Theoretical Aspects of Antibiotic Diffusion into Microbial Biofilms

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Antibiotic penetration into microbial biofilm was investigated theoretically by the solution of mathematical equations describing various combinations of the processes of diffusion, sorption, and reaction. Unsteady material balances on the antibiotic and on a reactive or sorptive biomass constituent, along with associated boundary and initial conditions, constitute the mathematical formulations. Five cases were examined: diffusion of a noninteracting solute; diffusion of a reversibly sorbing, nonreacting solute; diffusion of an irreversibly sorbing, nonreacting solute; diffusion of a stoichiometrically reacting solute; and diffusion of a catalytically reacting solute. A noninteracting solute was predicted to penetrate biofilms of up to 1 mm in thickness relatively quickly, within a matter of seconds or minutes. In the case of a solute that does not sorb or react in the biofilm, therefore, the diffusion barrier is not nearly large enough to account for the reduced susceptibility of biofilms to antibiotics. Reversible and irreversible sorption retards antibiotic penetration. On the basis of data available in the literature at this point, the extent of retardation of antibiotic diffusion due to sorption does not appear to be sufficient to account for reduced biofilm susceptibility. A catalytic (e.g., enzymatic) reaction, provided it is sufficiently rapid, can lead to severe antibiotic penetration failure. For example, calculation of β-lactam penetration indicated that the reaction-diffusion mechanism may be a viable explanation for failure of certain of these agents to control biofilm infections. The theory presented in this study provides a framework for the design and analysis of experiments to test these mechanisms of reduced biofilm susceptibility to antibiotics.

Persistent microbial infection is one of the leading causes of failure of indwelling medical devices. At the heart of these infections is a biofilm—microorganisms growing in dense aggregates on the surface of the biomaterial. The significance of the biofilm is that microorganisms in this mode of growth escape the host immune response and are much less susceptible to antibiotics than are their planktonically grown counterparts (7, 16). Two principal categories of explanation have been advanced to account for the relative resistance of microbial biofilms to antimicrobial chemotherapy (4, 7, 16). The first is a transport-based explanation positing that the biofilm acts as a barrier to antibiotic diffusion. The second is a physiology-based explanation attributing the reduced susceptibility of biofilm microorganisms compared to their freely suspended counterparts to physiological differences between the microorganisms in these two modes of growth. This article focuses on the question of antibiotic transport in biofilms.

The literature contains reports that argue both for (1, 3, 18–20, 31) and against (9, 12, 22–26) the presence of an antibiotic diffusion barrier in biofilms. It may be that both camps are correct, since the research has been conducted in diverse experimental systems. Indeed, one of the difficulties in interpreting the literature in this area is due to the common failure to report measurements in terms of fundamental parameters that can be extrapolated beyond a specific system. The purpose of this article is to provide a consistent, quantitative framework for the discussion and analysis of processes influencing antibiotic penetration into microbial biofilms. The value of such a theoretical base is that it identifies key parameters and explic-

itly states assumptions, both of which can guide experimental design. I follow in this effort the fine work of Nichols and coworkers (22–26) and a previous publication of mine (29).

THEORY

This section presents the assumptions and mathematical equations used to model diffusive transport in a biofilm. In addition to consideration of the simplest case of diffusion of a noninteracting solute, formulations that account for the modifying processes of sorption and reaction are described. All cases share certain assumptions. The biofilm is assumed to be a uniformly thick planar slab of thickness L_f with one side adjoining an impermeable boundary (i.e., the substratum) and the other side exposed to an aqueous medium. Fickian diffusion is assumed. Mass transfer resistance between the biofilm-fluid interface and the bulk fluid is neglected. The particulate constituents of the biofilm (e.g., cells or extracellular polysaccharide) are assumed to be homogeneously distributed. The biofilm contains cells (volume fraction ε_c), extracellular polysaccharide (volume fraction ε_p), and water (volume fraction ε_w, $\varepsilon_w = 1 - \varepsilon_p - \varepsilon_c$). Mathematical models with some of the same features as those described below have been derived to analyze solute diffusion in dental plaque (11), in biofilms occurring in wastewater treatment processes (13), and in biofilms implicated in microbially induced corrosion (27).

