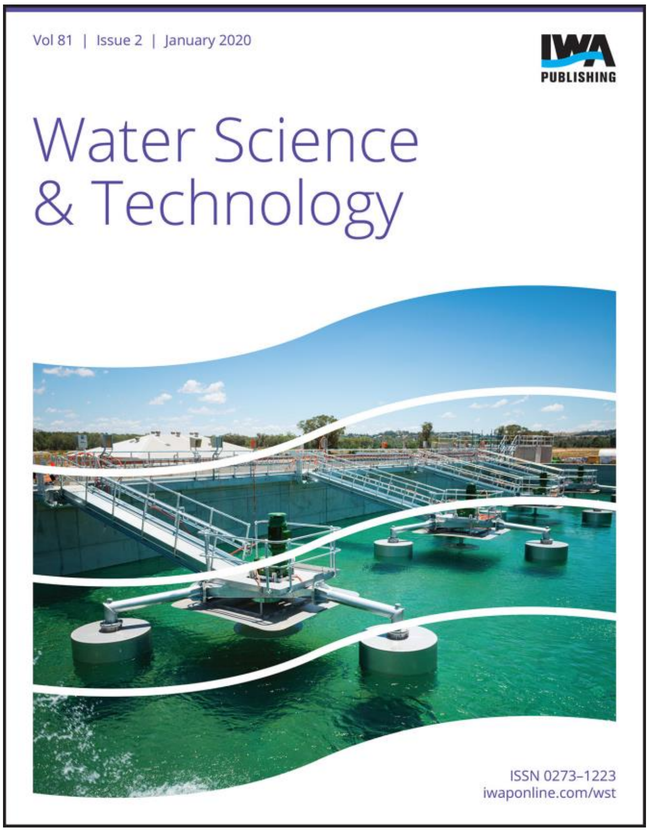
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Analyzing transient respirometric data by analytical algorithm for Monod kinetic parameters

Yeong-Shing Wu and Chow-Feng Chiang

ABSTRACT

This study aims to develop an analytical algorithm with oxygen update (O_u) data obtained from transient respirometric measurement. Based on Monod kinetics, this study formulates a novel two-phase analytical model for an oxygen uptake rate plot (OUR vs. O_u) obtained by respirometric techniques. The first phase is a hyperbolic equation relating to exogenous and endogenous respiration, while the second phase is a linear equation for endogenous respiration only.
An algorithm was therefore developed to analyze four Monod parameters by locating the best phase-separating point at which the absolute average relative error (ARE) of OUR is minimized. An analysis using test data on acetate verified that the algorithm is capable of transient kinetic parameter estimation with an ARE below 5–10%. A sensitivity analysis on domestic wastewater coupled with a Monte Carlo simulation concluded that the kinetic test must be conducted at a relatively high initial substrate level ($S_o/X_o \geqq 1$ and $S_o/K_s \geqq 10$) for reliable parameter estimation. Moreover, it is crucial to conduct the kinetic test with sufficient and acclimated seed culture for the degradation of substrate. The results of this study can be used to develop an automatic transient kinetic analyzer with modern programmable respirometers.

Key words | Monod kinetics, oxygen uptake rate, respirometer, transient kinetics

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INTRODUCTION

Analyzing kinetic parameters for microbial systems has long been of prime interest to scientists and engineers, since the pioneering work of Monod (1949). Based on Monod kinetics, researchers have proposed various expressions to describe the relationship between biomass (X) growth and substrate (S) utilization in a microbial system. They include defining different expressions for endogenous decay (Herbert 1958; Pirt 1965), differentiating active from inert biomass (Young 1981; Grady *et al.* 1999), and considering intermediate product (P) formation (Rittmann *et al.* 1987; Grady *et al.* 1989). This variety introduces more parameters into mathematical models and complicates the procedure for kinetic studies. Nevertheless, maximum specific growth rate (μ_m), half saturation constant (K_s), growth yield coefficient (Y_g), and decay coefficient (k_d) are the four kinetic parameters most widely used for kinetic studies.

The approach to data handling for estimating kinetic parameters also varies widely. The simplest one involves transforming the Monod expression into such a form that a linear regression method can be applied to chemostat data. The chemostat method is time-consuming and labor-intensive when used for sampling and analyzing substrate and biomass. Robinson & Tiedje (1983) proposed a non-linear regression algorithm to estimate kinetic parameters using transient data (S vs. t) obtained for a batch system. Berges *et al.* (1994) proposed a Monte Carlo approach to analyze test errors by propagating the uncertainty about model parameters and error component through the Michaelis-Menten equation. Hong *et al.* (2019) used Monod kinetics for the optimization of cold-water microbial-enhanced oil recovery (MEOR) in a homogenous reservoir.

With the advance of respirometric techniques developed over the past decades, Grady *et al.* (1989) proposed a non-linear iterative algorithm based on the transient oxygen uptake data (O_u vs. t). Although it gives a reliable estimate, Grady's algorithm is implicit (numerical) and may be limited to local optimization for kinetic estimation (Dang *et al.* 1989). Smets *et al.* (1996) suggested that the oxygen uptake rate plot (OUR vs. O_u) gives more distinct kinetic characteristics than the O_u vs. t plot. The OUR plot is characterized

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by a two-phase pattern. The first phase represents exogenous and endogenous respiration, while the second represents only endogenous respiration (Grady *et al.* 1989; Wu *et al.* 2003, 2004). However, to our knowledge, little work has been done to propose an analytical algorithm for the two-phase kinetic pattern. This study aims to develop an explicit (analytical) algorithm for the global optimization of the OUR plot obtained from transient respirometric measurement. The algorithm was evaluated by a Monte Carlo simulation for robustness and favorable test conditions. Further analysis was performed on experimental data to verify the parameter estimation capability of the proposed algorithm.

