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HYDRODYNAMICS AND KINETICS IN BIOFILM SYSTEMS – RECENT ADVANCES AND NEW PROBLEMS

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ABSTRACT

Application of microelectrode techniques, Nuclear Magnetic Resonance Imaging, and Confocal Laser Microscopy permitted analysis of hydrodynamics, kinetics, and internal structure in biofilm systems. The commonly accepted concept of one dimensional diffusion through a three compartment model (bulk solution, biofilm, and substratum) requires revision based on recent progress in understanding the internal structures of biofilms. Biofilms seem to form three dimensional porous structures with a network of interstitial voids filled with water, forming a network of channels connected with each other and with the biofilm surface. The basic unit of this structure appears to be a bacterial cluster (sometimes called microcolony).

KEYWORDS

Biofilm; hydrodynamics; Nuclear Magnetic Resonance Imaging; Confocal Laser Microscopy; microelectrodes.

INTRODUCTION

Transport of dissolved substrates to the biofilm surface is usually simplified to a one dimensional representation, where the substrate from the bulk solution crosses the boundary layer and enters the biofilm zone where it is consumed. Such a convenient model was always obscured by difficulty in determining the position of the biofilm-bulk water interface and lack of a theoretical description of flow over viscoelastic biopolymers. Consequently, the calculated kinetic parameters from dissolved substrate concentration profiles across the biofilm systems were always uncertain. Recently, new doubts were added to this picture. Laser Confocal Microscopy shows biofilms as three dimensional, porous structures with microbial clusters surrounded with a network of channels filled with water. This clearly contradicts the notion that biofilms are continuous and raises the question of what is actually represented by the diffusivity coefficient in biofilms.

Biofilm reaction kinetics. Microelectrodes are thus far the most accurate instruments for evaluating the kinetics of the chemical reactions in biofilm systems. Microsensors, usually in the form of microelectrodes, are increasingly popular (Whalen et al., 1969; Bungay et al., 1969; Revsbech and Jorgensen, 1986; Riethues et al., 1986; Baumgartl, 1987). Advanced biofilm research depends upon sensor miniaturization. A microelectrode with tip diameter less than 10 micrometers is manipulated within the biofilm system measuring the solute concentration at predetermined intervals. The result is a profile of substrate concentration versus distance. The shape of the profile is determined by

1 - microbial substrate uptake rate which is a function of microorganism concentration and their affinities for the substrate,
2 - substrate transport rate through the film which depends on substrate diffusivity through the biofilm,
3 - substrate transport rate to the biofilm which is a function of microbial substrate uptake rate, substrate diffusivity through the water and hydrodynamics near the biofilm surface.

The measured profile provides data to calculate kinetic parameters of the microbial reaction and the local microbial activity. The kinetic parameters of the microbial reaction are, however, buried in a complex network of relationships and have to be carefully extracted. Procedures for doing so are already available.

A profile of substrate concentration across a biofilm system consists of profiles in the bulk water and in the biofilm. The diffusivity difference between the water and the biofilm leads to a discontinuity in the substrate concentration profile at the biofilm surface (Lewandowski et al., 1990). This discontinuity arises because the mass transfer of substrate in the water and the biofilm are governed by different equations that are tied together by the requirement that the substrate flux at the fluid/biofilm interface be continuous at steady state. Each part of the concentration profile contains different information and should be analyzed separately.

Substrate profile above the biofilm surface. Each point of a concentration profile reflects an equilibrium between substrate transport and consumption. The transport of substrate in moving liquid is governed by molecular diffusion and convection. Transport of substrate to the biofilm interface is due to the combination of these processes. In stagnant water, transport of substrate would be almost entirely due to molecular diffusion, neglecting any contribution from natural convection. When the water starts to flow, convection exceeds diffusion. The substrate concentration gradient is maximum at the biofilm surface, where the flow velocity is minimum, turbulence is absent, and transport is entirely due to molecular diffusion.

