Accuracy analysis of a respirometer for activated sludge
dynamic modelling

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Abstract

The aim of the paper is to assess the experimental errors arising from the operation of a closed respirometer using autotrophic biomass. A closed, intermittent-flow device has been set-up for the measurement of oxygen uptake rate (OUR) and parameter calibration. After describing the device structure and operation, the factors affecting accuracy have been assessed. Inaccuracies may be caused by two groups of parameters: design parameters, including flow rate, volume, sampling time, numerical algorithm, sample injection and environmental parameters, concerning the physico-chemical conditions of the experiment, such as unwanted oxygen transfer, pH, and the influence of sludge condition on “start-up” behaviour. It is shown to what extent each of them affects the final accuracy of the OUR measurement. In the second part of the paper, the respirometric data are used to calibrate a two-step nitrification model and their impact on the accuracy of the estimation of model parameters is assessed. Confidence limits are derived for the identifiable parameter combinations and the practical identifiability assessed with the aid of trajectory sensitivity analysis. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Respirometry; Oxygen uptake rate; Activated sludge; Microbial kinetics; Nitrification; Mathematical modelling

1. Introduction

Respirometry is a widely used technique for the characterisation of wastewater and activated sludge and constitutes a well-established procedure to assess the state of microbial activity and for the calibration of microbial kinetic models. The principles of respirometry are thoroughly illustrated in Spanjers et al. [1], whereas its use as a tool for rapid characterisation of wastewater and activated sludge was proposed by Spanjers and Vanrolleghem [2], and Brouwer et al. [3]. Respirometry can also be used for the calibration of the activated sludge kinetic models of the IAWQ suite [4], as illustrated by Vanrolleghem et al. [5].

There are several engineering implementations of the respirometric principles. This paper describes a closed respirometer, composed of two separate vessels for aeration and respiration, operated in an intermittent-flow mode. The aim of the study is to assess the effect of system parameters on the accuracy of the oxygen uptake rate (OUR). They are grouped in two sets: design parameters, including flow rate, volume, sampling time and numerical algorithm for DO data processing, and environmental parameters, concerning the physico-chemical conditions of the experiment, including accidental air leak in the respiration vessel, pH, sludge condition and size of sample injection. Respiration connected to ammonium-N oxidation was selected as the test system for its simplicity and its interest in monitoring several nutrient-removal plants under the responsibility of the...
Wastewater Treatment Service of the Florence Municipality.

The paper is divided in two parts: in the first, it is shown how the design and environmental parameters can be tuned in order to maximise the accuracy of the respirometric result. In the next section, the OUR data are used to calibrate a two-step nitrification dynamics derived from the ASM1 model from which accuracy considerations and confidence limits are derived.

2. Experimental set-up

The experimental set-up illustrated in Fig. 1 was operated as a closed intermittent-flow respirometer. Substrate is added into the aerated vessel and is periodically transferred into the air-tight respiration vessel, where a fixed number of dissolved oxygen (DO) measurements are taken. The respirometer operates with a duty-cycle (i.e. the fill-and-stop sequence) of 20 s pumping, 10 s idle, 30 s respiration measurement, for a total cycle time of 60 s. This device is similar to the hybrid respirometer described by Petersen [6], which has a continuous circulation between the two chambers. The aerated vessel, a 1.5 l fermentor with a working volume of 1200 ml, is continuously stirred and thermally controlled at 20 °C. The 250 ml air-tight respiration vessel is fitted with a DO probe (WTW 3000, Weilheim, Germany). The vessel is placed on a magnetic stirrer with thermal regulation at 20 °C. Recycle is provided by a computer-controlled peristaltic pump (Watson–Marlow mod. 313U, Watson–Marlow Ltd., Falmouth, UK). Closed-loop control of pH is provided by an Applikon ADI 1030 Biocontroller, which maintains the pH within ±0.02 units around the set value, normally set between 7.5 and 8.5. The data acquisition and OUR computation are performed by proprietary software developed in LabVIEW 5.1 (National Instruments, Austin TX, USA). The analogue DO signal is sampled with a 12-bit Analogue/Digital converter (PC-MIO 16E1, National Instruments, Austin TX, USA) connected to the PC (Compaq Deskpro, Pentium II/166 MHz, Compaq Computer Corporation, Houston TX, USA) system bus via memory-mapping. The A/D converter was software-programmed for an input range of 1 V, corresponding to a resolution of $2^{-12} = 2.4414 \times 10^{-4}$ V. Since the probe constant is 14.922 mgO₂/l for 1 V, the final resolution in the

