Dissolved oxygen gradients near microbially colonized surfaces

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Section III
BIOCORROSION

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Zbigniew Lewandowski

I. INTRODUCTION

Microorganisms frequently colonize inert water-immersed surfaces, forming biofilms that range in thickness from several to a few hundred micrometers. The microorganisms bind to the surfaces by means of exopolysaccharide polymers. From the initial growth stage, biofilms remain tightly bound to their host surfaces. The cells divide, forming sister cells bound within the polymeric matrix. The process leads eventually to formation of a continuous biofilm on the colonized surface. The biofilms alter the near-surface environment by affecting both the bulk media and the growth surface (substratum). The result is a complex network of reactions which may have many consequences, including biodeterioration of the surface.

The magnitude of chemical environment modification near the biofilms is directly related to the biofilm substrate uptake rate. Experimental techniques for determining biofilm reaction kinetics depend on two types of analyses: (1) chemical analysis of bulk water and (2) chemical measurements inside the biofilm using microsensors. Biofilm systems are diffusion limited, and chemical conditions near and inside biofilms can vary dramatically over a distance of only a few micrometers. Consequently, the information obtained from analysis of bulk water is limited and must be closely scrutinized before drawing any conclusions with regard to the biofilm.

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Direct chemical measurements inside biofilms are severely restricted by the nature of the system. First, because biofilm thickness is usually quite small relative to the lateral surface dimensions, the space available for intrafilm instrumentation measurements is limited. Second, since biofilm respiration is diffusion limited, the substrate concentration varies across the film, forming concentration profiles. Each chemical constituent which is consumed or produced in the film forms a separate diffusivity-related profile. The heterogeneous and anisotropic nature of biofilm is enhanced by the fact that these films may be colonized at different locations by different microbial species. These spatial variations in surface coverage are particularly interesting when microorganisms colonize a metal substratum, which may result in the formation of local corrosion cells and contribute to a phenomenon called microbially influenced corrosion (MIC).

This chapter presents a technique of extracting kinetic parameters from substrate concentration profiles. The inherent heterogeneity of the biofilms causes the profiles taken at different locations to be different. Therefore, the kinetic parameters such as reaction rate, diffusivity, and half-saturation coefficient, evaluated at different locations, may contribute to describing this heterogeneity.

II. SUBSTRATE CONCENTRATION PROFILES USING MICROELECTRODES

Ion-selective and gas-sensing microelectrodes with tip diameters less than 10 μm are used for direct measurements of chemical constituents in biofilms. Advanced biofilm research depends upon sensor miniaturization. Microsensors, usually in the form of microelectrodes, are increasingly popular.[1–5] Microelectrodes are thus far the most accurate instruments for measuring the concentration profiles in biofilm systems. To make measurements effectively without disturbing the system to any significant extent, electrode tip diameter must be 10 μm or less. The size of the tip must be small because the electrode should penetrate the biofilm without physical damage to its structure. Larger electrodes would produce a dent in the biofilm, causing bulk water to penetrate and influence the measurement.

The dissolved oxygen (DO) profiles analyzed in this chapter were taken from a continuous-flow, open-channel reactor. A mixed-population biofilm was growing on a metal surface. Part of the deposits were abiotic corrosion products. The DO electrode was made of a 0.1-mm high-purity (99.99%) platinum wire etched electrochemically (with one end in KCN) to a tip diameter of about 2 μm. The wire was rinsed with concentrated HCl and ethanol and covered with soda-lime glass. The

