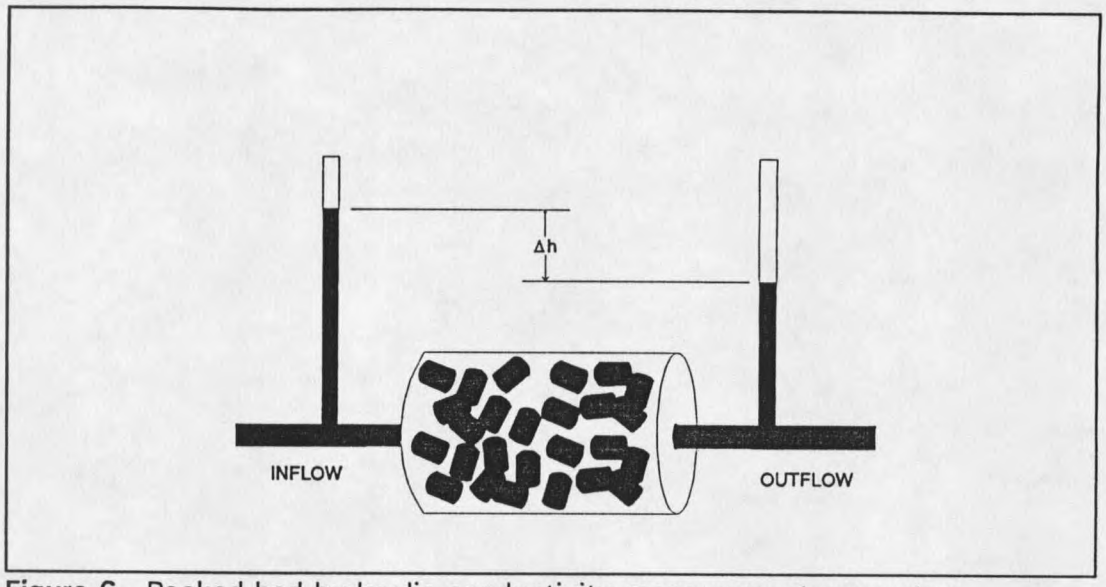


Figure 6. Packed-bed hydraulic conductivity measurement apparatus.

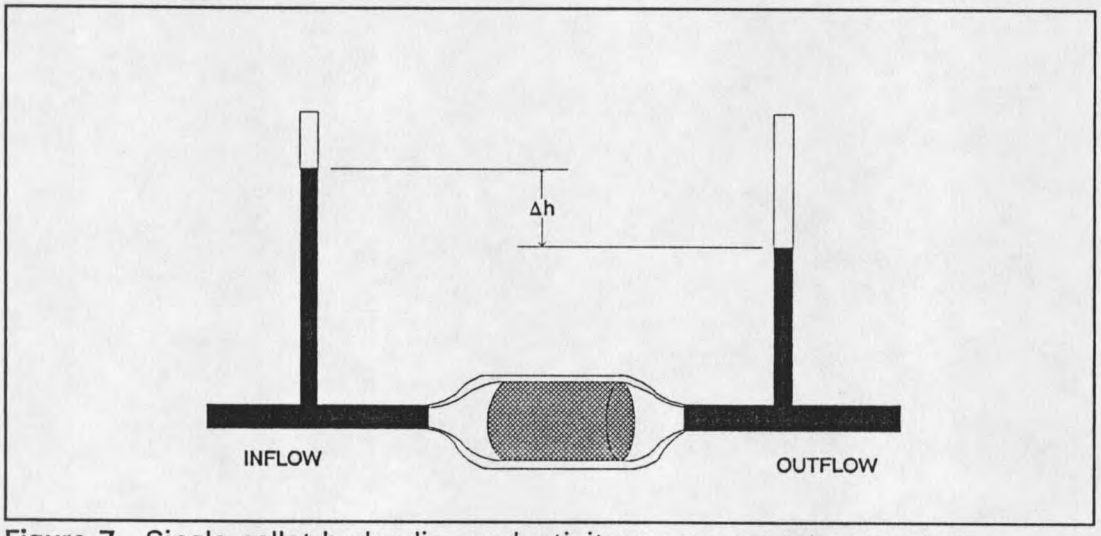


Figure 7. Single pellet hydraulic conductivity measurement apparatus.

2) The reactor dh/dl is then used in Equation 17, along with K_{pellet}, to solve for $Q/A = v_D$ (v_D = Darcy velocity).

3) Darcy velocity is related to actual pore velocity via

$$v_A = \frac{v_D}{e} \tag{18}$$

where: v_A = actual pore velocity (Lt^{-1})

e = pellet porosity (-)

Thus at any given reactor flow rate, an intrapellet advective flow velocity can be calculated.

Pellet and Reactor Porosity

Total pellet porosity was determined volumetrically by first saturating 250 ml of loosely packed pellets with water. The pellets were allowed to soak for 24 hours and then the excess water was poured off and measured. The weight of the saturated pellets was measured (in triplicate) on a Mettler (Highstown, NJ) balance and the arithmetic mean of the 3 measurements was used. Reactor macroporosity was determined as

$$E = \frac{V_{H_2O}}{V_{total}} \tag{19}$$

where: E = reactor macroporosity (interpellet) (-)

V_{H2O} = volume of water drained (L^3)

V_{total} = volume of water + pellets (L^3)

The saturated pellets were oven dried at 105°C for 24 hours, and weighed.

Pellet microporosity was calculated as

$$e = \frac{\frac{W_w - W_d}{\rho_w}}{V_{total} - V_{H_2O}} \qquad (20)$$

where: W_w = wet weight of pellets (M)

W_d = dry weight of pellets (M)

ρ_w = density of water (ML^{-3})

Pellet Dispersivity

As defined in Equation 15, dispersivity is a property of the porous medium, which here is an individual pellet. Dispersivity was calculated by solving Equation 15 for α. Hydrodynamic dispersion (D_l) was measured utilizing a step-function application of fluorescein dye in a constant head flow system (Figure 8). The system consisted of a constant head reservoir tank with an outlet at the base, to which a 2 cm length of tubing was attached. A single pellet was inserted in the tubing. 50 ml of concentrated fluorescein dye (5 mg l^{-1}) was pulsed into the stirred constant head tank at time t=0. Sample collection was done downstream from the pellet in 10 ml acid washed glass vials, and concentrations determined colorimetrically on a Varion DMS90 spectrophotometer at 493 nm.

Equation 14 can be solved for a step function input with the following

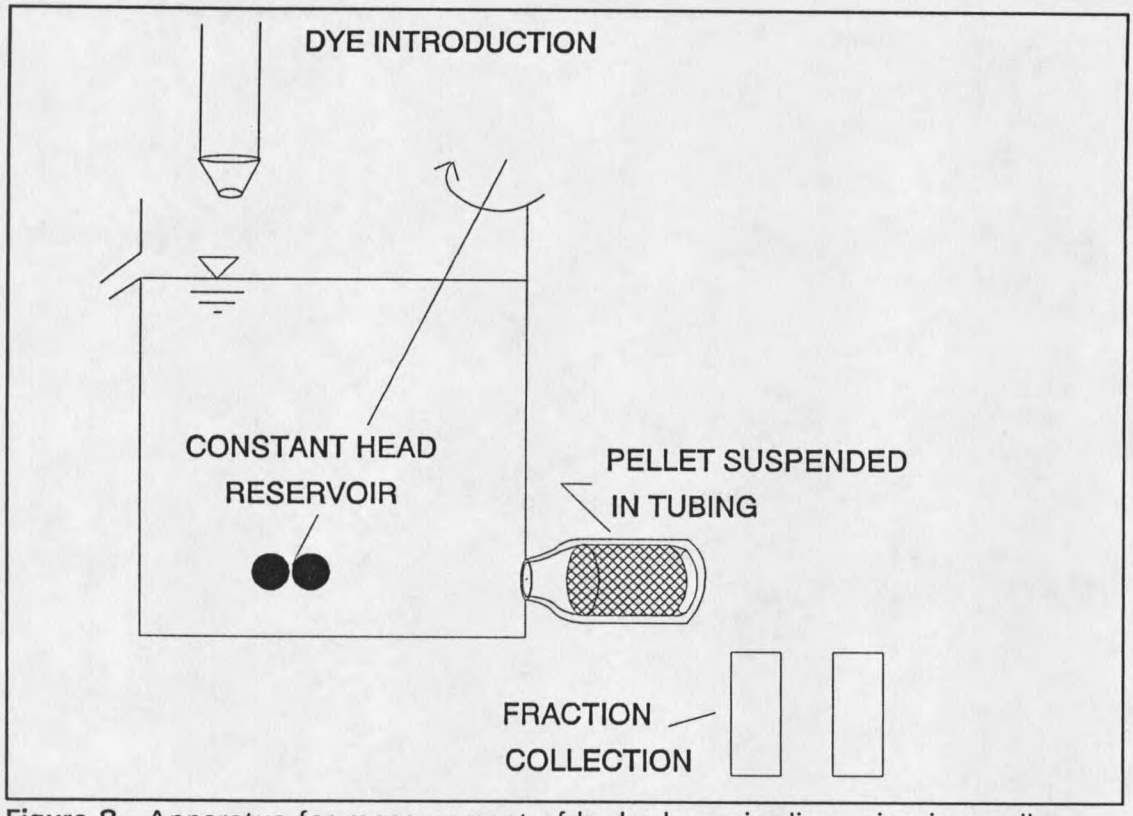


Figure 8. Apparatus for measurement of hydrodynamic dispersion in a pellet.

boundary conditions: $C=0$ at $t=0$, $C=C_0$ at $L=0$ & $t=0$. The solution for a saturated porous medium is (Ogata, 1970)

$$\frac{C}{C_0} = \frac{1}{2}\left[erfc\left(\frac{l-vt}{2\sqrt{D_l\, t}}\right) + \exp\left(\frac{vl}{D_l}\right) erfc\left(\frac{l+vt}{2\sqrt{D_l\, t}}\right)\right] \qquad (21)$$

where: erfc(x) = complementary error function of variable x

Measurement of dye breakthrough (C/C_0) as a function of time provided input values for the solution of this equation for D_l. Equation 15 can then be solved for α since u and D_{eff} for fluorescein dye are known.

Tyndall Air Force Base Experiments

An overview of experimental methods and apparatus used at Tyndall AFB is presented here. A more detailed account of these experiments is presented by Pettigrew (1991). Emphasis in this section will be placed on experiments conducted with pellets removed from bench-scale bioreactors set up at Tyndall AFB.

Reactor Configuration and Design.

A bench-scale bioreactor apparatus was constructed at Tyndall AFB, Florida, and used to degrade a mixture of aromatic compounds contained in a groundwater from Kelly Air Force Base, Texas. The bioreactor was a packed-bed system using Manville R-635 D.E. pellets as medium. Columns were operated in a submerged upflow mode and were fitted with sampling ports at the influent and effluent ends enabling removal of individual pellets. The flow system and reactor configuration were patterned after that used by Bouwer and McCarty (1982).

Initial Colonization.

The colonization procedure consists of loosely packing D.E. pellets into the reactors and initially colonizing with *Pseudomonas* sp. JS150. This pseudomonad was selected from sewage sludge for its capacity of growth on

chlorobenzene as its sole carbon source (Spain and Nishino, 1987). Reactor colonization involved filling the columns with a mineral salts cell culture medium, which was diluted 1:1 with tap water. This suspension was emptied and replaced at 24 hour intervals (3 times) to select for attached cells. Chlorobenzene was supplied to the column in the vapor phase during colonization by bubbling chlorobenzene saturated air through the column.

Reactor Operation.

Columns were operated in a plug-flow mode using groundwater from the contaminated site at Kelly AFB, Texas. After pretreatment with a water softener, this groundwater was mixed with mineral salts buffer in a 1:3 ratio.

Sampling.

During reactor operation, pellets were removed from the influent and effluent ends of the reactor at approximately 4 day intervals. Sampled pellets were suspended in mineral salts buffer and overnight mailed to the Center for Interfacial Microbial Process Engineering at Montana State University.

Analysis.

Scanning electron microscopy was performed on several pellets sent from Tyndall Air Force Base to gain qualitative evidence of cell penetration in the pellets. Pellet samples were sectioned with a sterilized razor blade at the

center to expose a radial face. Sections were dehydrated using successively stronger ethanol solutions as follows: 30%, 50%, 70%, 90%, 100% (each for 10 minutes). Samples were then critical point dried and gold sputter-coated. Observations were made with a JEOL JEM 100CX scanning electron microscope.

Cell Enumerations.

A method was devised which would allow quantification of cell colonization as a function of distance from the pellet surface. The method involved slicing radial cross-sections of the pellet with a sterile razor blade (Figure 9). Pellet section volume was measured by suspending the sliced section in 10 ml sterile water and measuring meniscus displacement with a micromanipulator. Pellet section samples were then further diluted with an additional 10 ml sterile distilled water. Blending solution (Camper et al., 1985) was added at 10 μl/ml, and the slurry was homogenized for 30 seconds at 20,000 rpm using a Tekmar tissuemizer. The homogenized mixture was then diluted and spread on plates

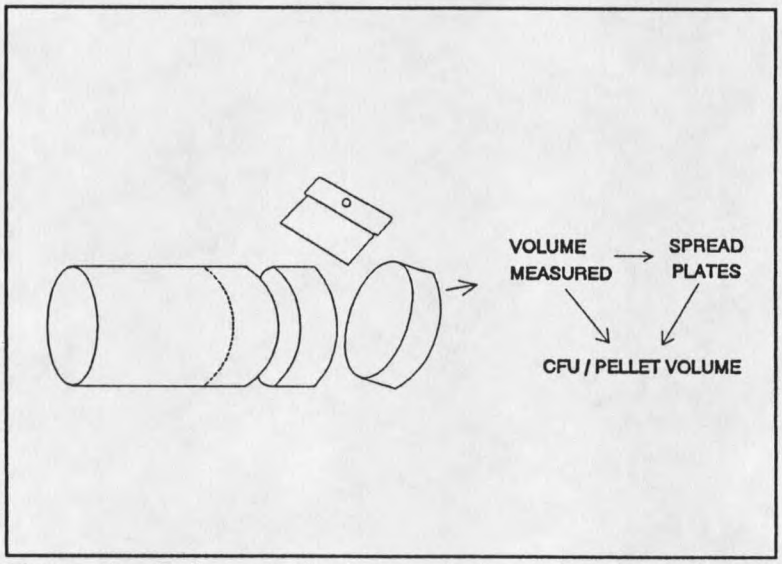


Figure 9. Pellet sectioning technique for Tyndall AFB experiments.

in triplicate on both nutrient (R2A, Difco) and carbon free, minimal salts (Noble, Difco) agars. The nutrient agar plates were incubated at room temperature for 2 days. The carbon free agar plates were incubated in a chlorobenzene and water saturated atmosphere for 10-14 days. Colonies were counted after the incubation period and the arithmetic mean of the three observations was used as the colony forming unit (cfu) count. Where possible, the dilution counted contained between 30 and 300 cfu per plate.

Competition Experiments

Experiments undertaken at M.S.U. sought to quantify bacterial penetration into pellet interiors and to explore the competitive phenomenon when an inoculated species is challenged by an invading species.

Reactor Configuration and Design:

A plug-flow reactor system with an attached chemostat (Figure 10) was used for these experiments. Manville R-635 D.E. pellets were loosely packed into the polycarbonate reactors (9 cm long, 3.4 cm diameter). All system pumps were peristaltic. The chemostat feed pump and the plug-flow reactor feed pump were operated at a flow rate of 0.6 ml min^{-1}, resulting in a hydraulic residence time of 45 minutes for the single species column and 23 minutes for

Table 4. Media Solution for Competition Studies.

Compound	Concentration (mg/l)
Glucose[a]	15
NH_4Cl	7.2
$MgSO_4*7H_2O$	2.0
$(NH_4)_6Mo_7O_{24}*4H_2O$	0.001
$ZnSO_4*7H_2O$	0.1
$MnSO_4*H_2O$	0.008
$CuSO_4*5H_2O$	0.002
$Na_2B_4O_7*10H_2O$	0.001
$FeSO_4*7H_2O$	0.112
$(HOCOCH_2)_3N$	0.4
$CaCl_2*2H_2O$	11.0
Na_2HPO_4	213
KH_2PO_4	204

[a] Added after sterilization

the competition column. The feed media (Table 4) was prepared and the entire apparatus was autoclaved at 121°C for 3-4 hours to ensure initial sterility.An experiment typically consisted of operating 3 columns in parallel. 1) an initial colonization control column. 2) a single species column which was operated in plug-flow mode but not subjected to a competing organism. 3) a competition column which was colonized, run in plug-flow for 4-5 days, then challenged with a competing organism for 5-15 additional days. The single species column and the competition column were dissected concurrently to determine the effects of competition on the resulting mixed population.

Pseudomonas aeruginosa and *Klebsiella pneumoniae* were chosen for the competition experiments because their growth rates differ by a factor of 5, *Pseudomonas* is motile and *Klebsiella* is not, and their kinetic coefficients have been well characterized (Table 6). Specific strains of both organisms have been

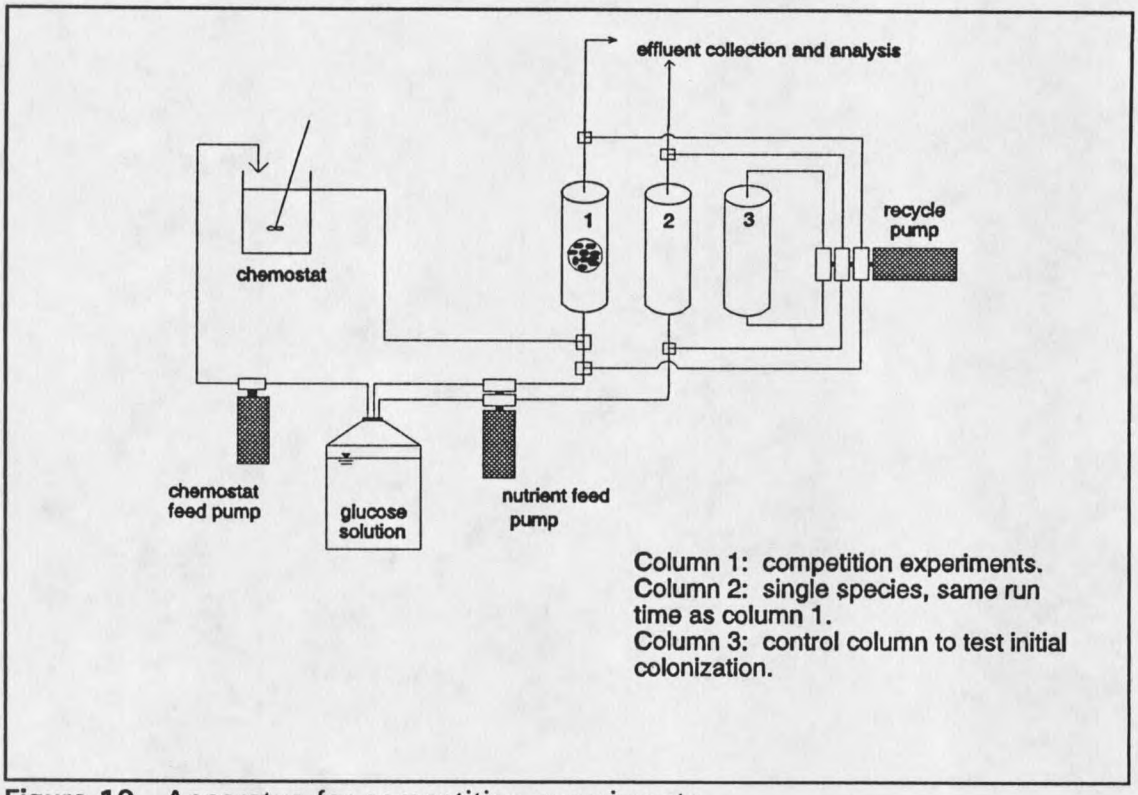


Figure 10. Apparatus for competition experiments.

extensively studied at the Center (Siebel, 1987).

Table 5. Mineral Salts Media.

Compound	Concentration (mg/l)
K_2HPO_4	700
KH_2PO_4	300
$(NH_4)_2SO_4$	100
$MgSO_4*7H_2O$	100

Initial Colonization.

Initial colonization was accomplished by injecting 2.5 ml of a cell suspension solution directly into the recycle loop of each reactor. The recycle loops were used for initial colonization only; no recycle flow occurred during column operation. The injected cell suspension was recycled through the pellet column in a mineral salts buffer (Table 5) for

3 days.

Cell suspensions for inoculation were made by mixing 0.1 ml frozen stock culture, 1 ml of 100 ppm glucose solution, and 100 ml sterile mineral salts buffer (pH 7) and incubating at 35°C for 48 hours. The cell slurry was then centrifuged for 20 minutes at 17000 xg in a Sorvall refrigerated centrifuge. The supernatant was discarded and the cell pellet was resuspended in 20 ml sterile mineral salts buffer which resulted in a cell concentration of approximately 10^9 cfu ml^{-1}.

Sampling.

During plug-flow operation reactors were sampled daily for effluent total organic carbon (TOC), effluent glucose and effluent cells. At the end of each experiment, pellets from the top, middle and bottom of each reactor were dissected. The dissection method used was a refinement over that described earlier. Instead of removing cross sectional slices as before, concentric shells of each pellet were removed (Figure 11). This technique allowed more accurate determination of interior colonization. Pellet section volume determination and homogenization were performed as described above. Viable plate counts were done in triplicate on both R2A and *Pseudomonas* selective agars. Colony types were distinctive on R2A agar and *Klebsiella* will not grow on *Pseudomonas* selective agar. Plates were incubated at room temperature for 1-2 days.

Table 6. Properties of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Property	*P. aeruginosa*	*K. pneumoniae*
motility	polar flagella[1]	non-motile
respiration	obligate aerobe[2]	facultative anaerobe[2]
metabolism	chemoorganotroph[3]	chemoorganotroph[3]
maximum growth rate	0.40 hr^{-1} [4]	2.00 hr^{-1} [5]
half-sat. coefficient	2.5 gm^{-3} [4]	1.43 gm^{-3} [5]

[1] Holt (1977)
[2] Buchanan and Gibbons (1974)
[3] Sutherland (1977)
[4] Characklis and Marshall (1990)
[5] Siebel (1987)

Effectiveness Factor Experiment

A single species experiment was undertaken to determine colonized pellet effectiveness factor and its dependence on nutrient loading. The reactor was colonized with *Pseudomonas aeruginosa* as described earlier, but competing organisms were not introduced.

After the 4 day colonization period in recycle flow, reactor flowrate was initially set at 0.6 ml/min. Effluent glucose concentration was monitored twice daily. Reactor flowrate was increased as soon as effluent glucose concentration stabilized, usually 24-48 hours. The reactor flowrate was incrementally increased to 2.0, 3.0, 4.0, 5.0, 6.4, 9.0, and 12.0 ml/min. Effluent cell counts were performed each 24 hour period by plating reactor

effluent on R2A agar. Pellets from this experiment were dissected as described earlier after 19 days of run time.

Analytical Methods

Total organic carbon samples were acidified to pH 2 with phosphoric acid, sparged for 5 minutes with oxygen, then injected into a Dohrmann DC80 Carbon Analyzer. Glucose samples were filtered with 0.2 μm polycarbonate filter (Whatman), and analyzed colorimetrically (Sigma Diagnostics, St.Louis, MO) with a Varian DMS 90 UV/visible spectrophotometer at 450 nm. Effluent cell counts were determined via heterotrophic plate counts on R2A agar (as described earlier). When effluent contained both *Klebsiella* and *Pseudomonas*, species were distinguished through concurrent plating on *Pseudomonas* selective and R2A agars.