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>C(i) (mgO₂/l)</th>
<th>dissolved oxygen concentration</th>
<th>$S_{NH}$ (mg NH₄-N/l)</th>
<th>added ammonium-N sample per unit volume (after dilution)</th>
</tr>
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<tbody>
<tr>
<td>$C_{0}$ (mgO₂/l)</td>
<td>total oxygen consumption per unit volume</td>
<td>$S_{NO₂}$ (mg NO₂-N/l)</td>
<td>nitrate concentration</td>
<td></td>
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<tr>
<td>$F$ (l/min)</td>
<td>pump flow rate</td>
<td>$S_{o}$ (mg O₂/l)</td>
<td>dissolved oxygen concentration</td>
<td></td>
</tr>
<tr>
<td>$K_{La}$ (min⁻¹)</td>
<td>dissolved oxygen mass transfer coefficient (uncontrolled)</td>
<td>$S_{o, sat}$ (mg O₂/l)</td>
<td>dissolved oxygen saturation concentration</td>
<td></td>
</tr>
<tr>
<td>$K_{NH}$ (mg NH₄-N/l)</td>
<td>ammonium oxidisers half-velocity concentration</td>
<td>$X_{A1}$ (mg COD/l)</td>
<td>ammonium oxidisers concentration</td>
<td></td>
</tr>
<tr>
<td>$K_{NO₂}$ (mg NO₂-N/l)</td>
<td>nitrite oxidisers half-velocity concentration</td>
<td>$X_{A2}$ (mg COD/l)</td>
<td>nitrite oxidisers concentration</td>
<td></td>
</tr>
<tr>
<td>$N$</td>
<td>number of data</td>
<td>$Y_{A1}$ (mg COD/mg N)</td>
<td>ammonium oxidisers yield factor</td>
<td></td>
</tr>
<tr>
<td>OC (gO₂/gN)</td>
<td>oxygen consumption = stoichiometric ratio of oxygen used per nitrogen consumed</td>
<td>$Y_{A2}$ (mg COD/mg N)</td>
<td>nitrite oxidisers yield factor</td>
<td></td>
</tr>
<tr>
<td>OURex (mg O₂/l min)</td>
<td>exogenous oxygen uptake rate</td>
<td>$V_{0}$ (l)</td>
<td>total reaction volume</td>
<td></td>
</tr>
<tr>
<td>OURend (mg O₂/l min)</td>
<td>endogenous oxygen uptake rate</td>
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<td></td>
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<tr>
<td>$S_{NH}$ (mg NH₄-N/l)</td>
<td>ammonia concentration</td>
<td></td>
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<tr>
<td>$S_{inj}$ (mg NH₄-N/l)</td>
<td>added ammonium-N sample per unit volume (before dilution)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$S_{o}$</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Greek letters</td>
<td>$\delta V_{inj}$ (l)</td>
<td>volume of the added sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\delta V_{buf}$ (l)</td>
<td>volume of the pH-correcting solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\mu_{maxA1}$ (min⁻¹)</td>
<td>ammonium oxidisers maximum growth rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\mu_{maxA2}$ (min⁻¹)</td>
<td>nitrite oxidisers maximum growth rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\tau$ (ms)</td>
<td>sampling time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#</td>
<td></td>
<td>denotes estimated quantity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DO scale is $3.643 \times 10^{-3}$ mgO$_2$/l, which is at least one order of magnitude better than the intrinsic probe accuracy.

3. Error sources

The first aim of the study is to assess the effect of system design on the accuracy of two key respirometric measurements: Oxygen uptake rate (OUR) and Oxygen consumption (OC) defined as the stoichiometric ratio of oxygen used per nitrogen consumed (gO$_2$/gN). For this purpose two parameter sets are considered

- **Design parameters**: including the sizing of the device (flow rate and volume), and its operation (sampling time, duty cycle, numerical algorithm for OUR estimation);
- **Environmental parameters**: concerning the physicochemical conditions of the experiment, i.e. uncontrolled oxygen transfer rate ($K_L a_o$), pH, sludge condition and size of sample injection.

3.1. Design parameters

These parameters are under the experimenter’s control and can be tuned to obtain maximum performance.

3.1.1. Flow rate and duty cycle

The device is operated in intermittent-flow mode and the sample is added in the aerated vessel. Therefore the speed with which the mixed liquor is transferred into the respiration vessel is a critical factor and the substrate transfer delay produces differing reaction rates in the two vessels, as shown in Fig. 2, obtained with a flow rate of 0.4 l/min and a pump duty-cycle (on-off proportion) of 20%. The jagged appearance of the variables is due to the intermittent flow. A similar result was obtained by Petersen [6] and Gernaey et al. [7] to show the different oxygen dynamics in the two vessels, but in their study the jags are missing since their device is continuous-flow. Both the flow rate and the on/off ratio (duty cycle) of the recycle pump contribute in making the two rates
different. The mathematical model described in the calibration section was used to determine the OC percentage error as a function of flow rate and duty-cycle, as shown in Fig. 3. This can be used to select a suitable operational compromise.

