

## Limitations of ASM1 and ASM3: a comparison based on batch oxygen uptake rate profiles from different full-scale wastewater treatment plants

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**Abstract** The two most popular models for the description of the biological COD removal are ASM1 and ASM3. However, some numerical inconsistencies arise when using these models to interpret the data obtained in short-term respirometric batch experiments. In this study, both models are fitted to four different respirometric batch profiles obtained with biomass from different WWTP. The parameter estimation results and the practical (local) identifiability are analysed, and the limitations of both models are discussed. The growth yield obtained by fitting ASM1 to the short-term respirometric batch profiles is higher than the default one, as well as the storage yield obtained by fitting ASM3 is lower than the default one. Based on these values, possible improvements to the modelling of the biological COD removal, such as the inclusion of simultaneous growth and storage on external substrate, are proposed.

**Keywords** Activated sludge models; biological COD removal; practical identifiability; respirometry; storage

### Introduction

In 1987, the International Water Association (IWA) introduced the Activated Sludge Model no. 1 (ASM1) for the description of the biological COD and nitrogen removal (Henze *et al.*, 2000). In this model, biomass was considered to grow solely on the external substrate present and the oxygen consumption after the external substrate depletion was explained with the decay of biomass. In the conventional activated sludge processes, the feed regime is highly variable and biomass is subjected to alternating conditions of external substrate availability (feast phase) and absence of external substrate (famine phase). Under these dynamic conditions, internal storage polymers play an important role in the substrate consumption (van Loosdrecht *et al.*, 1997). Recently, a new model for the COD removal (ASM3) has been developed mainly to take this storage phenomenon into account (Henze *et al.*, 2000). The main innovation of this model is the assumption that all the readily biodegradable organic substrates taken up under feast conditions are directly converted into stored material. These stored compounds become the carbon and energy source for growth purposes in the subsequent famine period. In ASM3, the decay processes are replaced with the endogenous processes. The conceptual basis of ASM3 has been largely criticized and alternative models taking into account simultaneous storage and growth processes were proposed (e.g. van Aalst-van Leeuwen *et al.*, 1997, Krishna and Van Loosdrecht, 1999; Beccari *et al.*, 2002, van Loosdrecht and Heijnen 2002, Karahan Gül *et al.*, 2003).

In this study, parameter estimation and identifiability issues of ASM3 in view of model calibration are addressed and compared with the well-studied ASM1 model. To this aim, oxygen uptake rate (OUR) measurements of biomass sampled from 3 different full-scale wastewater treatment plants (WWTPs) were used. The parameter estimation results of both models are interpreted and discussed in view of their possible (mechanistic) biological meaning. Further, the practical (local) identifiability of both models is

compared in view of unique parameter estimations. Based on the mechanistic meaning and the identifiability of the parameter estimates, possible improvements to modelling substrate conversion processes are discussed.

## Materials and methods

### Experimental set-up

The experimental work was performed in two different set-ups. On the one hand, experiments A and B were performed in an LFS type respirometer, which was developed in a previous work (Guisasola *et al.* 2003). On the other hand, experiments C and D were performed using the hybrid-respirometric set-up described in a previous study (Sin *et al.* 2003). The pH was controlled during these experiments to  $7.80 \pm 0.03$ .

In both set-ups, the biomass was first aerated overnight to reach the endogenous-state. Then, a first pulse of acetate was added to induce a “wake-up” effect on the biomass activity (Vanrolleghem *et al.* 1998). At the same time, ammonia in excess and ATU (30 mg/l) was added to avoid growth-limitation and nitrification, respectively. Activated sludge sampled from three different WWTP was used during experimental work: experiment D used biomass from the Maria Middlelares WWTP (Gent, Belgium), which performs COD removal and nitrification. Experiment C used biomass from Ossemeersen WWTP (Gent, Belgium), which performs COD removal, nitrification and denitrification the same way as Granollers WWTP (Catalonia, Spain) whose biomass was used for experiments A and B. These biomass samples were analysed for TSS and VSS according standard methods (APHA, 1995).

