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Microrespirometric model calibration applied to wastewater processes

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ABSTRACT

Microrespirometry is a recently developed method that combines classical respirometry and microreactor systems, for the characterization of microbial cultures. This method, which allows multiple simultaneous replicates from a single low-volume biomass sample, can improve and simplify the model calibration exercise by providing more and better experimental data. To follow up on the interest in microrespirometry, two model wastewater treatment processes were set up and used for model calibration: an aerobic degradation of 4-chlorophenol by acclimated sludge, and a synthetic wastewater treatment by activated sludge, which were well described by a Haldane model and a modified ASM3 model, respectively. For each process, the model parameters were estimated by model fitting, minimizing the objective function for each replicate independently, and minimizing the objective function for all replicates simultaneously (multiresponse approach). Parameter confidence intervals were determined for all parameter estimates, and the impacts of measurement errors, number of replicates, and parameter correlation were assessed. It was observed that the multiresponse approach presents several advantages, including single-step parameter estimation, independent of the number of replicates, and determination of confidence intervals that include the main sources of uncertainty. It was concluded that microrespirometry is a high-throughput and convenient method for model calibration.

1. Introduction

Estimation of kinetic and stoichiometric parameters of biokinetic models, also known as model calibration, is essential in the model building exercise [1]. In biotechnology, where complex biological systems are used, one of the main obstacles for model calibration is the acquisition of reliable experimental data, and the complexity of that task is further increased by the limited availability of methods to measure the key variables of the processes. In this regard, respirometry is a well-established experimental method, which is used to provide data for model calibration and for control strategies in activated sludge and other biological processes [2–4]. Respirometry is defined as the measurement of the exogenous oxygen uptake rate (OUR) in the liquid phase, under well-defined conditions [5]. In aerobic systems, the OUR reflects the microbial metabolism, including microbial growth, substrate uptake, maintenance, storage of polymers, among others.

Traditional respirometric methods, usually performed in batch stirred reactors on a liter scale, are susceptible to errors, resulting in high variability between replicates of experiments [6]. Several reasons for such variability have been suggested, among which the most important are the difficulty in maintaining accurate and constant mass transfer in the respirometer and the difficulty in reproducing the actual conditions prevailing in the biological system under study. For instance, Chu et al. [7] reported that low oxygen transfer rates in mechanical stirred respirometers can cause mass transfer limitations whereas high oxygen transfer rates obtained through high mixing can change the microbial aggregation; in both cases the OUR is modified, leading to errors in parameter estimation. Another common drawback of traditional respirometry is the use of electrochemical dissolved oxygen (DO) sensors, which are still the primary option for DO measurements. Electrochemical DO sensors (i) have a significant response time, which must be considered for data interpretation [8]; (ii) consume oxygen, which is undesirable when the samples are small

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or oxygen-deficient; (iii) require frequent calibration and maintenance; (iv) are susceptible to drift; and (v) are sensitive to several compounds, including H_2S, O_3, and Cl_2 [9].

Since the late 1990s, dynamic fluorescence quenching sensors have been developed. These sensors present several advantages, making them attractive for respirometry; these include: very short response time, low signal-to-noise ratio, no oxygen consumption, small size, and capability of non-invasive measurement through transparent vessel walls. The development of these sensors promoted the development of microreactor systems, which allow parallel cultivations in multiple independent wells. Microreactor systems have attracted much interest in high-throughput selection of culture media, strain screening, and process optimization [10]. The advantages of microreactors over bench-scale reactors have been listed by Kim et al. [11]. Recently, Esquivel-Rios et al. [12] and Ramirez-Vargas et al. [13] showed that the application of respirometry in microreactor systems – called microrespirometry – generates a high quantity and quality of respirometric data with potential for model calibration.

Whatever the determination method, including respirometry or microrespirometry, model calibration is potentially subject to identifiability problems because of the nonlinear nature of the microbial kinetics. The concept of identifiability refers to the possibility of finding unique parameter values for the calibration of a given model. Identifiability can be structural, i.e., related to the structure of the model; or practical, i.e., related to the information of the experimental data available for calibration [1]. An analysis of practical identifiability can reveal whether the information provided by the experiments leads to the accurate estimation of parameters; in addition, it provides information about the uncertainty of the estimated parameters in terms of parameter correlation.

During the model calibration, it is important to determine the confidence intervals of the estimated parameters, with the aim of quantifying the uncertainties associated with the estimation method. Parameters reported in literature rarely include confidence intervals, and when these are included, they often do not consider all sources of uncertainty, such as parameter correlation, measurement errors, and differences among replicates [14].

Different methods have been developed to determine the uncertainty caused by parameter correlation [1,15], and the Fisher Information Matrix (FIM) is the most common approach to evaluate this issue. Petersen et al. [16] showed that through optimal experimental design (OED) and a combination of respirometric and titrimetric data, the parameter correlation can be reduced substantially. With respect to the uncertainty caused by measurement errors, Liu and Zachara [17] as well as Guisasola et al. [18] have shown the importance of minimizing the measurement errors and increasing the data acquisition frequency to decrease parameter uncertainty. The uncertainty caused by the number of replicate experiments has received less attention, mainly due to the difficulty of achieving reproducible respirometric experiments. Some authors report the arithmetic mean of the parameter estimates from different replicates [19,20], while other authors report parameter estimates considering all replicates at the same time [21]. A third approach, called PRAMUS, was suggested by Magbanua et al. [6], who combined information retrieved from several parameters estimates in a single dataset prior to a new parameter estimation.

The use of a high-throughput method, such as microrespirometry, can simplify the model calibration task. This method can improve parameter identifiability by increasing the quality and quantity of the experimental data, obtained under reproducible conditions, during a single experiment. The aim of our work was to evaluate microrespirometry by comparing two methods of data analysis and applying an exhaustive error analysis. We selected two wastewater treatment processes: aerobic degradation of 4-chlorophenol and synthetic complex wastewater by activated sludge, which were described by a substrate inhibition model and a standard activated sludge model (ASM3), respectively. For each of these models, we compared two methods of data analysis for parameter estimation: one considering each replicate independently and the other one using all replicates simultaneously (the multi-response approach). We assessed the impact of measurement errors, number of replicates, and parameter correlation on the uncertainty of the parameters, by determining the parameter confidence intervals under different simulated conditions.

2. Materials and methods

2.1. Microbial cultures

Two processes were selected and set up (Table 1). The activated sludge degrading 4-chlorophenol as a single xenobiotic substrate was obtained from a sequencing batch reactor (SBR), previously described by Vital-Jacome et al. [22], and operated under steady-state conditions; i.e., the reactor was maintained under stable operation with the effluent substrate concentration remaining unchanged over time. The activated sludge process degrading complex synthetic wastewater was obtained from a continuous reactor operated under steady-state conditions, previously described by Esquivel-Rios et al. [12].

