

# Reliability of parameter estimation in respirometric models

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## Abstract

When modelling a biochemical system, the fact that model parameters cannot be estimated exactly stimulates the definition of tests for checking unreliable estimates and design better experiments. The method applied in this paper is a further development from Marsili-Libelli et al. [2003. Confidence regions of estimated parameters for ecological systems. *Ecol. Model.* 165, 127–146.] and is based on the confidence regions computed with the Fisher or the Hessian matrix. It detects the influence of the *curvature*, representing the distortion of the model response due to its nonlinear structure. If the test is passed then the estimation can be considered reliable, in the sense that the optimisation search has reached a point on the error surface where the effect of nonlinearities is negligible. The test is used here for an assessment of respirometric model calibration, i.e. checking the experimental design and estimation reliability, with an application to real-life data in the ASM context. Only dissolved oxygen measurements have been considered, because this is a very popular experimental set-up in wastewater modelling. The estimation of a two-step nitrification model using batch respirometric data is considered, showing that the initial amount of ammonium-N and the number of data play a crucial role in obtaining reliable estimates. From this basic application other results are derived, such as the estimation of the combined yield factor and of the second step parameters, based on a modified kinetics and a specific nitrite experiment. Finally, guidelines for designing reliable experiments are provided.

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## 1. Introduction

The structural and practical identifiability of Monod-based biochemical models has greatly progressed since the first studies of Pohjanpalo (1978) and Holmberg (1982) and is still a viable research topic (Kesavan and Law, 2005). Important contributions now exist both on the principles of estimation (Dochain et al., 1995; Vanrolleghem and Keesman, 1996; Petersen et al., 2000; Brun et al., 2002; Petersen et al., 2003a) and on its practical aspects (Vanrolleghem et al., 1995; Brouwer

et al., 1998; Petersen, 2000; Petersen et al., 2002), resulting in systematic estimation protocols (Petersen et al., 2003b; Sin, 2004; De Pauw, 2005). This evolution was also stimulated by the introduction of the ASM models (Henze et al., 2000) whose parameter identification is now considered such an important issue in wastewater treatment that the ASM3 model has replaced ASM1 not only for its better understanding of the biochemical mechanisms, but also for its improved identifiability (Gernaey et al., 2004). In this context, respirometry is a primary tool for model identification and a great deal of research has been devoted to providing uncertainty limits to parameter estimates and designing better experiments, especially

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Nomenclature			
ASM	activated sludge model	$r_{\text{end}}$	endogenous respiration rate (mg O <sub>2</sub> L <sup>-1</sup> min <sup>-1</sup> )
$C$	parameter covariance matrix, $\mathfrak{R}^{n_p \times n_p}$	$S_{\text{NH}}(0)$	initial concentration of ammonium-N (mg N-NH <sub>4</sub> L <sup>-1</sup> )
$C_J$	approximate covariance matrix based on the Fisher Information Matrix (FIM), $\mathfrak{R}^{n_p \times n_p}$	$S_{\text{NO}}(0)$	initial concentration of nitrite-N (mg N-NO <sub>2</sub> L <sup>-1</sup> )
$C_H$	approximate covariance matrix based on the Hessian matrix, $\mathfrak{R}^{n_p \times n_p}$	$S_{\text{NH}}$	ammonium-N concentration (mg N-NH <sub>4</sub> L <sup>-1</sup> )
$E(p)$	error functional for parameter estimation	$S_{\text{NO}}$	nitrite-N concentration (mg N-NO <sub>2</sub> L <sup>-1</sup> )
$F_{n_p, N-n_p}^{\alpha}$	$F$ statistics at the 100(1- $\alpha$ )% confidence level for $n_p$ parameters and $N-n_p$ degrees of freedom	$S_{P_j^y}$	output sensitivity to parameter $p_j$
$J$	Fisher Information Matrix (FIM), $\mathfrak{R}^{n_p \times n_p}$	$s^2$	estimated variance of the residuals (mg L <sup>-1</sup> ) <sup>2</sup>
$H$	Hessian (second derivative) matrix, $\mathfrak{R}^{n_p \times n_p}$	$t_{N-n_p}^{\alpha/2}$	two-tails Student's $t$ distribution for the given confidence level 100(1- $\alpha$ )% and $N-n_p$ degrees of freedom
$K_{\text{NH}}$	ammonium oxidisers half velocity constant (mg N-NH <sub>4</sub> L <sup>-1</sup> )	$X_{A1}$	ammonium oxidisers biomass concentration (mg COD L <sup>-1</sup> )
$K_{\text{NO}}$	nitrite oxidisers half velocity constant (mg N-NO <sub>2</sub> L <sup>-1</sup> )	$X_{A2}$	nitrite oxidisers biomass concentration (mg COD L <sup>-1</sup> )
$N$	number of experimental data	$Y_{A1}$	ammonium oxidisers biomass yield
NOD	nitrogen oxygen demand (mg O <sub>2</sub> L <sup>-1</sup> )	$Y_{A2}$	nitrite oxidisers biomass yield
$n_p$	number of estimated parameters	$\delta p_j$	individual parameter confidence interval estimated with the FIM approximation
$R_{\text{NH}}$	composite parameter related to the first oxidation stage (mg O <sub>2</sub> L <sup>-1</sup> min <sup>-1</sup> )	$\delta P_H$	individual parameter confidence interval estimated with the Hessian approximation
$R_{\text{NO}}$	composite parameter related to the second oxidation stage (mg O <sub>2</sub> L <sup>-1</sup> min <sup>-1</sup> )	$\mu_{\text{maxA1}}$	ammonium oxidisers maximum growth rate (min <sup>-1</sup> )
$r_{\text{NO}}$	synthesis oxygen uptake rate on nitrite-N (mg O <sub>2</sub> L <sup>-1</sup> min <sup>-1</sup> )	$\mu_{\text{maxA2}}$	nitrite oxidisers maximum growth rate (min <sup>-1</sup> )
$r_o^{\text{exp}}$	experimental respiration rate (mg O <sub>2</sub> L <sup>-1</sup> min <sup>-1</sup> )	$\tau$	time constant of nitrite-N modified respiration model (min)
$r_o$	model respiration rate (mg O <sub>2</sub> L <sup>-1</sup> min <sup>-1</sup> )		

when the model structure is a priori specified as in ASM models (Vanrolleghem et al., 1995; Vanrolleghem and Keesman, 1996; Brouwer et al., 1998; Petersen, 2000; Petersen et al., 2000; Marsili-Libelli and Tabani, 2002; Sin, 2004; De Pauw, 2005).