### Parameter estimation and confidence intervals

Modelling, simulation and parameter estimation were performed using MATLAB 6.5 (The MathWorks, Natick, MA). The differential equations were solved using an explicit Runge-Kutta (4,5) formula. Parameter estimation was carried out by using the Nelder-Mead Simplex search method, where the weighed sum  $J$  (equation 1) of squared errors between model outputs  $y(t_k, \theta)$  and the measured outputs  $y_M(k)$ , with  $Q_k$  as weighting matrix (equal to the inverse of the measurement error covariance matrix), is minimised:

$$J = \sum_{k=1}^N [y(t_k, \theta) - y_M(k)]^T Q_k [y(t_k, \theta) - y_M(k)] \quad (1)$$

where  $N$  is the number of measurements. Each of the output signals can be linearised in the neighbourhood of the optimal vector of parameters  $\theta_O$  (Dochain and Vanrolleghem, 2001):

$$y(t, \theta_O + \delta\theta) = y(t, \theta_O) + \left[ \frac{\delta y(t, \theta_O)}{\delta \theta^T} \right]_{\theta_O} \cdot \delta\theta = y(t, \theta_O) + Y_{\theta}^T(t) \delta\theta \quad (2)$$

where  $Y_{\theta}(t)$  is the so called output sensitivity function. If  $Q_k$  is the covariance matrix of the measurement noise, the Fisher Information Matrix (FIM) is defined as:

$$FIM = \sum_{k=1}^N Y_{\theta}^T(t_k) Q_k Y_{\theta}(t_k) \quad (3)$$

The FIM matrix summarises the quantity and quality of information obtained in each experiment because it considers the output sensitivity functions and the measurement errors of an experimental data (i.e. accuracy of an experiment). Assuming white measurement noise and no model mismatch, the inverse of the FIM provides the lower bound of

the parameter estimation error covariance matrix, which can be used for assessing the estimation uncertainty of  $\theta_O$  (equation 4).

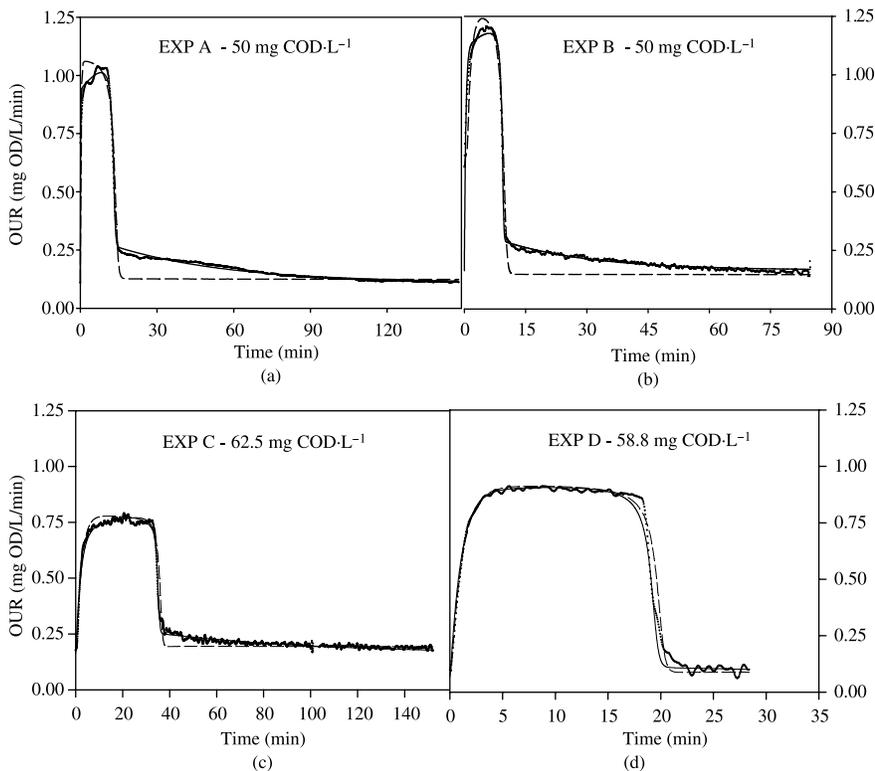
$$COV(\theta_O) \geq FIM^{-1} \quad (4)$$

Moreover, since output sensitivities of parameters with respect to measurement(s) are calculated using a model, the FIM also depends on the structure of the model. This property of FIM can be used to study the practical identifiability (local) of the model under the available experimental data (Dochain and Vanrolleghem, 2001).