2.2. Microrespirometric method

The two selected cultures were characterized by microrespirometry, performed in a microreactor system (Micro-24, Pall Corporation), as previously described by Ramirez-Vargas et al. [13]. In brief, the Micro-24 uses a 24-well cassette fixed on an orbital shaker. This system allows control of agitation speed, airflow, temperature, pH, and DO. The device uses DO fluorescence quenching sensors with a maximum data acquisition capacity of 2000 readings per hour. Cassettes designed for animal cell culture and with surface aeration were used (PRC, Pall Corporation, USA). Each well of the cassettes included a DO sensor, which are factory pre-calibrated. However, the sensors must be compensated for temperature and atmospheric pressure. As such, an additional calibration was done by injecting oxygen-free nitrogen and air at 0 and 100% saturation, respectively. The experimental conditions were the following: liquid volume, 4 mL; temperature, 25 °C; no airflow (surface aeration); agitation speed, 600 rpm for both activated sludge cultures. The system was not sealed and the filter of the caps (Type-D, Pall Corporation, USA) was removed to ensure the gas exchange between the headspace and the environment. A better surface aeration was thus ensured. Details of surface aeration in the microreactor system can be found in Ramirez-Vargas et al. [13]. The DO concentration was continuously recorded during the experiments, but not controlled, as usually done in dynamic respirometry.

Dynamic pulse respirometry was performed according to previously described methods for microreactors [12,13]. In brief: (i) biomass samples from each culture were centrifuged at 7000 rpm for 10 min (Centrifuge 5810, Eppendorf) and resuspended in culture media without a carbon source; (ii) each well of the microreactor cassette was filled with 3.9 mL of the biomass sample and aerated until endogenous respiration state was reached [23]; (iii) a pulse of 0.1 mL of known substrate concentration was injected in each well; (iv) DO readings were recorded until the system returned to endogenous respiration state; and (v) the oxygen mass transfer coefficient (K_La) was determined according to the method described by Badino et al. [24]. For each culture, the experiment was performed with 23 simultaneous replicates plus one negative control in which the substrate was replaced by a mineral

Table 1
Microbial cultures and their corresponding respirometric models.

Culture	Reactor operation	Substrate	Respirometric model
Activated sludge	Sequencing Batch Reactor	4-Chlorophenol	Haldane
Activated sludge	Continuous	Synthetic wastewater	ASM3

medium without any carbon source. Each respirometric test (one in each well) generated one respirogram, i.e., one DO concentration time series, and these series were used for parameter estimation by model fitting. In each assay, the concentration of biomass and the concentration of the solution used for pulse injection were determined as chemical oxygen demand (COD, mg L^{-1}) using the colorimetric reflux method [25].

3. Theory

3.1. Respirometric models

Two different respirometric models (one for each culture) were used to fit the experimental data (Table 2). Typically, parameter estimation by respirometry is based on model fitting to OUR data, determined from DO measurements. However, it has been shown that model fitting to OUR instead of DO may add some error that can be propagated to the estimated parameters and does not improve identifiability [26]. For that reason, in the present work, we used models based on DO as state variable and model output, which are shown in conventional matrix form in Table 2. For simplicity, these models only consider exogenous respiration processes (related to the consumption of exogenous substrates); endogenous respiration processes (related to maintenance and death) are neglected.

As shown in Table 1, the Haldane model was selected to describe the activated sludge culture degrading 4-chlorophenol. This model is a classical approach for substrate inhibition kinetics and has been previously used in respirometric experiments [27,28]. The Haldane model includes five parameters: (i) the maximum oxygen uptake rate (OUR_{max}), (ii) the half-saturation constant (K_S), (iii) the inhibition constant (K_I), (iv) the transient response time of the process (t_R) [29], and (v) the growth yield of heterotrophic biomass (Y_H). Only four of these parameters (OUR_{max}, K_S, K_I, and t_R) were included in the set for parameter estimation; the other one (Y_H) was determined from the area below the baseline DO concentration (C_b) according to Eq. (1), in which C is the measured DO concentration (mg L^{-1}); C_0 and C_f are the initial and final DO concentrations (mg L^{-1}), respectively; and S_p is the substrate concentration of the pulse (COD, mg L^{-1}).

$$Y_H = 1 - \frac{K_L a \cdot \int_0^t (C_b - C)\,dt + (C_0 - C_f)}{S_p} \quad (1)$$

The maximum specific growth rate (μ_{max}) was determined from OUR_{max}, Y_H, and the biomass concentration X (COD, mg L^{-1}) according to Eq. (2).

$$\mu_{max} = \frac{OUR_{max} \cdot Y_H}{X \cdot (1 - Y_H)} \quad (2)$$

The Activated Sludge Model No.3 (ASM3) was selected to describe the activated sludge culture degrading synthetic wastewater (Table 2). The standard ASM3 model includes 12 processes and numerous kinetic and stoichiometric parameters [30]. In the present work, we used a simplified ASM3 model that considers only two reactions: the conversion of the substrate (S) to storage material (X_{STO}), and the use of the storage material for biomass growth of heterotrophs (X) after depletion of substrate. During respirometric experiments, these reactions are called the feast and the famine phase, respectively (processes 3 and 4 in Table 2). The simplified ASM3 model includes seven parameters: (i) the maximum substrate storage rate (k_{STO}), (ii) the maximum specific growth rate on X_{STO} (μ_{max}), (iii) the storage material half-saturation constant (K_{STO}), (iv) K_S, (v) t_R, (vi) the storage yield (Y_{STO}), and (vii) the storage growth yield (Y_{XSTO}). The five kinetic parameters (i.e., μ_{max}, k_{STO}, K_{STO}, K_S, and t_R) were included in the set for parameter calibration; Y_{STO} and Y_{XSTO} were determined according to Eqs. (1), (3), and (4), in which C_{LSto} is the limit between oxygen consumption for storage and growth (see Ordaz et al. [31] for more details).

$$Y_{STO} = 1 - \frac{K_L a \cdot \int_0^t (C_{LSto} - C)\,dt}{S_p} \quad (3)$$

$$Y_{XSTO} = \frac{Y_H}{Y_{STO}} \quad (4)$$

The simplified model also includes four initial conditions: C_0, S_p, $X(0)$, and $X_{STO}(0)$, which is the initial concentration of storage material. In this work, $X_{STO}(0)$ was set to a fraction of $X(0)$ in such a manner that $X_{STO}(0)/X(0)$ was equal to 0.01. This assumption was based on previous reports of $X_{STO}(0)/X(0)$ in activated sludge processes [32] and on Krishna and Van Loosdrecht [33], who stated that under continuous feeding, activated sludge systems have a low storage capacity.

