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IMPLICATIONS OF REACTION–DIFFUSION THEORY FOR THE DISINFECTION OF MICROBIAL BIOFILMS BY REACTIVE ANTIMICROBIAL AGENTS

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Abstract—A theoretical framework was developed for analyzing the efficacy of antimicrobial agents when applied to microbial biofilms with which they react. Reaction–diffusion theory was adapted to investigate the potential for transport limitation of the overall rate of biofilm disinfection and the rate of antimicrobial penetration into the biofilm. Disinfection efficacy was investigated with simulations that assumed catalytic reaction of the antimicrobial agent with live and dead cells in a uniformly thick slab with simultaneous transformation of live to dead cells by an independent rate process (disinfection). The intrinsic rate of disinfection was assumed to follow first-order dependence on antimicrobial concentration. Zero- and first-order reaction kinetics of antimicrobial agent with biomass were analyzed. Microbial growth and external mass transfer resistance were neglected. Results show that antimicrobial efficacy, defined as the ratio of the observed rate of biofilm disinfection to the rate that would prevail in the absence of mass transport limitation, decreases sharply as the Thiele modulus exceeds one. The reduction in efficacy worsens when the antimicrobial dose is more concentrated or longer. A second case examined the penetration of an antimicrobial agent into a biofilm with which it reacts stoichiometrically, as would be expected with an oxidizing biocide such as chlorine or ozone. The antimicrobial agent eventually penetrates the biofilm by depleting the reactive biomass constituent, but the time scale for penetration can exceed the time scale for transient diffusion in the absence of reaction by orders of magnitude. These results provide a theoretical basis for explaining experimentally observed resistance of biofilms to chemical disinfectants.

INTRODUCTION

This article considers a novel application of classical reaction–diffusion theory: the efficacy of an antimicrobial agent when used against a microbial biofilm with which the agent reacts. A biofilm is a surface-associated aggregate of microorganisms, extracellular polymers produced by the microbes, and abiotic particles captured by the film. Biofilms are implicated in diverse problems such as fouling of heat exchangers and cooling water towers, contamination in food processing, microbially induced corrosion, and persistent infections associated with medical implants (Characklis, 1990). In each of these applications, antimicrobial agents are used to control biofilm accumulation and activity. It is now clear, however, that biocides and antibiotics are much less effective against biofilms than they are against the same microorganisms when freely suspended (Brown and Gilbert, 1993; Costerton *et al.*, 1987). The fundamental mechanisms of biofilm resistance have not been fully elucidated.

One attractive hypothesis to explain biofilm resistance to chemical challenge is failure of the antimicrobial compound to penetrate the biofilm due to a reaction–diffusion interaction. This idea has been aired in the microbiology literature (Costerton *et al.*, 1987; Nichols, 1989), although usually only in qualitative terms. An exception are papers by Nichols (1989) and Nichols *et al.* (1989) that analyze quantitatively the simultaneous reaction and diffusion of an antibiotic within a microbial colony. The application of computer modeling to this problem has recently been described (Stewart, 1994). Chen *et al.* (1991) presented evidence for diffusion limitation of monochloramine action against a *P. aeruginosa* biofilm by calculating an observable modulus that compared reaction and diffusion rates. On the experimental front, Tashiro *et al.* (1991) measure 'penetration depths' into an undefined population of the order of 100 μm for two biocides, methylene bis isothiocyanate and an isothiazolone mixture. These measurements were not made directly, so the validity of interpreting them in terms of depth of penetration is questionable. A chlorine microelectrode has been employed to measure profound chlorine concentration gradients within biofilms (de Beer *et al.*, 1994), which appear to be consistent with a reaction–diffusion interaction.