3.1.2. Filtering

The DO signal noise has several sources: the electric potential produced by the probe is inherently noisy given the motion of the liquid on the membrane surface and electromagnetic disturbances are picked up by the wires connecting the instrument to the process computer. The former is inevitable, since measuring the DO concentration in a still liquid would lead to major error due to oxygen depletion in the membrane neighbourhood [8]. The other noise source was minimised by providing accurate shielding of the wires and data pre-filtering prior to OUR computation. A median filter showed a better performance than low-pass or averaging filters for its inherent noise immunity. A general reference on digital filtering can be found in Oppenheim and Schafer [9]. The best trial-and-error compromise between data size and noise characteristics resulted in a filter rank of 12, meaning that the median was computed using the last 12 DO samples. Since the filtered data are time-centered, a lag of $12 \times 100/2 = 600$ ms was introduced, which represents a 1% error in the typical respiration window of 60s. It was found that the residuals $e = C_r - C$, between raw $C_r$ and filtered data $C$, had zero mean and a standard deviation $\sigma_e = 9.04 \times 10^{-3}$ mg O$_2$/l.

3.1.3. OUR estimation

Oxygen uptake rate was estimated as the slope of a regression line fitted to a series of DO filtered data as in

$$C(t_k) = -\text{OUR} t_k + C(0)$$

In Eq. (1) it is assumed that individual sampling intervals $\tau_i$ may differ as a consequence of slight timing perturbations introduced by LabVIEW™, running under Windows™ OS. This uncertainty is in the order of 1 ms and, being monitored on-line, makes each $\tau_i$ exactly known. The OUR value was computed using zero-mean quantities to minimise numerical errors

$$t_m = \frac{1}{N} \sum_{k=1}^{N} t_k \quad C_m = \frac{1}{N} \sum_{k=1}^{N} C(t_k)$$

$$\tilde{C}_k = C(t_k) - C_m \quad \tilde{t}_k = t_k - t_m$$

according to the least-square algorithm, with the time $\tilde{t}_k$ centered with respect to the median filter window,

$$\text{OUR} = \frac{\sum_{i=1}^{N} \tilde{C}_i \tilde{t}_i}{\sum_{i=1}^{N} \tilde{t}_i^2}$$

with estimation variance $\sigma_{\text{OUR}}^2 = \frac{\sigma_e^2}{\sum_{i=1}^{N} \tilde{t}_i^2}$

Fig. 4 shows the effect of median filtering on raw DO data and OUR estimation with Eqs. (2) and (3). Eq. (3) shows that the standard deviation is reduced by a factor $1/\sum_{i=1}^{N} \tilde{t}_i^2$ with respect to the variance of the residuals $\sigma_e^2$, which proved to be statistically uncorrelated. With the numerical values used in the study, this reduction factor is in the order of $0.5 \times 10^{-2}$.

The effectiveness of median filtering is confirmed by the numerical experiment of Fig. 5, using a synthetic
data set with the same statistics. It can be seen that the regression estimated with the filtered data is closer to the true one than that obtained with the raw data.

3.2. Environmental parameters

The consistency of these parameters is important to ensure that all measurements are taken under exactly the same operating conditions. Their influence in OUR and OC computation are now examined.

3.2.1. Respiration vessel $K_{L}a_0$

To assess the effect of accidental air leaks in the respiration vessel, which is supposed to be airtight, experimental data were collected with a leaky vessel and the oxygen dynamics calibrated with either $K_{L}a_0 \neq 0$ or $K_{L}a_0 = 0$. Fig. 6 shows that this leak produces a significant respirogram alteration.

After eliminating the air leaks, the two OC values were compared. In the case of an airtight vessel an $OC = 4.2843$ (gO$_2$/gN) was found, which is fairly close to the expected value $4.57 - (Y_{A1} + Y_{A2}) \approx 4.33$ (gO$_2$/gN), whereas in the case of air leak an unrealistically low value of $OC = 3.7665$ (gO$_2$/gN) was found. Air infiltration thus caused a 13% OC error. This fault could be detected by fitting the OUR data with $K_{L}a_0 = 0$. If a satisfactory fitting cannot be achieved, this could be interpreted as an indication of accidental air leak.