Based on the assumption that any given model structure is necessarily a simplified approximation of a complex reality, the paper applies a new estimation reliability test, derived from a previous method based on the approximate confidence regions (Marsili-Libelli et al., 2003), to a respirometric model based on ASM kinetics. Its aim is to determine under which conditions routine respirograms yield reliable estimates, without performing complex experiments with inhibitors (e.g. Nowak et al., 1995; Surmacz-Gorska et al., 1996) or combining respirometric and titrimetric measurements (Petersen, 2000; Ficara et al., 2002; Gernaey et al., 2002). After summarising the main results of Marsili-Libelli et al. (2003), the theory is taken a step further, presenting a new reliability test which is applied to the estimation of a two-step nitrification kinetics. Initially, the full two-step model is considered, showing that the

estimation of the second step parameters is critical and assessing the influence of the number of data. Later, a specific nitrite experiment is performed, leading to a better estimation of those parameters. Eventually, an estimation protocol is defined for the reliable estimation of the whole parameter set.

### 1.1. Theoretical identifiability of a two-step nitrification model

A two-step nitrification model derived from the ASM model suite (Henze et al., 2000) is considered, with two zero-growth autotrophic populations of ammonium oxidisers ( $X_{A1}$ ) and nitrite oxidisers ( $X_{A2}$ ). The zero-growth assumption is justified by the short duration of the experiments and the small amount of added substrate. A similar model was used by Petersen (2000) and Gernaey et al. (2001) for respirometric studies, whereas a more recent paper (Insel et al., 2003) includes hydrolysis. The endogenous respiration  $r_{\text{end}}$  is directly estimated by averaging the endogenous data. The identifiability of respirometric models without biomass

growth has been definitively assessed (Vanrolleghem et al., 1995; Petersen, 2000; Brun et al., 2002; Petersen et al., 2003a) and particularly by Brouwer et al. (1998), who estimated a model similar to Eq. (3) save for the yield coefficients which are supposed known. The following parameter combinations can be estimated

$$\begin{aligned} \vartheta_1 &= (3.43 - Y_{A1})R_{NH}, & \vartheta_4 &= (1.14 - Y_{A2})R_{NO}, \\ \vartheta_2 &= (3.43 - Y_{A1})S_{NH}(0), & \vartheta_5 &= (1.14 - Y_{A2})S_{NO}(0), \\ \vartheta_3 &= (3.43 - Y_{A1})K_{NH}, & \vartheta_6 &= (1.14 - Y_{A2})K_{NO}, \end{aligned} \quad (1)$$

where the two composite parameters  $R_{NH} = \hat{\mu}_{\max A1} X_{A1}/Y_{A1}$  and  $R_{NO} = \hat{\mu}_{\max A2} X_{A2}/Y_{A2}$  are introduced for identifiability reasons (Dochain et al., 1995). The first-step parameters ( $Y_{A1}, R_{NH}, K_{NH}$ ) are always identifiable if  $S_{NH}(0) \neq 0$ , whereas if  $S_{NO}(0) = 0$  only two of the three second-step parameters ( $Y_{A2}, R_{NO}, K_{NO}$ ) can be identified. In practice this condition implies a large estimation uncertainty of the second step parameters, as will be shown later. The aim of this paper is to assess the reliability of the estimates as a function of  $S_{NH}(0)$ , showing that this parameter plays a crucial role in the model identifiability, and suggesting appropriate values for this quantity.

## 1.2. Calibration of model parameters

A numerical optimisation search is used to minimise the sum of squared errors between respiration data  $r_o^{\text{exp}}$  and model responses  $r_o$  at the sampling times  $i = 1, 2, \dots, N$ ,

$$E(\mathbf{p}) = \sum_{i=1}^N (r_o^{\text{exp}}(i) - r_o(i))^2, \quad (2)$$

where  $\mathbf{p} = [Y_{A1} R_{NH} K_{NH} Y_{A2} R_{NO} K_{NO}]^T$  is the parameter vector,  $N$  is the number of respirometric data and the model output  $r_o$  is computed from Eq. (3)

$$\begin{aligned} r_o &= \frac{3.43 - Y_{A1}}{Y_{A1}} \hat{\mu}_{\max A1} \frac{S_{NH}}{K_{NH} + S_{NH}} X_{A1} \\ &+ \frac{1.14 - Y_{A2}}{Y_{A2}} \hat{\mu}_{\max A2} \frac{S_{NO}}{K_{NO} + S_{NO}} X_{A2} + r_{\text{end}}. \end{aligned} \quad (3)$$

The minimisation of the error functional Eq. (2) with the model constraint Eq. (3) is obtained with a combined search algorithm starting with a modified genetic algorithm (Marsili-Libelli and Alba, 2000) to determine the initial search region containing the global minimum, which is then refined with a modified simplex search (Marsili-Libelli, 1992). The calibration software was developed in the Matlab 6.5 platform (The Mathworks, Natick, MA, USA), using the stiff-Rosenbrock integration method with a relative accuracy of  $10^{-6}$ . The details of model implementation are described in Marsili-Libelli and Tabani (2002).

