Respirometric Assay for Biofilm Kinetics Estimation: Parameter Identifiability and Retrievability

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Abstract: Currently, no fast and accurate methods exist for measuring extant biokinetic parameters for biofilm systems. This article presents a new approach to measure extant biokinetic parameters of biofilms and examines the numerical feasibility of such a method. A completely mixed attached growth bioreactor is subjected to a pulse of substrate, and oxygen consumption is monitored by on-line measurement of dissolved oxygen concentration in the bulk liquid. The oxygen concentration profile is then fit with a mechanistic mathematical model for the biofilm to estimate biokinetic parameters. In this study a transient biofilm model is developed and solved to generate dissolved oxygen profiles in the bulk liquid. Sensitivity analysis of the model reveals that the dissolved oxygen profiles are sufficiently sensitive to the biokinetic parameters—the maximum specific growth rate coefficient ($\hat{\mu}$) and the half-saturation coefficient (K_s)—to support parameter estimation if accurate estimates of other model parameters can be obtained. Monte Carlo simulations are conducted with the model to add typical measurement error to the generated dissolved oxygen profiles. Even with measurement error in the dissolved oxygen profile, a pair of biokinetic parameters is always retrievable. The geometric mean of the parameter estimates from the Monte Carlo simulations prove to be an accurate estimator for the true biokinetic values. Higher precision is obtained for $\hat{\mu}$ estimates than for K_s estimates. In summary, this theoretical analysis reveals that an on-line respirometric assay holds promise for measuring extant biofilm kinetic parameters. © 1998 John Wiley & Sons, Inc. *Biotechnol Bioeng* **57:** 35–45, 1998.
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INTRODUCTION

Attached growth bioreactors are increasingly being used in place of suspended growth bioreactors because of their resistance to short-term toxic loads, their ability to perform at low influent substrate concentrations (oligotrophic conditions), and their high volumetric biomass concentrations, which allow small reactor volumes. To facilitate the design and operation of attached growth bioreactors, mathematical models have been developed that simulate substrate utilization and biomass growth in biofilms (Rittmann and McCarty, 1980a; Shieh and Keenan, 1986). One of the difficulties in using these models, however, is estimating the biokinetic parameters for bacterial growth and substrate removal.

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Current Biofilm Parameter Estimation

Several methods have been proposed for determining biofilm kinetic parameters. The simplest and most commonly used method consists of determining biokinetic growth parameters from batch experiments under suspended growth conditions and applying those values to attached growth systems (Livingston and Chase, 1989; Rittmann and McCarty, 1980b; Williamson and McCarty, 1976). Because the physiology and species composition of biofilm systems are different than suspended growth systems, it is unlikely that biokinetic parameters from suspended growth systems are accurate predictors of attached growth systems (Grady et al., 1996; Van Loosdrecht et al., 1990). Others have used biofilm grown cultures but measure the biokinetics after disruption of the biofilm structure that is then treated as a pseudo suspended growth culture with some incorporation of substrate transport kinetics (e.g, Cao and Alaerts, 1995; Jih and Huang, 1994). Several investigators have shown that bacteria develop a structure of cells and extracellular polymeric substances with channels and pores of water (de Beer et al., 1994; Murga et al., 1995). It is unclear whether biokinetic parameters are affected by disruption of this biofilm structure.

Few methods have been developed that measure biokinetic parameters from cultures with intact biofilms. In one such method, reactor influent and effluent substrate concentrations are measured, the resulting data are normalized and plotted, and the curves are visually compared with a series of design curves (Rittmann et al., 1986). Due to the inherent error in the sample analysis and visual comparison, it appears difficult to achieve accurate matches between the design curves and the experimental data (see Rittmann et al., 1986). Thus, the accuracy of the retrieved biokinetic parameters is uncertain. Furthermore, this method is time consuming, because each data point requires a different influent

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concentration condition, and the system must return to steady state between experiments. A batch method has also been developed for determining biokinetic parameters in fluidized bed reactors (Nguyen and Shieh, 1995). A reactor is operated in batch mode while substrate concentrations are recorded. Biokinetic parameters are determined through linearizations of the transformed data. In this approach, mass transfer limitations are lumped in the biokinetic parameters and are not accounted for explicitly. In addition, this method is time consuming, because separate batch experiments are required for each data point. The estimates have limited accuracy due to required sample analysis and linearizations that are performed in the parameter estimation step. Another technique determines biokinetic and substrate diffusion coefficients in water treatment biofilm systems (Zhang and Huck, 1996). By manipulating the steady-state biofilm equations developed by Rittmann and McCarty (1980a), the authors are able to derive an equation in two measurable variables [substrate flux into the biofilm (J) and substrate concentration in the bulk liquid (S_b)] and four model parameters: the diffusion coefficient for substrate in water (D_s), the minimum substrate concentration to maintain a steady-state biofilm ($S_{\min}$), the half-saturation coefficient (K_s), and the maximum growth rate ($\hat{\mu}X_f$, where $\hat{\mu}$ is the maximum specific growth rate coefficient and X_f is the biofilm density). While operating steady-state biofilm reactors at different influent concentrations, J and S_b are measured and the most probable values of D_s, $S_{\min}$, K_s, and $\hat{\mu}X_f$ are determined. The method is time consuming because separate steady states with different influent concentrations are necessary for each data point. Although error in the sample analysis is considered implicitly in determining the parameters, the final accuracy of the results is limited.

In summary, currently available techniques to estimate biokinetic parameters for biofilm systems are limited by three main factors. First, because these studies rely on measuring concentrations at steady-state conditions, long time periods are needed to bring the systems back to steady state between tests. For example, in the study by Nguyen and Shieh (1995) nine data points were collected and 1 week was required to return the reactor to steady state between each assay. Such a large time commitment often renders this method impractical for many design applications. Second, two of the techniques (Rittmann et al., 1986; Zhang and Huck, 1996) rely upon substrate specific analyses to determine the data points used in parameter estimation. Errors inherent in chemical specific analysis may lead to substantial experimental variability. This results in curve fits that are poor and produces biokinetic parameter values with limited accuracy. Third, a key concern of all available methods to date regards parameter identifiability. It has not been demonstrated for any of the presented techniques that parameters are identifiable and unique, or that reproducible parameter estimates can be obtained given the experimental variability. As a result, in the method by Zhang and Huck (1996) extremely large confidence intervals were reported: 0.731–5.58 l/day for $\hat{\mu}$ and −49.4–135 mg/L for K_s. Because of these limitations, the development of a more reliable, facile, and accurate technique for measuring biokinetic parameters in biofilm systems is warranted.