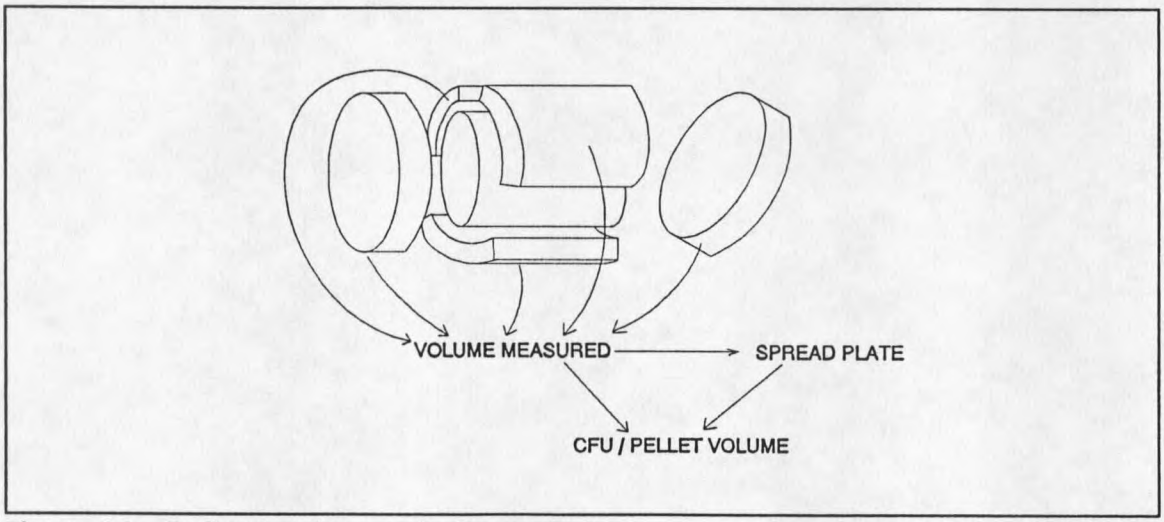


Figure 11. Refined pellet sectioning technique.

RESULTS

Experiments were conducted to determine pellet physical properties, and properties of packed-bed reactors using D.E. pellets. Raw data from these experiments are listed in Appendix A.

Pellets colonized with chlorobenzene degrader *Pseudomonas* sp. JS150 and exposed to actual contaminated groundwater for varying lengths of time were sent from Tyndall Air Force Base, Florida. Experiments performed on these pellets yielded insight into the persistence of the inoculated organism. Raw data from these experiments are listed in Appendix B.

Laboratory competition experiments sought to elucidate the roles of cell growth rate, motility, and order of inoculation on microbial persistence in the pellets. Raw data from these experiments are shown in Appendix C.

Pellet Physical Properties

Pellet and Reactor Porosity

Macroporosity in the loosely packed experimental columns used for the competition and effectiveness experiments was volumetrically determined to be 33% ± 3% of the total reactor volume. Intrapellet (micro-) porosity was similarly determined to be 50% ± 2% of total pellet volume.

Hydraulic Conductivity

Using Darcy's law, experimentally determined hydraulic conductivity (K) for both a loosely packed bed of D.E. pellets and an individual pellet were 18 cm/min. and 0.038 cm/min., respectively.

Dispersivity

A pellet 6mm in diameter and 7.5mm long was tightly suspended in tubing (Figure 11). The constant head flow through the pellet was 2.6 ml/min. corresponding to a pore velocity of 0.3 cm/sec. Fluorescein dye was introduced in step function fashion at time $t=0$, and effluent was collected. The dye breakthrough curve (Figure 12) permits the solution of Equation 28 by trial and error. At $t=2$ sec., $C/C_o \approx 0.35$. When these values are substituted into equation 21, solution for the coefficient of dispersion yields $D_l = 0.24$ $cm^2 s^{-1}$. Checking this value at $t=3$ sec yields $C/C_o \approx 0.75$, which is very close to the value predicted by the curve in Figure 12. The diffusivity of fluorescein dye can be estimated from a method outlined in Wilke and Chang (1955). This estimation sets $D_{eff} = 4.6 \times 10^{-6} cm^2\ s^{-1}$ for fluorescein dye. Further study of Equation 15 indicates that since $D_l \sim 10^{-2} cm^2\ s^{-1}$ and $v \sim 10^{-1} cm\ s^{-1}$, the contributions of D_{eff} to the dispersion coefficient can be ignored with no loss of accuracy. Solving Equation 15 for dispersivity then yields $\alpha = 0.08cm$.

Dispersivity can then be used to calculate a dispersion coefficient as a function of v for each important component of the experimental system. Relationships such as that shown in Figure 4 can be derived for each

component by plotting D_l/D_{eff} vs Peclet number. For a specific component and medium, *v* is the only variable (Figures 13-16) resulting in zones of diffusion dominated or transitory (diffusion and advection) dominated transport. Thus the value of *v* within the pellet for any flow regime permits determination of the dominant transport mechanism.

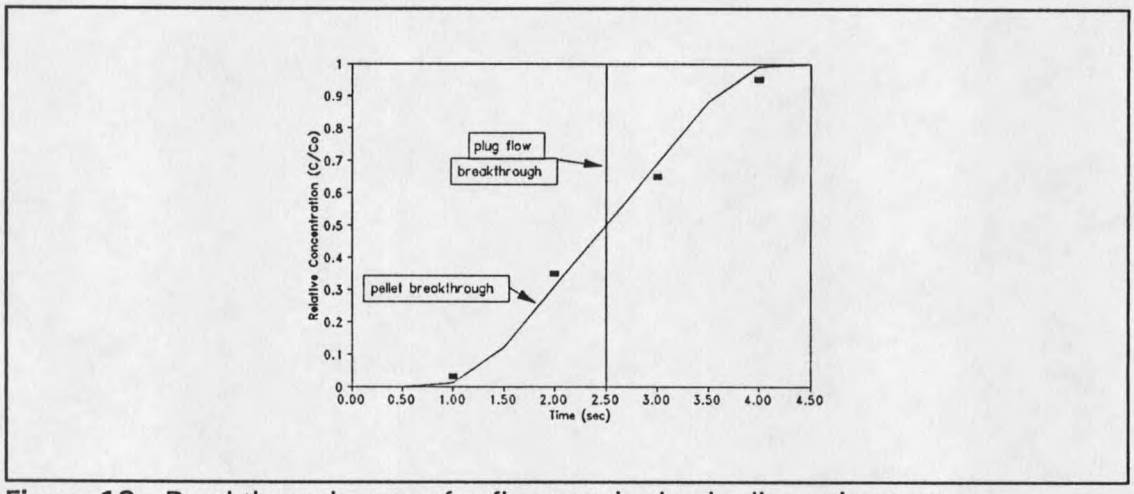


Figure 12. Breakthrough curve for fluorescein dye in dispersion test.

Intrapellet Velocity

Using K = 18 cm/min for a packed-bed reactor, Darcy's law is solved for dh/dl as follows:

$$\left(\frac{dh}{dl}\right)_{reactor} = \frac{Q_{reactor}}{K_{reactor}A_{reactor}} = \frac{0.5ml/\text{min}}{(18cm/\text{min})(9.08cm^2)} = 0.00306 \qquad (22)$$

considering this as the driving force, advective flow into a single pellet is found as:

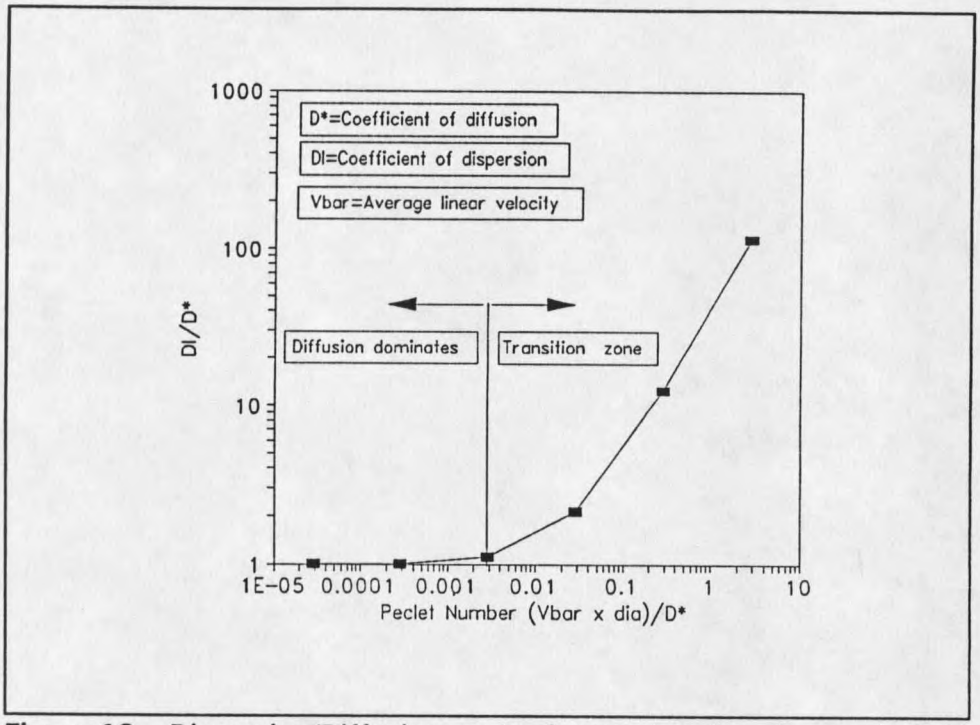


Figure 13. Dispersion/Diffusion vs Peclet number for glucose in D.E. pellets.

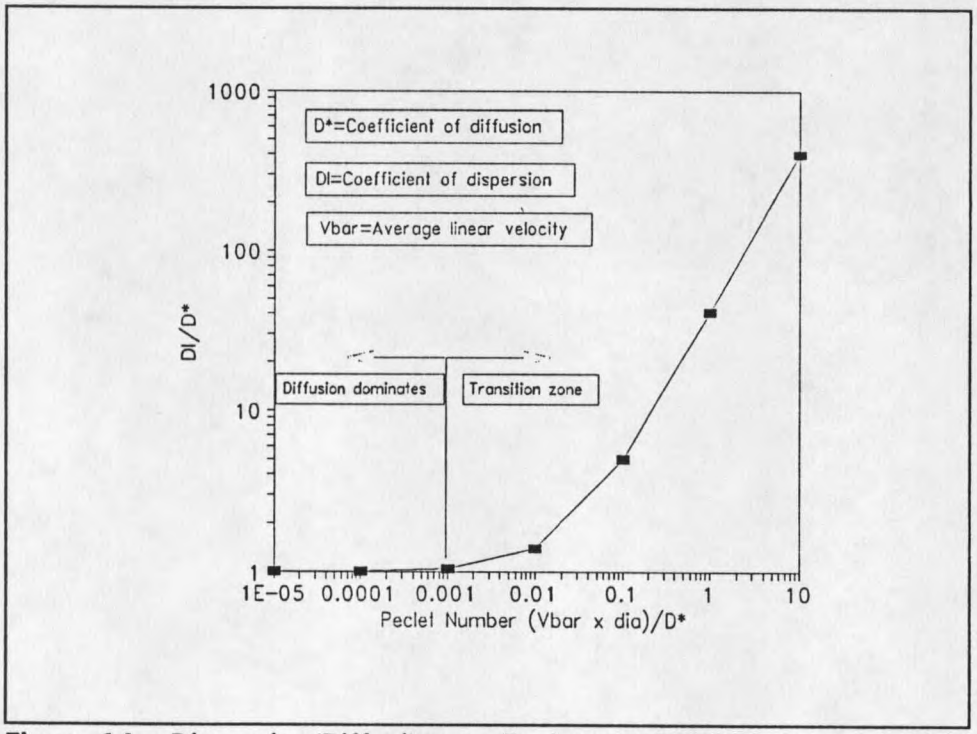


Figure 14. Dispersion/Diffusion vs Peclet number for oxygen in D.E. pellets.

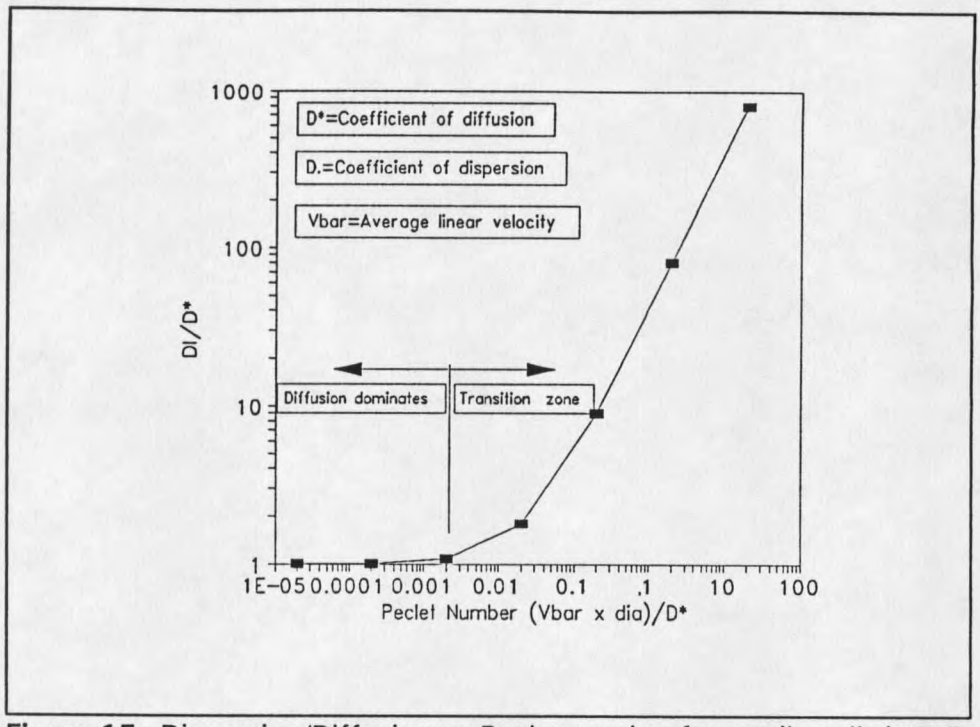


Figure 15. Dispersion/Diffusion vs Peclet number for motile cells in D.E. pellets.

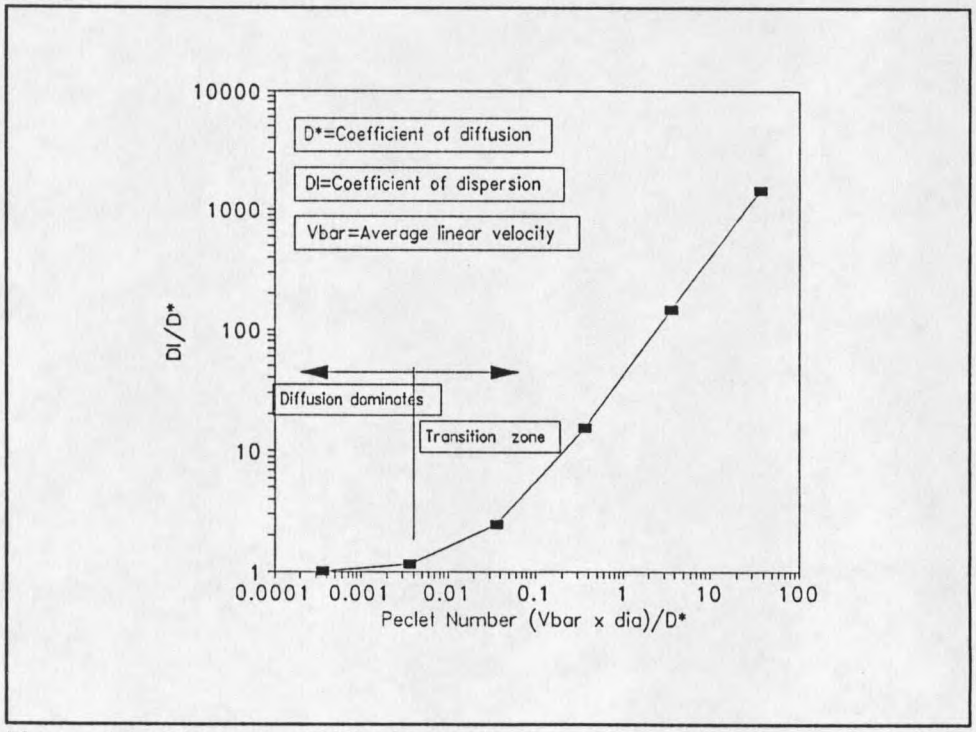


Figure 16. Dispersion/Diffusion vs Peclet number for non-motile cells in D.E. pellets.

$$(Q)_{pellet} = K_{pellet}A_{pellet}(\frac{dh}{dl})_{pellet} = (0.038cm/\text{min})(\frac{(.6cm)^2\pi}{4})(0.00306) = 3.46x10^{-5}ml/\text{min} \quad (23)$$

Intrapellet (pore) velocity is then found as:

$$v = \frac{Q_{pellet}}{eA_{pellet}} = \frac{3.46x10^{-5}}{(0.5)(\frac{(.6)^2\pi}{4})} = 0.0002cm/\text{min} \quad (24)$$

Under these flow conditions, the dominant transport mechanism for each component is shown in Table 7.

Table 7. Dominant transport mechanism within a pellet for major system components.

Component	Pe_{max} for diffusion dominated flow	Maximum *v* for diffusion dominated flow	Actual *v*	Dominant transport mechanism in pellets
Glucose	0.003	$1.1x10^{-5}$cm/s	$3.3x10^{-6}$cm/s	diffusive
Oxygen	0.001	$1.0x10^{-5}$cm/s	$3.3x10^{-6}$cm/s	diffusive
Mot+ cells	0.002	$1.0x10^{-5}$cm/s	$3.3x10^{-6}$cm/s	diffusive
Mot- cells	0.003	$8.3x10^{-9}$cm/s	$3.3x10^{-6}$cm/s	advective

Tyndall Air Force Base Experiments

Two bench-scale experiments were performed at Tyndall AFB, the first lasting 10 days and the second lasting 15 days. Scanning electron microscopy

performed on the first pellets received revealed qualitatively that organisms were penetrating to the center of the pellets (Figure 1).

Bench Scale Experiment 1

Over the 10 day duration of experiment 1, overall populations of chlorobenzene degrading organisms dropped from approximately 10^8 cfu ml^{-1} pellet to 10^5 cfu ml^{-1}. During this period, populations of total organisms remained constant at 10^9 cfu ml^{-1} (Figure 17). Intrapellet spatial distribution of total organisms and chlorobenzene degrading organisms remained constant from exterior pellet sections (0-1mm) to interior sections (2-3mm) for each time period (Figures 18,19).

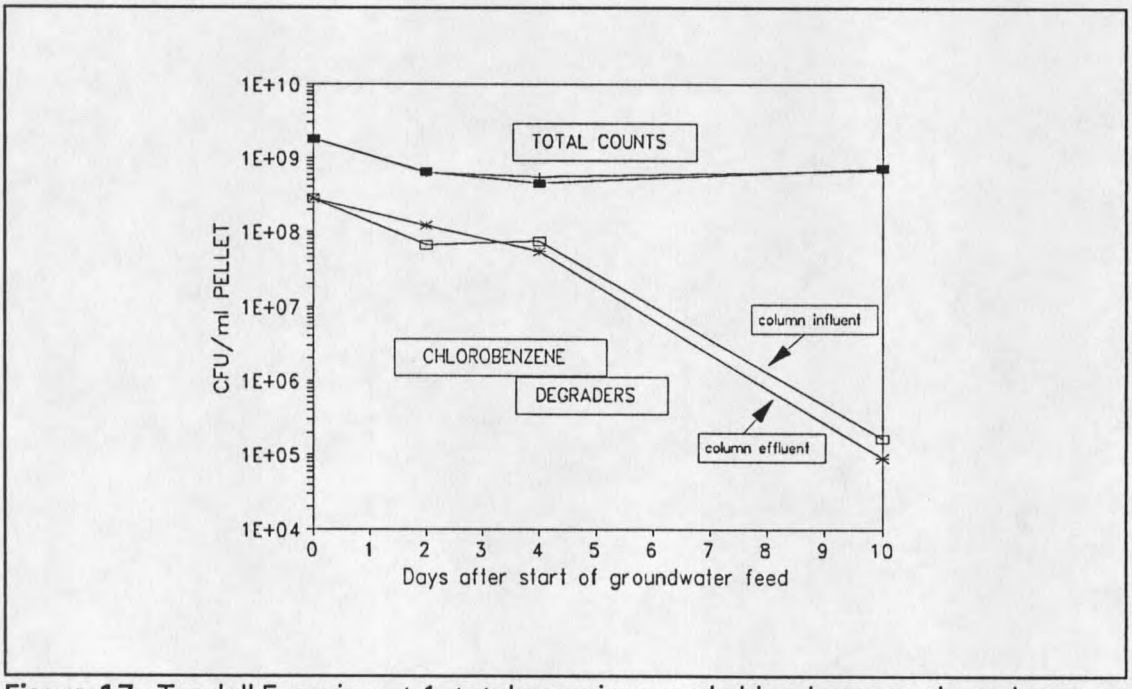


Figure 17. Tyndall Experiment 1, total organisms and chlorobenzene degraders over time (whole pellets).

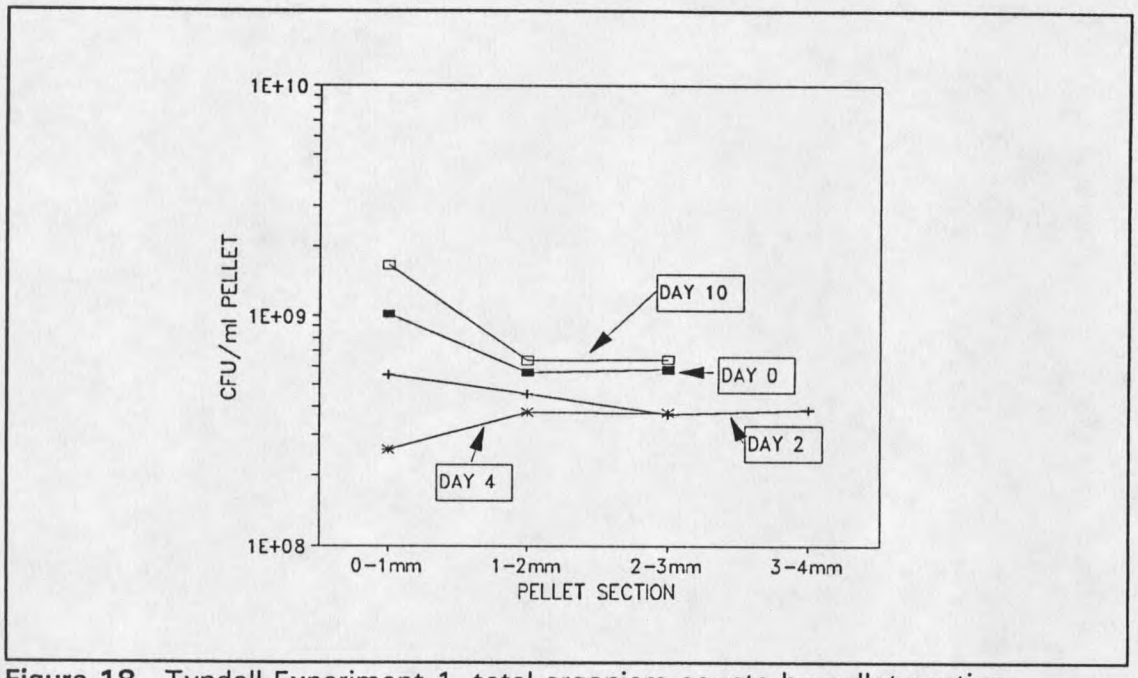


Figure 18. Tyndall Experiment 1, total organism counts by pellet section.

