Mobilization of Broad Host Range Plasmid from *Pseudomonas putida* to Established Biofilm of *Bacillus azotoformans.* II. Modeling

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Abstract: A strain of *Pseudomonas putida* that harbors plasmids RK2 and pDLB101 was exposed to a pure culture biofilm of *Bacillus azotoformans* grown in a rotating annular reactor. Transfer of the RK2 mobilizable pDLB101 plasmid to *B. azotoformans* was monitored over a 4-day period. Experimental results demonstrated that the broad host range, RSF1010 derivative pDLB101 was transferred to and expressed by *B. azotoformans.* In the companion article to this work, the rate of plasmid transfer was quantified as a function of the limiting nutrient, succinate, and as a function of the mechanism of transfer. A biofilm process simulation program (AQUASIM) was modified to analyze resultant experimental data. Although the AQUASIM package was not designed to simulate or predict genetic events in biofilms, modification of the rate process dynamics allowed successful modeling of plasmid transfer. For the narrow range of substrate concentrations used in these experiments, nutrient level had only a slight effect on the rate and extent of plasmid transfer in biofilms. However, further simulations using AQUASIM revealed that under nutrient poor conditions, the number of transconjugants appearing in the biofilm was limited. © 1998 John Wiley & Sons, Inc. *Biotechnol Bioeng* **57:** 280–286, 1998.
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INTRODUCTION

Biofilms can significantly influence industrial processes, wastewater treatment systems, medical systems, and natural ecosystems. Most bacteria in nature grow as biofilms at interfaces in soil or aquatic systems. These bacteria are responsible for maintaining the many nutrient cycles occurring in soil and water. Although microorganisms are naturally capable of degrading a wide range of organics, there is a great deal of interest in extending the range of their metabolic activity as well as improving their rates of transformation. Genetic engineering provides the tools necessary to create organisms with enhanced abilities, but current restrictions will not allow the release of genetically engineered microorganisms (GEMs) into an open environment because the effects of released recombinant DNA (rDNA) sequences on the indigenous microbial population are not completely understood. Despite the fact that adherent bacteria are responsible for the majority of open system microbial activity, most rDNA gene fate research is carried out in suspended cell cultures. With little information available on the dynamics of genetic exchange among biofilm microorganisms, improved knowledge of these transfer patterns will contribute to the understanding of the effect of GEMs in natural ecosystems.

In the first part of this study (Beaudoin et al., 1997) experiments were carried out to quantify the rate and extent of plasmid transfer between an introduced recombinant bacterial species and an established biofilm of a natural soil isolate. Results of these experiments indicated that plasmid transfer does occur from a released GEM to a recipient species in a biofilm community, even between Gram-negative and Gram-positive microorganisms. Consequently, special attention must be paid to the types of genes placed on plasmids designed for bioremediation. Because the *hok/sok* locus was inserted on the plasmid to ensure complete segregational stability of the plasmid, this study represents an extreme case of plasmid transfer.

The rate of plasmid transfer was measured as a function of the concentration of the primary carbon source fed to the reactor system for both donor and transconjugant initiated transfer (Beaudoin et al., 1997). Even though plasmid transfer between the same species is expected to be more efficient than transfer between two unrelated species, transconjugant mediated transfer was observed to be slower than donor mediated transfer at low nutrient concentrations. It is

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assumed that the slower transfer rates measured for the transconjugants may be related to an energy requirement associated with plasmid transfer and not to a bacterial species phenomenon. The transconjugants develop deeper into the biofilm than the donor species, exposing the transconjugants to a lower nutrient concentration.

In our companion article we showed that an introduced bacterial species will attach to and colonize an established biofilm of another species and will transfer its plasmid DNA to the established population (Beaudoin et al., 1997). Consequently, it is important to develop a spatially dynamic model that will predict the rate and extent of these events as well as determine values for the plasmid transfer kinetic constants. Early attempts to model species dynamics in biofilms were limited to specific microbial communities and reactor configurations (Alleman et al., 1982; Gonenc, 1982; Harremoes, 1982; Tanaka and Dunn, 1982; Watanabe et al., 1982, 1984; Young and McCarty, 1968). To create a more general biofilm model, Wanner and Gujer (1986) developed equations describing multiple species and multiple substrates biofilm formation using mass transport principles and a limited number of assumptions including 1-dimensional growth. The resulting set of partial and ordinary integral and differential equations were incorporated into a simulation package, AQUASIM (Reichert, 1994). Instead of confining the user to a specific set of system conditions, AQUASIM allows the user to define the number of microbial species in the biofilm, the number of substrates required for growth, the values of growth and transport parameters, and the chemical and biological transformation processes occuring in the biofilm. It is an effective tool for modeling bacterial growth and substrate conversion in various system geometries. This investigation uses the AQUASIM program to model both the the development of a mixed species biofilm and the subsequent plasmid transfer between the species.