Respirometry

For aerobic attached growth systems, many of the problems and limitations of the above methods can be eliminated by the indirect determination of the substrate uptake profile via the associated oxygen uptake profile. Oxygen consumption can be used as a surrogate measure for substrate consumption because of the stoichiometric link between the two processes for aerobic chemotrophic growth,

$$S + -(1 - Y)\ O_2 \rightarrow YX, \tag{1}$$

where S is the energy substrate concentration [M chemical oxygen demand (COD)/L^3], X is the biomass concentration [M COD/L^3], and Y is the biomass yield coefficient [M COD/M COD]. Equation (1) is general in that it represents the basic stoichiometry for all aerobic chemotrophic growth. In the case of chemoheterotrophic growth, S represents the carbon source that is oxidized to carbon dioxide. In the case of chemolithotrophic growth, such as nitrification, S would represent the NH_4^+—N that is oxidized to NO_2^-—N or the NO_2^-—N that is oxidized to NO_3^-—N. Because substrate and oxygen consumption are directly linked by this relationship, oxygen uptake profiles yield the same information as substrate depletion profiles, provided the yield (Y) can be estimated.

Measuring oxygen uptake profiles has several advantages over collecting substrate removal profiles (Rozich, 1992; Vanrolleghem et al., 1995). First, because dissolved oxygen probes are highly sensitive, very low dissolved oxygen concentrations and very small changes in dissolved oxygen concentrations can be measured with very little error. This sensitivity typically cannot be attained with chemical analysis for specific growth substrates (Ellis et al., 1996a). Second, because oxygen measurements can be made on-line, there is no need to disturb the reactor and remove mass, avoiding errors due to direct mass loss or volatilization (Naziruddin et al., 1995). Third, collection of dissolved oxygen profiles can be automated and performed continuously, providing many high quality data points with minimal experimental or analytical effort. Fourth, because aerobic chemotrophic microorganisms use oxygen as a terminal electron acceptor for any compound that serves as an electron donor, the same equipment and methodology can be used to determine biokinetic parameters for removal of any pure or even mixed compound substrate that serves as the carbon and energy source for growth (Ellis et al., 1996a). Fifth, respirometry allows the measurement of extant in addition to intrinsic biokinetic parameters (Ellis et al., 1996a; Smets et al., 1994), and the superiority of extant biokinetic parameters is increasingly being recognized (Ellis et al., 1996b; Grady et al., 1996; Nguyen and Shieh, 1995; Smets et al., 1994). Bacterial communities adapt to their environmental conditions by shifts in the community make-up and changes

in their physiological state, both of which are manifested in the community's observed kinetics (Grady et al., 1996). Because of this fact, it is important to obtain biokinetic parameters under conditions similar to the condition in the continuous reactor one is attempting to mimic. This can be achieved by minimizing physiological state changes during the biokinetic assay (Grady et al., 1996). In practice, this means that biokinetic assays should be performed with biomass taken from the continuous parent reactor, and the uptake of very small pulses of substrates should be monitored. Because respirometry is sensitive to small changes in oxygen concentration, biokinetic parameters can be determined from small pulses of substrate via respirometry while minimizing physiological state changes. Thus, respirometry is ideal for measuring extant biokinetic parameters.

This research suggests that a respirometric technique effective for measuring extant biokinetic parameters in suspended growth systems (Ellis et al., 1996a) can be modified to a new method for determining attached growth biokinetic parameters. With suspended growth systems, transient dissolved oxygen profiles are typically recorded as bacteria degrade a fixed mass of substrate. Those profiles are then matched with mathematical model predictions to determine the biokinetic parameters. In this research it is investigated whether it is numerically feasible to apply this method to attached growth bioreactors. Specifically, the four objectives of this research are: determine if a transient biofilm model can be solved in terms of a transient bulk dissolved oxygen profile; examine the sensitivity of the bulk dissolved oxygen profile to biofilm model parameters; develop a nonlinear optimization algorithm for determining biokinetic parameter values using a transient dissolved oxygen profile; and determine the accuracy of the parameter estimation algorithm when typical experimental error is incorporated in the transient dissolved oxygen profile.

MATERIALS AND METHODS

Transient Biofilm Model

Several models have been proposed to predict substrate and oxygen concentrations in a biofilm system. This research uses a variation of a conceptual biofilm model introduced by Rittmann and McCarty (1980a) and used extensively by others (Annachhatre and Khanna, 1990; Golla and Overcamp, 1990; Rittmann and Manem, 1990; Saéz and Rittmann, 1990; Suidan et al., 1987; Wanner and Gujer, 1986). The conceptual biofilm model incorporates the kinetics of cell growth, substrate removal, and substrate diffusional resistance. The model is illustrated in Figure 1. This construct assumes a continuous biofilm layer of equal local thickness surrounded by a stagnant water layer called the external transfer layer (ETL). Substrate and oxygen in the bulk liquid diffuse through the ETL and into the biofilm. Utilization of the substrate occurs only in the biofilm, and growth results in an increase in biofilm thickness. Growth in the suspended

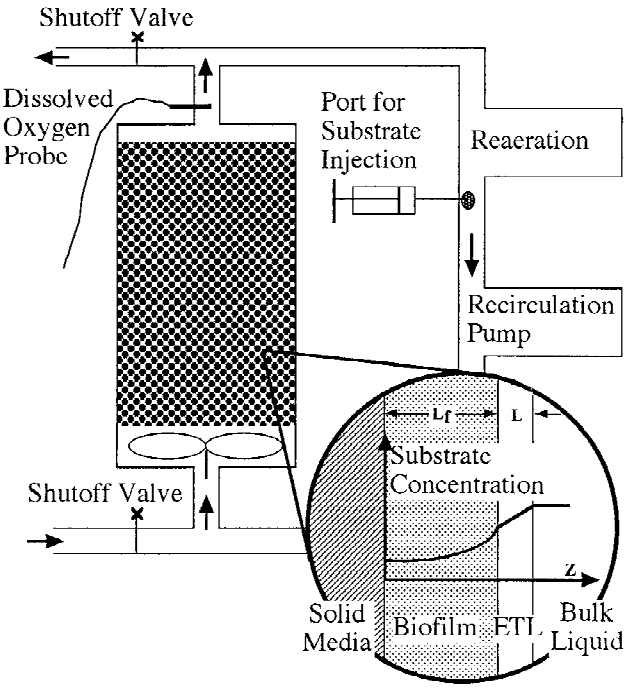

Figure 1. Hypothetical bioreactor and conceptual biofilm model.

phase is assumed to be negligible, and active biomass is removed from the biofilm by death and detachment and results in a constant biofilm thickness at steady state.

