ERROR PROPAGATION IN OPEN RESPIROMETRIC ASSAYS

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Abstract - This work deals with the calculation of the uncertainty of the exogenous respiration rate (R_{ex}) and the total oxygen consumed (OC_T) derived from a single open respirometric profile. Uncertainties were evaluated by applying a linear error propagation method. Results show that standard deviations (SD) of R_{ex} and OC_T depend not only on the SD of the dissolved oxygen (σ_C) and k_La (σ_{kLa}), but also on the SD of the derivative term (dC/dt) of the oxygen mass balance equation (σ_b). A Monte Carlo technique was employed to assess σ_b; a power law expression for the dependence of σ_b as a function of σ_C, the time window (t_w) and the sampling rate (Δt) was proposed. The equations obtained in the present work are useful to calculate suitable conditions (e.g., biomass concentration, k_La) that minimize the coefficient of variation corresponding to R_{ex} and OC_T.
Keywords: Respirometry; Error propagation; Respiration rate; Oxygen consumption.

INTRODUCTION

Respirometry is a well-established procedure in the field of biological wastewater treatment. Respirometric methods have been used to determine parameters of kinetic models that describe the aerobic biodegradation of wastewaters, single compounds, or mixtures of chemicals (Cokgor *et al.,* 2009; Orhon *et al.,* 2009). These techniques were also employed to evaluate the biodegradable fraction of wastewaters (Lagarde *et al.,* 2005), and to monitor the acclimation process of an activated sludge to a new environment (Carvalho *et al.,* 2004; Aktas, 2012). Additionally, several on-line respirometer-based biosensors in wastewater treatment plants have been developed. Based on the comparison between the actual respiration rate of the activated sludge with a given set point value (e.g., the historical respiration rate), these biosensors allow the early detection of a toxic wastewater (Sollfrank and Gujer, 1990; Vanrolleghem *et*

al., 1994). However, in order to avoid false alarms, the evaluation of the error associated with the measurement of the actual respiration rate is crucial to distinguish between a real event (e.g., a toxic wastewater) and aleatory fluctuations of the indicator used for toxicity (e.g., the decrease of the respiration rate).

The literature regarding respirometry has given a special emphasis to parameter identifiability, precision, and sensitivity (Dochain *et al.,* 1995; Vanrolleghem *et al.,* 1995, Petersen *et al.,* 2001; Guisasola *et al.,* 2006); conversely, less focus has been given with regard to the error associated with the estimation of some relevant respirometric parameters, such as the respiration rate (R), and the amount of oxygen consumed (OC) during the biodegradation process. Considering that not only R and OC, but also their uncertainties are essential for the appropriate design of aeration devices of wastewater treatment processes, the aim of this work was to

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obtain the expressions to estimate the error of the measurements of R and OC derived from a respirometric profile.

MATERIALS AND METHODS

Principle of the Open Respirometer

Batch respirometric tests are a powerful tool for the measurement of the respiration rate of both pure and mixture cultures, such as activated sludge. Although several respirometric principles are available (Vanrolleghem and Spangers, 1998), nowadays the open (flowing gas/static liquid) respirometer (Vanrolleghem, 2002) is one of the most used for research applications (Contreras *et al.*, 2008a,b; Lobo *et al.*, 2013).

Figure 1 shows an example of the dissolved oxygen profile (Fig. 1(a)) and the respiration rate (Fig. 1(b)) as a function of time during a typical respirometry. When a pulse of an oxidizable substrate is added (t = 0.05 h), C decreases to reach a minimum value C_{min} = 4.7 mgO_2 L^{-1} due to the increase of the respiration rate associated with the oxidation of the external substrate. After this transient period, the respiration rate remains almost constant up to the substrate depletion. In some cases, experimental conditions are selected to achieve a constant biomass concentration (X) throughout the experiment, simplifying the interpretation of the obtained respirometric profile. For example, the use of a culture medium devoid of the nitrogen source, or an initial substrate concentration (S_0) much lower than X, both prevent a significant increase of X during the experiment. Additionally, these conditions also lead to short assay times, minimizing the possible decay of biomass. If X remains constant throughout the experiment, the dissolved oxygen concentration after the substrate depletion will be equal to the dissolved oxygen concentration before the substrate addition.

