

## Development of a open-vessel single-stage respirometer

Marsili-Libelli, S., D'Ardes, V., Bondi, C.

University of Florence, Dept. of Systems and Computers, Via S. Marta 3, I-50139  
Florence, Italy  
(Email: [marsili@dsi.unifi.it](mailto:marsili@dsi.unifi.it))

**ABSTRACT** This paper describes the development and accuracy analysis of a single-stage respirometer which can be used both in the laboratory for wastewater characterization and in the plant as a process instrument. It is based on an accurate model of parasitic aeration, making the two-stage assumption unnecessary. Its operation is supervised by a real-time software, written in LabView, managing the various measurement procedures and estimating the wastewater characteristics. Its accuracy is assessed through sensitivity and error propagation analysis, proving superior to the conventional model. A laboratory implementation of the instrument was tested with readily degradable substrate, yielding consistent and accurate respirograms.

**Keywords** Respirometry, On-Line Process Control, Sensors, Parameter estimation, Sensitivity analysis.

### INTRODUCTION

Respirometry is now a major tool for assessing the viability of a microbial community (Spanjers and Vanrolleghem, 1995; Brouwer et al., 1998). and provide wastewater treatment plants with accurate on-line monitoring and control systems (Spanjers et al., 1998). One of the main factors affecting the accuracy of conventional two-stage respirometers is the stray air intrusion in the respiration chamber (Marsili-Libelli and Tabani, 2002). This paper presents the development of an open-vessel single-stage respirometer where the parasitic aeration is accounted for and estimated along with the other conventional parameters, producing a robust instrument which can be used for field and control studies. The identifiability of the instrument model is assessed using the approach outlined in Marsili-Libelli and Tabani (2002); Marsili-Libelli et al. (2003); Checchi and Marsili-Libelli (2005).

### STRUCTURE OF THE RESPIROMETER

In the present version, the instrument is composed of a respiration vessel with aerator and stirrer. The DO probe is inclined to minimize the effect of the ascending air bubbles. The DO meter (OXI 90, WTW, Weilheim, Germany) outputs an analogue signal which is 16-bit digitized by an ADAM 4018 (Advantech, Cincinnati, OH, USA) analogue-digital converter which communicates with the local PC via a RS-485 serial line through a protocol converter RS\_485/RS-232. The instrument is operated by a real-time control software developed in the LabView 6.1™ platform (National Instruments, Austin, TX, USA), which provides system monitoring, air switching and on-line parameter estimation.

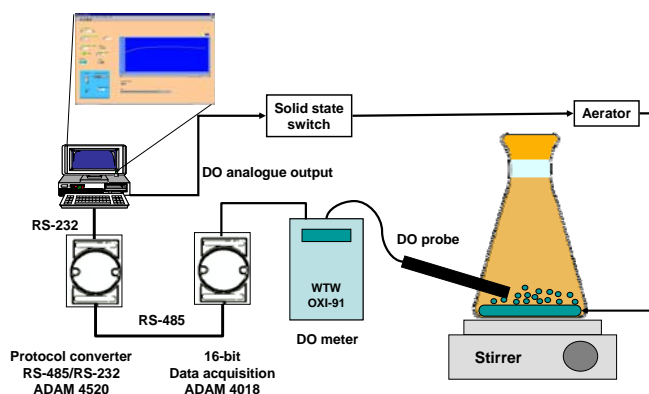


Figure 1 - Structure of the open-vessel, single-stage respirometer described in this paper. The instrument is supervised by a real-time control software developed in LabView™ 6.1.

The instrument can be operated either in the switching mode, turning the aeration on and off, or in the RODTOX mode (Vanrolleghem et al., 1990) with a single sweep, obtaining in either case the responses of Figure 2.