MODELING

Biofilm Equations

Biofilm formation and persistence are governed by mass transport processes and biological transformations. The extent of biofilm formation is set by bulk fluid dynamics that control not only the rate of nutrient supply to the biofilm but also the amount of biofilm detachment. Adsorption and desorption are the accumulation or depletion of molecules or cells on a solid substratum whereas attachment and detachment are the movement of cells between the bulk fluid phase and the biofilm matrix.

The biofilm itself consists of not only a solid phase of bacteria and their metabolic products, but also an interstitial liquid phase of bulk fluid. Within the biofilm itself, the processes comprising biofilm formation are the rates of mass transport of nutrients from the bulk fluid, the net rate of conversion of these substrates into new biomass, and bulk biofilm accumulation due to growth, replication, and polymer synthesis. Genetic events like plasmid transfer also take place within the biofilm.

The equation used by AQUASIM to describe 1-dimensional biofilm net accumulation for bacterial species i is given by

$$\frac{\partial X_i}{\partial t} = u\frac{\partial X_i}{\partial z} + \frac{1}{A}\frac{\partial}{\partial z}\left(AD_x\frac{\partial X_i}{\partial z}\right) + r_{x_i} - \frac{X_i}{1-\varepsilon}\sum^{x}\frac{r_{x_i}}{\rho_{s_i}}. \quad (1)$$

Equation (1) states that the time of change in a bacterial species at any point z is given by advection of the biofilm species due to cellular growth, diffusion of bacterial species within the biofilm, and the net production of biomass by each species. Net species biomass production includes both biological growth kinetics and the creation or destruction of certain strains due to plasmid transfer.

The mass balance on substrate j is given by

$$\frac{\partial S_j}{\partial t} = \frac{1-\varepsilon}{\varepsilon}u\frac{\partial S_j}{\partial z} + \frac{1}{\varepsilon A}\frac{\partial}{\partial z}\left(A\varepsilon D_s\frac{\partial S_j}{\partial z}\right) + \frac{1}{\varepsilon}\sum^{x}\frac{r_{x_i}}{\rho_{s_i}}S_j + \frac{1}{\varepsilon}\sum\frac{D_x}{\rho_{s_i}}\frac{\partial X_i}{\partial z}\frac{\partial S_j}{\partial z} + \frac{1}{\varepsilon}r_s. \quad (2)$$

The first term on the right of this equation describes the effect on the substrate concentration by convective expansion of the biofilm. The second term describes the diffusion of substrate from the bulk fluid into the biofilm. The third term represents the effect of substrate utilization by each bacterial species. The fourth term models the effect of diffusive transport of biomass within the biofilm. The last term accounts for the net production or consumption of substrate by the local species consumption.

The change in the biofilm liquid volume fraction is given by

$$\frac{\partial \varepsilon}{\partial t} = u\frac{\partial \varepsilon}{\partial z} - \frac{1}{A}\frac{\partial}{\partial z}\left(A\sum_i\frac{D_{x_i}}{\rho_s}\frac{\partial X_i}{\partial z}\right). \quad (3)$$

The first term in this equation takes into account changes in the liquid volume due to advection of the biofilm, and the second term results from transport of biomass from the bulk fluid into the biofilm.

Boundary conditions at $z = 0$ assume that there is no flux of substrate or biomass at the biofilm–substratum interface. At the biofilm–bulk fluid interface, $z = L$, the boundary conditions for the biomass and substrate are, respectively,

$$AuX - AD_x\frac{\partial X}{\partial z}\bigg|_{z=L} = A(k_{\text{det}}X - k_{\text{att}}X_{\text{bulk}}) \quad (4)$$

$$Au(1-\varepsilon)S_j - A\varepsilon D_s\frac{\partial S_j}{\partial z}\bigg|_{z=L} = Ak_{\text{mt}}(S_j - S_{j,\text{bulk}}). \quad (5)$$

Plasmid Transfer

AQUASIM has been used previously to predict the concentrations of substrates and bacterial species within a biofilm.