## 1.3. Approximations of confidence regions for the estimated parameters

Confidence regions represent the set of parameter values producing a model response within prescribed statistical boundaries. Being related to the error functional Eq. (2), any level  $E(\mathbf{p}) > E(\hat{\mathbf{p}})$  defines a region with a given degree of confidence. However, it is difficult to specify statistically significant levels of the increment  $\Delta E = E(\mathbf{p}) - E(\hat{\mathbf{p}})$  unless  $N$  is large, in which case  $\Delta E$  has the required  $\chi^2$  asymptotic properties to apply the  $F$  statistics (Seber and Wild, 1989). The numerical difficulty in estimating the exact confidence regions has been examined by Vanrolleghem and Keesman (1996) and Dochain and Vanrolleghem (2001) who, on the basis of a previous work by Lobry and Flandrois (1991), proposed a successive contraction method to find the value of  $E(\mathbf{p})$  corresponding to the prescribed  $F$  value. In this paper the approach of Marsili-Libelli et al. (2003) is used, based on linear or quadratic approximations, as suggested by Press et al. (1986) and Seber and Wild (1989). Confidence regions in the  $n_p$  parameter space can be expressed as a the quadratic form

$$(\mathbf{p} - \hat{\mathbf{p}})\mathbf{C}^{-1}(\mathbf{p} - \hat{\mathbf{p}})^T \leq n_p F_{n_p, N-n_p}^{\alpha}, \quad (4)$$

where the matrix  $\mathbf{C}$  is the equivalent of the parameter covariance matrix of the linear case (Ljung, 1999) and  $100(1-\alpha)\%$  is the required confidence level of the  $F$  statistics with  $n_p$  parameters and  $N-n_p$  degrees of freedom. This matrix can be approximated either by extending the results of the linear theory or through a second-order expansion of the error functional Eq. (2). The first approach, used by Petersen (2000) and Dochain and Vanrolleghem (2001), approximates  $\mathbf{C}$  with the inverse of the Fisher Information Matrix (FIM)  $\mathbf{J}$  (Ljung, 1999), defined as a quadratic form of the output sensitivity  $\partial y / \partial \mathbf{p}|_{\hat{\mathbf{p}}}$

$$\mathbf{C}_J = \mathbf{J}^{-1}, \quad \text{where } \mathbf{J} = \frac{1}{s^2} \sum_{k=1}^{N_{\text{exp}}} \left( \frac{\partial y(k)}{\partial \mathbf{p}} \right)^T \left( \frac{\partial y(k)}{\partial \mathbf{p}} \right). \quad (5)$$

The measurement error variance is estimated as  $s^2 = E(\hat{\mathbf{p}})/N - n_p$ . As an alternative,  $\mathbf{C}$  can be approximated by a second-order expansion of the objective error function in the neighbourhood of the minimum  $E(\hat{\mathbf{p}})$  (Press et al., 1986; Seber and Wild, 1989)

$$\mathbf{C}_H(\hat{\mathbf{p}}) \cong 2s^2 \mathbf{H}^{-1}, \quad \text{where } \mathbf{H} = \left. \frac{\partial^2 E(\mathbf{p})}{\partial \mathbf{p} \partial \mathbf{p}^T} \right|_{\hat{\mathbf{p}}}. \quad (6)$$

Substituting either  $\mathbf{C}_J$  or  $\mathbf{C}_H$  or in place of  $\mathbf{C}$  in Eq. (4) two differing approximate confidence ellipsoids are obtained. Numerical algorithms for computing these approximations are described in Press et al. (1986), Marsili-Libelli et al. (2003) and De Pauw (2005).

From Eqs. (5) or (6) the confidence interval of the individual parameter can be computed

$$\delta p_i = \pm t_{N-n_p}^{\alpha/2} \sqrt{C(i, i)}, \tag{7}$$

where  $t_{N-n_p}^{\alpha/2}$  is the two-tails Student's  $t$  distribution for the given confidence level  $100(1-\alpha)\%$  and  $N-n_p$  degrees of freedom. This statistics is consistent with the multivariate  $F$  distribution for  $n_p=1$ , since  $t_{N-n_p}^{\alpha/2} = \sqrt{F_{1, N-n_p}^{\alpha}}$ . Substituting  $C_J$  or  $C_H$  in place of  $C$  in Eq. (7) yields the approximate confidence bounds of the estimated parameters  $\hat{p}_i$ . It is important to notice that though  $\delta p_i$  refers to a single parameter, it takes into account the full  $n_p$ -dimensional confidence region through the matrix  $C$ .

1.4. A parameter estimation reliability test

In addition to yielding confidence regions, the two approximations Eqs. (5) and (6) provide a reliability test for the estimated parameters based on their inherent conceptual difference. The FIM approximation  $C_J$  is based on the sensitivities, whereas the Hessian approximation  $C_H$  depends on the shape of the error surface. For nonlinear systems  $C_H$  includes the effect of the curvature, reflecting the degree of nonlinearity induced by the model structure (Donaldson and Schnabel, 1987; Bates and Watts, 1988; Seber and Wild, 1989; Marsili-Libelli et al., 2003, Appendix B). Conversely  $C_J$ , being a linear approximation, does not contain this term. Since the curvature effect vanishes in the neighbourhood of the minimum of  $E(\mathbf{p})$ , comparing the two confidence regions yields a measure of the estimation reliability, because if the two regions diverge, this implies that the search terminated at a point where the effect of the curvature is still significant and therefore this cannot be the real minimum of  $E(\mathbf{p})$ . Conversely, if the two regions coincide the curvature effect is negligible and the identification can be considered reliable. In this case these regions coincide also with the exact confidence region determined on the basis of the error surface (Lobry and Flandrois, 1991). It should be reminded, however, that the curvature, amplifying the estimation errors, is an indicator of a failure to reach the minimum, but is not influenced by model inadequacy and residual characteristics. Its vanishing merely indicates that the residuals are orthogonal to the response surface in the neighbourhood of  $\hat{\mathbf{p}}$ , but does not attempt at characterizing them, e.g. whether they are gaussian and uncorrelated. This test is therefore an assessment of the quality of the optimisation and *not* of the model structure.