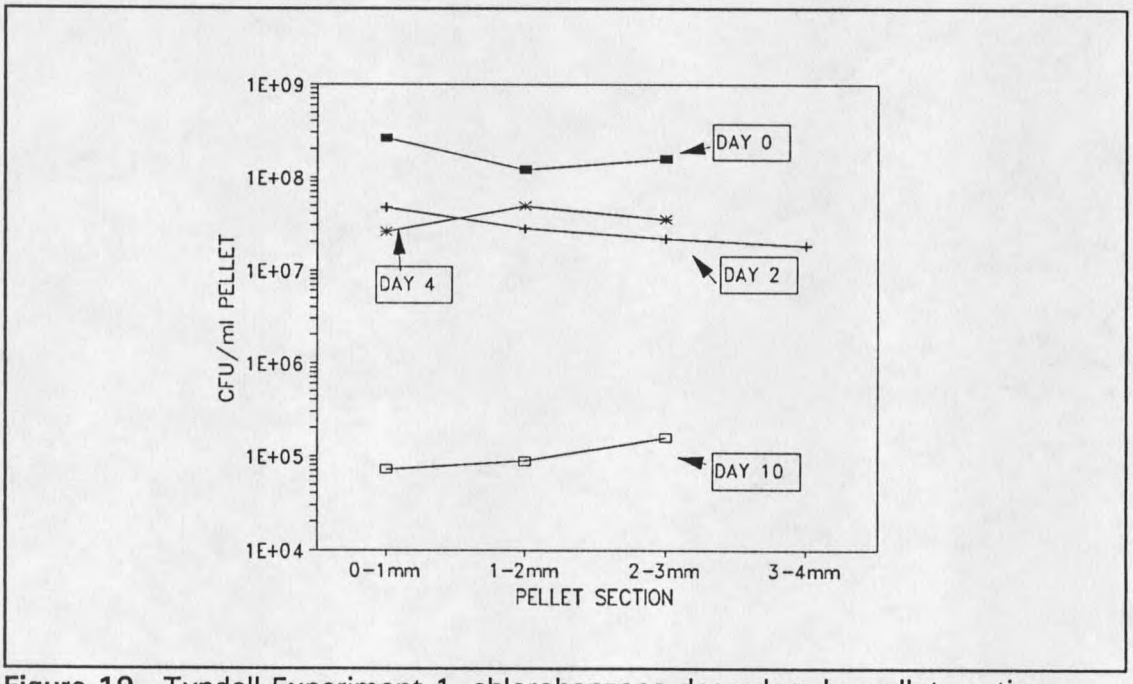


Figure 19. Tyndall Experiment 1, chlorobenzene degraders by pellet section.

Bench Scale Experiment 2

Total organism counts on whole pellets remained constant at 10^9 cfu/ml throughout the 15 day experiment at the effluent end of the reactor. At the reactor influent, total counts rose from 10^9 to 10^{10} cfu ml^{-1} at about day 10. Whole pellet chlorobenzene degrader counts remained constant at 10^6-10^7 cfu/ml over the duration of the experiment (Figure 20). In contrast to experiment 1, pellet sectioning showed chlorobenzene degrader colonization on pellet exterior sections was about 10 times that on interior sections (Figure 21). Total organisms remained fairly stable from interior to exterior sections (Figure 22).

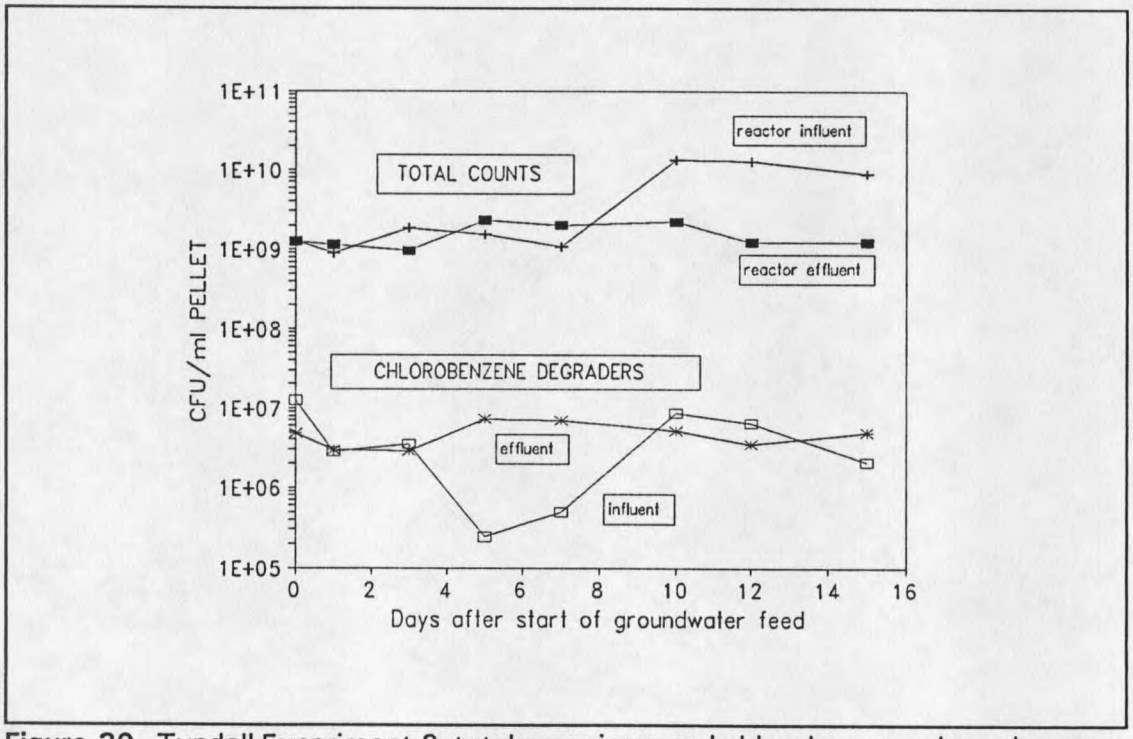


Figure 20. Tyndall Experiment 2, total organisms and chlorobenzene degraders over time.

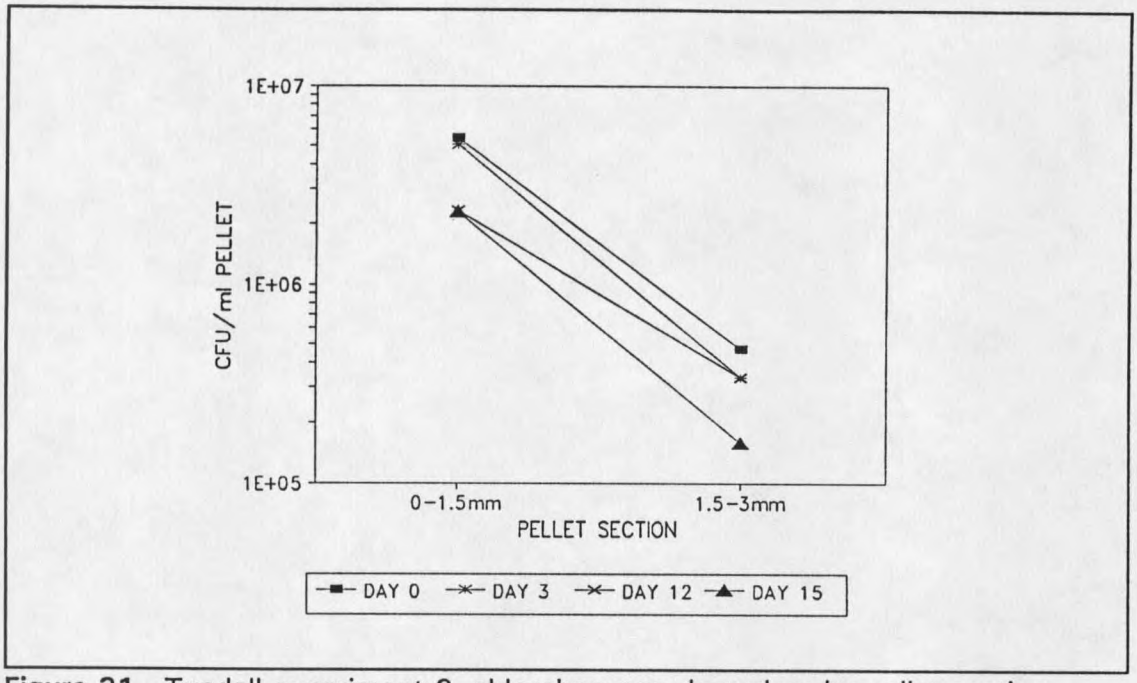


Figure 21. Tyndall experiment 2, chlorobenzene degraders by pellet section.

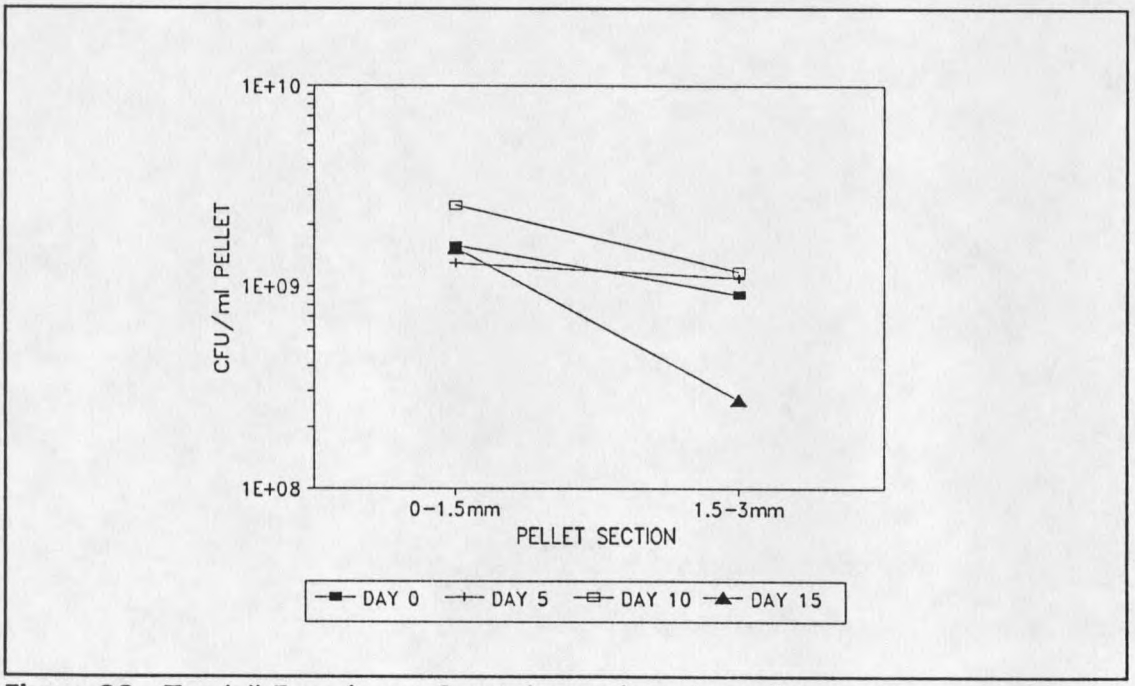


Figure 22. Tyndall Experiment 2, total organisms by pellet section.

Competition Experiments

Three experiments were performed to evaluate intrapellet competition. In experiments 1 and 2, pellets were colonized with *Pseudomonas aeruginosa* and challenged with *Klebsiella pneumoniae*. Experiment 1 ran for 10 days, experiment 2 for 21 days. In experiment 3, *Klebsiella* was the colonizing organism, *Pseudomonas* the challenger. This experiment ran for 10 days.

Cell Colonization Results

Competition Experiment 1. After 10 days of exposure to faster-growing *Klebsiella*, inoculated *Pseudomonas* numbers dropped by one order of magnitude in interior pellet sections, and by one-half order of magnitude in exterior sections. Invading *Klebsiella* colonized the outermost section only marginally less than the challenged *Pseudomonas* in the 10 day experiment. Interior colonization by *Klebsiella* was significantly less than challenged *Pseudomonas* (Figure 23).

Competition Experiment 2. This experiment was identical to experiment 1, but was allowed to run for 21 days. The longer time period resulted in more dense *Klebsiella* colonization than either challenged or unchallenged *Pseudomonas* at all pellet sections. *Klebsiella* not only outcompeted

Pseudomonas in the same reactor, but also showed more dense colonization than unchallenged *Pseudomonas*. *Klebsiella* colonization ranged from $3x10^8$ cfu/ml in exterior sections to 10^7 cfu ml^{-1} in the innermost section. *Pseudomonas* response to this competition was to decrease slightly in the 0-1mm and 1-2mm sections, but remain stable at the pellet center (2-3mm)(Figure 24).

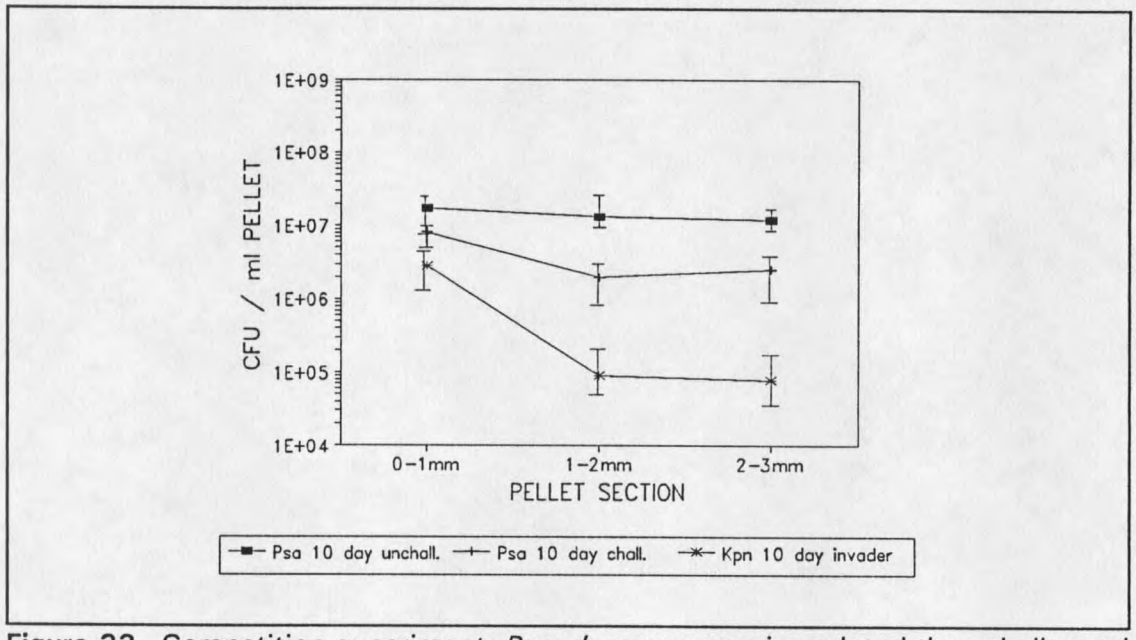


Figure 23. Competition experiment, *Pseudomonas* was inoculated then challenged with *Klebsiella*; experiment ran 10 days.

Competition Experiment 3. When *Klebsiella* was colonized first and challenged with *Pseudomonas*, there was very little change in *Klebsiella* cell numbers over the 10 days of the experiment. *Klebsiella* initially colonized all pellet surfaces at 10^7-10^8 cfu/ml, and stayed at this level despite *Pseudomonas* competition. *Pseudomonas* colonization was consistently 2 orders of

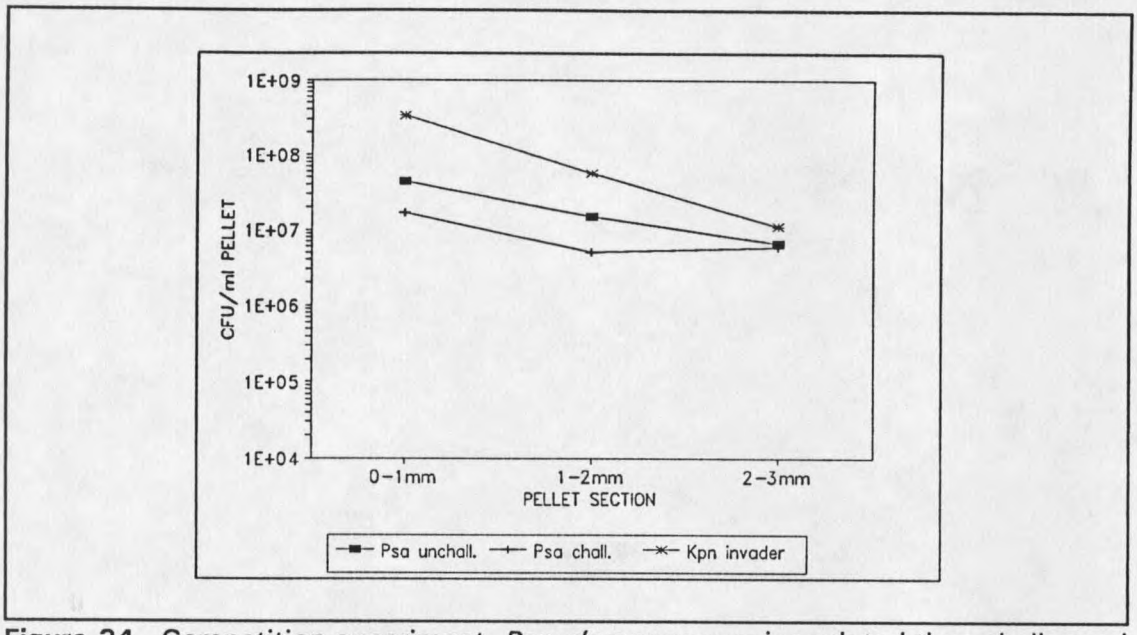


Figure 24. Competition experiment, *Pseudomonas* was inoculated then challenged with *Klebsiella*; experiment ran 21 days.

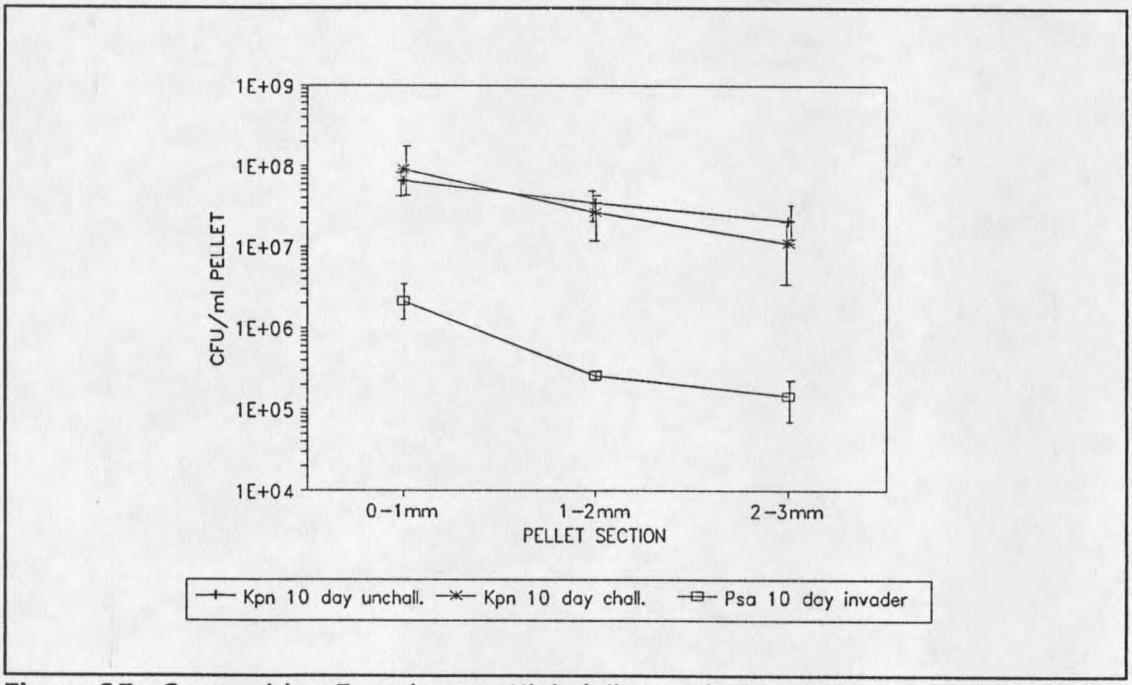


Figure 25. Competition Experiment, *Klebsiella* was inoculated and challenged with *Pseudomonas*. Experiment lasted 10 days.

magnitude below *Klebsiella* at all pellet sections (Figure 25).

Effluent Cell Results

For the competition experiments, effluent cell counts were taken for both reactor and chemostat effluents.

Experiments 1 and 2. Where *Pseudomonas* was colonized and subject to *Klebsiella* competition (via chemostat effluent), *Pseudomonas* effluent cell counts for the competition column were very close to those for the unchallenged column (Figures 26,27). In these experiments, *Klebsiella* counts in the reactor effluent were consistently up to 1 order of magnitude below the counts in the chemostat effluent which was fed into the reactor, indicating that up to 90% of *Klebsiella* cells in the reactor influent stayed in the reactor as attached cells.

Experiment 3. Where *Klebsiella* was colonized and subject to *Pseudomonas* competition, effluent *Klebsiella* counts for challenged and unchallenged columns showed more variation than seen in the *Pseudomonas* colonized experiments, but were always of the same order of magnitude (Figure 28). In contrast to invading *Klebsiella*, invading *Pseudomonas* effluent numbers were very close to chemostat effluent counts, indicating that relatively fewer *Pseudomonas* cells were attaching in the reactor.

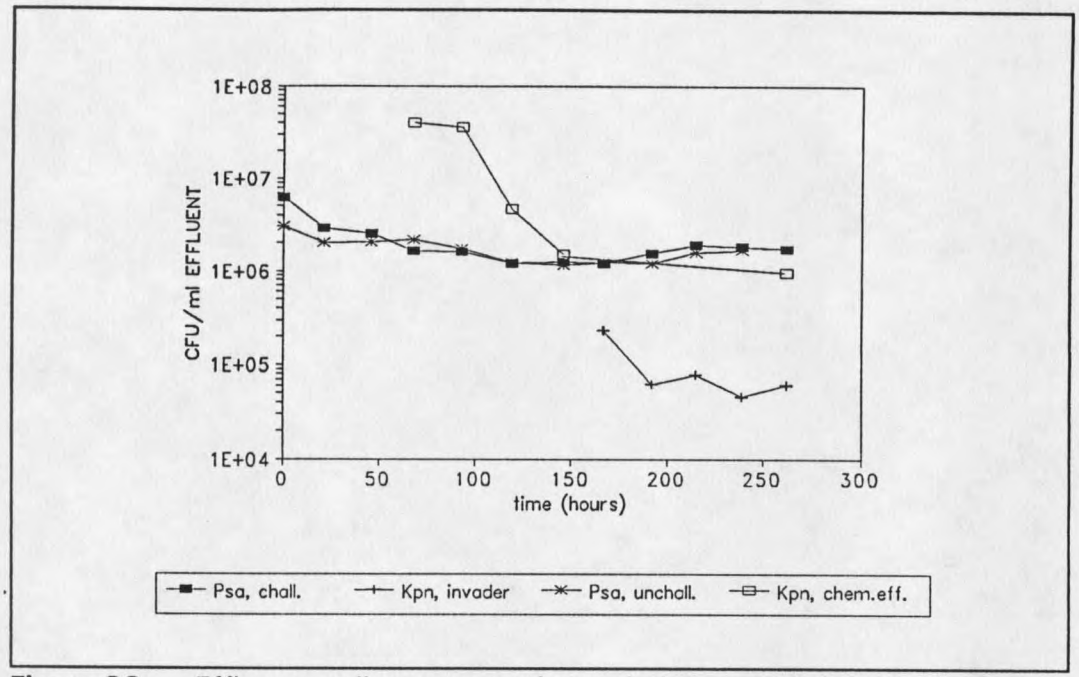


Figure 26. Effluent cell concentrations during competition experiment. *Pseudomonas* was inoculated then challenged with *Klebsiella*. Experiment lasted 10 days.