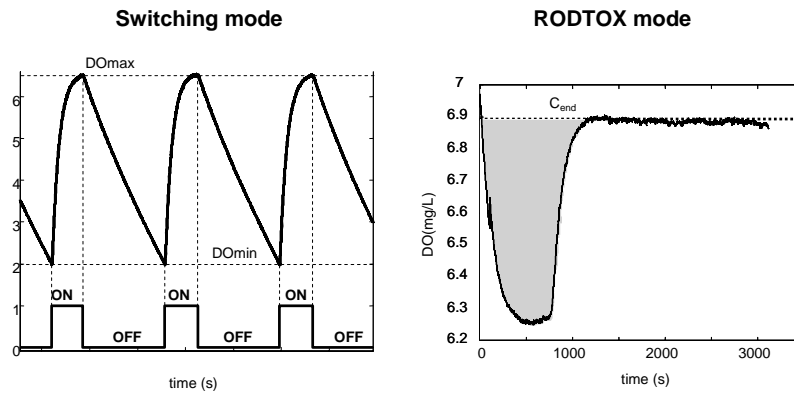


Figure 2 - The two possible operating modes of the instrument: multi-pass switching mode with intermittent aeration or single- pass RODTOX mode.

### RESPIROMETER MODEL

As mentioned in the introduction, the uncontrolled air diffusion from the head space of the respiration vessel into the solution is a major source of error (Marsili-Libelli and Tabani, 2002). The result is the loss of linearity in the dissolved oxygen (DO) decay. To account for this additional effect, the respiration model is rewritten as

$$\frac{dC}{dt} = K(C_{sat} - C) - r \quad \text{with} \quad K = \begin{cases} K_L a + K_L a_p & \text{ON} \\ K_L a_p & \text{OFF} \end{cases} \quad (1)$$

where  $C$  is the dissolved oxygen concentration (mg/L),  $r$  is the oxygen uptake rate (mg/L.s),  $K_L a$  (1/s) is the oxygen transfer through the aerator and  $K_L a_p$  (1/s) represent the parasitic oxygen transfer coefficient due to the uncontrolled aeration. During the OFF phase, oxygen transfer is not entirely discontinued due to the stray aeration. Equation (1) can be solved analytically from the initial condition  $C_o$  to obtain

$$C(t) = \underbrace{C_o(0)}_{\text{effect of initial conditions } C_o} e^{-K.t} + \underbrace{\left(C_{sat} - \frac{r}{K}\right)}_{\text{forced response}} \left(1 - e^{-K.t}\right) \quad (2)$$

From Eq. (2) it appears that during the respiration cycle the Do behaves as an exponential and the departure from linearity is directly proportional to the stray oxygen transfer rate  $K_L a_p$ , as shown in Figure 3.

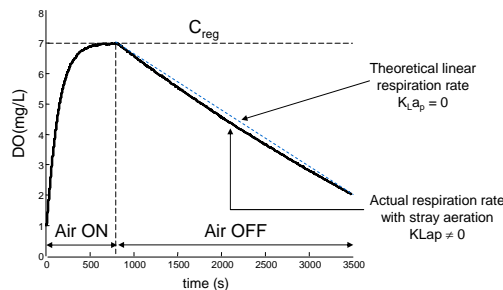


Figure 3 - Loss of linearity in the descending part of the respiration curve due to unwanted air diffusion in the respirometer vessel. The dashed line represents the theoretical DO decay due to respiration without stray aeration.

If the respiration rate  $r$  is constant, the DO concentration tends to the constant value

$$C_{reg} = \left( C_{sat} - \frac{r}{K} \right), \quad (3)$$

which holds strictly for aeration periods, because if  $K \rightarrow 0$  then  $C_{reg} \rightarrow -\infty$ .

### Structural identifiability of the respirometric model

Applying the Pohjanpalo test (Vanrolleghem et al., 1995) to the complete model (2)

$$\begin{cases} \frac{dC}{dt}(0) = -K \cdot \left( C_0 - C_{sat} + \frac{r}{K} \right) = v_1 \\ \frac{d^2C}{dt^2}(0) = K^2 \cdot \left( C_0 - C_{sat} + \frac{r}{K} \right) = v_2 \\ \frac{d^3C}{dt^3}(0) = -K^3 \cdot \left( C_0 - C_{sat} + \frac{r}{K} \right) = v_3 \end{cases} \Rightarrow \begin{cases} K = -\frac{v_2}{v_1} = \sqrt{\frac{v_3}{v_1}} \\ C_{sat} = C_0 - \frac{v_1}{v_2} \cdot (r + v_1) \end{cases} \Rightarrow C_{sat} = C_0 - K(r + v_1) \quad (4)$$