The purpose of this paper is to establish a general theoretical framework for analyzing the efficacy of antimicrobial agents when applied to biofilms with which they react. Application of reaction–diffusion theory to this problem differs from the formulations developed in the context of classical heterogeneous catalyst in two respects. First, the goal in antimicrobial treatment is to disinfect or remove the bio-

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film, whereas in catalysis the goal is to achieve productive reaction rates. The end focus in the current problem, therefore, will be a measure of overall disinfection rate rather than a measure of overall reaction rate. Second, it is possible when considering the interaction of an oxidizing biocide (e.g. chlorine, ozone) with biofilm to have stoichiometric consumption of biomass by reaction with the biocide. The analog in terms of traditional catalysis would be stoichiometric consumption of the catalyst by reaction with a reactant, a situation that is to be avoided.

THEORY

Let the biofilm be modeled as a uniformly thick planar aggregate of constant thickness L in which concentration gradients of dissolved species occur only in the direction perpendicular to the substratum. The biocide concentration in the bulk fluid is assumed to be constant in time. External mass transfer resistance is neglected. Within the biofilm, biocide is transported by Fickian diffusion only. There is no transport of biocide across the biofilm–substratum interface nor any reaction of biocide with the substratum.

Biomass within the biofilm is assumed to contain N constituents with which the biocide interacts, whose concentrations are denoted by X_i, $i = 1, \ldots, N$. Interactions between biocide and biomass constituents are of two types, reactions and disinfection transformations. Reactions involve consumption of both biocide and biomass constituents. Disinfection transformations convert one biomass constituent to another. The disinfection process depends on biocide concentration, but does not consume biocide. The kinetics of interactions between biomass constituents and biocide are assumed to be first order with respect to biomass constituent concentrations and zero or first order with respect to biocide concentration. Adsorption of antimicrobial agent by biomass is neglected, although this may be an important process with some antibiotics. Scant experimental data exist to guide the development of these kinetic expressions. The simple formulations we postulate, nevertheless, serve adequately to illustrate general phenomena.

Microbial growth is neglected. This assumption can be justified if the time scales for diffusion, biocide reaction, and disinfection are small compared to the time scale for growth. This appears to be the case: characteristic times for diffusion and disinfection are on the order of seconds or minutes, while microbial growth occurs on a time scale of hours.

These assumptions yield the mass balance equation on biocide concentration:

$$\frac{\partial B}{\partial t} = D_e \frac{\partial^2 B}{\partial z^2} - \sum_{i=1}^{N} k_i X_i B^{\alpha_i}, \quad 0 < z < L, \, 0 < t \tag{1}$$

$$\frac{\partial B(0,t)}{\partial z} = 0, \quad B(L,t) = B_b, \, 0 < t \tag{2}$$

$$B(z,0) = B^0(z), \quad 0 \leqslant z \leqslant L \tag{3}$$

where $\alpha_i = 0$ for zero-order kinetics and $\alpha_i = 1$ for first-order kinetics. The mass balance equations of reactive constituent concentrations are

$$\frac{\partial X_i}{\partial t} = \frac{-k_i X_i B^{\alpha_i}}{Y_{Bi}} + R_i, \quad X_i(z,0) = X_i^0(z),$$
$$0 \leqslant z \leqslant L, \, t > 0, \, i = 1, 2, \ldots, N \tag{4}$$

where Y_{Bi} is the stoichiometric coefficient relating the amount of biocide consumed per constituent i consumed and R_i is the net rate of disinfection transformations.

Case 1. Steady-state reaction with disinfection

To illustrate the theory we will consider a simplification of the general model in which there are only two biomass constituents, X_a and X_d, corresponding to viable and dead bacteria, respectively. Both biomass constituents react with biocide at the same rate; that is, the reaction with biocide is assumed to be independent of viability. This is a plausible assumption for oxidizing biocides. A single disinfection process occurs: the one-to-one transformation of live cells to dead cells. The mass balance equation for biocide concentration is

$$\frac{\partial B}{\partial t} = D_e \frac{\partial^2 B}{\partial z^2} - k_0 (X_a + X_d) B^{\alpha} \tag{5}$$

and the mass balances on bacterial cells are

$$\frac{\partial X_a}{\partial t} = \frac{-k_0}{Y_{Ba}} X_a B^{\alpha} - b X_a B \tag{6}$$

and

$$\frac{\partial X_d}{\partial t} = \frac{-k_0}{Y_{Bd}} X_d B^{\alpha} + b X_a B. \tag{7}$$