## Results and discussion

In this study, four different OUR profiles (Figure 1a–d) obtained from three different full-scale WWTPs were used to investigate the model-fit performance and identifiability for ASM1 and ASM3. These OUR profiles show different behaviours despite a pulse of the same substrate (acetate) was added (Figure 1), because the biomass used in each experiment came from a different WWTP. The differences appreciated among the OUR profiles are probably linked to the operational conditions of the plant i.e. alternating feed and famine conditions.

For instance, in experiments A, B and C two different phases can be easily distinguished. The first phase is related to the external substrate consumption, while the second phase corresponds to the consumption of the previously stored internal polymer (van Loosdrecht *et al.*, 1997). In Granollers and Ossemeersen WWTPs, both nitrification and denitrification take place and the biomass is subjected to alternating anoxic and aerobic conditions under the dynamic influent substrate/wastewater pattern. Under these conditions



**Figure 1** (a–d) Experimental data (dotted line) and modelled OUR profiles. The ASM1 and ASM3 fittings are depicted with short-dashed (---) and solid (—) lines respectively

of alternating external substrate availability, bacteria capable of storing substrate have a competitive advantage because they are able to balance their growth rate under continuously changing conditions. Under the periods of excess substrate, non-storage bacteria have to invest extra energy to grow faster in the presence of substrate and will deteriorate in the periods without substrate (van Loosdrecht *et al.* 1997). On the other hand, in experiment D only one phase can be distinguished which is a typical ASM1 type OUR profile. The biomass used in this experiment came from the Maria Middlelares WWTP, which is continuously aerated, and is probably subjected to rather stable influent dynamics. In other words, the feast and famine phases are probably less pronounced in this WWTP.

**Parameter estimation procedure**

The mathematical models used (see Table 1) to interpret the experimental data are simplified versions of ASM1 and ASM3 respectively: aerobic degradation of COD as substrate. The processes included in Table 1 are described in detail in Henze *et al.* (2000). An empirical factor was added in the kinetics of two processes (process numbers 1 and 4) to describe the fast transient period (1–3 minutes) in reaching the maximum OUR observed after the substrate addition. This phenomenon, known as “start-up”, can be mathematically described by a first order model (Guisasola *et al.* 2003; Vanrolleghem *et al.* 2004).

For the parameter estimation, the initial concentration of biomass,  $X_H(0)$  is estimated using the baseline endogenous OUR level prior to substrate addition, while fixing the decay rate coefficient ( $b_H$ ) to its default value assigned in the corresponding model. This approach was adopted since it is not possible to obtain unique values of both  $b_H$  and  $X_H(0)$  using OUR measurements alone. Hence, only one of the two parameters can be estimated and the other one should be fixed. In this study,  $b_H$  was fixed to its default value since it does not vary significantly among different WWTPs. The fittings of the models (ASM1 and ASM3) are given in Figure 1a–d and the results of the parameter estimation are given in Table 2. The parameter estimation errors obtained are quite good (see Table 2). This is because the method used to estimate these estimation errors is known to give too optimistic results due to autocorrelation in the OUR data (Dochain and Vanrolleghem, 2001).