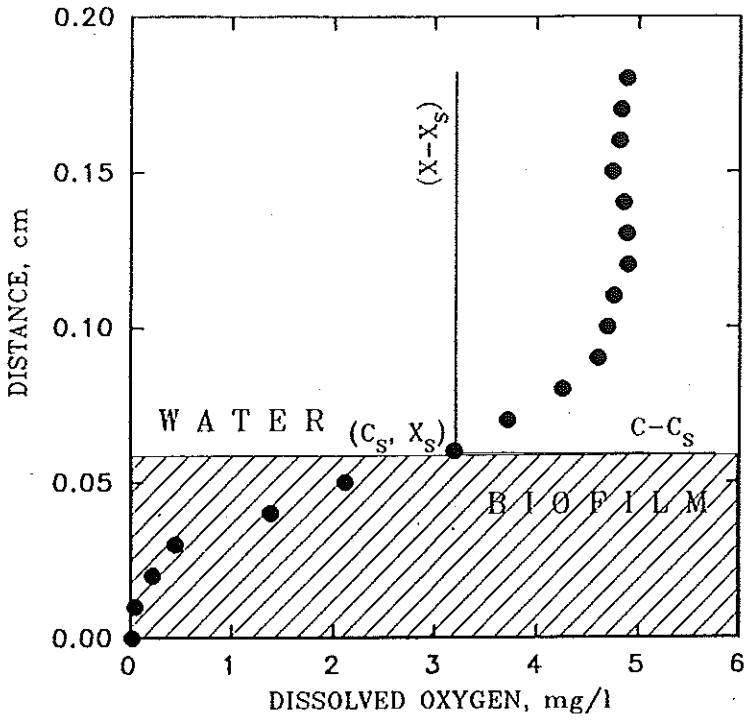

FIGURE 1. Profile of dissolved oxygen concentration across the biofilm system. The biofilm surface was positioned at the inflection point of the profile.

tip of the platinum wire was exposed by grinding on a rotating diamond wheel. The exposed platinum tip was subsequently etched in KCN to yield a recess of about 10 μm. Half of this recess was filled with gold by electrochemical plating. The operation was performed under a microscope with a mounted TV camera and observed on a video screen. The tip of the electrode was covered with a polymer (TePeX) to serve as the oxygen-permeable membrane. The measuring setup consisted of a picoammeter and a polarizing voltage source. The microelectrode was cathodically polarized to 800 mV against a silver-silver chloride reference electrode. The current in the circuit, in the range of picoamperes, is proportional to the concentration of DO. The electrode was calibrated in water by aeration and subsequent purging with pure nitrogen. Before the measurement the microelectrode was mounted on a micromanipulator and moved across the biofilm with predetermined increments. After each stop the current in the system was measured and compared with the calibration curve to calculate the DO concentration.

The result of measurement is a profile of DO vs. distance (Figure 1). The shape of the profile is determined by the following:

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 shape of the concentration profile is simultaneously influenced by all these factors. Consequently, the interpretation of the profiles is complex. Some progress in extracting information from concentration profiles has been achieved. A procedure of kinetic-parameters calculation based on the substrate concentration profile was presented by Lewandowski et al.[6] This procedure, however, required that the water be stagnant. Stagnant water above the biofilm resulted in a linear substrate concentration profile across the diffusion boundary layer and simplified calculations. The procedure described in this chapter is not limited by this condition.

III. CHEMICAL CONDITIONS AT BIOFILM SURFACE

Fixing the position of the biofilm surface at the substrate concentration profile is essential to any calculations in biofilm systems. Perhaps the most important advantage of knowing this position is that the substrate profile can be divided into two parts, one in water and one in the biofilm. The profile above the film contains information about substrate transport to the biofilm. The profile below the biofilm surface contains the information about the microbial activity.

It was shown[7] that the diffusivity difference between the water and the biofilm along with the 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 water and the biofilm are governed by different equations, which are tied together by the requirement that the substrate flux at the fluid-biofilm interface be continuous at a steady state.

Lewandowski et al.[7] have developed a fiber optic microprobe which detects the position of the biofilm surface while measuring substrate concentration. The biofilm is penetrated with a microprobe, 15 μm in diameter, which simultaneously measures the substrate concentration and optical density. A single-cable, glass fiber optic was used to fabricate the optical density sensor. The tip was etched in hydrofluoric acid (HF) to obtain a sharp tip of 10 μm in diameter. The DO microelectrode was coupled with the optical fiber to form a dual-sensor microprobe. Care was exercised to place the sensor tips in adjacent positions so they would be at the same level in the biofilm. Biofilm sample (growing on a trans-

parent polycarbonate) was placed in a petri dish. The probe was mounted in a motorized micromanipulator and positioned above the biofilm. A light-emitting diode (LED) was mounted below the petri dish in a hand-operated micromanipulator. The probe penetrated the biofilm at 5-μm increments and the DO concentration and light intensity were recorded at each stop. When the microprobe entered the biofilm the optical density of the environment changed, which was identified at the light intensity profile monitored by the fiber optic sensor. The change in optical density marked the position of the biofilm surface. At the same time the other part of the probe, the DO microelectrode, monitored changes in the DO concentration. Since the tips of both sensors were at the same level, the position of the biofilm surface evaluated from the optical density profile was superimposed on the DO profile. This technique, although accurate, was time consuming and work intensive.