These equations can be further simplified by considering the characteristic time scales for the reaction, disinfection and diffusion processes. If the consumption of biomass is slow compared to disinfection ($k_0 / b Y_{B_i} \ll 1$), then the first terms on the right-hand sides of eqs (6) and (7) can be omitted. In this case there is no net loss or gain of biomass and $X_a + X_d = X_0 =$ constant. If, furthermore, the characteristic time for diffusion is small compared to the duration of biocide treatment ($b B_b L^2 / D_e \ll 1$), then the steady-state equation for biocide concentration suffices. The differential equations are reduced to

$$D_e \frac{\partial^2 B}{\partial z^2} - k_0 X_0 B^{\alpha} = 0 \tag{8}$$

and

$$\frac{\partial X_a}{\partial t} = -b X_a B. \tag{9}$$

We will consider the two cases of first- and zero-order reaction kinetics ($\alpha = 1$ and $\alpha = 0$, respectively).

Case 1A. *First-order reaction kinetics*

For a first-order biocide reaction this system is modeled by the dimensionless equations

$$\frac{\partial^2 U}{\partial \zeta^2} - \phi^2 U = 0, \quad 0 < \zeta < 1 \tag{10}$$

$$\frac{\partial U(0)}{\partial \zeta} = 0, \quad U(1) = 1 \tag{11}$$

$$\frac{\partial X}{\partial \psi} = -XU, \quad X(\zeta, 0) = 1, \psi > 0, 0 \leqslant \zeta \leqslant 1 \tag{12}$$

where the dimensionless parameters are

$$U = \frac{B}{B_b}, \quad \zeta = \frac{z}{L}, \quad \phi^2 = \frac{k_0 X_0 L^2}{D_e},$$

$$X = \frac{X_a}{X_0}, \quad \psi = tbB_b.$$

The parameter ϕ is the Thiele modulus which is a measure of the relative rates of reaction and diffusion of the antimicrobial agent.

The solutions to the above equations can be shown to be

$$U(\zeta) = \frac{\cosh(\phi\zeta)}{\cosh(\phi)} \tag{13}$$

and

$$X(\zeta, \psi) = \exp[-\psi U(\zeta)]. \tag{14}$$

Case 1B. *Zero-order reaction kinetics*

For a zero-order biocide reaction the system in dimensionless form is

$$\frac{\partial^2 U}{\partial \zeta^2} - 2\phi^2 = 0, \quad 0 < \zeta < 1 \tag{15}$$

$$\frac{\partial U(0)}{\partial \zeta} = 0, \quad U(1) = 1 \tag{16}$$

$$\frac{\partial X}{\partial \psi} = -XU, \quad X(\zeta, 0) = 1, \psi > 0, 0 \leqslant \zeta \leqslant 1 \tag{17}$$

where the dimensionless parameters are the same as those defined for the first-order case with the exception of the Thiele modulus which is defined by

$$\phi^2 = \frac{k_0 X_0 L^2}{2D_e B_b}. \tag{18}$$

For $\phi \leqslant 1$ the solution to eqs (15) and (16) is given by

$$U(\zeta) = \phi^2(\zeta^2 - 1) + 1 \tag{19}$$

and for $1 \leqslant \phi$

$$U(\zeta) = \begin{cases} 0 & \text{if } 0 \leqslant \zeta < (1 - 1/\phi) \\ (\phi\zeta - \phi + 1)^2 & \text{if } (1 - 1/\phi) \leqslant \zeta \leqslant 1. \end{cases} \tag{20}$$

As in the first-order case,

$$X(\zeta, \psi) = \exp[-\psi U(\zeta)]. \tag{21}$$

Disinfection efficacy

The efficacy of biofilm disinfection has conventionally been assayed by scraping the biofilm off the surface, dispersing the cells, and enumerating survivors by growth on culture media. Usually reported is the ratio of the number of viable cells (per fixed area) after treatment to the number before treatment. The mathematical equivalent in our formulation is the average survival fraction of bacteria.