The substrate concentration profile above the biofilm surface can be described by the following exponential equation

$$\frac{C - C_s}{C_b - C_s} = 1 - \exp(-Bx) \tag{1}$$

where C is the local substrate concentration, C_b is the bulk substrate concentration, C_s is the concentration substrate at the biofilm surface, B is an experimental coefficient, and x is distance from biofilm surface (Lewandowski et al.,1993).

Substrate concentration profile below the biofilm surface. Substrate concentration profile below the biofilm surface is influenced by the molecular diffusivity and by the microbial reaction rate. Assuming one dimensional diffusion the profile of substrate in the biofilm is described as:

$$\left(\frac{dC}{dx}\right)_f = \sqrt{2\frac{V_{max}}{D_f}\left(C - C_o - K_s * Ln\,\frac{K_s + C}{K_s + C_o}\right)} \tag{2}$$

where D_f is the diffusion coefficient for the substrate in the biofilm, C is the substrate concentration at a point x, V_{max} and K_s have the usual meaning in the Michaelis-Menten equation, C_s is substrate concentration at the biofilm surface, and C_o is substrate concentration at the substratum surface.

Extracting kinetic parameters from concentration profiles. Figure 1 presents essential steps to extract

the kinetic parameters from a substrate concentration profile. The profile is first divided in two parts: water and biofilm. The usual procedure fixes the biofilm surface position at the inflection point of the concentration profile. A more precise determination uses a separate optical sensor (Lewandowski et al., 1990) to measure the position of this interface. Profile in the bulk solution is fitted to Equation 1 and the slope of the profile at the biofilm surface is calculated.