Results in a linear relationship among parameters, hence the model (2) is not structurally identifiable. On the other hand, if the estimation is split in two, identifying  $C_{reg}$  and  $K = K_{La} + K_{LaP}$  during the ON phase and only the respiration rate  $r$  and the parasitic aeration rate  $K_{LaP}$  during the OFF phase, then each of these two models is structurally identifiable. In fact, reparametrization of the ON model yields the following Pohjanpalo equations

$$\begin{cases} \frac{dC}{dt}(0) = K \cdot (C_{reg} - C_0) = v_1 \\ \frac{d^2C}{dt^2}(0) = -K^2 \cdot (C_{reg} - C_0) = v_2 \end{cases} \Rightarrow \begin{cases} K = -\frac{v_2}{v_1} \\ C_{reg} = C_0 - \frac{v_1^2}{v_2} \end{cases}, \quad (5)$$

which are linearly independent. A similar test proves the identifiability of the OFF model.

### Practical identifiability

Practical identifiability of the respirometric model (3.10) can be assessed via the sensitivity functions and the Fisher Information Matrix (FIM) (Dochain e Vanrolleghem, 2001) in relation to the estimation error functional  $J(P)$

$$J(P) = \sum_i [C_i(P) - C_i^{exp}]^T \cdot Q_i \cdot [C_i(P) - C_i^{exp}], \quad (6)$$

where  $C$  and  $C^{exp}$  are time-indexed model and experimental DO values. The FIM

$$F = \frac{1}{\sigma^2} \left[ \sum_i \left( \frac{\partial C_i}{\partial P} \right)^T \times \left( \frac{\partial C_i}{\partial P} \right) \right] \quad (7)$$

is a combination of the output sensitivity functions  $\frac{\partial C_i}{\partial P}$  and the measurement noise variance can be

estimated from the data as  $\sigma^2 = \frac{1}{N - n_p} \cdot J(\hat{P})$ . Considering the basic parametrization  $P = [C_{sat}, r, K]^T$ , with

$K = K_{La} + K_{LaP}$ , the FIM was computed using a set of about 500 experimental data (a typical respirogram length) the measurement uncertainty was estimated as  $\sigma^2 = 6.93 \times 10^{-4}$ . Analysis of the eigenvalues revealed a

considerable ill-conditioning, denoting poor identifiability. Further, Reich e Zinke (1974) proposed another method to assess the lack of identifiability, based on the redundancy matrix  $\mathbf{R}$  defined as

$$\mathbf{R} = \mathbf{D}^{-1} \cdot \mathbf{F} \cdot \mathbf{D}^{-1} \quad (8)$$

where  $\mathbf{D} = \text{diag}(\sqrt{F_{1,1}}, \sqrt{F_{2,2}}, \sqrt{F_{3,3}})$  is the similarity matrix between the FIM and  $\mathbf{R}$ . The criterion requires the computation of  $\det(\mathbf{R}^{-1})$  and considers the model scarcely identifiable if this quantity is greater than  $10^3 \div 10^4$ . This analysis confirmed the lack of identifiability of the original model. Conversely, the reduced parametrization of the ON model  $\mathbf{P}_{\text{ON}} = [C_{\text{reg}}, K]^T$  yields a better conditioned FIM. In particular, the ratio  $\lambda_{\text{max}}/\lambda_{\text{min}}$  is eleven orders of magnitude smaller than the previous one. It should be reminded that the extreme eigenvalue ratio is equal to the *mod* ( $\mathbf{E}$ ), one of the most used optimal experiment design criteria used in Checchi and Marsili-Libelli (2005). The redundancy matrix test yields a result well below the threshold set by Reich e Zinke (1974). The comparison of the identifiability parameters for the two parametrizations are summarized in Table 1. It can be concluded that the reparametrization had a beneficial effect on identifiability.

Table 1 - Summary of the identifiability results for the two models.

Identifiability indicator	Basic model	Reparametrized model
$\det(\mathbf{F})$	$5.82 \times 10^5$	$6.99 \times 10^9$
$\text{tr}(\mathbf{F})$	$2.41 \times 10^7$	$1.17 \times 10^7$
$\lambda_{\text{max}}/\lambda_{\text{min}}(\text{mod } \mathbf{E})$	$1.79 \times 10^{15}$	$1.98 \times 10^4$
$\det(\mathbf{R}^{-1})$	$2.33 \times 10^{11}$	1.84

Further, visual inspection of the sensitivity trajectory of the reparametrized model, in Figure 4, show that a long ON period is beneficial for estimating  $C_{\text{reg}}$  but not for  $K$ , which has a sensitivity peak shortly after the start of the aeration. Likewise, in the OFF phase the estimation accuracy of  $r$  and  $K_L a_p$  increases almost linearly with the phase length.