However, it has never been used nor was it intended to predict the concentrations of various bacterial strains or species resulting from genetic events like plasmid transfer or even segregational plasmid instability. To account for the conversion of recipient species to transconjugants, Equation (1) was modified as follows to include the kinetics of plasmid transfer.

For the case of conjugational transfer, there are three biomass species: donors, recipients, and transconjugants. Substituting into Equation (1), the respective microbial mass balances in the biofilm are

$$\frac{\partial D}{\partial t} = u\frac{\partial D}{\partial z} + \frac{1}{A}\frac{\partial}{\partial z}\left(AD_x\frac{\partial D}{\partial z}\right) + r_D - \frac{D}{1-\varepsilon}\sum^{x}\frac{r_x}{\rho_s}, \quad (6)$$

$$\frac{\partial R}{\partial t} = u\frac{\partial R}{\partial z} + \frac{1}{A}\frac{\partial}{\partial z}\left(AD_x\frac{\partial R}{\partial z}\right) + r_R - \frac{R}{1-\varepsilon}\sum^{x}\frac{r_x}{\rho_s}, \quad (7)$$

$$\frac{\partial T}{\partial t} = u\frac{\partial T}{\partial z} + \frac{1}{A}\frac{\partial}{\partial z}\left(AD_x\frac{\partial T}{\partial z}\right) + r_T - \frac{T}{1-\varepsilon}\sum^{x}\frac{r_x}{\rho_s}, \quad (8)$$

The rates of formation of these species are

$$r_D = \frac{\mu_m SD}{K+S}, \quad (9)$$

$$r_R = \frac{\mu_m SR}{K+S} - k_1 DR - k_2 TR, \quad (10)$$

$$r_T = \frac{\mu_m ST}{K+S} + k_1 DR + k_2 TR, \quad (11)$$

Because there are three distinct bacterial strains, the values of μ_m, K, and Y are different for each cell type. To simplify the boundary conditions describing bacterial exchange at the biofilm–bulk fluid interface, only the donor species attaches to the biofilm from the bulk fluid and detachment is treated as a global process, not an individual strain process. During these experiments, all three strains compete for the same limiting nutrient, succinate, leaving only one substrate mass balance to be solved. The resulting set of three bacterial species mass balances, the substrate balance, and liquid volume fraction equation can be solved simultaneously using AQUASIM.

SIMULATION RESULTS

The kinetic expressions listed above were entered into the AQUASIM program along with initial conditions, influent concentrations, and model parameter values. AQUASIM then automatically solved the mass balance equations, Equations (1)–(8), as a function of time and biofilm depth using the specified inputs for the donor, recipient, transconjugant, and substrate concentrations.

Model Parameters

The model of plasmid transfer in biofilms includes the 20 parameters listed in Table I. Several parameters are based on the experimental conditions and are known. Monod growth parameters are set at values measured in independent experiments conducted over the range of substrate concentrations used in these experiments. Succinate diffusivity was set at 80% of that measured in water. The initial biofilm thickness came from images taken of the cryoembedded and cryosectioned samples taken before donor addition. The thicknesses used were mean values of several measurements in order to take into account variations due to biofilm heterogeneities. Initial concentration and distribution of the recipients were determined from cell

Table I. Parameter values for AQUASIM simulation of plasmid transfer in biofilms.

Symbol	Description	Value	Units	Source
A	Biofilm surface area	1345.5	cm^2	Set
D_s	Substrate diffusivity	0.03	cm^2/h	Bennett and Myers (1982)
D_x	Bacterial diffusivity	0.0079	cm^2/h	Estimate
r_{det}	Rate of bacterial detachment	$0.99 * u$	cm/h	Parameter fit
D_{feed}	Concentration of donors in the bulk fluid	3×10^{-3} or 2×10^{-5}	g/cm^3	Set
k_{att}	Attachment rate constant for donors	1×10^{-6}	cm/h	Parameter fit
k_1	Donor transfer coefficient	20	$cm^3/g\ h$	Parameter fit
k_2	Transconjugant transfer coefficient	15	$cm^3/g\ h$	Parameter fit
K_D	Monod half-saturation constant for donors	1.2×10^{-5}	g/cm^3	Measured
K_R	Monod half-saturation constant for recipients and transconjugants	1.2×10^{-5}	g/cm^3	Measured
L_o	Initial biofilm thickness	0.001–0.002	cm	Measured
$\mu_{max,don}$	Max. specific growth rate for donors	0.36	1/h	Measured
$\mu_{max,rec}$	Max. specific growth rate for recip. and trans.	0.365	1/h	Measured
Q	Volumetric flow rate of feed	500	cm^3/h	Set
ρ_s	Bacterial density in biofilm	0.05	g/cm^3	Estimate
R_o	Initial recipient concentration	$0.9*z + 1.9 \times 10^{-6}$	g/cm^3	Measured
S_{feed}	Concentration of limiting substrate in feed	1.25×10^{-5} to 7.5×10^{-5}	g/cm^3	Set
V	Reactor volume	500	cm^3	Set
Y_D	Yield coefficient for donors	0.25		Estimate
Y_R	Yield coefficient for recip. and trans.	0.2		Estimate