Adhering to the conceptual biofilm model as proposed by Rittmann and McCarty (1980a), the relevant differential equations that can be derived describe the fate of substrate and oxygen in the biofilm during the proposed respirometric assay. The governing equations as proposed by Rittmann and McCarty (1980a) are modified to accommodate transient conditions for substrate and oxygen concentrations. Transport of substrate and oxygen through the ETL is described by

$$\frac{\partial S}{\partial t} = D_s \frac{\partial^2 S}{\partial z^2}, \tag{2}$$

$$\frac{\partial O}{\partial t} = D_o \frac{\partial^2 O}{\partial z^2}, \tag{3}$$

Transport and utilization of substrate and oxygen in the biofilm is described by

$$\frac{\partial S}{\partial t} = D_{f,s} \frac{\partial^2 S}{\partial z^2} - \frac{\hat{\mu}}{Y} X_f \frac{S}{K_s + S}, \tag{4}$$

$$\frac{\partial O}{\partial t} = D_{f,o} \frac{\partial^2 O}{\partial z^2} - \frac{1 - Y}{Y} \hat{\mu} X_f \frac{S}{K_s + S} - bX_f, \tag{5}$$

where b is the endogenous decay coefficient [$1/t$], D_s is the diffusion coefficient for substrate in water [L^2/t], D_o is the diffusion coefficient for oxygen in water [L^2/t], $D_{f,s}$ is the diffusion coefficient for substrate in the biofilm [L^2/t], $D_{f,o}$ is the diffusion coefficient for oxygen in the biofilm [L^2/t],

K_s is the half-saturation coefficient [M COD/L^3], $\hat{\mu}$ is the maximum specific growth rate coefficient [1/t], $O(z,t)$ is the concentration of oxygen [M O_2/L^3], $S(z,t)$ is the concentration of substrate [M COD/L^3], t is the time [t], X_f is the biofilm density [M COD/L^3], Y is the growth yield coefficient [M COD/M COD], and z is the distance from the solid surface [L] (see Fig. 1).

A simple linear model is used to represent the oxygen uptake due to endogenous decay [$-bX_f$ in (5)]. Although detachment plays a role in maintaining a steady-state biofilm thickness, it is assumed to be negligible during the assay. If detachment needs to be accounted for, it can impact the biofilm thickness, L_f, which can directly impact the oxygen uptake profile.

Typically, the solid surface is nonreactive, such that the flux of oxygen and substrate at the solid surface equals zero. It is also evident that the flux and concentration of oxygen and substrate must be continuous across the ETL–biofilm interface. These statements translate into boundary conditions at the solid surface ($z = 0$),

$$\frac{\partial S}{\partial z} = 0, \tag{6}$$

$$\frac{\partial O}{\partial z} = 0, \tag{7}$$

and at the biofilm–ETL interface ($z = L_f$),

$$D_s \left(\frac{\partial S}{\partial z}\right)_{\text{ETL}} = D_{f,s} \left(\frac{\partial S}{\partial z}\right)_{\text{biofilm}}, \tag{8}$$

$$D_o \left(\frac{\partial O}{\partial z}\right)_{\text{ETL}} = D_{f,o} \left(\frac{\partial O}{\partial z}\right)_{\text{biofilm}}, \tag{9}$$

$$(S)_{\text{ETL}} = (S)_{\text{biofilm}}, \tag{10}$$

$$(O)_{\text{ETL}} = (O)_{\text{biofilm}}, \tag{11}$$

where L_f is the biofilm thickness [L].

The model development assumes an experimental setup similar to that used by Rittmann et al. (1986) to measure biofilm parameters, as shown in Figure 1. In that research, attached bacteria were grown on 3-mm glass beads in a completely mixed, flow-through column. In addition, this experimental setup assumes on-line measurement of the dissolved oxygen concentration (e.g., via a dissolved oxygen probe placed in the recycle line) and reaeration to supply dissolved oxygen to the recycle line. The reactor specifications and measured system parameters from the Rittmann et al. (1986) study were used for simulations in this research.

A major challenge in the use of a transient mathematical formulation is the necessity of stating the initial conditions of the system. The steps in the assay were designed to provide known initial concentrations for oxygen and substrate everywhere in the system. In preparation for the assay, biofilm will be grown in the reactor under steady-state, flow-through conditions while oxygen is supplied in excess (see Fig. 1). Once the biofilm has reached steady state, the assay will be initiated. The flow will be altered so that the reactor operates with a complete recycle and without substrate or oxygen input. The reactor will be monitored to determine when the oxygen uptake profile attains linearity. This condition indicates that all residual substrate has been consumed, and the remaining oxygen consumption is only due to endogenous decay. Thus, the substrate concentration is zero throughout the reactor, and the initial conditions for substrate can be specified by $S(z, 0) = 0$ for $0 \le z < L_f + L$, where L is the ETL thickness [L].

To set up an initial condition for oxygen, the bulk liquid is then reaerated until a steady-state bulk liquid oxygen concentration is attained. The oxygen concentration in the bulk liquid will be kept constant to ensure that the oxygen concentration in the ETL and biofilm attains a steady-state profile. The steady-state oxygen concentration in the ETL can then be determined by equating the transient term in Equation (3) to zero, resulting in the initial condition in the ETL,

$$\frac{\partial^2 O}{\partial z^2} = 0, \tag{12}$$

while equating the transient term and the substrate concentration in Equation (5) to zero, resulting in the initial condition in the biofilm,

$$\frac{\partial^2 O}{\partial z^2} = \frac{bX_f}{D_{f,o}}, \tag{13}$$

with boundary conditions (7), (9), and (11), and at the ETL–bulk liquid interface as

$$O = O_o, \tag{14}$$

where O_o is the initial concentration of oxygen in the bulk liquid [M O_2/L^3]. When the initial conditions for substrate and oxygen have been met, reaeration will cease and a fixed amount of substrate will be injected into the reactor. Dissolved oxygen concentrations in the bulk liquid will then be recorded over time as the substrate is consumed. The substrate injection is represented by the initial condition $S(L_f + L, \text{O}) = S_o$, where S_o is the initial substrate concentration in the bulk liquid of the reactor due to the pulse injection at the start of the experiment [M COD/L^3].

Finally, a boundary condition is required for the ETL–bulk liquid interface. Because the reactor will be operated as a closed system, the mass of substrate and oxygen in the reactor can be tracked. The mass flux of substrate and oxygen into the ETL must equal the mass loss rate in the bulk liquid. Assuming that the substrate and oxygen concentrations are uniform in the bulk liquid, this can be expressed as a boundary condition at the ETL–bulk liquid interface ($z = L_f + L$),

$$\frac{\partial S}{\partial t} hV = D_s \frac{\partial S}{\partial z} aV, \tag{15}$$

$$\frac{\partial O}{\partial t} hV = D_o \frac{\partial O}{\partial z} aV, \tag{16}$$

where a is the specific surface area of media in the reactor [L^2/L^3], h is the volume fraction of bulk liquid (excluding the ETL liquid) per total reactor volume [L^3/L^3], and V is the volume of the reactor [L^3].

This formulation of the transient substrate and oxygen profile in the biofilm assumes that the biofilm thickness does not change over time. Provided that the amount of substrate added to the reactor is small enough to result in negligible biofilm growth (i.e., $S_o\ hV \ll X_f L_f aV$), the assumption of negligible biofilm growth is acceptable. Similarly, because of the short duration of the assay and the minimal growth in biomass, detachment is assumed to be negligible. When combined these two assumptions suggest a fixed biofilm thickness for the assay. These conditions can be promoted by the injection of small pulses of substrate so that changes in biomass physiology and structure, including thickness, will be minimal, and true extant biokinetics will be measured (Ellis et al., 1996a). In addition, this formulation assumes that oxygen is never rate limiting so that its removal is stoichiometrically related to the removal of the rate limiting substrate. This assumption can be ensured by maintaining high bulk liquid oxygen concentrations at the experimental onset. Assuming tests are conducted with an initial bulk liquid oxygen concentration of 10 mg/L, simulations with the model show that the minimum oxygen concentration within the biofilm during the course of an assay with the test problem is greater than 7.5 mg/L.

