REVIEW

A Review of Experimental Measurements of Effective Diffusive Permeabilities and Effective Diffusion Coefficients in Biofilms

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Received 14 August 1997; accepted 2 December 1997

Abstract: Experimental measurements of effective diffusive permeabilities and effective diffusion coefficients in biofilms are reviewed. Effective diffusive permeabilities, the parameter appropriate to the analysis of reaction-diffusion interactions, depend on solute type and biofilm density. Three categories of solute physical chemistry with distinct diffusive properties were distinguished by the present analysis. In order of descending mean relative effective diffusive permeability ($D_e/\mathcal{D}_{aq}$) these were inorganic anions or cations (0.56), nonpolar solutes with molecular weights of 44 or less (0.43), and organic solutes of molecular weight greater than 44 (0.29). Effective diffusive permeabilities decrease sharply with increasing biomass volume fraction suggesting a serial resistance model of diffusion in biofilms as proposed by Hinson and Kocher (1996). A conceptual model of biofilm structure is proposed in which each cell is surrounded by a restricted permeability envelope. Effective diffusion coefficients, which are appropriate to the analysis of transient penetration of nonreactive solutes, are generally similar to effective diffusive permeabilities in biofilms of similar composition. In three studies that examine diffusion of very large molecular weight solutes (>5000) in biofilms, the average ratio of the relative effective diffusion coefficient of the large solute to the relative effective diffusion coefficient of either sucrose or fluorescein was 0.64, 0.61, and 0.36. It is proposed that large solutes are effectively excluded from microbial cells, that small solutes partition into and diffuse within cells, and that ionic solutes are excluded from cells but exhibit increased diffusive permeability (but decreased effective diffusion coefficients) due to sorption to the biofilm matrix. © 1998 John Wiley & Sons, Inc. *Biotechnol Bioeng* **59**: 261–272, 1998.

Keywords: effective diffusive permeability; diffusion coefficient; biofilm; cell density; review

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Contract grant sponsors: Center for Biofilm Engineering at Montana State University; National Science Foundation; The Center for Biofilm Engineering at Montana State University Industrial Associates

Contract grants numbers: EEC-8907039

INTRODUCTION

Solutes are transported in microbial biofilms by a combination of advection and diffusion. The heterogeneous structure of many biofilms permits convective transport within voids and water channels permeating the biofilm (de Beer et al., 1994; de Beer and Stoodley, 1995; Lewandowski et al., 1995). Within cell aggregates or clusters, however, molecular diffusion is still recognized as the predominant mode of mass transport (de Beer and Stoodley, 1995). The rate of the diffusion process within biofilm cell clusters is characterized by a single phenomenological parameter: a diffusion coefficient.

Because biofilms are mostly water, the starting point in evaluating a biofilm diffusion coefficient is an estimate of the value of the diffusion coefficient of the solute of interest in water. The presence of microbial cells, extracellular polymeric substances (EPS), and inorganic materials impedes diffusion in the biofilm and reduces the diffusion coefficient from its value in pure water. This reduction is characterized by the ratio of the effective diffusion coefficient in the biofilm to the diffusion coefficient in the medium bathing the biofilm. Experimental measurements of this ratio are the subject of this review.

Here it is important to distinguish between two parameters that are both commonly referred to as effective diffusion coefficients, but which are, in fact, different. Adopting the terminology of Libicki et al. (1988), let $\mathcal{D}_e$ denote the effective diffusion coefficient and D_e the effective diffusive permeability. Both parameters are defined by statements of Fick's first law, but with different definitions of the solute concentration in the concentration gradient.

To appreciate the difference between $\mathcal{D}_e$ and D_e, consider a solute diffusing in a biofilm comprised of N components. The first of these components is the extracellular aqueous phase ($i = 1$ denoted by subscript aq), and the remaining components could include cells, extracellular polymeric

CCC 0006-3592/98/030261-12

substances, precipitates, corrosion products, silt or fibrous material, and gas bubbles. For purposes later in this article, the non-aqueous components will be collectively termed the biomass phase. Assuming that the solute distributes rapidly and reversibly between the various biofilm components, the concentration in each component phase can be related to the fluid phase concentration, C_{aq}, by a partition coefficient:

$$C_i = C_{aq}\gamma_i \tag{1}$$

where γ_i are the respective partition coefficients. The relationship between the total volume-averaged concentration and the fluid phase concentration is therefore

$$C_{tot} = C_{aq}\sum_{i=1}^{N}\epsilon_i\gamma_i \tag{2}$$

where ϵ_i denotes the respective volume fraction occupied by the *i*-th component. The effective diffusion coefficient is defined by

$$J = -\mathcal{D}_e\nabla C_{tot} \tag{3}$$

where J is the solute flux. The effective diffusive permeability is defined by

$$J = -D_e\nabla C_{aq} \tag{4}$$

From Equation (2), the relationship between the two parameters is

$$\mathcal{D}_e = \frac{D_e}{\sum_{i=1}^{N}\epsilon_i\gamma_i} \tag{5}$$

In a system in which the composition of the biofilm is uniform in space, $\mathcal{D}_e$ and D_e differ by a system-specific constant. If there is significant sorption to the biomass phase ($\gamma_i \gg 1$), then $\mathcal{D}_e$ can be less than D_e. On the other hand, if the solute is largely excluded from the biomass phase, then $\mathcal{D}_e$ will be greater than D_e.

Regardless of whether Equation (3) or (4) is used as a starting point, an unsteady differential material balance on the solute within the biofilm leads to

$$\frac{\partial C}{\partial t} = -\mathcal{D}_e\nabla^2 C \tag{6}$$

where C may be C_{tot}, C_{aq}, or any other consistent sum of component phase concentrations. Thus, when transient data are analyzed, it is the effective diffusion coefficient, $\mathcal{D}_e$, that is ordinarily extracted.

Experimental measurements of a steady state flux, either directly, as with a diffusion cell, or indirectly through a reaction-diffusion analysis, generate an estimate of the effective diffusive permeability, D_e. The reason for this is that these analyses are invariably framed in terms of the aqueous-phase concentration, C_{aq}. It is the aqueous-phase concentration that applies in kinetic expressions or that is measured on two sides of a diaphragm cell.