The change of the dissolved oxygen concentration (C) as a function of time (Fig. 1(a)) is determined by two competing processes, namely, the oxygen supply and the microbial respiration. Generally, aeration conditions (e.g., air flow rate, agitation) are constants during a typical experiment; however, the respiration rate depends on the availability of an oxidizable substrate. Two processes are responsible for the observed respiration rate. In the absence of substrates, the microbial oxygen uptake rate reflects the endogenous respiration rate (R_{en}) associated with maintenance processes; in this case, the biomass itself serves as the oxidizable substrate.
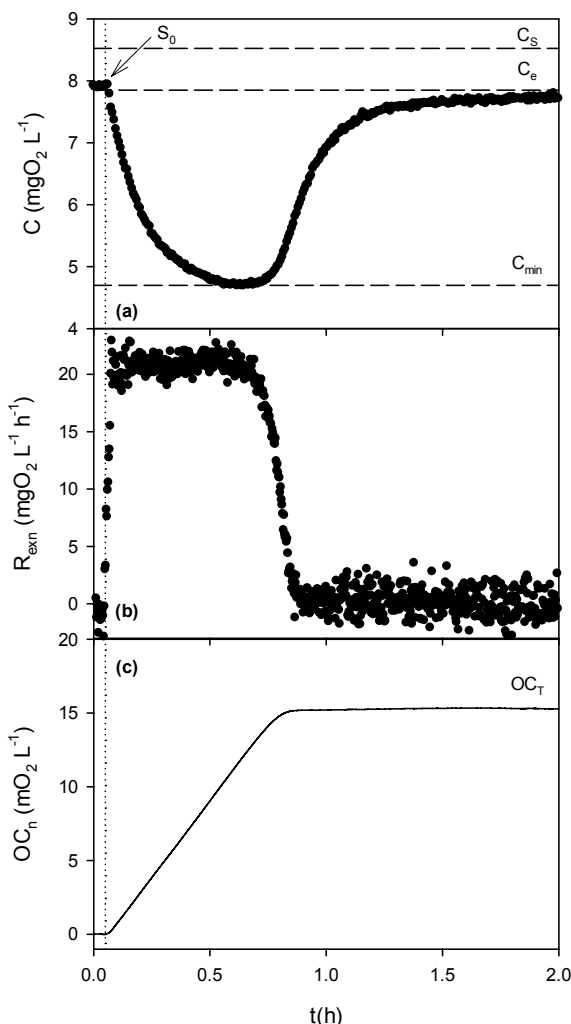

Figure 1: Example of a typical respirogram: a) dissolved oxygen (C), b) exogenous respiration rate (R_{exn}), and c) oxygen consumed (OC_n) as a function of time. Dotted line indicates the addition of 4-hydroxybenzoate. Experimental conditions: pH = 7, X = 1.8 gTSS L^{-1}, S_0 = 20 mg L^{-1}, C_S = 8.5 mgO_2 L^{-1}, $k_L a$ = 6.8 h^{-1}, C_e = 7.9 mgO_2 L^{-1}, Δt = 1 s, t_w = 60 s.

Conversely, the exogenous respiration rate (R_{ex}) is defined as the oxygen uptake rate related to the oxidation of an external substrate (Contreras *et al.*, 2008a,b; Lobo *et al.*, 2013). Considering these processes, the dissolved oxygen mass balance in the respirometer is:

$$\frac{dC}{dt} = k_L a \left(C_S - C \right) - R_{en} - R_{ex} \qquad (1)$$

where $k_L a$ is the volumetric oxygen mass transfer coefficient, and C_S is saturation dissolved oxygen concentration under the experimental conditions. In

the absence of substrate $R_{ex} = 0$; thus, C reaches a constant value (C_e) due to the equilibrium between oxygen transfer and endogenous respiration:

$$R_{en} = k_L a \left(C_S - C_e \right) \qquad (2)$$

Combination of Eqs. (1) and (2) yields the following expression:

$$R_{ex} = k_L a \left(C_e - C \right) - \frac{dC}{dt} \qquad (3)$$

In the absence of an external substrate ($R_{ex} = 0$), the actual values of $k_L a$, C_S, and C_e in an open respirometer can be obtained by several methods. For example, by stopping the oxygen supply, the first term on the right hand side of Eq. (1) becomes zero. Then, at a certain $t = t_0$ the air supply is recommenced and C increases according to Eq. (1) (Ros, 1993; Vanrolleghem, 2002). Considering the two above mentioned aeration conditions and assuming that $k_L a$, C_S, and R_{en} are constants within the tested time interval, integration of Eq. (1) leads to the following expression:

$$C = \begin{cases} C_S - R_{en} \left(\dfrac{1}{k_L a} + t \right) & t < t_0 \\[4mm] C_S - R_{en} \left[\dfrac{1}{k_L a} + t_0 e^{-k_L a (t - t_0)} \right] & t \geq t_0 \end{cases} \qquad (4)$$

Thus, $k_L a$, C_S, and R_{en} values can be obtained by fitting Eq. (4) to the data corresponding to dissolved oxygen concentration as a function of t before and after t_0; then, from these parameters, C_e can be calculated using Eq. (2). To determine R_{ex} by means of Eq. (3), a numerical differentiation, such as a moving regression window, is required:

$$R_{exn} = k_L a \left(C_e - C_n \right) - b_{t_w, \Delta t} \qquad (5)$$

where R_{exn} is the exogenous respiration rate evaluated around the point n, which corresponds to the process time $t = (n-1) \Delta t$, Δt is the interval between two successive data (e.g., the sampling frequency), t_w is the time window, and $b_{t_w, \Delta t}$ is the slope of the regression line within t_w.