The model requires a numerical solution due to the nonlinearity of the growth rate expressions in Equations (4) and (5) and the coupling of Equations (4) and (5) by the S variable. A numerical solution was developed using finite differences that were implicit in time. The nonlinear growth terms in Equations (4) and (5) and the derivatives in the boundary conditions (15) and (16) were lagged one time step to yield a system of linear equations at each time step. The initial dissolved oxygen concentration profile in the reactor was determined by solving Equations (12), (13), and (14) that analytically result in expressions for oxygen concentration in the ETL as

$$O(z) = \frac{bX_f L_f}{D_o}(z - L_f - L) + O_o, \tag{17}$$

and in the biofilm as

$$O(z) = \frac{bX_f}{D_o}\left(z^2 + L_f^2 - (L_f + L)L_f - \frac{L_f^2 D_o}{2D_{f,o}}\right) + O_o. \tag{18}$$

Figure 2 illustrates the numerical solution for the substrate and oxygen profiles [Equations (2)–(5)] predicted by this model for a test case using parameters measured in Rittmann et al. (1986): $a = 1.2 \times 10^3$ l/m, $b = 1.2 \times 10^{-7}$ l/s, $D_o = 2.5 \times 10^{-9}$ m^2/s, $D_{f,o} = 1.9 \times 10^{-9}$ m^2/s, $D_s = 9.3 \times 10^{-10}$ m^2/s, $D_{f,s} = 7.2 \times 10^{-10}$ m^2/s, $h = 0.107$, $L = 8.6 \times 10^{-5}$ m, $L_f = 1.6 \times 10^{-4}$ m, $\hat{\mu} = 0.22$ L/day, $V = 4.91 \times 10^{-5}$ m^3, $X_f = 58{,}000$ g COD/m^3, $Y = 0.45$ g COD/g COD. A larger K_s value of 1.0 g COD/m^3 was used to represent higher concentration systems, rather than the oligotrophic conditions tested in Rittmann et al. (1986). Oxygen and substrate concentrations were determined every 0.05 s at 200 nodes along the z direction spanning the biofilm and ETL. The substrate and oxygen concentrations in the bulk liquid are plotted versus time, and the substrate pulse injection occurs 40 s after the onset of the experiment. The rapid decrease in substrate concentration after injection is mirrored by a decrease in the oxygen concentration, demonstrating that the oxygen uptake profile is linked to substrate uptake. This corroborates that the oxygen uptake profile alone may provide enough information to estimate the biokinetic growth parameters. Figure 2 also illustrates a linear decrease in oxygen concentration prior to substrate injection and after substrate depletion, which is due to oxygen demand from endogenous decay. This linear profile provides a means to estimate b. For the test case investigated, the assay takes approximately 4 min to complete.

Parameter Identification

Several steps were taken to determine if biokinetic parameters are identifiable and retrievable from the generated dissolved oxygen profile. Initially it was tested whether the oxygen profiles were sufficiently sensitive to the biokinetic parameters or whether the error in the assumed model parameters dwarf the sensitivity of the oxygen profiles to the biokinetic parameters. A sensitivity analysis of the transient biofilm model was conducted by varying one model parameter while holding the remaining parameters constant. Sensitivity was judged by the effect these perturbations had on the resulting dissolved oxygen profiles. This was performed

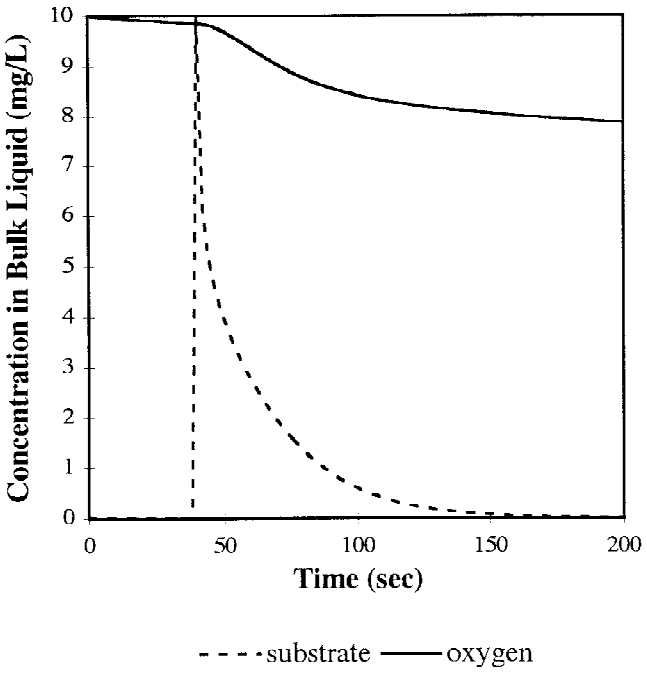

Figure 2. Simulated dissolved oxygen concentration and substrate concentration in the bulk liquid over time.

with the parameters of interest $\hat{\mu}$ or K_s and parameters D_s, $D_{f,s}$, D_o, $D_{f,o}$, L_f, L, and X_f, which are assumed to be known for the estimation procedure.

Parameter retrievability was tested by developing a numerical algorithm to determine the biokinetic parameters from a synthetic dissolved oxygen profile generated by the transient biofilm model. This algorithm consists of varying the parameter values ($\hat{\mu}$ and K_s) until the synthetic oxygen uptake profile is matched by the predicted oxygen profile as illustrated in Figure 2. This is achieved by determining the parameter values that minimize the deviation of the measured profile from the predicted profile. The parameter identification problem was formulated as a least squares problem of the form

$$\text{minimize } f(\hat{\mu}, K_s) = (\tilde{O}_{obs} - \tilde{O}_{model})^t \mathbf{V}^{-1} (\tilde{O}_{obs} - \tilde{O}_{model}), \quad (19)$$

where $\tilde{O}_{obs}$ is a vector of observations in the experimental oxygen profile, $\tilde{O}_{\text{model}}$ is a vector of observations in the model simulation oxygen profile that depends on the biokinetic parameters $\hat{\mu}$ and K_s, and $\mathbf{V}$ is the covariance matrix of the experimental data (Marsili–Libelli, 1992). In the absence of true experimental data, $\mathbf{V}$ was set to the identity matrix. In this step, typical measurement error was not added to the synthetic dissolved oxygen profile.