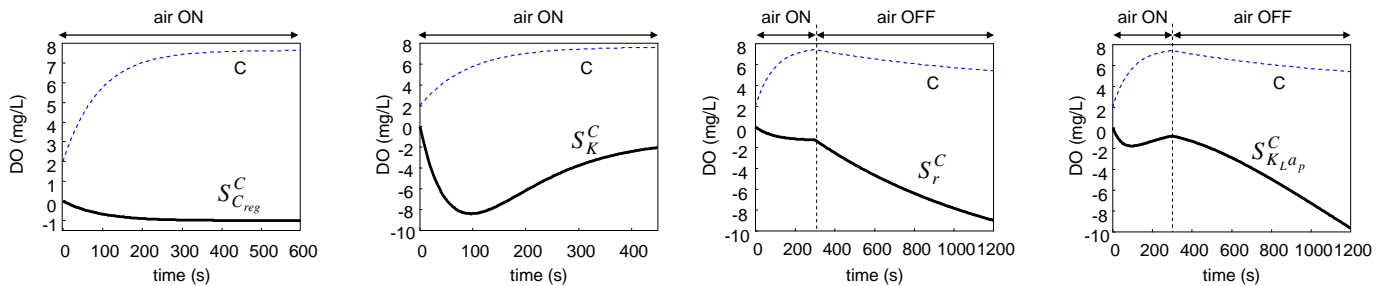


Figure 4 - Sensitivity trajectories of the reparametrized model obtained with optimal perturbation factors. The left figures refer to the air ON model, whereas the two right ones to the air OFF model.

### Computation of parameter uncertainty

The FIM can be used to compute the parameter covariance matrix  $\mathbf{C}$  as  $\mathbf{F}^{-1}$  (see e.g. Dochain and Vanrolleghem, 2001). For the two models, with the original and reduced parametrization  $\mathbf{C}$  is computed as follows

$$\mathbf{C} = \begin{pmatrix} 5.62 \cdot 10^4 & 6.83 \cdot 10^2 & -4.44 \cdot 10^{-10} \\ 6.83 \cdot 10^2 & 8.29 & 1.23 \cdot 10^{-10} \\ -4.44 \cdot 10^{-10} & 1.23 \cdot 10^{-10} & 1.08 \cdot 10^{-10} \end{pmatrix} \quad \mathbf{C}_I = \begin{pmatrix} 1.16 \cdot 10^{-6} & -7.61 \cdot 10^{-9} \\ -7.61 \cdot 10^{-9} & 1.09 \cdot 10^{-10} \end{pmatrix} \quad (9)$$

showing that the reduced model is more accurate. Further, the uncertainty for  $C_{\text{reg}}$  in the reduced model is ten orders of magnitude lower than in the original one and lower than the observed DO variance  $\sigma^2 = 6.93 \times 10^{-4}$ . Hence estimating  $C_{\text{reg}}$  with the model is more accurate than measuring it directly through DO

measurements. Also, measuring  $C_{reg}$  implies keeping the DO level high, which can be detrimental both for the probe and the biomass in the respirometer. As a last step, the correlation matrix of the two parametrizations are computed

$$\Gamma = \begin{pmatrix} 1 & 0.99 & -1.79 \cdot 10^{-7} \\ 0.99 & 1 & 4.08 \cdot 10^{-6} \\ -1.79 \cdot 10^{-7} & 4.08 \cdot 10^{-6} & 1 \end{pmatrix} \quad \Gamma_1 = \begin{pmatrix} 1 & -0.67 \\ -0.67 & 1 \end{pmatrix} \quad (10)$$

showing much less correlation in the reduced model. In fact, while in the traditional model,  $C_{sat}$  and  $r$  are highly correlated, whereas  $K$  is almost uncorrelated, in the reduced one the residual ( $C_{reg}$ ,  $K$ ) correlation is due to the fact that the steady-state oxygen concentration depends on  $K$ .

This analysis demonstrated the advantage of using the partitioned model with the reduced parametrization during the aerated phase. The next step is to evaluate the accuracy with which the original parameters [ $C_{sat}$ ,  $r$ ,  $K_{La}$ ,  $K_{La_p}$ ] can be computed back.

