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ORIGINAL PAPER

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Identifiability and retrievability of unique parameters describing intrinsic Andrews kinetics

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Abstract A key factor contributing to the variability in the microbial kinetic parameters reported from batch assays is parameter identifiability, i.e., the ability of the mathematical routine used for parameter estimation to provide unique estimates of the individual parameter values. This work encompassed a three-part evaluation of the parameter identifiability of intrinsic kinetic parameters describing the Andrews growth model that are obtained from batch assays. First, a parameter identifiability analysis was conducted by visually inspecting the sensitivity equations for the Andrews growth model. Second, the practical retrievability of the parameters in the presence of experimental error was evaluated for the parameter estimation routine used. Third, the results of these analyses were tested using an example data set from the literature for a self-inhibitory substrate. The general trends from these analyses were consistent and indicated that it is very difficult, if not impossible, to simultaneously obtain a unique set of estimates of intrinsic kinetic parameters for the Andrews growth model using data from a single batch experiment.

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Introduction

The quantitative analysis and design of microbial processes requires estimation of the parameters in the kinetic expression chosen to represent the process of interest, such as microbial growth and substrate depletion. Of particular interest in this work is estimation of the parameters for the Andrews growth kinetics model (Andrews 1968), which has been found by several researchers, e.g., (D'Adamo et al. 1984; Edwards 1970; Hill and Robinson 1975; Pawlowsky and Howell 1973; Yang and Humphrey 1975) to describe the biodegradation kinetics for self-inhibitory substrates:

$$q = \frac{\mu}{Y} = \left(\frac{\mu_{\max}}{Y}\right)\left(\frac{S}{K_s + S + \frac{S^2}{K_i}}\right) \quad (1)$$

Here, q is the specific substrate removal rate, μ is the specific growth rate, Y is the true growth yield, $\mu_{\max}$ is the maximum specific growth rate, K_s is the half-maximum rate coefficient, K_i is the inhibition coefficient, and S is the substrate concentration. Determination of Andrews kinetic parameters is challenging in part because it must be done at relatively high and inhibitory substrate concentrations. Under these conditions, maintaining stable steady-states may not be possible in continuous systems (e.g., chemostats; Andrews 1968). Therefore, some researchers estimate inhibition kinetic parameters in continuous cultures maintained at unstable steady-states (e.g., Schröder et al. 1997), but non-steady-state conditions in batch assays or washout experiments are more typically used.

Unfortunately, standard procedures for determining kinetic parameters in non-steady-state batch assays do not exist. Consequently, the kinetic parameter values reported in the literature vary widely for a given compound, even with pure cultures (e.g., Monteiro et al. (2000). This variability appears to be attributable to three key factors (Grady et al. 1996): (1) culture history, i.e., the type and duration of the environmental conditions imposed on the culture prior to measuring the kinetics, which may

influence enzyme expression and the physiological state and composition of a mixed culture, (2) parameter identifiability, which refers to the ability of the mathematical routine used for parameter estimation to provide unique estimates of the individual parameter values, and (3) the ratio of the initial substrate concentration, S_o, to the initial biomass concentration, X_o, used, which impacts the influence of the culture history and parameter identifiability.

To help reduce the confusion associated with reported kinetic parameters, Grady et al. (1996) proposed terminology to distinguish between different types of kinetic measurements. "Intrinsic kinetics" refers to kinetic parameters obtained from batch experiments with a high S_o/X_o ratio [e.g., $S_o/X_o>20$, with both concentrations as chemical oxygen demand (COD)] and S_o exceeding the anticipated K_s. The kinetics measured under such conditions represent the maximum capability of the microbial community members with the fastest growth kinetics. "Extant kinetics" refer to kinetic parameters measured in batch experiments with very low S_o/X_o values (e.g., $S_o/X_o<0.025$, both concentrations as COD), or when an inhibitor of protein synthesis is present. Kinetics measured under these conditions represent the microbial culture's capability in the continuous culture reactor that served as the source of inoculum. Frequently, batch kinetic parameters reported in the literature were measured at intermediate S_o/X_o values; and the meaning of these transitional kinetics is not clear.

Of the factors contributing to the variability in (and limiting the usefulness of) reported kinetic values, the focus in this work is on parameter identifiability. The ability of a mathematical routine to obtain unique independent estimates of the individual parameter values can be assessed analytically by examining the behavior of the sensitivity equations (Robinson 1985; Robinson and Tiedje 1983). Sensitivity equations are the first derivatives of the dependent variable with respect to a model parameter and, thus, describe the sensitivity of the measured variable (e.g., substrate utilization) to change in each of the model parameters. When the sensitivity equations depend on one or more of the other model parameters, the model is defined as being nonlinear in its parameters. Unique parameter estimates cannot be obtained for a nonlinear model, i.e., more than one combination of parameters may describe the same data set, when the sensitivity equations are multiples of one another. However, even when a model is a priori identifiable, based on the sensitivity analysis, it may not be possible to retrieve unique parameter estimates in practice, due to noise (i.e., measurement errors) in the data (Robinson 1998).

