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BIOFILM SURFACE POSITIONING

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Abstract.

A method is described by which the position of the interface between a biofilm and the bulk fluid can be determined. This method is performed using a 5 micrometer oxygen microelectrode coupled with a 10 micrometer optical density sensor. This probe simultaneously measures oxygen concentration and optical density at 2.5 micrometer intervals within a biofilm. The data obtained permits superimposing the biofilm surface on a dissolved oxygen profile.

Introduction.

Advanced biofilm research depends upon sensor miniaturization. Microsensors, usually in the form of microelectrodes, are increasingly popular for biofilm research (Whalen et al.,1969, Bungay et al., 1969, Revsbech and Jorgensen, 1986, Riethues et al, 1986, Revsbech, 1989, Lewandowski et al. 1989). Microelectrodes with tip diameters of less than 10 micrometers are the most accurate instruments for measuring the kinetics of biofilm chemical reactions. The electrodes, to effectively take measurements and not disturb the biofilm structure, must be reduced to tip diameters of less than 10 micrometers, since biofilm thicknesses are typically in the range of a few to a few hundred micrometers. Figure 1 shows a dissolved oxygen microelectrode penetrating a biofilm. Application of microelectrodes allows in situ measurements of ionic and dissolved gas concentration profiles in biofilms. Chemical reactions in biofilms are usually diffusion controlled and the result of microsensor measurement is a profile of concentration across the biofilm.

Technical problems associated with manufacturing sensors with tip diameters in the range of micrometers, problems with their movement through the biofilm, and difficulties with measuring very small currents or potential changes often

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Figure 1. Dissolved oxygen microelectrode penetrating a biofilm.

overwhelm questions associated with interpretation of the obtained results.

There has been no analytical procedure for incorporating results of measurements into existing biofilm models. Without such procedures the profiles remain only pictures obtained with great effort, using time and money consuming techniques. The most serious limitation of any theoretical consideration is the uncertainty in locating the biofilm-bulk water interface using the profile of substrate concentration alone. The result of the measurement is a function of concentration versus distance. For theoretical applications, a function of concentration versus biofilm depth is needed. However, concentration profiles have had no reference point to compare one profile to another. Using the biofilm-substratum interface as a reference point will not work if the substrate is depleted before this position. Another reference point is the biofilm-fluid interface. To accurately describe concentration changes as a function of biofilm depth, the surface of the biofilm must be superimposed on the substrate concentration profile.

A commonly accepted, idealized model of a biofilm by Williamson and McCarty (1976) describes the biofilm system as a three layer model: bulk liquid, diffusion layer and biofilm. The flux of substrate across the diffusion layer is described as:

$$J_o = \frac{D}{L}(S - S_o) \quad (1)$$

where:
J_o = substrate flux through the diffusion layer, $mg.cm^{-2}.h^{-1}$
S_o = substrate concentration at the surface of the biofilm, $mg.l^{-1}$
S = bulk liquid substrate concentration, $mg.cm^{-3}$
L = thickness of the diffusion layer, cm
D = diffusion coefficient for the substrate in the bulk liquid, cm2.h-1

The steady state substrate flux into a biofilm is achieved when consumption in the film is equal to the rate of transport due to molecular diffusion:

$$D_x \frac{d^2S_x}{dz^2} = \frac{u}{Y} X_x \quad (2)$$

where:
D_x = diffusion coefficient for the substrate within the biofilm, cm^2h^{-1}
S_x = substrate concentration at a point x within the biofilm, $mg.cm^{-3}$
u = microorganism's specific growth rate, h^{-1}
z = distance between the biofilm surface and point x, depth, cm
Y = microbial yield
X_x = biofilm concentration (or density), $mg.cm^{-3}$

Equations (1) and (2) both assume existence of a distinguishable biofilm surface. Equation (2) also assumes that the substrate diffusion coefficient does not vary within the biofilm, which further implies uniform distribution in terms of the biofilm density. A biofilm is actually a highly heterogenous material, as proven by many published scanning electron microphotographs; however, the assumption that the biofilm is homogeneous is a necessary simplification.

Surface location.

An attempt to describe the dimensions of the diffusive boundary layer based on a dissolved oxygen profile has been made by Jorgensen and Revsbech (1985). The authors analyzing the shape of the dissolved oxygen profile in sediments and detritus used a rather simplified technique to determine the position of the surface: "... as the electrode tip was hardly visible in the water, the exact level where the electrode first touched the solid surface during an oxygen profile measurement had to be determined by slightly vibrating the electrode; the surface was then defined as the level where surface particles started to vibrate as well."

Our effort to locate the biofilm surface utilizing only a substrate microelectrode has led to a mathematical analysis of the transport processes. A material balance at the biofilm-liquid interface will show the flux of substrate out of the bulk water must be equal to the flux of substrate into the biofilm at steady state.