Simplified procedures for biofilm surface positioning are used in routine measurements. A typical DO profile across a biofilm system is presented in Figure 1. The profile was measured in an open-channel biofilm reactor. A mixed-population biofilm was accumulated using a feeding solution based on glucose and mineral salts. The biofilm surface was positioned at the inflection point of the substrate concentration profile. This procedure is certainly less accurate than using a separate sensor, but is much more practical. How this experimental simplification influences the results of kinetic calculations remains to be tested.

IV. BIOFILM REACTION KINETICS

Assuming one-dimensional diffusion, the mathematical expression for the rate of change of substrate concentration in the biofilm is given as

$$\left[\frac{\partial C}{\partial t}\right]_f = D_f\left[\frac{\partial^2 C}{\partial x^2}\right]_f - \frac{V_{max}\, C}{K_s + C} \tag{1}$$

where D_f is the diffusion coefficient for the DO in the biofilm ($cm^2 \cdot s^{-1}$), C is the DO concentration at a point x ($mg \cdot l^{-1}$), V_{max} ($mg \cdot l^{-1} s^{-1}$), and K_s ($mg \cdot l^{-1}$) have the usual meaning in the Michaelis-Menten equation.

The steady-state concentration within a biofilm $(dC/dt = 0)_f$ is achieved when the consumption rate is equal to the rate of transport due to diffusion. Disregarding time dependence allows the removal of the partial derivatives, giving

$$0 = D_f\left[\frac{d^2 C}{dx^2}\right]_f - \frac{V_{max}\, C}{K_s + C} \tag{2}$$

This nonlinear equation cannot be solved exactly. However, as presented by Frank-Kamenetski,[8] it is often beneficial to integrate it once. The procedure follows:

$$p = \left[\frac{dC}{dx}\right] \tag{3}$$

$$\frac{d^2C}{dx^2} = \frac{dp}{dx} = \frac{dC}{dx}\frac{dp}{dC} = p\frac{dp}{dC} \tag{4}$$

since

$$\frac{1}{2}p^2\frac{d}{dC} = \frac{1}{2}\frac{d}{dp}p^2\frac{dp}{dC} = p\frac{dp}{dC} \tag{5}$$

then

$$\frac{d^2C}{dx^2} = \frac{1}{2}p^2\frac{d}{dC} \tag{6}$$

Substituting from Equation 2:

$$\frac{1}{2}p^2\frac{d}{dC} = \frac{V_{max}}{D_f}\frac{C}{K_s + C} \tag{7}$$

which combined with (3) yields:

$$\left[\frac{dC}{dx}\right]_f = \sqrt{2\frac{V_{max}}{D_f}\int \frac{C}{K_s + C}dC} \tag{8}$$

Evaluation of the integral in Equation 8 gives

$$\int \frac{C}{K_s + C}\,dC = C - K_s \ln(K_s + C) + \text{const} \tag{9}$$

The integration constant in Equation 9 can be determined from the boundary conditions. If the film is not totally penetrated by the constituent, then $(dC/dx)_f = 0$ for $C = 0$ (i.e., there is no mass transport beyond this depth). If the biofilm is totally penetrated, and the concentration at the bottom equals C_0, then $(Dc/dx)_f = 0$ for $C = C_0$ and there is no penetration at the substratum surface. For partially penetrated biofilms, the constant is

$$\text{const} = K_s \ln K_s \tag{10}$$

which, when substituted into Equation 9 and combined with Equation 8, gives

$$\left[\frac{dC}{dx}\right]_f = \sqrt{2\frac{V_{max}}{D_f}\left[C - K_s \ln\frac{K_s + C}{K_s}\right]} \tag{11}$$

Likewise, for totally penetrated films, the constant is

$$\text{const} = K_s \ln(K_s + C_0) - C_0 \tag{12}$$

which, through the same substitutions, gives

$$\left[\frac{dC}{dx}\right]_f = \sqrt{2\frac{V_{max}}{D_f}\left[C - C_0 - K_s \ln\frac{K_s + C}{K_s + C_0}\right]} \tag{13}$$

The reaction rate (R) can be directly calculated from the above equations by recognizing that it is equal to the rate of mass transfer across the biofilm surface,

$$R = F \cdot D_f \left[\frac{dC}{dx}\right]_f \tag{14}$$

where F is the biofilm surface area. Equation 13 can then be considered a general form of Equation 11. This means that the partially penetrated biofilms should be considered a specific case of totally penetrated biofilms. The reaction rate at the biofilm surface is given as

$$R = F\sqrt{2V_{max}D_f\left[C_s - C_0 - K_s \ln\frac{K_s + C_s}{K_s + C_0}\right]} \tag{15}$$

where C_s is the substrate concentration at the biofilm surface, F is the biofilm surface area. When the biofilm is totally penetrated by the substrate ($C_0 = 0$), Equation 15 is reduced to the case described by Equation 11.