Importantly, in a batch experiment, the behavior of the sensitivity equations is a function of the initial conditions. The influence of initial conditions on the identifiability of the Monod (1949) model parameters has been evaluated under intrinsic (e.g., Robinson and Tiedje 1983) and extant (e.g., Ellis et al. 1996) conditions. However, less work has been reported on parameter identifiability for the Andrews kinetic model. Ellis et al. (1996) performed a sensitivity analysis for determination of extant Andrews kinetics using batch experiments and found that $S_o/K_s \geq 1.0$ was needed to reduce the correlation between μ_{max} and K_s. In addition, the correlation between μ_{max} and K_i decreased as the S_o/K_i ratio decreased and the K_s/K_i ratio increased. However, in assessing the accuracy of their parameter estimation routine in the presence of their experimental noise, Ellis et al. (1996) determined that, in general, all three parameters, μ_{max}, K_s, and K_i were assessed accurately with $S_o/K_i \geq 0.5$ for $S_o/K_s \geq 1$. K_i could not be retrieved at low S_o/K_i ratios, probably because, at low S_o or high K_i, the self-inhibitory effects of the substrate were minimal.

The overall goal of this work was to evaluate the parameter identifiability of intrinsic kinetic parameters describing the Andrews growth model that are obtained from non-steady-state batch assays. This evaluation had three components. First, an identifiability analysis was performed by visually inspecting the sensitivity equations. Second, the practical retrievability of the parameters in the presence of measurement errors was evaluated for the estimation routine used. These analyses were used to identify the initial batch experimental conditions that allow determination of unique and accurate estimates of the intrinsic Andrews kinetic parameters. Finally, the results of these analyses were tested using a published data set for a self-inhibitory substrate.

Materials and methods

Batch Andrews kinetic model

For a batch culture, the change in substrate concentration, assuming Andrews kinetics (Eq. 1) is described by:

$$\frac{dS}{dt} = -\left(\frac{\mu_{max}}{Y}\right)\left(\frac{S}{K_S + S + \frac{S^2}{K_i}}\right)X \tag{2}$$

Assuming there is no biomass decay, the equation for the change in biomass concentration in a batch culture can be expressed as:

$$\frac{dX}{dt} = -Y\left(\frac{dS}{dt}\right) \tag{3}$$

Separating the variables in Eq. 3 and integrating gives:

$$X = Y(S_o - S) + X_o \tag{4}$$

Substituting Eq. 4 into Eq. 2 results in:

$$\frac{dS}{dt} = -\left(\frac{\mu_{max}}{Y}\right)\left[\frac{S[Y(S_o - S) + X_o]}{K_S + S + \frac{S^2}{K_i}}\right] \tag{5}$$

Equation 5 can be integrated to give (Hill and Robinson 1975):

$$\left[\frac{K_i K_s + K_i\left(S_o + \frac{X_o}{Y}\right) + \left(S_o + \frac{X_o}{Y}\right)^2}{K_i\left(S_o + \frac{X_o}{Y}\right)}\right]\ln\left[\frac{Y(S_o - S) + X_o}{X_o}\right] + \left(\frac{K_s}{S_o + \frac{X_o}{Y}}\right)\ln\left(\frac{S_o}{S}\right) - \left(\frac{1}{K_i}\right)(S_o - S) = \mu_{max} t \tag{6}$$

Sensitivity equations and analysis

An a priori parameter identifiability analysis was performed using the sensitivity equations for Andrews kinetics (i.e., the first derivatives of S with respect to μ_{max}, K_s, K_i, Y), which were derived from Eq. 6 by using implicit differentiation (equations not shown). The differentiation was performed using Mathematica ver 1.2 for Macintosh (Wolfram Research, Champaign, Ill.). Note that the sensitivity equations are all functions of K_s, K_i, and Y; and, therefore, the integrated Andrews equation is nonlinear with respect to its parameters (Robinson 1985).

The following kinetic parameter values were used in the sensitivity analysis: μ_{max}=0.1 h^{-1}, K_s=5 mg l^{-1}, Y=0.2 mg X mg S^{-1} (Robinson and Tiedje 1983), and K_i=50 mg l^{-1}. All masses are expressed as COD units. Three S_o/X_o values (0.1, 20, 250) were evaluated in the sensitivity equations by using different X_o values (Robinson and Tiedje 1983). Furthermore, the sensitivity analysis included six different S_o values that resulted in S_o/K_s ratios representing very different regions along the Andrews μ vs S curve, as follows: (1) S_o/K_s=0.04 (first-order case), (2) S_o/K_s=2.0 (mixed-order case), (3) $S_o/K_s=S^*/K_s$=3.16 [maximum inhibited μ case, where $S^*=(K_sK_i)^{1/2}$], (4) S_o/K_s=4.47 (case with S_o between S^* and K_i), (5) S_o/K_s=12.0 (case with S_o close to K_i), and (6) S_o/K_s=40.0 (case with $S_o>K_i$). Therefore, a total of 18 cases were analyzed. In each case, S_o/K_s was set and the S_o needed to get the desired S_o/K_s ratio was calculated and used to calculate X_o, based on the selected S_o/X_o ratio.