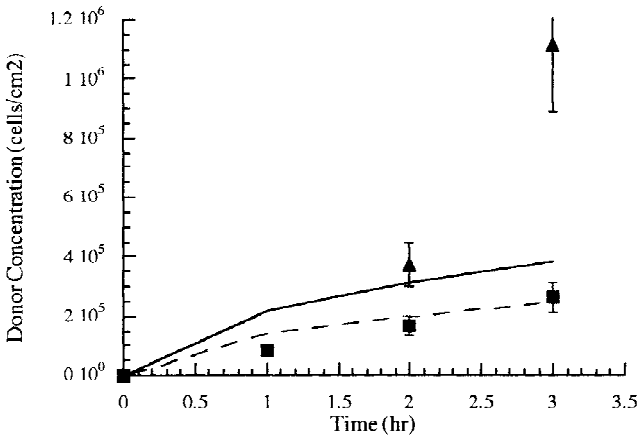

Figure 1. Comparison of experimental and predicted number of donor cells, *P. putida* PB2440 (RK2, pDLB101), in the biofilm during the first 3 h of contact: (▲) 12.5 μg/mL succinate experiment and (■) 25 μg/mL succinate. (—) AQUASIM predicted values for 12.5 μg/mL succinate and (- - -) AQUASIM predicted values for 25 μg/mL succinate.

counts and from the images taken of the biofilm before donor addition. Bacterial diffusivity and density are estimates based on other AQUASIM simulations (Reichert, 1994). Values used for the yield coefficients are assumed to be similar to literature values for succinate consumption by other bacteria (Bailey and Ollis, 1986). Consequently, only four parameters, k_{att} det, k_1, and k_2, remained, which must be fit to the data.

Donor Attachment Velocity (k_{att})

The rate of bacterial attachment, r_{att}, is given by

$$r_{att} = k_{att} * D_b \tag{12}$$

The attachment velocity is a function of particle size, fluid velocity, motility, mixing, and diffusion. For a given bacterial species under the same reactor operating conditions, the rate of attachment will vary according to the concentration of cells in the bulk, but the attachment velocity should be a constant.

The specific rate for donor attachment was fit to the number of donors measured in the biofilm during the 3-h contact between the recipient biofilm and the donors in the bulk fluid. Using the known growth parameters for the donor species, k_{att} was adjusted and AQUASIM simulations of the first 3 h were carried out until the measured and predicted number of donors in the biofilm were in agreement.

Figure 1 illustrates the number of donors in the biofilm resulting from two different experimental conditions. One case is for a succinate concentration of 12.5 μg/mL while the other case represents the base substrate concentration (25 μg/mL succinate) employed in experiments but at a smaller concentration of donor cells in the bulk fluid (10^7 cells/mL instead of 10^9 cells/mL). In both cases the attachment velocity that best fit both sets of data was a value of 1×10^{-6} cm/h. As the plot indicates, this value gave donor concentrations close to the measured values except for the 3-h sample taken in the first case. This point is so far off from the rest of the data that it probably can be neglected because its exponential increase is indicative of the onset of cell replication. As expected, having fewer donors in the bulk fluid results in fewer donors attaching and colonizing the biofilm. Substrate concentration, however, does not appear to affect the rate of attachment.