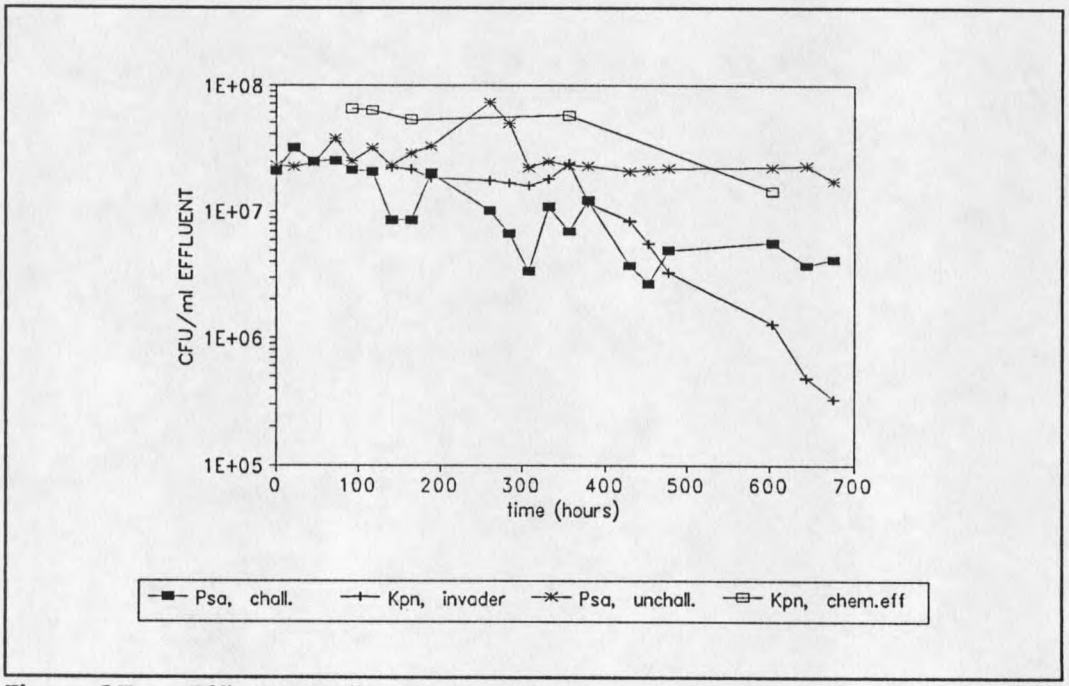


Figure 27. Effluent cell concentrations during competition experiment. *Pseudomonas* was inoculated then challenged with *Klebsiella*. Experiment lasted 21 days.

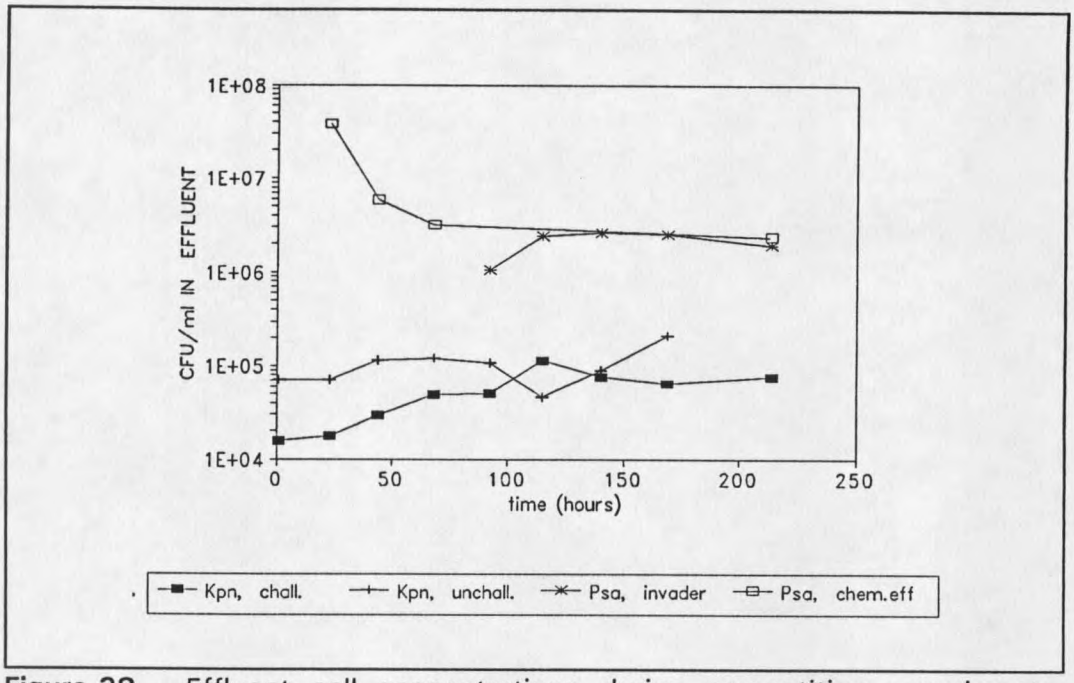


Figure 28. Effluent cell concentrations during competition experiments. *Klebsiella* was inoculated then challenged with *Pseudomonas*. Experiment lasted 10 days.

Effectiveness Factor Experiment

The reactor configuration used for both the competition and effectiveness factor experiments proved very efficient at removing feed solution glucose. In all competition experiments, effluent glucose concentration was zero. Similarly, in effectiveness factor experiments at the initial flowrate of 0.6 ml min^{-1},reactor effluent contained no measurable glucose. In fact, significant concentrations of glucose were not measured in reactor effluent until the flowrate was raised to 4 ml/min and above (Figure 29). Above a flowrate of 4 ml/min, effluent glucose concentration increased monotonically with flowrate (Figure 30).

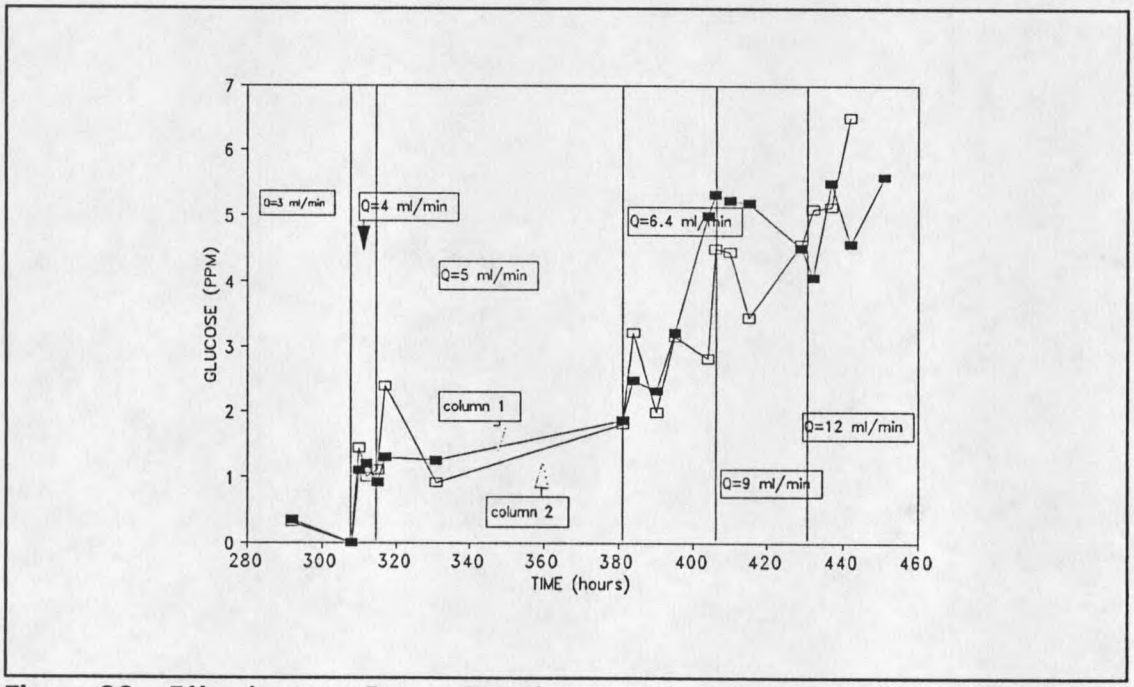


Figure 29. Effectiveness Factor Experiment, effluent glucose concentration over time (flowrate was varied as shown).

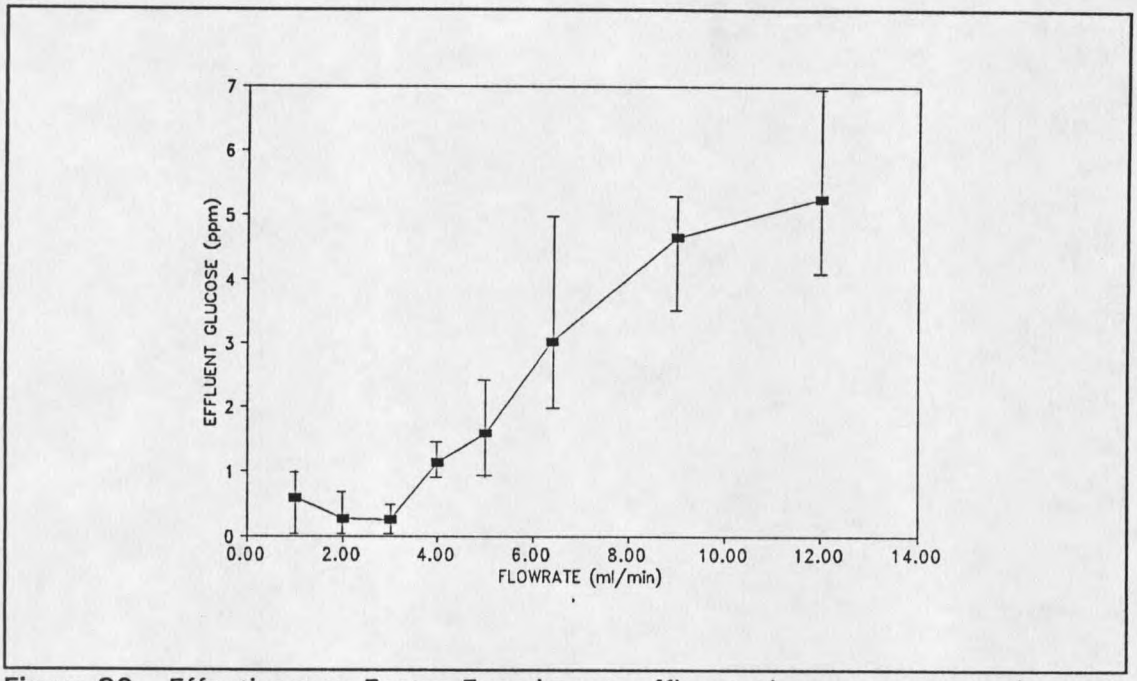


Figure 30. Effectiveness Factor Experiment, effluent glucose concentration as a function of flowrate.

DISCUSSION

Pellet Physical Properties

Diatomaceous earth pellets are hydrodynamically similar to a homogeneous soil which contains many macropores between colloids. An intrapellet hydraulic conductivity of 0.038 cm min^{-1} is on the same order as a limestone core, whereas a loosely packed bed of D.E. pellets is hydraulically comparable to gravel (Todd, 1980).

The measured dispersivity of 0.08 cm is at the low end of values for geologic materials. Klotz and Moser (1974) measured values in the range of 0.01 -2 cm, and observed dispersivity to be a function of grain size and grain size distribution rather than shape and angularity. The highly uniform pore structure of these pellets is reflected in the low measured dispersivity.

Tyndall Air Force Base Experiments

In bench scale experiment 1, inoculated chlorobenzene degrading organisms were originally colonized at 10^8 cfu ml^{-1} pellet while total counts were at 10^9 cfu ml^{-1}. Chlorobenzene degrader populations remained stable until sometime between day 3 and day 10 (when the experiment was terminated) at which point they dropped to 10^5-10^6 cfu ml^{-1}. Experiment 2 shows a similar

pattern with regard to total organisms, but differs with regard to chlorobenzene degraders. Total counts started at 10^9 cfu ml^{-1}, like experiment 1, and remained stable until day 10, when they jumped to 10^{10} cfu ml^{-1} at the reactor influent. This sudden jump could have been caused by an influx of cells from the influent groundwater solution, or by a particularly prolific growth period brought on by an increase in easily assimilable organic material in the reactor influent. Neither influent cells nor non-contaminant organics were measured in column influent during these experiments.

In the second experiment, chlorobenzene degraders were initially colonized at 10^6-10^7 cfu ml^{-1}, and stayed at this level for the duration of the 15 day experiment. No overall decrease was observed, as with experiment 1. Initial colonization did not seem to be as effective during this experiment, and at the lower level of colonization, the competitive effects of the other organisms were not apparent. While not increasing their numbers, the chlorobenzene degraders were not diminished either. Several factors could account for the observed drop in chlorobenzene degraders in experiment 1 and their stability in experiment 2: 1) The maximum sustainable level of chlorobenzene degraders could be approximately 10^6 cfu ml^{-1}. This would account for the drop in experiment 1, and the maintenance of chlorobenzene degraders at this level in experiment 2. This upper limit of chlorobenzene degraders could be caused by substrate limitations in reactor influent. 2) The sudden loss of chlorobenzene degraders in experiment 1 may have been caused

by the onset of anaerobic conditions due to an excess of organics to the system. Though not recorded, the risk of low dissolved oxygen levels was high because of the carbon-rich nature of the groundwater. 3) The inoculated organism (*Pseudomonas* sp JS150) may account for little or none of the chlorobenzene degraders measured in these experiments. Pettigrew (1991) reported similar volatile organic compound removals from both inoculated and uninoculated reactors, indicating that there were indigenous chlorobenzene degraders present in the groundwater. The rapid drop in experiment 1 could have been due to the total (or near total) loss of the inoculum. The maintenance of chlorobenzene degraders at a level of 10^6 cfu ml^{-1} after day 3 in experiment 1 and throughout experiment 2 could have been due to indigenous organisms. For this to be the case, the inoculation in experiment 2 would have had to been a near total failure, otherwise a higher initial chlorobenzene degrader population should have been observed in experiment 2.

Chlorobenzene degrading organisms did not persist in pellet interior sections to a greater extent than exterior sections in either experiment. On the contrary, in experiment 2 (which utilized the more refined pellet sectioning technique) chlorobenzene degraders appeared to be 10 times more abundant at exterior sections than interior for all days tested. This result suggests that interior colonized chlorobenzene degraders were at a relative disadvantage, due to substrate or electron acceptor conditions.

Competition Experiments

Colonization. Examination of equations 22-24 indicates that intrapellet pore velocity (v) is a linear function of reactor flowrate (Q). The flowrate used in the competition experiments resulted in an intrapellet pore velocity of 3.3×10^{-6} cm s^{-1}. This low advective velocity resulted in diffusive flux as the dominant mass transport mechanism for substrate, electron acceptor, and motile cells. The lower calculated diffusivity of non-motile cells resulted in advective transport dominating their movement. Determination of the dominant transport mechanism for motile and non-motile cells allows calculation of the theoretical time necessary for each cell type to move from pellet exterior to interior sections. For motile cells, the diffusive velocity of 1.0×10^{-5} cm s^{-1} results in a time of 8 hours for cells to move the 0.3 cm from the surface to the center of the pellets. For non-motile cells, the advective velocity controls transport, which results in a transport time of about 25 hours. These figures reflect the assumption of a pellet tortuosity of 1, which is probably understated by a factor of 3 or 4. Actual times may be closer to 1 day for motile cells and 4 days for non-motile cells. An idea of the magnitude of these transport rates is helpful in interpreting the data from the competition experiments.

Although transport rates are important in pellet colonization, growth rates of the organisms may be of greater significance. Competition experiments 1 and 2 offer dramatic evidence of *Klebsiella*'s ability to overtake and outcompete

slower-growing *Pseudomonas*. The results from these experiments (Figures 23 and 24) show that despite its lack of motility, *Klebsiella* surpassed *Pseudomonas* in colonization numbers somewhere between the second and third weeks of exposure. In comparing these two figures, it is interesting to note that although overshadowed by *Klebsiella*, challenged *Pseudomonas* numbers did not drop significantly in experiment 3 compared to experiment 2. *Klebsiella* numbers were steadily increasing, but *Pseudomonas* numbers remained stable at 10^6-10^7 cfu ml^{-1} pellet.

Pellet section (distance from edge) did not seem to have a dramatic effect on *Pseudomonas* survival. In both experiments there was less than 1 order of magnitude difference in interior sections colonization compared to exterior. It appears that *Pseudomonas* cells are remaining viable in the pellet interior sections, but are not actively reproducing. *Klebsiella* is apparently growing in the pellet because of its ability to replicate under lower substrate concentrations as evidenced by its lower K_s and higher μ_{max} (Table VI).

Unchallenged *Pseudomonas* numbers increased slightly from the 10 day to the 21 day experiment. Virtually all of this growth occurred in the exterior sections (0-1mm) while the 1-2mm and 2-3mm sections were almost identical for unchallenged *Pseudomonas* in the two experiments (Figures 23,24). In pellet interior sections, unchallenged *Pseudomonas* did not grow to a cell density greater than $\sim 10^7$ cfu ml^{-1}. When subject to *Klebsiella* competition, challenged *Pseudomonas* does not drop much below this level either. In the

innermost pellet section, invading *Klebsiella* outgrows *Pseudomonas*, but does not displace it.

The third competition experiment shows that *Pseudomonas* is unable to significantly challenge pre-colonized *Klebsiella* in a 10 day experiment even though *Pseudomonas* was continuously inoculated. Two interesting comparisons can be made with the first competition experiment (*Pseudomonas* vs *Klebsiella*, 10 days). First, while challenged *Pseudomonas* numbers dropped in comparison with unchallenged *Pseudomonas* during the first experiment (Figure 23), there was virtually no difference between challenged and unchallenged *Klebsiella* during the third experiment (Figure 25). Therefore, *Pseudomonas* competition did not have as dramatic an effect on colonized *Klebsiella* as vice versa. Second, the plots for *Klebsiella* 10 day invader (Figure 23) and *Pseudomonas* 10 day invader (Figure 25) are very similar. Exterior section colonization is the same at $2x10^6$ cfu/ml, and interior section colonization is slightly greater for *Pseudomonas*, as would be expected due to its motility.

If the results from the competition experiments were extrapolated to predict organism behavior for longer periods, two situations are indicated. 1) when a faster growing organism continuously challenges a slower growing inoculated organism, the challenger will overtake and outcompete the inoculum. While not completely disappearing, the inoculated species will exist in a background role, possibly at cell densities several orders of magnitude below

the faster growing challenger. 2) when a slower growing organism continuously challenges a faster growing inoculated organism, the disparities in growth rate (and attachment efficiency) will determine the extent to which the challenger can compete. Where the growth rate differs by a factor of 5 (as in this case), the inoculum will maintain a dominant position.

The first case above describes the conditions of the bench scale reactor at Tyndall AFB, which resulted in cell counts analogous to the competition experiments where *Pseudomonas* was outcompeted by challenging *Klebsiella*.

Cells in Reactor Effluent. In the competition experiments, effluent cell enumerations for the inoculated species were the same for both the challenged and unchallenged columns during the first 10 days, though effluent *Pseudomonas* in the challenged column is approximately one-tenth that of unchallenged *Pseudomonas* after 10 days (Figure 27). This result is consistent with the aforementioned observation that competition resulted in a slight drop in *Pseudomonas* numbers (less than 1 order of magnitude), and virtually no change in *Klebsiella*, when these species were the inoculated organism.

Klebsiella's greater tendency to attach within the reactor may be due to its higher rate of formation of extracellular products. Siebel (1987) found *Klebsiella*'s rate of product formation to be approximately 3 times that of *Pseudomonas* in both suspended growth and biofilm systems. *Klebsiella*'s greater ability to adhere to surfaces may also be indicated by the fact that

effluent cell counts for both the challenged and unchallenged columns show *Klebsiella* at a concentration on the order of 10^4-10^5 cfu ml^{-1}. This is almost 2 orders of magnitude less than *Pseudomonas* under similar circumstances (Figures 26-28). This apparent attachment advantage may be of equal importance as growth rate in determining organism distribution in the pellets.

MATHEMATICAL MODEL

Prediction of biomass growth and substrate utilization within an individual pellet necessitates the development of a model which utilizes the equations for mass flux developed in first chapter. For the sake of simplicity, the model presented here will describe a single species system where one substrate (glucose) limits growth. The model is developed from the flux balance across a differential element of a pellet for cells and substrate. Spherical geometry is assumed.

Equation 13 describes the substrate concentration profile over the radius of a pellet. Boundary conditions for this equation are: $C_A = C_{Abulk}$ at $r = R$ and $dC_A/dr = 0$ at $r = 0$. Equation 13 uses the variable X to represent cell density per unit volume of pellet. Within the pellet, X can be further divided into X_s (suspended cells) and X_a (attached cells). The flux balance on total cells is developed similarly to that on substrate:

$$\frac{d^2X_s}{dr^2} + \frac{2}{r}\frac{dX_s}{dr} = \frac{1}{D_{eff}}\left[(X_a+X_s)\left(\frac{\mu_{max}C_A}{K_A+C_A}\right)\right] \qquad (25)$$

boundary conditions: $X_s = X_{effluent}$ at $r = R$, $dX_s/dr = 0$ at $r = 0$

For attached cells, the diffusive flux terms go to zero, leaving only the reaction term

$$0 = -4\pi r^2 \Delta r[(\frac{\mu_{max} C_A}{K_A + C_A}) X_a - K_d X_a^2] \quad (26)$$

where: K_d = coefficient of detachment

This equation assumes a second order dependence of detachment on biomass accumulation. Solving Equation 26 for X_a yields

$$X_a = \frac{\mu_{max} C_A K_d}{K_A + C_A} \quad (27)$$

This value is then substituted into Equation 25.

Equation 13 was solved using a Matlab iterative program (Appendix D). Because of difficulty in coupling the substrate and biomass flux equations, an equation for the observed biomass accumulation as a function of pellet radius within an individual pellet was used.

Data from the effectiveness factor experiment can be analyzed to yield an average substrate flux into a pellet for each flowrate. The substrate flux is calculated as

$$N_{A_R} = \frac{Q(C_{Ainf} - C_A)}{A_p} \quad (28)$$

where: Q = reactor flowrate ($L^3 t^{-1}$)

C_{Ainf} = influent concentration of A

C_A = effluent concentration of A

A_P = total exterior surface area of all pellets in reactor

Using Fick's first law (Equation 3), the substrate gradient at the pellet surface (dC_A/dr) can be calculated. This substrate gradient offers a valuable tool for model validation. Given an input function of cell density as a function of radial distance from pellet center, the model provides a substrate profile in the pellet. The slope of this profile at r = R is the substrate gradient at the pellet surface.