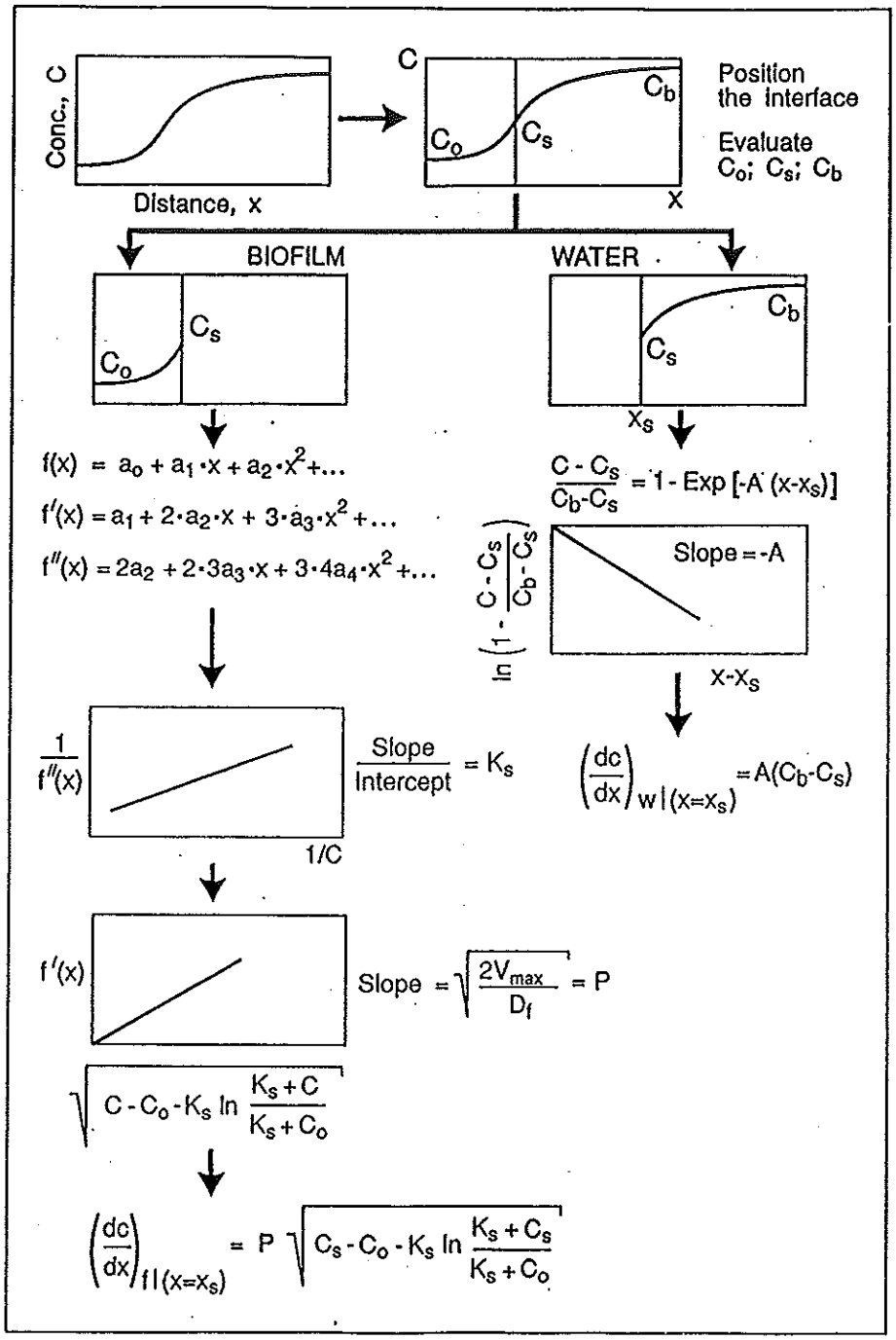

Figure 1. Extracting kinetic information from substrate concentration profiles.

The profile below the biofilm surface is described by Equation 2. This is, however, still in differential form and a simplified procedure is used to find the coefficients. The profile is approximated by a third order polynomial from which the first and the second derivatives are calculated. The slope at the biofilm surface is calculated from the biofilm side. This slope is compared with the slope calculated from the bulk solution side. The ratio of these slopes is equal to the ratio of diffusivities in the water and in the biofilm. Since the diffusivity in water is known or can be easily measured the only unknown is the diffusivity in the biofilm. The half saturation coefficient K_s is calculated graphically from the

following equation which relates diffusion with reaction:

$$\left(\frac{d^2C}{dx^2}\right)^{-1} = \frac{D_f * K_s}{V_{max}} \frac{1}{C} + \frac{D_f}{V_{max}} \quad (3)$$

The numerical example is presented elsewhere (Lewandowski, 1993).

Flow velocity near biofilm surface NMRI. Previously, we measured velocity in several bioreactors (Lewandowski, et al., 1992; Lewandowski, et al., 1993) using NMR phase methods (Caprihan and Fukushima, 1990). These studies showed the feasibility of the new techniques and demonstrated that the flow produced in some reactors is much more complicated than one might initially expect. We also learned that the microelectrodes do not introduce large disturbances into the flow. Our most recent experiments used a reactor of rectangular cross-section (2.0 cm wide by 0.25 cm deep by 38 cm long). We designed an NMR velocity imaging sequence with anisotropic spatial resolution which produced images with approximately 80 pixels spanning the 0.25 cm depth of the reactor. The velocity field develops as a viscous flow as expected, and is fully developed at our test section, 18 cm from the inlet. For Re_h (Vh/v, based on average velocity V, kinematic viscosity v and reactor depth h) ranging from 30 to 58, the flow was essentially uniform across the width of the reactor and parabolic across the depth. Thin viscous layers appeared at the side walls so that the velocity field satisfied the no-slip condition along the entire boundary, as expected. Measurements during day 3 and day 4 of biofilm growth were made at this location.