Effective diffusion coefficients and effective diffusive permeabilities have different applications. For analysis of phenomena involving reaction-diffusion interactions, the effective diffusive permeability is the correct parameter. The effective diffusion coefficient is appropriate to the analysis of unsteady behavior of a non-reactive solute, for example, the penetration of a stain or migration of a plasmid.

In preparing this review, I drew on earlier summaries presented by Libicki et al. (1988), Fan et al. (1990), and Hinson and Kocher (1996). Twenty-one additional studies have been added to those reviewed by these researchers. Reports published after 1996 were not included in the analysis (Beyenal et al., 1997; Converti et al., 1997; de Beer et al., 1997; Vrany et al., 1997). Also excluded were indirectly determined diffusivities, for example, those calculated from a pore structure model based on a porosity measurement (Zhang and Bishop, 1994; Zhang et al., 1995). The present survey considered investigations of biofilms and flocs, but not of gel-entrapped cells because the latter may not realistically represent the properties of naturally aggregated cells. Diffusion coefficients in immobilized cell systems have been reviewed elsewhere (Riley et al., 1996; Westrin and Axelsson, 1991).

METHODS

Unless otherwise noted, comparisons of statistical significance were performed using a two-sample-two-sided *t*-test assuming unequal variances (Freedman et al., 1980). These tests were implemented using canned functions in a QuattroPro spreadsheet.

REVIEW

Relative Effective Diffusive Permeabilities, $D_e/\mathcal{D}_{aq}$

Experimental measurements of the ratio $D_e/\mathcal{D}_{aq}$ are compiled in Table I. An early question to ask of such a data set is whether the experimental method employed influenced the result obtained. I examined three aspects of the experimental methods: (1) reactivity of the diffusing solute, (2) external mass transfer resistance effects, and (3) preparation of the biofilm. With regard to biofilm preparation, I was particularly interested in whether simulated biofilms prepared by filtering cells or flocs yielded results comparable to those obtained using intact biofilms. The mean $D_e/\mathcal{D}_{aq}$ value for intact biofilm was 0.38 ($n = 85$) whereas for artificial biofilm the mean was 0.61 ($n = 25$). The difference between these two groups was statistically significant ($p = 0.0005$). This result suggests that artificial biofilms, in which native biofilm structure has been disrupted, tend to lead to unrealistically elevated estimates of effective diffusive permeability. Data from studies using artificial biofilms were therefore omitted from all subsequent analyses. Analysis of the abridged data set revealed no statistically detectable difference between measurements made with reactive solutes vs. those in which the solute was non-reactive or the

Table I. Biofilm relative effective diffusive permeabilities.[a]

Biomass density (g/L)	$D_e/\mathcal{D}_{aq}$	Solute	Method	Biofilm	Reference
20	0.72	O_2	RI	*A. niger* pellet	Yano et al., 1961
35	0.13				
45	0.27				
50	0.50				
65	0.20				
80	0.49				
90	0.20				
130	0.45				
145	0.35				
170	0.13				
40	0.55	O_2	RI	Mixed microbial	Tomlison and Snaddon, 1966
400	0.09	O_2	REI	*Z. ramigera* floc	Mueller et al., 1968
390	0.08	Glucose	RI	*Z. ramigera* floc	Baillod and Boyle, 1970
—	1.10	Glucose	RI	Mixed microbial	Atkinson and Davies, 1974
	0.70	NH_4^+			
—	0.37	Glucose	DEA	Mixed microbial	Pipes et al., 1974
—	0.20	Glucose	RI	Mixed microbial	Lamotta, 1976a
94	0.35	Glucose	REI	Mixed microbial	Lamotta, 1976b
—	0.54	O_2	DEA	Mixed microbial	Matson and Characklis, 1976
	0.30	Glucose			
73	0.80	NH_4^+	DEA	Nitrifying	Williamson and McCarty, 1976
58	0.87				
63	0.86	NO_2^-			
61	0.86				
71	0.93	NO_3^-			
50	1.00				
84	0.85	O_2			
71	0.85				
19	0.53	O_2	REI	Fungal pellet	Ngian and Lin, 1976 Miura et al., 1975
24	0.50	O_2	RI	Mixed microbial	Fujie et al., 1979
	0.50	Glucose			
62	0.46	NO_3^-	REI	Denitrifying	Mulcahy et al., 1980 Mulcahy et al., 1981
69	0.34	Valerate	RI	Denitrifying	Andrews and Tien, 1981
—	0.21	Acetate	DEI	Dental plaque	Dibdin, 1981
	0.20	Propionate			
	0.20	Lactate			
	0.22	Glucose			
	0.22	Fructose			
	0.19	Sucrose			
	0.31	^3H_2O			
—	0.68	NO_3^-	RI	Denitrifying	Arvin and Kristensen, 1982
	0.33	CO_2			
	0.49	HCO_3^-			
—	0.75	O_2	DEA	Mixed bacterial	Onuma and Omura, 1982
	0.73	Glucose			
	0.75	NH_4^+			
74	0.12	O_2	RI	Denitrifying	Wang and Tien, 1984
	0.68	Valerate			
29	0.31	O_2	DEA	Mixed microbial floc	Smith and Coackley, 1984
42	0.34				
65	0.33				
84	0.36				
—	0.39	O_2	RI	Mixed mixed microbial	la Cour Jensen et al., 1985
	0.25	Glucose			
	0.44	Methanol			
	0.30	Acetate			
	0.54	NO_3^-			