3.2.2. pH control

Autotrophic metabolism is considerably impaired if pH is outside its optimal value, which some authors place in the 7.2–8.5 interval (see e.g. [10]). In the present set-up pH control is obtained with an Applikon ADI 1030 Biocontroller, adding the exact amount of acid or alkali to maintain pH in a narrow range ($\pm 0.02$ units) around the optimal value for nitrifiers. The volume increase due to reagent addition is accounted for in the OC computation. Also, avoiding inorganic carbon (IC) depletion due to CO$_2$ stripping and nitrifiers growth is important in order to maintain the growth medium integrity. This can be obtained by using gaseous CO$_2$ as the acid reagent and adding a stoichiometric amount of HCO$_3^-$ with each NH$_4^+$ injection. This quantity was determined assuming an average yield factor $Y_{A1} + Y_{A2} \approx 0.24$ mg COD/mg N and C$_5$H$_7$O$_2$N as the standard elemental composition of the biomass [6,11].

3.2.3. Start-up behaviour

Start-up phenomena in short-term biokinetic experiments are well known and reported in the literature (see e.g. [12]) and may be caused by intracellular transport
and conversion processes. Also, when a batch of activated sludge kept in starvation/endogenous conditions for a long time (e.g. high SRT process conditions or prolonged aeration periods in a batch reactor) is used in a respirometer, a variable initial behaviour is observed following the first substrate injection. An example of this is shown in Fig. 7 where three successive respiromograms were obtained from previously starved activated sludge. Autotrophs “awakening” is clearly visible, with OUR increasing with each experiment, and denotes adaptation to the new condition.

In order to enhance the consistency of the respirometric experiments, the microorganisms were acclimated to the substrate with a series of preliminary small pulses prior to the respirometric experiment. Furthermore, an initial “shoulder” in the respiromograms can be seen in the experiments of Fig. 8, as a consequence of the two-step ammonia oxidation process. In fact, initially \( S_{\text{NH}} \) is at its maximum value, whereas \( S_{\text{NO}_2} \) is zero and so is its conversion rate. As ammonia is oxidised, a build-up of \( S_{\text{NO}_2} \) follows and its conversion rate increases accordingly. Since the experimental OUR is the sum of the two rates, the initial build-up produces the observed “rounded” leading edge shown in Fig. 8. The slope and the extent of this shoulder are a function of the half-velocity constant and the maximum conversion rate of the nitrite oxidizers, increasing with the value of \( K_{\text{NO}_2} \).

### 3.2.4. Volume variations and sample injection procedure

The sample injection procedure plays a key role in respirometric accuracy causing volume variations. The added substrate should be enough to attain maximum growth rate. Therefore, the injected concentration \( S_{\text{inj}} \) should be high enough to allow full development of nitrification kinetics and to produce a visible second nitrification step, after dilution in the aerated vessel. This is shown in Fig. 8, obtained with different initial substrate concentrations. It can be seen that when \( S_{\text{inj}} \) is small the second step of the reaction is hardly discernible and respiration rate never reaches its saturation value.

On the other hand, the more the substrate is added, the larger is the reagent quantity needed for pH control. If \( \delta V_{\text{inj}} \) is the volume of the sample injection with concentration \( S_{\text{inj}} \) and \( \delta V_{\text{buf}} \) the required volume of buffer solution injected by the pH controller, the total volume in the respirometer to be considered for OC computation will be \( V_{\text{tot}} = V_0 + \delta V_{\text{inj}} + \delta V_{\text{buf}} \), where \( V_0 \) is the volume prior to the addition. The actual ammonium-N concentration is then

\[
S_{\text{NH}}' = \frac{S_{\text{inj}} \delta V_{\text{inj}}}{V_0 + \delta V_{\text{inj}} + \delta V_{\text{buf}}} \tag{4}
\]

Being exactly known, this dilution effect is not strictly a random error, nevertheless it may constitute a systematic inaccuracy if neglected.

Fig. 7. Successive respirograms produced with a series of acclimation pulses applied to a starved culture prior to the main respirometric experiment.

Fig. 8. Experimental effect of varying initial amounts of ammonia. The \( S_{\text{NH}}' \) figures account for the dilution according to Eq. (4). The endogenous respiration has been omitted.
3.2.5. Estimation of yield factors

A linear relationship is observed between the amount of biodegradable added substrate and the amount of consumed oxygen expressed as short-term BOD (BOD$_s$), namely BOD$_s$ = (1 - Y$_A$)S$_{NH}$ [13]. Injecting differing amounts of substrate S$_{NH}$ and evaluating the corresponding area under the respiration curve (BOD$_s$), a regression line can be drawn whose slope $m = 1 - Y_A$ is related to the yield factor. A large number of respirograms were obtained in the period January—August 1999. For each experiment a fresh sludge sample was used. After a starving period different amounts of ammonium-N, corrected for the dilution effect, were added and the corresponding BOD$_s$ values were fitted with a regression line through the origin, as shown in Fig. 9. The sludge was taken from two different municipal wastewater treatment plants: S. Giusto, treating normal domestic effluent, and Torre, which in addition to a relatively light domestic loading, is subject to random high ammonium-N shock loading. The regression results are listed in Table 1. The high values of the correlation coefficient $R^2$ indicate a high consistency for each batch. However, a relevant variability of the yield coefficients from batch to batch was observed and the results are generally greater than the theoretical value of 0.24 often found in the literature (see e.g. [11]).