Visual inspection of the agreement or divergence between the confidence regions involves a fair amount of subjectivity. To obtain an objective test the curvature radii are considered, which are related to the curvature

(Bates and Watts, 1988). Consider the maximum and minimum curvature radii  $r_{\max} = (1 - \lambda_{\max})^{-1/2}$  and  $r_{\min} = (1 - \lambda_{\min})^{-1/2}$ , where  $\lambda_{\max}$  and  $\lambda_{\min}$  are the maximum and minimum eigenvalues of the matrix  $B$  related to the Hessian and FIM matrices by the relationship (Seber and Wild, 1989)

$$B = I_{n_p} - \frac{1}{2} K^T H K, \tag{8}$$

where  $K = R^{-1}$  and  $R^T R$  is the upper-triangular decomposition of  $s^2 J$ . Eq. (8) is the result of a coordinate transformation in the parameter space which attempts at reducing the confidence ellipsoid to a sphere (Seber and Wild, 1989). In the absence of curvature both  $r_{\max}$  and  $r_{\min}$  tend to 1, therefore the extent to which their ratio exceeds unity yields an indication of the residual curvature in the neighbourhood of  $\hat{\mathbf{p}}$ . A normalized threshold value for the ratio  $r_{\max}/r_{\min}$  can be obtained in the form

$$\bar{\rho} = 1 + \sqrt{\frac{n_p}{N - n_p} F_{n_p, N - n_p}^{\alpha}}, \tag{9}$$

where the square root represents the tolerance to keep into account the effective ellipsoids dimensions in the tangent plane (Bates and Watts, 1988; Seber and Wild, 1989) normalized to be insensitive to scaling errors. Further, Quinn et al. (2005) recently demonstrated that the  $F$  statistics can be used to determine the confidence limits of a parameter function such as Eq. (8). If  $r_{\max}/r_{\min} < \bar{\rho}$  the estimation can be considered reliable at the  $100(1-\alpha)\%$  level, implying that the search terminated at a point on the error surface where the effect of the curvature is negligible. This test is more practical than the mere visual comparison between ellipses because it does not involve the subjectivity of the examiner and it takes into account the full  $n_p$  dimension of the parameter space rather than the 2-dimensional subspace of the projected ellipsoids.

1.5. Optimal experiment design (OED) criteria

Other estimation accuracy indexes may be obtained as scalar functions of the covariance matrix  $C$ . Each of them emphasises differing aspects of the confidence ellipsoid, such as its volume or axes. Based on these indicators, a theory of optimal experiments has been developed (Fedorov, 1972) with the aim of minimising the estimates covariance. Table 1 illustrates the main criteria used in this and similar studies (see e.g. Atkinson and Donev, 1992; Versyck et al., 1998; Petersen, 2000; De Pauw, 2005). Of all the criteria mentioned in Table 1, only  $D$  has the property of being scale invariant, whereas all the others are scale sensitive, particularly mod  $E$ . The least sensitive of all appears to be mod  $A$ .

Table 1  
Optimal experiment design criteria based on FIM

Criteria	Method	Effect
<i>A</i>	$\min(\text{tr}(\mathbf{J}^{-1}))$	Minimization of the arithmetic mean of parameter errors.
mod <i>A</i>	$\max(\text{tr}(\mathbf{J}))$	Same as <i>A</i> , but insensitive to FIM ill-conditioning
<i>D</i>	$\max(\det(\mathbf{J}))$	Minimizes the volume of the confidence ellipsoid
<i>E</i>	$\max(\lambda_{\min}(\mathbf{J}))$	Minimizes the length of the largest axis of the confidence ellipsoid
mod <i>E</i>	$\min(\lambda_{\max}/\lambda_{\min})$	Minimizes the ill-condition number

## 2. Calibration of the respirometric model

The purpose of this section is to investigate the influence of the initial amount of substrate  $S_{\text{NH}}(0)$  on the identifiability of the model parameters  $\mathbf{p} = [Y_{\text{A1}} R_{\text{NH}} K_{\text{NH}} Y_{\text{A2}} R_{\text{NO}} K_{\text{NO}}]^T$ , using three data sets obtained with the respirometer described in Marsili-Libelli and Tabani (2002). The validity of the identified parameters is assessed through several tests: sensitivity, curvature radii and OED criteria.

### 2.1. Assessment of estimated parameters

Though the estimation results, shown in Fig. 1, appear visually acceptable in all cases, the inspection of the parameter values and confidence intervals of Table 2 reveals that under certain experimental conditions the results may be seriously flawed. As a preliminary test, parameter values should always check for physical constraints; in this sense the negative values  $Y_{\text{A2}}$  for low and medium  $S_{\text{NH}}(0)$  indicates an unreliable estimation, though its wide confidence interval encompasses reasonable values. On the other hand, imposing positivity constraints to the simplex algorithm may disrupt the search and produce awkward numerical results, as demonstrated by the results of Alfonso and da Conceição Cunha (2002), later criticized by De Pauw et al. (2004). For this reason it was preferred to use an unconstrained method, but rather use the confidence regions approach to design reliable experiments. The most serious flaws appearing in Table 2 are the negative values of the yield coefficients for low and medium initial  $S_{\text{NH}}(0)$  and the abnormally large values  $R_{\text{NO}}$  and  $K_{\text{NO}}$  for  $S_{\text{NH}}(0) = 5 \text{ mg L}^{-1}$ . Further, since the duration of the three experiments differs, the number of available data is not the same and this may affect the estimation accuracy. To obtain a fair comparison among the three experiments, the data sets for  $S_{\text{NH}}(0) = 5$  and  $10 \text{ mg L}^{-1}$  were under-sampled selecting every other data, in order to have approximately the same experimental basis for all of them. The results were rather counterintuitive: not only the estimation did not get worse, but rather it improved considerably for  $S_{\text{NH}}(0) = 5 \text{ mg L}^{-1}$ , particularly for the yield coefficients and the critical second-step parameters. The radii test Eq. (9) was also passed,