3.2. Parameter estimation

The model parameters were estimated by minimization of the objective function (Eq. (5)) using the fminsearch (Nelder–Mead simplex method) function of MATLAB (R2015a). In Eq. (5), $y_{ik}(\theta)$ are the model outputs for a given parameter set (θ), and y_{ik} are the measured outputs for a given number of data ($Ndat$) and a given number of replicates ($Nrep$). Note that Eq. (5) is an unweighted function and therefore assumes that each replicate has the same statistical weight; this approach will be discussed further on. The differential equations derived from the models in Table 2 were solved using the ode45 function of MATLAB.

$$J(\theta) = \sum_{k=1}^{Nrep} \left(\sum_{i=1}^{Ndat} \left(y_{ik}(\theta) - y_{ik}\right)^T \cdot \left(y_{ik}(\theta) - y_{ik}\right) \right) \quad (5)$$

In each microrespirometric experiment, the respirogram provided by each well of the microreactor system was considered a replicate with a unique set of oxygen–time data. From the complete data set obtained from each microrespirometric experiment, two different kinds of parameters can be estimated: first, the mean parameters (MPs), i.e., parameters estimated by minimizing the objective function for each replicate independently ($Nrep$ = 1) prior to the determination of the arithmetic mean; second, the multiple replicate parameters (MRPs), i.e., parameters estimated from all the replicates' data, minimizing the objective function (Eq. (5)) for all data analyzed simultaneously ($Nrep$ = 1–23) by the use of a multiresponse estimation approach.

3.3. Confidence intervals

The confidence intervals of the MP values were calculated using Eq. (6) for a confidence level of 95% (α = 0.05) from a Student t dis-

Table 2
Matrix form of the respirometric models.

Process	Component				Kinetics
	S (mg COD L^{-1})	X (mg COD L-1)	X_{STO} (mg COD L^{-1})	C (mg O_2 L^{-1})	
1. Oxygen transfer rate Haldane model				1	$K_La \cdot (C_b - C)$
2. Aerobic growth on S	$-\frac{1}{(1-Y_H)}$			−1	$OUR_{max} \cdot \left(\frac{S}{K_S+S+\frac{S^2}{K_I}}\right) \cdot (1 - e^{-t/t_R})$
Simplified ASM3 model					
3. Aerobic storage of S	$-\frac{1}{Y_{STO}}$		1	$-\frac{(1-Y_{STO})}{Y_{STO}}$	$k_{STO} \cdot \left(\frac{S}{K_S+S}\right) \cdot X \cdot (1 - e^{-t/t_R})$
4. Aerobic growth on X_{STO}		1	$-\frac{1}{Y_{XSTO}}$	$-\frac{(1-Y_{XSTO})}{Y_{XSTO}}$	$\mu_{max} \cdot \left(\frac{\frac{X_{STO}}{X}}{K_{STO}+\frac{X_{STO}}{X}}\right) \cdot X$

tribution, where n is the number of replicates and SD is the standard deviation of the MP values.

$$\theta_i \pm t_{\alpha;n-1}\frac{\mathrm{SD}}{\sqrt{n}} \tag{6}$$

Meanwhile, the estimation of the confidence intervals for the MRP values was based on the Fisher information matrix (FIM). To that end, first the FIM was calculated from Eq. (7) [1]; where Y_θ^y are the output sensitivity functions of the parameters, given by Eq. (8), which represent the derivative of the output variable (y_j) with respect to each parameter (θ_i). Calculation of these sensitivity functions (Eq. (8)) is the central task in the practical identifiability analysis. Matrix Q_i is the inverse of the measurement error covariance matrix, which can be assumed to be a $p \times p$ identity matrix when only a single state variable is measured, with p being the number of parameters. To allow graphical analysis of sensitivity functions on the same scale, normalized sensitivity functions (S_θ^y) were estimated by multiplying the sensitivity functions by the ratio of the parameter value over the value of the output variable (Eq. (9)).

$$\mathrm{FIM} = \sum_{i=1}^{N}\left(Y_\theta^y\right)^{\mathrm{T}} \cdot Q_i \cdot Y_\theta^y \tag{7}$$

$$Y_\theta^y = \frac{\partial y_j}{\partial \theta_i} = \lim_{\Delta\theta\to 0}\frac{y_j\left(\theta_i\right) - y_j\left(\theta_i + \Delta\theta_i\right)}{\Delta\theta_i} \tag{8}$$

$$S_\theta^y = \frac{\theta_i}{y_j} \cdot \frac{\partial y_j}{\partial \theta_i} \tag{9}$$

Then, the parameter error covariance matrix COV was determined by taking the inverse of the FIM (Eq. (10)).

$$\mathrm{COV} = \mathrm{FIM}^{-1} \tag{10}$$

Considering a single state variable, the standard error of the parameters was determined from the square root of each diagonal element of the covariance matrix according to Eq. (11).

$$\sigma\left(\theta_i\right) = s\sqrt{\mathrm{COV}_{ii}} \tag{11}$$

The residual mean square (s) was calculated from Eq. (12), in which $J_{\mathrm{opt}}(\theta)$ is the value of the objective function evaluated with the optimal parameter set (θ), N is the total number of data, and p is the number of parameters.

$$s^2 = \frac{J_{\mathrm{opt}}\left(\theta\right)}{N-p} \tag{12}$$

Finally, the confidence interval for each parameter was calculated using Eq. (13) for a confidence level of 95% ($\alpha = 0.05$).

$$\theta_i \pm t_{\alpha;N-p}\sigma\left(\theta_i\right) \tag{13}$$

3.4. Impact of data quality, data quantity and number of replicates on parameter estimation

To estimate the error caused by the noise of the DO sensors, the impact of the signal-to-noise ratio (SNR) on the confidence intervals was evaluated. The SNR was determined during the endogenous respiration state; i.e., a pseudo-steady state during which the DO concentration is relatively constant. For each well, the SNR was determined according to Eq. (14), in which $\bar{C}$ and σ are the average DO concentration and the standard deviation of the DO measurements, respectively.