Another important parameter that can be derived from a typical respirometric profile is the total oxygen consumed (OC_T) to oxidize a given amount of the tested substrate (Lobo *et al*., 2013). By

definition, the oxygen consumed (OC) as a function of time during the degradation of the exogenous substrate is

$$OC = \int_0^t R_{ex} dt \qquad (6)$$

Combining Eqs. (3) and (6), and considering that at $t = 0$, $C_1 = C_e$, the following equation was obtained:

$$\begin{aligned} OC &= \int_0^t \left[k_L a \left(C_e - C \right) - \frac{dC}{dt} \right] dt \\[3mm] &= k_L a \left(C_e t - \int_0^t C \, dt \right) - C + C_e \end{aligned} \qquad (7)$$

Taking into account $t = (n-1) \Delta t$, and applying the trapezoidal rule (Euler method) to the integral in Eq. (7):

$$\begin{aligned} OC_n &= k_L a \Delta t \left[C_e (n-1) - \frac{1}{2} \left(C_e + C_n \right) - \sum_{i=2}^{n-1} C_i \right] \\[2mm] &\quad - C_n + C_e \end{aligned} \qquad (8)$$

where OC_n corresponds to OC evaluated at the point n. When the exogenous substrate is depleted, R_{ex} is zero, thus, $C_n = C_e$. Based on these considerations, the total oxygen consumed (OC_T) can be calculated as follows:

$$\begin{aligned} OC_T &= k_L a \Delta t \left[(N-2) C_e - \sum_{i=2}^{N-1} C_i \right] \\[2mm] &\cong k_L a \Delta t \left(N C_e - \sum_{i=2}^{N-1} C_i \right) \end{aligned} \qquad (9)$$

where $N \gg 2$ is the total number of data. For example, taking into account that the sampling interval (Δt) used to obtain the respirometric profile shown in Figure 1(a) was 1 s, and that the total assay time was 2 h, this assay consisted of $N = 7200$ data. Figure 1(c) shows that OC increased as a function of time up to the substrate depletion, and then it remained constant; in this example, the total oxygen consumed (OC_T) was 15.8 mgO$_2$ L^{-1}.

RESULTS AND DISCUSSION

Uncertainty of Respirometric Measurements

Equations (5) and (9) demonstrate that sources of error in the calculation of R_{ex} and OC_T are the error associated with the measurements of C, k_La, and $b_{t_W,\Delta t}$. Uncertainty of R_{ex}, and OC_T can be evaluated by applying the linear error propagation method to Eqs. (5), and (9), respectively. Uncertainties were extrapolated from first-order local sensitivity functions using the following expression (Lucas and Prinn, 2005; Pasternack *et al.*, 2006):

$$\sigma_V^2 = \sum_{i=1}^{M}\left(\frac{\partial V}{\partial \theta_i}\right)^2 \sigma_{\theta_i}^2 \qquad (10)$$

where $\dfrac{\partial V}{\partial \theta_i}$ is the first-order sensitivity of some variable V (e.g., R_{ex}, OC_T) to the parameter θ_i (e.g., C, k_La), σ_V^2 and $\sigma_{\theta_i}^2$ are the variances of V and θ_i respectively, and the summatory is over the M parameters of the equation used to calculate V (Saltelli *et al.*, 2005).