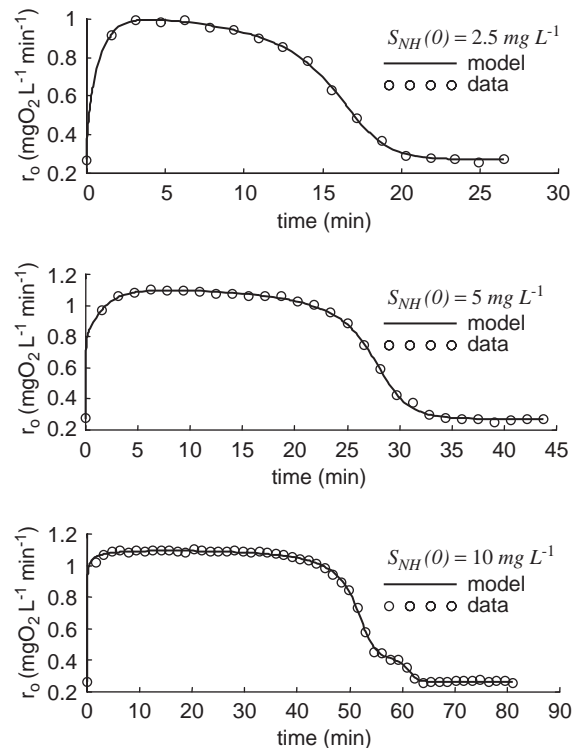


Fig. 1. Fitting the model Eq. (3) to the batch respirometric data.

whereas the full data set failed. This results is in agreement with De Pauw (2005), who found that under-sampling may results in an improved accuracy and reduced parameter correlation.

### 2.2. Sensitivity analysis

The sensitivity trajectories for the three initial substrate conditions  $S_{\text{NH}}(0)$  are shown in Fig. 2. They were computed with an incremental method similar to that described by De Pauw and Vanrolleghem (2003) and De Pauw (2005). The sensitivity to  $Y_{\text{A1}}$  and  $Y_{\text{A2}}$  is very small for all initial  $S_{\text{NH}}(0)$ . This is in agreement with the results of Table 2 and suggest that the best way to identify the yield coefficients is to estimate their sum

Table 2  
Estimated parameters for the respirometric experiments, confidence intervals and reliability indicators

$S_{NH}(0)$	No. of data	$Y_{A1}$	$R_{NH}$	$K_{NH}$	$Y_{A2}$	$R_{NO}$	$K_{NO}$	$Y_A = Y_{A1} + Y_{A2}$	$r_{end}$	$r_{max}/r_{min}$	$\bar{\rho}$	
2.5	18	$\hat{\rho}$	2.5288	0.1909	0.2124	-2.2039	2.4849	1.8595	0.3249	0.2725	36.4446	2.3900
		$\delta p_j$	$\pm 5.9618$	$\pm 0.0110$	$\pm 0.0498$	$\pm 5.7584$	$\pm 13.9352$	$\pm 12.1508$	$\pm 0.2115$			
		$\delta p_H$	$\pm 5.2857$	$\pm 0.0077$	$\pm 0.0280$	$\pm 5.1212$	$\pm 1.7371$	$\pm 1.3545$	$\pm 0.1721$			
5.0	15	$\hat{\rho}$	0.1973	0.2063	0.2006	-0.0258	0.2036	0.1371	0.1714	0.2710	1.1565	2.6387
		$\delta p_j$	$\pm 0.7820$	$\pm 0.0078$	$\pm 0.0351$	$\pm 0.7577$	$\pm 0.0513$	$\pm 0.2092$	$\pm 0.0492$			
		$\delta p_H$	$\pm 0.7023$	$\pm 0.0072$	$\pm 0.0352$	$\pm 0.6809$	$\pm 0.0473$	$\pm 0.1893$	$\pm 0.0478$			
5.0	29	$\hat{\rho}$	0.7340	0.2032	0.2088	-0.4982	13.4362	24.443	0.2358	0.2710	202.6126	1.9416
		$\delta p_j$	$\pm 0.7867$	$\pm 0.0037$	$\pm 0.0273$	$\pm 0.7582$	$\pm 584.9534$	$\pm 1081.8783$	$\pm 0.0451$			
		$\delta p_H$	$\pm 0.5556$	$\pm 0.0022$	$\pm 0.0207$	$\pm 0.5379$	$\pm 3.8382$	$\pm 6.3424$	$\pm 0.0393$			
10.0	21	$\hat{\rho}$	0.0657	0.2061	0.2440	0.1557	0.1777	0.0819	0.2214	0.2657	1.6819	1.8830
		$\delta p_j$	$\pm 0.0998$	$\pm 0.0015$	$\pm 0.0214$	$\pm 0.0986$	$\pm 0.0097$	$\pm 0.0513$	$\pm 0.0232$			
		$\delta p_H$	$\pm 0.0946$	$\pm 0.0014$	$\pm 0.0216$	$\pm 0.0939$	$\pm 0.0069$	$\pm 0.0340$	$\pm 0.0232$			
10.0	42	$\hat{\rho}$	0.0388	0.2058	0.2416	0.1936	0.1824	0.1103	0.2324	0.2657	1.3155	1.6205
		$\delta p_j$	$\pm 0.0870$	$\pm 0.0013$	$\pm 0.0196$	$\pm 0.0875$	$\pm 0.0079$	$\pm 0.0467$	$\pm 0.0205$			
		$\delta p_H$	$\pm 0.0820$	$\pm 0.0013$	$\pm 0.0208$	$\pm 0.0829$	$\pm 0.0066$	$\pm 0.0389$	$\pm 0.0204$			

$Y_A = Y_{A1} + Y_{A2}$  and perform a separate experiment for calibrating  $Y_{A2}$ , as will be shown later. For  $S_{NH}(0) = 2.5 \text{ mg L}^{-1}$ , the proportional sensitivities of the second step parameters  $R_{NO}$  and  $K_{NO}$  denote poor identifiability. This effect decreases as  $S_{NH}(0)$  increases, but still underlines the critical identifiability of the second step parameters. It should also be noticed that the shape of the  $R_{NO}$  trajectory is largely influenced by  $S_{NH}(0)$ , exhibiting a large peak at the beginning of the second step for  $S_{NH}(0) = 10 \text{ mg L}^{-1}$ . This indicates that the transition between the first and the second step is a critical event for parameter estimation and it will be investigated further with a specific nitrite experiment.