Rate of Bacterial Detachment (r_{det})

The detachment of bacteria from the biofilm was fit to the biofilm thickness measured over the course of the experiment and the results were compared to the number of donors, recipients, and transconjugants measured in the effluent over the course of the same experiment. Following other researchers who used AQUASIM to simulate biofilm processes (Wanner and Reichert, 1996), we assumed that the overall rate of detachment, detach, was a fraction of biofilm advection, *u:*

$$r_{det} = a * u. \tag{13}$$

Using the known growth parameters for each species, the fraction *a* was adjusted until the measured and simulated biofilm thicknesses were in agreement. Figure 2 shows the resulting biofilm thicknesses from three different succinate concentrations: 25, 12.5, and 75 μg/mL. The detachment rate that best fits the data was $0.99 * u$, indicating that the biofilm reached a steady-state thickness.

Plasmid Transfer Rate Constant for Donors (k_1)

Initially, the majority of transfer is from the donors to the recipients. Therefore, the value of k_1 was fit to the measured numbers of recipients and transconjugants in the biofilms

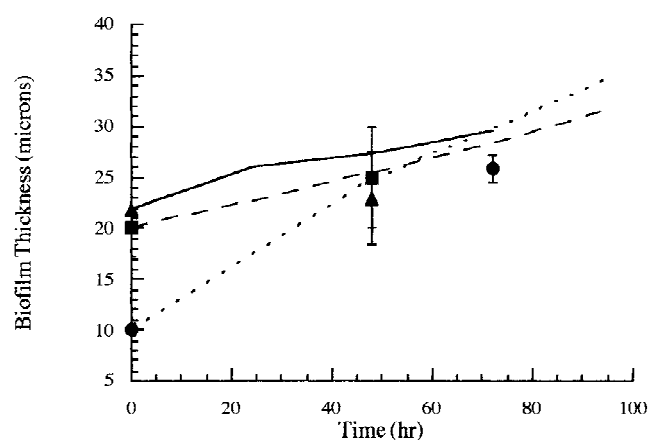

Figure 2. Comparison of experimental and predicted biofilm thicknesses resulting from experiments of plasmid transfer between *P. putida* PB2440 and *B. azotoformans:* (●) 75 μg/mL succinate, (▲) 12.5 μg/mL succinate, and (■) 25 μg/mL succinate. (—) AQUASIM predicted values for 12.5 μg/mL succinate, (- - -) AQUASIM predicted values for 25 μg/mL succinate, and (· · ·) AQUASIM predicted values for 75 μg/mL succinate.

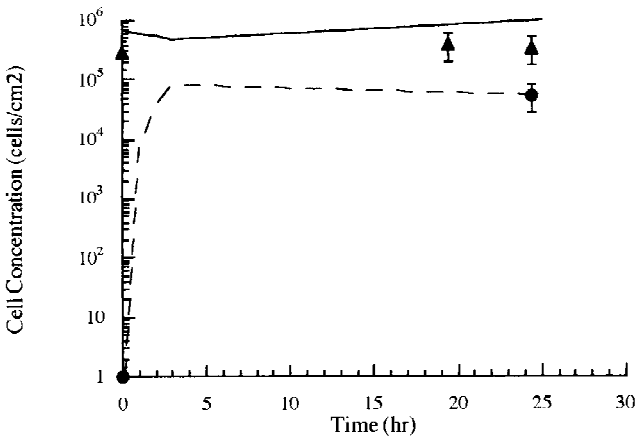

Figure 3. Experimental and simulated concentration profiles for plasmid transfer from *P. putida* PB2440 (RK2, pDLB101) to *B. azotoformans* using 25 μg/mL succinate: (●) transconjugants and (▲) recipients. (- - -) AQUASIM transconjugants and (—) AQUASIM recipients.

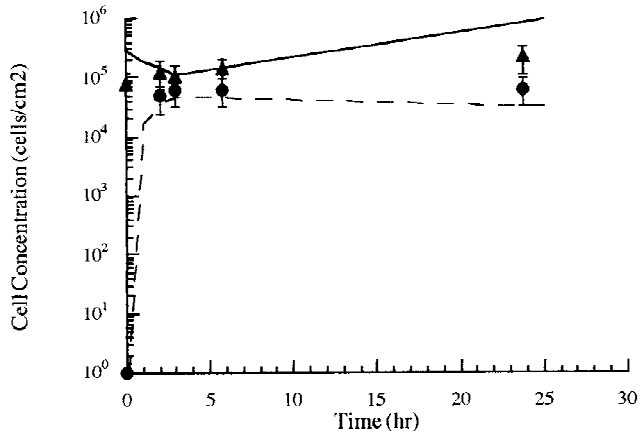

Figure 4. Comparison of measured and predicted concentration profiles for plasmid transfer from *P. putida* PB2440 (RK2, pDLB101) to *B. azotoformans* using 12.5 μg/mL succinate: (●) transconjugants and (▲) recipients. (- - -) AQUASIM transconjugants and (—) AQUASIM recipients.

for the three different substrate concentrations over the first 24 h of each experiment.