In order to use Eq. (10) to evaluate the variances corresponding to R_{ex}, ($\sigma_{R_{exn}}^2$) and OC_T ($\sigma_{OC_T}^2$), an estimation of the variances associated with the measurements of C (σ_C^2), k_La ($\sigma_{k_La}^2$), and $b_{t_W,\Delta t}$ (σ_b^2) is necessary. With regard to the measurement of the dissolved oxygen concentration (C), we must distinguish between signal noise and accuracy of the dissolved oxygen probe. While signal noise can be reduced using several smoothing techniques (Hardle, 1991), accuracy depends on the probe characteristics. According to the specifications provided by several manufacturers, the accuracy of a typical optical dissolved oxygen probe within the range 0 - 10 mgO_2 L^{-1} is ±0.1 mgO_2 L^{-1} (YSI, 2013). Besides, the literature has given less attention to the error associated with the volumetric oxygen mass transfer coefficient. Gogate and Pandit (1999) studied several of the methods that are commonly used for the measurement of the mass transfer coefficient; these authors concluded that, within the range of the operating conditions encountered in fermentor operation, errors less than 10% of k_La can be expected. Similar results were reported by Puskeiler and Weuster-Botz (2005). However, at the laboratory scale, k_La errors are usually lower. Moreover, the error associated with k_La can be assumed to be proportional to its value, the proportionality constant being the coefficient of variation of k_La (CV_{kLa}). For example, Figure 2 shows that CV_{kLa} measured in our respirometer (500 mL working volume) was approximately 6% of the measured value. Based on the above mentioned considerations, the following standard deviations were assumed in this work: $\sigma_C = 0.1$ mgO_2 L^{-1}, $\sigma_{kLa} = CV_{kLa}$ k_La, where $CV_{kLa} = 0.06$.
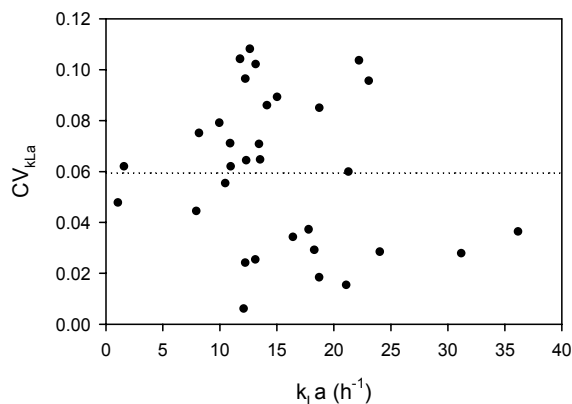

Figure 2: Coefficient of variation of k_La (CV_{kLa}) as a function of the k_La value in a 500-mL respirometer. All measurements are triplicates at least. The dotted line indicates the overall mean value of CV_{kLa}.

Evaluation of the Standard Deviation of the Derivative Term ($b_{t_W,\Delta t}$) by a Monte Carlo Technique

The estimation of the standard deviation of the derivative term $b_{t_W,\Delta t}$ (σ_b) was assessed using a Monte Carlo technique. For a given set of values corresponding to the time window (t_w), and the interval between two successive data (Δt), a noiseless profile of the dissolved oxygen concentration (C) was calculated assuming a linear decrease of C as a function of t with a constant slope (b) within t_w. Then, for each C value, a Gaussian noise with $\sigma_C = 0.1$ mgO_2 L^{-1} was added. Finally, from the linear regression of the dissolved oxygen concentration with the added noise as a function of time, the slope $b_{t_w,\Delta t}$ was calculated (Figure 3). This procedure was repeated 10000 times to obtain the distribution of $b_{t_w,\Delta t}$, statistical relevant estimations of the mean, and the standard deviation of $b_{t_w,\Delta t}$ within the tested conditions (the routine was implemented in Sigma Plot 10.0 and is available on request).
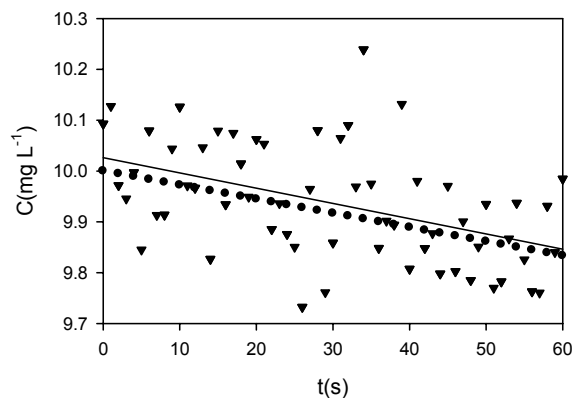
Figure 3: Example of the implementation of the Monte Carlo method to assess the standard deviation of $b_{t_w,\Delta t}$ (σ_b^2). Conditions: $t_w = 60$ s, $\Delta t = 1$ s, $b = 10$ mgO_2 L^{-1} h^{-1}, $\sigma_C = 0.1$ mgO_2 L^{-1}. Circles: noiseless dissolved oxygen data. Tringles: data with a Gaussian noise. The continuous line represents the first order regression of the data with Gaussian noise to obtain $b_{t_W,\Delta t}$.

Figure 4 shows that a Gaussian distribution of the standard deviation of $b_{t_w,\Delta t}$ (σ_b) was obtained by the Monte Carlo method.
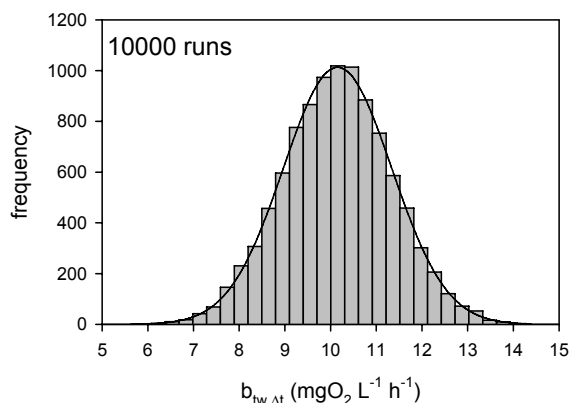

Figure 4: Distribution of $b_{t_w,\Delta t}$ obtained with 10000 Monte Carlo runs. Tested conditions were the same as in Fig. 3. The continuous line represents the Gaussian curve. In this case, $\sigma_b = 2.6$ mgO_2 L^{-1} h^{-1}.