$$\mathrm{SNR} = \frac{\bar{C}}{\sigma} \tag{14}$$

The impact of the data acquisition frequency on parameter confidence intervals was also determined, by artificially reducing the number of data available in each respirogram for parameter estimation. Given the vector of experimental data $\boldsymbol{Y}$, wherein each element y_i is the value of the state variable measured at time t_i (Eq. (15a)), new vectors $\boldsymbol{W}$ (Eq. (15b)) were built with selected elements from $\boldsymbol{Y}$; k is an integer that sets the data interval. The corresponding measurement time interval is defined by Eq. (15c). Several sub–data sets were constructed with this method, and the parameter estimation was performed from each one of these subsets.

$$\boldsymbol{Y} = [y_1, \ldots, y_n]^{\mathrm{T}} \tag{15a}$$

$$\boldsymbol{W} = [y_1, y_{1+k}, y_{1+2k}, \ldots, y_{1+mk}]^{\mathrm{T}} \tag{15b}$$

$$\Delta t = \left(t_j - t_{j-1}\right), j = 1, 1+k, \ldots, 1+mk \tag{15c}$$

The impact of the number of replicates on the confidence intervals of the MRPs was assessed by varying the number of respirograms, i.e., the number of vectors $\boldsymbol{W}$ available for parameter estimation, from 1 to 23 replicates.

4. Results and discussion

4.1. Model calibration

4.1.1. Haldane model

The degradation kinetics of 4-chlorophenol were well described by the Haldane model, the most common model for describing substrate inhibition kinetics in respirometry. Table 3 shows the kinetic parameters estimated from substrate pulses of 185 (COD, mg L^{-1}). The parameters are in the same range as those previously reported for 4-chlorophenol by Sahinkaya and Dilek [34]. Indeed, these authors reported a μ_{max} ranging from 0.48 to 3.14 d^{-1}, a K_S of 1.1 mg L^{-1}, and a K_I of 194 mg L^{-1}. The four parameters were estimated as MPs and MRPs, and some differences were observed among them, which can be explained by variations among replicates and calibration approaches. Fig. 1A shows an example of ten respirogram replicates, as well as the model output based on MPs and MRPs estimated from these replicates. Despite significant

Table 3
Parameter estimation results for the Haldane model ($K_La = 31\ h^{-1}$; $S_p = 185$ mg COD L^{-1}; $X(0) = 1.45$ g COD L^{-1}).

Parameter	MP ± CI[a]	Confidence Interval[b] (%)	MRP ± CI[a]	Confidence interval[b] (%)
Parameters estimated				
OUR_{max} (mg O_2 L^{-1} h^{-1})	73.31 ± 1.99	2.7	79.38 ± 1.66	2.1
K_S (mg COD L^{-1})	3.26 ± 0.16	4.9	6.47 ± 0.49	7.6
K_I (mg COD L^{-1})	180.17 ± 2.69	1.5	151.44 ± 7.14	4.7
t_R (min)	1.36 ± 0.07	5.2	1.35 ± 0.12	8.9
Parameters calculated				
Y_H (g COD g^{-1} COD)	0.65			
μ_{max} (day^{-1})	2.25 2.44			

[a] Parameter estimates are reported together with their corresponding confidence interval, i.e., the confidence region with a confidence level of 95% ($\alpha = 0.05$).
[b] In these columns, the confidence intervals are expressed in percentage, relative to the parameter value.

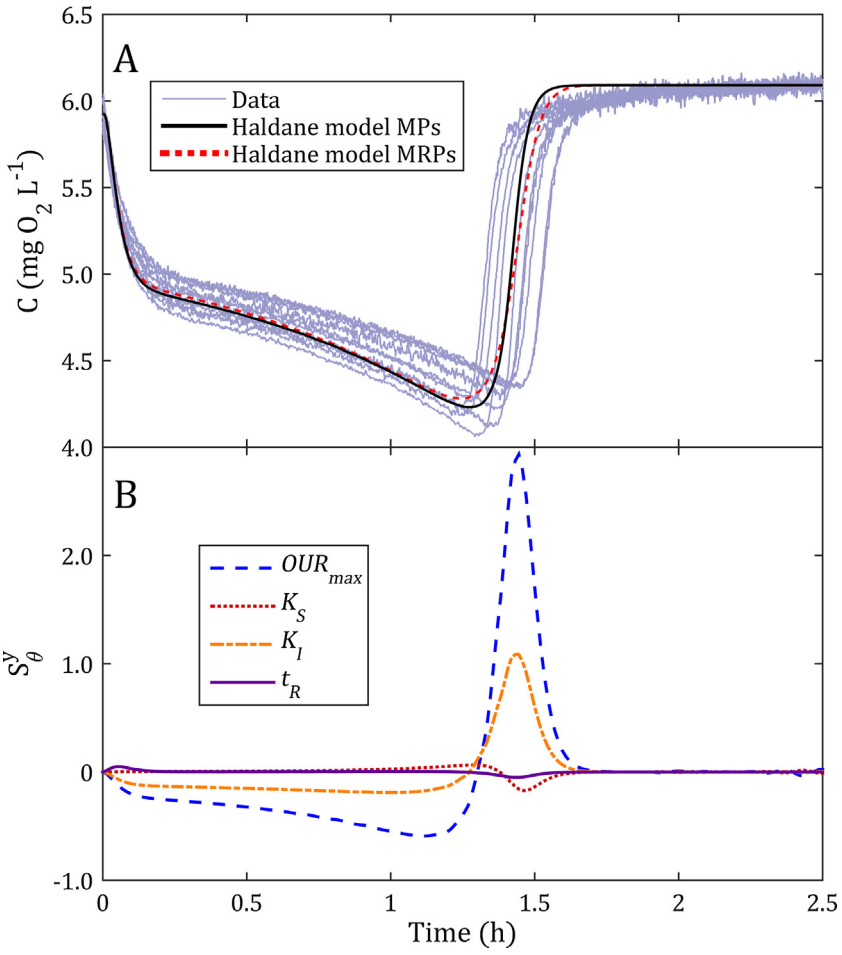

Fig. 1. (A) Simultaneous model fitting of replicates. (B) Normalized sensitivity functions of the Haldane model.

differences between the MPs and the MRPs, their corresponding model outputs show little difference.

Table 3 also shows the confidence intervals for each parameter estimation, ranging from 1.5% to 8.9%. Except for OUR_{max}, the confidence intervals are higher for the MRPs than for the MPs. Two explanations support this difference. First, the confidence intervals for the MRP estimates are based on FIM, which includes parameter sensitivity functions (Eq. (8)) and therefore considers the uncertainty caused by parameter correlation, whereas the MP confidence intervals do not include this correlation. Second, the confidence intervals for the MRP estimation reflect variations in mass transfer between wells. Indeed, the MRP estimation is based on a single K_La value, whereas the MP estimation is based on an individual K_La value for each well. Ramirez-Vargas et al. [13] showed a coefficient of variation (CV) for K_La between wells of 9.1%, whereas the K_La determination for a single well was characterized by a CV of 6.4%.