Cell enumerations performed on pellets from the effectiveness factor experiment (Figure 31) showed cell colonization which adhered to the following function

$$X = X_0 e^{(r\,(0.18+0.21C_A))} \qquad (29)$$

where: X = cell density (g cells m^{-3} pellet volume)

X_0 = base cell density (at pellet center)

r = radial distance from pellet center (L)

C_A = bulk substrate concentration (g m^{-3})

X_0 was observed to be 10^7 cfu ml^{-1} pellet. This converted into mg/l as follows:

$$(3x10^{-13}\frac{gram}{cell})(10^7\frac{cell}{ml\ pellet})(10^3\frac{mg}{gram})(10^3\frac{ml}{l\ pellet}) = 3.0\frac{mgcells}{l\ pellet} \qquad (30)$$

Model predictions of substrate profile for this biomass function yielded a significantly lower substrate flux at r = R than experimentally observed for all

flow situations (Figure 31). The probable reason for this difference is that the observed cell function (Equation 29) does not account for the buildup of biomass on the outermost surface of the pellet. This phenomena, observed in pellets sent from Tyndall AFB, is characterized by a continuous biofilm on the pellet surfaces. Such a film would greatly enhance the substrate flux at the pellet surface because the substrate gradient would be much higher. The pellet sectioning technique used was not sensitive enough to detect a large biomass accumulation at the pellet surface.

To ascertain model sensitivity to the biomass profile, the function presented in Equation 29 was changed to reflect a surface biomass accumulation 10 times greater than that measured in the 0-1mm section of the pellet. This function is

$$X = X_0 \, e^{(r\,(0.95+0.21C_A))} \qquad (31)$$

When this function is used in the model, the resulting substrate flux at r=R is considerably greater than the earlier model (Figure 31). Even at this level, however, substrate flux is still not as high as experimental observations would indicate, though they are of the same order of magnitude. Actual pellet colonization (Figure 32) is dependent on radial distance from pellet center, particularly at high glucose feed rates.

When the kinetic coefficients (μ_{max} and K_s) for *Klebsiella pneumoniae* are substituted into the model, the resulting substrate profile is steeper than that for *Pseudomonas aeruginosa*, and the substrate concentration drops to zero in

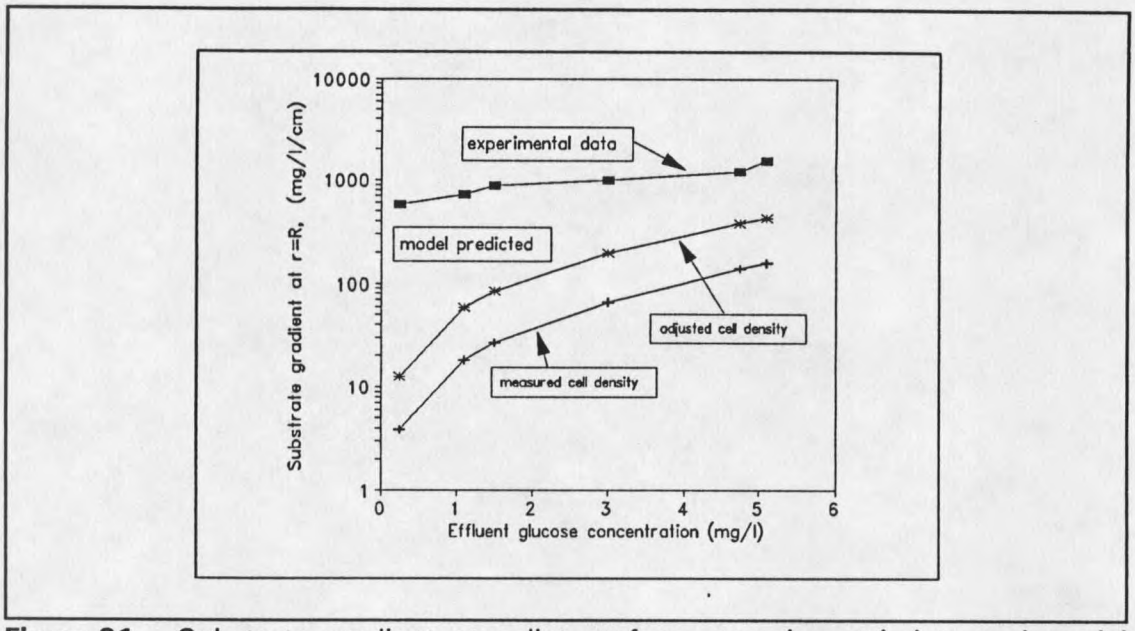


Figure 31. Substrate gradient at pellet surface, experimental data and model predicted for observed and adjusted cell densities (*Pseudomonas aeruginosa*).

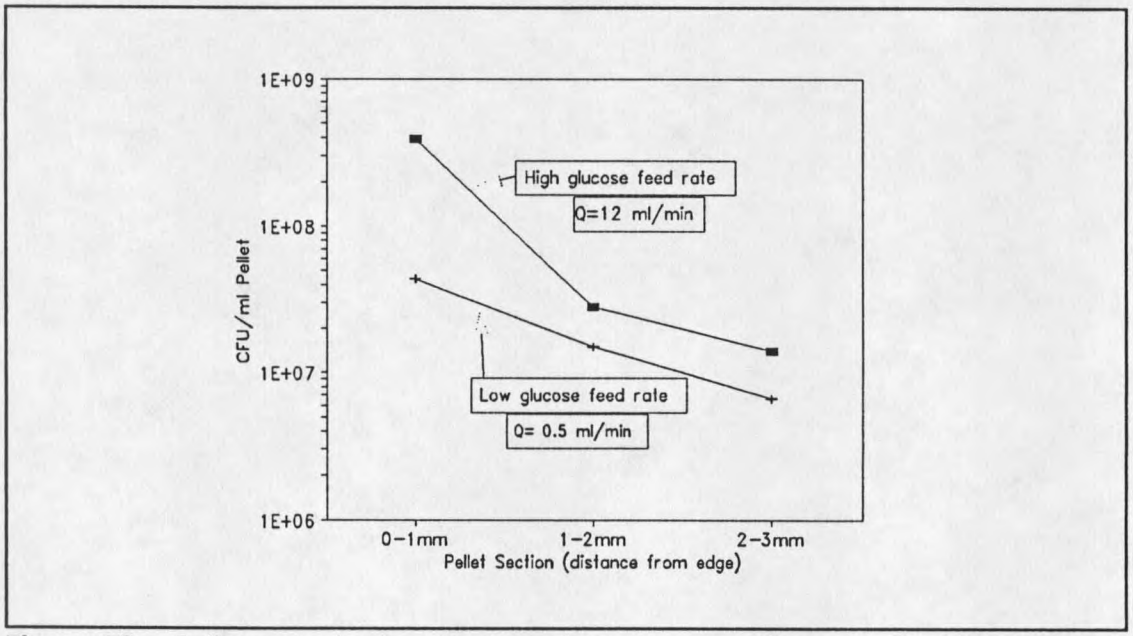


Figure 32. Pellet colonization by *Pseudomonas aeruginosa* under high and low glucose feed rates.

0.25mm as opposed to 1mm for *Pseudomonas* (Figures 33,34).

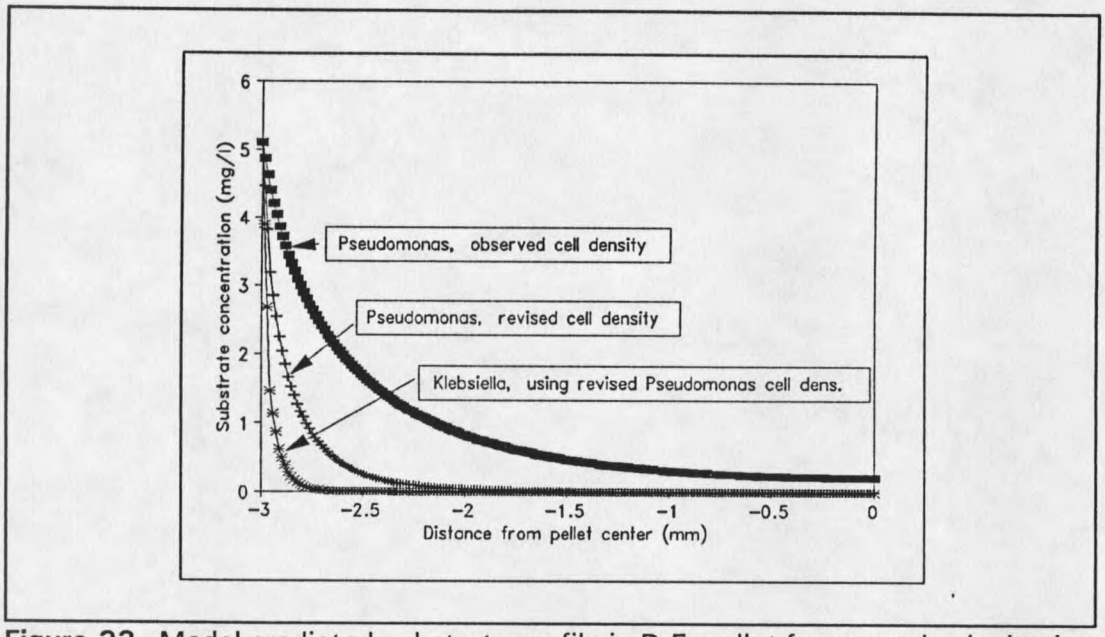


Figure 33. Model predicted substrate profile in D.E. pellet for several colonization conditions.

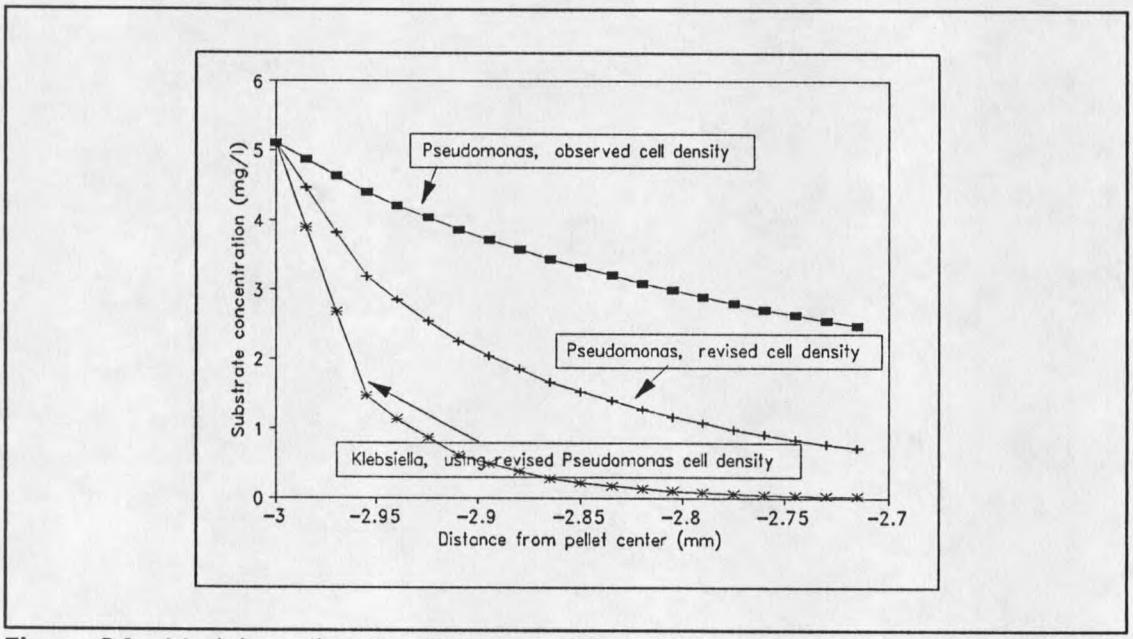


Figure 34. Model predicted substrate profile in D.E. pellet, detail of pellet edge.

EFFECTIVENESS FACTOR DETERMINATIONS

Determination of overall pellet effectiveness factor is dependent on the substrate profile within the pellet. Coupling the substrate function calculated with the mathematical model presented above and the Monod equation yields an organism growth rate for the entire pellet. When this growth rate is compared with the rate which would occur if all pellet surfaces were exposed to bulk fluid substrate concentrations, the overall pellet effectiveness factor can be calculated. This effectiveness factor makes the assumption that biomass density within the pellet is a constant function of pellet radius; even if pellet interior sections were exposed to bulk fluid substrate concentrations, no increase in biomass density would result. This effectiveness factor is represented by

$$\eta = \frac{\int_{r=0}^{r=R} X(r) \frac{C_A(r) * \mu_{max}}{Y(K_A + C_A(r))} r^2 dr}{\int_{r=0}^{r=R} X(r) \frac{C_{A_{bulk}} * \mu_{max}}{Y(K_A + C_{A_{bulk}})} r^2 dr} \qquad (32)$$

where: $C_A(r)$ = substrate concentration at r within pellet (described by model)

C_{Abulk} = bulk fluid substrate concentration

Since $C_A(r)$ is unknown, but graphically well represented, Riemann sums may be used to closely approximate η. As the cell density profile was adjusted

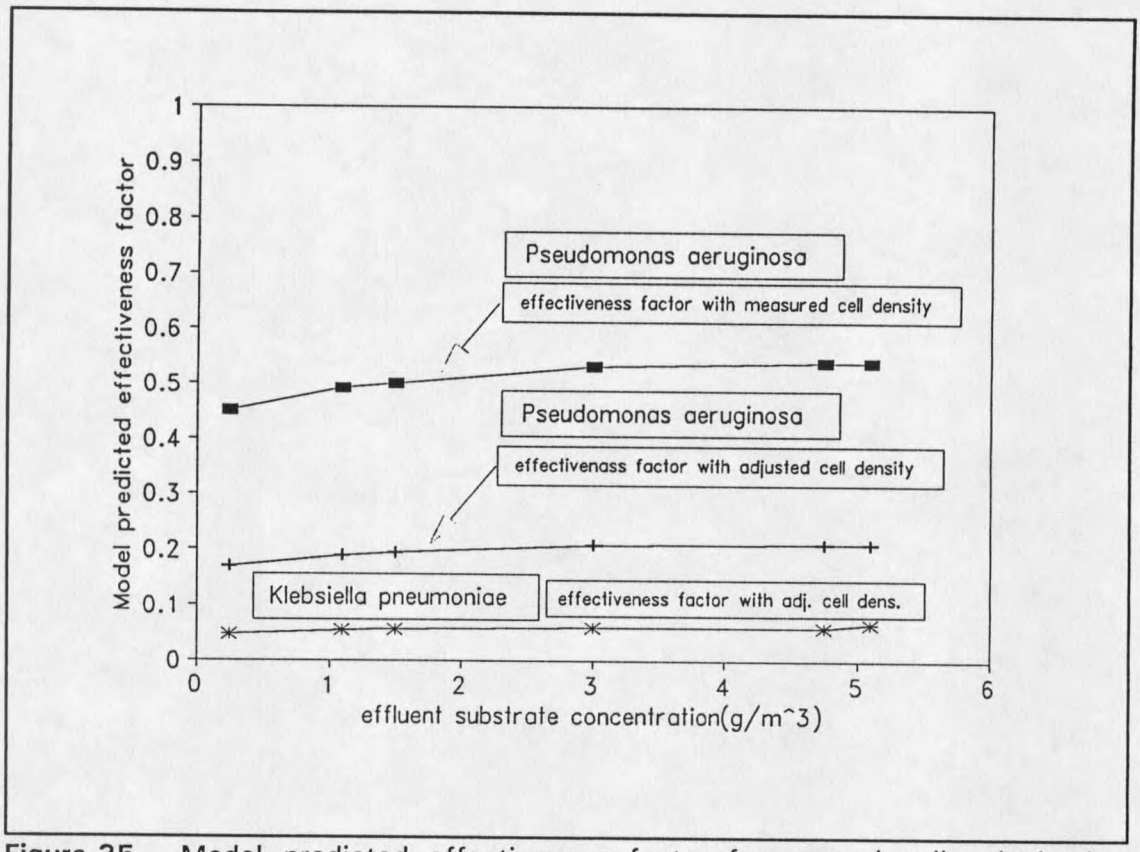


Figure 35. Model predicted effectiveness factor for several cell colonization conditions and cell types.

from the observed data to a more realistic approximation, η decreased (Figure 35). When kinetic coefficients for faster growing *Klebsiella* were substituted for *Pseudomonas*, η decreased further. The actual η within the experimental system is probably lower still, since the observed glucose flux is greater than that predicted by the model, even when *Klebsiella* kinetic coefficients were used. This suggests strongly that actual pellet effectiveness for this system is below 0.10.

Effectiveness factor changes little as effluent substrate concentration increases. This is because the model predicts the substrate concentration

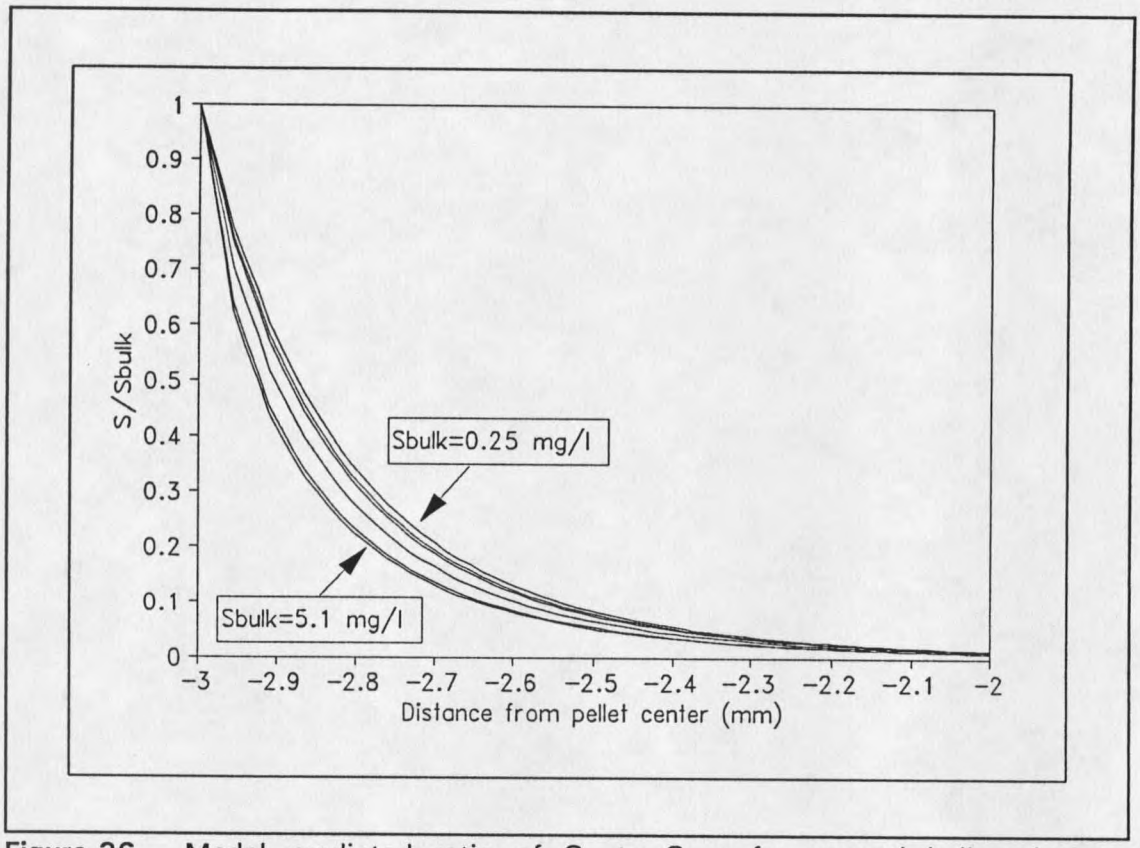


Figure 36. Model predicted ratio of C_A to C_{Abulk} for several bulk substrate concentrations, as a function of distance from pellet center.

within the pellet to go to zero at about the same radial distance from the surface regardless of C_{Abulk}. Because the ratio of C_A to C_{Abulk} remains constant as C_{Abulk} varies from 0.25 to 5.1 mg l^{-1} (Figure 36), the effectiveness factor must also remain fairly constant.

CONCLUSIONS

The following conclusions can be drawn from the experiments conducted both at Tyndall AFB, and at the Engineering Research Center. Recognizing that many interrelated factors contribute to cell behavior, and that the pellet system used is inherently complex, these conclusions are valid within the range of experimental conditions employed.

1. Diatomaceous earth pellets contain an extensive interconnected micropore structure which can be colonized by motile and non-motile microorganisms.

2. Organism growth rate is a more important factor than either order of introduction or motility in determining species predominance within the pellets.

3. Despite a slower growth rate, *Pseudomonas* was not completely displaced by *Klebsiella* in the time period observed. Although *Pseudomonas* numbers decrease after exposure to *Klebsiella*, complete washout does not occur.

4. Both motile and non-motile microorganisms colonize exterior pellet

surfaces to a much greater extent than interior surfaces. This colonization gradient makes the pellet exterior surface much more influential in substrate transformation than interior surfaces.

5. Model representation of substrate profiles within the pellets indicate that pellet effectiveness factor is significantly less than 1 for both bacterial species tested.

NOMENCLATURE

A	total reactor cross sectional area (L^2)
A_{pellet}	cross sectional void area within a pellet (L^2)
C_A	concentration of constituent A (ML^{-3})
C_{Abulk}	bulk fluid concentration of constituent A (ML^{-3})
C_{Ainf}	influent concentration of constituent A (ML^{-3})
$C_A(r)$	concentration of constituent A at radius r within a pellet (ML^{-3})
D_{eff}	effective diffusivity (L^2t^{-1})
D_l	coefficient of dispersion (L^2t^{-1})
d	average pore diameter in a pellet (L)
dh/dl	head loss (-)
E	reactor macroporosity (-)
e	pellet porosity (-)
erfc(x)	complementary error function of variable x
K	hydraulic conductivity (Lt^{-1})
K_A	half saturation coefficient of constituent A (ML^{-3})
K_d	coefficient of detachment (variable)
k	reaction rate constant (variable)
m	order of reaction (-)
N_{Ar}	flux of constituent A at radial distance r from pellet center ($ML^{-2}t^{-1}$)
Pe	Peclet number (-)
Q	reactor volumetric flow rate (L^3t^{-1})
R	pellet radius (L)
R_A	reaction rate of constituent A ($ML^{-3}t^{-1}$)
R_{Amax}	maximum reaction rate of constituent A ($ML^{-3}t^{-1}$)
r	pellet radius at some inner shell (L)
S_v	pore surface area per volume (L^{-1})
V	volume of pellet (L^3)

NOMENCLATURE-Continued

V_{H2O}	volume of water drained from reactor (L^3)
V_{total}	volume of water and pellets (L^3)
v	average linear pore velocity along a flowpath (Lt^{-1})
v_A	actual pore velocity (Lt^{-1})
v_D	Darcy velocity (Lt^{-1})
W_d	dry weight of pellets (M)
W_w	wet weight of pellets (M)
X	cell mass per unit pellet volume (ML^{-3})
X_0	base cell density used for model (ML^{-3})
X_a	attached cell density (ML^{-3})
X_s	suspended cell density (ML^{-3})
Y	substrate yeild ($M_{cells}/M_{substrate}$)
α	dispersivity (L)
η	effectiveness factor (-)
μ	specific growth rate (t^{-1})
μ_{max}	maximum specific growth rate (t^{-1})
ρ_w	density of water (ML^{-3})
ϕ	Thiele diffusion modulus (-)

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APPENDICES

APPENDIX A

Pellet Physical Properties

Determination of Pellet and Reactor Porosity:

	Trial 1	2	3	AVG.
Saturated volume (ml)	250	250	250	
Water drained (ml)	83	80	85	
Weight of pellets-wet (gm)	226.74	227.10	219.35	
Weight of pellets-dry (gm)	139.06	142.60	138.50	
Weight of water in pellets (gm)	87.68	84.50	80.85	
Pellet porosity	0.525	0.497	0.490	0.504
Bulk reactor porosity	0.33	0.32	0.34	0.33

Determination of Hydraulic Conductivity:

Trial	Flow (ml/min)	headloss (dh/dl)	KA (cm_3/min)		K (cm/min)
1	1.5	41	0.037		0.036
2	2.32	58	0.040		0.039
3	3.04	84.8	0.036		0.035
4	3.46	99.8	0.038		0.037
5	5.47	128	0.043		0.042
6	6.20	154.7	0.040		0.039
				AVG =	0.038

Determination of Reactor Hydraulic Conductivity:

Trial	Flow (ml/min)	headloss (dh/dl)	KA (cm_3/min)		K (cm/min)
1	29.6	0.55	53.6		17.9
2	4.3	0.08	55.24		18.5
3	9.7	0.18	52.45		17.6
				AVG =	18.0

Determination of Pellet Dispersivity:

Time (sec)	Trial#	C/C_o 1	2	3	AVG
1		0.03	0.02	0.03	0.03
2		0.33	0.37	0.38	0.36
3		0.64	0.68	0.63	0.65
4		0.92	0.94	0.99	0.95

APPENDIX B

Tyndall Air Force Base Experiments-Raw Data

Table 8. Tyndall Air Force Base Experiment 1-Raw Data.