**Evaluation of the quality of the fit of ASM1 and ASM3**

A first glance at Figure 1 shows that ASM1 is not able to describe the tail observed in experiments A and B, where the storage effect is emphasized. Many respirograms can be found in the literature with this tail, and the main criticism that ASM1 may receive is that these tails are not predicted when the feed solely contains readily biodegradable substrate. In contrast, when using typical raw wastewater the effect of storage would be lumped in

**Table 1** The simplified ASM1 and ASM3 models used in this work (M stands for the Monod kinetics of the corresponding parameter: e.g.  $M_O = S_O/S_O + K_O$ )

	$X_H$	$X_{STO}$	$X_S$	$S_S$	$S_O$	Kinetics
<b>ASM1 processes</b>						
Growth on $S_S$	1			$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{Y_H}$	$\mu_H M_S \cdot M_O \cdot X_H \cdot (1 - e^{-t/\tau})$
Biomass decay	-1		(1-fp)			$b_H X_H$
Hydrolysis			-1	1		$k_H M_{X_S/X_H} \cdot M_O \cdot X_H$
<b>ASM3 processes</b>						
$S_S$ Storage		1		$-\frac{1}{Y_{STO}}$	$-\frac{1-Y_{STO}}{Y_{STO}}$	$k_{STO} \cdot M_S \cdot M_O \cdot X_H \cdot (1 - e^{-t/\tau})$
Growth on $X_{STO}$	1	$-\frac{1}{Y_{GSTO}}$			$-\frac{1-Y_{GSTO}}{Y_{GSTO}}$	$\mu_H \cdot M_{X_{STO}/X_H} \cdot M_O \cdot X_H$
Endogenous respiration	-1				-1	$b_H \cdot M_O \cdot X_H$
$X_{STO}$ respiration		-1			-1	$b_{STO} \cdot M_O \cdot X_H$

**Table 2** Parameter estimation results and confidence intervals (COD<sub>X</sub> – COD biomass, COD<sub>S</sub> – COD external substrate, COD<sub>P</sub> – COD PHA)

	EXP A	EXP B	EXP C	EXP D
ASM1 fittings				
$\mu_H$ (d <sup>-1</sup> )	3.876 ± 0.003	4.112 ± 0.009	1.020 ± 0.001	2.951 ± 0.001
$Y_H$ (g COD <sub>X</sub> g <sup>-1</sup> COD <sub>S</sub> )	0.757 ± 0.001	0.792 ± 0.001	0.666 ± 0.001	0.726 ± 0.001
$K_S$ (mg COD·L <sup>-1</sup> )	1.789 ± 0.005	1.63 ± 0.02	0.558 ± 0.008	0.718 ± 0.005
$\tau$ (min)	0.240 ± 0.007	0.95 ± 0.05	2.072 ± 0.006	1.065 ± 0.007
$X_H(0)$ (mg COD <sub>X</sub> L <sup>-1</sup> )	1250	1800	2300	1250
SSE	2.386	2.192	1.673	0.966
ASM3 fittings				
$k_{STO}$ (d <sup>-1</sup> )	4.88 ± 0.009	4.679 ± 0.009	1.056 ± 0.002	3.027 ± 0.007
$Y_{STO}$ (g COD <sub>P</sub> g <sup>-1</sup> COD <sub>S</sub> )	0.796 ± 0.006	0.831 ± 0.006	0.715 ± 0.005	0.75 ± 0.01
$K_S$ (mg COD·L <sup>-1</sup> )	0.80 ± 0.02	0.91 ± 0.02	0.69 ± 0.02	0.79 ± 0.02
$\mu_H$ (d <sup>-1</sup> )	28.1 ± 0.5	64 ± 2	19.8 ± 0.4	51 ± 32
$Y_{GSTO}$ (g COD <sub>X</sub> g <sup>-1</sup> COD <sub>P</sub> )	0.804 ± 0.002	0.921 ± 0.002	0.838 ± 0.002	0.96 ± 0.01
$\tau$ (min)	0.123 ± 0.005	0.34 ± 0.01	2.21 ± 0.03	1.02 ± 0.03
$X_H(0)$ (mg COD <sub>X</sub> L <sup>-1</sup> )	1000	1500	2000	1000
SSE	0.560	0.744	0.999	0.755

the hydrolysis process and, hence, ASM1 could describe correctly the experimental OUR profile. As shown in Table 2, ASM3 better describes all the experimental profiles when comparing the sum of squared errors (SSE). This fact was expected since more parameters are estimated in ASM3 (7 versus 5). The more parameters to be estimated, the more chances to obtain better fittings.