Regarding the identifiability analysis, Fig. 1B shows the normalized parameter sensitivity functions of the MRPs. The maximum parameter sensitivity is observed around 1.5 h, when the substrate is depleted and the oxygen concentration returns rapidly to the endogenous respiration state. Moreover, around 1.5 h, the parameter sensitivity functions show proportionality, which indicates a strong parameter correlation and suggests possible identifiability problems, as has been previously reported for the Haldane model [28,35].

In order to distinguish between MPs and MRPs, the parameters were used to plot the corresponding OUR-S curves (Fig. 2), which were compared with the OUR-S curves of the 23 replicates in a

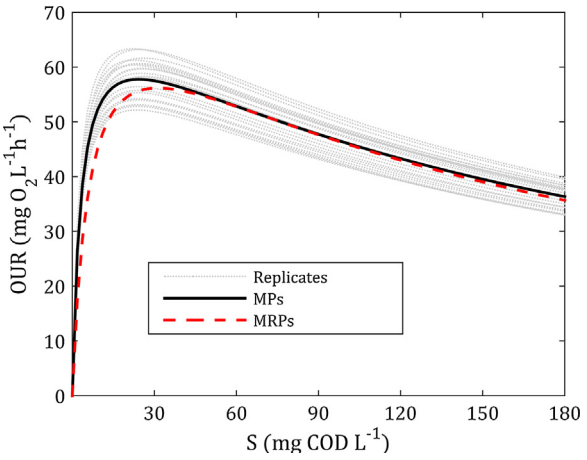

Fig. 2. Comparison of OUR-S curves for MRP and MP values and OUR-S curves for parameter sets of individual replicates.

manner similar to that previously suggested by Magbanua et al. [6]. Fig. 2 shows that the MPs better reflect the overall pattern of the replicate curves, especially at low *S*, where MRPs show a systematic deviation. However, at high *S*, both parameter sets describe the same overall kinetics. In addition, the deviation of the MRPs from the overall kinetics as observed in Fig. 2 may be partially caused by the high variation among replicates, especially around 1.5 h, when S is depleted and maximum parameter sensitivity and correlation occur (Fig. 1B).

Notwithstanding sensitivity and parameter correlation, the confidence intervals stayed below 9%, which suggests that the identifiability problems were overcome by the large respirometric data sets and the number of replicates, together with S_p high enough to observe the substrate inhibition phenomenon. These results validate the interest in microrespirometry for calibration of the Haldane model.

4.1.2. ASM3 model

The biodegradation of synthetic wastewater was selected as an example of typical organic carbon removal processes. The ASM3 model has been used extensively in activated sludge processes and is considered as an improved version of previous ASM models. As mentioned in Section 3, in the simplified ASM3 model the most common approach describing the consumption of X_{STO} during the famine phase is through saturation kinetics, assuming that X_{STO} is density dependent, i.e., proportional to biomass concentration. However, it has been shown that this assumption leads to strong correlation among parameters and thus to identifiability problems [36]. In a first stage of this research, we performed the parameter estimation considering saturation kinetics (process 4 in Table 2). The model fit the experimental data well (Fig. 3A), but the sensitivity function showed a strong correlation between μ_{max} and K_{STO} (Fig. 3B), resulting in erratic μ_{max} and K_{STO} values as well as large confidence intervals for both MPs and MRPs (Table 4). In addition to the latter, the FIM calculated during the determination of the MRP confidence intervals was almost singular or ill-conditioned, which confirms the presence of identifiability problems [37]. Consequently, it was concluded that unique μ_{max} and K_{STO} values cannot be identified with this model, as previously discussed by Guisasola et al. [36], regardless of the type of parameters estimated (MP or MRP). To allow the simplified ASM3 model calibration, the original approach was modified, assuming a first-order model to describe the consumption of X_{STO} during the famine phase (Eq. (16)), instead of saturation kinetics. The first-order approach has been suggested and previously used to model the kinetics of X_{STO} consumption by Beun et al. [38] and Van Loosdrecht and Heijnen [39]. This modification reduces the number of parameters to be estimated from five to four, as the kinetic constant of the first-order model (k_P) is estimated instead of μ_{max} and K_{STO}. For clarity, this model will be called the modified ASM3 model hereinafter.

$$r = k_P \left(X_{STO}/X\right) \tag{16}$$

The modified ASM3 model fit the experimental data well, with no difference in model output between MPs and MRPs, and giving the same output as that previously observed with the simplified ASM3 model (Fig. 3A). The results for the MRP and MP values from the modified ASM3 model are shown in Table 4. Both types of parameters present small confidence intervals compared with those of the simplified ASM3 model, ensuring the identification of all the parameters. Additionally, no significant differences were observed between MPs and MRPs (Table 4) except for t_R. Regarding the identifiability analysis, Fig. 3C shows the observed sensitivity functions of the modified ASM3 model for the MRPs, in which no strong correlation among parameters occurred. The difference between MP and MRP values of t_R can be attributed to small variations among respirograms at the beginning of the experiments, because this parameter is more sensitive in this region (Fig. 3C). Most of these parameters are within the same range as those reported by Guisasola et al. [36]. For instance, the ranges of k_{STO}, t_R, Y_{STO} and Y_{XSTO} reported by these authors were 1.056–4.88 d^{-1}, 0.123–2.21 min, 0.715–0.831 g COD g^{-1} COD, and 0.804–0.960 g COD g^{-1} COD, respectively. On the contrary, K_S estimated in the present work, was significantly higher than the K_S estimated by Guisasola et al. [36], ranging from 0.69–0.80 mg L^{-1}. The differences in K_S might be due to the nature of the wastewater used, as Guisasola et al. [36] used real wastewater, while synthetic wastewater was preferred in the present work. The last parameter, estimated in the present work (k_P), cannot be compared to the previous work, due to the modification of the ASM3 model structure that we made, in addition to identifiability issues reported by Guisasola et al. [36] and in the present work. From the results obtained, it is confirmed that a modified ASM3 model can be calibrated by microrespirometry and that the parameters can be estimated as MPs or MRPs, without significant difference between parameter values.

4.2. MPs vs. MRPs

As shown, the microrespirometry method allowed the estimation of both MP and MRP parameters for both the Haldane and the modified ASM3 models. MRP estimation presents some advantages over the more traditional MP estimation. First, the MRP approach allows estimation of parameters in a single step, independent of the number of replicates, whereas the MP approach requires the parameter estimation process to be repeated for each replicate; this results in a significant reduction of the data treatment effort. Second, the MRP approach allows the determination of parameter confidence intervals that combine the uncertainty caused by measurement errors, replicated experiments, and parameter correlations from all replicates; this means that it is possible to consider all these uncertainties at the same time in one single analysis.