	DIL TO	Z1	Z2	dZ	VOL	TOTAL ORGANISMS					CHLOROBENZENE DEGRADERS				
SECTION	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml	DIL	COUNTS			CELLS/ml
TO WHOLE	100	0.054	0.084	0.03	0.124	4	223	221	230	1.8e+09	3	228	369	447	2.8e+08
T2T WHOLE	100	0.119	0.148	0.029	0.118	4	87	80	63	6.5e+08	3	178	139	121	1.2e+08
T4T WHOLE	100	0.286	0.334	0.047	0.195	4	104	85	75	4.5e+08	3	115	108	93	5.40e+07
T10T WHOLE	100	0.312	0.374	0.062	0.254	5	17	16	22	7.2e+08	2	1	1	5	9.18e+04
TO WHOLE	100	0.054	0.084	0.03	0.124	4	223	221	230	1.8e+09	3	228	369	447	2.8e+08
T4B WHOLE	100	0.048	0.094	0.046	0.191	4	130	118	111	6.3e+08	3	127	130	122	6.63e+07
T2B WHOLE	100	0.045	0.079	0.033	0.137	4	81	68	77	5.5e+08	3	79	118	108	7.40e+07
T10B WHOLE	100	0.05	0.094	0.044	0.18	4	122	119	120	6.7e+08	2	4	3	2	1.66e+05
T0 0-1	20	0.028	0.033	0.006	0.024	3	1324	1313	1027	1.0e+09	2	2800	3300	3300	2.6e+08
T0 1-2	20	0.076	0.085	0.009	0.036	3	1065	1111	861	5.6e+08	2	2500	3000	1000	1.2e+08
T0 2-3	20	0.055	0.063	0.008	0.033	3	951	1024	896	5.8e+08	2	2000	1700	4100	1.6e+08
T2T 0-1	20	0.045	0.058	0.013	0.052	4	146	165	121	5.5e+08	3	123	103	141	4.67e+07
T2T 1-2	20	0.065	0.076	0.011	0.045	4	85	130	94	4.5e+08	3	65	69	57	2.81e+07
T2T 2-3	20	0.072	0.083	0.011	0.045	4	78	77	94	3.7e+08	3	62	30	54	2.16e+07
T2T 3-4	20	0.056	0.068	0.013	0.052	4	100	111	89	3.9e+08	3	54	39	49	1.84e+07

Table 8-Continued.

	DIL TO	Z1	Z2	dZ	VOL	TOTAL ORGANISMS					CHLOROBENZENE DEGRADERS				
SECTION	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml	DIL	COUNTS			CELLS/ml
T4T 0-1	20	0.026	0.04	0.014	0.058	4	88	69	69	2.6e+08	3	82	80	65	2.60e+07
T4T 1-2	20	0.048	0.058	0.01	0.042	4	84	91	61	3.8e+08	3	125	90	89	4.86e+07
T4T 2-3	20	0.022	0.036	0.015	0.06	4	106	71	162	3.8e+08	3	92	114	112	3.52e+07
T10T 0-1	20	0.059	0.073	0.013	0.055	5	47	44	46	1.7e+09	2	2	4	0	7.29e+04
T10T 1-2	20	0.014	0.025	0.011	0.045	4	152	150	133	6.4e+08	2	4	2	0	8.82e+04
T10T 2-3	20	0.03	0.039	0.009	0.038	4	123	130	117	6.4e+08	2	4	4	1	1.56e+05

Table 9. Tyndall Air Force Base Experiment 2-Raw Data.

	DIL	Z1	Z2	dZ	TOTAL ORGANISMS					CHLOROBENZENE DEGRADERS				
SECTION	(ml)	•	•		DIL	COUNTS			CELLS/ml	DIL	COUNTS			CELLS/ml
T0T WHOLE	100	0.044	0.075	0.031	4	36	47	35	1.26e+09	2	17	13		4.81e+06
T1T WHOLE	100	0.115	0.147	0.032	4	36	37	36	1.12e+09	2	5	14		2.93e+06
T3T1 W	100	0.048	0.071	0.023	3	247	210	211	9.60e+08	2	2	8	10	2.87e+06
T5T WHOLE	100	0.117	0.188	0.072	4	177	153		2.31e+09	2	52			7.27e+06
T7T WHOLE	100	0.099	0.208	0.109	4	206	227		1.98e+09	2	75			6.87e+06
T10T WHOLE	100	0.01	0.1	0.09	4	208	189		2.22e+09	2	46			5.14e+06
T12T WHOLE	100	0.185	0.316	0.132	4	162	158		1.21e+09	2	46			3.49e+06
T15T WHOLE	100	0.172	0.24	0.068	4	69	87	69	1.21e+09	2	28	39		4.96e+06
T0B WHOLE	100	0.099	0.132	0.033	4	51	40	37	1.30e+09	2	36	45		1.23e+07
T1B WHOLE	100	0.204	0.244	0.039	4	37	33	35	8.88e+08	2	9	13		2.79e+06
T3B1 W	100	0.016	0.037	0.021	4	38	42	35	1.63e+09	1	67	65	66	3.48e+06
T5B WHOLE	100	0.111	0.233	0.122	4	194	179		1.53e+09	2	3			2.46e+05
T7B WHOLE	100	0.036	0.158	0.122	4	133	133		1.09e+09	2	6			4.91e+05
T10B WHOLE	100	0.081	0.148	0.067	4	932	839		1.33e+10	2	57			8.55e+06
T12B WHOLE	100	0.186	0.292	0.106	4	1352			1.20e+10	2	68			6.43e+06
T15B WHOLE	100	0.182	0.274	0.092	5	74	81	86	8.77e+09	2	21	17		2.07e+06

Table 10. Tyndall Air Force Base Experiment 2-Raw Data.

	DIL	Z1	Z2	dZ	CHLOROBENZENE DEGRADERS				
SECTION	(ml)	*	*		DIL		COUNT		CELLS/ml
T0T O	40	0.079	0.1	0.021	2	26	32		5.45e+06
T0T I	20	0.1	0.11	0.011	2	2	3		4.72e+05
T3T O	40	0.053	0.085	0.033	2	38	40	44	5.01e+06
T3T I	20	0.05	0.057	0.007	1	10	17	10	3.38e+05
T12T O	40	0.483	0.521	0.038	2	22			2.33e+06
T12T I	20	0.231	0.238	0.007	1	12			3.38e+05
T15T O	40	0.195	0.238	0.043	2	27	23		2.32e+06
T15T I	20	0 218	0.235	0.016	1	13			1.60e+05

Table 11. Tyndall Air Force Base Experiment 2-Raw Data

	DIL	Z1	Z2	dZ	TOTAL ORGANISMS				
SECTION	(ml)	*	*		DIL		COUNT		CELLS/ml
T0B O	40	0.111	0.142	0.031	4	72	93	77	1.06e+09
T0B I	20	0.078	0.086	0.008	4	38	26	38	8.83e+08
T5B O	40	0.059	0.1	0.04	4	139	144		1.40e+09
T5B I	20	0.07	0.082	0.012	4	48	72		9.92e+08
T10B O	40	0.16	0.208	0.048	4	482			4.01e+09
T10B I	20	0.02	0.042	0.022	4	145	143		1.32e+09
T15B O	40	0.186	0.216	0.03	4	502	563	580	7.36e+09
T15B I	20	0.162	0.171	0.009	4	89	102	81	1.99e+09

APPENDIX C

Competition Experiments-Raw Data

Table 12. Competition Experiment 1-Raw Data, *Pseudomonas* initial colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Pseudomonas aeruginosa				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T1 0-1	20	0.023	0.039	0.0165	0.0681	3	68	82		2.20e+07
T1 1-2	20	0.056	0.063	0.0071	0.0293	3	24	18	26	1.55e+07
T1 2-3	20	0.161	0.176	0.0151	0.0623	3	66	41	53	1.71e+07
T2 0-1	20	0.187	0.197	0.0095	0.0392	3	58	53	43	2.62e+07
T2 1-2	20	0.161	0.179	0.0183	0.0755	3	22	21	26	6.09e+06
T2 2-3	20	0.177	0.185	0.0081	0.0334	3	16	16	18	9.98e+06
M 0-1	20	0.132	0.14	0.0077	0.0318	3	48	55	45	3.11e+07
M 1-2	20	0.114	0.121	0.0074	0.0305	2	167	182	170	1.13e+07
M 2-3	20	0.104	0.116	0.0118	0.0487	3	32	33	39	1.42e+07
B 0-1	20	0.188	0.198	0.01	0.0412	3	29	28	40	1.57e+07
B 1-2	20	0.144	0.153	0.009	0.0371	2	114	240	205	1.00e+07
B 2-3	20	0.174	0.179	0.0045	0.0186	2	144	144	126	1.49e+07
T 0-1	20	0.106	0.132	0.0263	0.1085	3	91	113	97	1.85e+07
T 1-2	20	0.097	0.113	0.0165	0.0681	3	136	124	105	3.58e+07
T 2-3	10	0.155	0.16	0.0055	0.0227	3	81	45	43	2.48e+07

Table 13. Competition Experiment 1-Raw Data, unchallenged *Pseudomonas* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Pseudomonas aeruginosa				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T1 0-1	20	0.069	0.084	0.0152	0.0627	3	83	136	84	3.22e+07
T1 1-2	20	0.069	0.082	0.0137	0.0565	3	26	37	25	1.04e+07
T1 2-3	20	0.18	0.187	0.007	0.0289	2	113	165	157	1.00e+07
M1 0-1	20	0.221	0.25	0.0292	0.1204	3	97	120	87	1.68e+07
M1 1-2	20	0.194	0.204	0.0096	0.0396	3	20	23	43	1.45e+07
M1 2-3	20	0.199	0.206	0.0074	0.0305	2	119	133	120	8.13e+06
M2 0-1	20	0.156	0.198	0.0425	0.1753	3	117	143	128	1.48e+07
M2 1-2	20	0.128	0.148	0.0207	0.0854	3	35	35		8.20e+06
M2 2-3	20	0.151	0.156	0.0054	0.0223	2	85	107	111	9.07e+06
B2 0-1	20	0.046	0.078	0.0321	0.1324	3	134	112		1.86e+07
B2 1-2	20	0.06	0.176	0.116	0.4785	3	43	35	35	1.57e+06
B2 2-3	20	0.076	0.084	0.0082	0.0338	2	132	150	101	7.55e+06
B 0-1	20	0.105	0.127	0.0218	0.0899	3	38	51	53	1.05e+07
B 1-2	20	0.1	0.12	0.0201	0.0829	3	73	62	40	1.41e+07
B 2-3	10	0.108	0.12	0.0116	0.0478	3	90	111	97	2.08e+07

Table 14. Competition Experiment 1-Raw Data, challenged *Pseudomonas* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Pseudomonas aeruginosa				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.159	0.2	0.0415	0.1712	3	34	42	31	4.17e+06
T 1-2	20	0.1	0.119	0.0191	0.0788	3	3	4	8	1.27e+06
T 2-3	20	0.116	0.121	0.0051	0.021	2	24	26	18	2.16e+06
M 0-1	20	0.055	0.099	0.044	0.1815	3	46	46	56	5.44e+06
M 1-2	20	0.057	0.068	0.0104	0.0429	3	2	0	3	7.77e+05
M 2-3	10	0.056	0.058	0.0017	0.007	2	6	8	5	9.03e+05
B 0-1	20	0.035	0.082	0.0468	0.193	3	108	122		1.19e+07
B 1-2	20	0.065	0.075	0.0106	0.0437	3	6	9	10	3.81e+06
B 2-3	10	0.067	0.072	0.0047	0.0194	2	51	46	37	2.30e+06
M 0-1	20	0.109	0.147	0.0381	0.1572	3	47	31	49	5.39e+06
M 1-2	20	0.104	0.117	0.0133	0.0549	2	41	49	38	1.56e+06
M 2-3	10	0.102	0.107	0.0054	0.0223	2	73	78	76	3.40e+06
B 0-1	20	0.134	0.175	0.0407	0.1679	3	110	113	120	1.36e+07
B 1-2	20	0.099	0.118	0.0186	0.0767	2	89	98	101	2.50e+06
B 2-3	20	0.103	0.111	0.0081	0.0334	2	68	80	57	4.09e+06

Table 15. Competition Experiment 1-Raw Data, invading *Klebsiella* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Klebsiella pneumoniae				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.159	0.2	0.0415	0.1712	3	11	12	8	1.21e+06
T 1-2	20	0.1	0.119	0.0191	0.0788	3	0	0	0	0.00
T 2-3	20	0.116	0.121	0.0051	0.021	2	0	1	0	3.17e+04
M 0-1	20	0.055	0.099	0.044	0.1815	3	15	19	18	1.91e+06
M 1-2	20	0.057	0.068	0.0104	0.0429	3	0	0	0	0.00
M 2-3	10	0.056	0.058	0.0017	0.007	2	0	0	0	0.00
B 0-1	20	0.035	0.082	0.0468	0.193	3	22	38	45	3.63e+06
B 1-2	20	0.065	0.075	0.0106	0.0437	3	0	0	0	0.00
B 2-3	10	0.067	0.072	0.0047	0.0194	2	0	0	0	0.00
M 0-1	20	0.109	0.147	0.0381	0.1572	2	28	28	33	3.78e+05
M 1-2	20	0.104	0.117	0.0133	0.0549	1	5	6	6	2.07e+04
M 2-3	10	0.102	0.107	0.0054	0.0223	1	5	6	1	1.80e+04
B 0-1	20	0.134	0.175	0.0407	0.1679	2	41	33	24	3.89e+05
B 1-2	20	0.099	0.118	0.0186	0.0767	1	8	9	11	2.43e+04
B 2-3	20	0.103	0.111	0.0081	0.0334	1	1	6	2	1.80e+04

Table 16. Competition Experiment 2-Raw Data, unchallenged *Pseudomonas* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Pseudomonas aeruginosa				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.073	0.109	0.0366	0.151	3	128	141		1.78e+07
T 1-2	20	0.085	0.104	0.0189	0.078	3	53	44	44	1.21e+07
T 2-3	20	0.127	0.136	0.0091	0.0375	2	76	81	65	3.94e+06
M 0-1	20	0.095	0.125	0.0301	0.1242	3	77	49	42	9.02e+06
M 1-2	20	0.079	0.097	0.0189	0.078	2	129	140	108	3.22e+06
M 2-3	20	0.071	0.076	0.0059	0.0243	2	30	21	28	2.16e+06
B 0-1	20	0.073	0.113	0.0408	0.1683	4	91	85		1.05e+08
B 1-2	20	0.074	0.108	0.0342	0.1411	3	194	230		3.01e+07
B 2-3	20	0.067	0.077	0.0097	0.04	3	25	23	35	1.38e+07

Table 17. Competition Experiment 2-Raw Data, challenged *Pseudomonas* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Pseudomonas aeruginosa				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.315	0.338	0.0227	0.0936	4	9	6	3	1.28e+07
T 1-2	20	0.079	0.096	0.0167	0.0689	3	16	20	26	6.00e+06
T 2-3	20	0.036	0.049	0.0131	0.054	2	253	208	198	8.13e+06
M 0-1	20	0.129	0.156	0.0266	0.1097	4	13	10	6	1.76e+07
M 1-2	20	0.138	0.161	0.0233	0.0961	3	32	29	24	5.90e+06
M 2-3	20	0.036	0.043	0.0073	0.0301	1	260	261	258	1.72e+06
B 0-1	20	0.114	0.147	0.0333	0.1374	3	137	127	143	1.98e+07
B 1-2	20	0.112	0.132	0.0207	0.0854	3	16	14	14	3.44e+06
B 2-3	20	0.055	0.062	0.0075	0.0309	3	11	14	11	7.76e+06

Table 18. Competition Experiment 2-Raw Data, invading *Klebsiella* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Klebsiella pneumoniae				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.315	0.338	0.0227	0.0936	4	93	97	85	1.96e+04
T 1-2	20	0.079	0.096	0.0167	0.0689	3	20	27	22	6.68e+03
T 2-3	20	0.036	0.049	0.0131	0.054	1	139	107	98	4.24e+04
M 0-1	20	0.129	0.156	0.0266	0.1097	4	156	150	140	2.71e+04
M 1-2	20	0.138	0.161	0.0233	0.0961	3	116	130	141	2.68e+04
M 2-3	20	0.036	0.043	0.0073	0.0301	1	227	204	239	1.48e+05
B 0-1	20	0.114	0.147	0.0333	0.1374	4	382	325	372	5.24e+04
B 1-2	20	0.112	0.132	0.0207	0.0854	4	56	61	54	1.34e+04
B 2-3	20	0.055	0.062	0.0075	0.0309	3	48	43	56	3.17e+04

Table 19. Competition Experiment 3-Raw Data, *Klebsiella* initial colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Klebsiella pneumoniae				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.287	0.314	0.0266	0.1097	4	55	62	49	1.01e+08
T 1-2	20	0.104	0.122	0.0175	0.0722	3	111	134	117	3.34e+07
T 2-3	20	0.17	0.181	0.0109	0.045	2	644	630	605	2.79e+07
M 0-1	20	0.078	0.11	0.0323	0.1332	4	49	31	28	5.40e+07
M 1-2	20	0.129	0.14	0.0112	0.0462	3	92	95	69	3.69e+07
M 2-3	20	0.117	0.125	0.0078	0.0322	2	554	659	624	3.81e+07
B 0-1	20	0.081	0.1	0.0182	0.0751	4	82	129	101	2.77e+08
B 1-2	20	0.094	0.113	0.0189	0.078	3	250	275	227	6.43e+07
B 2-3	20	0.111	0.12	0.0084	0.0346	2	358	438	422	2.34e+07
T 0-1	20	0.111	0.145	0.0336	0.1386	2	2400			3.46e+07
T 1-2	20	0.122	0.141	0.0194	0.08	2	219	181	198	4.98e+06
T 2-3	20	0.179	0.195	0.0164	0.0676	2	99	107	89	2.91e+06
M 0-1	20	0.11	0.144	0.0347	0.1431	2	4000			5.59e+07
M 1-2	20	0.129	0.145	0.0162	0.0668	2	952			2.85e+07
M 2-3	20	0.093	0.103	0.0092	0.0379	2	153	114	110	6.62e+06

Table 20. Competition Experiment 3-Raw Data, unchallenged *Klebsiella* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Klebsiella pneumoniae				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.178	0.216	0.0376	0.1551	4	54	53	55	6.96e+07
T 1-2	20	0.111	0.135	0.0239	0.0986	3	233	193	203	4.25e+07
T 2-3	20	0.108	0.116	0.0084	0.0346	3	30	38	42	2.12e+07
M 0-1	20	0.17	0.198	0.028	0.1155	3	179	198	224	3.47e+07
M 1-2	20	0.071	0.093	0.0224	0.0924	3	106	88	110	2.19e+07
M 2-3	20	0.104	0.115	0.0101	0.0417	2	248	254	235	1.18e+07
B 0-1	20	0.115	0.137	0.022	0.0907	4	33	45	38	8.52e+07
B 1-2	20	0.161	0.183	0.0217	0.0895	3	165	169	179	3.82e+07
B 2-3	20	0.047	0.064	0.0171	0.0705	3	55	59	57	1.62e+07
T 0-1	20	0.1	0.141	0.041	0.1691	4	95	99	126	1.26e+08
T 1-2	20	0.084	0.106	0.0228	0.094	4	28	29	40	6.88e+07
T 2-3	20	0.072	0.081	0.0087	0.0359	2	230	211	209	1.21e+07
M 0-1	20	0.06	0.09	0.0301	0.1242	3	270	209		3.86e+07
M 1-2	20	0.076	0.095	0.019	0.0784	3	63	69	65	1.68e+07
M 2-3	20	0.106	0.117	0.0114	0.047	3	64	48	48	2.27e+07
B 0-1	20	0.126	0.172	0.0464	0.1914	4	32	29	30	3.17e+07
B 1-2	20	0.095	0.107	0.0124	0.0511	3	51	58	55	2.14e+07
B 2-3	20	0.052	0.059	0.0068	0.028	3	56	56	56	3.99e+07

Table 21. Competition Experiment 3-Raw Data, challenged *Klebsiella* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Klebsiella pneumoniae				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.12	0.157	0.0364	0.1501	3	185	213	213	2.71e+07
T 1-2	20	0.099	0.13	0.0311	0.1283	3	56	54	67	9.20e+06
T 2-3	20	0.182	0.194	0.0113	0.0466	2	74	65	65	2.92e+06
M 0-1	20	0.155	0.188	0.0329	0.1357	4	52	56	54	7.96e+07
M 1-2	20	0.115	0.138	0.0224	0.0924	3	70	75	72	1.57e+07
M 2-3	20	0.148	0.154	0.0065	0.0268	2	151	129	136	1.03e+07
B 0-1	20	0.152	0.17	0.0186	0.0767	4	25	33	37	8.26e+07
B 1-2	20	0.162	0.184	0.0223	0.092	3	91	86	99	2.00e+07
B 2-3	20	0.093	0.102	0.0091	0.0375	3	29	34	34	1.72e+07
T 0-1	20	0.225	0.269	0.044	0.1815	4	161	183	150	1.81e+08
T 1-2	20	0.095	0.117	0.022	0.0907	4	35	39	29	7.57e+07
T 2-3	20	0.1	0.108	0.0085	0.0351	3	49	36		2.42e+07
M 0-1	20	0.087	0.126	0.0398	0.1642	4	70	71	66	8.41e+07
M 1-2	20	0.075	0.1	0.0249	0.1027	3	55	53	66	1.13e+07
M 2-3	20	0.085	0.097	0.0125	0.0516	2	39	45	32	1.50e+06

Table 22. Competition Experiment 3-Raw Data, invading *Pseudomonas* colonization.