Hence, the clearer the storage effect is, the higher the improvement of using ASM3 instead of ASM1. This improvement is even observed in the experiment D, where no storage can be appreciated. However, once a good fitting is obtained, an analysis on the mechanistic meaning of the parameter estimation results is required. In the following, the analysis of the of the parameter estimation results of both models is developed.

#### Evaluation of the parameter estimation results of the models

In the experiments with apparent storage (A and B) two different shoulders can be easily distinguished. According to ASM1, the direct growth on external substrate is the cause of the first shoulder, whereas the ASM3 model links this first consumption to the storage of substrate into internal polymer. These processes have different default yield values: 0.67 for the growth yield in ASM1 and 0.85 for the storage yield in ASM3, because less energy is required to store external substrate than to produce new cells. When fitting experimental data to ASM1, the growth yields obtained (0.76 and 0.79) are higher than 0.67. This finding indicates the storage presence because less oxygen consumption is observed while the majority of the substrate flux is incorporated into biomass (e.g. as new cells in ASM1 or internal storage products + new cells ASM3).

On the other hand, the storage yields obtained by fitting ASM3 (0.79 and 0.83) are a bit lower than the default one of ASM3 (0.85), probably reflecting that not all the acetate consumed is stored. Yield values for storage with acetate in this range are also experimentally observed in other similar works (van Aalst-van Leeuwen *et al.* 1997 (0.75), Krishna and van Loosdrecht 1999 (0.73), Koch *et al.* 2000 (0.72), Karahan-Gül *et al.* 2003 (0.78)). These observations i.e. higher growth yield in ASM1 and lower storage yields in ASM3, agree with the fact that both growth and storage processes occur simultaneously and part of acetate is used for growth and the rest is stored.

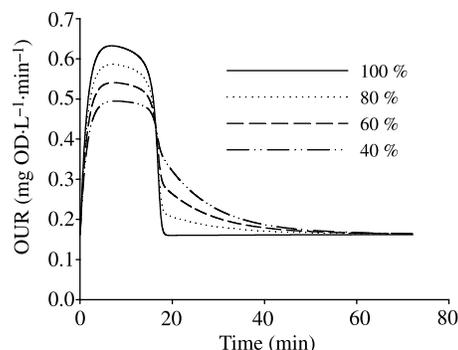
Although the tail is accurately fitted by ASM3, the mechanistic meaning of the parameters related to this tail is highly questionable. First of all, the parameter estimation

error of  $\mu_H$  is the highest of all the parameters (especially in experiment where no storage effect is observed). Moreover, both the maximum growth rate ( $\mu_H$ ) and the growth yield ( $Y_{G_{STO}}$ ) estimated by ASM3 (see Table 2) are noticeably higher than the default ones,  $2\text{ d}^{-1}$  and 0.63 respectively. The reason for these high values could be that the real production of  $X_{STO}$  (e.g. PHA) during the experiment is less than the one predicted by the model. This is not surprising since ASM3 considers that all the acetate is stored. Hence, the experimentally observed tail (see Figure 1a) is much smaller than the one predicted by the ASM3 with its default values. From a model-fit point of view, the  $\mu_H$  must be increased so that the endpoint of the PHA consumption can be correctly predicted. From a parameter identifiability point of view, however,  $Y_{G_{STO}}$  is correlated with  $\mu_H$  (see below). Therefore an increase in  $\mu_H$  is compensated by an increase in the estimate of  $Y_{G_{STO}}$  so that the total oxygen consumed is correctly predicted. High values of the growth yield,  $Y_{G_{STO}}$ , are also observed in the literature when fitting ASM3 to experimental data (Koch *et al.* 2000; Karahan-Gül *et al.* 2003; Beccari *et al.* 2002). This is however contradicting the conceptual basis of ASM3 since the predicted growth yield does not have any longer mechanistic meaning. On the other hand ASM1 is not able to predict the tail often observed in OUR obtained from batch experiments. However, for a profile with low storage effect (as experiment C) an increase in the  $b_H$  value could result in better model-fit.