The average survival fraction (ASF) of bacteria is obtained by integrating X over the thickness of the biofilm,

$$\mathrm{ASF}(\psi) = \int_0^1 \exp[-\psi U(\zeta)]\, d\zeta. \tag{22}$$

We define an efficacy parameter, η_B, by

$$\eta_B(\psi) = \frac{\ln(\mathrm{ASF})}{\ln(\mathrm{ASF}_T)} \tag{23}$$

where ASF_T is the theoretical average survival fraction in the absence of mass transport limitations. This parameter can be viewed as the observed rate of disinfection compared to the rate of disinfection that would prevail for suspended cells: η_B is analogous to the effectiveness factor traditionally employed in analyzing reaction rates in heterogeneous catalysts. For our case

$$\mathrm{ASF}_T = \exp(-\psi) \tag{24}$$

hence

$$\eta_B(\psi) = \frac{\ln(\mathrm{ASF})}{-\psi}. \tag{25}$$

For case 1A we were unable to compute an asymptotic series for η_B as $\phi \to \infty$. However we were able to obtain the following estimates independent of ψ:

$$\frac{1}{\cosh(\phi)} \leqslant \eta_B \leqslant \frac{\tanh(\phi)}{\phi}. \tag{26}$$

It can be shown that $1/\cosh(\phi)$ is the greatest lower-bound for η_B, i.e. this represents the worst possible. For Case 1B

$$\eta_B \approx \frac{1}{\phi\psi} \quad \text{as } \phi \to \infty.$$

Case 2. *Transient reaction*

As a second case we will consider a single reactive constituent, X_r, that is depleted by a reaction that is first order with respect to both biocide and biomass constituent concentrations. This situation is modeled by the following system of equations:

$$\frac{\partial B}{\partial t} = D_e \frac{\partial^2 B}{\partial z^2} - kX_r B, \quad 0 < z < L, t > 0 \tag{27}$$

$$\frac{\partial B}{\partial z}(0, t) = 0, \quad B(L, t) = B_b, t > 0 \tag{28}$$

$$B(z,0) = B_0(z), \quad 0 \leqslant z \leqslant L \tag{29}$$

$$\frac{\partial X_r}{\partial t} = -\frac{k}{Y_{BX_r}} BX_r, \quad X_r(z,0) = X_r^0(z), \quad t > 0, \quad 0 \leqslant z \leqslant L. \tag{30}$$

The equations in dimensionless form are

$$\frac{\partial U}{\partial \tau} = \frac{\partial^2 U}{\partial \zeta^2} - \phi^2 UX, \quad 0 < \zeta < 1, \tau > 0 \tag{31}$$

$$\frac{\partial U}{\partial \zeta}(0,\tau) = 0, \quad U(1,\tau) = 1, \quad \tau > 0 \tag{32}$$

$$U(\zeta,0) = U_0(\zeta), \quad 0 \leqslant \zeta \leqslant 1 \tag{33}$$

$$\frac{\partial X}{\partial \tau} = -\frac{\phi^2}{\rho} UX, \quad X(\zeta,0) = 1, \quad 0 \leqslant \zeta \leqslant 1, \quad \tau > 0 \tag{34}$$

with dimensionless parameters

$$U = \frac{B}{B_b}, \quad X = \frac{X_r}{X_r^0}, \quad \zeta = \frac{z}{L}, \quad \tau = \frac{tD_e}{L^2},$$

$$\phi^2 = \frac{X_r^0 k L^2}{D_e}, \quad \rho = \frac{Y_{BX_r} X_r^0}{B_b}.$$

The parameter ρ is a measure of the reactive capacity of the biofilm.

To obtain simulation data, eq. (22) was numerically integrated using an adaptive Newton–Cotes quadrature rule. The system of eqs (31)–(34) was numerically solved using an explicit finite difference method. Time step sizes were suitably chosen to ensure stability of the difference method.