However, the MRP approach may also present some drawbacks compared with standard MP estimation. The main drawback is probably that MRP estimation is based on a single set of experimental conditions for all the replicates, discarding any potential differences between individual wells of the microrespirometer. For instance, as discussed earlier, K_La is a parameter that varies among wells and cannot be considered during MRP estimation. Other potential differences among wells include the liquid volume, the biomass concentration, the temperature, and the time at which the substrate pulse is injected. Assuming that these variations can be corrected, they could be taken into account in MP estimation but not under the MRP approach. Nevertheless, except for K_La, the

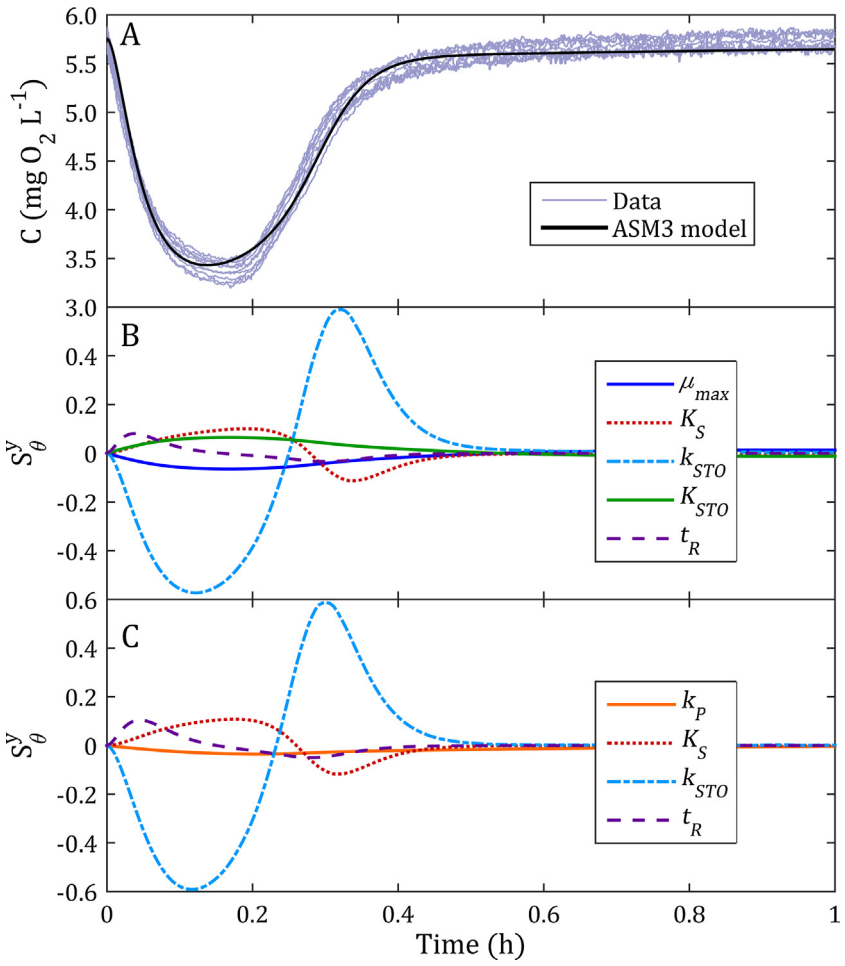

Fig. 3. (A) Simultaneous model fitting of replicates. (B) Normalized sensitivity functions of the simplified ASM3 model. (C) Normalized sensitivity functions of the modified ASM3 model.

Table 4
Parameter estimation results for the ASM3 model ($K_La = 30\ h^{-1}$; $S_p = 86$ mg COD L^{-1}; $X(0) = 3.99$ g COD L^{-1}). See text for details regarding the differences between the simplified and modified models.

Parameter	MP ± CI[a]	Confidence interval[b] (%)	MRP ± CI[a]	Confidence interval[b] (%)
Parameters estimated (Simplified ASM3 model)				
μ_{max} (day^{-1})	$7.18 \times 10^8 \pm 5.82 \times 10^8$	81.1	$2.87 \times 10^8 \pm 4.82 \times 10^{12}$	1.68×10^6
K_S (mg COD L^{-1})	9.73 ± 0.47	4.9	9.99 ± 20	200
k_{STO} (day^{-1})	2.12 ± 0.05	2.4	2.14 ± 0.01	0.5
K_{STO} (g COD g^{-1} COD)	$1.26 \times 10^7 \pm 1.06 \times 10^7$	84.1	$7.55 \times 10^6 \pm 1.27 \times 10^{11}$	1.68×10^6
t_R (min)	0.65 ± 0.04	6.8	0.75 ± 1.5	200
Parameters estimated (Modified ASM3 model)				
k_P (g COD g^{-1} COD h^{-1})	0.87 ± 0.04	4.6	0.87 ± 0.05	5.8
K_S (mg COD L^{-1})	10.36 ± 0.57	5.5	10.33 ± 0.59	5.7
k_{STO} (day^{-1})	2.16 ± 0.05	2.3	2.15 ± 0.01	0.5
t_R (min)	1.32 ± 0.05	3.8	0.91 ± 0.01	1.1
Parameters calculated				
Y_H (g COD g^{-1} COD)	0.78			
Y_{STO} (g COD g^{-1} COD)	0.82			
Y_{XSTO} (g COD g^{-1} COD)	0.94			
Parameters assumed				
$X_{STO}(0)/X(0)$(g COD g−1 COD)	0.01			

[a] Parameter estimates are reported together with their corresponding confidence interval, i.e., the confidence region with a confidence level of 95% (α = 0.05).
[b] In these columns, the confidence intervals are expressed in percentage, relative to the parameter value.

microreactor systems commercially available are well designed to minimize variations and to optimize reproducibility.