Initially attempts were made to solve this nonlinear optimization problem using the method of steepest descent and the quasi-Newton Broyden–Fletcher–Goldfarb–Shanno method (Luvenberger, 1984). As often occurs with steepest descent, the method converged too slowly to be practically useful. Additionally, the Broyden–Fletcher–Goldfarb–Shanno method was successful at retrieving values for the true parameters only from certain starting points. A narrow valley in the response surface proved to be intractable for these methods. When the search point moved into the valley, both techniques were unable to move along the valley toward the true solution. This is due to the fact that both methods rely upon derivative approximations in the coordinate directions. The steep walls in the valley prevent derivative approximations with sufficient accuracy to determine a descent direction in the direction of the valley. Rosenbrock's method of rotating coordinate axes was found to be an appropriate alternative for this problem (Rosenbrock, 1960). This algorithm was developed specifically for functions with ridges and valleys by rotating the search axes at the end of every stage to orient one of the search directions in the valley direction. Furthermore, this method does not require approximation of the derivatives.

A second set of retrievability analyses used synthetic data sets with the introduction of typical experimental error. Although experiments have not been conducted with the proposed assay for biofilm systems, analogous tests have been conducted using suspended growth bacterial cultures (e.g., Ellis et al., 1996a). Because a similar dissolved oxygen measurement technique will be used in the proposed biofilm assay, the measurement error observed in the suspended growth experiments should be representative of measurement error in the biofilm assay. A typical oxygen profile measured from a batch suspended growth assay was used to approximate the measurement error in a biofilm experiment. This oxygen profile was fit with the Monod equation (Monod, 1949) to estimate biokinetic parameters per the method of Ellis et al. (1996a). The difference between the measured oxygen profile and the best fit profile (from 333 data points) was set equal to the error for each measurement. A histogram of these differences is shown in Figure 3 along with a best fit normal distribution. Pearson's χ^2 goodness of fit test indicates that the normal distribution provides a good fit to the data (Devore, 1987). The data set has a mean of 0.00037 mg/L, a standard deviation of 0.011 mg/L, and a skew of 0.077 $(\text{mg/L})^{-3}$. Uncorrelated, random, normally distributed error with the calculated mean and standard deviation was subsequently added to the simulated oxygen profile created with the transient biofilm model. Monte Carlo simulations were then conducted to determine the means and standard deviations of the parameter estimations for dissolved oxygen profiles with specified normally distributed error.

RESULTS AND DISCUSSION

A primary concern with parameter estimation is the identifiability of the biokinetic parameters from experimental data (Beck and Arnold, 1977; Currie, 1982; Dochain et al., 1995; Holmerg, 1982; Robinson, 1985). This research addressed several aspects of this issue. First, the oxygen profiles were tested for their sensitivity to the biokinetic parameters that are being estimated. Sensitivity analyses were performed with a base case defined by the parameters used in Figure 2. Figure 4 shows predicted oxygen profiles for different values of the biokinetic parameters, $\hat{\mu}$ and K_s. This figure

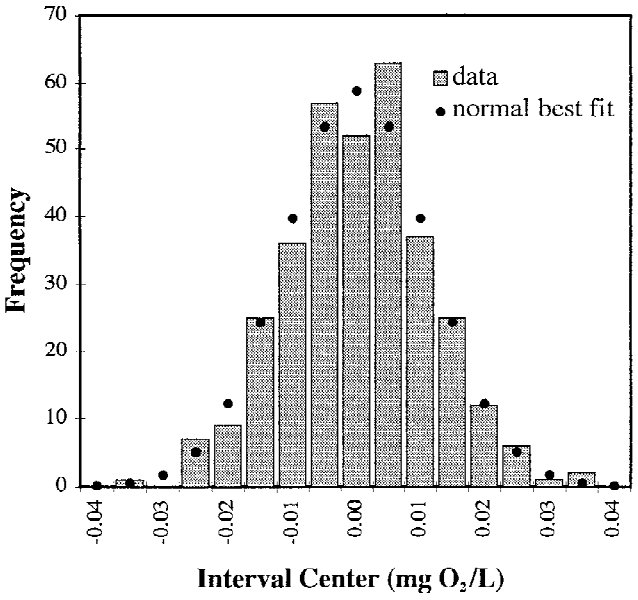

Figure 3. Histogram of typical experimental error in a suspended growth respirometric assay and best fit to the normal distribution.

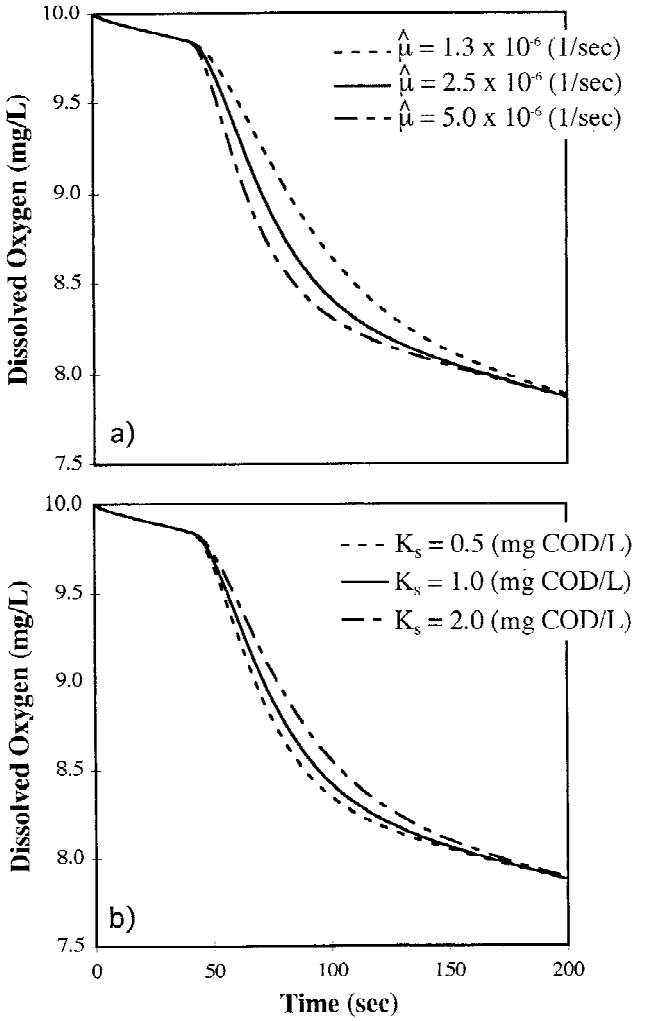

Figure 4. Sensitivity of dissolved oxygen profile to (a) $\hat{\mu}$ and (b) K_s.