RESULTS

When a reactive disinfectant is used against a microbial biofilm, the observed rate of disinfection depends strongly on the Thiele modulus as shown in Fig. 1. In the limit of small Thiele modulus ($\phi < 0.3$), disinfection proceeds at essentially the same rate as it would in the absence of any transport limitation. The observed rate of killing declines sharply near $\phi = 1$. This dependence of observed disinfection rate on ϕ can also be expressed in terms of an efficacy factor, which is an analog of the traditional effectiveness factor. This presentation takes the form of η_B vs ϕ plots with ψ as a parameter (Fig. 2). The family of curves have the same limit of $\eta_B \to 1$ as ϕ tends to 0. The curves diverge for $\phi > 1$ with lower efficacy observed for larger ψ. The magnitude of the reduction in the observed disinfection rate can be quite large even for modest values of ϕ. For example with $\phi = 2$ and $\psi = 10$, the observed rate of disinfection is reduced

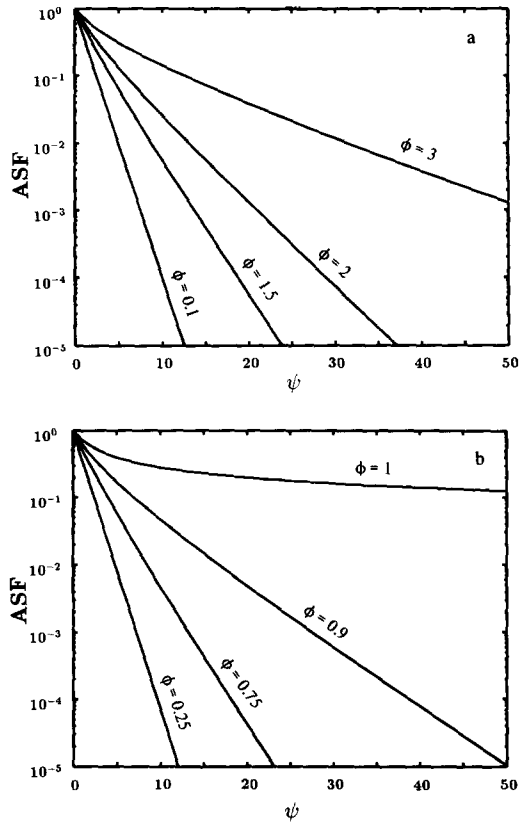

Fig. 1. Average survival fraction as a function of dimensionless time ψ for (a) first-order reaction kinetics and (b) zero-order reaction kinetics.

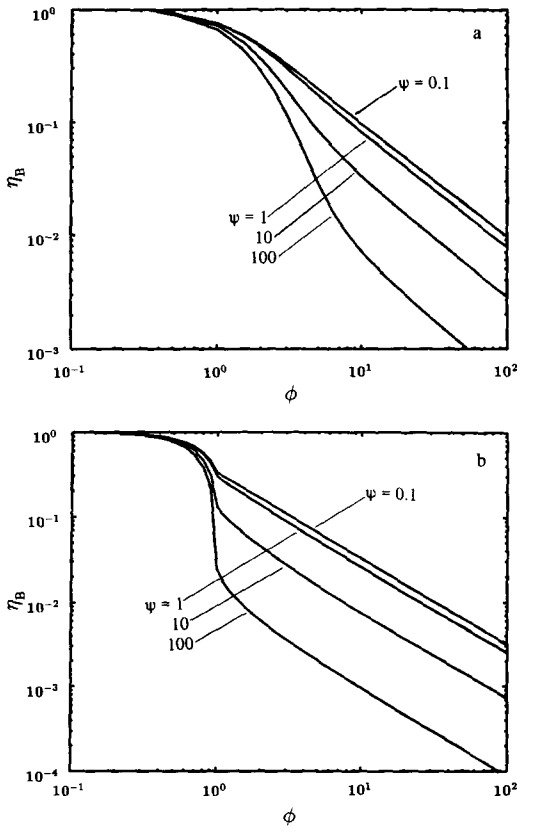

Fig. 2. Biocide efficacy parameter as a function of Thiele modulus for (a) first-order reaction kinetics and (b) zero-order reaction kinetics.

from the intrinsic rate by a factor of 2.7 in the case of first-order reaction kinetics and by more than 20 in the case of zero-order kinetics.