Simulations show that σ_b did not depend on the dissolved oxygen concentration (C), or on the actual slope (b). Conversely, Figure 5(a) shows a linear increase of σ_b as a function of σ_C. Moreover, Figures 5(b) and 5(c) show that, in the cases when $t_w/\Delta t$ was higher than 10 (e.g., more than 10 points were used to calculate the slope $b_{t_w,\Delta t}$ of the regression line), σ_b has a power law dependence on t_w, and Δt. Based
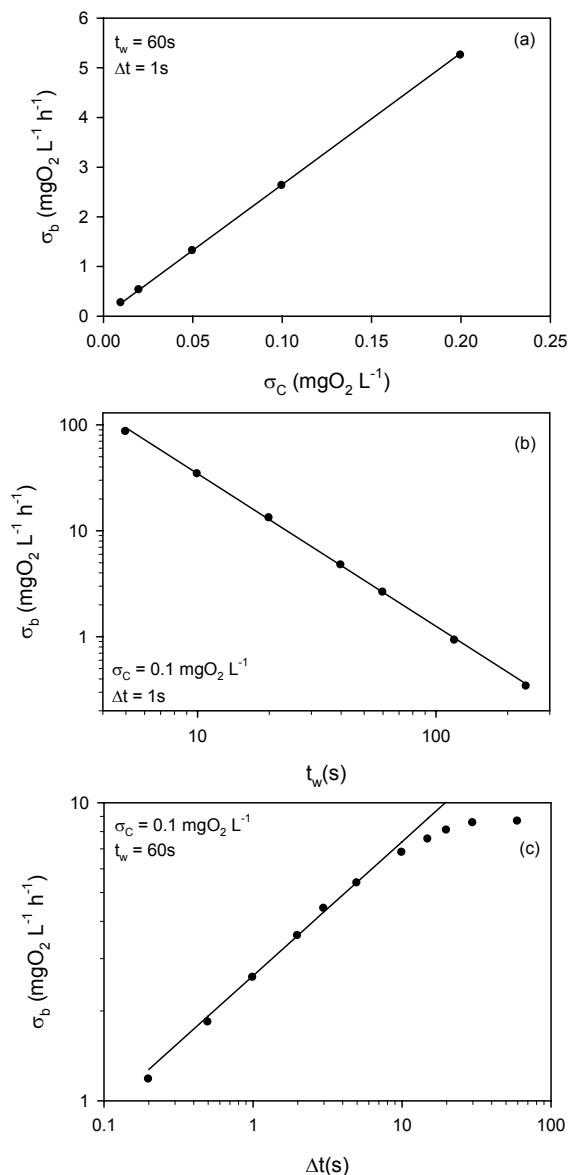

Figure 5: Effect of a) σ_C, b) t_w, and c) Δt, on σ_b. Each point corresponds to σ_b of 10000 Monte Carlo runs. Lines indicate σ_b values calculated by Eq. (11).

on these considerations, the following empirical equation was proposed to calculate σ_b as a function of σ_C, t_w, and Δt:

$$\sigma_b = \alpha \, \sigma_C \, t_w^\beta \, \Delta t^\gamma \qquad (11)$$

where σ_b, σ_C, t_w, and Δt were expressed in mgO_2 L^{-1} h^{-1}, mgO_2 L^{-1}, and s, respectively. Equation (11) was fitted to the data generated using the Monte Carlo method and the following coefficients were obtained: $\alpha = 9543$, $\beta = -1.44$, $\gamma = 0.45$ ($r^2 = 0.9950$). This equation is useful for calculating the standard

deviation (σ_b) of the slope $b_{t_w,\Delta t}$ obtained from the regression line of the dissolved oxygen (C) as a function of the standard deviation of C (σ_C), the time window (t_w) and the sampling rate ($1/\Delta t$).

While σ_C is an intrinsic feature of the DO probe, t_w, and Δt can be selected to minimize σ_b. Because of the aleatory noise of C, if the time window is small, then large variations on the derivative (therefore, on the respiration rate) are obtained. In most cases, this problem may be overcome by using digital filters or larger time windows (Savitzky and Golay, 1964; Bromba and Zlegler, 1981). Although there are several methods to select the optimal window size of digital filters (Vivo-Truyols and Schoenmakers, 2006), the selection of very large values of t_w may produce a flattened effect on R_{ex} curves, masking the actual behavior of the respiration rate as a function of time. Moreover, large t_w values in combination with a high sampling rate (e.g., a very low Δt value) produce very large data files, which are difficult to process due to computer limitations. The optimal selection of t_w and Δt to evaluate the derivative arises from a compromise between data noise characteristics, size of the data file, and accuracy in the estimation of the respiration rate (Marsili-Libelli and Tabani, 2002). It must be emphasized that, once t_w, and Δt are selected, σ_b only depends on σ_C; thus, because the accuracy of a typical optical dissolved oxygen probe is constant when operating in the range 0 - 10 mgO_2 L^{-1}, σ_b is also a constant.