![Theoretical slope](image)

Fig. 9. Fitted regression lines between injected ammonium and consumed oxygen for several respiration experiments. The theoretical slope of 4.33 is shown for comparison.

<table>
<thead>
<tr>
<th>Date and place</th>
<th>Y$_A$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 June 1999 S. Giusto</td>
<td>0.2964</td>
<td>0.9999</td>
</tr>
<tr>
<td>8 June 1999 S. Giusto</td>
<td>0.2581</td>
<td>0.9999</td>
</tr>
<tr>
<td>9 June 1999 S. Giusto</td>
<td>0.2979</td>
<td>0.9995</td>
</tr>
<tr>
<td>12 June 1999 Torre</td>
<td>0.2927</td>
<td>0.9989</td>
</tr>
<tr>
<td>16 June 1999 S. Giusto</td>
<td>0.2763</td>
<td>0.9986</td>
</tr>
<tr>
<td>17 June 1999 S. Giusto</td>
<td>0.2826</td>
<td>0.9988</td>
</tr>
</tbody>
</table>

4. Calibration of kinetic parameters

4.1. Two-step nitrification model

A two-step nitrification model derived from ASM1 is considered, with two autotrophic populations of ammonium oxidisers (Y$_A$) and nitrite oxidisers (Y$_A$). No growth is assumed during the respiration experiments. A similar model was used by Petersen [6], Petersen et al. [14], Gernaey et al. [7] for respirometric studies, save for the intermittent flow.

A combined two-stage model was used, which takes into account the mass transfers before each respiration and the intermittent mode of operation (i.e. $q_r = 0$ during the DO measurement).

**Ammonium-N (aeration chamber):**

$$\frac{dS_{NH}^a}{dt} = -\frac{1}{Y_{A1}}\mu_{maxA1}\frac{S_{NH}^a}{K_{NH} + S_{NH}^a}Y_{A1} + q_a(S_{NH}^a - S_{NH}).$$

(5)

**Nitrite-N (aeration chamber):**

$$\frac{dS_{NO}^a}{dt} = -\frac{1}{Y_{A1}}\mu_{maxA1}\frac{S_{NH}^a}{K_{NH} + S_{NH}^a}Y_{A1}$$

$$-\frac{1}{Y_{A2}}\mu_{maxA2}\frac{S_{NO2}^a}{K_{NO2} + S_{NO2}^a}Y_{A2} + q_a(S_{NO2}^a - S_{NO2}).$$

(6)

**Ammonium-N (respiration chamber):**

$$\frac{dS_{NH}^r}{dt} = -\frac{1}{Y_{A1}}\mu_{maxA1}\frac{S_{NH}^r}{K_{NH} + S_{NH}^r}Y_{A1} + q_r(S_{NH}^r - S_{NH}).$$

(7)

**Nitrite-N (respiration chamber):**

$$\frac{dS_{NO}^r}{dt} = -\frac{1}{Y_{A1}}\mu_{maxA1}\frac{S_{NH}^r}{K_{NH} + S_{NH}^r}Y_{A1}$$

$$-\frac{1}{Y_{A2}}\mu_{maxA2}\frac{S_{NO2}^r}{K_{NO2} + S_{NO2}^r}Y_{A2} + q_r(S_{NO2}^r - S_{NO2}).$$

(8)

**Dissolved oxygen in the respiration chamber:**

$$\frac{dS_O^r}{dt} = K_Lq_d(S_{O_2sat}^r - S_O) - \text{OUR}_{ex}$$

$$-\text{OUR}_{end} - q_r(S_O^r - S_O).$$

(9)

**Oxygen Uptake Rate in the respiration chamber:**

$$\text{OUR} = \frac{3.43 - Y_{A1}}{Y_{A1}}\mu_{maxA1}\frac{S_{NH}^r}{K_{NH} + S_{NH}^r}Y_{A1}$$

$$+ \frac{1.14 - Y_{A2}}{Y_{A2}}\mu_{maxA2}\frac{S_{NO2}^r}{K_{NO2} + S_{NO2}^r}Y_{A2} + \text{OUR}_{end}.$$
In Eq. (10) the endogenous OUR OUR\textsubscript{end} is left as an unstructured parameter.