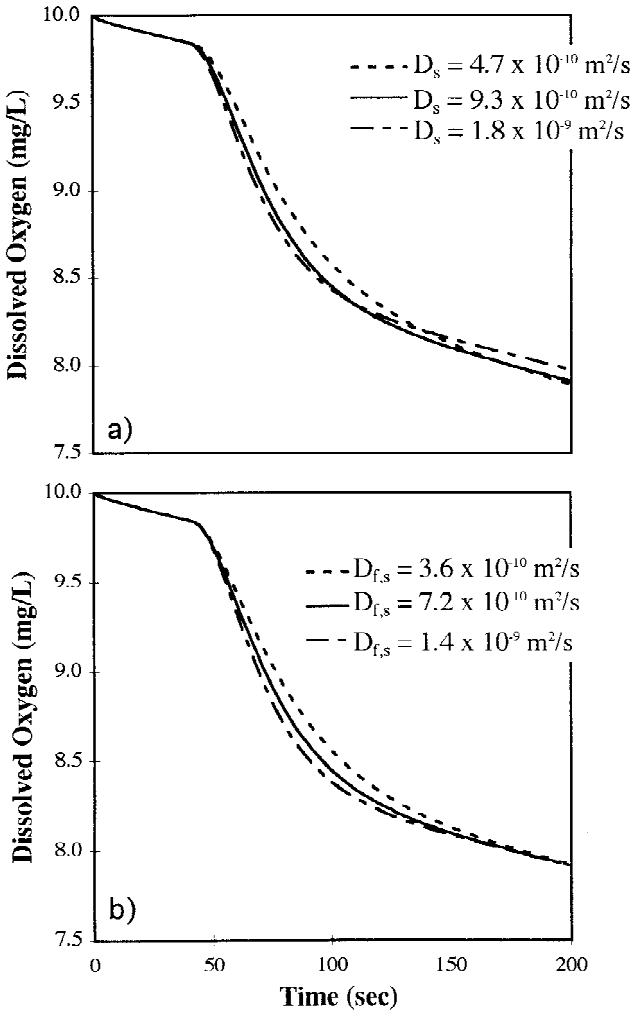

Figure 5. Sensitivity of dissolved oxygen profile to (a) D_s and (b) $D_{f,s}$.

clearly illustrates that the oxygen profiles are sensitive to changes in values of parameters $\hat{\mu}$ and K_s. During the period of the experiment when oxygen profiles are changing most rapidly (i.e., while the substrate is being utilized), the predicted oxygen profiles differ by as much as 0.4 mg/L per time point. Typical measurement errors for dissolved oxygen probes are around 0.01 mg/L, far below the sensitivity observed in Figure 4 for twofold changes in parameter values. This confirms that the predicted oxygen profiles are sensitive to the biokinetic parameters over the tested range and strongly corroborates the potential of the proposed respirometric technique to measure the biokinetic parameters.

Equally important to the sensitivity of the sought parameters is that the system is sufficiently insensitive to assumed model parameters. Sensitivity analysis were conducted with all parameters in the transient biofilm model, excluding those that are easily measured (a, b, h, O_o, S_o, V, and Y). The parameters tested were D_s, D_o, $D_{f,s}$, $D_{f,o}$, X_f, L_f, and L; and the sensitivity of the transient biofilm model to these parameters is shown in Figures 5–7. The bulk dissolved oxygen profile is insensitive to the oxygen diffusion coefficients in the biofilm, $D_{f,o}$, and liquid, D_o, (results not

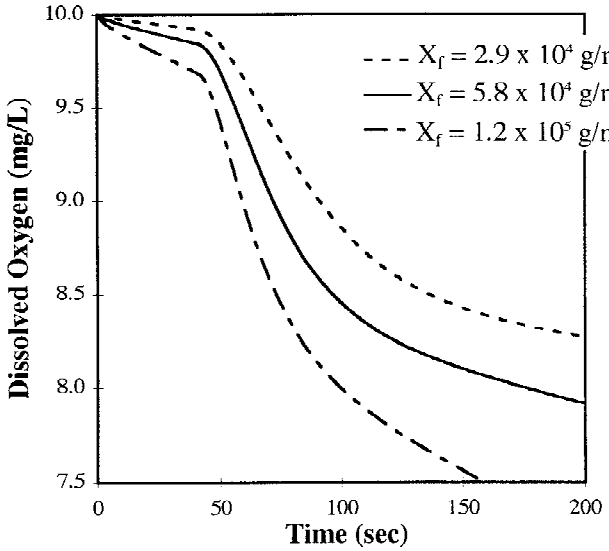

Figure 6. Sensitivity of dissolved oxygen profile to X_f.

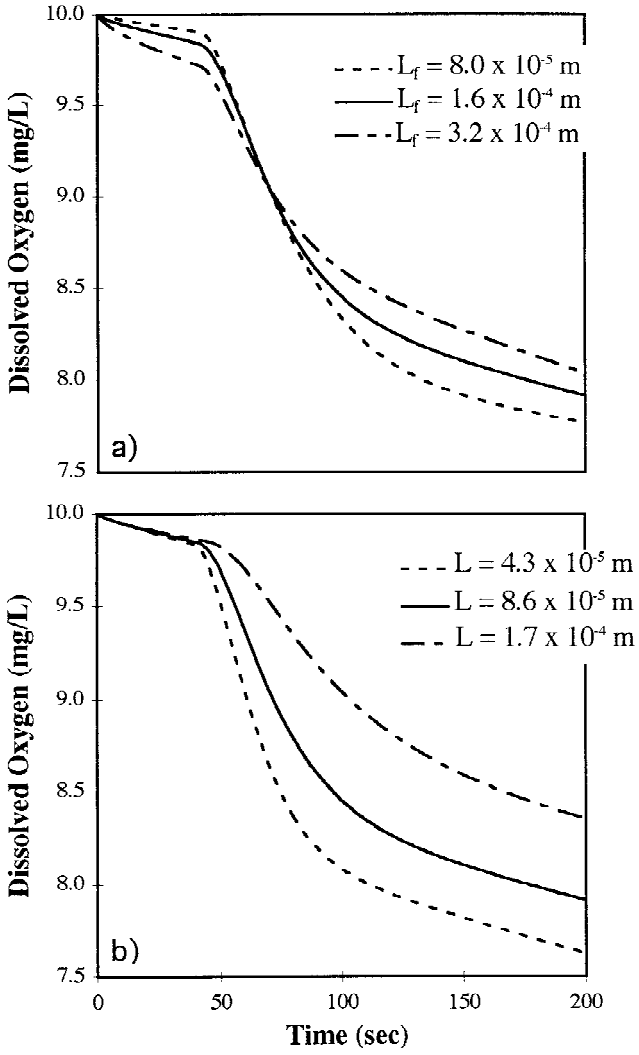

Figure 7. Sensitivity of dissolved oxygen profile to (a) L_f and (b) L.

shown). On the other hand, the profile is sensitive to changes in the substrate diffusion coefficients. Comparison of Figure 5 with Figure 4 reveals, however, that the oxygen profiles are less sensitive to twofold changes in the substrate diffusion coefficients than to twofold changes in the $\hat{\mu}$ value and equally sensitive to twofold changes in the K_s value. As a result, small errors in the estimates of D_s and $D_{f,s}$ are not likely to affect the estimates for $\hat{\mu}$, although K_s estimates may be affected. Because of the profile's sensitivity to D_s and $D_{f,s}$, good estimates of these parameters are deemed necessary to maximize the accuracy of the $\hat{\mu}$ and K_s estimates. D_o and D_s can be calculated from published chemical data or measured with accuracy; several methods have been published for measuring $D_{f,o}$ and $D_{f,s}$ (Hinson and Kocher, 1996; Lawrence et al., 1994; Williamson and McCarty, 1976).