4.3. Impact of data quality

Because of the nature of the quenching fluorescence sensors and the manufacturing process for the microreactor cassettes, each well

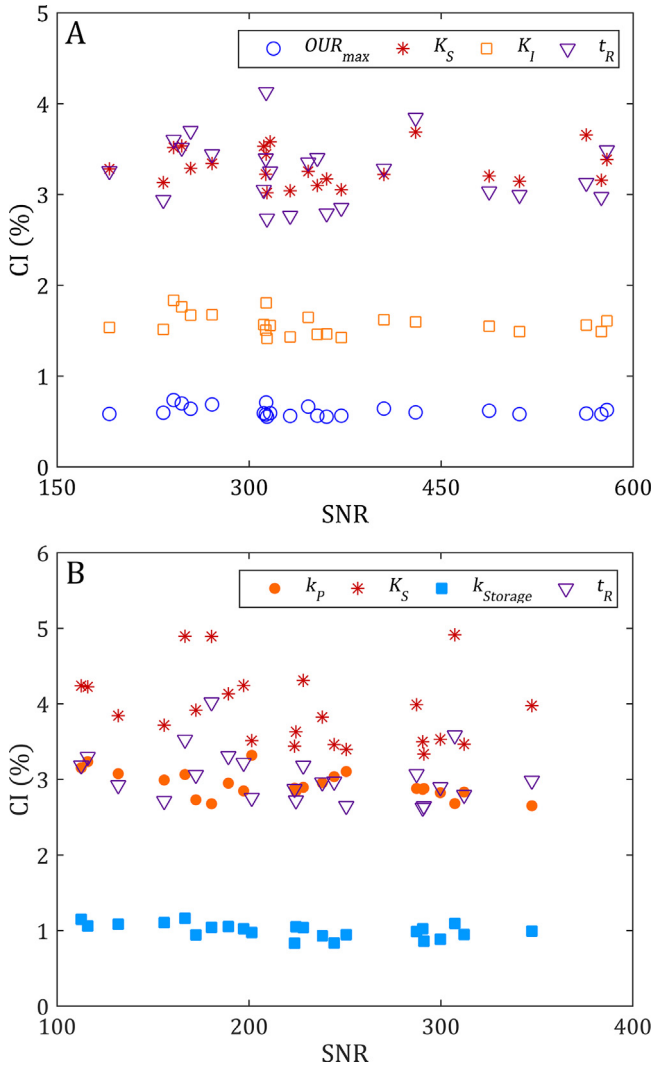

Fig. 4. Effect of the signal-to-noise ratio on MRP confidence intervals of (A) the Haldane model and (B) the simplified ASM3 model.

of the microreactor system has a unique sensor, with corresponding signal and noise levels. The noise level can be measured by the SNR, which can be considered a measure of the quality of the DO experimental data, related to the accuracy of the parameter estimation and the practical identifiability [1]. Due to the unique SNR of each sensor and the variation among them, an analysis of each respirometric replicate through the FIM can provide an assessment of the impact of the DO measurement error on parameter confidence intervals, and this analysis was performed.

Fig. 4A and B shows the confidence intervals of the Haldane model and the modified ASM3 model, respectively, versus the SNR of the corresponding sensor. The measured SNR was between 100 and 600, with an average of 361 and 237 for the cassette used with the 4-chlorophenol (Haldane model) and the synthetic wastewater (modified ASM3 model) processes, respectively. Fig. 4A and B shows that there was no effect of the SNR on the confidence interval of any parameter. This result indicates that, in terms of DO measurements, differences between sensors are without effect and that all replicates are equally reliable. Additionally, this result supports our choice of an unweighted objective function for the multiresponse approach (Eq. (5)).

4.4. Impact of data quantity and number of replicates

Another important factor affecting the uncertainty of parameter estimation is the quantity of experimental data available. The quantity of data obtained in each experiment depends on the measurement interval for DO, which can be modified directly from the control software. In most commercial microreactor systems, DO data can be recorded with measurement intervals from 2 to 3 s to several minutes, and the data acquisition frequency has an important effect on the uncertainty of the parameters. In addition, the uncertainty of the parameters depends on the number of replicates considered during the estimation. In the case of MPs, the effect of the number of replicates on the uncertainty is well described by Eq. (6), but in the case of MRPs, this effect is not obvious. To analyze together the effect of data frequency and the number of replicates on MRPs, several estimations were performed, artificially varying the measurement interval and the number of replicates (by randomly selecting them from a single data set) used to minimize the objective function. In each case, the confidence interval was determined by the FIM. Figs. 5 and 6 show the results obtained for the Haldane model and the modified ASM3 model, respectively.

On the one hand, as expected, the confidence intervals increased with the measurement interval [18], but on the other hand, the number of replicates had a more complex impact. Indeed, in both models and for all MRPs, having a small number of replicates apparently caused smaller confidence intervals. This is misleading, because at low numbers of replicates, the FIM reflects more the uncertainty caused by parameter correlation than the uncertainty caused by differences among replicates, which cannot be discriminated. By increasing the number of replicates, the uncertainty caused by their differences increases in importance until no major impact is further observed. These results suggest that the data frequency is of utmost importance and that a very large number of replicates increase the confidence intervals. Therefore, depending on the model, about five microrespirometric replicates might be considered optimal for parameter estimation. As most of the microreactor systems include at least 24 wells, some wells are available for simultaneous parameter estimation under different experimental conditions. As suggested by Dochain and Vanrolleghem [1], this is of interest because it can be advantageous to perform multiple experiments under slightly different conditions with the aim of estimating a common set of parameters.

5. Conclusions

Calibration of activated sludge models by microrespirometry provides several advantages compared with standard respirometry. First, it offers a significant reduction of the experimental effort by multiplying the number of replicates in a single experiment, using a single low-volume biomass sample, which also reduces the errors by differences among samples. Second, microrespirometry allows to estimate multiple replicate parameters (MRPs) that present several benefits compared with the standard mean parameters (MPs). The MRP approach allows to estimate the parameters in a single step, independent of the number of replicates, with a reduction in the data treatment effort. The MRP approach also allows to determine confidence intervals that include the main sources of uncertainty (parameter correlation, experimental errors, and differences among replicates). We also showed that there was no effect of the SNR on the confidence interval of any parameter, that five replicates are enough to ensure the determination of the MRP confidence intervals, and that the increase in the number of replicates may improve but it does not guarantee the identification of the parameters. Altogether, we conclude that microrespirometry combined with MRP estimation is a high-throughput and