It is hoped that the results of this study will prompt the development of an automatic kinetic analyzer by incorporating the analytical and speedy algorithm into modern programmable respirometers. Kinetic analysis can be used for short-term biological oxygen demand (BOD) testing, biomass activity testing, compatible analysis, biological reagent study, and respirogram analysis, which are essential for the design and operation of many wastewater treatment and biochemical production processes (Wu *et al.* 2004).

MATERIALS AND METHODS

The respirometric system

The respirometric system on which the mathematical expression of the kinetic study was based is described below. This apparatus normally consists of a series of closed reaction vessels (300–1,000 mL) that operates in batch mode with sufficient mixing. Microbial seeds are normally cultured in a separate master reactor with nutrients and acclimatized to the target substrates at a mean cell residence time (MCRT) of 5–10 days. Two operating procedures are normally used. The first involves feeding a fixed volume of substrate into reaction vessels containing acclimated seed cultures (substrate feeding procedure). The second requires dosing a fixed amount of acclimated seed cultures into reaction vessels containing a substrate solution (seed dosing procedure). Either procedure involves only one replacement so that it measures the true kinetic characteristics of seed cultures associated with their growth history. Modern programmable respirometers normally allow for on-line acquisition of oxygen uptake data in a relative short reaction time, such as 10–60 seconds.

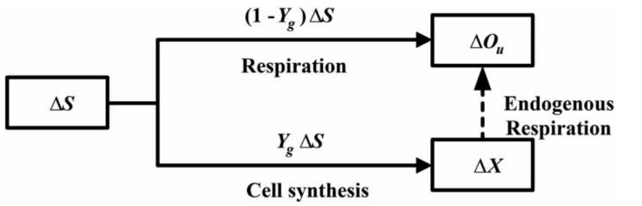

Figure 1 | The conceptual mass-balance model for this kinetic study. The ΔS, ΔX, and ΔO_u are substrate utilization, biomass decay, and oxygen uptake, respectively; Y_g is growth yield coefficient.

Kinetic equations and expressions

The basic scheme of McCarty's two-pathway model (McCarty 1969) was adopted for this study, as shown in Figure 1. Respirometric oxygen demand was used as the basis of model derivation due to its advantage of being a direct measure.

Based on the microbial system described above, five expressions are defined below:

Biomass growth rate: $\mathrm{d}X/\mathrm{d}t = (\mu - k_d)X$ (1)

Substrate utilization rate: $\mathrm{d}S/\mathrm{d}t = -\mu X/Y_g$ (2)

or, $X = -Y_g(\mathrm{d}S/dt)/\mu$ (2a)

Monod kinetics: $\mu = \mu_m S/(K_s + S)$ (3)

Oxygen demand (OD) balance: $\mathrm{d}O_u/\mathrm{d}t = -\mathrm{d}S/\mathrm{d}t - \mathrm{d}X/\mathrm{d}t$ (4)

Initial conditions (IC): $O_u = 0, S = S_o, X = X_o$, at $t = 0$ (5)

where μ is the specific growth rate and μ_m is the maximum μ; and S_o and X_o are the initial substrate and biomass concentration, respectively. It should be noted that S_o is the concentration in the reactor bulk solution after all the additions of seed cultures and nutrient solution, and is different from the feed concentration (S_f). The substrate concentration (S), biomass concentration (X), and half saturation constant (K_s) are all expressed in mg/L of oxygen equivalent. The growth yield coefficient (Y_g) is a dimensionless ratio of active biomass synthesis to substrate removal. Although it might be desirable to express biomass as volatile suspended solids (VSS) for practical use, the relationship between the active biomass and VSS can be conveniently determined by performing a respirometric analysis on the biomass under the endogenous condition.

Derivation of governing equations

By using the above kinetic expressions (Equations (1)–(4)) and initial conditions (Equation (5)), two governing equations for the respirometric system are derived below. The basic approach taken in this derivation is to obtain S and X in terms of O_u and system parameters. In order to eliminate the $\mathrm{d}X/\mathrm{d}t$ and X terms in Equations (1) and (4), Equations (1) and (2a) are substituted into Equation (4) and μ is replaced with Equation (3) to yield:

$$\mathrm{d}O_u/\mathrm{d}t = [Y_g[1-k_d(K_s+S)/(\mu_m S)]-1]\mathrm{d}S/\mathrm{d}t \tag{6}$$

Integrating of Equation (6) at the IC of Equation (5) yields:

$$O_u = [Y_g(1-k_d/\mu_m)-1](S-S_o)-Y_g k_d K_s/\mu_m \times \ln(S/S_o) \tag{7}$$

The term 'ln(S/S_o)' in Equation (7) can be replaced by a Taylor expansion as follows:

$$\ln(S/S_o) = (S-S_o)/S_o-[(S-S_o)/S_o]^2/2+\ldots + (-1)^{n-1}[(S-S_o)/S_o]^n/n + R_{n+1} \tag{8}$$

where R_{n+1} is the residual term of the Taylor series. By using a linearization technique (Himmelblau & Bischoff 1968) at S approaching S_o, Equation (8) can be approximated by taking only the first term of Equation (8):

$$\ln(S/S_o) = (S-S_o)/S_o \tag{9}$$

Substituting of Equation (9) into Equation (7) yields:

$$O_u = [Y_g(1-k_d/\mu_m)-1](S-S_o)-(Y_g k_d/\mu_m)(S-S_o)/(S_o/K_s) \tag{10}$$

The approximation of ln(S/S_o) by Equation (9) may introduce errors in estimating O_u using Equation (10). However, the error (between Equations (7) and (10)) would not be of major concern as S_o/K_s in the second term of Equation (10) is sufficiently large (or S_o is large relative to K_s in the second term of Equation (7)) so that the second term in both equations becomes relatively small. The appropriateness of this approximation will be further evaluated later under different test conditions. Equation (10) is further rearranged to give a linear relation between O_u and S:

$$O_u = \eta(S-S_o) \tag{11}$$

where

$$\eta = Y_g[1-k_d(K_s+S_o)/(\mu_m S_o)]-1 \tag{11a}$$

Integrating Equation (4) at the IC of Equation (5) and rearranging yields:

$$X = X_o-O_u+S_o-S \tag{12}$$

By substituting Equations (1) and (2) into the mass balance equation (Equation (4)) and replacing μ with Equation (3), dO_u/dt is derived as a function of S and X:

$$\mathrm{d}O_u/\mathrm{d}t = [(1/Y_g-1)\mu_m S/(K_s+S)+k_d]X \tag{13}$$

By substituting the S and X expression (Equations (11) and (12)) into Equation (13), $\mathrm{d}O_u/\mathrm{d}t$ can be further derived as a function with respect only to O_u:

$$\mathrm{d}O_u/\mathrm{d}t = (\lambda_1 O_u+\lambda_2)(\lambda_3 O_u+X_o)/(O_u+\lambda_4) \tag{14}$$

where

$$\lambda_1 = (1/Y_g-1)\mu_m + k_d \tag{14a}$$

$$\lambda_2 = -(\lambda_1 S_o + k_d K_s)\times\eta \tag{14b}$$

$$\lambda_3 = -(1+1/\eta)-1 \tag{14c}$$

$$\lambda_4 = -(K_s+S_o)\times\eta \tag{14d}$$

Equation (14) can be rewritten as:

$$\mathrm{d}O_u/\mathrm{d}t = (\alpha_1 O_u^2+\alpha_2 O_u+\alpha_3)/(O_u+\alpha_4) \tag{15}$$

where

$$\alpha_1 = \lambda_1\lambda_3 \tag{15a}$$

$$\alpha_2 = \lambda_1 X_o+\lambda_2\lambda_3 \tag{15b}$$

$$\alpha_3 = \lambda_2 X_o \tag{15c}$$

$$\alpha_4 = \lambda_4 \tag{15d}$$

Equation (15) is essentially a hyperbolic function with $\mathrm{d}O_u/\mathrm{d}t$ being the y-axis and O_u being the x-axis to describe the first phase on OUR vs. O_u plot. Equation (15) can be further transformed into a form of Equation (16), so that a multiple regression method can be used for the estimation of kinetic parameters:

$$[O_u \times \mathrm{OUR}] = \alpha_1[O_u^2] + \alpha_2[O_u] + \alpha_3 - \alpha_4[\mathrm{OUR}] \quad (16)$$

In the above equation, the OUR is dO_u/dt. For the second phase, Equations (12) and (13) can be simplified into Equation (17), at S approaching zero:

$$\mathrm{OUR} = \beta_1 O_u + \beta_2 \quad (17)$$

where

$$\beta_1 = -k_d \quad (17a)$$

$$\beta_2 = k_d(X_o + S_o) \quad (17b)$$

Equation (17) is a linear form and can be directly solved for k_d by using a simple linear regression method. For this the oxygen uptake reaction must reach the endogenous phase and the uptake data must be collected for a sufficient period of time for reliable parameter estimation. Equations (16) and (17) are the two analytical equations that can be used to analyze the OUR vs. O_u plot obtained by the respirometric test for the kinetic parameters estimation.

Kinetic parameters estimation algorithm

To estimate the coefficients of the two governing equations (Equations (16) and (17)) as derived above, a computational algorithm is proposed in Table 1. The algorithm first locates the best separation point (SP) between the first and second phases by sweeping SP from the initial point at i_{sp} of 5 to the final point at i_{sp} of n–3, as illustrated in Figure 2. An i_{sp} of at least 5 must be used to estimate the four parameters (α_1–α_4) in the exogenous phase (Equation (16)). This algorithm is therefore called the 'SP-sweeping method', as compared to the 'grid-searching method' proposed by Grady *et al.* (1989). As described in Table 1, the best SP and kinetic parameters are determined by minimizing an objective function of the sum of the absolute average relative errors ($\mathrm{ARE} = (\Sigma_i|\mathrm{OUR}^e - \mathrm{OUR}|/\mathrm{OUR})/n \times 100\%$) between the measured and the estimated OUR (OUR^e).

Table 1 | The kinetic parameter estimation algorithm and Monte Carlo evaluation procedure of the kinetic model proposed in this study

Step	Description
Kinetic parameters estimation algorithm	
1	*Generate* OUR *vs.* O_u *plot:* obtain 600–800 (n) data points for the OUR plot (solving Equations (1)–(4) simultaneously for this study).
2	*Select the starting SP* (i_{sp}): among the 600–800 data points, select the separating point of i_{sp} starting from Point 5 on the OUR plot.
3	*Solve for* β_1 and β_2: perform simple linear regression using Equation (17) to minimize the objective function $\Sigma_i(\mathrm{OUR}^e - \mathrm{OUR})^2$.
4	*Solve for* α_1, α_2, α_3, and α_4: calculate $\mathrm{OUR} \times O_u$ and apply multiple linear regression to Equation (16) by minimizing the objective function $\Sigma_i(O_u^e \times \mathrm{OUR}^e - O_u \times \mathrm{OUR})^2$.
5	*Solve for* k_d: use the β_1 and β_2 obtained in Step 3 to solve Equation (17a) and (17b) simultaneously.
6	*Solve for* μ_m, Y_g, and K_s: with the α_1, α_2, α_3, and α_4 obtained in Step 4, substitute Equation (14a)–(14d) into Equation (15a)–(15d) and to solve the four equations simultaneously.
7	*Estimate* O_u *and* OUR (O_u^e, OUR^e): use the parameters estimated in Steps 5–6 to compute Equations (1)–(4) for O_u^e vs. t and transform it into OUR^e vs. O_u^e.
8	*Determine the best* i_{sp} *and the kinetic parameters*: repeat Steps 1–7 until Point n – 3 to determine the best SP and the corresponding parameters by minimizing the objective function of absolute ARE of $(\Sigma_i|\mathrm{OUR}^e - \mathrm{OUR}|/\mathrm{OUR})/n \times 100\%$.