Table I. Continued

Biomass density (g/L)	$D_e/\mathcal{D}_{aq}$	Solute	Method	Biofilm	Reference
25	0.60	O_2	REI	Nitrifying	Siegrist and Gujer, 1987
72	0.25	Phenol	REI	Mixed microbial	Tang and Fan, 1987
78	0.16				
152	0.10				
151	0.086				
36	1.00	N_2O	NEA	*E. coli* aggregates	Libicki et al., 1988
92	0.66				
98	0.63				
204	0.37				
290	0.28				
299	0.27				
8	1.00	O_2	REI	Pseudomonad	Wagner and Hempel, 1988
	1.00	Napthalene-2-sulfonate			
141	0.88	Phenol	REI	Mixed microbial	Livingston and Chase, 1989
195	0.30				
217	0.05				
219	0.16				
223	0.12				
—	0.11	Ethanol	REI	Methanogenic gloc	Ozturk et al., 1989
	0.23	H_2			
	0.11	Acetate			
122	0.28	Li^+	NEA	Methanogenic floc	Nilsson and Karlsson, 1989
—	0.51	O_2	DEI	Photosynthetic	Revsbech, 1989
97	0.25	Phenol?	RI	Mixed microbial	Fujie as cited in Fan et al., 1990
135	0.18				
144	0.17				
180	0.13				
199	0.07				
—	0.88	O_2	REI	Mixed microbial	Lewandowski et al., 1991
—	0.07	Li^+	NEI	Methanogenic	Kitsos et al., 1992
—	0.69	O_2	DEI	Photosynthetic	Glud et al., 1992
—	0.78	O_2	DEI	Photosynthetic	Kühl and Jørgensen, 1992
87	0.65	Lactose	REI	Acidogenic	Yu and Pinder, 1993
—	0.18	Lactate	DEI	Dental plaque	Dibdin, 1993
	0.26	^3H_2O			
—	0.7	O_2	REI	Mixed microbial	Lewandowski, 1993
—	0.31	Acetate	REI	Methanogenic	Yu and Pinder, 1994
	0.41	Propionate			
	0.28	Butyrate			
35	0.48	O_2	REI	*Z. ramigera*	Beyenal and Tanyolaç, 1994
53	0.36				
80	0.31				
86	0.29				
35	0.34	Glucose			
53	0.23				
80	0.17				
86	0.13				
35	0.96	NH_4^+			
53	0.81				
80	0.62				
86	0.48				
—	0.68	N_2O	DEI	Cyanobacterial mat	Glud et al., 1995
	0.55	O_2			

[a]Method codes are: R = reactive solute; N = nonreactive solute; D = deactivated biofilm; E = external mass transfer resistance addressed; I = intact biofilm; A = articial biofilm.

biofilm was deactivated ($p = 0.55$). There was also no discernable difference between studies in which external mass transfer resistance was explicitly addressed and those in which it was not ($p = 0.87$). Sometimes in examining historical data, jumps or trends in time can be observed that reflect the introduction of new techniques or refinement of existing methods. No such time trend could be discerned for effective diffusive permeabilities ($p = 0.96$).

Much of the variation in measurements of relative effective diffusive permeabilities in intact biofilm studies, in which $D_e/\mathcal{D}_{aq}$ ranged from 0.05 to 1.1, can be attributed to differences in biofilm composition and in the chemical properties of the solute. I will address the role of the physical-chemical nature of the solute first.

The degree to which a solute is excluded by the biomass phase and the degree to which a solute sorbs to biofilm constituents will affect $D_e/\mathcal{D}_{aq}$ for that solute. A solute that is excluded from the particulate constituents of the biofilm (e.g., microbial cells, gas bubbles) would be expected to diffuse more slowly than a solute that partitions freely into the dispersed phase and diffuses within that phase. Glucose is an example of a solute that is likely to be excluded by microbial cell membranes, whereas oxygen is an example of a solute that could potentially cross cell membranes and diffuse within the cell. An important parameter in analyzing this phenomenon is the relative diffusive permeability of the particulate phase, which I will denote by $D_c/\mathcal{D}_{aq}$. A solute that partitions into the biomass phase and is mobile in that phase will exhibit a higher relative effective diffusive permeability than a solute that does not sorb.

Table II summarizes average $D_e/\mathcal{D}_{aq}$ values for solutes for which data from two or more independent studies were available. This group of solutes includes oxygen, glucose, phenol, acetate, lactate, propionate, NH_4^+, and NO_3^-. The two inorganic ions exhibit the highest relative effective diffusive permeabilities, oxygen is intermediate, and organic solutes are the lowest.

Table II. Mean relative effective diffusive permeability ($D_e/\mathcal{D}_{aq}$) and relative effective diffusion coefficient ($\mathcal{D}_e/\mathcal{D}_{aq}$) values in biofilm for individual solutes and solute categories.[a]

Solute	$D_e/\mathcal{D}_{aq}$	$\mathcal{D}_e/\mathcal{D}_{aq}$
NH_4^+	0.71	—
NO_3^-	0.56	—
O_2	0.45	0.48
Propionate	0.31	—
Glucose	0.24	—
Acetate	0.23	0.23
Phenol	0.21	—
Sucrose	—	0.19
Lactate	0.19	0.17
Ionic	0.58	0.14
Small	0.43	0.46
Large	0.29	0.39

[a]Individual solutes were included if there were data from two or more independent studies. The mean values for solute categories incorporate all data for that category.

I defined three categories of solute physical chemistry based on this observation. The first category included small non-polar solutes with molecular weights of 44 or less (here termed small solutes). This category included such species as O_2, H_2, N_2O, CO_2, ^3H_2O, and methanol. The second category included inorganic ions, such as NH_4^+, Li^+, HCO_3^-, and NO_3^- (ionic solutes). The third category included species with molecular weights of 45 or greater (large solutes). In this category were such solutes as sugars and fatty acids. Fatty acids could also be categorized as ionic solutes, depending on the pH of the biofilm milieu and the pKa of the acid, but their diffusive properties seem to align more closely with other organic solutes of comparable size. Mean relative effective diffusive permeabilities and standard deviations for these categories (Table II) were, in descending order, ionic solutes (0.58 ± 0.24), small solutes (0.43 ± 0.22), and large solutes (0.29 ± 0.24). Mean $D_e/\mathcal{D}_{aq}$ values for these three categories are statistically distinguishable from each other at the 95% confidence level with the exception of the small solute-ionic solute comparison for which $p = 0.11$. I retain the distinction between these latter two categories based on additional analyses discussed below.