SECT.	DIL	Z1	Z2	dZ	VOL	Pseudomonas aeruginosa				
	(ml)	•	•	•	(ml)	DIL	COUNTS			CELLS/ml
T 0-1	20	0.12	0.157	0.0364	0.1501	2	117	113	96	1.45e+04
T 1-2	20	0.099	0.13	0.0311	0.1283	2	20	17	15	2.70e+03
T 2-3	20	0.182	0.194	0.0113	0.0466	1	16	7	14	5.29e+03
M 0-1	20	0.155	0.188	0.0329	0.1357	2	83	79	63	1.11e+04
M 1-2	20	0.115	0.136	0.0224	0.0924	2	3	0	0	2.16e+02
M 2-3	20	0.148	0.154	0.0065	0.0268	1	1	0	0	2.49e+02
B 0-1	20	0.152	0.17	0.0186	0.0767	2	76	65	76	1.89e+04
B 1-2	20	0.162	0.184	0.0223	0.092	2	8	15	12	2.54e+03
B 2-3	20	0.093	0.102	0.0091	0.0375	1	12	24	17	9.41e+03
T 0-1	20	0.225	0.269	0.044	0.1815	3	37	37	30	3.62e+03
T 1-2	20	0.095	0.117	0.022	0.0907	3	8	9	6	1.69e+03
T 2-3	20	0.1	0.108	0.0085	0.0351	2	13	9	12	6.47e+03
M 0-1	20	0.087	0.126	0.0398	0.1642	3	20	22	20	2.52e+03
M 1-2	20	0.075	0.1	0.0249	0.1027	3	1	1	2	2.60e+02
M 2-3	20	0.085	0.097	0.0125	0.0516	2	7	8	8	2.97e+03

Table 23. Competition Experiment 1-Raw Data, reactor effluent cell concentrations.

hour	chall.Psa	invading Kpn	unchall.Psa	Kpn,chem.eff.
0	6.20e+06		3.00e+06	
21	2.87e+06		2.00e+06	
46	2.53e+06		2.05e+06	
68	1.64e+06		2.17e+06	4.00e+07
93	1.61e+06		1.73e+06	3.60e+07
119	1.21e+06		1.22e+06	4.70e+06
146	1.25e+06		1.16e+06	1.50e+06
167	1.21e+06	2.33e+05	1.22e+06	
192	1.54e+06	6.17e+04	1.21e+06	
215	1.90e+06	7.80e+04	1.56e+06	
239	1.84e+06	4.60e+04	1.66e+06	
262	1.71e+06	6.03e+04		9.50e+05

Table 24. Competition Experiment 2-Raw Data, reactor effluent cell concentrations.

hour	chall.Psa	invading Kpn	unchall.Psa	Kpn,chem.eff.
0	2.1e+07		2.3e+07	
21	3.1e+07		2.2e+07	
45	2.5e+07		2.4e+07	
72	2.5e+07		3.8e+07	
92	2.1e+07		2.5e+07	6.5e+07
117	2.0e+07		3.2e+07	6.3e+07
141	8.6e+06	2.2e+07	2.3e+07	
165	8.6e+06	2.2e+07	2.9e+07	5.3e+07
188	2.0e+07	1.8e+07	3.2e+07	
260	1.0e+07	1.8e+07	7.3e+07	
284	6.7e+06	1.7e+07	4.9e+07	
308	3.4e+06	1.6e+07	2.2e+07	
332	1.1e+07	1.8e+07	2.5e+07	
356	7.0e+06	2.4e+07	2.3e+07	5.7e+07
379	1.2e+07	1.2e+07	2.3e+07	
429	3.7e+06	8.4e+06	2.1e+07	
452	2.7e+06	5.6e+06	2.1e+07	
477	5.0e+06	3.3e+06	2.2e+07	
603	5.6e+06	1.3e+06	2.2e+07	1.4e+07
644	3.6e+06	4.8e+05	2.3e+07	
676	4.2e+06	3.3e+05	1.7e+07	

Table 25. Competition Experiment 3-Raw Data, reactor effluent cell concentrations.

hour	chall.Kpn	invading Psa	unchall.Kpn	Psa,chem.eff.
1				
23	1.57e+04		7.10e+04	
44	1.77e+04		7.10e+04	3.80e+07
68	2.97e+04		1.15e+05	5.90e+06
92	4.97e+04		1.22e+05	2.30e+06
115	5.07e+04	1.04e+06	1.08e+05	
140	1.14e+05	2.43e+06	4.67e+04	
169	7.60e+04	2.62e+06	8.87e+04	
214	6.50e+04	2.50e+06	2.11e+05	
238	7.67e+04	1.95e+06		2.40e+06

Table 26. Effectiveness Factor Experiment-Raw Data, cells in reactor effluent.

		COLUMN 1		COLUMN 2	
		Eff.	Eff.cell	Eff.	Eff.cell
	FLOW	Gluc.	concent.	Gluc.	concent.
hour	ml/min	(ppm)	(CFU/ml)	(ppm)	(CFU/ml)
1	1	10.90	1.1e+07	10.80	7.1e+06
21	1	0.95	2.0e+07	1.10	1.7e+07
45	1	0.77	2.9e+07	0.48	2.4e+07
72	1	0.19	2.5e+07	0.00	1.7e+07
92	2	0.60	1.2e+07	0.13	1.4e+07
117	2	0.19	8.8e+06	0.31	1.1e+07
141	2	0.00	1.1e+07	0.37	1.3e+07
165	2	0.48	1.5e+07	0.00	1.3e+07
188	2	0.40	2.3e+07	0.10	2.3e+07
213	2	0.40	5.6e+07	0.2	7.4e+07
236	3	0.49	2.9e+07	0.2	2.5e+07
261	3	0.31	3.2e+07	0.49	2.6e+07
284	3	0.2	2.7e+07	0 08	2.1e+07
292	3	0.34		0.31	
308	3	0		0	
310	4	1.1		1.44	
312	4	1.2		1	
315	4	0.91		1.1	
317	5	1.3		2.4	
331	5	1.25	1.5e+07	0.912	1.6e+07
361	5	1.87	1.4e+07	1.81	1.6e+07
384	6.4	2.48		3.22	
390	6.4	2.318		1.98	
395	6.4	3.218		3.162	
404	6.4	5		2.82	
406	9	5.33		4.5	
410	9	5.23		4.45	
415	9	5.19		3.44	
429	9	4.512		4.568	
432	12	4.062		5.1	
437	12	5.5		5.131	
442	12	4.568		6.5	

Table 27. Effectiveness Factor Experiment-Raw Data, *Pseudomonas aeruginosa* colonization in pellets.

						Pseudomonas aeruginosa				
SECT.	DIL	Z1	Z2	dZ	VOL	DIL	COUNTS			CELLS/ml
	(ml)	-	-	-	(ml)					
T 0-1	20	0.091	0.119	0.0278	0.1147	5	39	38	36	6.57e+08
T 1-2	20	0.063	0.088	0.0249	0.1027	4	12	13	22	3.05e+07
T 2-3	20	0.088	0.103	0.0142	0.0586	3	29	27	28	9.56e+06
M 0-1	20	0.127	0.162	0.0356	0.1468	5	20	27	32	3.59e+08
M 1-2	20	0.126	0.152	0.0262	0.1081	4	21	17	19	3.52e+07
M 2-3	20	0.1	0.11	0.0104	0.0429	3	36	44	29	1.69e+07
B 0-1	20	0.328	0.366	0.0377	0.1555	4	123	113	114	1.50e+08
B 1-2	20	0.095	0.115	0.0201	0.0829	4	5	7	11	1.85e+07
B 2-3	20	0.099	0.106	0.0071	0.0293	2	212	248	231	1.57e+07

APPENDIX D

Mathematical Model

Table 28. Computer model code.

```
%engr2.m
%this is a matlab file which uses a finite element method to
%solve -(r^2u')' =   f(u)
%       u(-.3) =   Sb
%       u'(0) = 0
%   accesses func2.m and a canned nonlinear system solver
%
clg
clear
M=input('no of basis functions?');
mu = 2;
Ks = 1.43;
d = 2*10^(-6);
Sb=1.1;
c=ones(M+1,1);
DETAILS= [0 1 1 0 0 100];
FPARAM = [];
JAC = [];
SCALE = [];
[XF,TERMCODE]=nesolve('func2',c,DETAILS,FPARAM,JAC,SCALE);
%calculate u
c=XF;
h=.3/(M+1);
x=[0:h:.3]';
xf=[0:.0015:.3]';
PHI=zeros(201,M+1);
phi0 = -(xf-x(2))/h;
phi0=max(phi0,zeros(xf));
for i = 2:M+1;
        phi_i=min((xf-x(i-1))/h,(x(i+1)-xf)/h);
        phi_i=max(phi_i,zeros(xf));
        PHI(:,i-1)=phi_i;
end
phim = (xf-x(M+1))/h;
phim = max(phim,zeros(xf));
PHI(:,M+1)=phim;
u_M=PHI*c+phi0;
u_m=Sb*u_M;
% plot results
t=sprintf('Approximate solution to S.  mu = %1.2f Ks = %1.2f D=%1.4e',mu,Ks,d);
plot(xf-.3,u_m,'-'),xlabel ('radius'),ylabel('S(r)'),
    title(t);
print;
%save results in a file
diary paul8.m
disp(mu);
disp(Ks);
disp(d);
disp(u_m);
diary off
end;
```

Table 28-Continued.

```
%func2.m
%accessed from nonlinear solver used in engr2.m
% this evaluates the right-hand side of the equation
% and builds the nonlinear system to be solved.
%
function[F]=func2(c);
M=max(size(c))-1;
mu = 2;
Ks = 1.43;
d = 2*10^(-6);
Sb=1.1;
Kappa =3*mu/(60*60*.30*d);
h=.3/(M+1);
x=[-.3:h:0]';
x_interior=x(2:M+1);
x_mid=[-.3+h/2;(x_interior + h/2)];
n=[1:1:M];
end
% calculate left-hand side of equation
for i = 2:M+1;
maindiag(i-1) = (x(i+1)^3-x(i-1)^3)/(3*h^2);
subdiag(i-1)=-(x(i+1)^3-x(i)^3)/(3*h^2);
end;
maindiag(M+1)=(x(M+2)^3-x(M+1)^3)/(3*h^2);
A=diag(maindiag) + diag(subdiag,-1) + diag(subdiag,1);
% calculate last approximation for u
PHI=zeros(M+1,M+1);
phi0 = -(x_mid-x(2))/h;
phi0=max(phi0,zeros(x_mid));
for i = 2:M+1;
        phi_i=min((x_mid-x(i-1))/h,(x(i+1)-x_mid)/h);
        phi_i=max(phi_i,zeros(x_mid));
        PHI(:,i-1)=phi_i;
end
phim=(x_mid-x(M+1))/h;
phim = max(phim,zeros(x_mid));
PHI(:,M+1)=phim;
u=PHI*c+phi0;
% evaluate right-hand side of equation
f = -Kappa*((x_mid(1:M).^2)).*exp((-10*x_mid(1:M)).*(.95+.21*Sb*u(1:M))).*u(1:M);
f=f./(Ks+Sb*u(1:M));
f                                                    2                                                    =
-Kappa*((x_mid(2:M+1)).^2).*exp((-10*x_mid(2:M+1)).*(.95+.21*Sb*u(2:M+1))).*u(2:
M+1);
f=f+f2./(Ks+Sb*u(2:M+1));
f(M+1)=-Kappa*x_mid(M+1)^2*exp(-10*x_mid(M+1)*(.95+.21*Sb*u(M+1)))*u(M+1);
f(M+1)=f(M+1)/(Ks+Sb*u(M+1));
f=f*h/2;
bt1=(x(2)^3-x(1)^3)/(3*h^2);
e = zeros(f);
e(1)=-bt1;
f=f-e;
% put equation into form for newton's solver
F=A*c-f;
end
```

Table 29. Model results using *Pseudomonas* kinetics and measured cell density. C_{Abulk} varies from 0.25-5.1 mg L^{-1}.

This model data generated using Xo=3mg/l, K1=0.18						
PSEUDOMONAS KINETICS						
Mu(max) (1/hr)=0.4		Ks (mg/l)=2.5		Deff (cm^2/sec)=2x10^-6		
radius (mm)	glucose (mg/l)					
3	5.1	4.75	3	1.5	1.1	0.25
2.985	4.4601	4.1779	2.7048	1.3742	1.0112	0.2312
2.97	3.8202	3.6058	2.4097	1.2484	0.9224	0.2124
2.955	3.1803	3.0337	2.1145	1.1226	0.8336	0.1936
2.94	2.8506	2.726	1.9243	1.0319	0.7681	0.1791
2.925	2.5466	2.4403	1.7429	0.9442	0.7045	0.1651
2.91	2.2427	2.1546	1.5615	0.8564	0.6409	0.151
2.895	2.0374	1.9596	1.4298	0.7893	0.5917	0.1398
2.88	1.85	1.7811	1.3073	0.726	0.5451	0.1292
2.865	1.6626	1.6025	1.1848	0.6627	0.4984	0.1186
2.85	1.5216	1.4676	1.0895	0.6121	0.4609	0.1089
2.835	1.3945	1.3457	1.0025	0.5653	0.4261	0.1019
2.82	1.2675	1.2239	0.9155	0.5185	0.3913	0.0938
2.805	1.1656	1.126	0.8446	0.4788	0.3624	0.087
2.79	1.075	1.0388	0.7808	0.4446	0.3361	0.0808
2.775	0.9843	0.9515	0.7171	0.4095	0.3098	0.0746
2.76	0.9084	0.8784	0.6632	0.3795	0.2873	0.0693
2.745	0.8417	0.814	0.6154	0.3528	0.2672	0.0645
2.73	0.775	0.7497	0.5677	0.326	0.2471	0.0597
2.715	0.7171	0.6939	0.5261	0.3026	0.2294	0.0555
2.7	0.6669	0.6454	0.4898	0.282	0.2139	0.0518
2.685	0.6167	0.5969	0.4535	0.2615	0.1984	0.0481
2.67	0.572	0.5537	0.421	0.243	0.1844	0.0447
2.655	0.5336	0.5166	0.3931	0.2271	0.1724	0.0418
2.64	0.4952	0.4794	0.3651	0.2111	0.1603	0.0389
2.625	0.4602	0.4456	0.3395	0.1965	0.1492	0.0362
2.61	0.4305	0.4168	0.3178	0.184	0.1398	0.034
2.595	0.4008	0.3881	0.296	0.1715	0.1303	0.0317

Table 29-Continued.

2.58	0.373	0.3613	0.2757	0.1598	0.1214	0.0295
2.565	0.3498	0.3388	0.2586	0.15	0.114	0.0277
2.55	0.3266	0.3163	0.2415	0.1401	0.1065	0.0259
2.535	0.3045	0.2949	0.2253	0.1308	0.0994	0.0242
2.52	0.2862	0.2772	0.2118	0.123	0.0935	0.0228
2.505	0.2678	0.2594	0.1983	0.1151	0.0876	0.0213
2.49	0.2501	0.2423	0.1852	0.1076	0.0818	0.0199
2.475	0.2355	0.2282	0.1744	0.1014	0.0771	0.0188
2.46	0.221	0.2141	0.1637	0.0951	0.0724	0.0176
2.445	0.2066	0.2002	0.1531	0.089	0.0677	0.0165
2.43	0.196	0.1889	0.1445	0.084	0.0639	0.0156
2.415	0.1833	0.1776	0.1359	0.079	0.0601	0.0146
2.4	0.1717	0.1663	0.1273	0.074	0.0563	0.0137
2.385	0.1623	0.1572	0.1203	0.07	0.0532	0.013
2.37	0.1528	0.1482	0.1134	0.066	0.0502	0.0122
2.355	0.1435	0.1391	0.1064	0.0619	0.0471	0.0115
2.34	0.1358	0.1316	0.1007	0.0586	0.0446	0.0109
2.325	0.1282	0.1242	0.0951	0.0553	0.0421	0.0103
2.31	0.1206	0.1169	0.0895	0.0521	0.0396	0.0097
2.295	0.1142	0.1107	0.0847	0.0493	0.0375	0.0091
2.28	0.108	0.1047	0.0801	0.0466	0.0355	0.0087
2.265	0.1019	0.0987	0.0755	0.044	0.0335	0.0082
2.25	0.0965	0.0935	0.0716	0.0417	0.0317	0.0077
2.235	0.0915	0.0886	0.0679	0.0395	0.0301	0.0073
2.22	0.0864	0.0837	0.0641	0.0373	0.0284	0.0069
2.205	0.082	0.0794	0.0608	0.0354	0.0269	0.0066
2.19	0.0778	0.0754	0.0577	0.0336	0.0256	0.0062
2.175	0.0737	0.0714	0.0546	0.0318	0.0242	0.0059
2.16	0.07	0.0678	0.0519	0.0302	0.023	0.0056
2.145	0.0665	0.0645	0.0493	0.0287	0.0218	0.0053
2.13	0.0631	0.0611	0.0468	0.0272	0.0207	0.0051
2.115	0.06	0.0581	0.0445	0.0259	0.0197	0.0048
2.1	0.0571	0.0553	0.0424	0.0247	0.0188	0.0046

Table 29-Continued.

2.085	0.0543	0.0526	0.0403	0.0234	0.0178	0.0044
2.07	0.0516	0.05	0.0383	0.0223	0.017	0.0041
2.055	0.0492	0.0477	0.0365	0.0213	0.0162	0.0039
2.04	0.0469	0.0454	0.0348	0.0202	0.0154	0.0038
2.025	0.0446	0.0433	0.0331	0.0193	0.0147	0.0036
2.01	0.0427	0.0413	0.0316	0.0184	0.014	0.0034
1.995	0.0407	0.0394	0.0302	0.0176	0.0134	0.0033
1.98	0.0388	0.0376	0.0288	0.0167	0.0127	0.0031
1.965	0.0371	0.036	0.0275	0.016	0.0122	0.003
1.95	0.0354	0.0343	0.0263	0.0153	0.0116	0.0028
1.935	0.0338	0.0328	0.0251	0.0146	0.0111	0.0027
1.92	0.0324	0.0314	0.024	0.014	0.0107	0.0026
1.905	0.031	0.03	0.023	0.0134	0.0102	0.0025
1.89	0.0296	0.0287	0.022	0.0128	0.0097	0.0024
1.875	0.0284	0.0275	0.0211	0.0123	0.0093	0.0023
1.86	0.0272	0.0264	0.0202	0.0118	0.009	0.0022
1.845	0.0261	0.0252	0.0193	0.0113	0.0086	0.0021
1.83	0.025	0.0243	0.0186	0.0108	0.0082	0.002
1.815	0.024	0.0233	0.0178	0.0104	0.0079	0.0019
1.8	0.023	0.0223	0.0171	0.0099	0.0076	0.0018
1.785	0.0221	0.0215	0.0164	0.0096	0.0073	0.0018
1.77	0.0213	0.0205	0.0158	0.0092	0.007	0.0017
1.755	0.0204	0.0198	0.0151	0.0088	0.0067	0.0016
1.74	0.0197	0.0191	0.0146	0.0086	0.0065	0.0016
1.725	0.0189	0.0183	0.014	0.0082	0.0062	0.0015
1.71	0.0182	0.0176	0.0135	0.0079	0.006	0.0015
1.695	0.0175	0.017	0.013	0.0076	0.0058	0.0014
1.68	0.0169	0.0164	0.0125	0.0073	0.0056	0.0014
1.665	0.0163	0.0158	0.0121	0.007	0.0053	0.0013
1.65	0.0157	0.0152	0.0116	0.0068	0.0052	0.0013
1.635	0.0151	0.0147	0.0112	0.0065	0.005	0.0012
1.62	0.0146	0.0141	0.0108	0.0063	0.0048	0.0012
1.605	0.0141	0.0137	0.0105	0.0061	0.0046	0.0011

Table 29-Continued.

1.59	0.0136	0.0132	0.0101	0.0059	0.0045	0.0011
1.575	0.0131	0.0127	0.0098	0.0057	0.0043	0.0011
1.56	0.0127	0.0123	0.0094	0.0055	0.0042	0.001
1.545	0.0123	0.0119	0.0091	0.0053	0.004	0.001
1.53	0.0119	0.0115	0.0088	0.0051	0.0039	0.001
1.515	0.0115	0.0111	0.0085	0.005	0.0038	0.0009
1.5	0.0111	0.0108	0.0083	0.0048	0.0037	0.0009
1.485	0.0108	0.0105	0.008	0.0047	0.0035	0.0009
1.47	0.0105	0.0101	0.0078	0.0045	0.0034	0.0008
1.455	0.0101	0.0098	0.0075	0.0044	0.0033	0.0008
1.44	0.0098	0.0095	0.0073	0.0042	0.0032	0.0008
1.425	0.0095	0.0092	0.0071	0.0041	0.0031	0.0008
1.41	0.0093	0.009	0.0069	0.004	0.003	0.0007
1.395	0.009	0.0087	0.0067	0.0039	0.003	0.0007
1.38	0.0087	0.0085	0.0065	0.0038	0.0029	0.0007
1.365	0.0085	0.0082	0.0063	0.0037	0.0028	0.0007
1.35	0.0083	0.008	0.0061	0.0036	0.0027	0.0007
1.335	0.008	0.0078	0.006	0.0035	0.0026	0.0006
1.32	0.0078	0.0076	0.0058	0.0034	0.0026	0.0006
1.305	0.0076	0.0074	0.0056	0.0033	0.0025	0.0006
1.29	0.0074	0.0072	0.0055	0.0032	0.0024	0.0006
1.275	0.0072	0.007	0.0054	0.0031	0.0024	0.0006
1.26	0.007	0.0068	0.0052	0.003	0.0023	0.0006
1.245	0.0068	0.0066	0.0051	0.003	0.0022	0.0005
1.23	0.0067	0.0065	0.005	0.0029	0.0022	0.0005
1.215	0.0065	0.0063	0.0048	0.0028	0.0021	0.0005
1.2	0.0064	0.0062	0.0047	0.0027	0.0021	0.0005
1.185	0.0062	0.006	0.0046	0.0027	0.002	0.0005
1.17	0.0061	0.0059	0.0045	0.0026	0.002	0.0005
1.155	0.0059	0.0057	0.0044	0.0026	0.0019	0.0005
1.14	0.0058	0.0056	0.0043	0.0025	0.0019	0.0005
1.125	0.0057	0.0055	0.0042	0.0024	0.0019	0.0005
1.11	0.0056	0.0054	0.0041	0.0024	0.0018	0.0004

Table 29-Continued.

1.095	0.0054	0.0053	0.004	0.0023	0.0018	0.0004
1.08	0.0053	0.0052	0.0039	0.0023	0.0017	0.0004
1.065	0.0052	0.005	0.0039	0.0022	0.0017	0.0004
1.05	0.0051	0.0049	0.0038	0.0022	0.0017	0.0004
1.035	0.005	0.0048	0.0037	0.0022	0.0016	0.0004
1.02	0.0049	0.0048	0.0036	0.0021	0.0016	0.0004
1.005	0.0048	0.0047	0.0036	0.0021	0.0016	0.0004
0.99	0.0047	0.0046	0.0035	0.002	0.0016	0.0004
0.975	0.0046	0.0045	0.0034	0.002	0.0015	0.0004
0.96	0.0046	0.0044	0.0034	0.002	0.0015	0.0004
0.945	0.0045	0.0043	0.0033	0.0019	0.0015	0.0004
0.93	0.0044	0.0043	0.0033	0.0019	0.0014	0.0004
0.915	0.0043	0.0042	0.0032	0.0019	0.0014	0.0003
0.9	0.0043	0.0041	0.0032	0.0018	0.0014	0.0003
0.885	0.0042	0.0041	0.0031	0.0018	0.0014	0.0003
0.87	0.0041	0.004	0.0031	0.0018	0.0014	0.0003
0.855	0.0041	0.0039	0.003	0.0018	0.0013	0.0003
0.84	0.004	0.0039	0.003	0.0017	0.0013	0.0003
0.825	0.0039	0.0038	0.0029	0.0017	0.0013	0.0003
0.81	0.0039	0.0038	0.0029	0.0017	0.0013	0.0003
0.795	0.0038	0.0037	0.0028	0.0017	0.0013	0.0003
0.78	0.0038	0.0037	0.0028	0.0016	0.0012	0.0003
0.765	0.0037	0.0036	0.0028	0.0016	0.0012	0.0003
0.75	0.0037	0.0036	0.0027	0.0016	0.0012	0.0003
0.735	0.0036	0.0035	0.0027	0.0016	0.0012	0.0003
0.72	0.0036	0.0035	0.0027	0.0015	0.0012	0.0003
0.705	0.0035	0.0034	0.0026	0.0015	0.0012	0.0003
0.69	0.0035	0.0034	0.0026	0.0015	0.0011	0.0003
0.675	0.0035	0.0034	0.0026	0.0015	0.0011	0.0003
0.66	0.0034	0.0033	0.0025	0.0015	0.0011	0.0003
0.645	0.0034	0.0033	0.0025	0.0015	0.0011	0.0003
0.63	0.0033	0.0032	0.0025	0.0014	0.0011	0.0003
0.615	0.0033	0.0032	0.0025	0.0014	0.0011	0.0003

Table 29-Continued.