### 2.3. Evaluation of design criteria

The OED indicators of Table 1 were evaluated for the calibrated model of Fig. 1 and the results of Table 3 were obtained. In agreement with the confidence intervals of Table 2, the uncertainty affecting the estimates of  $R_{NO}$  and  $K_{NO}$  is not monotonic and this reflects into the OED criteria of Table 3, save for mod A, which is not affected by FIM ill-conditioning (Petersen, 2000) and shows a monotonic increase with  $S_{NH}(0)$ . In fact it was found that if  $R_{NO}$  and  $K_{NO}$  are not estimated all the OED indexes exhibit a monotonic behaviour. Further, all the OED criteria were only slightly affected by data decimation.

### 2.4. Estimation of the yield factors

Fig. 3 shows the fitted regression line nitrogen oxygen demand (NOD) =  $(4.57 - Y_A) S_{NH}(0)$  between the initial

amount  $S_{NH}(0)$  and the consumed oxygen expressed as NOD, where  $Y_A$  is the yield factor of the two steps combined and a fourth experiment with  $S_{NH}(0) = 1 \text{ mg L}^{-1}$  was added for improved accuracy. The resulting value  $Y_A = 0.245$  is very close to 0.24, which is generally indicated as the typical yield factor for autotrophs (Orhon and Artan, 1994; Henze et al., 2000; Petersen, 2000; Sin, 2004).

### 2.5. Respiration on nitrite

The estimation of the second step has always posed special problems and in the past the estimation of both steps was achieved by introducing selective inhibitors (Nowak et al., 1995; Surmacz-Gorska et al., 1996) such as Allylthiourea (ATU) or sodium chloride. However, the use of inhibitors, in addition to requiring a sample of fresh biomass for each experiment, is not advisable for their lack of selectivity. On another front, Ficara et al. (2002) characterize the two steps by measuring pH or performing two separate experiments, if only DO measurements are considered. To investigate further the dynamics of the second step, a nitrite respirogram with  $S_{NO}(0) = 2.7 \text{ mg L}^{-1}$  was performed. According to Eq. (3) the pertinent model should be

$$r_{NO} = (1.14 - Y_{A2})R_{NO} \frac{S_{NO}}{K_{NO} + S_{NO}} + r_{end}. \quad (10)$$

This, however, does not take into account the gradual response of the nitrite oxidisers observed by Vanrolleghem et al. (1998) and Vanrolleghem et al. (2004). To account for this biochemical fact, an exponential term is

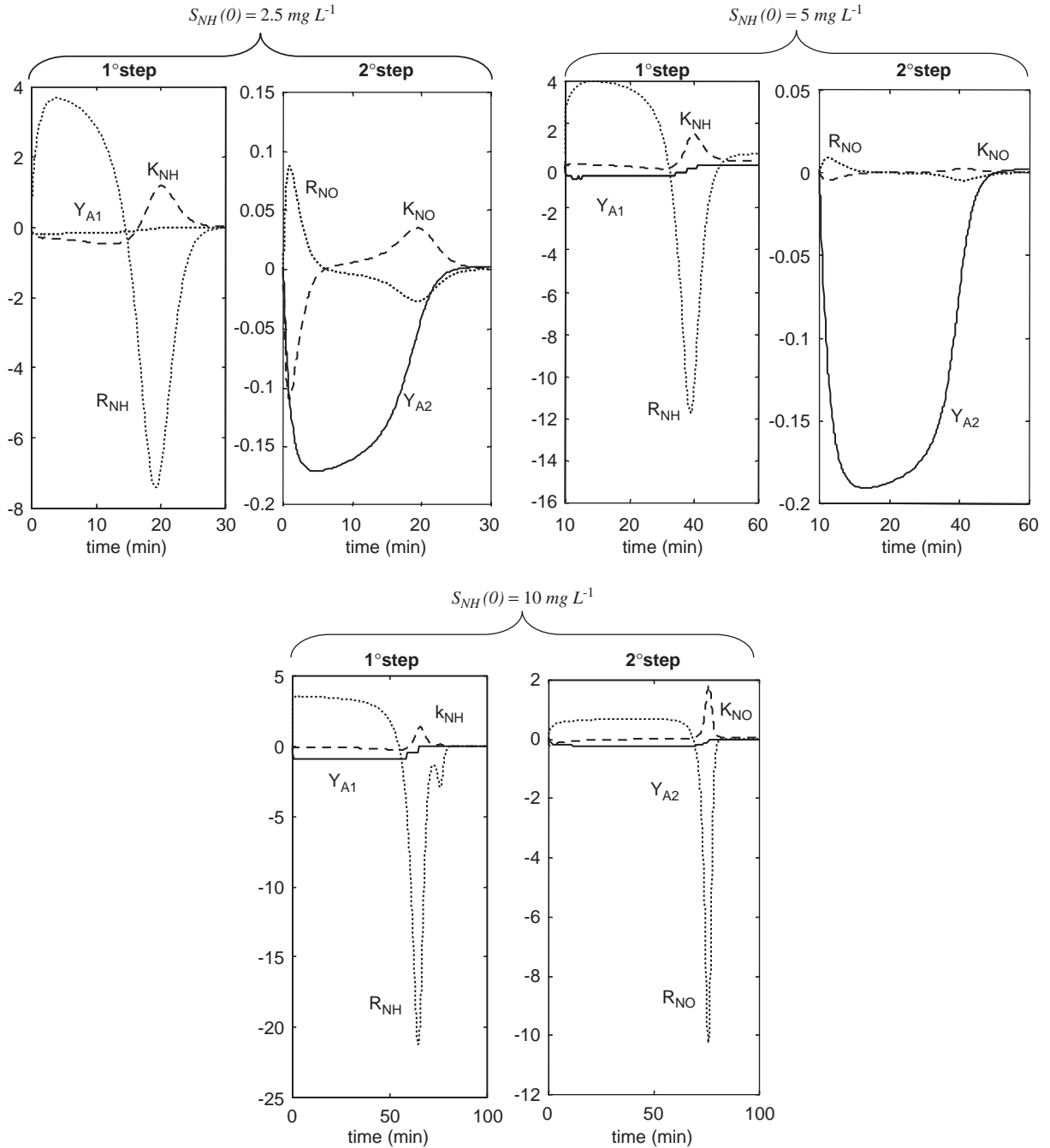


Fig. 2. Sensitivity trajectories of the respirometric model Eq. (3) for the three values of  $S_{NH}(0)$  over the two steps. The solid lines refer to  $Y$ 's, the dotted lines to  $R$ 's and the dashed lines to  $K$ 's.