The preceding results (Figs 1 and 2) were based on the assumption of a steady gradient in biocide concentration within the biofilm. If in fact the reaction between biocide biomass results in the depletion of a reactive constituent of the biofilm, then biocide concentrations in the biofilm will continually increase while the concentration of the reactive biomass steadily decreases. This transient situation is depicted for an illustrative case in Fig. 3.

One way to quantify the time required for biocide to permeate the biofilm in this case is to measure the time required for the antimicrobial agent concentration at the base of the biofilm to reach a specific level. We define a dimensionless penetration time, τ_{50}, as the time required for the biocide concentration at the substratum to attain 50% of its bulk fluid concentration divided by the time required for this degree of penetration in the absence of biocide reaction ($0.38L^2/D_e$). This ratio is plotted as a function of

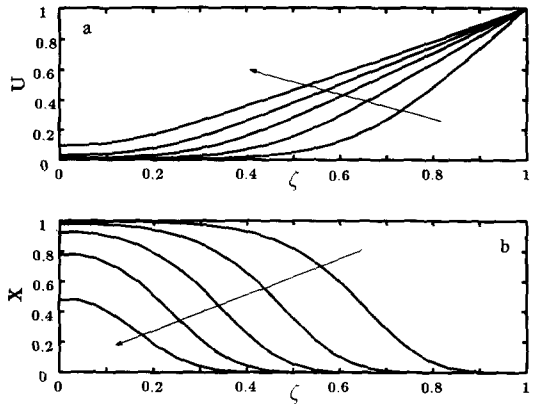

Fig. 3. Transient biocide concentration (a) and biomass concentration (b) profiles with $\phi = 10$ and $\rho = 10$ at times $\tau = 0.85, 1.7, 2.55, 3.4, 4.25$. The arrow indicates increasing time.

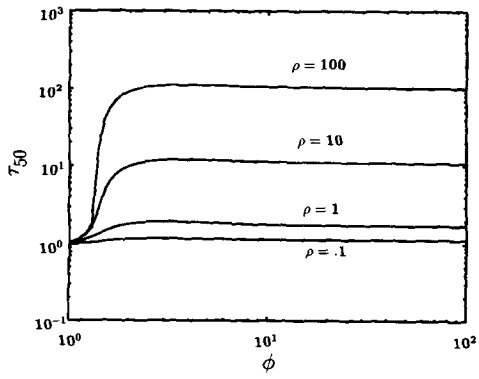

Fig. 4. Dimensionless biocide penetration time, τ_{50}, as a function of Thiele modulus and biofilm reactive capacity.

ϕ and of the biofilm reactive capacity, ρ, in Fig. 4. For $\rho < 1$, the penetration time is within a factor of 2 of the time for transient diffusion alone. For $\rho > 1$ and $\phi > 2$, penetration time exceeds the transient diffusion time in direct proportion to ρ. Penetration time is largely independent of Thiele modulus for ϕ greater than approximately 2.

DISCUSSION

We have analyzed the disinfection capacity of a reactive biocide or antibiotic against microbial biofilms and found that antimicrobial efficacy decreases sharply as the Thiele modulus exceeds one. This reduction in efficacy worsens when the antimicrobial dose is more concentrated or longer (i.e. ψ is larger). In practical situations, it is estimated that the magnitude of ψ is of the order of 10 or greater. The theory suggests that reductions in antimicrobial efficacy of an order of magnitude or more arise easily. These results provide a theoretical basis for explaining experimentally observed resistance of biofilm microorganisms to chemical disinfectants.