Evaluation of the Variances Corresponding to R_{exn}, and OC_T

Once the standard deviations of C (σ_C,), k_La (σ_{k_La}), and $b_{t_w,\Delta t}$ (σ_b) are known, variances corresponding to R_{exn}, and OC_T were calculated by applying Eq. (10) to Eqs. (5), and (9), respectively:

$$\sigma_{R_{exn}}^2 = (CV_{kLa}\, k_La)^2 (C_e - C_n)^2$$
$$+2(\sigma_C\, k_La)^2 + \sigma_b^2 \tag{12}$$

$$\sigma_{OC_T}^2 = (CV_{kLa}\, OC_T)^2 + 2(\sigma_C k_La\, \Delta t\, N)^2 \tag{13}$$

The first term in Eq. (12) represents the contribution of the error in k_La to the total variance of R_{exn}. This term changes throughout the experiment from zero ($C_n = C_e$) to a given maximum (at $C_n = C_{min}$). The second term arises from the imprecision

of the DO. The third term in Eq. (12) represents the inaccuracy resulting from the estimation of the derivative and it depends on t_w and Δt, as was discussed previously. Equation (13) shows that the variance of the total oxygen consumed (OC_T) is comprised of two terms. The first one depends on the variance of k_La and is proportional to OC_T; the second term depends on the variance of C and is proportional to k_La and to the product $\Delta t\, N$, which represents the total time of the respirogram. For example, considering the experimental conditions used in the respirogram shown in Figure 1 (k_La = 6.8 h^{-1}, C_e = 7.9 mgO_2 L^{-1}, t_w = 60 s, Δt = 1 s), according to Eq. (12), σ_b = 2.6 mgO_2 L^{-1} h^{-1}; thus, $\sigma_{R_{exn}}$ ranges from 2.8 (at $C_n = C_e$) to 3.1 (at $C_n = C_{min}$) mgO_2 L^{-1} h^{-1}. Figure 1(c) shows that OC reaches a maximum value of 15.8 mgO_2 L^{-1} at t = 1 h due to the substrate depletion and then it remains constant; at this time, the product $\Delta t N$ = 1 h, resulting in σ_{OC_T} = 1.4 mgO_2 L^{-1} (Eq. (13)).

From a practical standpoint, the parameters that can be selected by the operator to obtain the most suitable results using an open respirometer are the initial substrate concentration (S_0), the biomass concentration (X), the oxygen transfer coefficient (k_La), and the dissolved oxygen sampling interval (Δt). Note that not all parameters are independent of each other. Equation (13) shows that, among others, the variance of OC_T depends on the value of OC_T, and on the product $\Delta t\, N$, which represents the total time of the experiment. Because both OC_T and the total time are proportional to S_0, σ_{OC_T} increases as a function of S_0. As an example, the following case study is used to derive the relation between these parameters.

Case Study: the Monod Model

The most used equation to represent the dependence of the substrate consumption rate as a function of the initial exogenous substrate concentration is the Monod equation. This equation can be employed to interpret the dissolved oxygen profile obtained from an open respirometric assay. In some cases, such as the example shown in Figure 1, it can be assumed that $K_S \ll S$ for most of the assay time. If the biomass concentration (X) can be considered constant throughout the experiment (e.g., a culture medium devoid of the nitrogen source, or initial substrate concentration much lower than X), then the dynamics of the respirometer can be described by the following equations:

$$\frac{dS}{dt} \approx -q_{Sm}X \tag{14}$$

$$\frac{dC}{dt} \approx k_L a\left(C_S - C\right) - \left(q_{en} + Y_{O/S}q_{Sm}\right)X \tag{15}$$

where S is the substrate concentration that limits the respiration rate, q_{Sm} is the specific maximum substrate consumption rate, q_{en} is the specific endogenous respiration rate, and $Y_{O/S}$ is the substrate oxidation coefficient. Before the addition of S, the dissolved oxygen concentration in the absence of substrate (C_e) is:

$$C_e = C_S - \frac{q_{en}X}{k_L a} \tag{16}$$

After the susbtrate addition, the respiration rate increases up to a maximum value ($R_{ex\text{-}max}$)

$$R_{ex-max} = Y_{O/S}q_{Sm}X \tag{17}$$

and C reaches a minimum (C_{min})

$$C_{min} = C_S - \left(q_{en} + Y_{O/S}q_{Sm}\right)\frac{X}{k_L a} \geq C_C \tag{18}$$

In most respirometric assays, experimental conditions are usually selected to maintain the dissolved oxygen above a certain critical concentration (e.g., $C_C = 2$ mgO_2 L^{-1}) to avoid the oxygen limitation of the respiration rate. Thus, Eq. (19) imposes a severe restriction on the suitable values of X and $k_L a$.