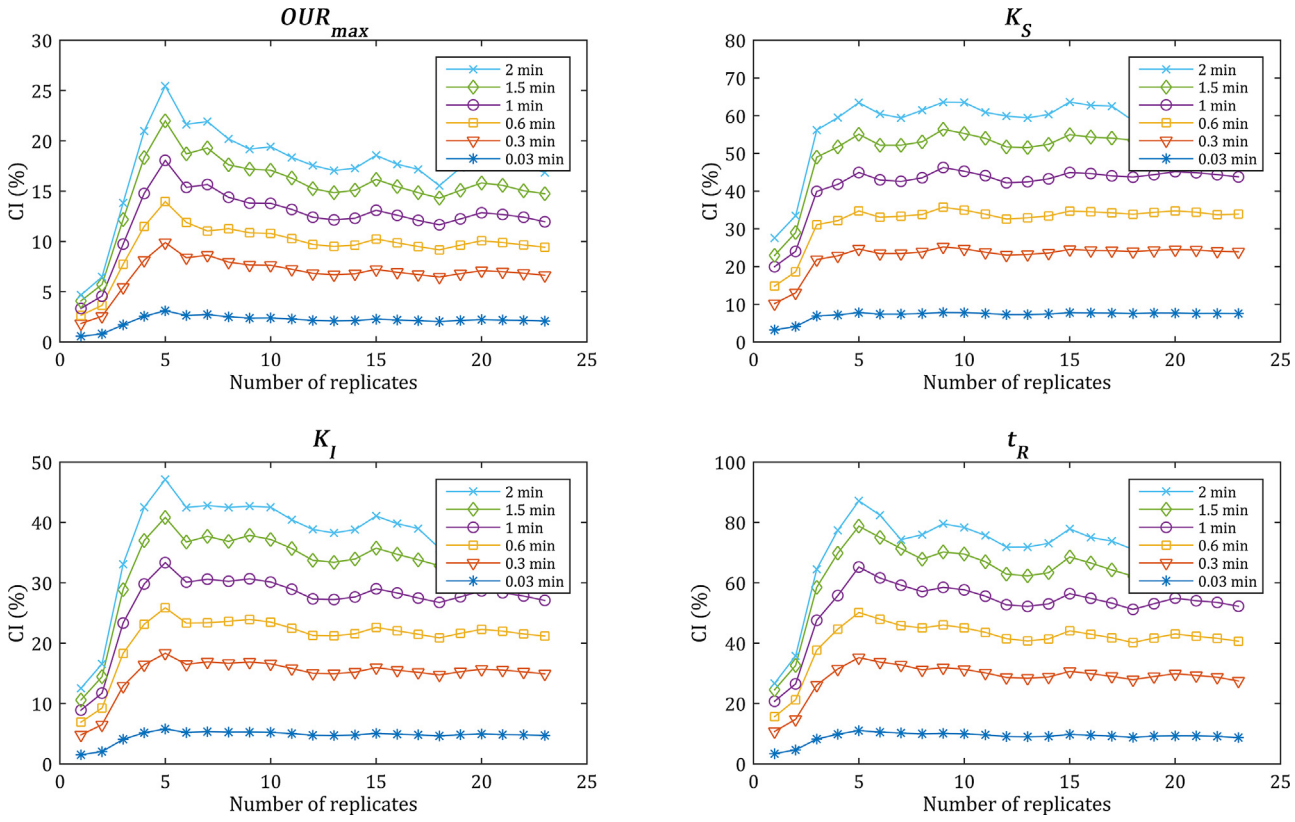

Fig. 5. Combined effect of the measurement interval and number of replicates on MRP confidence intervals for the Haldane model.

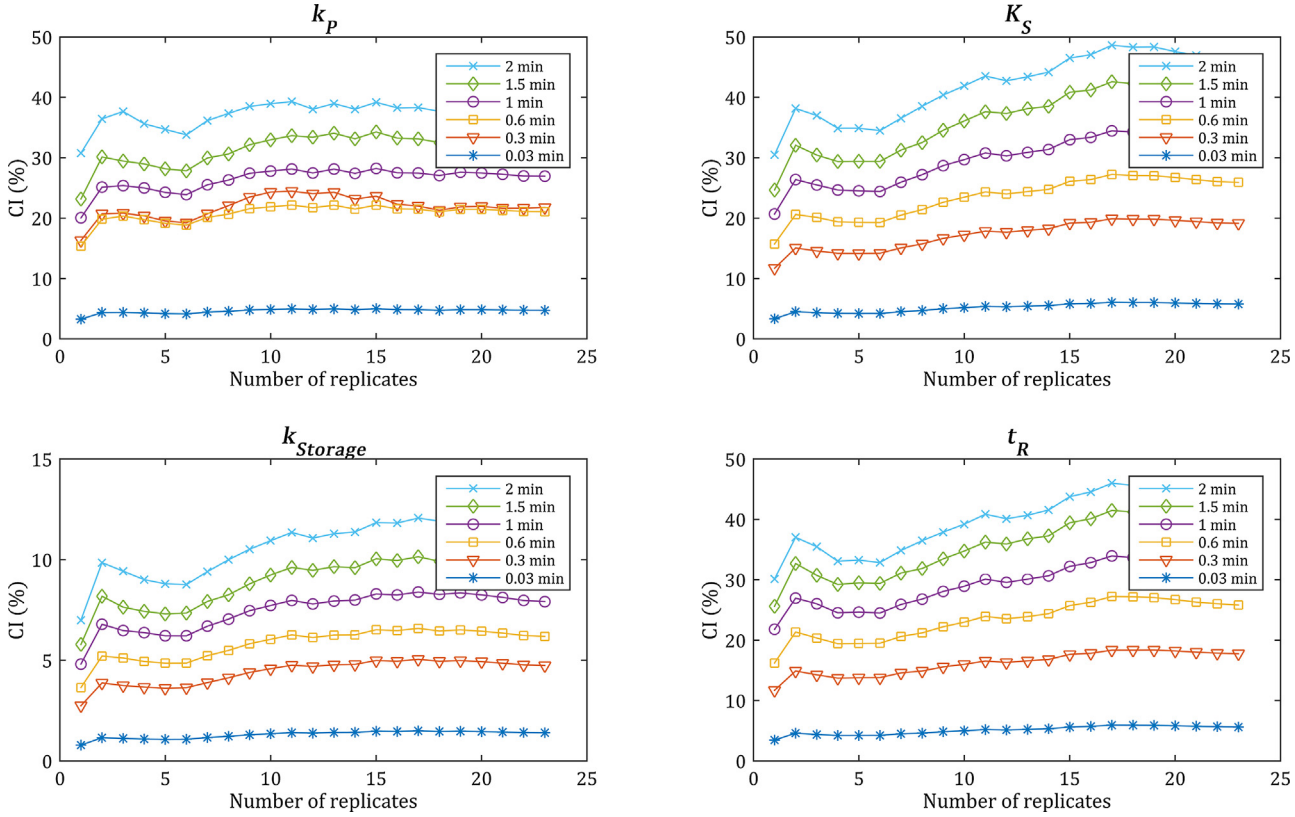

Fig. 6. Combined effect of the measurement interval and number of replicates on MRP confidence intervals for the modified ASM3 model.

convenient method for model calibration applied to wastewater processes, as well as for other models and applications.

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