### Error propagation and estimation accuracy of the original model parameters

In the previous section it was concluded that the reparametrized model in which  $C_{reg}$  and  $K$  are estimated during the air ON phase, whereas  $r$  and  $K_{La_p}$  in the air OFF phase is better identifiable than the conventional model in which all the parameters are jointly estimated.

Now the next step is evaluate the estimation error of the original (secondary) parameters from that of the (primary) parameters in the two sub-models, provided that these can be obtained from the primary ones with a negligible error.

A set of 700 simulated respirograms was generated perturbing the model parameters around their mean value with a gaussian noise of variance comparable to that of experimental estimations. This set of noisy data was used to calibrate the primary parameters  $P_{ON} = [C_{reg} \ K]$  and  $P_{OFF} = [r \ K_{La_p}]$ .

Assuming that the primary parameters can be expressed as a mean value and a random estimation error, i.e.

$$\begin{cases} C_{reg} = \bar{C}_{reg} + e_{C_{reg}} \\ K = \bar{K} + e_K \\ r = \bar{r} + e_r \\ K_{La_p} = \overline{K_{La_p}} + e_{K_{La_p}} \end{cases} \quad (11)$$

The propagation of these errors to the secondary parameters

$$K_{La} = K - K_{La_p} \text{ and } C_{sat} = C_{reg} + \frac{r}{K} \quad (12)$$

can be computed as

$$K_{La} = \bar{K} + e_K - \overline{K_{La_p}} - e_{K_{La_p}} = (\bar{K} - \overline{K_{La_p}}) + (e_K - e_{K_{La_p}}) \quad (13)$$

$$C_{sat} = \overline{C_{reg}} + e_{C_{reg}} + \frac{\bar{r} + e_r}{\bar{K} + e_K} \quad (14)$$

From the random simulations the following values were obtained

$$\begin{cases} \bar{\mu}_{K_{La}} = \bar{\mu}_K - \bar{\mu}_{K_{La}p} \cong 10^{-8} - 10^{-6} \cong -10^{-6} \\ \sigma_{K_{La}}^2 = \sigma_K^2 + \sigma_{K_{La}p}^2 \cong 10^{-13} + 10^{-10} \cong 10^{-10} \end{cases} \quad (15)$$

where the most important aspect is that the estimation of  $K_{La}$  is affected by the error on  $K_{La}p$ , whereas the estimation error of  $K$  has a negligible influence, being two orders of magnitude smaller.

In the same way, the estimation error of  $C_{sat}$  can be evaluated considering  $e_K \ll \bar{K}$  to obtain

$$C_{sat} = \overline{C_{reg}} + e_{C_{reg}} + \frac{\bar{r}}{\bar{K}} + \frac{e_r}{\bar{K}} = \left( \overline{C_{reg}} + \frac{\bar{r}}{\bar{K}} \right) + \left( e_{C_{reg}} + \frac{e_r}{\bar{K}} \right) = \overline{C_{sat}} + e_{C_{sat}} \quad (16)$$

with mean and variance given by (using again the values obtained from the simulations)

$$\bar{\mu}_{C_{sat}} = \bar{\mu}_{C_{reg}} + \frac{1}{\bar{K}} \cdot \bar{\mu}_{OUR} \cong 10^{-5} + \frac{1}{10^{-2}} \cdot 10^{-5} \cong 10^{-3} \quad (17)$$

$$\sigma_{C_{sat}}^2 = \sigma_{C_{reg}}^2 + \frac{1}{\bar{K}^2} \cdot \sigma_{OUR}^2 \cong 10^{-8} + \frac{1}{10^{-4}} \cdot 10^{-8} \cong 10^{-4} \quad (18)$$

which confirm the importance of the magnitude of the mass transfer in adversely affecting the estimation accuracy of  $C_{sat}$ .

Table 2 compares the estimation accuracy for the basic and reparametrized models, from which it can be seen that the latter model can be estimated with lower relative errors.

Table 2 - Comparison of relative estimation accuracy of the two models.