For further calculations, the first and the second derivatives should be known. Therefore, the part of the substrate concentration profile below the biofilm surface is extracted from Figure 1 and approximated using a third-order polynomial.

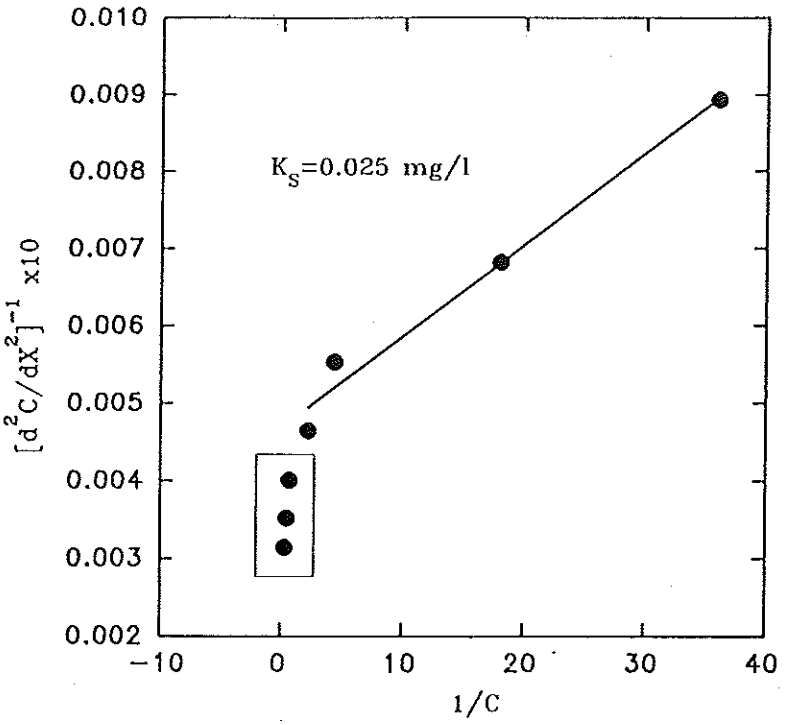

FIGURE 2. Evaluation of the half-saturation coefficient.

V. HALF SATURATION COEFFICIENT, K_s

Inversion of Equation 2 permits estimation of the K_s value:

$$\left[\frac{d^2C}{dx^2}\right]_f^{-1} = \frac{D_f * K_s}{V_{max}} \frac{1}{C} + \frac{D_f}{V_{max}} \tag{16}$$

The slope divided by the intercept in the plot (Figure 2) of the inverse of the second derivative against the inverse of the concentration gives K_s. The second derivative is calculated from the third-order polynomial representation of the DO profile in the biofilm. The data in Figure 2 indicate that the procedure of linearizing is efficient when using the DO concentrations near the K_s value. When the concentration of DO becomes much higher than the K_s (small 1/C value), the profile in Figure 2 becomes nonlinear. Although this explanation is by no means conclusive it is possible that the problem is similar to that encountered in planktonic cultures. It is well known from evaluating the K_s in planktonic cultures of microorganisms that the substrate concentration should not be much different than the K_s itself, otherwise the result may be meaningless.[9] Using the concentration values near the bottom of the biofilm yielded a "reasonable" value of $K_s = 0.025$ mg·l^{-1}. This value is similar to what may be expected from planktonic cultures.

VI. SUBSTRATE PROFILES ACROSS THE DIFFUSION BOUNDARY LAYER

The substrate molecules, before reaching the biofilm surface, must travel across the diffusion boundary layer. In an idealized, perfectly stagnant water the process would be entirely dependent on molecular diffusion. Consequently, the profile of substrate concentration would be linear. In natural systems, convection occurs. The magnitude of convection depends on turbulence, which in turn depends on flow velocity. The flow velocity is maximum away from the biofilm. It decreases near the biofilm and probably reaches zero at the surface or just below the biofilm surface. The velocity profile near the biofilm surface is nonlinear, and so is the convective transport rate. As a result, the concentration profile above the biofilm surface is also nonlinear.