The total time necessary to achieve the susbtrate depletion ($\Delta t\,N$) can be calculated by integration of Eq. (14):

$$\Delta t\,N = \frac{S_0}{q_{Sm}X} \tag{19}$$

where S_0 is the initial substrate concentration. Finally, assuming that the substrate oxidation coefficient ($Y_{O/S}$) is a constant, the total oxygen consumed (OC_T) is:

$$OC_T = Y_{O/S}S_0 \tag{20}$$

Equations (16) to (20) can be combined with Eqs. (12) and (13) to obtain the following:

$$\sigma^2_{Rex-max} = \left(CV_{kLa}\,Y_{O/S}q_{Sm}X\right)^2 + 2\left(\sigma_C\,k_L a\right)^2 + \sigma^2_b \tag{21}$$

$$\sigma^2_{OC_T} = \left(CV_{kLa}\,Y_{O/S}\,S_0\right)^2 + 2\left(\sigma_C\,k_L a\,\frac{S_0}{q_{Sm}X}\right)^2 \tag{22}$$

Equation (21) shows that $\sigma^2_{Rex-max}$ is a function of the biomass concentration (X), and of the oxygen transfer coefficient ($k_L a$); moreover, $\sigma^2_{OC_T}$ (Eq. 22) also depends on the initial substrate concentration (S_0). Although equations (21) and (22) are a function of $Y_{O/S}$ and q_{Sm}, these coefficients can be easily obtained from a respirogram:

$$Y_{O/S} = \frac{OC_T}{S_0} \tag{23}$$

$$q_{Sm} = \frac{R_{ex-max}}{Y_{O/S}X} \tag{24}$$

For example, according to Figure 1, $R_{ex\text{-}max} = 22$ mgO_2 L^{-1} h^{-1}, and $OC_T = 15.8$ mgO_2 L^{-1}; considering that $S_0 = 20$ mgS L^{-1}, and $X = 1.8$ $gTSS$ L^{-1}, according to Eqs. (23) and (24), values corresponding to $Y_{O/S}$ and q_{Sm} are 0.79 mgO_2 mgS^{-1}, and 15.5 mgS $gTSS^{-1}$ h^{-1}, respectively.

Combining Eqs. (21) to (24), the expressions for the coefficients of variation corresponding to $R_{ex\text{-}max}$, and OC_T (CV_R, CV_{OC}) can be obtained:

$$CV_R = \frac{\sigma_{Rex-max}}{R_{ex-max}} = \sqrt{CV^2_{kLa} + 2\left(\frac{\sigma_C\,k_L a}{Y_{O/S}q_{Sm}X}\right)^2 + \left(\frac{\sigma_b}{Y_{O/S}q_{Sm}X}\right)^2} \tag{25}$$

$$CV_{OC} = \frac{\sigma_{OC_T}}{OC_T} = \sqrt{CV^2_{kLa} + 2\left(\frac{\sigma_C\,k_L a}{Y_{O/S}q_{Sm}X}\right)^2} \tag{26}$$