To evaluate the identifiability of intrinsic Andrews kinetics, the sensitivity equations were scaled appropriately, plotted, and visually inspected to see whether they were proportional (Robinson 1985).

Computational method for parameter estimation

The practical retrievability of the parameters in the presence of experimental error was evaluated by generating synthetic data sets, using a FORTRAN program (GENCURVE) employing a fourth-order Runge Kutta expression. The coupled differential Eqs. 2, 3 were simultaneously solved to predict profiles of substrate and biomass concentrations over time for a batch assay. However, for this analysis, biomass decay was included for completeness and modeled using an inverse Monod function, as documented by Dang et al. (1989), so that Eq. 3 became:

$$\frac{dX}{dt} = (\mu_{max})\left(\frac{S}{K_s + S + \frac{S^2}{K_i}}\right)X - \left(\frac{S}{K_s + S}\right)bX \quad (7)$$

Here, b is the first-order biomass decay coefficient. Profiles of oxygen consumption were computed by substituting the resulting time-dependent substrate and biomass concentrations into the following equation for oxygen consumption, O_u, at any time in a batch reactor, based on an electron balance (Dang et al. 1989):

$$O_u = (S_o - S) - (X - X_o) \quad (8)$$

Here, the concentrations of all constituents are expressed as COD.

For all simulations used in the practical retrievability analysis, the initial conditions and kinetic parameters were the same as assumed in the sensitivity analysis, with the addition of the first-order decay coefficient, b=0.01 h^{-1}. Finally, error was added to the synthetic experimental data for oxygen uptake. To simulate chemical specific experiments, a constant average relative error was assumed. The maximum amplitude of the error was set at 5% and a random number (between −1 and +1) generator was used to attribute a relative error to each synthetic data point.

Non-linear parameter estimation (NPE) for the synthetic data sets was performed by means of a FORTRAN program (NVOLMA), using the differential equations and numerical technique described for the synthetic data generation. The estimation routine took the resulting time-dependent predictions for S and X and substituted the values into Eq. 8, to obtain a batch oxygen-consumption curve for comparison with the synthetic data. The best-fit estimates for μ_{max}, K_s, and K_i were determined using the complex searching routine of Box (Kuester and Mize 1973). In view of the error used to generate the synthetic data, a relative residual sum of squares criterion was used. Each data set was subjected to four rounds of parameter estimation, by employing initial parameter estimates of 0.25×, 0.5×, 1.0×, and 2.0× the true parameter estimates used to generate the data; and the resulting kinetic parameters were compared with the true values.

Experimental data used for method evaluation

The results of the identifiability and retrievability analyses were evaluated using the experimental data of Brown et al. (1990). These data were selected for evaluation because they were obtained by a research group that has traditionally given great care to the issues of parameter identifiability and retrievability. Brown et al. (1990) used respirometric oxygen uptake data to experimentally determine kinetic parameters for 4-chlorophenol (4-CP) biodegradation at 25 °C with nominal S_o values of 20 mg COD l^{-1} and 100 mg COD l^{-1}. Each experiment was performed with a ratio of S_o/X_o (as COD) of approximately 20. Brown et al. (1990) observed substrate inhibition when 4-CP was added at 100 mg COD l^{-1} and used the Andrews model to fit the parameter values for the two different initial substrate concentrations. The final parameter values were determined by Brown et al. (1990), using the same basic parameter estimation approach as described above for the synthetic data sets. The differential equations expressing the mass balances for substrate, biomass, and soluble microbial products (SMPs) in a batch reactor were simultaneously solved using numerical techniques. The calculated concentrations for S, X, and SMP over time were substituted into an electron balance similar to Eq. 8, to obtain a theoretical oxygen consumption curve for comparison with their experimental values. The best parameter estimates were obtained by using a grid search routine and minimizing the residual sum of square error. The resulting parameters for 4-CP are summarized in Table 1.

In this study, the GENCURVE program was used to simulate the oxygen uptake curve resulting from the S_o and X_o values and best-fit Andrews kinetic parameters reported by Brown et al. (1990). Relative error with a maximum amplitude of 5% was added to each synthesized data point, as discussed above for the retrievability analysis. However, similar results were obtained when these analyses were run with a maximum relative error amplitude of 2% (data not shown). The resulting simulated curves had the same shape as the original data reported by Cooper (1989). The simulated experimental data sets for S_o = 100 mg COD l^{-1} and 20 mg COD l^{-1} were subjected to several rounds of least-square parameter estimation, using the NVOLMA program . Initial parameter estimates were set at 0.25×, 0.5×, 1.0×, 1.25×, and 2.0× the original parameter estimates reported by Brown et al. (1990) for S_o = 100 mg COD l^{-1} and at 0.25×, 0.5×, and 1.0× the original parameter estimates for S_o = 20 mg COD l^{-1}. The fitted parameters were μ_{max}, K_s, and K_i. The product yield, Y_P, and Y values were obtained from Brown et al. (1990; Table 1) and assumed to be constant.

The results of these analyses were confirmed using another NPE program written with Scientist ver 2.01 (MicroMath, St. Louis, Mo.; data not shown).