Experiment D seems to be the only OUR profile which is in agreement with ASM1 because the typical storage tail is not observed. However, the estimated growth yield (0.73) (see Table 2) is still higher than the default value in ASM1 (0.67). This observation strongly suggests the presence of storage phenomenon and as such, it again supports the aforementioned observation of simultaneous storage and growth. Concerning the fit of ASM3 to experiment D, non-reliable/non-mechanistic parameter estimates were obtained (see Table 2), especially the values referring to the growth on storage product (e.g.  $\mu_H$ ) is around  $50\text{ d}^{-1}$  for the same reason explained below, i.e. the actually experimental produced  $X_{STO}$  is lower than what the ASM3 predicts.

#### Simultaneous growth and storage on external substrate

In general, more reliable parameter values would be obtained if the model could describe that part of the acetate was used directly for growth. In this case, the model would predict less PHA production and the predicted tail would be lower and, then, closer to the experimental data. Moreover, a decrease on the values of  $\mu_H$  and  $Y_{G_{STO}}$  would be necessary to describe the tail. The reduction of the tail as a function of a percentage of the acetate used directly for growth is depicted in Figure 2. In this figure, four simulations with a model coming from a combination of ASM1 and ASM3 are performed.

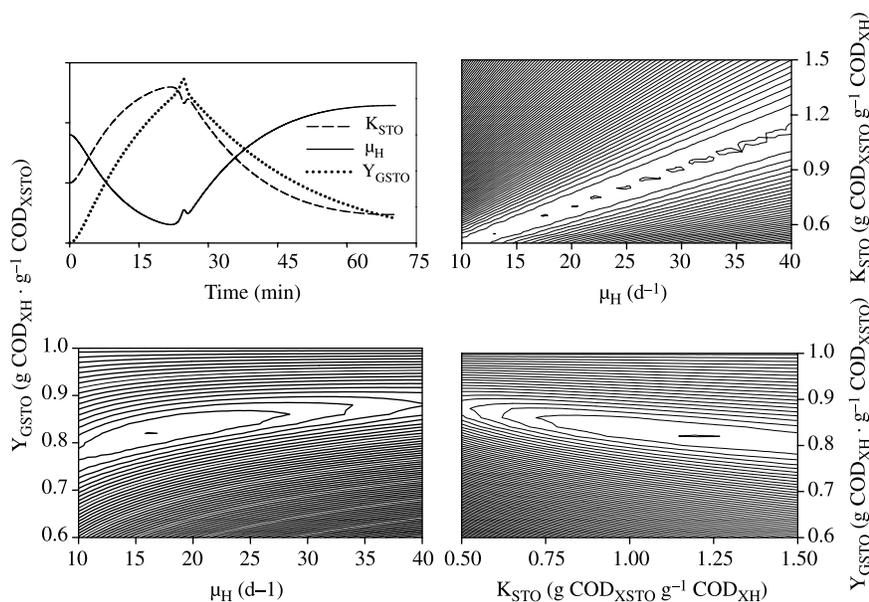


**Figure 2** Simulation of the effect of a percentage of the acetate being used directly for growth

This observation has been developed in some metabolic models (van Aalst-van Leeuwen *et al.* 1997) and, recently, in the works of van Loosdrecht and Heijnen (2002) and Karahan-Gül *et al.* (2003). Apart from a more reliable description of the reality, taking into account the growth on external substrate can overcome another described failure of ASM3. ASM3 fails in predicting the maximum growth rate profile on these short-term respirometric batch experiments. Krishna and van Loosdrecht (1999) pointed out the presence of a discontinuity on this profile. In other words, the growth rate observed in the feast phase is higher than the one observed in the famine phase. ASM1 correctly describes this observation, because the oxygen consumption is solely related to the growth process, so both the OUR and the growth rate profiles have the same trend. In contrast, according to ASM3, the growth rate must be constant and continuous along the experiment corresponding to the maximum growth rate on storage product. Two different growth rates are predicted by taking into account the simultaneous growth and storage on external substrate and, hence, the model describes more accurately the reality.