4.2. Parameter identifiability

The respirometric results obtained with the equipment described in the previous sections can be used to calibrate some combinations of parameters of the model (5–10). The structural identifiability of nonlinear models based on Monod kinetics has already been investigated by Pohjanpalo [15], Holmberg [16], Marsili–Libelli [17] and Dochain et al. [18]. In particular the identifiability of the model (5–10) has already been assessed by Petersen [6] and Petersen et al. [14]. The calibration procedure described in this section is based on these results, under the assumption that no bacterial growth occurs and that only respirometric measurements are available. Under these conditions the following parameter combinations can be estimated:

\[
\begin{align*}
\vartheta_1 &= \frac{3.43 - Y_{A1}}{Y_{A1}} \mu_{\text{max}, A1} X, \quad \vartheta_4 = \frac{1.14 - Y_{A2}}{Y_{A2}} \mu_{\text{max}, A2} Y, \\
\vartheta_2 &= (3.43 - Y_{A1}) S_{\text{NH}}^{\text{a}}, \quad \vartheta_5 = (1.14 - Y_{A2}) S_{\text{NO}_2}, \\
\vartheta_3 &= (3.43 - Y_{A1}) \tilde{K}_{\text{NH}}, \quad \vartheta_6 = (1.14 - Y_{A2}) \tilde{K}_{\text{NO}_2}.
\end{align*}
\]

(11)

Introducing the composite quantities \( R_{\text{NH}} = \hat{\mu}_{\text{max}, A1} X / Y_{A1} \) and \( R_{\text{NO}} = \hat{\mu}_{\text{max}, A2} X / Y_{A2} \) the following parameters can be obtained:

\[
\begin{align*}
Y_{A1} &= 3.43 - \frac{\vartheta_2}{S_{\text{NH}}^{\text{a}}}, \\
Y_{A2} &= \begin{cases} 
4.57 - Y_{A1} - m & \text{if } S_{\text{NO}_2}^{\text{a}} = 0, \\
1.14 - \frac{\vartheta_5}{S_{\text{NO}_2}} & \text{if } S_{\text{NO}_2}^{\text{a}} \neq 0,
\end{cases} \\
R_{\text{NH}} &= \vartheta_1 Y_{A1}, \quad R_{\text{NO}} = \vartheta_4 Y_{A2}, \\
K_{\text{NH}} &= \frac{3.43 - Y_{A1}}{1.14 - Y_{A2}}, \quad K_{\text{NO}_2} = \frac{\vartheta_6}{1.14 - Y_{A2}}.
\end{align*}
\]

(12)

There are two possible ways to determine \( Y_{A2} \) depending on the type of experiment: if no nitrite is initially present, then \( \vartheta_3 = 0 \) and \( Y_{A2} \) must be computed through the regression coefficient \( m \), since \( S_{\text{NO}_2}^{\text{a}} = 0 \) is usually zero.

4.3. Trajectory sensitivity

Sensitivity is important in assessing to what extent the observed data influence the accuracy of parameter estimation. It has been demonstrated [16,17,19,20] that trajectory sensitivity indicates in which dynamical condition each parameter has the highest influence on system response. A sensitivity system can be derived from the previous model written in terms of the identifiable parameter combinations, with Eq. (10) as the output equation.

\[
\begin{align*}
\frac{dS_{\text{NH}}}{dt} &= -\frac{R_{\text{NH}} S_{\text{NH}}^{\text{a}}}{K_{\text{NH}} + S_{\text{NH}}^{\text{a}}} + q_\delta (S_{\text{NH}} - S_{\text{NH}}^{\text{a}}), \\
\frac{dS_{\text{NO}_2}}{dt} &= \frac{R_{\text{NO}} S_{\text{NO}_2}^{\text{a}}}{K_{\text{NO}_2} + S_{\text{NO}_2}^{\text{a}}} X_{A2} + q_\delta (S_{\text{NO}_2} - S_{\text{NO}_2}^{\text{a}}), \\
\frac{dS_{\text{NH}}^{\text{a}}}{dt} &= -\frac{R_{\text{NH}} S_{\text{NH}}^{\text{a}}}{K_{\text{NH}} + S_{\text{NH}}^{\text{a}}} + q_\delta (S_{\text{NH}} - S_{\text{NH}}^{\text{a}}), \\
\frac{dS_{\text{NO}_2}^{\text{a}}}{dt} &= \frac{R_{\text{NO}} S_{\text{NO}_2}^{\text{a}}}{K_{\text{NO}_2} + S_{\text{NO}_2}^{\text{a}}} X_{A2} + q_\delta (S_{\text{NO}_2} - S_{\text{NO}_2}^{\text{a}}).
\end{align*}
\]

(13)