Results

Parameter identifiability

The scaled sensitivity equations for S_o/X_o values of 0.1, 20, and 250 were plotted for visual inspection of parameter estimate correlation. As an example, the

Table 1 Kinetic parameters describing the biodegradation of 4-CP at 25 °C with nominal S_o values of 20 mg l^{-1} and 100 mg l^{-1} (as COD; Cooper 1989). See Materials and methods for all parameters. A fixed value of the product yield, Y_p equal to the average was used for all parameter sets. *RSSE* Residual sum of squares for error

Flask	S_o (mg COD l^{-1})	μ_{max} (h^{-1})	K_s (mg COD l^{-1})	K_i (mg COD l^{-1})	b (h^{-1})	Y (mg mg^{-1})	Y_p (mg mg^{-1})	RSSE $\times 10^5$ (mg l^{-1})^2
L1	23.6	0.25	2.20	172	0.010	0.44	0.08	4.6
L2	23.6	0.32	5.80	196	0.005	0.42	0.08	31.3
L3	23.6	0.21	1.62	56	0.004	0.39	0.08	2.7
L4	23.6	0.24	1.00	32	0.008	0.38	0.08	1.4
Mean ±SD		0.25±0.05	2.66±2.15	114±82	0.007±0.003	0.41±0.03	0.08	10.0±14.3
H1	108.9	0.31	8.40	24	0.000	0.29	0.06	6.3
H2	109.6	0.25	5.00	60	0.000	0.32	0.06	3.3
H3	114.5	0.28	7.60	64	0.000	0.33	0.06	2.5
H4	112.1	0.32	7.80	44	0.000	0.32	0.06	3.3
Mean ±SD		0.29±0.03	7.2±1.51	48±18	0.000±0.000	0.31±0.02	0.06	3.9±1.7

sensitivity equation plots for S_o/X_o =20 are presented in Fig. 1.

At S_o/X_o=0.1, there is good separation between all four sensitivity equations over most of the progress curve for S_o/K_s values of 2.0, 3.16, and 4.47. However, consistent with previous observations by Robinson and Tiedje (1983) using the Monod sensitivity equations, the sensitivity equations for μ_{max} and Y are more correlated in the first-order region (S_o/K_s=0.04). In addition, as S_o/K_s increases above 12.0 (S_o/K_i>1.2), the μ_{max}, K_i, and K_s sensitivity equations start to become more proportional.

In comparison, the sensitivity equations tend to be more highly correlated for S_o/X_o=20 (Fig. 1) and S_o/X_o =250. In both cases, the K_i and Y sensitivity equations are nearly proportional over most of the progress curve for all S_o/K_s, although the degree of correlation is somewhat lower at S_o/X_o=20 for S_o/K_s values of 2.0, 3.16, and 4.47. As S_o/K_s increases, K_i, μ_{max}, and Y become increasingly correlated at both S_o/X_o ratios. At S_o/X_o=20, the three sensitivity equations are nearly proportional along the progress curve for $S_o/K_s \geq 4.47$ ($S_o/K_i \geq 0.45$), while at S_o/X_o=250, they are nearly proportional for $S_o/K_s \geq 2.0$ ($S_o/K_i \geq 0.2$). The K_s sensitivity equations also become increasingly correlated with the other equations as S_o/K_s increases. For $S_o/K_s \geq 12.0$ ($S_o/K_i \geq 1.2$), the four parameter sensitivity equations are nearly proportional over most of the progress curve at both S_o/X_o=20 and S_o/X_o=250.

In summary, this analysis suggests that unique estimates of all four of the Andrews parameters can be obtained from a single batch assay only within a narrowly defined combination of initial conditions, i.e., S_o/X_o=0.1 and $S_o < K_i$, but above the first-order range (S_o/K_s>0.04). In addition, if an independent estimate of Y is available, i.e., Y does not need to be estimated by NPE (e.g., Ellis et al. 1996), these results suggest it may be possible to get unique parameter estimates both when S_o/X_o has a ratio of 0.1, 20, or 250 and S_o is in the first-order region and when S_o/X_o=20 and $S_o \leq S^*$.

Parameter retrievability

Practical problems associated with determining unique and accurate estimates of intrinsic Andrews kinetics were evaluated by simulating the parameter estimation procedure in the presence of experimental error. The results of all parameter estimation runs using the synthetically generated data sets are illustrated in Fig. 2. For each of the scenarios evaluated, the average and standard deviation of the four best-fit parameter estimates for μ_{max}, K_s, and K_i are presented in panels a, b, and c, respectively. Each set of three columns represents one S_o/K_s scenario. Within a set of columns, the first, second, and third columns represent S_o/X_o ratios of 0.1, 20, and 250, respectively. In general, the S_o/K_s ratio appears to impact the parameter retrievability more than the S_o/X_o ratio. In addition, high standard deviations characterize many of the parameter estimates, suggesting poor identifiability.