Another means of examining the effect of solute type on the effective diffusive permeability of biofilm is to compare the permeabilities of different solutes measured in the same experimental biofilm system. This eliminates the variability associated with differing methods and biofilm compositions. Ratios of $D_e/\mathcal{D}_{aq}$ values for pairs of solutes derived from such internal comparisons are summarized in Table III. Small solutes including oxygen, methanol, tritiated water, and hydrogen display higher relative effective diffusive permeabilities than large solutes by a mean factor of approximately 1.5. The ratio of relative effective diffusive permeabilities for small solutes to large solutes from the overall averages compiled in Table II is 1.48. In five out of six internal comparisons, ionic solutes had higher relative effective diffusive permeabilities than large solutes with an average value of 2.7 (Table III). The ratio of effective diffusive permeabilities for ionic solutes to large solutes from the overall averages compiled in Table II is 2.00. Diffusive permeability ratios from internal comparisons for the small-large, ionic-large, and small-ionic categories were statistically distinguishable from unity ($p < 0.05$) by both a one-sided, one-sample *t*-test and by the nonparametric Wilcoxon's ranked sign test. Comparisons of solute diffusive permeabilities in the same biofilm system further support, therefore, the definition of three separate categories of solute type.

The effect of biofilm density, or more specifically biomass volume fraction, on relative effective diffusive permeabilities is shown in Figure 1. In only a few instances was an independent measurement of volume fraction made. In most cases, the biomass volume fraction ($\epsilon_c + \epsilon_p$) was estimated herein as $\epsilon_c + \epsilon_p = X_b/\rho_x$, where X_b is the biofilm density (mass per biofilm volume) and ρ_x is the intrinsic density of biomass particulate matter (mass per hydrated

Table III. Comparison of biofilm relative effective diffusivities between different solutes.[a]

Solute	Ratio	$D_e/\mathcal{D}_{aq}$	$\mathcal{D}_e/\mathcal{D}_{aq}$	Reference
NH_4^+/glucose	0.64	0.70		Atkinson and Davies, 1974
Oxygen/glucose	(1.83)	(0.30)		Matson and Characklis, 1976
NH_4^+, NO_3^-, NO_2^-/oxygen	(1.04)	(0.85)		Williamson and McCarty, 1976
NO_3^-/methanol	0.46	—		Riemer and Harremoës, 1978
Oxygen/glucose	1.00	0.50		Fujie et al., 1979
^3H_2O/lactate, sugars	1.48	0.21		Dibdin, 1981
NO_3^-, HCO_3^-/CO_2	1.77	0.33		Arvin and Kristensen, 1982
Oxygen/glucose	(1.03)	(0.73)		Onuma and Omura, 1982
NH_4^+/glucose	(1.03)	(0.73)		Onuma and Omura, 1982
Oxygen/valerate	0.18	0.12		Wang and Tien, 1984
Oxygen/glucose, acetate	1.43	0.28		la Cour Jensen et al., 1985
Methanol/glucose, acetate	1.60	0.28		la Cour Jensen et al., 1985
NO_3^-/glucose, acetate	1.96	0.28		la Cour Jensen et al., 1985
Hydrogen/actetate, ethanol	2.17	0.11		Ozturk et al., 1989
^3H_2O/lactate	1.44	0.18		Dibdin, 1993
Oxygen/glucose	2.23	0.13		Beyenal and Tanyolaç, 1994
	1.82	0.17		
	1.57	0.23		
	1.41	0.34		
NH_4^+/glucose	3.69	0.13		Beyenal and Tanyolaç, 1994
	3.65	0.17		
	3.52	0.23		
	2.82	0.34		
Nitrous oxide/oxygen	1.24	0.55		Glud et al., 1995
HCO_3^-/fatty acids, sucrose	0.49		0.10	Tatevossian, 1979
^3H_2O/acetate	1.03		0.29	Dibdin, 1993

[a]The ratio is the ratio of $D_e/\mathcal{D}_{aq}$ (or $\mathcal{D}_e/\mathcal{D}_{aq}$) values for the pair of solutes. The value of $D_e/\mathcal{D}_{aq}$ (or $\mathcal{D}_e/\mathcal{D}_{aq}$) tabulated is that of the solute representing the denominator of the ratio. Values in parentheses indicate a study performed with artificial biofilm; these were not included in graphs or analyses.

particulate volume). Unless experiment-specific information was available, the intrinsic cell density, ρ_x, was taken to be 210 g/L for fungal aggregates (Bakken and Olsen, 1983), 315 g/L for *E. coli* (Bratbak and Dundas, 1984; Ju and Ho, 1988; Stewart and Robertson, 1989), and 350 g/L for general bacterial systems (Bakken and Olsen, 1983). Because *Zoogloea ramigera* flocs exceeded densities of 350 g/L, the higher estimate of 438 g/L of Bratbak and Dundas, which is an average of three bacterial species, was used in this case. Density measurements were made in only about half of the studies, so Figure 1 reflects a subset of the investigations presented in Table I. $D_e/\mathcal{D}_{aq}$ decreases sharply as biomass volume fraction increases.

Westrin and Axelsson (1991) proposed the following model of effective diffusive permeabilities in gel-immobilized cell particles

$$\frac{D_e}{\mathcal{D}_{aq}} = \left(\frac{D_e}{D_{eo}}\right)\left(\frac{D_{eo}}{\mathcal{D}_{aq}}\right) \tag{7}$$

where D_{eo} is the effective diffusive permeability of the extracellular matrix. This model treats the resistances posed by particulates (e.g., cells) and EPS as multiplicative reductions in diffusive permeability. The first term of Equation (7) which describes the effect of the cells, is predicted by Maxwell's result (Maxwell, 1892) for a suspension of spheres of relative permeability $D_c/\mathcal{D}_{aq}$ in a medium of relative permeability $D_{eo}/\mathcal{D}_{aq}$:

$$\frac{D_e}{D_{eo}} = \frac{2\frac{\mathcal{D}_{aq}}{D_c} + \frac{\mathcal{D}_{aq}}{D_{eo}} - 2\epsilon_c\left(\frac{\mathcal{D}_{aq}}{D_c} - \frac{\mathcal{D}_{aq}}{D_{eo}}\right)}{2\frac{\mathcal{D}_{aq}}{D_c} + \frac{\mathcal{D}_{aq}}{D_{eo}} + \epsilon_c\left(\frac{\mathcal{D}_{aq}}{D_c} - \frac{\mathcal{D}_{aq}}{D_{eo}}\right)} \tag{8}$$

The permeability of the EPS-containing medium in the space between cells is modeled by

$$\frac{D_{eo}}{\mathcal{D}_{aq}} = \frac{\left(1 - \frac{\epsilon_p}{1-\epsilon_c}\right)^3}{\left(1 + \frac{\epsilon_p}{1-\epsilon_c}\right)^2} \tag{9}$$

after Westrin and Axelsson (1991). I have chosen to express biofilm composition in terms of the fraction of the biomass volume occupied by EPS, which I denote by *b*:

$$b = \frac{\epsilon_p}{\epsilon_c + \epsilon_p} \tag{10}$$

Two examples of curves predicted by the model comprised by Equations (7)–(10) are plotted in Figure 1A. As Hinson and Kocher (1996) have concluded, this model cannot capture the experimentally observed steep decline in $D_e/\mathcal{D}_{aq}$ at relatively low biomass volume fractions.