The biofilm surface was located at the inflection point of the substrate concentration profile (Figure 1). For further considerations this point constitutes an origin of a new system of coordinates, (x_s,C_s). The new system of coordinates divides the profile into two parts. The part in the biofilm, below the interface, contains information about biofilm reaction rate and substrate diffusivity through the film. The part of the profile above the interface is analyzed to reveal information on substrate diffusivity through water and hydrodynamics. To calculate the diffusivity of DO in the biofilm, the profile just above the biofilm surface (bulk water side) is compared to the profile just below the biofilm surface (biofilm side). Since the flux of DO across this interface must be preserved, the first derivatives of concentration along the distance multiplied by the relevant diffusivities must be equal on both sides of the water-biofilm interface. The first derivative of concentration along the distance from the biofilm side can be calculated from Equation 11 or 13. The first derivative of DO concentration along the distance from the bulk water side can be calculated from the following analysis.

It was found that the DO profile above the biofilm surface can be adequately described by an empirical exponential function,

$$\frac{C - C_s}{C_b - C_s} = 1 - \exp[-B(x - x_s)] \tag{17}$$

where C is the local substrate concentration, C_b is the bulk substrate concentration, B is an experimental coefficient, and x is the distance in the new system of coordinates. This equation can be linearized to conveniently find the coefficient B from experimental data:

$$\ln\left[1 - \frac{C - C_s}{C_b - C_s}\right] = -B(x - x_s) \tag{18}$$

Coefficient B, calculated as the slope of the line when presenting the data in coordinates $(x-x_s)$ vs. $\ln[1-(C-C_s)/(C_b-C_s)]$, equals 54 cm^{-1} (Figure 3a). The model adequately reflects the distribution of the experimental data (Figure 3b).

The first derivative of concentration along the distance at the biofilm surface from the bulk water side can be calculated as follows:

$$\left[\frac{dC}{dx_{(x=x_s)}}\right]_w = B(C_b - C_s) \tag{19}$$

Substituting the numerical values of C_b = 4.9 mg/l and C_s = 3.2 mg/l yields $(dC/dx)_w$ = 92 $mg \cdot l^{-1} \cdot cm^{-1}$.

VII. DIFFUSION COEFFICIENT

The biofilm is partially penetrated with oxygen (Figure 2). Thus the first derivative dC/dx should be linearly related to the $\{C-K_s \ln[(C + K_s)/K_s]\}^{1/2}$ (Figure 4). The regression line is as follows:

$$\left[\frac{dC}{dx}\right]_f = 75.2\sqrt{C - K_s * \ln\frac{K_s + C}{K_s}} \tag{20}$$

$(dC/dx)_f$ at the biofilm-water interface can be calculated from Equation 11. For the DO concentration at the biofilm-water interface, $C = C_s$ = 3.2 mgl^{-1} and K_s = 0.025 mgl^{-1}, $(dC/dx)_f$ = 132 $mgl^{-1}cm^{-1}$.

The flux of oxygen through the biofilm-water interface is

$$J_f = D_f\left[\frac{dC}{dx}\right]_f \tag{21}$$

where subscript "f" stands for "film". The flux of oxygen through the diffusion layer is

$$J_w = D_w\left[\frac{dc}{dx}\right]_w \tag{22}$$

where subscript "w" stands for "water". The flux continuity must be preserved at the biofilm-water interface ($J_f = J_w$):