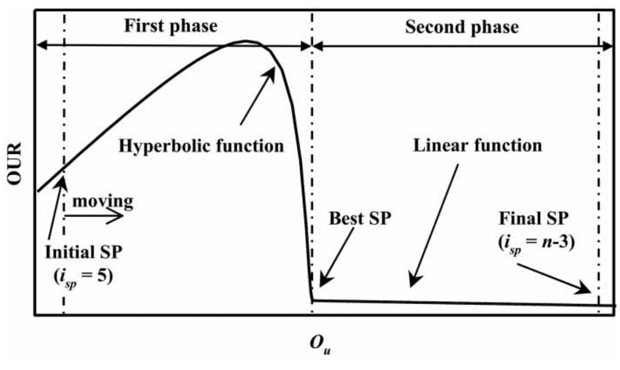

Figure 2 | A conceptual diagram illustrating the separation-point (SP) sweeping method for kinetic parameter estimation proposed in this study.

Experimental validation

Two sets of respirometric data were obtained from Young (2001) to illustrate the parameter estimation capability. Figure 3 shows a schematic diagram of the respirometric apparatus system used in this study. The effective volume

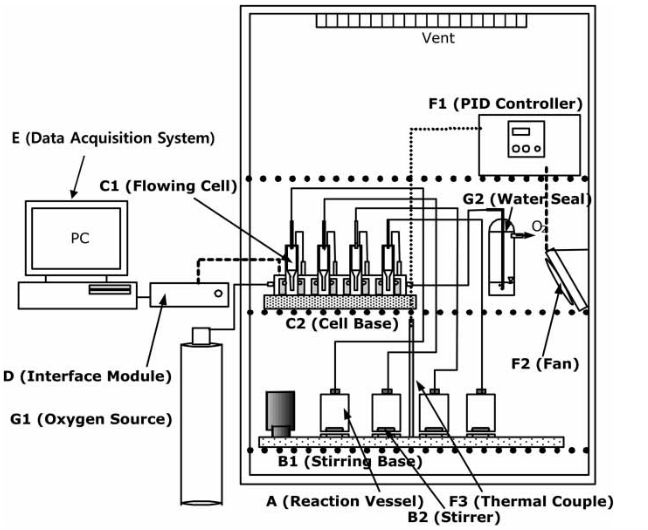

Figure 3 | The respirometric apparatus used in this study, consisting of six major systems: reaction vessel (A), mixing system (B1 = stirring base, B2 = magnetic stirrer), flowing cell (C1) with cell base (C2), interface module (D), data acquisition system (E), and temperature control system (F1 = PID controller, F2 = Fan, and F3 = thermal couple; G1 = oxygen source and G2 = water seal bottle.

of test vessel was 500 mL and acetate was used as the substrate at an initial concentration (S_o) of 300 mg chemical oxygen demand (COD)/L. An acclimated seed culture (approximately 2,000 mg VSS/L) was dosed once into each vessel at a volume of 25 mL and 100 mL. The test was operated in a batch mode at 20 °C. The detailed procedure can be found in Young (2001).

Monte Carlo analysis

In order to evaluate the algorithm for robustness to test errors, a Monte Carlo procedure was used to generate simulated test data for each condition. Four kinetic parameter data typical of domestic wastewater were first selected from the literature: $\mu_m = 3.6$ 1/d, $K_s = 70$ mg/L of BOD, $Y_g = 0.7$, $k_d = 0.06$ 1/d (Metcalf & Eddy 1991) to generate a set of O_u vs. t data (by Equations (1)–(4)) for each test condition. The OUR vs. O_u data were then calculated by a method of central difference.

For a sequential batch operation, the initial cell concentration can be set as a finite number as $X_o = \chi$, while the initial substrate concentration can be calculated as $S_o = S_f \times D/F$, in which S_f is the feed substrate concentration in mg/L, D is daily dilution rate in 1/d, and F is daily feeding frequency in #/d. The inverse of D/F is also defined as the total feed number per cycle ($N_f = F/D$) so that S_o is also calculated as S_f/N_f. The robustness evaluation procedure was evaluated at three operating factors, each at one low and one high level: 2 and 6 # for N_f, 6 and 18 days for $1/D$, and 1,000 and 3,000 mg/L for S_f. A version of the Monte Carlo technique with Gaussian distribution was employed to generate random errors at four levels of variation coefficients (Cv = 0, 5, 10, and 15%) for each point in the OUR data set. A total of 32 sets (2^3 level$^{\text{factor}} \times 4$ Cv) of data were obtained for error-free and error-generated OUR vs. O_u plots. In order to compute a reliable mean ARE value for error-imposed OUR data, 10 Monte Carlo simulations were performed for each set of test conditions, each simulating 10 replicate tests with an error produced by the computer random number generator.

RESULTS AND DISCUSSION

Illustration by experimental study

Table 2 gives stepwise computation results for 59 data points as proposed in Table 1 for kinetic parameter estimation. The best SP was located at Point 30 with the minimum ARE of 7.6%. Figure 4(a)–4(d) show the experimental data and simulated curves of O_u, OUR vs. t and OUR vs. O_u, and the simulated curves of S and X vs. t, at two different seed volumes (25 and 100 mL). These curves illustrate the excellent curve fitting capability of the proposed model with the distinct two-phase characteristics. As shown in Figure 4(a) and 4(b), the substrate was exhausted at 12 hours when the biomass was entering the decay phase and O_u and OUR were entering the second phase.