Equations (25) and (26) demonstrate that, in order to minimize CV_R and CV_{OC}, high values of X and low values of $k_L a$ are necessary. However, these conditions may also lead to dissolved oxygen values lower than the critical value (Eq. 18) (Fig. 6). Thus, the equations obtained in the present work are useful to obtain the suitable operative conditions (X, $k_L a$) that minimize CV values corresponding to $R_{ex\text{-}max}$, and OC_T.
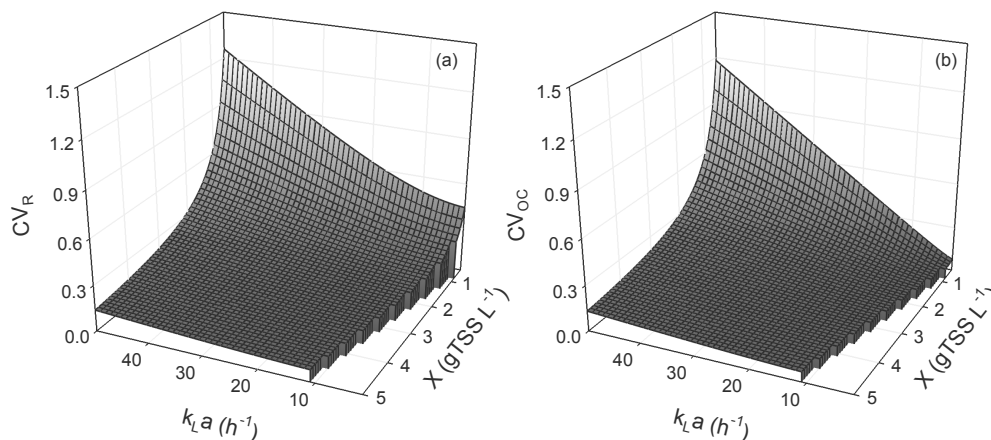
Figure 6: Effect of the volumetric mass transfer coefficient ($k_L a$) and the biomass concentration (X) on the coefficient of variation corresponding to (a) R_{ex-max}, and (b) OC_T calculated using Eqs. (25) and (26), respectively. Conditions: $CV_{kLa} = 0.06$, $\sigma_C = 0.1$ mgO$_2$ L^{-1}, $\sigma_b = 2.6$ mgO$_2$ L^{-1} h^{-1}, $C_s = 8.5$ mgO$_2$ L^{-1}, $q_{en} = 2$ mgO$_2$ gTSS^{-1} h^{-1}, $Y_{O/S} = 0.79$ mgO$_2$ mgS^{-1}, $q_{Sm} = 15.5$ mgS gTSS^{-1} h^{-1}. Discontinuities indicate the ($k_L a$, X) pairs for which $C_{min} < C_C = 2$ mgO$_2$ L^{-1} (Eq. 18).

CONCLUSIONS

▪ In this work, expressions were obtained for the evaluation of the standard deviation (SD) corresponding to the exogenous respiration rate (R_{ex}) and the total oxygen consumed (OC_T) derived from a single open respirometric profile.

▪ Standard deviations of R_{ex} and OC_T not only depend on the SD of the dissolved oxygen (σ_C) and $k_L a$ (σ_{kLa}), but also on the SD corresponding to the derivative term (σ_b) of the oxygen mass balance equation of the respirometer.

▪ The implemented Monte Carlo technique allowed calculating σ_b; an empirical power law expression for the dependence of σ_b as a function of σ_C, the time window (t_w) and the sampling rate (Δt) was obtained.

▪ The expressions obtained in the present work demonstrate that high values of X and low values of $k_L a$ are necessary to minimize the coefficient of variation (CV) of R_{ex-max} and OC_T. However, these experimental conditions may also lead to dissolved oxygen concentrations that could limit the substrate oxidation rate. For this reason, the equations obtained are useful to achieve the suitable operative conditions that minimize the CV of R_{ex-max}, and OC_T.

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NOMENCLATURE

Latin Letters

$b_{t_w,\Delta t}$	slope of the regression line	mgO$_2$ L^{-1} h^{-1}
C	dissolved oxygen concentration	mgO$_2$ L^{-1}
C_C	critical dissolved oxygen concentration	mgO$_2$ L^{-1}
C_e	equilibrium dissolved oxygen concentration	mgO$_2$ L^{-1}
C_{min}	minimum dissolved oxygen concentration	mgO$_2$ L^{-1}
C_S	saturation dissolved oxygen concentration	mgO$_2$ L^{-1}
CV_{kLa}	coefficient of variation of $k_L a$	
CV_{OC}	coefficient of variation of OC_T	
CV_R	coefficient of variation of R_{ex-max}	
$k_L a$	volumetric oxygen mass transfer coefficient	h^{-1}
N	total number of data	
OC	oxygen consumed	mgO$_2$ L^{-1}
OC_T	total oxygen consumed	mgO$_2$ L^{-1}
q_{en}	specific endogenous respiration rate	mgO$_2$ L^{-1} h^{-1}

q_{Sm}	maximum specific substrate consumption rate	mgS gTSS^{-1} h^{-1}
R_{en}	endogenous respiration rate	mgO$_2$ L^{-1} h^{-1}
R_{ex}	exogenous respiration rate	mgO$_2$ L^{-1} h^{-1}
S_0	initial substrate concentration	mgS L^{-1}
t	time	h
t_w	time window used for the regression line	s
X	biomass concentration	gTSS L^{-1}
$Y_{O/S}$	substrate oxidation coefficient	mgO$_2$ mgS^{-1}

Greek Letters

α, β, γ	empirical coefficients of Eq. (11)	
Δt	time interval between two data	s
σ_b	standard deviation of the slope of the regression line	mgO$_2$ L^{-1} h^{-1}
σ_C	standard deviation of C	mgO$_2$ L^{-1}
σ_{kLa}	standard deviation of k_La	h^{-1}
σ_{OC_T}	standard deviation of OC$_T$	mgO$_2$ L^{-1}
σ_{Rexn}	standard deviation of R$_{ex}$ at the point n	mgO$_2$ L^{-1} h^{-1}

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