0.6	0.0033	0.0032	0.0024	0.0014	0.0011	0.0003
0.585	0.0033	0.0032	0.0024	0.0014	0.0011	0.0003
0.57	0.0032	0.0031	0.0024	0.0014	0.0011	0.0003
0.555	0.0032	0.0031	0.0024	0.0014	0.001	0.0003
0.54	0.0032	0.0031	0.0023	0.0014	0.001	0.0003
0.525	0.0031	0.003	0.0023	0.0014	0.001	0.0003
0.51	0.0031	0.003	0.0023	0.0013	0.001	0.0002
0.495	0.0031	0.003	0.0023	0.0013	0.001	0.0002
0.48	0.0031	0.003	0.0023	0.0013	0.001	0.0002
0.465	0.003	0.003	0.0023	0.0013	0.001	0.0002
0.45	0.003	0.0029	0.0022	0.0013	0.001	0.0002
0.435	0.003	0.0029	0.0022	0.0013	0.001	0.0002
0.42	0.003	0.0029	0.0022	0.0013	0.001	0.0002
0.405	0.003	0.0029	0.0022	0.0013	0.001	0.0002
0.39	0.003	0.0029	0.0022	0.0013	0.001	0.0002
0.375	0.0029	0.0028	0.0022	0.0013	0.001	0.0002
0.36	0.0029	0.0028	0.0022	0.0013	0.001	0.0002
0.345	0.0028	0.0028	0.0022	0.0013	0.001	0.0002
0.33	0.0029	0.0028	0.0021	0.0012	0.0009	0.0002
0.315	0.0029	0.0028	0.0021	0.0012	0.0009	0.0002
0.3	0.0028	0.0028	0.0021	0.0012	0.0009	0.0002
0.285	0.0029	0.0028	0.0021	0.0012	0.0009	0.0002
0.27	0.0028	0.0028	0.0021	0.0012	0.0009	0.0002
0.255	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.24	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.225	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.21	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.195	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.18	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.165	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.15	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.135	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.12	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002

Table 29-Continued.

0.105	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.09	0.0028	0.0027	0.0021	0.0012	0.0009	0.0002
0.075	0.0028	0.0027	0.002	0.0012	0.0009	0.0002
0.06	0.0028	0.0027	0.002	0.0012	0.0009	0.0002
0.045	0.0028	0.0027	0.002	0.0012	0.0009	0.0002
0.03	0.0028	0.0027	0.002	0.0012	0.0009	0.0002
0.015	0.0028	0.0027	0.002	0.0012	0.0009	0.0002
0	0.0028	0.0027	0.002	0.0012	0.0009	0.0002

Table 30. Model results using *Pseudomonas* kinetics and revised cell density. C_{Abulk} varies from 0.25-5.1 mg L^{-1}.

PSEUDOMONAS KINETICS						
Mu(max) (1/hr) = 0.4		Ks (mg/l) = 2.5		Deff (cm^2/sec) = 2x10^-6		
radius (mm)	glucose (mg/l)					
3	5.1	4.75	3	1.5	1.1	0.25
2.985	4.8633	4.542	2.9008	1.46	1.0721	0.2442
2.97	4.6267	4.3339	2.8017	1.42	1.0442	0.2384
2.955	4.39	4.1259	2.7025	1.3801	1.0163	0.2326
2.94	4.2093	3.9634	2.6177	1.3441	0.991	0.2273
2.925	4.0332	3.8047	2.5342	1.3084	0.9658	0.2219
2.91	3.8671	3.646	2.4506	1.2728	0.9407	0.2166
2.895	3.7128	3.5143	2.3771	1.2402	0.9175	0.2116
2.88	3.5742	3.3874	2.3055	1.2083	0.8948	0.2067
2.865	3.4356	3.2605	2.2338	1.1763	0.872	0.2019
2.85	3.3167	3.1508	2.1695	1.1468	0.8509	0.1973
2.835	3.2036	3.0461	2.1073	1.118	0.8302	0.1927
2.82	3.0905	2.9415	2.0451	1.0892	0.8095	0.1882
2.805	2.9902	2.8482	1.9883	1.0624	0.7901	0.184
2.79	2.8956	2.76	1.9337	1.0363	0.7713	0.1798
2.775	2.801	2.6718	1.8792	1.0103	0.7525	0.1757
2.76	2.7149	2.5913	1.8286	0.9858	0.7348	0.1717
2.745	2.6343	2.5156	1.7804	0.9622	0.7176	0.1679
2.73	2.5536	2.44	1.7322	0.9386	0.7004	0.1641
2.715	2.4788	2.3697	1.6868	0.9162	0.6841	0.1604
2.7	2.409	2.304	1.6439	0.8947	0.6684	0.1569
2.685	2.3393	2.2382	1.601	0.8733	0.6527	0.1533
2.67	2.2736	2.1762	1.5601	0.8527	0.6377	0.1499
2.655	2.2126	2.1186	1.5217	0.8332	0.6233	0.1467
2.64	2.1516	2.0609	1.4832	0.8136	0.609	0.1434
2.625	2.0933	2.0057	1.4463	0.7947	0.5951	0.1402
2.61	2.0395	1.9547	1.4117	0.7768	0.5819	0.1372
2.595	1.9856	1.9036	1.3771	0.759	0.5687	0.1342
2.58	1.9336	1.8543	1.3435	0.7415	0.5559	0.1313
2.565	1.8858	1.8088	1.3123	0.7252	0.5438	0.1285

Table 30-Continued.

2.55	1.8379	1.7633	1.281	0.7088	0.5317	0.1257
2.535	1.7912	1.7189	1.2504	0.6928	0.5198	0.123
2.52	1.7484	1.6781	1.2221	0.6778	0.5087	0.1204
2.505	1.7056	1.6373	1.1937	0.6628	0.4976	0.1178
2.49	1.6634	1.5972	1.1658	0.6479	0.4866	0.1153
2.475	1.6249	1.5604	1.14	0.6341	0.4763	0.1129
2.46	1.5864	1.5237	1.1142	0.6204	0.4661	0.1105
2.445	1.5481	1.4872	1.0886	0.6067	0.4559	0.1081
2.43	1.5133	1.4539	1.065	0.594	0.4464	0.1059
2.415	1.4785	1.4207	1.0415	0.5813	0.437	0.1037
2.4	1.4437	1.3875	1.018	0.5686	0.4275	0.1015
2.385	1.4122	1.3573	0.9964	0.5569	0.4188	0.0995
2.37	1.3806	1.3271	0.9749	0.5452	0.41	0.0974
2.355	1.349	1.2969	0.9534	0.5335	0.4013	0.0954
2.34	1.3201	1.2692	0.9335	0.5227	0.3932	0.0935
2.325	1.2914	1.2417	0.9138	0.5119	0.3851	0.0916
2.31	1.2626	1.2142	0.894	0.5011	0.3771	0.0897
2.295	1.236	1.1887	0.8757	0.491	0.3695	0.0879
2.28	1.2098	1.1635	0.8575	0.481	0.3621	0.0861
2.265	1.1836	1.1384	0.8394	0.4711	0.3546	0.0844
2.25	1.1591	1.1149	0.8224	0.4617	0.3476	0.0827
2.235	1.1351	1.0919	0.8057	0.4525	0.3407	0.0811
2.22	1.1111	1.0689	0.789	0.4433	0.3338	0.0795
2.205	1.0885	1.0472	0.7732	0.4345	0.3272	0.0779
2.19	1.0665	1.026	0.7579	0.426	0.3208	0.0764
2.175	1.0444	1.0049	0.7425	0.4175	0.3144	0.0749
2.16	1.0235	0.9848	0.7279	0.4094	0.3083	0.0734
2.145	1.0033	0.9654	0.7137	0.4015	0.3024	0.072
2.13	0.9831	0.946	0.6995	0.3936	0.2964	0.0706
2.115	0.9637	0.9274	0.6859	0.386	0.2907	0.0693
2.1	0.9451	0.9095	0.6728	0.3787	0.2852	0.0679
2.085	0.9264	0.8916	0.6598	0.3714	0.2797	0.0666
2.07	0.9085	0.8743	0.647	0.3643	0.2744	0.0654

Table 30-Continued.

2.055	0.8913	0.8578	0.6349	0.3575	0.2683	0.0642
2.04	0.8741	0.8413	0.6227	0.3507	0.2642	0.0629
2.025	0.8574	0.8252	0.6109	0.3441	0.2592	0.0618
2.01	0.8416	0.81	0.5997	0.3378	0.2545	0.0606
1.995	0.8257	0.7947	0.5885	0.3315	0.2497	0.0595
1.98	0.8102	0.7798	0.5775	0.3253	0.2451	0.0584
1.965	0.7955	0.7657	0.5671	0.3195	0.2407	0.0573
1.95	0.7808	0.7516	0.5567	0.3136	0.2363	0.0563
1.935	0.7664	0.7377	0.5464	0.3079	0.2319	0.0553
1.92	0.7528	0.7246	0.5368	0.3024	0.2278	0.0543
1.905	0.7392	0.7115	0.5271	0.297	0.2237	0.0533
1.89	0.7258	0.6986	0.5175	0.2916	0.2197	0.0523
1.875	0.7132	0.6865	0.5086	0.2865	0.2159	0.0514
1.86	0.7006	0.6744	0.4996	0.2815	0.212	0.0505
1.845	0.688	0.6623	0.4907	0.2764	0.2082	0.0496
1.83	0.6763	0.651	0.4823	0.2717	0.2047	0.0487
1.815	0.6646	0.6398	0.474	0.267	0.2011	0.0479
1.8	0.6529	0.6286	0.4656	0.2623	0.1976	0.047
1.785	0.6421	0.6181	0.4579	0.2579	0.1943	0.0463
1.77	0.6312	0.6076	0.4501	0.2536	0.191	0.0455
1.755	0.6204	0.5972	0.4424	0.2492	0.1877	0.0447
1.74	0.6102	0.5874	0.4351	0.2451	0.1846	0.0439
1.725	0.6001	0.5777	0.4279	0.241	0.1815	0.0432
1.71	0.5901	0.568	0.4207	0.2369	0.1784	0.0425
1.695	0.5806	0.5588	0.4139	0.2331	0.1755	0.0418
1.68	0.5712	0.5498	0.4072	0.2293	0.1726	0.0411
1.665	0.5618	0.5408	0.4005	0.2255	0.1698	0.0404
1.65	0.5529	0.5322	0.3941	0.2218	0.1671	0.0397
1.635	0.5442	0.5238	0.3879	0.2183	0.1644	0.0391
1.62	0.5356	0.5154	0.3816	0.2148	0.1617	0.0385
1.605	0.5271	0.5074	0.3757	0.2114	0.1592	0.0378
1.59	0.519	0.4996	0.3699	0.2081	0.1567	0.0372
1.575	0.5109	0.4918	0.364	0.2048	0.1542	0.0367

Table 30-Continued.

1.56	0.5031	0.4843	0.3585	0.2016	0.1518	0.0361
1.545	0.4955	0.477	0.353	0.1985	0.1495	0.0355
1.53	0.488	0.4697	0.3476	0.1955	0.1471	0.035
1.515	0.4807	0.4627	0.3424	0.1925	0.1449	0.0344
1.5	0.4736	0.4559	0.3373	0.1896	0.1427	0.0339
1.485	0.4666	0.4491	0.3323	0.1868	0.1406	0.0334
1.47	0.4598	0.4425	0.3274	0.184	0.1385	0.0329
1.455	0.4532	0.4362	0.3226	0.1813	0.1364	0.0324
1.44	0.4466	0.4298	0.3179	0.1786	0.1344	0.0319
1.425	0.4402	0.4237	0.3133	0.176	0.1325	0.0314
1.41	0.4341	0.4178	0.3089	0.1735	0.1306	0.031
1.395	0.428	0.4119	0.3046	0.171	0.1287	0.0305
1.38	0.422	0.4061	0.3002	0.1686	0.1268	0.0301
1.365	0.4163	0.4006	0.2962	0.1663	0.1251	0.0297
1.35	0.4106	0.3951	0.2921	0.1639	0.1233	0.0293
1.335	0.405	0.3897	0.288	0.1616	0.1216	0.0288
1.32	0.3997	0.3846	0.2842	0.1595	0.12	0.0285
1.305	0.3943	0.3795	0.2804	0.1573	0.1183	0.0281
1.29	0.3891	0.3744	0.2766	0.1552	0.1167	0.0277
1.275	0.3841	0.3696	0.273	0.1531	0.1152	0.0273
1.26	0.3792	0.3649	0.2695	0.1511	0.1136	0.0269
1.245	0.3743	0.3601	0.2659	0.1491	0.1121	0.0266
1.23	0.3696	0.3557	0.2626	0.1472	0.1107	0.0262
1.215	0.365	0.3512	0.2593	0.1453	0.1093	0.0259
1.2	0.3604	0.3468	0.256	0.1435	0.1079	0.0256
1.185	0.3561	0.3426	0.2529	0.1417	0.1065	0.0252
1.17	0.3518	0.3385	0.2498	0.1399	0.1052	0.0249
1.155	0.3475	0.3343	0.2467	0.1382	0.1039	0.0246
1.14	0.3435	0.3305	0.2438	0.1366	0.1026	0.0243
1.125	0.3395	0.3266	0.241	0.1349	0.1014	0.024
1.11	0.3355	0.3228	0.2381	0.1333	0.1002	0.0237
1.095	0.3317	0.3191	0.2354	0.1317	0.099	0.0234
1.08	0.328	0.3155	0.2327	0.1302	0.0979	0.0232

Table 30-Continued.

1.065	0.3243	0.312	0.23	0.1287	0.0967	0.0229
1.05	0.3208	0.3086	0.2275	0.1273	0.0956	0.0226
1.035	0.3173	0.3052	0.225	0.1259	0.0946	0.0224
1.02	0.3138	0.3019	0.2225	0.1244	0.0935	0.0221
1.005	0.3105	0.2987	0.2202	0.1231	0.0925	0.0219
0.99	0.3073	0.2956	0.2179	0.1218	0.0915	0.0216
0.975	0.3041	0.2925	0.2155	0.1205	0.0905	0.0214
0.96	0.3011	0.2896	0.2133	0.1192	0.0896	0.0212
0.945	0.2981	0.2867	0.2112	0.118	0.0886	0.021
0.93	0.2951	0.2838	0.209	0.1168	0.0877	0.0207
0.915	0.2922	0.2811	0.207	0.1156	0.0868	0.0205
0.9	0.2895	0.2784	0.205	0.1145	0.086	0.0203
0.885	0.2867	0.2757	0.203	0.1134	0.0851	0.0201
0.87	0.284	0.2732	0.2011	0.1123	0.0843	0.0199
0.855	0.2815	0.2707	0.1993	0.1112	0.0835	0.0197
0.84	0.2789	0.2683	0.1974	0.1102	0.0827	0.0195
0.825	0.2764	0.2659	0.1956	0.1092	0.082	0.0194
0.81	0.2741	0.2636	0.194	0.1082	0.0813	0.0192
0.795	0.2717	0.2613	0.1923	0.1073	0.0805	0.019
0.78	0.2694	0.2591	0.1906	0.1063	0.0798	0.0188
0.765	0.2673	0.257	0.189	0.1054	0.0791	0.0187
0.75	0.2651	0.2549	0.1875	0.1045	0.0785	0.0185
0.735	0.263	0.2529	0.1859	0.1037	0.0778	0.0184
0.72	0.261	0.2509	0.1845	0.1028	0.0772	0.0182
0.705	0.259	0.249	0.1831	0.102	0.0766	0.0181
0.69	0.257	0.2471	0.1817	0.1012	0.076	0.0179
0.675	0.2552	0.2454	0.1803	0.1005	0.0754	0.0178
0.66	0.2533	0.2436	0.179	0.0997	0.0749	0.0177
0.645	0.2515	0.2418	0.1777	0.099	0.0743	0.0175
0.63	0.2499	0.2402	0.1765	0.0983	0.0738	0.0174
0.615	0.2482	0.2386	0.1753	0.0976	0.0733	0.0173
0.6	0.2465	0.237	0.1741	0.097	0.0728	0.0172
0.585	0.245	0.2356	0.173	0.0963	0.0723	0.017

Table 30-Continued.

0.57	0.2435	0.2341	0.1719	0.0957	0.0718	0.0169
0.555	0.242	0.2326	0.1709	0.0951	0.0713	0.0168
0.54	0.2406	0.2313	0.1699	0.0945	0.0709	0.0167
0.525	0.2392	0.23	0.1689	0.094	0.0705	0.0166
0.51	0.2379	0.2287	0.1679	0.0934	0.0701	0.0165
0.495	0.2366	0.2275	0.167	0.0929	0.0697	0.0164
0.48	0.2354	0.2263	0.1661	0.0924	0.0693	0.0163
0.465	0.2341	0.2251	0.1652	0.0919	0.0689	0.0162
0.45	0.233	0.224	0.1644	0.0914	0.0686	0.0162
0.435	0.2319	0.223	0.1636	0.091	0.0683	0.0161
0.42	0.2308	0.2219	0.1628	0.0906	0.0679	0.016
0.405	0.2298	0.2209	0.1621	0.0901	0.0676	0.0159
0.39	0.2289	0.22	0.1614	0.0897	0.0673	0.0158
0.375	0.2279	0.2191	0.1607	0.0894	0.067	0.0158
0.36	0.227	0.2182	0.1601	0.089	0.0667	0.0157
0.345	0.2262	0.2174	0.1595	0.0886	0.0665	0.0156
0.33	0.2253	0.2166	0.1589	0.0883	0.0662	0.0156
0.315	0.2246	0.2158	0.1583	0.088	0.066	0.0155
0.3	0.2238	0.2152	0.1578	0.0877	0.0658	0.0155
0.285	0.2231	0.2145	0.1573	0.0874	0.0656	0.0154
0.27	0.2224	0.2138	0.1568	0.0871	0.0653	0.0154
0.255	0.2218	0.2132	0.1564	0.0869	0.0651	0.0153
0.24	0.2212	0.2127	0.1559	0.0866	0.065	0.0153
0.225	0.2207	0.2121	0.1555	0.0864	0.0648	0.0152
0.21	0.2202	0.2116	0.1552	0.0862	0.0646	0.0152
0.195	0.2197	0.2112	0.1548	0.086	0.0645	0.0152
0.18	0.2192	0.2107	0.1545	0.0858	0.0643	0.0151
0.165	0.2189	0.2104	0.1542	0.0856	0.0642	0.0151
0.15	0.2185	0.21	0.1539	0.0855	0.0641	0.0151
0.135	0.2181	0.2096	0.1537	0.0853	0.064	0.0151
0.12	0.2178	0.2094	0.1535	0.0852	0.0638	0.015
0.105	0.2176	0.2091	0.1533	0.0851	0.0638	0.015
0.09	0.2173	0.2088	0.1531	0.085	0.0637	0.015

Table 30-Continued.

0.075	0.2171	0.2087	0.153	0.0849	0.0637	0.015
0.06	0.2169	0.2085	0.1528	0.0849	0.0636	0.015
0.045	0.2167	0.2083	0.1527	0.0848	0.0636	0.015
0.03	0.2166	0.2082	0.1526	0.0847	0.0635	0.0149
0.015	0.2165	0.2081	0.1525	0.0847	0.0635	0.0149
0	0.2164	0.208	0.1525	0.0846	0.0635	0.0149

Table 31. Model results using *Klebsiella* kinetics and revised cell density. C_{Abulk} varies from 0.25-5.1 mg l^{-1}.

This model data generated using Xo = 3mg/l, K1 = 0.95						
KLEBSIELLA KINETICS						
Mu(max) (1/hr) = 2		Ks (mg/l) = 1.43		D_{eff} (cm^2/sec) = 2x10^-6		
radius (mm)	glucose (mg/l)					
3	5.1	4.75	3	1.5	1.1	0.25
2.985	3.8874	3.6466	2.3767	1.2109	0.8908	0.2031
2.97	2.6748	2.5432	1.7534	0.9218	0.6816	0.1561
2.955	1.4621	1.4398	1.1301	0.6326	0.4724	0.1092
2.94	1.131	1.1196	0.8958	0.5062	0.3786	0.0876
2.925	0.8733	0.8646	0.6938	0.3934	0.2943	0.0681
2.91	0.6156	0.6097	0.4919	0.2805	0.21	0.0485
2.895	0.4875	0.4828	0.39	0.2227	0.1668	0.0385
2.88	0.3829	0.3792	0.3064	0.175	0.131	0.0303
2.865	0.2783	0.2757	0.2228	0.1272	0.0952	0.022
2.85	0.2193	0.2172	0.1756	0.1002	0.075	0.0173
2.835	0.174	0.1723	0.1393	0.0795	0.0595	0.0137
2.82	0.1286	0.1274	0.1029	0.0587	0.0439	0.0101
2.805	0.1007	0.0998	0.0806	0.046	0.0344	0.0079
2.79	0.0806	0.0798	0.0645	0.0368	0.0275	0.0063
2.775	0.0604	0.0599	0.0484	0.0276	0.0206	0.0047
2.76	0.0471	0.0466	0.0377	0.0215	0.0161	0.0037
2.745	0.038	0.0376	0.0304	0.0173	0.0129	0.003
2.73	0.0289	0.0286	0.0231	0.0132	0.0098	0.0023
2.715	0.0224	0.0222	0.0179	0.0102	0.0076	0.0018
2.7	0.0182	0.018	0.0146	0.0083	0.0062	0.0014
2.685	0.014	0.0139	0.0112	0.0064	0.0048	0.0011
2.67	0.0109	0.0108	0.0087	0.0049	0.0037	0.0008
2.655	0.0089	0.0088	0.0071	0.004	0.003	0.0007
2.64	0.0069	0.0069	0.0055	0.0032	0.0024	0.0005
2.625	0.0054	0.0053	0.0043	0.0024	0.0018	0.0004
2.61	0.0044	0.0044	0.0035	0.002	0.0015	0.0003
2.595	0.0035	0.0034	0.0028	0.0016	0.0012	0.0003

Table 31-Continued.

2.58	0.0027	0.0027	0.0021	0.0012	0.0009	0.0002
2.565	0.0022	0.0022	0.0018	0.001	0.0008	0.0002
2.55	0.0018	0.0018	0.0014	0.0008	0.0006	0.0001
2.535	0.0014	0.0014	0.0011	0.0006	0.0005	0.0001
2.52	0.0011	0.0011	0.0009	0.0005	0.0004	0
2.505	0.0009	0.0009	0.0007	0.0004	0.0003	0
2.49	0.0007	0.0007	0.0006	0.0003	0.0002	0
2.475	0.0006	0.0006	0.0005	0.0003	0.0002	0
2.46	0.0005	0.0005	0.0004	0.0002	0.0002	0
2.445	0.0004	0.0004	0.0003	0.0002	0.0001	0
2.43	0.0003	0.0003	0.0003	0.0001	0.0001	0
2.415	0.0003	0.0003	0.0002	0.0001	0	0
2.4	0.0002	0.0002	0.0002	0	0	0
2.385	0.0002	0.0002	0.0001	0	0	0
2.37	0.0001	0.0001	0.0001	0	0	0
2.355	0.0001	0.0001	0	0	0	0
2.34	0	0	0	0	0	0

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