Hinson and Kocher argue that the shape of biofilm effective diffusivity vs. cell density data suggests that solutes are transported in biofilms as if they were subject to serial re-

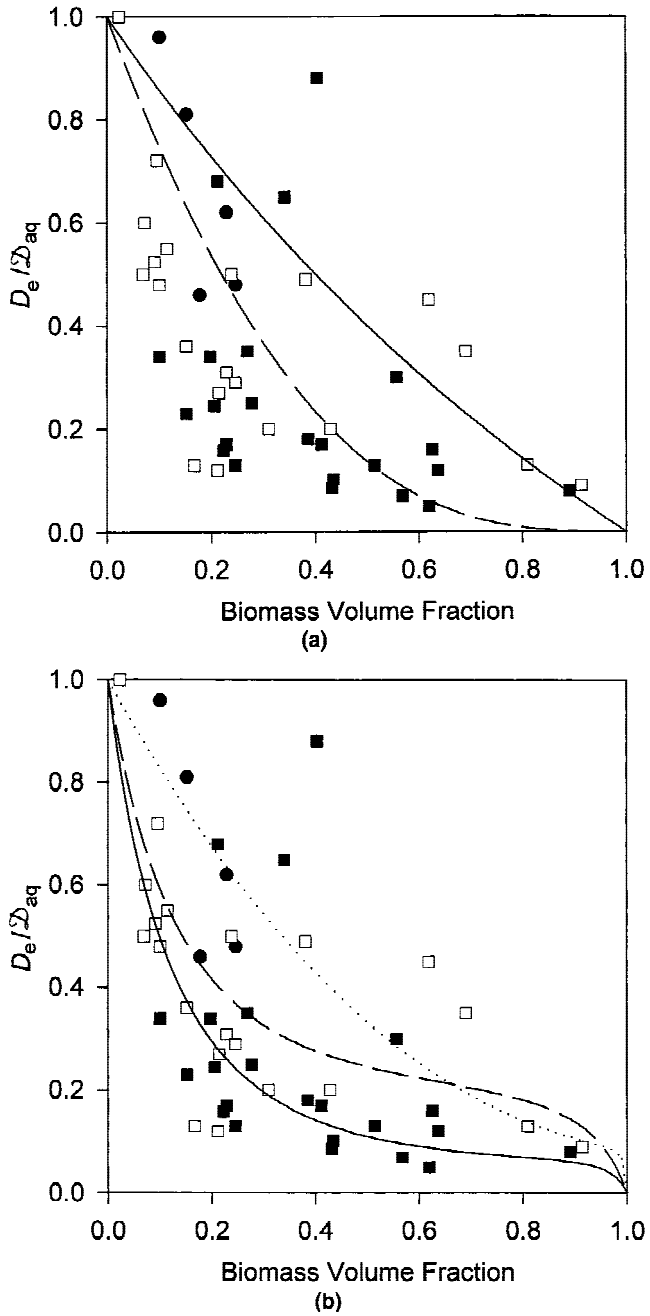

Figure 1. Effect of estimated biomass volume fraction on relative effective diffusive permeabilities for large (■), small (□), and ionic (●) solutes. The curves in **(a)** were generated by the model of Westrin and Axelsson with the following parameter values: $b = 0$, $D_c/\mathcal{D}_{aq} = 0$, (—); $b = 0.5$, $D_c/\mathcal{D}_{aq} = 0$, (– –). Curves in **(b)** are fits to the model of Hinson and Kocher using parameter values listed in Table IV and representing large (—), small (– –), and ionic (· · · ·) solutes.

sistances. Working with cells and EPS as the two resistance components, they propose that Equation (9) be replaced by

$$\frac{D_{eo}}{\mathcal{D}_{aq}} = \epsilon_{aq}\left(\epsilon_p \frac{\mathcal{D}_{aq}}{D_p} + \epsilon_{aq}\right)^{-1} \tag{11}$$

Here D_p is the effective diffusive permeability of the pure EPS phase and ϵ_{aq} is the water volume fraction, which in this construct is subject to the constraint:

$$\epsilon_{aq} + \epsilon_c + \epsilon_p = 1 \tag{12}$$

Qualitative fits of Hinson and Kocher's empirical model [Eqs. (7), (8), (10)–(12)] to data for solutes in each of the three categories are shown in Figure 1B. Model parameter values for these curves are given in Table IV. The model succeeds in describing the general shape of relative effective diffusive permeability vs. volume fraction data, at least for large and small solutes. Data for ionic solutes are sparse and the fit in this case is unconvincing.

The Hinson-Kocher model predicts that EPS volume fraction, ϵ_p, very strongly influences diffusive permeability. EPS content of biofilm is rarely measured, however, so it is impossible at this time to make good independent estimates of b or ϵ_p. Variation in the actual proportion of cells and extracellular polymeric substances (which I have fixed subjectively at $b = 0.15$) from biofilm to biofilm may account for some of the remaining scatter apparent in Figure 1B. More detailed characterization of biofilm composition is required in future investigations to enable better tests of the Hinson-Kocher and other models of biofilm diffusive permeability.

It is worth mentioning at this point, that cell densities and volume fractions may change with position inside a biofilm. Literature reports suggest that biomass density increases with biofilm depth (Christensen and Characklis, 1990; Okabe et al., 1997; Zhang and Bishop, 1994; Zhang et al., 1995). This means that the effective diffusive permeability within a particular biofilm may not be accurately characterized by a single value, but may require a position-dependent formulation (Zhang and Bishop, 1994).