Diffusion of a noninteracting solute. The transient diffusion of a solute into a slab following a step change in the bulk fluid concentration of that solute from zero to a finite level, C_o, is given by

$$\varepsilon_w \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} \tag{1}$$

$$at\ z = L_f, C = C_o \text{ for all } t > 0 \tag{2}$$

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$$\text{at } z = 0, \frac{\partial C}{\partial z} = 0 \text{ for all } t > 0 \tag{3}$$

$$\text{at } t \leq 0, C = 0 \text{ for all } 0 \leq z \leq L_f \tag{4}$$

Equation 1 is the diffusion equation, which constitutes an unsteady mass balance on the solute. The term on the lefthand side represents the local accumulation (or, if negative in sign, disappearance) of the solute, and the term on the righthand side represents the net change in concentration due to diffusion. Equations 2 and 3 are boundary conditions that impose constant concentration at the biofilm-bulk fluid interface and a no-flux condition at the substratum, respectively. Equation 4 is an initial condition that specifies zero concentration throughout the biofilm at time zero. This formulation assumes that there is no interaction (e.g., sorption or reaction) between the solute and any constituent of the biofilm.

The parameter D_e is the effective diffusion coefficient in the biofilm. The numerical value of D_e is expected to be somewhat smaller than the value of the diffusion coefficient in water, D_{aq}, because of the physical obstruction posed by polymer and cells. Diffusion in porous media and polymer gels has been extensively studied, so there exist theories and correlations that can be used to estimate the reduction of diffusion coefficient that occurs in a biofilm. Westrin and Axelsson (32) suggest that the effect on the diffusion coefficient of the physical presence of microbial cells can be accounted for adequately by the relation

$$\frac{D_e}{D_{aq}} = \left(\frac{1 - \varepsilon_c}{1 + \varepsilon_c/2}\right)\left(\frac{(1 - \varepsilon_p{}^*)^3}{(1 + \varepsilon_p{}^*)^2}\right) \tag{5}$$

where $\varepsilon_p{}^*$ is the polymer volume fraction in the intercellular space, which is just $\varepsilon_p/(1 - \varepsilon_c)$. In equation 5 the first term on the righthand side, which is attributed to James Maxwell, of electricity and magnetism fame, accounts for the reduction in diffusion coefficient due to the cells. It assumes that the solute is completely excluded from the cell. The second term in equation 5 is a semiempirical relationship that accounts for the reduction in diffusion coefficient due to the extracellular polysaccharide. This estimate of the effective diffusion coefficient accounts for the effects of having the medium partially occupied by cells and polymer. It does not account for any reduction in the apparent diffusion coefficient due to sorption, uptake, or reaction of the solute.

Diffusion of a reversibly sorbing, nonreacting solute. Consider a solute that sorbs reversibly to some fixed constituent of the biofilm according to a linear isotherm, that is,

$$S = \kappa C \tag{6}$$

where S is the sorbed concentration (mass per nonwater volume of the biofilm) and κ is a partition coefficient quantifying the equilibrium distribution of solute between the aqueous and biofilm phases. The partition coefficient is dimensionless. Possible examples of reversible sorption could be sorption to extracellular polysaccharides or cell surfaces. Any real sorption process is likely to be saturable, which would lead to a nonlinear isotherm. The linear approximation is valid for low concentrations, and the impact of sorption at higher concentrations will likely be overestimated.

The mass balance on the solute, considering both dissolved and sorbed components, is

$$\varepsilon_w \frac{\partial C}{\partial t} + (1 - \varepsilon_w)\frac{\partial S}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} \tag{7}$$

The terms, from left to right, represent accumulation of dissolved solute, accumulation of sorbed solute, and the effect of diffusion. Here it is assumed that only the dissolved solute is transported. By using the isotherm, equation 7 can be written

$$[\varepsilon_w + (1 - \varepsilon_w)\kappa]\frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} \tag{8}$$

Boundary and initial conditions for this problem are identical to those for the preceding case; that is, equations 2 through 4 apply. The assumption of reversibility requires that the time scale for sorption and desorption be small compared to the time scale for diffusion ($L_f{}^2/D_e$).