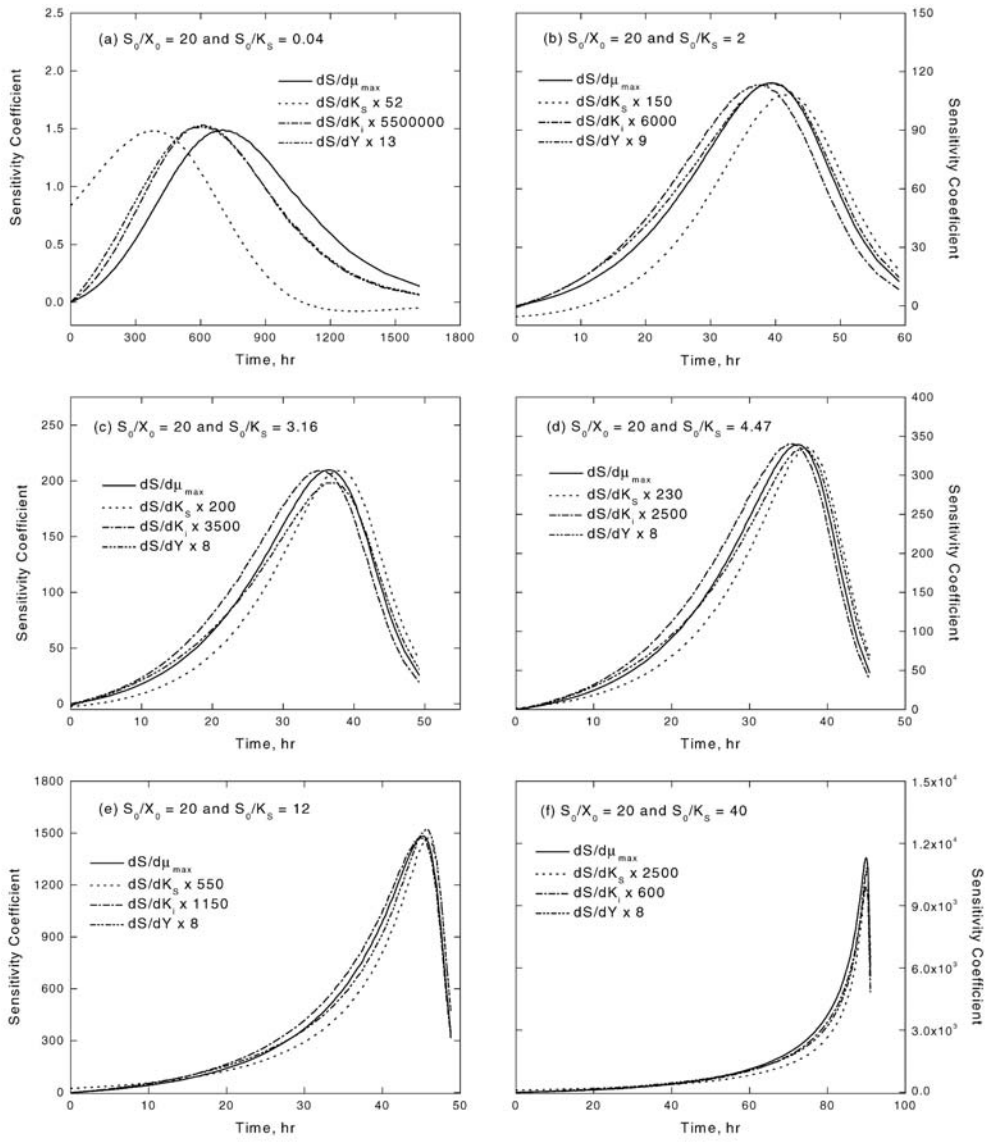

Fig. 1a–f Sensitivity equations for S_o/X_o=20 with: **a** S_o/K_s=0.04, **b** S_o/K_s=2.0, **c** S_o/K_s=3.16, **d** S_o/K_s=4.47, **e** S_o/K_s=12.0, and **f** S_o/K_s=40.0. See Materials and methods for all parameters. *hr* Hours

Analysis of experimental data

The results of the identifiability and retrievability analyses were tested by fitting the Andrews model to the synthesized data obtained by applying the original best-fit parameters from Brown et al. (1990). All of the experiments performed by Brown et al. (1990) were conducted under conditions appropriate for intrinsic batch kinetics (S_o/X_o=20). Using the original mean fitted parameters from Brown et al. (1990) and a nominal S_o of 100 mg l^{-1}, the following values were calculated: S_o/K_s=15.5, S_o/K_i=2.32, and S^*=18.6 mg l^{-1}. As discussed above, these conditions are not expected to allow simultaneous retrieval of unique independent estimates of μ_{max}, K_s, and K_i, but, as examined below, should permit discrimination between the Monod and Andrews models. Similarly, using the mean fitted parameters from Brown et al. (1990) and a nominal S_o of 20 mg l^{-1}, the following values were calculated: S_o/K_s=8.87, S_o/K_i=0.207, and S^*=17.4 mg l^{-1}. Again, retrieval of unique independent estimates of all three parameters is not expected. Further, under the second set of conditions, it may not be possible to discriminate between the Monod and Andrews kinetic models. In fact, Brown et al. (1990) corroborate this, noting that it was difficult to detect substrate inhibition from examination of their raw and transformed oxygen uptake data for S_o=20 mg l^{-1}.