At the seed volume of 25 mL, the four kinetic parameters (μ_m, Y_g, K_s, and k_d) were estimated to be 0.37 1/h, 0.40, 10 mg/L, 0.066 1/h, and the two initial concentrations (S_o and X_o) to be 285 and 3.78 mg/L. At the seed volume of 100 mL, the estimated parameters were $\mu_m = 0.20$ 1/h, $Y_g = 0.66$, $K_s = 4.9$ mg/L, $k_d = 0.032$ 1/h, $S_o = 450$ mg/L, and $X_o = 124$ mg/L. The higher S_o for the 100-mL dose run suggests that additional substrate was carried over from the seed addition. This situation should be minimized when conducting a transient kinetic study by harvesting seed cultures as the substrate is exhausted at the end of a feed cycle. The two substrate ratios of S_o/X_o and S_o/K_s were calculated to be 75 and 29 for the seed volume of 25 mL, and 3.7 and 93 for the seed volume of 100 mL, respectively, both satisfying the test criteria of $S_o/X_o \geqq 1$ and $S_o/K_s \geqq 10$ as concluded by Wu *et al.* (2003). However, the test data gave a much lower ARE of 5.2% for the seed volume of 100 mL compared to 32% for the seed volume of 25 mL. Although the added biomass ratio between the

Table 2 | Illustration of the parameter estimation algorithm proposed in Table 1, showing stepwise computation results across *i* sets of OUR vs. O_u data points with the minimum ARE of 7.6% located at the 30th points; test data obtained from Young (2001) for acetate degradation for a seed dose volume of 100 mL

Step #	1–2		3		4		5		6		7	8
i (#)	O_u (mg/L)	OUR (mg/L-h)	β_1	β_2	α_1	α_2–α_4	k_d (1/h)	μ_m (1/h)	Y_g (-)	K_s (mg/L)	ARE (%)	Min. ARE
1–4							NA	NA	NA	NA	NA	
5	33.0	64.8	−0.4	111	0.29		0.400	0.06	−0.32	−81.1	NA	
6–23	(Data not shown)											
24	179	84.8	−1.3	316	0.23		1.325	3.04	1.51	136.0	NA[a]	
25	187	85.0	−1.3	305	0.25		1.277	1.61	0.25	14.2	47.9	
26	196	82.0	−1.1	264	0.27		1.098	1.76	0.49	70.7	60.7	
27	203	65.6	−0.8	191	0.24		0.780	1.06	0.27	10.2	30.5	
28	209	47.0	−0.5	118	0.24		0.467	0.72	0.24	9.3	18.0	
29	212	29.0	−0.2	64	0.24		0.236	0.49	0.23	9.5	10.5	
30	215	13.4	−0.1	36	0.24		0.114	0.36	0.26	10.3	7.6	Min
31	216	10.6	−0.1	32	0.24		0.096	0.34	0.28	10.9	7.9	
32	217	11.2	−0.1	32	0.24		0.099	0.35	0.28	11.3	8.5	
33–45	(Data not shown)											
46	231	10.0	0.0	9	0.21		0.001	0.21	0.95	126.9	13.3	
47	232	9.75	0.1	−4	0.20		−0.054	−4.72	1.01	−3,731	NA[a]	
48–51	(Data not shown)											
52	236	8.05	−0.2	47	0.17		0.157	0.32	0.21	1.0	11.7	
53	237	10.4	−0.4	107	0.16		0.408	0.84	0.74	97.4	96.2	
54	238	10.3	−0.4	111	0.14		0.424	0.82	0.74	89.5	95.5	
55	239	9.75	−0.3	89	0.13		0.333	0.75	0.76	111.6	99.5	
56	240	8.60	−0.1	41	0.12		0.132	1.35	0.93	285.2	95.5	
57	241	8.85	−0.3	86	0.11		0.320	0.69	0.76	106.8	99.9	
58–59	(Data not shown)											

[a]Not applicable, if $Y_g > 1$ and/or negative value for any k_d, μ_m, Y_g, or K_s.

two seed volumes was only 4 (100/25), the active biomass ratio between the two estimated X_o was as large as 33 (124/3.75). This reveals that only 12% (4/33) of activity was developed in the test with 25-mL seed volume compared to the test with 100-mL seed volume. Consequently, more kinetic information was developed for the 100-mL test to yield more reliable parameter estimation. While maintaining a high substrate ratio is important, it is also crucial to conduct kinetic studies with enough seed culture for reliable parameter kinetic estimation.

Torretta *et al.* (2014) also conducted an acetate kinetic study by respirometer, which was seeded with the activated sludge from a municipal wastewater treatment plant. Their study showed the four kinetic parameters (μ_m, Y_g, K_s, and k_d) to be 0.018–0.069 1/h, 0.59–0.74, 1.0–2.9 mg/L, 0.015 1/h, respectively. And their two initial concentrations (S_o and X_o) were 20–120 and 638–967 mg/L. Compared to the parameters determined by our study, the value of Y_g is consistent, but the values of μ_m, K_s, and k_d are very different. Beside the difference in the seed culture, it is notable that their test criteria of $S_o/X_o = 0.02$–0.14 and $S_o/K_s =$ 39–52 do not fully satisfy the test criteria of $S_o/X_o \geqq 1$ and $S_o/K_s \geqq 10$ as proposed by this study and Wu *et al.* (2003). The comparison shows that estimated kinetic parameters depend on seed culture and test criteria as also suggested by Grady *et al.* (1996). They proposed three types of kinetic parameters associated with initial test conditions: intrinsic ($S_o/X_o \geqq 20$), extant ($S_o/X_o \leqq 0.025$), and pseudo-intrinsic ($0.025 \leqq S_o/X_o \leqq 20$) with defined test conditions. For conducting the maximum achievable kinetics, $S_o/X_o \geqq 20$ should be used. For assessing the existing kinetics, as in activated sludge processes, the S_o/X_o value should be limited, as