introduced to model the initial time lag  $\tau$ .

$$r_{NO} = (1 - e^{-t/\tau})(1.14 - Y_{A2})R_{NO} \frac{S_{NO}}{K_{NO} + S_{NO}} + r_{end}. \quad (11)$$

From the identification viewpoint, the initial rising data are incompatible with the Monod term, which cannot fit the OUR initial increase. It is therefore fair to omit them and calibrate the model Eq. (10) only with the data which it can explain. The technique of eliminating

Table 3

Optimal experiment design criteria evaluated for the three respirograms. The FIM results are in italic, the Hessian in bold

$S_{NH}(0)$	$A$	mod $A$	$D$	$E$	mod $E$
2.5	$8.6480 \times 10^1$	$1.9961 \times 10^6$	$7.1748e \times 10^{12}$	$1.3325 \times 10^{-2}$	$1.4888 \times 10^8$
	<b><math>1.2432 \times 10^1</math></b>	<b><math>1.9859 \times 10^6</math></b>	<b><math>7.9761 \times 10^{14}</math></b>	<b><math>8.5083 \times 10^{-2}</math></b>	<b><math>2.3194 \times 10^7</math></b>
5	$3.5347 \times 10^5$	$4.7588 \times 10^6$	$7.4034 \times 10^9$	$2.8290 \times 10^{-6}$	$1.6758 \times 10^{12}$
	<b><math>1.2983 \times 10^1</math></b>	<b><math>4.6728 \times 10^6</math></b>	<b><math>3.5911 \times 10^{14}</math></b>	<b><math>9.7351 \times 10^{-2}</math></b>	<b><math>4.7818 \times 10^7</math></b>
10	$4.4101 \times 10^{-3}$	$1.5971 \times 10^7$	$2.9120 \times 10^{27}$	$2.6742 \times 10^2$	$5.3342 \times 10^4$
	<b><math>3.8515 \times 10^{-3}</math></b>	<b><math>1.6782 \times 10^7</math></b>	<b><math>4.9547 \times 10^{27}</math></b>	<b><math>3.0053 \times 10^2</math></b>	<b><math>4.9163 \times 10^4</math></b>

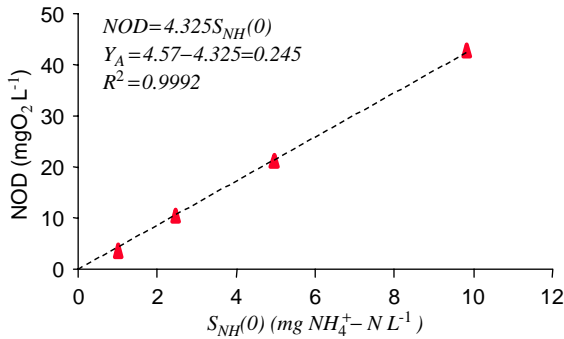


Fig. 3. Computation of the combined yield factor  $Y_A$  from respirograms. For improved accuracy a fourth experiment with  $S_{NH}(0) = 1 \text{ mg L}^{-1}$  was considered.

the initial respiration data has been frequently used in the literature (Vanrolleghem et al., 1995; Vanrolleghem and Coen, 1995; Vanrolleghem and Keesman, 1996; Ossenbruggen et al., 1996; Ubay Çokgör et al., 1998; Mathieu and Etienne, 2000) in estimating a Monod kinetics. Conversely, Eq. (11), including both start-up and nitrification, can explain all the experimental data and the introduction of the exponential term in Eq. (11) is fully supported by experimental evidence and biochemical theory (Vanrolleghem et al., 1998; Petersen, 2000; Baeza et al., 2002; Vanrolleghem et al., 2004). Further, this additional term does not pose special estimation problems given the sensitivity separation with the other parameters  $Y_{A2}$ ,  $R_{NO}$  or  $K_{NO}$ . In fact the trajectories of Fig. 4 indicate two high-sensitivity zones: at the beginning of the experiment, both the time lag  $\tau$  and the yield factor  $Y_{A2}$  are the most sensitive parameters, whereas in the last part of the respirogram the two most critical parameters are  $R_{NO}$  and  $K_{NO}$ . The introduction of the time lag allows the preservation of the biological meaning of  $Y_{A2}$ ,  $R_{NO}$  or  $K_{NO}$ . The relative fit of the two models is shown in Fig. 5 and the estimated parameters of the two models are compared in Table 4. It is evident from the parameter

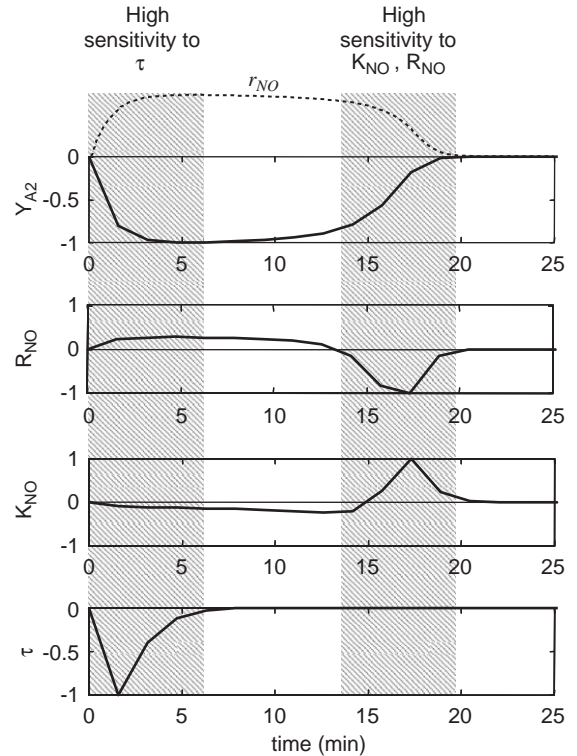


Fig. 4. Sensitivity analysis of the nitrite respirogram. The dotted line represents the reference  $r_{NO}$  trajectory.

correlation matrix  $R$

$$R = \begin{bmatrix} Y_{A2} & R_{NO} & K_{NO} & \tau \\ 1.0000 & -0.1859 & -0.2803 & -0.4453 \\ -0.1859 & 1.0000 & 0.8912 & 0.3143 \\ -0.2803 & 0.8912 & 1.0000 & 0.2863 \\ -0.4453 & 0.3143 & 0.2863 & 1.000 \end{bmatrix} \begin{matrix} Y_{A2} \\ R_{NO} \\ K_{NO} \\ \tau \end{matrix} \quad (12)$$

that apart from the couple  $(R_{NO}, K_{NO})$ , all other correlations are moderate.