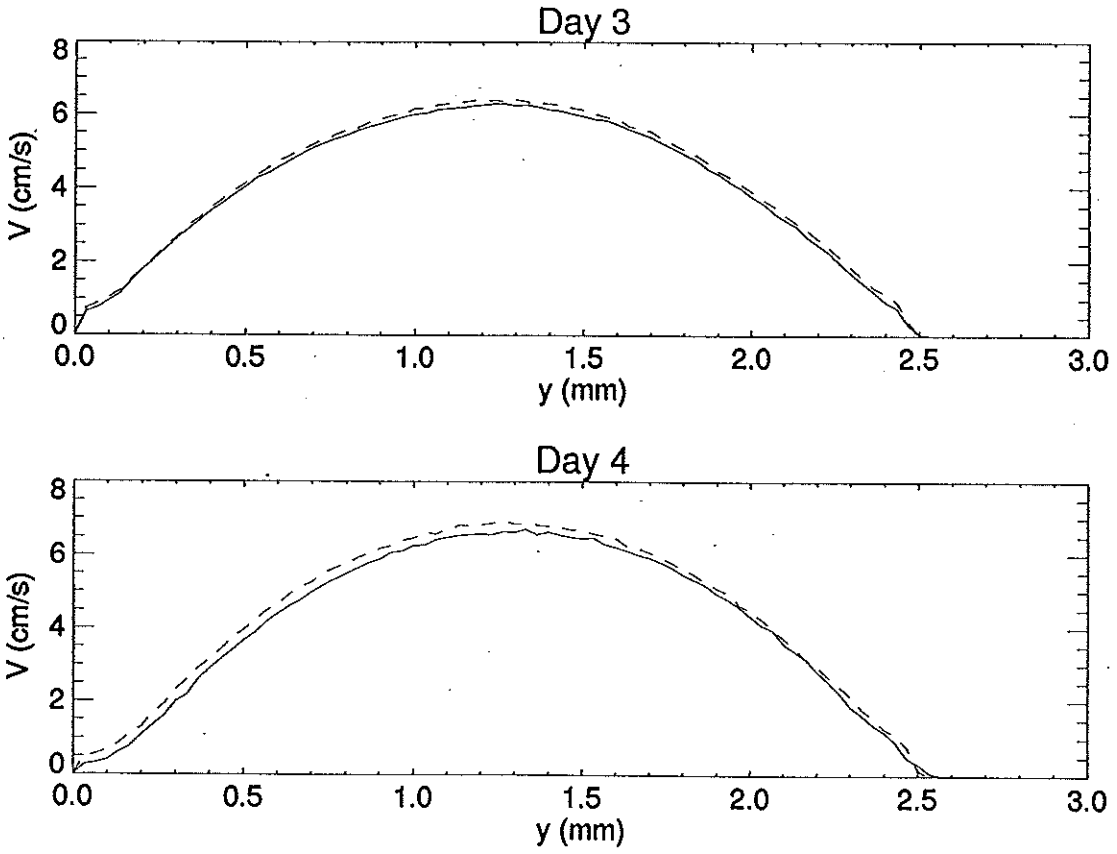

Figure 2. NMR Velocity profiles across the 2.5 mm depth to the bioreactor.

Figure 2 shows average velocity profiles for the left half (solid lines) and the right half (dotted lines) for day 3 and day 4. The bottom of the channel is labeled y=0. Small non-uniformities ("kinks") are apparent near the reactor walls. The distance from the reactor substratum to the kinks, which is of the order of the expected thickness of the biofilm, increased from day 3 to day 4. Comparison of the

profiles near the bottom shows that there is a thicker layer of retarded flow on day 4. As previously described, the flow velocity distribution near biofilm surface can be described by an exponential function

$$\frac{v}{v_b} = 1 - \exp(-Ax) \qquad (4)$$

where x is the distance measured from the biofilm surface.