#### Practical (local) identifiability ASM3 using OUR

An important issue that should be considered in modeling, particularly in view of calibration, is the identifiability of the models. The identifiability of the ASM1 model based on short-term respirometric profiles is already discussed in detail in Dochain and Vanrolleghem (2001). ASM3 introduces the storage process in addition to the growth process for the description of the tail. The growth process on  $X_{\text{STO}}$  of ASM3 is a totally different model structure, which contains three parameters:  $\mu_{\text{H}}$ ,  $K_{\text{STO}}$  and  $Y_{\text{GSTO}}$  (see Table 1). As shown in Figure 3 top left, the output sensitivity functions of  $\mu_{\text{H}}$  and  $K_{\text{STO}}$  (calculated for exp C) are correlated with each other. This implies that both parameters cannot be identified uniquely. The correlation between these two parameters becomes clear when the shape of the objective function,  $J$ , (Equation 1) is calculated around an optimum as a function of  $\mu_{\text{H}}$  and  $K_{\text{STO}}$ . The shape of objective function (see Figure 3 top



**Figure 3** Sensitivity functions of  $\mu_{\text{H}}$  (solid line),  $K_{\text{STO}}$  (short-dashed line) and  $Y_{\text{GSTO}}$  (dotted line) (upleft) and correlation of  $\mu_{\text{H}}$  and  $K_{\text{STO}}$  (upright),  $\mu_{\text{H}}$  and  $Y_{\text{GSTO}}$  (downleft) and  $K_{\text{STO}}$  and  $Y_{\text{GSTO}}$  (downright)

right) shows a flat valley with a certain direction in the plane ( $\mu_H$  and  $K_{STO}$ ). This has often been observed in Monod-type models (e.g. Dochain and Vanrolleghem, 2001). This means that several different combinations of  $\mu_H$  and  $K_{STO}$  can fit the experimental data equally well. This observation was also confirmed when both parameters were considered for parameter estimation. In that case, the parameter estimation error (uncertainty in parameter estimation) of  $\mu_H$  and  $K_{STO}$  increased considerably, up to 300% of relative errors, indicating no reliable estimates for both parameters are possible.

On the other hand, the shape of the objective function as a function of  $\mu_H$  and  $Y_{GSTO}$  depicted in Figure 3 bottom left does not show linearity in the plane ( $\mu_H$  and  $Y_{GSTO}$ ). However, the contour plots of the objective function are rather large which indicates that still a high correlation exists between these two parameters. The same conclusion can be obtained from the plot of the objective function as a function of  $K_{STO}$  and  $Y_{GSTO}$  (Figure 3 bottom right). This implies the existence of a severe correlation between  $K_{STO}$  and  $Y_{GSTO}$ . In this study,  $K_{STO}$  was not estimated together with  $\mu_H$  and  $Y_{GSTO}$  and it was fixed to its default value in ASM3 i.e.  $1 \text{ g COD}_{XSTO} \cdot \text{g}^{-1} \text{COD}_{XH}$ .

### Conclusions

- ASM3 better describes all the experimental profiles when comparing the sum of squared errors. However, it has to be taken into account that seven parameters are estimated in this model in contrast with ASM1, where only five parameters are estimated.
- In experiments with considerable storage, ASM1 is not able to predict the tail observed due to the internal polymer consumption. In contrast, ASM3 can describe this second tail accurately, but non-mechanistic parameters are obtained.
- The growth yield ( $Y_H$ ) obtained by fitting ASM1 to the short-term respirometric batch profiles is higher than the default one (0.67) and the storage yield ( $Y_{STO}$ ) obtained by fitting ASM3 is lower than the default one (0.85). These values agree with the observation of simultaneous storage and growth on external substrate already developed in other works (e.g. van Loosdrecht and Heijnen, 2002). The introduction of this hypothesis would also help to improve the mechanistic meaning of the estimated parameters.
- From a practical identifiability point of view, this study shows the difficulty to obtain reliable values of the parameters related to the ASM3-growth process because  $\mu_H$  and  $K_{STO}$  are not identifiable, and high correlation exists between  $Y_{GSTO}$  and  $\mu_H$ , and  $Y_{GSTO}$  and  $K_{STO}$ .
- Future model developments should take into account the identifiability issues. Non-identifiable model structures should be avoided to improve the mechanistic meaning of model parameters thereby facilitating model validation tasks.