There are few experimental studies in which sufficient information is reported to be able to estimate the magnitude of the Thiele modulus. In three cases summarized in Table 1, estimates of ϕ range from approximately 0.5 to 20, indicating the potential for significant reduction of antimicrobial efficacy against biofilm. The antimicrobial agents in the examples range from an enzymatically hydrolyzed β-lactam antibiotic to a powerful oxidizing agent, free chlorine. It is unfortunate that the vast majority of experimental studies report only disinfection data. Data on the degradation kinetics of the antibiotic are essential to evaluate reaction–diffusion limitations.

An antimicrobial agent that reacts with the biofilm stoichiometrically rather than catalytically will eventually penetrate the biofilm by depleting the biomass. The time scale for this penetration, however, can exceed the time scale for transient diffusion alone by an order of magnitude or more. For example, de Beer *et al.* report a time series of microelectrode measurements of chlorine concentration profiles in biofilm that nicely mirror the theoretical results shown in Fig. 3(a) [their Fig. 3(b)]. From these experimental results the time needed for the biocide concentration at the substratum to reach 50% of the bulk fluid concentration was estimated to be approximately 50 min. The corresponding time for transient diffusion alone was estimated to be approximately 0.5 min. The ratio of these times yields a τ_{50} (see Fig. 4) value of 100. The theory presented here may be useful for estimating appropriate dose durations.

If indeed a reaction–diffusion mechanism is responsible for limiting antibiotic efficacy in many instances, then the process of selecting antimicrobial agents for use against biofilms must be rethought. The efficacy of the antimicrobial agent will, in this scenario, be influenced not only by the strength of its action as a disinfectant, but also by its reactivity with the target microbial system. It is possible in comparing two

Table 1. Estimated Thiele moduli for reactive antimicrobial agents in experimental biofilm systems

System	Assumed kinetic order	D (m^2/s)	k_0X_0 or† k_0X_0/B_b (s^{-1})	L (m)	ϕ	Source
Tobramycin vs *P. aeruginosa*	First	3.8×10^{-10}	3×10^{-4} 9×10^{-2}	5×10^{-4}	0.44 7.7	Nichols *et al.* (1989)
Chlorine vs *P. aeruginosa* and *K. pneumoniae*	First	1.4×10^{-9}	2.3×10^{-1} 1.2×10^{1}	2×10^{-4}	2.6 18.2	de Beer *et al.* (1994)
Monochloramine vs *P. aeruginosa*	Zero	1.9×10^{-9}	3×10^{-1} 2.4×10^{-1} $>1.7\times10^{-1}$	3.5×10^{-5}	0.76 1.2 >1.7	Griebe *et al.* (1993)

† The first formulation applies for first-order kinetics, the second for zero-order kinetics.

biocides that the weaker disinfectant may be more effective against biofilms if it is also considerably less reactive. Exactly this scenario may explain experimental reports of superior performance of monochloramine over free chlorine in biofilm systems (Griebe *et al.*, 1993; LeChevallier, 1990; LeChevallier *et al.*, 1988).

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NOTATION

ASF average survival fraction
b_i, b disinfection transformation rate parameter, $L^3M^{-1}T^{-1}$
B biocide concentration, ML^{-3}
B_b biocide concentration in bulk liquid compartment, ML^{-3}
B^0 initial biocide concentration, ML^{-3}
D_e effective biocide diffusivity in biofilm, L^2T^{-1}
k_i reaction rate parameter, $L^3M^{-1}T^{-1}$
L biofilm thickness, L
R rate of disinfection transformation, $ML^{-3}T^{-1}$
t time, T
U dimensionless biocide concentration
X_i reactive constituent concentration, ML^{-3}
X_i^0 initial concentration of reactive constituent, ML^{-3}
X dimensionless reactive constituent concentration
Y_{Bi} stoichiometric coefficient, MM^{-1}
z distance coordinate, L

Greek letters

α, α_i kinetic exponents
ζ dimensionless spatial variable
η_B efficacy parameter
ρ biofilm reactive capacity
τ dimensionless time variable for case 2
τ_{50} dimensionless penetration time
ϕ Thiele modulus
ψ dimensionless time variable for case 1

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