The output trajectory sensitivity \( S_{\text{p}}^{\text{OUR}} \) is generated by the dynamical system

\[
\frac{dS_{\text{p}}^{\text{OUR}}}{dt} = \frac{\partial f}{\partial \mathbf{x}} |_{\mathbf{x}_n} S_{\text{p}}^{\text{x}} + \frac{\partial g}{\partial \mathbf{p}} |_{\mathbf{p}_n},
\]

(17)

where \( S_{\text{p}}^{\text{x}} \) is the sensitivity of the state system \( \mathbf{x} = [S_{\text{NH}}^{\text{a}}, S_{\text{NO}_2}^{\text{a}}, S_{\text{NO}_2}^{\text{a}}, S_{\text{NH}}^{\text{a}}, S_{\text{NO}_2}^{\text{a}}, S_{\text{NO}_2}^{\text{a}}]^\top \) to each component of the parameter vector \( \mathbf{p} = [Y_{A1} R_{\text{NH}} K_{\text{NH}} Y_{A2} R_{\text{NO}} K_{\text{NO}_2}]^\top \), \( \partial f / \partial \mathbf{x} \) is the Jacobian of system Eqs. (13)–(16) and \( \partial g / \partial \mathbf{p} \) is the linearisation of output Eq. (10), all computed along the nominal trajectory. The initial conditions of the sensitivity system (17) are \( S_{\text{p}}^{\text{x}}(0) = 0 \). From Eqs. (13)–(17) the trajectory sensitivities of Fig. 10 were obtained for the respiration chamber. The identifiability considerations of the previous sections are confirmed since trajectories are not proportional to one another. The relative magnitude of the sensitivity functions are very different, with the rate parameters \( R_{\text{NH}} \) and \( R_{\text{NO}} \) exhibiting the highest values and the yield factors \( Y_{A1} \) and \( Y_{A2} \) the lowest. The fact that these latter trajectories are not proportional to one another implies that \( Y_{A1} \) and \( Y_{A2} \) can indeed be estimated individually. However, given the small values of the sensitivity functions, weak identifiability and large uncertainty are to be expected. Regarding \( Y_{A2} \), the trajectories of Fig. 10 indicate that even the data from the first nitrification step are relevant to its estimation.

The composite parameter \( R_{\text{NH}} \) exhibits a high sensitivity throughout the respiration experiment with two peaks appearing at the end of each oxidation step, whereas \( R_{\text{NO}} \) has a single sharp peak at the same time as \( K_{\text{NO}_2} \) when the nitrite oxidation is completed. This suggests that data collected during the “knee” phase of the respirogram are most important for the estimation.
of the second step, but overweighing of these data will lead to biased residuals as shown in the next section.

4.4. Parameter estimation

Determining the optimal parameter vector \( \hat{\mathbf{P}} = [Y_{A1}R_{NH}K_{NH}Y_{A2}R_{NO}K_{NO}2]^T \) implies minimisation of the sum of squared errors between OUR data (OURexp) and model responses (OUR)

\[
\hat{\mathbf{P}} = \arg \min_{[Y_{A1}R_{NH}K_{NH}Y_{A2}R_{NO}K_{NO}2]^T} \sum_{i=1}^{N} w_i (\text{OUR}_{\text{exp}}^i - \text{OUR}_i)^2, \tag{18}
\]

where \( N \) is the number of OUR data, \( n_p \) the number of estimated parameters and \( w_i \) are data weights. The minimisation of objective function (18) is performed via an optimised Simplex search described elsewhere [19].

4.5. Residual statistics

The variance of the estimated parameters was computed assuming that the observation errors are normally distributed and uncorrelated. However, since (18) is in practice a \( \chi^2 \) estimator, the following results can be considered valid even if the normality condition fails [21]. Under these assumptions the covariance matrix is given by

\[
\Gamma = \text{Cov}[(\mathbf{P} - \hat{\mathbf{P}}) \cdot (\mathbf{P} - \hat{\mathbf{P}})^T] = 2\sigma^2 \left[ \frac{\partial^2 E(\mathbf{P})}{\partial \mathbf{P} \cdot \partial \mathbf{P}^T} \right]^{-1}, \tag{19}
\]

with \( \hat{\mathbf{P}} \) denoting the estimated parameter vector. An estimate of \( \sigma^2 \) can be obtained from the definition of the error functional (18)

\[
\sigma^2 = \frac{1}{N - n_p} \cdot E(\hat{\mathbf{P}}). \tag{20}
\]

Substituting into Eq. (19) yields

\[
\hat{\mathbf{P}} = \frac{2}{N - n_p} \cdot E(\hat{\mathbf{P}}) \cdot \left[ \frac{\partial^2 E(\mathbf{P})}{\partial \mathbf{P} \cdot \partial \mathbf{P}^T} \right]^{-1} \bigg|_{\mathbf{P}=\hat{\mathbf{P}}}
\]

\[
= \frac{2}{N - n_p} \cdot E(\hat{\mathbf{P}}) \cdot \mathbf{H}(\hat{\mathbf{P}})^{-1}. \tag{21}
\]