Diffusion of an irreversibly sorbing, nonreacting solute. When sorption is fast but desorption is negligibly slow, the mathematical statement of the transport problem is

$$\varepsilon_w \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} - k_s C X_s \tag{9}$$

$$\frac{\partial X_s}{\partial t} = -\frac{k_s C X_s}{Y_{cx}} \tag{10}$$

$$\text{at } z = L_f, C = C_o \text{ for all } t > 0 \tag{11}$$

$$\text{at } z = 0, \frac{\partial C}{\partial z} = 0 \text{ for all } t > 0 \tag{12}$$

$$\text{at } t \leq 0, C = 0 \text{ and } X_s = X_s{}^o \text{ for all } 0 \leq z \leq L_f \tag{13}$$

These equations describe material balances on the solute (equation 9) and its biofilm binding site (equation 10) along with boundary conditions (equations 11 and 12) and initial conditions (equation 13) analogous to those formulated in the preceding cases. The terms in equation 9, from left to right, represent solute accumulation, diffusion, and sorption. The rate of sorption is assumed to be first order in both the dissolved solute concentration and the concentration of free binding sites (X_s); k_s is the rate coefficient for this process. The parameter Y_{cx} is a yield coefficient describing the mass of solute sorbed per mass of biofilm in which binding sites have been saturated. Irreversible sorption could be the case if the solute is taken up into the bacterial cytoplasm.

Diffusion of stoichiometrically reacting solute. This case considers the fate of solute that reacts with some constituent of the biofilm. The solute is irreversibly destroyed in the reaction, as is the reactive constituent of the biofilm. Solute and reactive biomass are depleted in the stoichiometric proportion given by Y_{cx}, which in this case is interpreted as the mass of solute neutralized per mass of biofilm reacted. Material balance equations and boundary and initial conditions for this problem are mathematically identical to those in the previous case of irreversible sorption. For clarity, the sorption rate coefficient, k_s, is replaced with a reaction rate coefficient, k_r.

Diffusion of a catalytically reacting solute. Suppose alternatively that a reaction occurs in which the solute is irreversibly destroyed but the reactive biofilm constituent is not. This would be the case, for example, with an enzymatically catalyzed reaction. For a first-order reaction, the material balance on the solute is given by equation 14, with boundary and initial conditions given by equations 2 through 4.

$$\varepsilon_w \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial z^2} - k_r C \tag{14}$$

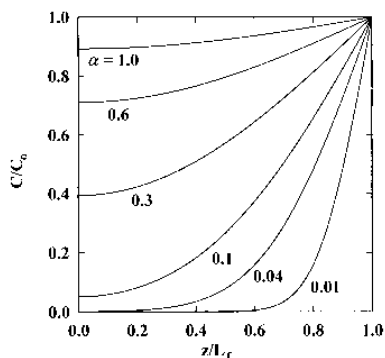The terms in equation 14 represent, reading from left to right, accumulation, diffusion, and reaction.

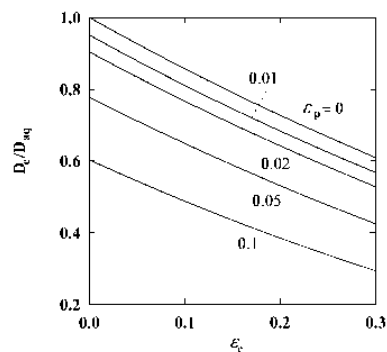FIG. 1. Concentration profiles for unsteady-state diffusion in a slab of uniform thickness L_f. The parameter α is dimensionless time.

FIG. 2. Dependence of the ratio of the effective diffusion coefficient within biofilm to the diffusion coefficient in water on cell (ε_c) and polymer (ε_p) volume fraction.

RESULTS AND DISCUSSION

Noninteracting solute. An analytical series solution is available for equations 1 through 4 (2):

$$\frac{C}{C_o} = 1 - 2 \sum_{n=0}^{\infty} \frac{(-1)^n}{(n + 1/2)\pi} \exp[-(n + 1/2)^2\pi^2\alpha] \qquad (15)$$

$$\cos\left((n + 1/2)\frac{\pi z}{L_f}\right)$$

This solution is reproduced graphically in Fig. 1. In this case, the penetration attained depends on a single parameter, α, given by

$$\alpha = \frac{tD_e}{\varepsilon_w L_f^2} \qquad (16)$$

The penetration of a noninteracting solute as a function of time can thus be readily calculated and depends only on the biofilm thickness, the effective diffusion coefficient, and the volume fraction of water in the biofilm.