Another way to test the Hinson-Kocher model is by comparing measured ratios of relative effective diffusive permeabilities of solutes from different categories (Table III) with the ratio predicted by the respective Hinson-Kocher model fits (Fig. 2). The ratio of relative effective diffusive permeabilities of solutes in different categories increases with decreasing $D_e/\mathcal{D}_{aq}$ (except at very high volume fractions) which is the expected behavior for solutes with differing biomass diffusive permeabilities. Good quantitative agreement between model and experiment is observed for the small solute-large solute comparison. The fit between model and theory is qualitatively, but not quantitatively, acceptable for the ionic-large solute comparison.

Table IV. Parameter values used in the Hinson-Kocher model to fit $D_e/\mathcal{D}_{aq}$ and $\mathcal{D}_e/\mathcal{D}_{aq}$ as a function of biomass volume fraction.

Solute category	$D_p/\mathcal{D}_{aq}$	b	$\mathcal{D}_c/\mathcal{D}_{aq}$
$D_e/\mathcal{D}_{aq}$, large	0.02	0.15	0.1
$D_e/\mathcal{D}_{aq}$, small	0.025	0.15	0.5
$D_e/\mathcal{D}_{aq}$, ionic	0.2	0.15	0.1
$\mathcal{D}_e/\mathcal{D}_{aq}$, large	0.025	0.15	0.3
$\mathcal{D}_e/\mathcal{D}_{aq}$, small	0.025	0.15	0.3

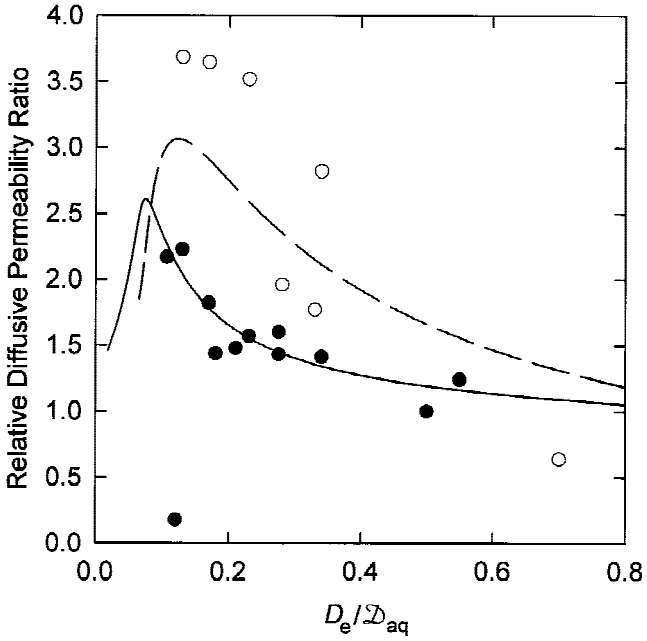

Figure 2. Ratio of $D_e/\mathcal{D}_{aq}$ for pairs of solutes as a function of $D_e/\mathcal{D}_{aq}$ for the second, generally less permeable, solute. The curves are the theoretical ratios predicted by the Hinson-Kocher model fits shown in Figure 1**B.** Curves and symbols represent ionic solutes compared to large solutes, (○, – –); small solutes compared to large solutes, (●, —).

The notion of serial resistances imposed by cells and EPS is at first difficult to reconcile with the known structure of biofilms. Microbial cells and extracellular matrix materials are thought to be heterogeneously, but approximately randomly, distributed in space. Never has a structure been suggested in which cells and EPS occupy distinct lamellae. One resolution to this apparent contradiction between the structure of biofilm suggested by diffusion measurements and the structure determined by direct microscopic examination may be arrived at by considering the situation experienced by a reactive solute that ends its journey inside a cell. In this case, one resistance to transport is imposed by the cells and extracellular matrix, and a second resistance is imposed by a restricted permeability layer surrounding the cell itself (Fig. 3). Cell membranes and capsular polymers may contribute to the reduced permeability envelope around each cell. If this conceptual model is correct, then reactive and non-reactive solutes would be expected to exhibit different diffusive permeabilities in biofilm, at least in the case of solutes that are normally excluded from the cell proper. Studies using non-reactive solute or deactivated biofilm might overestimate the apparent effective diffusive permeability of biofilm. Existing data are insufficient to test this assertion conclusively. New experimental studies and novel approaches to modeling simultaneous reaction and diffusion in multiphase media would be required to test this conceptual model.

Relative Effective Diffusion Coefficients, $\mathcal{D}_e/\mathcal{D}_{aq}$

Measurements of relative effective diffusion coefficients in biofilms, $\mathcal{D}_e/\mathcal{D}_{aq}$, are recorded in Table V. Relative effective diffusion coefficients of ionic solutes were statistically distinct from those of small and large solutes. Small and large solute-relative effective diffusion coefficients could not be statistically distinguished in this case ($p = 0.45$). A fit to the model of Hinson and Kocher of $\mathcal{D}_e/\mathcal{D}_{aq}$ vs. biomass volume fraction is shown in Figure 4 with model parameter values given in Table IV. The model does a reasonable job of capturing the shape of this relationship.

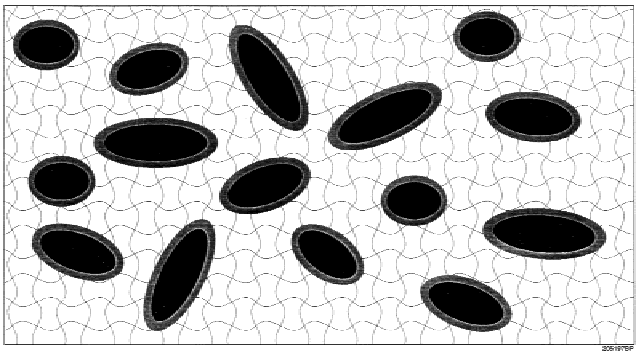

Figure 3. Conceptual model of the structure of a biofilm cell cluster. Microbial cells (black) are surrounded by reduced permeability envelopes (grey) and embedded in an EPS matrix (net).