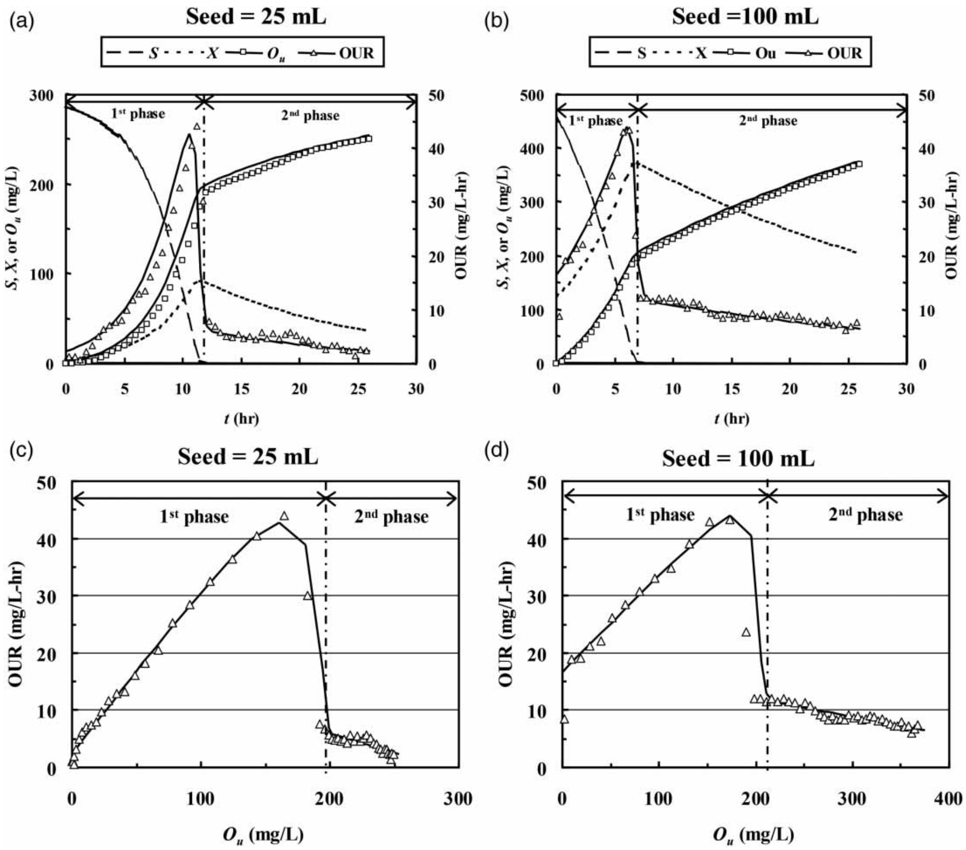

Figure 4 | Comparison of experimental data and simulated curves for the plots of S, X, O_u and OUR vs. t, at a seed volume of 25 mL for plots (a) and (b) and 100 mL for plots of (c) and (d).

they are normally operated near the endogenous phase to meet the effluent standards. Based on this study, minimum ratios of $S_o/X_o = 1$ and $S_o/K_s = 10$ are suggested for reliable parameter estimation. The use of the criterion of $S_o/K_s \geqq 10$ could allow this proposed algorithm to accommodate different types of substrates with different levels of biodegradability, as a substrate with a higher K_s is associated with being more refractory to degradation, particularly for many industrial wastewaters. More studies on different types of substrates are suggested.

Verification by error free data

Table 3 shows AREs that were calculated from the verification procedure for the 32 sets of test conditions in this study. The first eight sets of analyses were performed with error-free data (Cv = 0%), showing a range of 0.43–5.0% for AREs. This analysis clearly indicates that the proposed algorithm exhibits a better curve-fitting capability at the low feeding number of 2 # (ARE = 0.43–1.6%) than at the high feeding number of 6 # (ARE = 0.88–5.0%). It should be noted that feeding at low N_f will result in high S_o as required for deriving Equation (6a). Also, the highest error (ARE) occurred at the lowest ratio of S_o/K_s (2.4) among the eight error-free runs. Nevertheless all of the ARE errors were well below 5.0%, suggesting the kinetic model and algorithm are properly developed.

Monte Carlo simulation

The algorithm was further evaluated with error-generated OUR data obtained from a Monte Carlo simulation at three Cv values of 5, 10, and 15%. The results in Table 3 indicate that at 5% Cv, ARE only increases to a certain limit of 4.0–4.2%, except in Runs #10 and #12 (the lowest S_o/K_s). It appears that the robustness of the proposed algorithm is not affected significantly by the OUR error at a Cv up to 5%. The exceptionally high AREs (76–81%) for Runs #10 and #12 occurred at the lowest ratio of S_o/K_s.