The primary aim of this experiment is the direct estimation of  $Y_{A2}$ , and in this sense model Eq. (11) is definitely superior to Eq. (10), in which the large estimated value for  $Y_{A2}$  can be explained observing that the oxygen consumption is given by  $NOD = (1.14 - Y_{A2}) S_{NO}(0)$ . If the respirogram area decreases, as a consequence of discarding the initial four data, NOD decreases and therefore the right-hand-side must decrease. Since  $S_{NO}(0)$  is constant, this can only be accomplished by increasing  $Y_{A2}$ , which leads to the observed error. This value is clearly unfeasible, being larger than the sum of  $Y_{A1}$  and  $Y_{A2}$  of Table 2 for  $S_{NH}(0) = 10 \text{ mg L}^{-1}$  whereas its confidence interval is unrealistically narrow, indicating a great confidence in a wrong estimate. On the other hand, the parameter values of Eq. (11) are in good agreement with those of Table 2 for  $S_{NH}(0) = 10 \text{ mg L}^{-1}$  and are consistent with the literature values (Orhon and Artan, 1994; Petersen, 2000). They confirm, however, the large inherent uncertainty, about 36%, in the estimation of  $Y_{A2}$ , in line with the results found by Chandran and Smets (2001) by an indirect method based on electron balance. Respirometric experiments based on titrimetric and off-gas analysis (Gapes et al., 2003) report a similar range (0.02–0.07). As expected, both models satisfy the curvature criterion Eq. (9), confirming that this test is capable of detecting only error in the optimisation and should not be used to discriminate among model structures. From these experiments it appears that the best way to estimate  $Y_{A2}$  is to compute the sum  $Y_A =$

$Y_{A1} + Y_{A2}$  with the area method of Section 2.4 and then perform a separate nitrite respirogram.

### 3. Conclusion

This paper has advanced the results of a previous study to obtain an estimation reliability test, which was applied to a respirometric model to assess its estimates and design efficient experiments. A test (Eq. (9)) is presented, based on the computation of the approximate confidence regions, from which a discriminating threshold is derived: if the ratio of the curvature radii is below a given value, then the estimation can be considered reliable in the sense that the estimation procedure ended in a well-behaved region of the error functional, corresponding to acceptable parameters. This test is based on the effect of curvature on parameter estimation and detects possible obstacles preventing the successful termination of the search, but is not influenced by model inadequacy and residual characteristics, therefore it should not be directly used to discriminate among model structures. In the paper this test has been used to assess the role played by the initial amount of ammonium-N  $S_{NH}(0)$  in identifying a two-step respirometric model and the effect of decimating the data, which may produce more accurate estimates. Increasing the amount of  $S_{NH}(0)$  produces a general decrease in the uncertainty of the estimated parameters. However, it is necessary to take into account other factors which limit this quantity: (1) the model does not consider biomass growth (as in Nowak et al., 1995; Brun et al., 2002) and therefore the experiment must have a rather short duration; (2) the nitrification process is very sensitive to several factors: not only pH and temperature, but also a high ammonium-N concentration as an inhibiting agent. This conflicts with the requirement of a high  $S_{NH}(0)$  for good identifiability, though a high  $S_{NH}(0)$  produces more data, which increase the estimation accuracy. Therefore one of the aims of the paper has been that of determining the best compromise for producing a well-identifiable two-step respirogram without sacrificing either the accuracy of the first step with an insufficient  $S_{NH}(0)$  or inhibiting the process with a too large  $S_{NH}(0)$ . It was also shown that a nitrite experiment could be used to estimate the second step alone, adding an exponential term to take into account the gradual nitrite uptake by the biomass. The

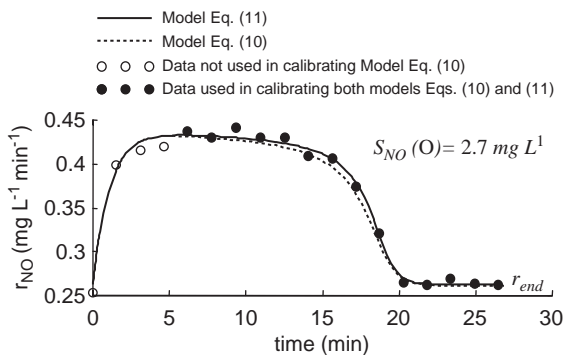


Fig. 5. Calibration of the second step with nitrite data. The initial transient data (hollow circles) were not used in the calibration of model Eq. (10).

Table 4  
Estimated parameters of the nitrite respiration models

	$Y_{A2}$	$R_{NO}$	$K_{NO}$	$r_{end}$	$\tau$	$r_{max}/r_{min}$	$\bar{\rho}$
Model Eq. (10)	$0.3615 \pm 0.0155$	$0.236 \pm 0.007$	$0.145 \pm 0.0138$	0.263	—	1.1330	1.9891
Model Eq. (11)	$0.0637 \pm 0.0233$	$0.1665 \pm 0.066$	$0.0921 \pm 0.0428$	0.263	$1.0694 \pm 0.3425$	1.2358	1.9430

estimation of the yield factors, given their strong correlation, is best accomplished by computing the global yield  $Y_A = Y_{A1} + Y_{A2}$  obtained from the oxygen consumption and then perform a separate nitrite respirogram for  $Y_{A2}$  using the modified Eq. (11) which includes an exponential term for taking into account the initial start-up data.

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