$\mathcal{D}_e/\mathcal{D}_{aq}$ is generally comparable to $D_e/\mathcal{D}_{aq}$ for similar solutes (Table II), except for ionic solutes. Whereas ionic solutes had the highest effective diffusive permeabilities of any solute category, they exhibit the lowest effective diffusion coefficients. The difference between the $\mathcal{D}_e/\mathcal{D}_{aq}$ and $D_e/\mathcal{D}_{aq}$ group means for ionic solutes is statistically significant ($p = 0.0002$) while it is not for solutes in the other two categories. Internal comparisons of relative effective diffusive permeabilities for different solutes measured in the same experimental system indicated higher values for ionic solutes than for large solutes (Table III). In the one comparison of this type available for a relative effective diffusion coefficient, the ionic solute was less mobile than large solutes by a factor of two (Table III). The only way that $\mathcal{D}_e$ can be less than D_e is if the solute absorbs to biofilm constituents. This demonstrates that inorganic ions, both cationic and anionic, sorb significantly to some constituent of biofilm.

The unexpected behavior exhibited by ionic solutes invites further investigation. The mobilities of ionic solutes in an aqueous milieu are electrostatically coupled and, therefore, depend on the background ionic and strength and composition, effects I have not attempted to analyze here. There are some handsome modeling efforts that incorporate electrostatic effects by including a local charge balance (Dibdin, 1992; Flora et al., 1993).

In contrast to sorbing ions, solutes that do not sorb and that are excluded from the particulate phase of the biofilm would be expected to display effective diffusion coefficients greater than effective diffusive permeabilities. This was the case for large solutes, for which $\mathcal{D}_e/\mathcal{D}_{aq}$ was approximately 35% greater than $D_e/\mathcal{D}_{aq}$ on average (Table II).

There are some interesting measurements of $\mathcal{D}_e/\mathcal{D}_{aq}$ for

Table V. Biofilm relative effective diffusion coefficients.[a]

Biomass density (g/L)	$\mathcal{D}_e/\mathcal{D}_{aq}$	Solute	Method	Biofilm	Reference
—	0.048	O_2	REI	Mixed microbial	Bungay et al., 1969
15	0.58	O_2	REI	*A. niger* pellet	Huang and Bungay, 1973
—	0.46	Xe	NEI	Dental plaque	McNee et al., 1979
—	0.05	HCO_3^-	REI	Dental plaque	Tatevossian, 1979
	0.09	Acetate			
	0.09	Lactate			
	0.12	Butyrate			
	0.11	Sucrose			
—	0.13	SO_4^-	DEI	Cyanobacterial mat	Jørgensen et al., 1979
	0.16	H_2S			
—	0.23	NaF	NEI	Dental plaque	McNee et al., 1980
—	0.60	O_2	REI	Mixed microbial	Chen and Bungay, 1981
180	0.43	Sucrose	DEI	Dental plaque	McNee et al., 1982
	0.31	Acetate			
	0.31	Lactate			
20	0.5	Glucose, Na^+,	NEI	Mixed bacterial	Siegrist and Gujer, 1985
14	0.6	Br^-			
20	0.5				
25	0.5				
22	0.6				
23	0.7				
19	0.8				
26	0.5				
26	0.5				
200	0.08	Lactate	DEI	Dental plaque	Tatevossian, 1985a
200	0.045	Sucrose	DEI	Dental plaque	Tatevossian, 1985b
—	0.71	O_2	DEI	Cyanobacterial mat	Revsbech et al., 1986
182	0.26	Phenol	DEI	Mixed microbial	Fan et al., 1990
130	0.38				
170	0.13				
178	0.39				
—	0.29	Acetate		Dental plaque	Dibdin, 1993
	0.30	^3H_2O			
13	0.69	O_2	DEI		Fu et al., 1994
39	0.56				
81	0.44				
99	0.23				
—	0.023	Fluorescein	NI	Mixed bacterial	Lawrence et al., 1994
—	0.97	Fluorescein	NEI	Mixed bacterial	de Beer and Stoodley, 1995
—	0.91	Fluorescein	NEI	*P. putida*	Bryers and Drummond, 1996

[a]Data for solutes with molecular weights less than 1000 are included.

solutes of very large molecular weights (Birmingham et al., 1995; Bryers and Drummond, 1996; de Beer and Stoodley, 1995; Lawrence et al., 1994; Tatevossian, 1979). No trend of $\mathcal{D}_e/\mathcal{D}_{aq}$ with molecular weight can be discerned by examining all of the relative effective diffusion coefficient data (Fig. 5). Internal comparisons of solutes of molecular weights greater than 1000 with smaller solutes showed that $\mathcal{D}_e/\mathcal{D}_{aq}$ decreased with increasing molecular weight in three of four cases. The average ratio of $\mathcal{D}_e/\mathcal{D}_{aq}$ values for very large solutes to the value of $\mathcal{D}_e/\mathcal{D}_{aq}$ for either sucrose or fluorescein, was 0.64, 0.61, and 0.36 in these three studies (Bryers and Drummond, 1996; de Beer and Stoodley, 1995; Tatevossian, 1979). The results of the fourth study (Lawrence et al., 1994) are suspect on two counts. First, the reported values of $\mathcal{D}_e/\mathcal{D}_{aq}$ are anomalously low (compare the last three rows of Table V). Second, $\mathcal{D}_e/\mathcal{D}_{aq}$ actually increases for the highest molecular weight solute, a dependence that is unlikely. Inadequate analysis of external mass transfer resistance may be responsible for these artifacts.

SUMMARY AND RECOMMENDATIONS

Experimental measurements of relative effective diffusive permeabilities, $D_e/\mathcal{D}_{aq}$, in biofilms range widely, but most of the variation can be attributed to differences in solute physical chemistry and in biofilm density (or better, cell and EPS volume fractions). Three categories of solute physical chemistry were distinguished by the present analysis. In order of descending relative effective diffusive permeability, these were ionic solutes (inorganic anions or cations),