Hydrodynamics and kinetics near biofilm surfaces. The concentration profile is simultaneously influenced by hydrodynamics and kinetics. Figure 3 shows Equations 1, 2, 3, and 4 in a model biofilm system. The question mark at the biofilm surface indicates the uncertainty of the velocity profile near the biofilm surface.

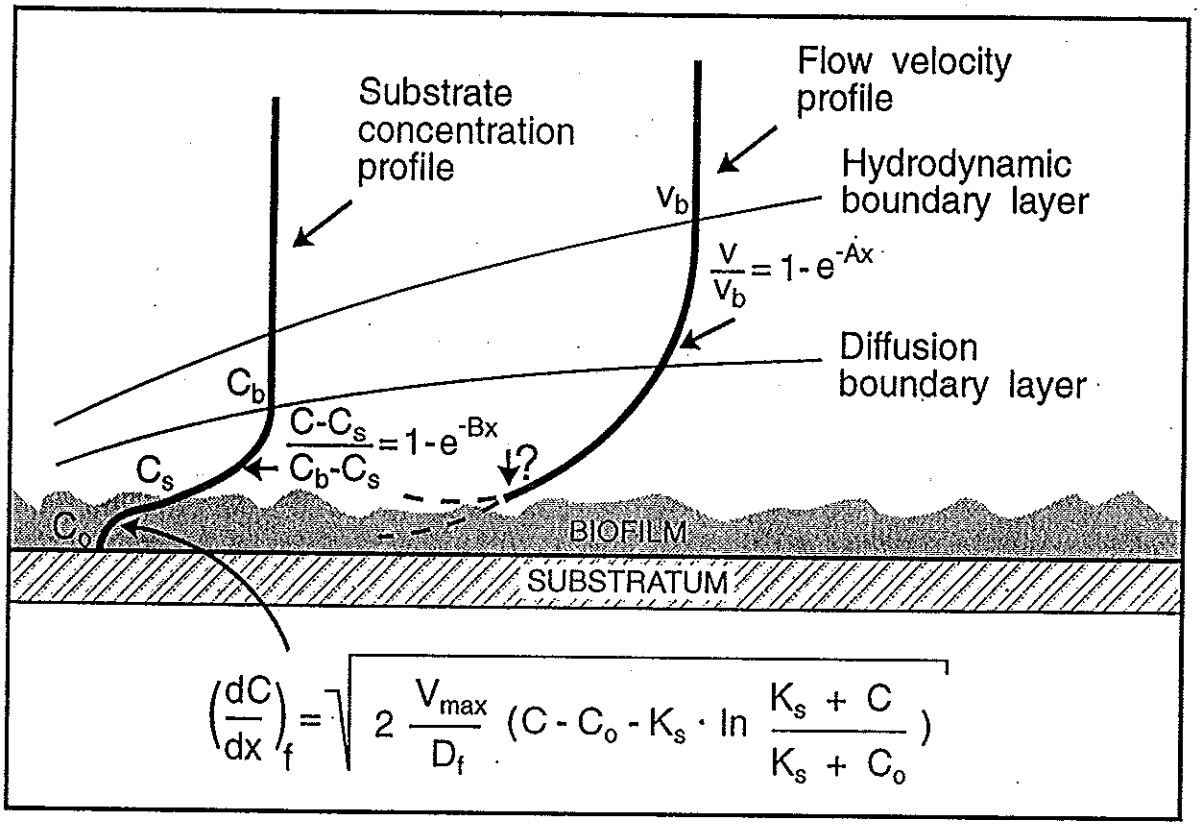

Figure 3. Hydrodynamics and kinetics in a biofilm system.

Internal structure of biofilms. It is well known that the intrafilm kinetic measurement results vary from place to place (Lewandowski et al., 1991). The accepted explanation for this fact was that the microorganisms in the biofilm are non-uniformly distributed. Recent application of Confocal Laser Microscopy to study the biofilm structure shows that the biofilms form a porous, three dimensional structure. The structure includes a network of interconnected channels. An example of such structure is presented in Figure 4. A slice of a biofilm is 26 micrometers from the substratum by Laser Confocal Microscopy. Total thickness of the biofilm was evaluated as 140 micrometers. The dark areas in Figure 4 represent microbial clusters and the light areas the interstitial voids. The interstitial voids form a network of channels connected with the bulk liquid.