$$D_f\left[\frac{dC}{dx}\right]_f = D_w\left[\frac{dC}{dx}\right]_w \tag{23}$$

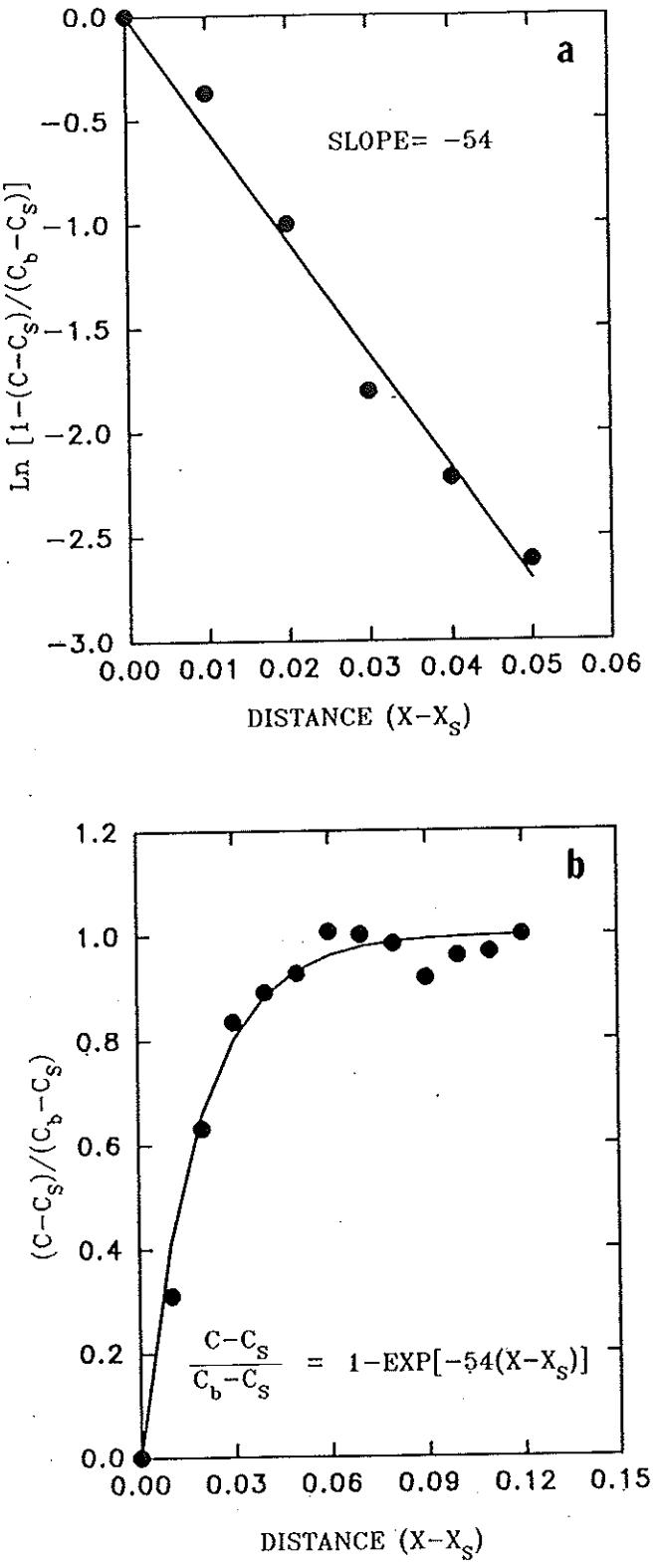

FIGURE 3. (a) Linearized dissolved oxygen profile (above the biofilm surface) together with (b) model and experimental data.