The effective diffusion coefficient in the biofilm can be measured experimentally, though doing this measurement well is not simple. D_e can also be estimated from correlations. The starting point for calculation of D_e is an estimate of the diffusion coefficient of the solute in water. Values of aqueous diffusion coefficients for selected antibiotics at 37°C (Table 1) vary by perhaps a factor of 2, with a typical value of approximately 6×10^{-6} cm^2/s. The effective diffusion coefficient in biofilm is reduced from the aqueous value because of the presence of cells and polymer. Using the formulation given in equation 5, for a range of cell and polymer volume fractions

TABLE 1. Diffusion coefficients of selected antibiotics in water at 37°C calculated by the Wilke-Chang correlation[a]

Agent	MW (g/mol)[b]	D_{aq} (10^{-6}) (cm^2/s)
Ciprofloxacin	330.4	6.87
Benzylpenicillin	376.4	6.69
Tobramycin	465.5	5.56
Gentamicin	477.6	6.20
Piperacillin	517.6	5.25
Ceftazidime	548.6	5.12
Vancomycin	1,468.3	2.83

[a] Reference 28.
[b] MW, molecular weight.

thought to be characteristic of biofilms, one finds D_e to lie in the range of 30 to 90% of the value in pure water (Fig. 2).

D_e values predicted by the above procedure are compared with experimental determinations of this parameter in Table 2. The average absolute error between the predicted and measured values is 46%. The predicted value is higher in eight instances and lower in two, indicating that the theory may tend to overestimate the actual effective diffusion coefficient or that experimental methods tend to underestimate the actual value.

One can now readily estimate the order of magnitude of the penetration time for solute diffusion into a biofilm. Consider, for example, the time needed to attain, at the substratum, 50% of the bulk fluid concentration (which corresponds to α of 0.379). For typical values of D_{aq} equalling 6×10^{-6} cm^2/s, D_e/D_{aq} equalling 0.8, and ε_w equalling 0.85, 50% penetration times of 5.4 and 537 s are obtained for biofilms of 100 and 1,000 µm thicknesses, respectively. It is clear from this calculation that a noninteracting solute moves into the biofilm quickly, in a matter of seconds or minutes. This duration is much shorter than the duration of antimicrobial chemotherapy. In the case of a solute that does not sorb or react in the biofilm, therefore, the diffusion barrier is not nearly large enough to account for the reduced susceptibility of biofilms to antibiotics. This is the same conclusion reached by Nichols (23, 24).

Reversibly sorbing, nonreacting solute. The mathematics of this case are identical to those of the previous problem, except that the definition of the parameter α must be modified (modified version indicated by α_s) to incorporate the partitioning of antibiotic between the aqueous and solid phases of the biofilm:

$$\alpha_s = \frac{tD_e}{[\varepsilon_w + (1 - \varepsilon_w)\kappa]L_f^2} \qquad (17)$$

The solution to the transport problem can be read, as before, from Fig. 1 by merely substituting α_s for α. The effect of partitioning is to retard penetration. As the partition coefficient, κ, increases, α_s is reduced, and the extent of penetration is likewise reduced.

Experimental measurements from which κ can be calculated are few. Data reported by Darouiche et al. for vancomycin suggest a value for κ of approximately 32, which, assuming a biofilm water fraction of 80% ($\varepsilon_w = 0.8$), translates to retardation by about a factor of 7 (9). Investigating tobramycin sorption to alginate, Nichols et al. calculated retardation by about a factor of 3 (26). If these two cases are representative, then reversible sorption could perhaps increase the penetra-

TABLE 2. Comparison of experimentally measured and predicted D_e of selected antibiotics in polymer gels[a]

Agent	T (°C)	Expt D_e (10^{-6}) (cm^2/s)	Predicted D_e (10^{-6}) (cm^2/s)	Conditions	Source
Benzylpenicillin	25	3.38	3.01	10% porcine gastric mucus	5
Tobramycin	37	3.00	5.03	2% agar	15
Gentamicin	25	2.52	2.78	10% porcine gastric mucus	5
	37	2.20	5.61	2% agar	15
Piperacillin	37	3.70	4.75	2% alginate	15
	37	4.00	4.75	2% agar	15
Ceftazidime	25	3.42	2.30	10% porcine gastric mucus	5
	37	2.90	4.63	2% alginate	15