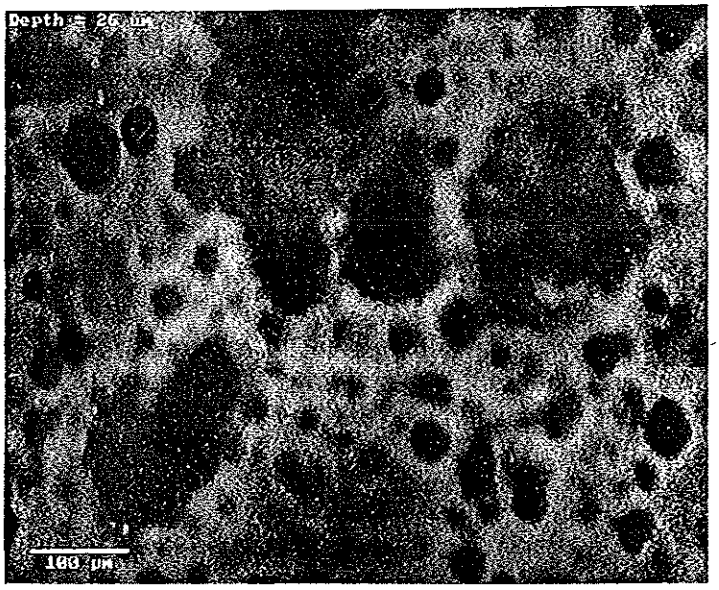

Figure 4. Section of biofilm imaged by a Laser Confocal Microscope. The biofilm thickness was 140 micrometers. The presented section was at a distance of 26 micrometers from the substratum.

DISCUSSION AND CONCLUSIONS

The hydrodynamics and kinetics in biofilm systems are intimately related by the fact that most biofilm reactions are diffusion limited. Consequently, the shapes of the concentration gradients are determined by the molecular diffusivity and convection. Simplifying assumptions permit the calculation of kinetic parameters from microelectrode measurements while hydrodynamic parameters are obtained from Nuclear Magnetic Resonance Imaging measurements. The calculations require the assumptions that the biofilm is continuous (can be described by a single diffusion coefficient) and that the biofilm surface can be precisely localized. Recent investigation of the biofilm internal structure using Confocal Laser Microscopy indicates that biofilms are porous structures consisting of microbial clusters separated by interstitial voids. It is not clear to what extent this fact influences the transport of solute to the biofilm. The obvious implication is that the biofilm surface is much more developed than the surface of the substratum can indicate. The diffusivity coefficient through biofilms measured by many authors reflected the diffusivity through the pores and channels rather than through the microbial clusters. It is imperative to investigate the nature of the microbial clusters.

It is inviting to identify the "kinks" observed in the NMR velocity profiles with some feature of the biofilm, but this correspondence has not been established,yet. The slice thickness in the longitudinal direction was 5 mm, so each pixel in the NMR image represents an average of the conditions existing over this thickness. At a location near one of the reactor walls, this 5 mm distance may be partially filled with biofilm (and the fraction of the slice inhabited may change with the flow condition). This implies that velocity components represent an average over the slice thickness, so that an answer to the question "Is there flow in the biofilm?" cannot be given without precise knowledge of the spatial distribution of the biofilm. [Preliminary NMR data roughly indicate the spatial distribution based on the difference in the nuclear spin-lattice relaxation time T1 of water in and out of the film. The relatively poor spatial resolution of our low field apparatus limits the usefulness of this technique for now.] The current results do show that there is motion of the bulk solution at a level which is partially occupied by the biofilm.

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