Figure 3 shows the initial numbers of recipients and transconjugants resulting from plasmid transfer using 25 μg/mL succinate. For this set of conditions, the best plasmid transfer rate constant for the donors, k_1, was 20 cm^3/g h. The numbers of recipients and transconjugants resulting from plasmid transfer using 12.5 μg/mL succinate is shown in Figure 4. Once again, the plasmid transfer constant that best fits this case was a value of k_1 equal to 20 cm^3/g h. The overprediction of the recipient concentration at 24 h was due to the initiation of transconjugant mediated plasmid transfer.

Plasmid Transfer Rate Constant for Transconjugants (k_2)

With all other parameters now known or fit, the transconjugant's plasmid transfer rate constant, k_2, was fit to the measured numbers of recipients and transconjugants in the biofilms using data for the three different substrate concentrations from 24 h until the end of the experiment.

Figures 5 and 6 illustrate the numbers of recipients and transconjugants resulting from plasmid transfer using 75 and 12.5 μg/mL succinate, respectively, after 24 h of contact. For both cases, the value of k_2 that predicted both the recipient and transconjugant populations well was 15 cm^3/g h. The transconjugant concentration was overpredicted at the 24- and 48-h sample points for the 75 μg/mL succinate case, but predicts the final 72-h sample point well.

Effect of Substrate Concentration on Plasmid Transfer

It has been shown experimentally that the rates of plasmid transfer in biofilms (Beaudoin et al., 1996) and in suspension (MacDonald et al., 1992) are a function of the limiting substrate concentration. However, in the simulations presented above, the plasmid transfer rate parameter for a given bacterial and plasmid system was found to be independent of substrate concentration. Even with a constant value for this parameter, the rate and extent of transconjugant production differed for each concentration of succinate because the rate of plasmid transfer was also a function of the concentration of each species. The highest concentration of transconjugants was reached using the lowest nutrient concentration. The lower succinate concentrations also achieved a steady-state concentration of transconjugants whereas with 3× succinate the transconjugant population was still increasing after 100 h of contact.

With all parameters known or fit, AQUASIM is a convenient tool for correlating the effects of various process conditions on dependent variables. To see the extent that nutrient concentration affects plasmid transfer, simulations were run in which the amount of substrate in the feed was

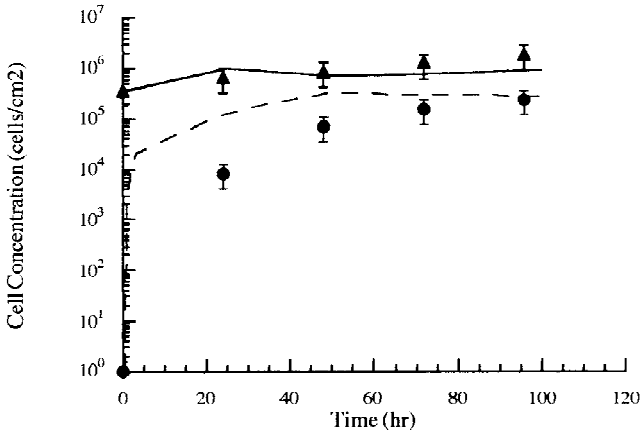

Figure 5. Experimental and simulated concentration profiles for plasmid transfer from *P. putida* PB2440 (RK2, pDLB101) to *B. azotoformans* using 75 μg/mL succinate: (●) transconjugants and (▲) recipients. (- - -) AQUASIM transconjugants and (—) AQUASIM recipients.

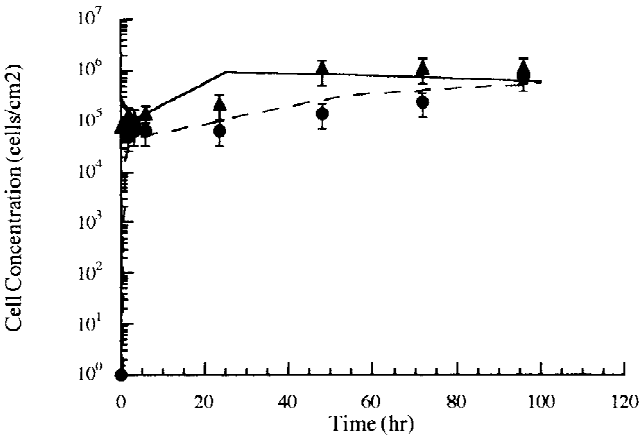

Figure 6. Experimental and simulated concentration profiles for plasmid transfer from *P. putida* PB2440 (RK2, pDLB101) to *B. azotoformans* using 12.5 μg/mL succinate: (●) transconjugants and (▲) recipients. (- - -) AQUASIM transconjugants and (—) AQUASIM recipients.