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### References

- APHA (1995). *Standard Methods for the Examination of Water and Wastewater*, 19th ed, American Publishers Health Ass.
- Beccari, M., Dionisi, D., Giuliani, A., Majone, M. and Ramadori, R. (2002). Effect of different carbon sources on aerobic storage by activated sludge. *Wat. Sci. Tech.*, **45**(6), 157–168.

- Dochain, D. and Vanrolleghem, P.A. (2001). *Dynamical Modelling and Estimation in Wastewater Treatment Processes*, IWA Publishing, London, UK.
- Guisasola, A., Baeza, J.A., Carrera, J., Casas, C. and Lafuente, J. (2003). An off-line respirometric procedure to determine inhibition and toxicity of biodegradable compounds in biomass from an industrial WWTP. *Wat. Sci. Tech.*, **48**(11-12), 267–275.
- Henze, M., Gujer, W., Mino, T. and van Loosdrecht, M.C.M. (2000). Activated Sludge Models ASM1, ASM2, ASM2d and ASM3. IWA Scientific and Technical Report no 9 IWA. London.
- Karahan-Gül, Ö., van Loosdrecht, M.C.M. and Orhon, D. (2003). Modification of Activated Sludge no 3 considering direct growth on primary substrate. *Wat. Sci. Tech.*, **47**(11), 219–225.
- Koch, G., Kühni, M., Gujer, W. and Siegrist, H. (2000). Calibration and validation of activated sludge model no 3 for swiss municipal wastewater. *Wat. Res.*, **34**(14), 3580–3590.
- Krishna, C. and van Loosdrecht, M.C.M. (1999). Substrate flux into storage and growth in relation to activated sludge modelling. *Wat. Res.*, **33**(14), 3149–3161.
- Sin, G., Malisse, K. and Vanrolleghem, P. (2003). An integrated sensor for the monitoring of aerobic and anoxic activated sludge activities in biological nitrogen removal plants. *Water. Sci. Tech.*, **47**(2), 141–148.
- Van Aalst-van Leeuwen, M.A., Pot, M.A., van Loosdrecht, M.C.M. and Heijnen, J.J. (1997). Kinetic modelling of poly( $\beta$ -hydroxybutyrate) production and consumption by *Paracoccus pantotrophus* under dynamic substrate supply. *Biotechnol. Bioeng.*, **55**(5), 773–782.
- Van Loosdrecht, M.C.M., Pot, M.A. and Heijnen, J.J. (1997). Importance of bacterial storage polymers in bioprocesses. *Wat. Sci. Tech.*, **35**(1), 41–47.
- Van Loosdrecht, M.C.M. and Heijnen, J. (2002). Modelling of activated sludge processes with structured biomass. *Wat. Sci. Tech.*, **45**(6), 12–23.
- Vanrolleghem P.A., Gernaey K., Coen F.O., Petersen B., De Clerq B., Ottoy J.P. (1998). Limitations of short-term experiments designed for identification of activated sludge biodegradation models by fast dynamic phenomena. In: *Proceedings 7<sup>th</sup> IFAC Conference on Computer Applications in Biotechnology. Osaka. Japan. May 31-June 4. 1998.*
- Vanrolleghem, P., Sin, G. and Gernaey, K. (2004). Transient response of aerobic and anoxic activated sludge activities to sudden concentration changes. *Biotechnol. Bioeng.*, **86**(3), 277–290.