[a] The predicted value was determined from equation 5 by using the values of D_{aq} given in Table 1 and was corrected for temperature as appropriate. D_e, effective diffusion coefficients.

tion time by an order of magnitude. Considering once again biofilms of 100- and 1,000-μm thicknesses, the 50% penetration time would be extended to approximately 1 and 90 min, respectively. By itself, this degree of retardation is still not sufficient to explain biofilm recalcitrance.

Irreversibly sorbing, nonreacting solute. Irreversible sorption can profoundly retard the penetration of a solute into a biofilm. The time required to penetrate in this case is dependent on three parameters: α, defined previously; ρ, which can be viewed as a measure of the capacity of the biofilm to bind the antimicrobial agent relative to the bulk concentration of the agent, where

$$\rho = \frac{X_s^o \, Y_{cx}}{\varepsilon_w C_o} \quad (18)$$

and ϕ, where

$$\phi = \left(\frac{k_s X_s^o L_f^2}{D_e}\right)^{1/2} \quad (19)$$

The parameter ϕ is known as a Thiele modulus and in this case allows comparison of the relative rates of sorption and diffusion. Concentration profiles for an illustrative case are shown in Fig. 3; parameter values for this case are given in Table 3. In this case, the time to attain 50% of the bulk fluid concentration at the substratum occurs at an α of 57, which is more than 100 times longer than in the case of a noninteracting solute ($\alpha =$

0.38). In dimensional terms, for this specific illustrative case, the penetration times are 0.23 and 22.4 h for 100- and 1,000-μm-thick biofilms, respectively. This extent of retardation occurs even though the biofilm can only bind antibiotic in the amount of 0.2% of its mass ($Y_{cx} = 0.002$). It is conceivable, therefore, that irreversible sorption could retard antibiotic penetration sufficiently to account for the reduced susceptibility of biofilm.

Penetration time depends, in this case, on binding capacity, ρ. As shown in Fig. 4, the relationship $\alpha_{50}/\rho = 0.6$ gives a good (accuracy within about 15%) estimate of the penetration time for a wide range of values of ρ greater than 1 and ϕ greater than 2. In other words, penetration time is directly proportional to binding capacity, ρ. When ϕ is less than 1, there is no transport limitation for any value of ρ.

Since the level of retardation experienced by a penetrating antibiotic depends on whether sorption is reversible or irreversible, determination of this characteristic should be an important part of any study of antibiotic binding to cells or other biofilm components. The data presented by Darouiche et al. (for vancomycin [9]) and Nichols et al. (for tobramycin [26]) suggest reversible sorption. One of the most conclusive ways to assess reversibility is to conduct desorption experiments. Suci et al. report such data (for ciprofloxacin) that suggest that there may be slow desorption of this agent in their experimental system (31).

Diffusion of stoichiometrically reacting solute. The case of an antimicrobial agent that is neutralized by a chemical reac-
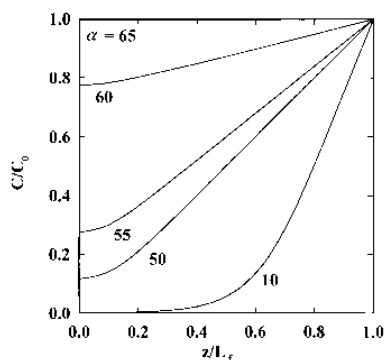

FIG. 3. Concentration profiles for unsteady-state penetration of a diffusing solute into a biofilm in which irreversible sorption or stoichiometric reaction occurs. The parameter α is dimensionless time. Illustrated is the case for reactive capacity, ρ, equalling 111 and the Thiele modulus, ϕ, equalling 10.