All the quantities in Eq. (21) can be numerically approximated. In particular the Hessian matrix \( \mathbf{H}(\mathbf{P}) \) evaluated in the neighbourhood of the estimated parameter vector \( \hat{\mathbf{P}} \) can be approximated with finite differences. The generic \( j, k \)th component is evaluated as

\[
[H(\hat{\mathbf{P}})]_{j,k} = \frac{1}{\delta_j \delta_k} \left[ E(\hat{\mathbf{P}} + \delta_j \mathbf{P} + \delta_k) - E(\hat{\mathbf{P}} + \delta_k) - E(\hat{\mathbf{P}} + \delta_j) + E(\hat{\mathbf{P}}) \right]. \tag{22}
\]

where \( \delta_j \) and \( \delta_k \) are finite increments of \( p_j \) and \( p_k \).

Two important quantities can be obtained from the covariance matrix \( \Gamma \): the parameter confidence intervals and the joint confidence regions at the confidence level \( (1 - \alpha)/2\% \). The individual confidence limits can be estimated from a two-tails Student’s \( t \)-test for the given confidence level and \( N - n_p \) degrees of freedom

\[
p_i : \{ p_i = \hat{p}_i \pm t_{\alpha/2} \left( \frac{1}{\chi^2_{N - n_p}} \right) \} \quad i = 1, \ldots, n_p. \tag{23}
\]

Applying the algorithm to the data used to estimate the parameters of the respirometric model (10, 13–16)
The sum of the two yield factors $\hat{Y}_A + \hat{Y}_A = 0.2957$ is reasonably close to the value determined with the regression method yielding the values of Table 1 (0.2979). It can also be seen that the yield coefficients have the largest confidence intervals, which is consistent with their low sensitivity values, denoting a practical estimation difficulty. Their cross correlation is also high ($\Gamma = 0.9722$), exceeded only by the $(R_{NO}, K_{NO2})$ pair with 0.9737.

The joint confidence region for $g$ parameters can be approximated with the ellipsoids bounded by the $(1 - \alpha)\%$ level of the $F$ distribution [20,21]

$$P : (\mathbf{P} - \mathbf{\hat{P}})^T \Gamma (\mathbf{P} - \mathbf{\hat{P}}) \leq \frac{1}{1 - \gamma} F_{10 - 1, 9}$$

In the sequel only two-dimensional confidence ellipsoids ($g = 2$) will be considered. These loci are important to appreciate the degree of correlation between parameter couples, as shown in Fig. 11, where the ellipsoids for the most relevant parameter combinations are drawn.

A last word of caution regarding data weighting. Since the transition from the first to the second nitrification step is the most difficult to fit, there is the temptation to put more emphasis on the data in this

Fig. 11. Joint 95% confidence ellipsoids for parameter couples, with cross-hairs representing the estimated values.
transition zone to get a better fit. However, increasing the weight of data around the second step results in marginal accuracy improvement (Fig. 12), whereas it induces a considerable bias in the residuals, as the autocorrelogram of Fig. 13 shows. The zero-autocorrelation confidence limits of 95% were computed as $t_{N-n_p}^{1-0.05/2} / \sqrt{N - \tau}$ for each value of the lag $\tau$.

### 5. Conclusion

This paper has assessed the error sources of an intermittent-flow closed respirometer and their effect on parameter estimation of a kinetic model. The influence of design and operation parameters has been considered, with the aid of an experimental set-up and a two-step kinetics derived from the ASM1 model. For the design accuracy, a guideline is proposed to select flow rate, duty cycle, sampling time, number of samples and numerical filter characteristics, in order to achieve a prescribed tolerance for the oxygen consumption. For the environmental accuracy, the effect of parasitic air infiltration in the respiration chamber was considered and a detection method was proposed. Other sensitive aspects regarded constant-pH operation and sludge conditioning, in relation to the “start-up” behaviour. Finally, the sizing of ammonium-N sample was addressed. The estimation of the yield factor through linear regression concluded the first part.

In the second part, the calibration of a two-step nitrification kinetics derived from the ASM1 model was considered and practical identifiability limits were found through sensitivity analysis. Also, one and two-dimensional confidence regions were determined and parameter correlations assessed. Both techniques confirmed strong correlation between estimates of $(Y_{A1}, Y_{A2})$ and of $(R_{NO}, K_{NO2})$. It was also shown that selective data weighting to enhance fitting around particular areas of the respirogram, such as the second nitrification step, should be avoided as it may produce biased residuals.

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