Figures 6 and 7 illustrate the expected strong sensitivity of the transient biofilm model to the parameters X_f, L_f, and L. Twofold changes in these parameters cause shifts in the dissolved oxygen profile that exceed shifts caused by twofold changes in $\hat{\mu}$ and K_s. It seems unlikely that $\hat{\mu}$ and K_s

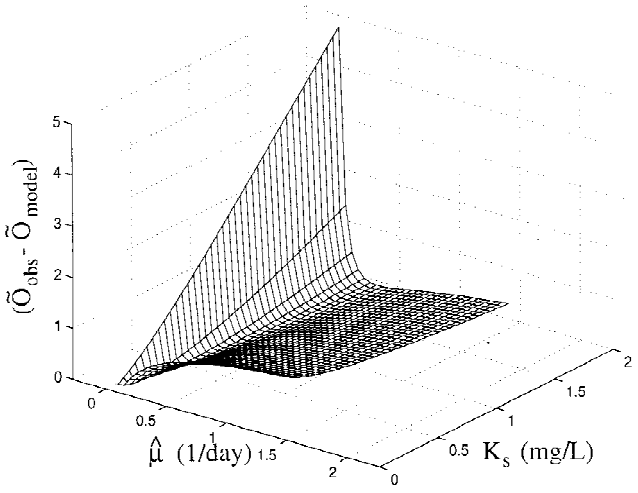

Figure 8. Orthographic projection of the parameter estimation response surface with no error.

can be estimated with accuracy without good estimates of X_f, L_f, and L. Rittmann and McCarty (1980a) list formulae based on porous media hydrodynamics to estimate L, and several methods have been identified to measure L_f (Livingston and Chase, 1989; Mirpuri et al., 1997; Rittmann et al., 1986) and X_f (Hinson and Kocher, 1996; Rittmann et al., 1986).

Parameter retrievability was investigated by examination of the parameter estimation response surface. The response surface resulting from the least squares formulation is shown in Figures 8 and 9 over a range of values of the biokinetic parameters. This surface was generated by calculating the oxygen profile for a base case ($\tilde{O}_{obs}$) with the same parameters used for Figure 2. Each data point on the surface was then computed by calculating the oxygen profile for a new $\hat{\mu}$, K_s pair ($\tilde{O}_{model}$) and evaluating the function using Equation (19). Note that estimating the biokinetic parameters from a dissolved oxygen profile is equivalent to

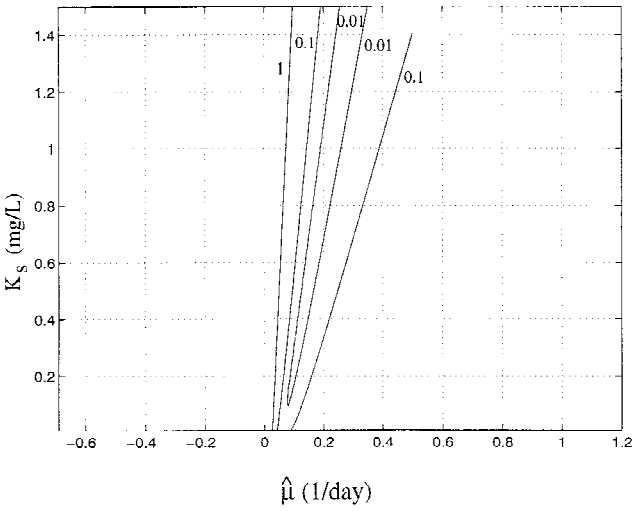

Figure 9. Contour plot of the parameter estimation response surface with no error.

Table I. Starting points for parameter estimation algorithm.

$\hat{\mu}$ (1/day)	K_s (mg/L)	$\hat{\mu}$ (1/day)	K_s (mg/L)
0.10	0.5	0.05	2.5
1.00	0.1	0.10	4.0
2.00	0.5	2.00	4.0
1.00	2.0	1.20	6.0
4.00	2.0	4.00	6.0

finding the ($\hat{\mu}$, K_s) point with the minimum value on this surface. The response surface consists of a single, unidirectional valley with steep walls and a slightly sloped valley floor and is very similar to the response surface for suspended growth batch experiments exhibiting Monod type biodegradation kinetics (Vanrolleghem et al., 1995). The function itself is smooth and well behaved. Optimization runs using Rosenbrock's method of rotating coordinate axes were conducted starting from the ten different points listed in Table 1. These starting points cover all four quadrants around the true solution. All of the optimization simulations converged to the same minimum ($\hat{\mu}$ = 0.22 l/day and K_s = 1.0 mg/L), suggesting that a single minimum exists on this surface. Thus, in data without error the minimum of the surface can be determined; i.e., the true parameters can be retrieved.

Parameter identifiability of this assay was further investigated by determining if the true parameters are identifiable with data containing typical measurement error. A synthetic dissolved oxygen profile was generated using the parameters described for Figure 2 and an uncorrelated, normally distributed error with a standard deviation of 0.01 mg/L was added. Optimization runs were again conducted from the 10 starting points listed in Table I. All simulations converged to the point ($\hat{\mu}$ = 0.180 l/day, K_s = 0.734 mg/L). In addition, the least squares value at the minimum (0.0055) was less than the least squares value at the true parameter values (0.0056). This confirms that the true parameter set is no longer the minimum location on the surface generated with typical measurement error. These results indicate that the addition of the measurement error moved the location of the minimum of the surface. However, it appears that the surface retains a single minimum. Thus, even dissolved oxygen profiles with measurement error yield retrievable parameter estimates, although the accuracy of those estimates is diminished.

Monte Carlo simulation was subsequently used to determine the distribution of estimated parameter values that would result from random measurement error. Two hundred and fifty realizations of dissolved oxygen profiles containing random measurement error were created, and biokinetic parameters were estimated from each of those realizations. This ensemble was created with uncorrelated, normally distributed error with a mean of zero and a standard deviation of 0.01 mg/L. Histograms of the $\hat{\mu}$ and K_s parameters estimated from the profiles are shown in Figure 10 and Figure 11 along with fitted lognormal distributions. Both of these

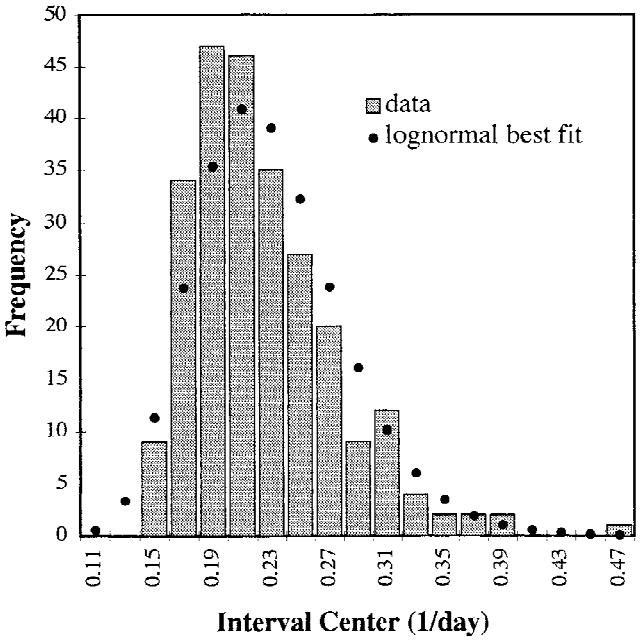

Figure 10. Histogram of $\hat{\mu}$ estimation results using a dissolved oxygen profile with normally distributed error (standard deviation = 0.01 mg/L) and best fit to the lognormal distribution.

data sets exhibit a strong positive skew, possibly due to the fact that $\hat{\mu}$ and K_s must be greater than zero. Pearson's χ^2 goodness of fit tests were conducted on both sets of data (Devore, 1987).