As the Cv error increased further to 10%, test runs at the low N_f (2 #) appear to be more reliable than at the high N_f (6 #) for parameter estimation. The ARE increases

Table 3 | Results of Monte Carlo simulation for robustness evaluation as indicated in ARE with three operating factors, each at one high and one low level (2 # and 6 # for N_f, 1 and 18 days for $1/D$ and 1,000 and 3,000 for S_f) and four Cv values (0, 5, 10, 15%) associated with OUR errors

Run	N_f (#)[a]	$1/D$ (d)[a]	S_f (mg/L)[a]	Cv (%)	S_o/X_o (–)	S_o/K_s (–)	ARE (%)[b]
#1	6	18	3,000	0	0.62	7.1	0.88
#2	6	18	1,000	0	0.62	2.4	2.3
#3	6	6	3,000	0	0.40	7.1	1.6
#4	6	6	1,000	0	0.40	2.4	5.0
#5	2	18	3,000	0	3.4	21	0.43
#6	2	18	1,000	0	3.4	7.1	1.0
#7	2	6	3,000	0	2.0	21	0.56
#8	2	6	1,000	0	2.0	7.1	1.6
#9	6	18	3,000	5	0.62	7.1	4.2
#10	6	18	1,000	5	0.62	2.4	81
#11	6	6	3,000	5	0.40	7.1	4.2
#12	6	6	1,000	5	0.40	2.4	76
#13	2	18	3,000	5	3.4	21	4.1
#14	2	18	1,000	5	3.4	7.1	4.1
#15	2	6	3,000	5	2.0	21	4.0
#16	2	6	1,000	5	2.0	7.1	4.2
#17	6	18	3,000	10	0.62	7.1	42
#18	6	18	1,000	10	0.62	2.4	80
#19	6	6	3,000	10	0.40	7.1	102
#20	6	6	1,000	10	0.40	2.4	76
#21	2	18	3,000	10	3.4	21	15
#22	2	18	1,000	10	3.4	7.1	11
#23	2	6	3,000	10	2.0	21	23
#24	2	6	1,000	10	2.0	7.1	29
#25	6	18	3,000	15	0.62	7.1	61
#26	6	18	1,000	15	0.62	2.4	74
#27	6	6	3,000	15	0.40	7.1	103
#28	6	6	1,000	15	0.40	2.4	78
#29	2	18	3,000	15	3.4	21	46
#30	2	18	1,000	15	3.4	7.1	38
#31	2	6	3,000	15	2.0	21	110
#32	2	6	1,000	15	2.0	7.1	82

[a] S_f is feed substrate concentration in mg/L, D is daily dilution rate in 1/day, F is daily feeding frequency in #/day, N_f (inverse of D/F) is total feeding number per cycle in #/cycle.
[b] ARE $= \Sigma_i(|OUR^e - OUR|/OUR)/n \times 100\%$; the value is a mean estimated from 10 replicates of simulation.

to a reasonable limit of 11–29% at the low feeding number. The algorithm was further evaluated at a Cv of 15%. Among all the eight test runs at Cv of 15%, the model robustness is unlikely to be maintained at an ARE of 30% or less, even for runs tested at high substrate biomass ratios.

Based on the above analysis, it can be concluded that the new algorithm gives better parameter estimation at a lower N_f and higher S_f, and consequently at higher S_o/K_s ratios among all the test runs. The kinetic parameters estimated from the two preferred levels ($N_f = 2$ # and $S_f =$ 3,000 mg/L) and a $1/D$ of 18 days were then used to generate OUR vs. O_u plots (Figure 5(a)–5(d)) at the four Cv levels for further visual verification of the model robustness. At a Cv up to 10%, the OUR plot clearly shows fair robustness with a typical shape pattern distinctly separated into two phases. The first phase is also characterized by a typical pattern of a gradual rise followed by a rapid decline in OUR. However, the model estimation capacity drastically collapsed as the Cv reached 15%.

As discussed previously, the derivation of the first-phase governing equation (Equation (6a)) requires the assumption of sufficiently high initial substrate concentrations. The effect of the two substrate ratios of S_o/K_s and S_o/X_o (Table 3) on ARE was further examined. In general, test runs at higher substrate ratios gave lower AREs: 1% for a Cv of 0%, 5% for a Cv of 5%, and 20% for a Cv of 10%. It can be concluded that a minimum substrate ratio of 10 for S_o/K_s and 1 for S_o/X_o is required for the proposed algorithm to give an acceptable ARE of 5% or less (Wu *et al.* 2003).

CONCLUSIONS

A novel model was successfully developed in this study to analyze the OUR vs. O_u plot obtained from transient respirometric data. Under the assumption of a high initial substrate ratio (S_o/K_s), the model can be derived into two analytical equations (Equations (16) and (17)), a hyperbolic function describing the first phase of exogenous and endogenous respiration, and a linear function of endogenous respiration describing the second phase on the OUR plot. A novel algorithm was also proposed to assess four kinetic coefficients (μ_m, K_s, Y_g, and k_d) by sweeping for the separating point across the entire range of the observed time span until a minimum average relative error (ARE_o) of OUR is reached. It was concluded that the algorithm is capable of parameter estimation at an ARE_o below 5–30% for variation coefficients (Cv) on OUR up to 5–10%. The algorithm is unlikely to maintain its robustness for all the test runs at a Cv up to 15%. The testing conditions must be designed in favor of high substrate ratios (S_o/K_s) to improve the parameter estimating capability of the proposed algorithm. Minimum ratios for S_o/X_o of 1.0 and for S_o/K_s of 10 are suggested.