In the water phase, transport of substrate is governed by equation 3.

$$D \frac{d^2S_x}{dz^2} = 0 \qquad (3)$$

In the biofilm equation 2 holds. The diffusivity of a substrate in the biofilm is in general not equal to the diffusivity in water. This diffusivity difference and substrate utilization in the biofilm lead to a discontinuity in the substrate concentration profile at the biofilm surface. This discontinuity arises because the mass transfer of substrate in the fluid and the biofilm are governed by different equations tied together by the requirement that the substrate flux at the fluid-film interface be continuous at steady state. Thus, the substrate profile will be piecewise continuous with a discontinuity at the fluid-film interface.

With discrete data, as taken with the dissolved oxygen microelectrode, a small discontinuity in the oxygen profile is difficult to find. However, by smoothing the data and taking the first derivative, the location of the discontinuity can be determined (Figure 2).

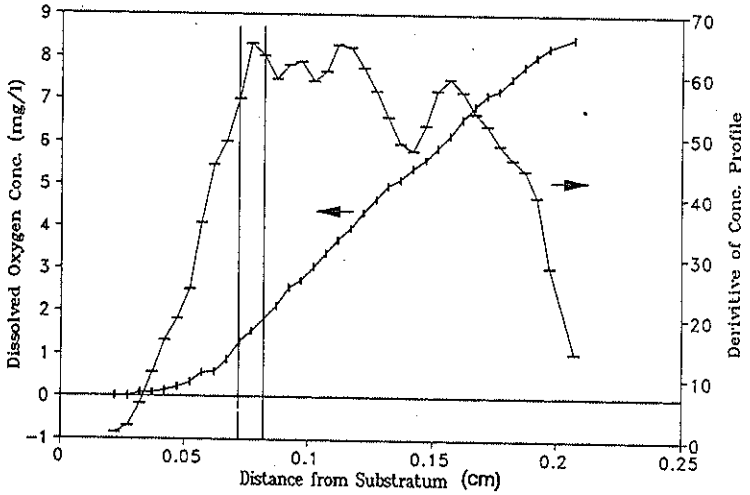

Figure 2. Substrate profile and first derivative. The vertical lines indicate the position of the discontinuity and consequently the biofilm surface.

Experiments by Wittler et al. 1986, utilizing microorganisms immobilized in pellets may require reinterpretation based on this procedure. The dissolved oxygen profile inflection point (discontinuity) was always found to be inside the pellet and not at its surface. Wittler, however, positioned the pellet surface using a dissolved oxygen microprobe. He "drained the medium to just under the

apex point of the pellet, then the microprobe mounted on a micromanipulator was lowered until an increase in current was sensed when the probe just touched the wet pellet". This description suggests the authors detected the water surface attached to the pellet rather than the pellet surface itself. This may explain the shift in dissolved oxygen profile inflection point toward the center of the pellet.

Methods.

We have chosen to locate the biofilm surface with an independent measurement. A simultaneous penetration of the biofilm with a microsensor measuring the substrate concentration profile and a microsensor detecting changes in optical density was undertaken to locate the biofilm surface. The combined probe consists of a dissolved oxygen microelectrode of tip diameter 5 micrometers, and a single fiber, fiber optic cable, tapered to a tip diameter of 10 micrometers. The two sensors were coupled with their tips at the same level (Figure 3). The fiber optic sensor monitored changes in frequency modulated infrared light (940 nm) penetrating the biofilm through a transparent substratum. The probe was mounted on a motorized micromanipulator and penetrated the sample at 2.5 micrometers per second after which there was 2.5 second period during which the measurements of light intensity and dissolved oxygen concentration were made. The instruments were interfaced to a computer data acquisition system and the profiles of dissolved oxygen concentration and changes in light absorption were simultaneously displayed on the screen and recorded. Based on the measured changes in light intensity, the biofilm surface and the substratum surface were located. Superimposing biofilm and substratum surfaces on the dissolved oxygen profile allows for the description of changes in dissolved oxygen concentration as a function of biofilm depth.

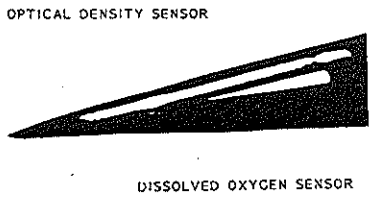

Figure 3. Microprobe for measuring both optical density and dissolved oxygen concentration.

Interpretation of Results.

Based on the measured changes in optical density, the biofilm and substratum surfaces were identified. Since the tips of the sensors were level, the biofilm surface and the substratum surface could be located in the dissolved

oxygen profile. An example of interpreted data is presented in Figure 4. Two abrupt changes in the light intensity profiles can be seen. The first, marked ("1"), appears where the probe enters the biofilm, the second, marked ("2"), appears where the probe touches the substratum. When the probe enters the biofilm a change in optical density is recorded. When the probe touches the substratum the fiber optic sensor bends slightly, which is detected as a decrease in light intensity. Point "2" was identified as the substratum surface and the light intensity recorded at this position was accepted as the incident radiation intensity for further calculations.