The $\hat{\mu}$ data set failed goodness of fit tests for normal, γ, and lognormal distributions, although the lognormal distribution gave the closest match. The data set had a mean of

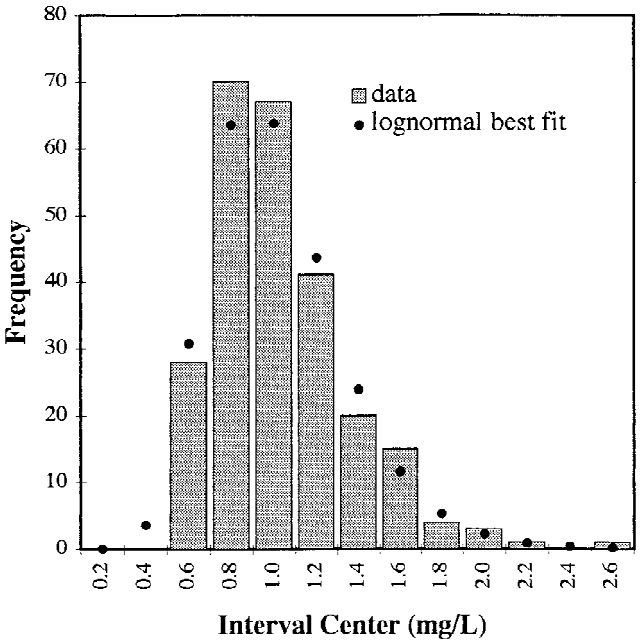

Figure 11. Histogram of K_s estimation results using a dissolved oxygen profile with normally distributed error (standard deviation = 0.01 mg/L) and best fit to the lognormal distribution.

0.225 l/day, a standard deviation of 0.050 l/day, and a skew of 1.26 $(l/day)^{-3}$. The synthetic dissolved oxygen profiles were created with a $\hat{\mu}$ of 0.22 l/day, indicating an error of 2.3% when the arithmetic mean was used as an estimator for the expected value. It is evident, however, that the data set more closely matched a lognormal distribution than a normal distribution, which implies that the geometric mean may be a better estimator for the expected value. The geometric mean of this data set was 0.2202 l/day, which very closely matched the expected value of the population mean and reduced the error of the estimator to 0.09%. Thus, addition of normally distributed error to the synthetic dissolved oxygen profile does not appear to affect the expected value of the $\hat{\mu}$ parameter estimated from the profile. In addition, the low standard deviation of 0.050 l/day and a concomitant coefficient of variation of 22% indicated that fair precision may be expected when using this method to estimate $\hat{\mu}$.

The lognormal distribution provided a good fit to the K_s data set. The data set had a mean of 1.03 mg/L, a standard deviation of 0.33 mg/L, and a skew of 1.26 $(mg/L)^{-3}$. The synthetic dissolved oxygen profiles were created with a K_s of 1.0 mg/L, indicating an error of 3.3% when the arithmetic mean was used as an estimator for the expected value. Because the data set was lognormally distributed, the geometric mean was the proper estimator for the expected value. The geometric mean of this data set was 0.9877 mg/L, which closely matched the expected value of the population mean and reduced the error of the estimate to 1.2%. Addition of normally distributed error to the dissolved oxygen profile did not appear to affect the expected value of the K_s parameter estimated from the profile. The coefficient of variation for this data set (33%) was higher than the coefficient of variation for the $\hat{\mu}$ data set (22%), indicating lower precision in the K_s estimate. Still, K_s values are typically difficult to measure with precision and a coefficient of variation of 33% represents adequate precision for this parameter.

This research indicates that estimating biokinetic parameters of attached growth bioreactors using respirometry is numerically feasible. However, additional work needs to be conducted to determine if this method is effective in the laboratory. Although the conceptual model used in this work has been cited often in the literature, it is not clear whether this model has sufficient accuracy for this application. Failure of any of the assumptions necessary for this model, such as uniform biofilm thickness, uniform biofilm density, completely mixed bulk liquid, etc., may result in failure of the method. Additionally, several detailed steps at the initiation of the method were described that are necessary to establish known initial conditions in the reactor: i.e., a substrate concentration of zero throughout the reactor and a steady-state oxygen profile throughout the reactor. It must be examined whether these conditions can be achieved experimentally without affecting the physiology of the biofilm itself. These remaining issues need to resolved in the next phase of the laboratory experiments. This research does indicate, however, that with accurate model parameters and a system that obeys the conceptual model, the biokinetic parameters $\hat{\mu}$ and K_s can be estimated with good accuracy using the proposed respirometric assay, even in the presence of typical measurement error.

CONCLUSIONS

This research has shown that a transient biofilm model can be solved to yield transient bulk dissolved oxygen profiles. These profiles are sufficiently sensitive to the biokinetic parameters $\hat{\mu}$ and K_s to support parameter estimation if accurate estimates of other model parameters can be obtained, in particular D_s, $D_{f,s}$, X_f, L_f, and L. In addition, the sensitivity of these profiles to biokinetic parameters exceeds the expected measurement error. Even with expected measurement error in the dissolved oxygen profile, a single pair of biokinetic parameters is always retrievable. Simulations with biokinetic parameters of $\hat{\mu} = 0.22$ l/day and $K_s = 1.0$ mg/L and with typical uncorrelated, normally distributed experimental error (standard deviation of 0.01 mg/L) result in estimates of K_s that are lognormally distributed. The $\hat{\mu}$ estimates could not be statistically fit to the normal, lognormal, or γ distributions, although they most closely match the lognormal distribution. The geometric mean proved to be an accurate estimator for the expected value with geometric means of 0.2202 l/day for $\hat{\mu}$ and 0.9877 mg/L for K_s. The coefficient of variation for the $\hat{\mu}$ estimates (22%) indicates fair precision in these estimates. The coefficient of variation for the K_s estimates (33%) indicates a greater spread in these estimates, although the estimates still have adequate precision for this parameter. In summary, this theoretical analysis reveals that a respirometric assay to measure extant biofilm kinetic parameters is promising.

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