The synthesized data for the cases of S_o=100 mg COD l^{-1} and 20 mg COD l^{-1} are presented in Fig. 3a and b, respectively. Because the data were obtained by simulation, no lag period is shown, although a lag was observed in the real experiments of Brown et al. (1990). The fitted curves for the cases of S_o=100 mg COD l^{-1} and 20 mg COD l^{-1} are also presented in Fig. 3a and b, respectively. The estimated parameter values obtained from the least-square fitting with each different initial guess are provided in Table 2.

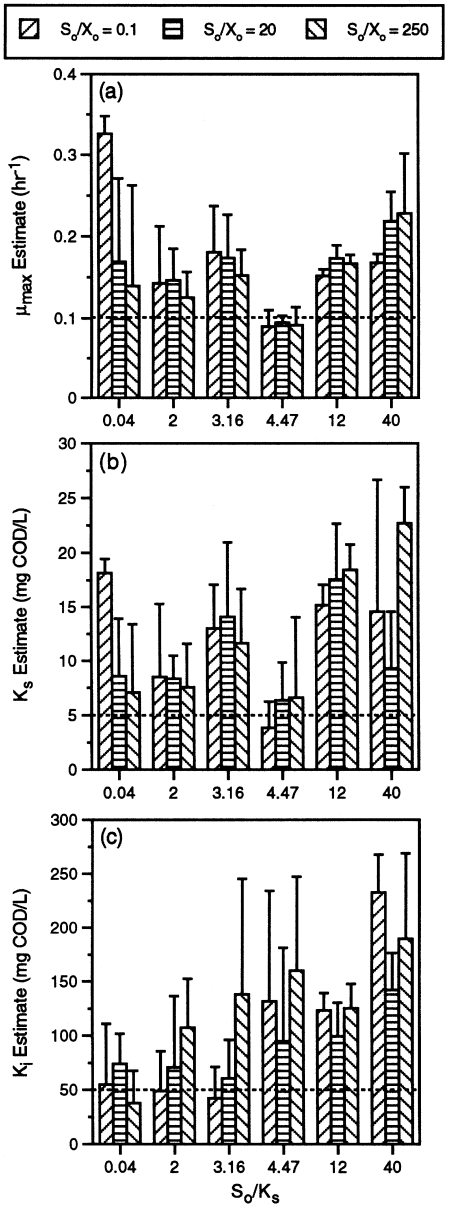

Fig. 2a–c Best-fit estimates of: **a** μ_{max}, **b** K_s, and **c** K_i from synthetic data sets generated with different initial S_o/K_s and S_o/X_o ratios. *Columns* represent the average of the four estimates and *error bars on the columns* represent one standard deviation. *Dashed lines* indicate the true parameter value used to generate the synthetic data set. *L* Liter

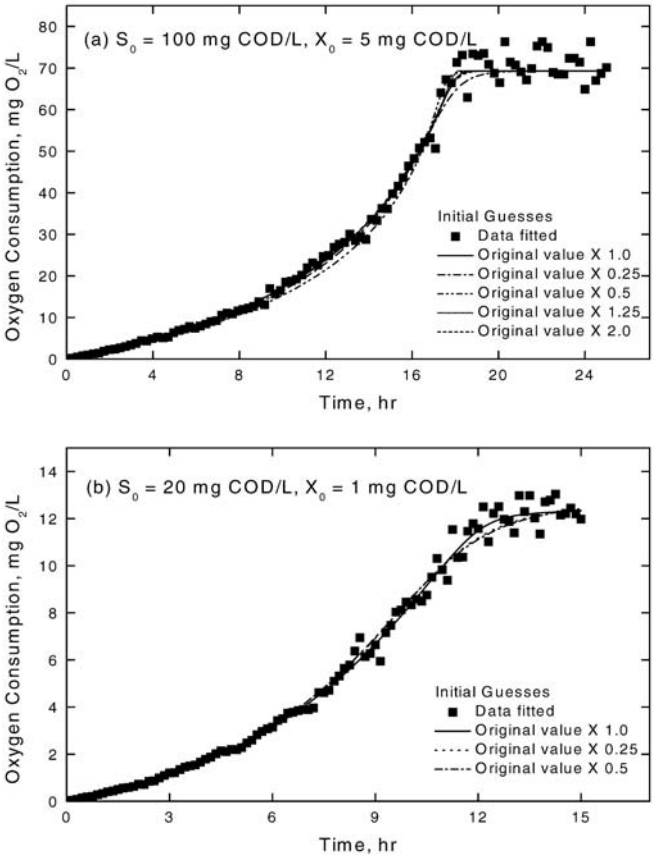

Fig. 3a, b Simulated oxygen uptake data using the best-fit parameters of Brown et al. (1990) for a nominal S_o of: **a** 100 mg chemical oxygen demand (COD) l^{-1} and **b** 20 mg COD l^{-1}, along with best-fit curves for the Andrews model that were generated using different initial parameter estimates