Parameter	Basic model	Reparametrized model
$C_{sat} \approx 7.5$	$e_r \approx 10^{-1}$	$e_r \approx 10^{-3}$
$r \approx 10^{-3}$	$e_r \approx 10^{-1}$	$e_r \approx 10^{-2}$
$K_{La} \approx 10^{-2}$	$e_r \approx 10^{-1}$	$e_r \approx 10^{-3}$
$K_{La}p \approx 10^{-4}$	$e_r \approx 10^{-2}$	$e_r \approx 10^{-2}$

## SOFTWARE IMPLEMENTATION AND PERFORMANCE EVALUATION

The software controlling the respirometer was developed in the LabView 6.1 platform. In the first implementation, the basic respiration model was used and the estimation results are shown in Figure 5. It can be seen that the noise affecting the estimates is still considerable, as a consequence of directly using the basic parametrization. A second implementation was then developed, using the reparametrized model and splitting the estimation in two steps. Depending on the current operating phase (air ON or OFF) the data are routed to the pertinent section, where a nonlinear Levenberg-Marquardt least-squares estimation is performed. Then the original parameters are computed back and the results are written on the hard disk as an ASCII file. The front panel and diagram of the LabView Virtual Instrument supervising the respirometer is shown in Figure 6. A first application of the improved instrument was to re-estimate the endogenous respiration parameters, as shown in Figure 7, where a lower noise level resulted, as expected from the previous accuracy analysis. The discrepancy between the  $K_{La}$  values depend from a modified aeration system, since the original one proved too powerful and created problems with the biomass, stimulating an excessive development of filamentous bacteria. Then several respirograms were produced, both on a readily degradable substrates, either carbonaceous (sodium acetate) or mixed nitrogen/carbon (ammonium acetate). Figure 8 shows three of these respirograms, where the stability of the endogenous level can be appreciated.

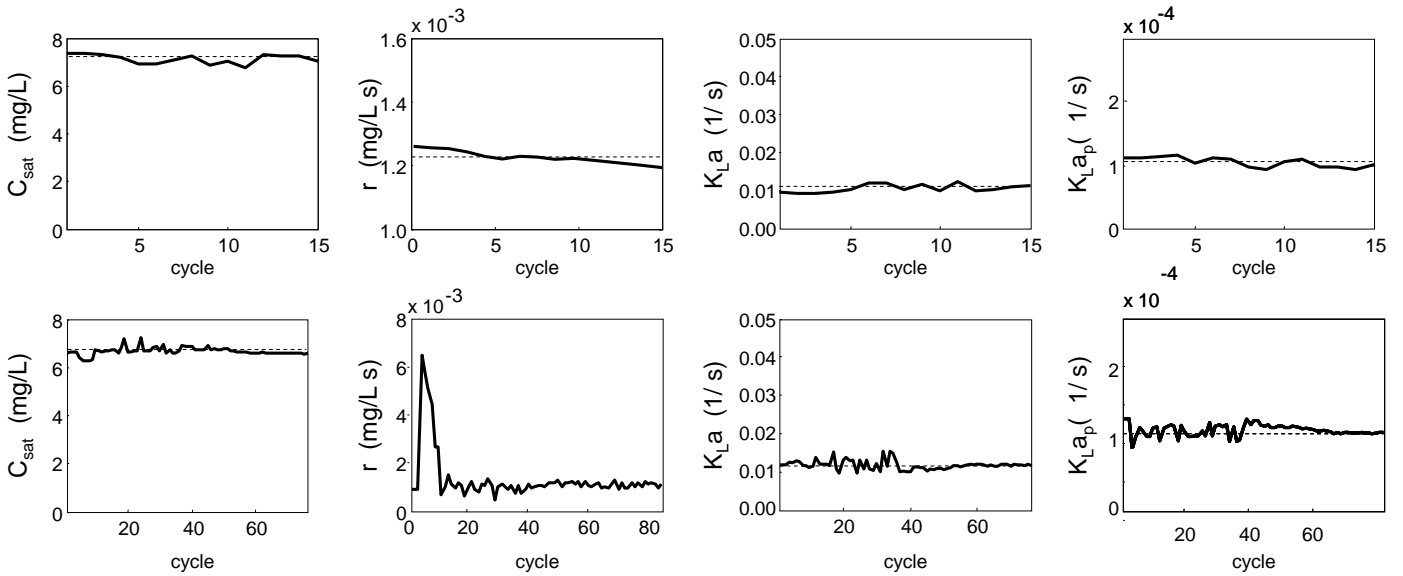


Figure 5 - Parameter estimation with the basic model in endogenous (above) and synthesis (below) conditions. The noise level of the estimates is still considerable, especially that on  $K_{La_p}$ . The exogenous respirogram was obtained with a small injection of sodium acetate.