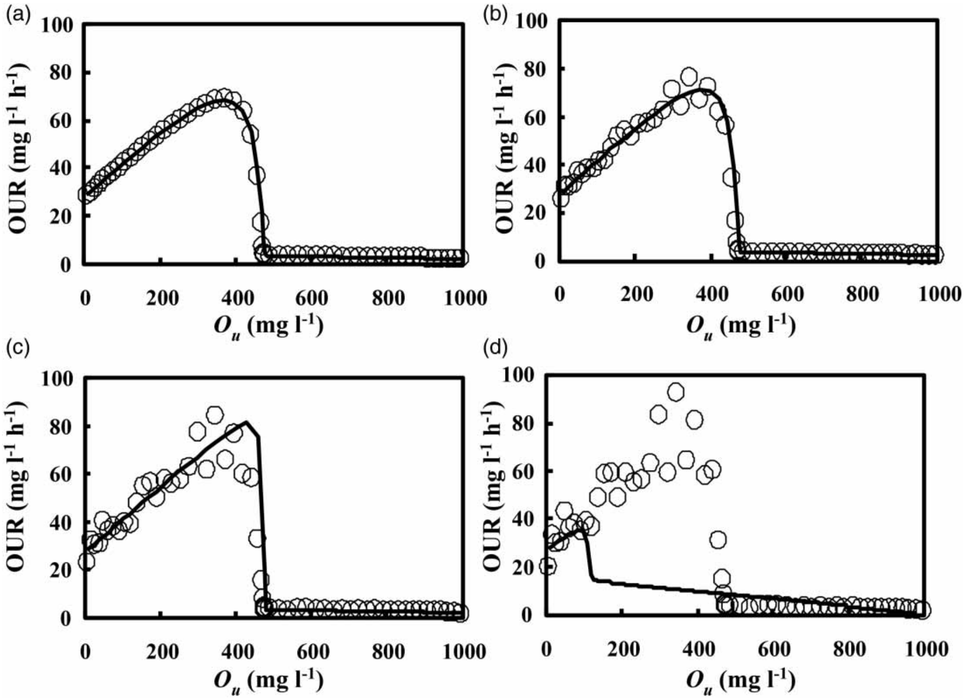

Figure 5 | Comparison of test data and simulated lines for the four OUR plots generated at four Cv values associated with OUR errors for the three preferred operating levels (N_f of 2 # per SRT, SRT of 18 days, and S_f of 3,000 mg/L of oxygen demand) from Table 2, with (a) 0%, (b) 5%, (c) 10%, and (d) 15% for the Cv values.

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REFERENCES

Berges, J. A., Montagnes, D. J. S., Hurd, C. L. & Harrison, P. J. 1994 Fitting ecological and physiological data to rectangular hyperbolae – a comparison of methods using Monte-Carlo simulations. *Marine Ecology Progress Series* **114** (1), 175–183.

Dang, J. S., Harvey, D. M., Jobbagy, A. & Grady, C. P. L. 1989 Evaluation of biodegradation kinetics with respirometric data. *Research Journal of the Water Pollution Control Federation* **61** (11–12), 1711–1721.

Grady Jr., C. P. L., Dang, J. S., Harvey, D. M., Jobbagy, A. & Wang, X. L. 1989 Determination of biodegradation kinetics through use of electrolytic respirometry. *Water Science and Technology* **21** (8–9), 957–968.

Grady Jr., C. P. L., Smets, B. F. & Barbeau, D. S. 1996 Variability in kinetic parameter estimates: a review of possible causes and a proposed terminology. *Water Research* **30** (3), 742–748.

Grady, C. P. L., Digger, G. T. & Lim, H. C. 1999 *Biological Wastewater Treatment*. Marcel Dekker, New York, USA.

Herbert, D. 1958 Some principles of continuous culture. In: *Recent Progress in Microbiology* (G. Tunevall, ed.). Almqvist & Wiksell, Stockholm, pp. 381–396.

Himmelblau, D. M. & Bischoff, K. B. 1968 *Process Analysis and Simulation: Deterministic Systems*. John Wiley & Sons, New York, USA.

Hong, E., Jeong, M. S., Kim, T. H., Lee, J. H., Cho, J. H. & Lee, K. S. 2019 Development of coupled biokinetic and thermal model to optimize cold-water microbial enhanced oil recovery (MEOR) in homogenous reservoir. *Sustainability* **11** (6), 1652.

McCarty, P. L. 1969 The 5th Rudolf Research Conference. In: *Energetics and bacterial growth*, July 2. Rutgers. The State University, New Brunswick, New Jersey, USA.

Metcalf &Eddy 1991 *Wastewater Engineering Treatment, Disposal, and Reuse*. McGraw-Hill, New York, USA.

Monod, J. 1949 The growth of bacterial cultures. *Annual Review of Microbiology* **3** (1), 371–394.

Pirt, S. J. 1965 The maintenance energy of bacteria in growing cultures. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **163** (991), 224–231.

Rittmann, B. E., Bae, W., Namkung, E. & Lu, C. J. 1987 A critical-evaluation of microbial product formation in biological processes. *Water Science and Technology* **19** (3–4), 517–528.

Robinson, J. A. & Tiedje, J. M. 1983 Nonlinear estimation of Monod growth kinetic parameters from a single substrate depletion curve. *Applied Environmental Microbiology* **45** (5), 1453–1458.

Smets, B. F., Jobbagy, A., Cowan, R. M. & Grady, C. P. L. 1996 Evaluation of respirometric data: identification of features that preclude data fitting with existing kinetic expressions. *Ecotoxicology and Environmental Safety* **33** (1), 88–99.

Torretta, V., Ragazzi, M., Trulli, E., De Feo, G., Urbini, G., Raboni, M. & Rada, E. 2014 Assessment of biological kinetics in a conventional municipal WWTP by means of the oxygen uptake rate method. *Sustainability* **6** (4), 1833–1847.

Wu, Y. S., Chiang, C. F. & Lu, C. J. 2003 Dimensional analysis for establishing the testing criteria of kinetic study with respirometry. *Water Science and Technology* **47** (11), 275–280.

Wu, Y. S., Chiang, C. F. & Lu, C. J. 2004 Respirometric evaluation by graphical analysis for microbial systems. *Environmental Monitoring and Assessment* **92** (1–3), 137–152.

Young, J. C. 1981 Specific oxygen-demand as an operating parameter for activated-sludge processes. *Water Science and Technology* **13** (10), 397–403.

Young, J. C. 2001 The 7th annual industrial wastes technical and regulatory conference. In: *Impact of Cleaning and Disinfecting Agents on Biological Treatment Processes*, 12–15 August, Charleston, USA.

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