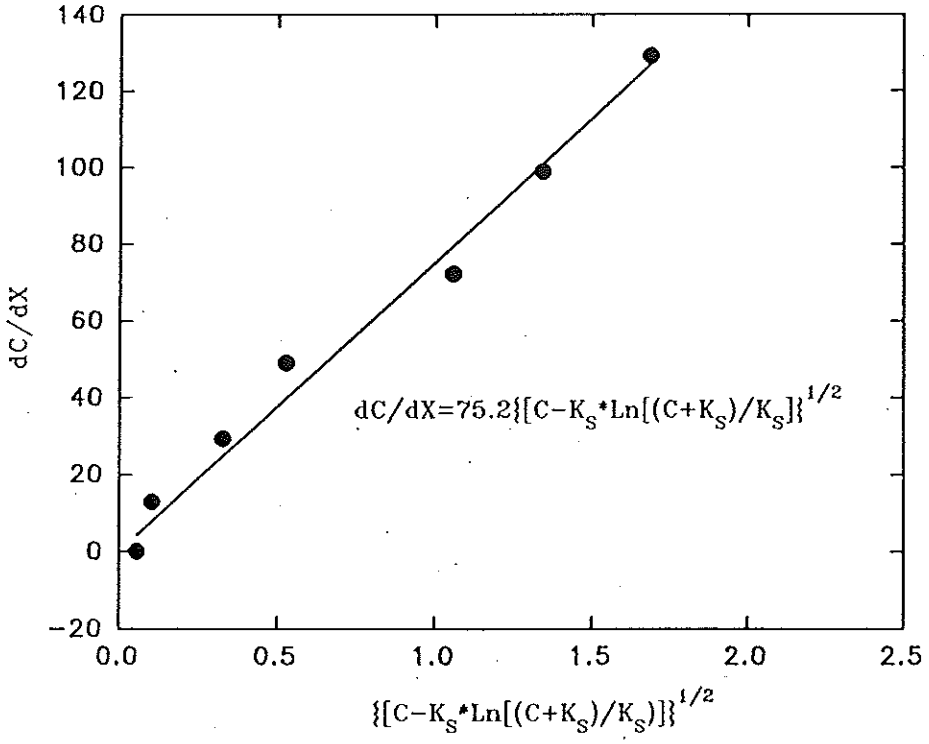

FIGURE 4. Linearized dissolved oxygen profile (below the biofilm surface).

The $(dC/dx)_w$ calculated from Equation 19 equals 91.8 $mgl^{-1}cm^{-1}$.

The biofilm diffusion coefficient can be calculated as follows:

$$D_f = D_w \frac{\left[\frac{dC}{dx}\right]_w}{\left[\frac{dC}{dx}\right]_f} \tag{24}$$

Substituting the calculated values, D_f is estimated as $0.70D_w$.

Using the DO diffusion coefficient in water at 21°C[10], $D_w = 2.0 \times 10^{-5}$ cm^2s^{-1}, the DO diffusion coefficient in the biofilm is $D_f = 1.31 \times 10^{-5}$ cm^2s^{-1}.

VIII. OTHER KINETIC PARAMETERS

By substituting the known values of D_f, $(dC/dx)_w$, $(dC/dx)_f$, and K_s, other important parameters can be calculated from the presented equations. For example, flux of oxygen equals $D_f(dC/dx)_f = 1.73 \times 10^{-6}$ $mg{\cdot}cm^{-2}{\cdot}s^{-1}$.

IX. DISCUSSION AND CONCLUSIONS

Kinetic parameters of biofilm reaction can be evaluated from the substrate concentration profile for flowing water systems. The result is site specific, which means that the evaluation is valid only for the site penetrated by the microsensor.

The measurements presented in this chapter stress the importance of the conditions at and near biofilm surfaces. All calculations are related to the position of the biofilm surface. Such treatment requires that the biofilm surface is precisely positioned. Biofilms, however, lack precisely positioned surfaces. In fact, the biofilm-water interface is a layer of variable thickness rather than a defined plane, something existing models do not tolerate. In extreme cases when the biofilm surface is colonized with filamentous microorganisms the position of the surface can be a function of flow velocity. The higher the flow velocity, the more the filamentous microorganisms will yield to the flow and smooth the surface, resulting in a simultaneous change in the outer biofilm boundary. The biofilm-water interface is not a clearly defined concept, and problems arise when this fact is imposed on existing models of diffusion and hydrodynamics. Because of all these limitations, the analytical solutions obtained can only have an approximate character. The precision of the solutions obtained is directly related to the precision with which the biofilm-water interface can be located. New approaches to the concept of the biofilm-water interface are needed before further progress can be obtained.

The calculated DO diffusivity in the biofilm considered in this study is $D_f = 1.31 \times 10^{-5}$ cm^2s^{-1}. This result is very close to the diffusivity of DO in marine sediments reported by Revsbech et al.[10] In sediment cores, incubated under 0.1 *M* solution of $HgCl_2$ for 3 weeks to eliminate microbial activity, Revsbech measured $D_f = 1.40 \times 10^{-5}$ cm^2s^{-1}. The results are very close, but the technique described here does not require any treatment to exclude the microbial activity and the measurement can be conducted *in situ*.

The locally measured values of substrate concentration are subject to experimental error which, considering the complexity of the equipment, can also be a function of many variables. Because of high impedance of microelectrodes, such measurements are always subject to electromagnetic noise. Noise elimination without signal distortion is an activity which is continuously exercised while using microelectrodes. How precisely the local conditions can be described depends on many factors and cannot be generalized.

The procedures presented here yield the kinetic parameters of the biofilm system as diffusivity, maximum reaction rate, half-saturation coefficient, and DO flux. The results are obtained from *in situ* micro-

electrode measurements. The experimental and theoretical limitations, however, are imposed on their validity.

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