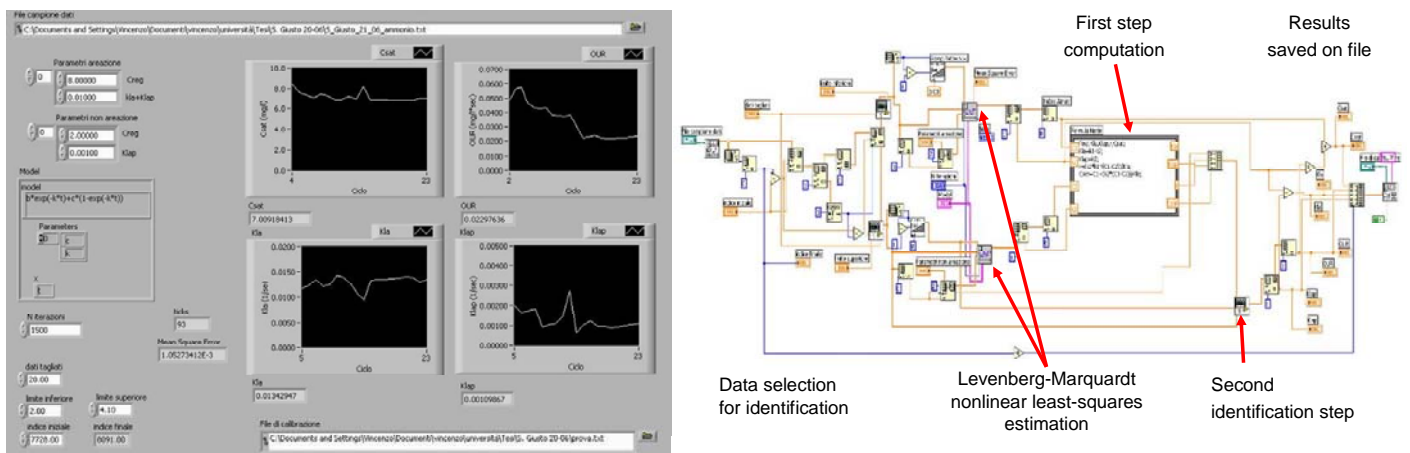


Figure 6 - LabView implementation of the respirometer. The graphical user interface (front panel), where the user can input the estimation and experimental conditions, is shown on the left, whereas the operational diagram performing the computation is shown on the right.

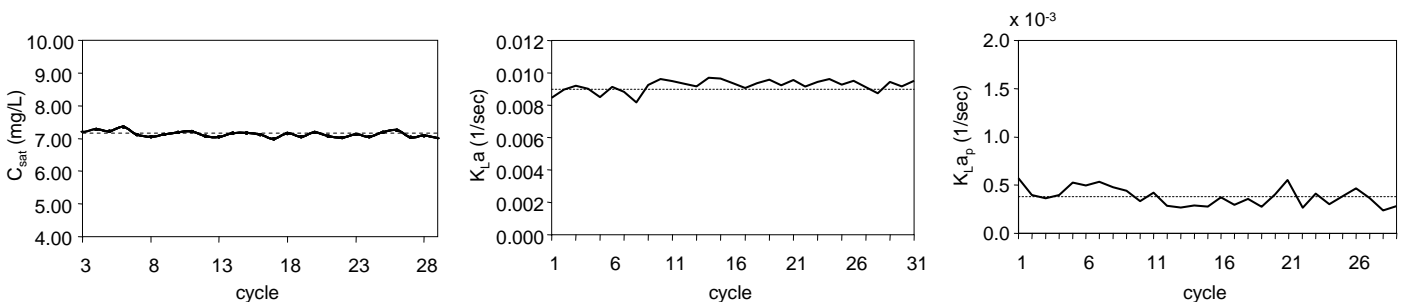


Figure 7 - Endogenous respiration estimation using the reparametrized model and the implementation of Figure 6. Notice the lower noise level affecting the estimates. The lower  $K_{La}$  value compared with that of Figure 5 is a consequence of a different air diffusion system.