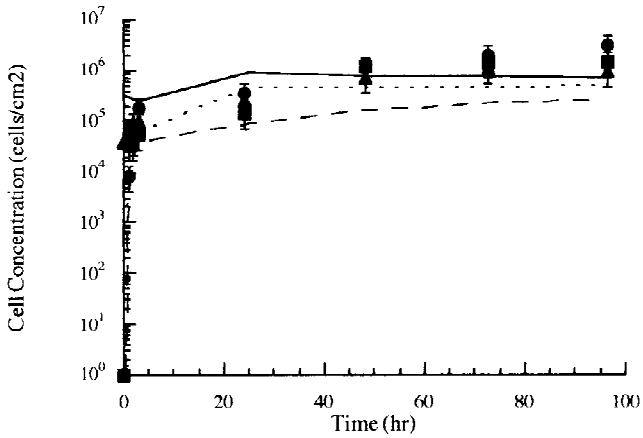

Figure 8. Comparison of experimental and predicted concentration profiles for plasmid transfer from *P. putida* PB2440 (RK2, pDLB101) to *B. azotoformans* using 25 μg/mL succinate and a small donor inoculum: (●) measured transconjugants, (▲) measured recipients, and (■) measured donors. (- - -) predicted transconjugants, (—) predicted recipients, and (· · ·) predicted donors.

varied over a 6 order of magnitude range. Figure 7 shows the number of transconjugants resulting from these simulations as a function of time as well as biofilm depth. Very little plasmid transfer occurred at a substrate concentration of 2.5×10^{-6} mg/mL succinate. There was a dramatic increase in the number of transconjugants when 2.5×10^{-4} mg/mL succinate was fed to the system, and the number of transconjugants was still increasing after 200 h of contact. There appeared to be no difference in the number of transconjugants formed when either 0.025 or 2.5 mg/mL succinate was applied to the biofilm.

Validity and Accuracy of Model

With all model parameters now known or fit and an expression for the kinetics of plasmid transfer developed, one more simulation was performed and compared with the experimental results to test the accuracy of the model. In this case, the normal level of succinate (25 μg/mL) was used, but a donor inoculum of only 10^7 cells/mL was exposed to the recipient biofilm. Figure 8 shows the predicted and measured concentration profiles for the case of a small donor inoculum. All three populations were predicted relatively well by AQUASIM. The transconjugant population was underpredicted everywhere, but the right trends were seen.

As another test of the accuracy of the model, the spatial profile resulting from the above simulation can be compared to a cryosectioned and stained biofilm sample run under the same conditions. Figure 9 shows the concentration profile of each species as a function of biofilm depth after 70 h of contact. As indicated by the graph, the recipient concentration is highest near the substratum and decreases to zero at

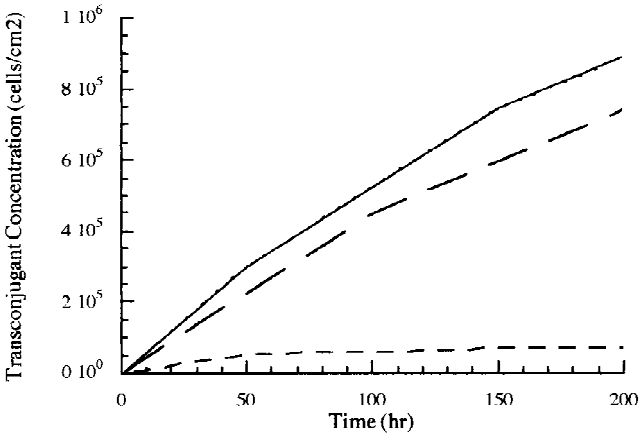

Figure 7. Predicted transconjugant profiles from simulations of plasmid transfer from *P. putida* PB2440 (RK2, pDLB101) to *B. azotoformans* using various feed concentrations of succinate: (- - -) 2.5×10^{-6} mg/mL, (– – –) 2.5×10^{-4} mg/mL, (—) 0.025 mg/mL, and (· · ·) (overlapped by solid line) 2.5 mg/mL.