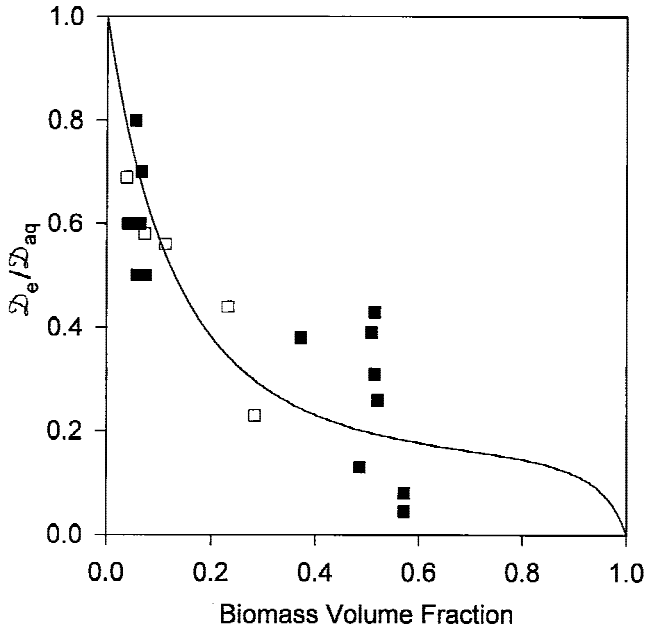

Figure 4. Effect of estimated biomass volume fraction on relative effective diffusive permeabilities for large (■) and small (□) solutes. The curve is a fit to the model of Hinson and Kocher using parameter values listed in Table IV.

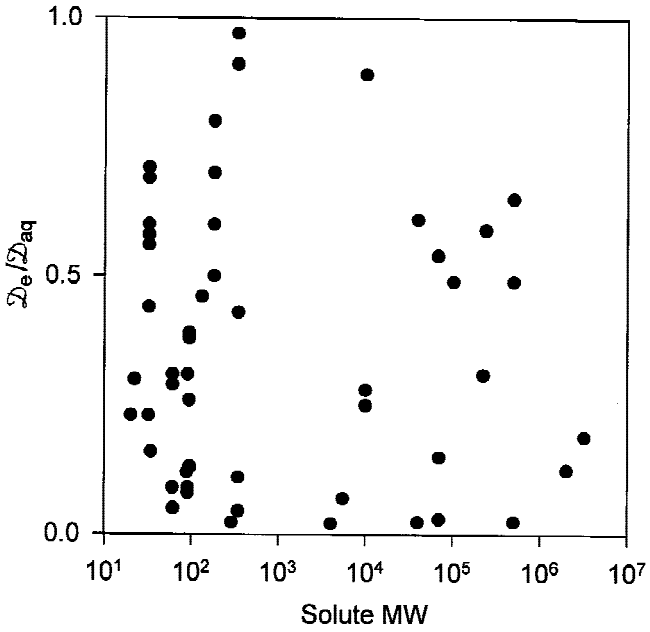

Figure 5. Relative effective diffusion coefficients in biofilms as a function of solute molecular weight.

small solutes (nonpolar solutes with molecular weights of 44 or less), and large solutes (organic solutes of molecular weight greater than 44). It is proposed that large solutes are effectively excluded from microbial cells, that small solutes partition into and diffuse within cells, and that ionic solutes are excluded from cells but exhibit increased diffusive permeability (but decreased effective diffusion coefficients) due to sorption to the biofilm matrix. Effective diffusive permeabilities decrease sharply with increasing biomass volume fraction suggesting a serial resistance model of diffusion in biofilms as proposed by Hinson and Kocher (1996). A conceptual model of biofilm structure is proposed in which each cell is surrounded by a restricted permeability envelope. New theoretical and experimental investigations of diffusion and reaction in multiphase media are needed to test these concepts. More detailed characterization of biofilm composition, particularly with regard to the EPS volume fraction, is also now essential.

If an estimate of biofilm density or volume fraction is available, $D_e/\mathcal{D}_{aq}$ or $\mathcal{D}_e/\mathcal{D}_{aq}$ can be predicted using the Hinson-Kocher model with appropriate parameter values from Table IV. To predict $D_e/\mathcal{D}_{aq}$ or $\mathcal{D}_e/\mathcal{D}_{aq}$ when no biomass volume fraction is at hand, one could search Tables I or V, respectively, for studies in which properties such as microbial speciation or primary metabolic activity are similar to the one of interest, and extract comparative values. In doing so, the user should remember that ionic, small, and large solutes display different effective diffusive permeabilities, even in the same biofilm. Representative values of relative effective diffusive permeabilities and relative effective diffusion coefficients for selected biofilm categories are presented in Table VI.

Table VI. Representative effective diffusive permeabilities and effective diffusion coefficients in selected biofilm types.

	$D_e/\mathcal{D}_{aq}$			$\mathcal{D}_e/\mathcal{D}_{aq}$		
Biofilm type	Ionic solutes	Small solutes	Large solutes	Ionic solutes	Small solutes	Large solutes
Methanogenic	—	0.3	0.2	—	—	—
Dental	—	0.3	0.2	0.15	0.3	0.2
Denitrifying	0.5	(0.4)	0.25	—	—	—
Fungal pellet	—	0.45	(0.35)	—	(0.6)	—
Photosynthetic	—	0.65	(0.6)	0.15	0.7	(0.6)
Other	(0.7)	0.45	0.35	—	—	—
All	0.6	0.45	0.3	0.15	0.45	0.4

[a]Values in parentheses indicate an estimate based on very limited data. Dashes indicate that data were lacking or too varied to arrive at a consensus value.

I thank Martin Hamilton for patient and considered advice regarding statistical analyses.

NOMENCLATURE

b	fraction of the biomass phase that is EPS
C	solute concentration
C_{aq}	solute concentration in the aqueous phase
C_{tot}	volume-averaged solute concentration in the biofilm
$\mathscr{D}_{\mathrm{aq}}$	diffusion coefficient in water
$\mathscr{D}_{\mathrm{e}}$	effective diffusion coefficient in biofilm
D_c	effective diffusive permeability of the pure particulate (cells and abiotic materials) phase
D_{e}	effective diffusive permeability of biofilm
D_{eo}	effective diffusive permeability of the extracellular matrix
D_p	effective diffusive permeability of pure EPS phase
J	solute flux
N	number of phases in the biofilm
t	time
X_b	biomass density within biofilm

Greek Letters

γ_i	partition coefficient for the i-th solute with respect to the aqueous phase
ϵ_c	volume fraction occupied by cells and abiotic particulate matter within the biofilm
ϵ_p	volume fraction occupied by EPS within the biofilm
ϵ_{aq}	volume fraction occupied by water within the biofilm
ρ_x	intrinsic biomass density

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