Discussion

Parameter identifiability

The sensitivity analysis defined the conditions under which it is possible to obtain unique estimates of the Andrews kinetic parameters. However, to be useful in this study, those conditions must also: (1) correspond to intrinsic kinetics and (2) allow discrimination between the Andrews kinetic model and expressions for noninhibitory kinetics, such as the Monod equation. Grady and co-workers (e.g., Brown et al. 1990; Dang et al. 1989) used S_o/X_o=20 (on a COD basis) in experiments for evaluating intrinsic Monod and Andrews kinetics to ensure not only that the estimated parameters are unique, but also that biodegradation is coupled to biomass growth. Unfortunately, based on the discussion above, for S_o/X_o=20 and S_o/X_o=250, unique estimates of intrinsic Andrews kinetics can only be obtained in the first-order region, or for S_o/X_o=20 with $S_o \leq S^*$, and then only when there is an independent estimate of Y. In contrast, there is good separation of the four sensitivity equations at S_o/X_o=0.1 for S_o/K_s values of 2.0, 3.16, and 4.47. However, the assumption of substrate biodegradation coupled to biomass growth is not appropriate under this condition. In fact, simulations for biomass at S_o/X_o=0.1 indicate that complete substrate depletion resulted in only a slight (2%) increase in biomass when decay was ignored and a 17% decrease in biomass when decay was included (b=0.01 h^{-1}). Thus, for the conditions evaluated here, when S_o/X_o=0.1, the kinetics being measured are approaching the extant kinetics. Consis-

Table 2 Best-fit parameter values from parameter estimations with different initial guesses. The five values given are factors multiplied by the original parameter values to get the initial guess for each case

	Original value	Best-fit parameters from different initial guesses					Average	Standard deviation
		0.25	0.5	1.0	1.25	2.0		
Nominal S_o = 100 mg COD l^{-1} and X_o = 5 mg COD l^{-1}								
μ_{max} (h^{-1})	0.29	2.1	2.7	0.29	0.34	0.23	1.13	1.17
K_s (mg COD l^{-1})	7.2	190	88	7.2	12.3	1.15	59.7	80.9
K_i (mg COD l^{-1})	48	4.9	3.4	48	39	65.8	32.2	27.4
Nominal S_o = 20 mg COD l^{-1} and X_o = 1 mg COD l^{-1}								
μ_{max} (h^{-1})	0.25	0.77	3.03	0.25	–	–	1.35	1.48
K_s (mg COD l^{-1})	2.66	22	104	2.66	–	–	42.9	53.8
K_i (mg COD l^{-1})	114	10.7	2.16	114	–	–	42.3	62.3

tent with these results at S_o/X_o=0.1, the sensitivity analysis for extant kinetics conducted by Ellis et al. (1996) indicated that $S_o/K_s \geq 1$ was necessary to reduce the correlation between μ_{max} and K_s; and the correlation between μ_{max} and K_i decreased as S_o/K_i decreased and K_s/K_i increased (K_s/K_i=0.1 for all cases in this study).

Discrimination between competing kinetic models (and mechanisms) can be achieved under test conditions that accentuate the differences between the proposed models and put them in jeopardy of failing (Berthouex and Brown 1994; Robinson 1985). Therefore, to distinguish the Andrews and Monod kinetic models, studies must be made at a relatively high concentrations that result in inhibition (Berthouex and Brown 1994; Ellis et al. 1996), i.e., $S_o > S^* = (K_s K_i)^{1/2}$ (D'Adamo et al. 1984). Based on the sensitivity equation analysis, it appears that it is difficult, if not impossible, to use a batch assay to simultaneously obtain unique estimates of the Andrews parameters for intrinsic kinetics ($S_o/X_o \geq 20$) under conditions allowing discrimination between the Andrews and Monod models [$S_o > (K_s K_i)^{1/2}$].

Parameter retrievability

Several key points regarding parameter retrievability can be made, based on Fig. 2. First, at high S_o/K_s ratios of 12.0 and 40.0, all three average parameter estimates were inaccurate. This is consistent with the sensitivity equation analysis, which indicated that these three parameters are generally correlated under these conditions. Second, as S_o/K_s decreased to 4.47 (S_o/K_i=0.45), the average estimates of μ_{max} and K_s were most accurate, although in the case of K_s the standard deviation is relatively large, suggesting the identifiability of K_s is at issue. These results are also consistent with the sensitivity equation analyses, which showed that μ_{max} and K_s were not strongly correlated under these conditions. The K_i average estimates at S_o/K_s=4.47 were poor for all three S_o/X_o ratios, probably due to the correlation between μ_{max} and K_i at S_o/X_o=20 and S_o/X_o=250. However, the sensitivity equations were not proportional at S_o/X_o=0.1. Third, at the lower S_o/K_s ratios of 0.04, 2.0, and 3.16 (S_o/K_i=0.004, 0.2, 0.32), the estimates for μ_{max} and K_s were generally inaccurate, even though the sensitivity equations were not proportional. In contrast, the average K_i estimates were most accurate under these initial S_o/K_s conditions for S_o/X_o=0.1. However, the standard deviations of these K_i estimates were relatively large, which again suggests that parameter identifiability may be an issue. The K_i estimates were generally not as good at the three lowest S_o/K_s ratios for S_o/X_o=20 and S_o/X_o=250. These findings are consistent with the sensitivity analysis, which indicated good separation of the K_i sensitivity equation under conditions with no growth (S_o/X_o=0.1), but an increasing correlation between μ_{max} and K_i for S_o/X_o=20 and S_o/X_o=250, especially at S_o/K_s>0.04. Interestingly, although Ellis et al. (1996) also showed little correlation between K_i and the other parameters at low S_o/K_i in their sensitivity analysis of extant Andrews kinetics, K_i could not be retrieved under these conditions, as noted above. The authors found that S_o/K_i>0.5 was necessary for accurate assessment of K_i. It is not clear why the K_i retrievability results are not the same in the two studies, although it could be due to the use of different parameter estimation routines or differences in the magnitude of the measurement noise.