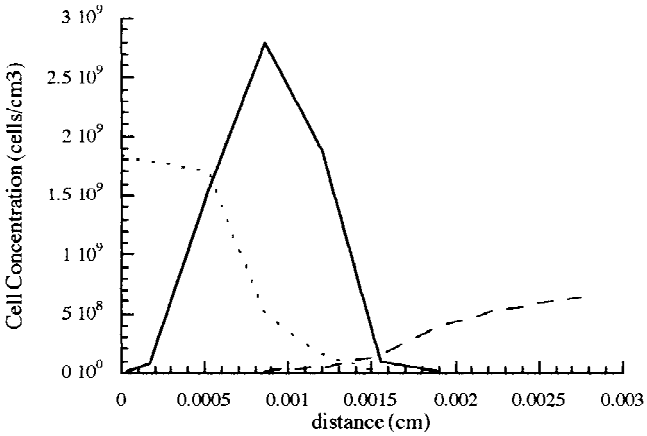

Figure 9. AQUASIM's predicted spatial concentration profile for each species in the biofilm resulting from plasmid transfer between *P. putida* PB2440 (RK2, pDLB101) and *B. azotoformans* using 25 μg/mL succinate and a small inoculum of donor cells after 70 h of contact: (—) transconjugants, (· · ·) recipients, and (- - -) donors.

the center of the biofilm (15 μm). A few transconjugants have formed near the substratum with a maximum concentration appearing at 10 μm above the substratum. The donor cells do not reach as high a concentration as the recipients or the transconjugants, but comprise more than half of the 28-μm biofilm thickness. 4,6-Diamidino-2-phenylindole (DAPI) and di-β-galactopyranoside (FDG) stained biofilm samples taken 72 h after the first contact between the donors and recipients (photos not shown) indicate that the biofilm grown in this case reaches an average thickness of 22 μm, which is close to what AQUASIM predicts. The images also show that almost all of the biofilm has *lac*Z activity. Between expression by the donors as well as the transconjugants, the simulation also indicates activity in all of the biofilm. The results of this simulation indicate that this model provides an accurate description of plasmid transfer in biofilms.

CONCLUSIONS

Simple mass action models have been developed to describe plasmid transfer in suspension. These models have been used to measure rate constants for transfer using various bacterial species and plasmids. The dynamics of biofilm accumulation do not allow these constants to be determined easily from experimental data.

AQUASIM is a simulation tool that has been widely used to model bacterial growth and substrate conversion in various system geometries, but it has never been used to model genetic events like plasmid transfer. Comparison of simulations to experimental data indicate that AQUASIM can be a useful tool to simulate and predict genetic events such as plasmid transfer in a biofilm. This study also shows that AQUASIM may be used to evaluate attachment of a new species to an established biofilm and the resulting growth of the different species. AQUASIM not only predicts the relatively correct concentrations of each species over time, but also predicts the correct spatial distribution of each species.

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NOMENCLATURE

A	biofilm surface area (cm^2)
D_s	substrate diffusivity (cm^2/h)
D_x	bacterial diffusivity (cm^2/h)
ϵ	liquid volume fraction
K_i	Monod half saturation constant for strain i (g substrate/cm^3)
k_1	donor transfer coefficient (cm^3/g biomass/h)
k_2	transconjugant transfer coefficient (cm^3/g biomass/h)
k_{att}	cellular attachment coefficient (cm/h)
k_{det}	biofilm detachment coefficient (cm/h)
k_{mt}	substrate mass transfer coefficient (cm/h)
μ_m	maximum specific growth rate (L/h)
ρ_s	biofilm bacterial density (g/cm^3)
r_s	consumption of substrate (g/cm^3/h)
r_x	bacterial growth or transformation (g/cm^3/h)
S	substrate concentration (g substrate/cm^3)
S_j	substrate j concentration in the biofilm (g/cm^3)
Sj,b	substrate j concentration in the bulk fluid (g/cm^3)
u	advective velocity due to biofilm growth (cm/h)
X	biomass concentration (g/cm^3)
X_b	biomass concentration in the bulk fluid (g/cm^3)
X_i	biomass i concentration (g/cm^3)
Y	yield coefficient
z	distance from biofilm substratum (cm)

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