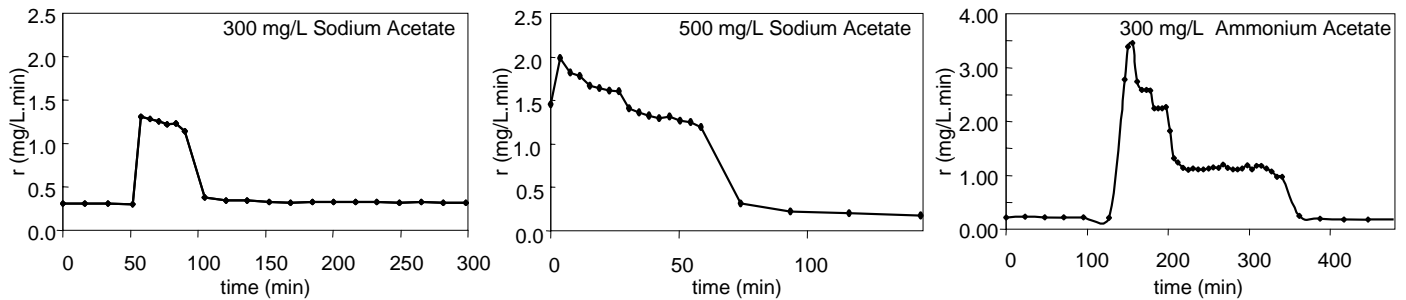


Figure 8 - Respirograms obtained with the reparametrized model on differing substrate. Notice the lower noise in the endogenous level and the nitrification double-step following the injection of sodium acetate (right).

## CONCLUSIONS

A single-stage low-cost respirometer was developed for laboratory and process control applications. It is composed of a single open vessel equipped with magnetic stirrer, air diffuser and oxygen probe. The core of the instrument is represented by a LabView-based Virtual Instrument supervising the instrument operation (ON/OFF air switching) and on-line parameter estimation. The main idea is to account for stray air infiltration and estimate this uncontrolled input along with normal operating parameters. To improve the estimation accuracy, a reparametrized model was proposed and its accuracy proved to be higher than the conventional one. Later, the implementation aspects have been discussed, showing the instrument laboratory use with test injections. The next step will be an outdoor implementation with remote data acquisition, for process control use.

## REFERENCES

- Brouwer, H., Klapwijk, A. and Keesman, K.J. (1998). Identification of activated sludge characteristics using respirometric batch-experiments. *Wat. Res.*, **32** (4): 1240 - 1254.
- Dochain, D. and Vanrolleghem, P.A. (2001). *Dynamical modelling and estimation in wastewater treatment processes*. IWA Publishing, London.
- Checchi, N. and Marsili-Libelli, S. (2005). Reliability of parameter estimation in respirometric models. *Wat. Res.* **39**: 3686 - 3696.
- Marsili-Libelli, S. and Tabani, F. (2002). Accuracy analysis of a respirometer for activated sludge dynamic modelling. *Wat. Res.* **36**: 1181 - 1192.
- Marsili-Libelli, S., Guerrizio, S. and Checchi, N. (2003). Confidence regions of estimated parameters for ecological systems. *Ecol. Model.* **165**: 127 - 146.
- Reich, J.G. and Zinke, I. (1974). Analysis of kinetic and binding measurements. *Studia Biophysica*, **43** (2): 91-107.
- Spanjers H. and Vanrolleghem P. A. (1995). Respirometry as a tool for rapid characterisation of wastewater and activated sludge. *Wat. Sci. Tech.* **31** (2): 105 - 114.
- Spanjers H., Vanrolleghem P.A., Olsson G. and Dold P.L. (1998). Respirometry in control of the activated sludge process: principles. *IAWQ Scientific and Technical Report n. 7*.
- Vanrolleghem P.A., Dries D. and Verstraete W. (1990). RODTOX: Biosensor for rapid determination of the biochemical oxygen demand and the on-line monitoring of the toxicity of wastewaters. In: *Proceedings Fifth European Congress on Biotechnology*. Copenhagen, Denmark, July 8-13 1990. Vol. 1, 161-164.
- Vanrolleghem, P.A., Van Daele, M. and Dochain, D. (1995). Practical identifiability of a biokinetic model of activated sludge respiration. *Wat. Res.*, **29**: 2561-2570.