Consistent with the identifiability analysis, the trends from the retrievability analysis indicate that it is difficult to simultaneously obtain unique and accurate estimates of μ_{max}, K_s, and K_i from a single batch experiment, with S_o/X_o ratios approaching intrinsic conditions ($S_o/X_o \geq 20$) and S_o/K_s ratios for which the Andrews model can be distinguished from the Monod model ($S_o/K_s \geq S^*/K_s$, $S_o/K_i \geq 0.32$). Based on these findings, it is not surprising that several investigators previously reported difficulties associated with the estimation of Andrews kinetic parameters using batch experiments and the application of such kinetic parameters. For example, the application of NPE to estimate Andrews kinetic parameters using data obtained in batch tests with high S_o/X_o ratios has been shown to be sensitive to the initial guesses for the parameter values and, in some cases, the resulting numerical parameter estimates are not meaningful (e.g., D'Adamo et al. 1984; Hill and Robinson 1975; Luong

1987). In addition, other reports in the literature indicate that Andrews kinetic parameters obtained in batch tests with high S_o/X_o ratios tend to overpredict the steady-state substrate concentrations in continuous bioreactor systems (e.g., Rozich and Gaudy 1985).

Analysis of experimental data

As shown in Fig. 3a and b, based on a visual inspection, all the fitted curves describe the synthesized data relatively well for S_o=100 mg and 20 mg COD l^{-1}, respectively. However, in both cases, the estimated values for each parameter are very different, depending on the initial guesses (Table 2). For the case of a nominal S_o=100 mg COD l^{-1} (Table 2), when the estimation process is performed with initial guesses that are larger than the original values, the best-fit μ_{max}, K_i, and K_s remain relatively close to the original values. In contrast, with smaller initial guesses, the best-fit μ_{max} and K_s become larger and the best-fit K_i values become smaller, respectively, than the original values. The standard deviations of the estimated values for each parameter are 85–136% of the average values.

Similarly, for the nominal S_o=20 mg COD l^{-1} case (Table 2), all parameter values exhibit wide ranges and depend on the initial guess values. Again, the standard deviations of the estimated values for each parameter are high, ranging across 110–147% of the average values. Parameter estimations with initial guesses of 1.25× and 2.0× the original values were performed, but are not shown because, for these initial guesses, the criterion for the computation of parameter estimates could not be met. In addition, the K_i value used in this specific case was sufficiently large for the S_o/K_i term in the Andrews model to become relatively small, i.e., S_o/K_i=0.18. In this case, it is difficult to discriminate whether the data being studied follow the Monod or the Andrews model.

The average best-fit parameter values obtained in this study also do not correspond well to the original values obtained by Brown et al. (1990), nor are these average values reasonable. For example, for S_o=100 mg COD l^{-1}, the average K_s is almost two times greater than K_i; and, for S_o=20 mg COD l^{-1}, K_s is approximately the same as K_i. These observations suggest that, as predicted based on the identifiability and retrievability analyses, it is not possible to simultaneously obtain a unique and accurate set of intrinsic Andrews parameters with the batch assay conditions used by Brown et al. (1990). It should be noted that an alternative approach for evaluating replicate sets of extant Andrews kinetic parameters other than the use of arithmetic mean parameter values has been proposed by Magbanua et al. (1998). However, whatever technique is used, in the case of intrinsic Andrews kinetic parameters, the identifiability issues discussed above remain. This is illustrated by the fact that widely varying parameter estimates (Table 2) result in very similar oxygen consumption curves (Fig. 3).

In summary, the general trends from the identifiability and retrievability analysis were consistent and indicate that it is very difficult, if not impossible, to simultaneously obtain a unique and accurate set of intrinsic kinetic parameters for the Andrews model using a single batch experiment. We applied the parameter identifiability and retrievability analyses to evaluate the initial conditions used by other researchers to obtain example data sets for a self-inhibitory substrate. Based on these analyses, it was predicted that retrieval of unique parameter estimates and/or discrimination between the Monod and Andrews kinetic models would be difficult using these initial conditions. Consistent with the predictions, the average best-fit parameter values we obtained were not accurate, unique, or reasonable.

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