TABLE 3. Parameter input values for modeling transient penetration of an irreversibly sorbing (or stoichiometrically reacting solute) into biofilm[a]

Parameter	Symbol	Value
Sorption rate kinetic coefficient	k_s	7.5 day^{-1}
Yield coefficient	Y_{cx}	0.002 μg μg^{-1}
Bulk fluid antibiotic concentration	C_o	10 μg ml^{-1}
Water volume fraction	ε_w	0.90
Initial cell biofilm density	X_s^o	5,000 μg ml^{-1}
Biofilm thickness	L_f	400 μm
Antibiotic diffusion coefficient	D_{aq}	6 × 10^{-6} cm^2 s^{-1}
Biofilm/bulk diffusivity ratio	D_e/D_{aq}	0.81

[a] Simulation results are plotted in Fig. 3. Sources of estimates of some of the parameters can be found in reference 29; values of k_s and Y_{cx} are purely hypothetical.
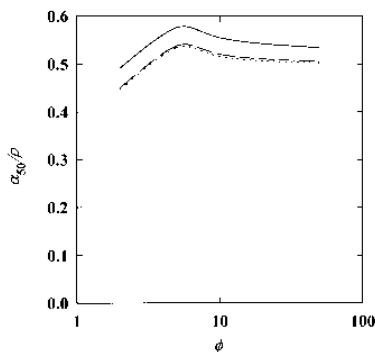
FIG. 4. Dimensionless penetration time (α_{50}) as a function of the Thiele modulus (ϕ) and reactive capacity (ρ). Values of ρ are as follows: ——, 11; – –, 111; · · ·, 1,110.

tion with the biofilm in a stoichiometric manner is identical to the preceding case of irreversible sorption. The only difference is a conceptual one. In the case of a stoichiometric reaction, the antibiotic and its biofilm reaction site are both permanently destroyed when they meet. In the case of irreversible sorption, the antibiotic and its biofilm binding site, though intact, are permanently sequestered. The preceding discussion of irreversible sorption can also be understood in terms of stoichiometric reaction by the appropriate substitution of terms. In particular, the yield coefficient, Y_{cx}, can be viewed in this case as the mass of antimicrobial agent destroyed per biofilm mass in which neutralizing moieties are depleted. There is now good experimental evidence that a stoichiometric reaction-diffusion mechanism is important in determining biofilm recalcitrance to certain industrial biocides, such as chlorine (6, 10, 33).

Catalytically reacting, nonsorbing solute. In the case of a catalytically reacting solute, persistent gradients in solute concentration can be maintained within the biofilm. Figure 5 illustrates the solution to the steady-state version of equation 14. The steady-state solution is approached rapidly. The time scale to approach steady state is just α; in dimensional terms, the time to reach steady state is anticipated to be on the order of seconds or minutes, depending on the biofilm thickness. The degree of penetration depends on a single parameter, ϕ, the Thiele modulus, where

$$\phi = \left(\frac{k_r L_f^2}{D_e}\right)^{1/2} \qquad (20)$$

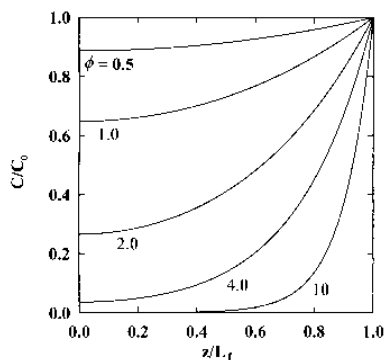This dimensionless group compares the rates of reaction and

FIG. 5. Concentration profiles for steady-state penetration of a diffusing solute into a biofilm with which a catalytic reaction occurs.

diffusion. When ϕ is small, diffusion is fast compared to reaction, and the solute penetrates well. When ϕ is large (≥ 1), diffusion is slow compared to reaction, and the biofilm is never fully penetrated by the solute. A catalytic reaction can readily explain the reduced susceptibility of biofilms to antimicrobial agents, provided the antimicrobial-neutralizing reaction is sufficiently fast (30).