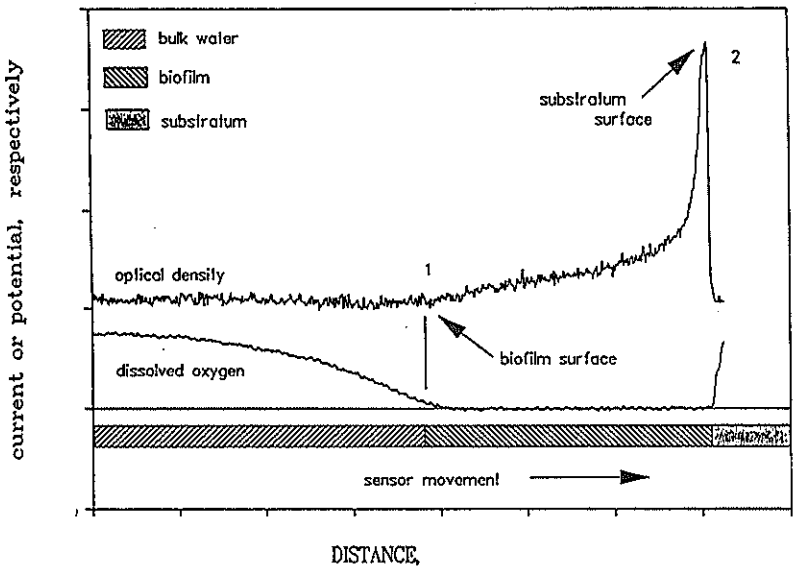

Figure 4. Interpretation of data used to locate the biofilm-substratum interface.

Figure 5 presents the data collected by the data acquisition system. The results of the optical density measurements were calculated according to Lambert's law: $\log(I_o/I)=EL$, where I_o is the intensity of the incident radiation (maximum radiation monitored at place "2", Figure 2), I is the emergent intensity, E is the extinction coefficient and L is the layer thickness. The calculated $\log(I_o/I)$ values were presented as a function of the distance (L). The dissolved oxygen concentration was calculated based on the microelectrode calibration curve.

Typical Results of Measurement.

Typical results of measurements calculated according to the above procedures are presented in Figure 5. The dissolved oxygen concentration in the investigated system decreased from 8 mgl^{-1} in bulk water to zero in biofilm.

The biofilm surface was detected at a distance of 0.7 mm from the substratum. Dissolved oxygen concentration at the biofilm surface was 1.2 mgl^{-1}. Diffusion layer thickness was 1.33 mm. The decrease in dissolved oxygen concentration within the diffusion layer was 6.8 mgl^{-1}.

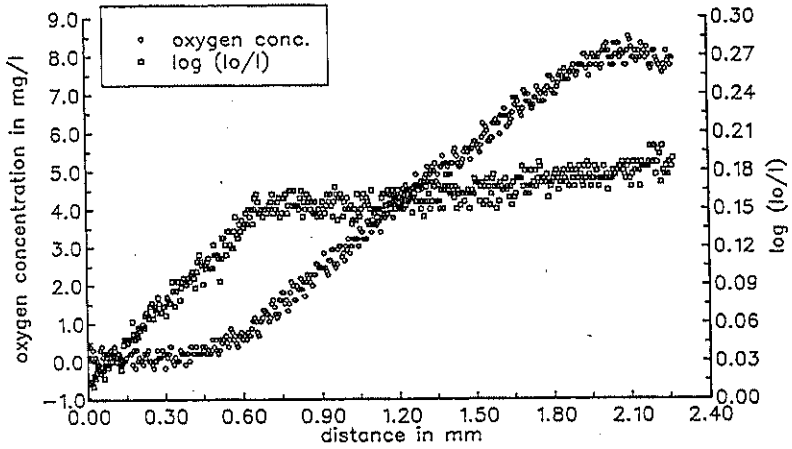

Figure 5. Dissolved oxygen concentration and calculated log(Io/I) with distance.

Conclusions and implications.

The position of the biofilm surface has been located on a dissolved oxygen concentration profile. The microprobe simultaneously measures the substrate concentration profile and the optical density profile across a biofilm. The optical density profile allows detection the biofilm surface. Superimposing the position of the biofilm surface on the dissolved oxygen profile allows determination of the oxygen concentration at the biofilm surface. Further utilization of the results is possible. The substrate profile within the biofilm can be separated from the substrate profile in the fluid. Based on this, theoretical models describing diffusion-controlled reactions can be solved. Substrate flux, diffusion coefficient and utilization rate in microbial films can be calculated. The procedure can be applied for any chemical compound, ion or dissolved gas for which a concentration profile can be measured across the biofilm. Availability of the microsensors is the obvious limitation of the procedure. No simplifying assumptions are necessary to get the raw concentration profiles and locate the biofilm-fluid interface. The measurement is non-destructive and can be totally automatic.

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