In a medical context, the enzymatic degradation of β-lactam antibiotics is an example of such a catalytic reaction. Nichols et al. have considered the penetration of β-lactam antibiotics into microbial aggregates in which a β-lactamase is expressed (26). This was done theoretically by a formulation essentially identical to equation 14. They concluded that the reaction rate by β-lactamase (in their case, with cefsulodin) would not be sufficiently fast to account for failure of the antibiotic to penetrate a biofilm unless the biofilm were as thick as 1 mm. The maximum reaction rate they evaluated, for example, when applied to a 100-μm-thick biofilm corresponded to a Thiele modulus of 1.54. At steady state, the antibiotic concentration at the base of the biofilm would be 41% of the bulk fluid concentration (Fig. 5). These calculations were predicated on β-lactamase activities ranging from 0.08 to 6 nmol mg^{-1} min^{-1}. Since cefsulodin is a poor substrate for the chromosomally coded enzyme of *Pseudomonas aeruginosa*, actual β-lactamase-specific activities in some induced organisms may be higher than this. Giwercman et al. (14) and Hewinson and Nichols (17) have reported enzyme activities as high as 1,200 (nitrocefin) and 6,500 (cephalosporin C) nmol mg^{-1} min^{-1}, respectively (the former converted from published units for the purpose of comparison, assuming 0.5 mg of protein per mg of dry mass). These two substrates are hydrolyzed at rates admittedly higher than those that would be seen with representative antipseudomonal β-lactams. In addition, these activity assays were performed after freeze-thawing of the bacteria to release the enzyme. Thus, these rates do not reflect the throttling of the reaction rate that may occur because of outer membrane permeability restriction and must be regarded as upper bounds. With these higher reaction rates, one can readily attain values of ϕ of the order of magnitude of 10. This is sufficient to leave the bottom half of the biofilm entirely unexposed to antibiotic (Fig. 5). A reaction-diffusion mechanism may be a viable explanation for failure of certain β-lactam agents to control biofilm infections if it can be shown that the necessary high rates of antibiotic degradation are actually attainable under physiological conditions.

The transport models presented in this article neglect two features of real biofilms that modify transport rates: external mass transfer resistance and biofilm structural heterogeneity. External mass transfer resistance, which refers to the resistance to transport of a solute as it moves from the bulk fluid to the biofilm surface, further retards penetration. External mass transfer can be easily accounted for, provided one can estimate an appropriate mass transfer coefficient, by replacing equation 2 with a matching flux boundary condition. This condition requires that the solute flux from the bulk fluid to the biofilm surface be equal to the flux of solute into the biofilm. Structural heterogeneity, now recognized as a common feature of microbial biofilms (8), would tend to enhance the rate of solute penetration in most instances. The significance of structural heterogeneity on solute transport can be largely accounted for by replacing the biofilm thickness, L_f, with the biofilm volume-to-surface-area ratio. A fuller understanding of the significance of these aspects of biofilm structure and function for antibiotic penetration awaits further investigation.

This theoretical investigation of antibiotic penetration into microbial biofilm suggests that a diffusion barrier would only

be tenable as an explanation for reduced biofilm susceptibility to antimicrobial chemotherapy if the diffusion process is modified by significant irreversible sorption or fast reaction of the antibiotic within the biofilm matrix. On the basis of limited data available in the literature at this point, reversible sorption does not appear to be sufficient to account for failure of antibiotics to penetrate biofilms. Although rapid reaction of certain antibiotics, such as the β-lactams, could explain their inability to penetrate, for the majority of antibiotics there is no evidence of such rapid degradation. Nor is there evidence in the literature to date of profound irreversible sorption of antibiotics to biofilm constituents. I infer from this that, while transport limitations may impinge on the efficacy of selected antibiotics when used against biofilm infections, some other mechanism of reduced biofilm susceptibility must be at work. This conclusion is essentially identical to that of Nichols (23, 24). Physiology-based explanations, such as the possible presence within biofilms of slowly growing or phenotypically altered and hence less susceptible microorganisms, afford attractive alternative hypotheses (4, 21).

Progress in understanding the role of transport limitation of antibiotic penetration into biofilm now depends on experimental measurements of antibiotic sorption and reaction in biofilms. To elucidate the role of sorption, it must be determined whether sorption is reversible or irreversible and both the rate and extent of sorption must be measured. To assess the significance of reaction in impacting solute transport into biofilms, it is essential to establish whether the reaction is catalytic in nature or, alternatively, involves stoichiometric depletion of a biofilm constituent. Reaction rates and extents (stoichiometry) must likewise be quantified. The theory presented in this article provides a framework for design and analysis of experiments to test these mechanisms of reduced biofilm susceptibility to antibiotics.

ACKNOWLEDGMENTS

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