Estimation of stoichiometric and kinetic coefficients of ASM3 under aerobic and anoxic conditions via respirometry

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Abstract Activated Sludge Model No.1 (ASM1) has been used extensively for the design and simulation of biological treatment systems. Batch respirometric experiments have been described in the model for the determination of model coefficients and respirometric studies have been proved to be useful in kinetic parameter estimation and wastewater characterisation for ASM1. Activated Sludge Model No. 3 (ASM3) has also introduced a number of kinetic and stoichiometric coefficients with the new processes defined in the model, also suggesting some default values. Recent studies on the application of ASM3 are limited to special cases in terms of parameter determination. Proper calibration of ASM3 parameters can be a difficult task without involving respirometric procedures as experimental tools. Respirometric batch tests were conveniently used in this study in order to estimate ASM3 parameters and the main kinetic and stoichiometric model coefficients were successfully and uniquely determined for aerobic and anoxic conditions for acetate.
Keywords Activated Sludge Model No. 3 (ASM3); correction factors under anoxic conditions; growth; kinetic and stoichiometric coefficients; respirometry; storage

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Introduction

Activated sludge behaviour is now studied using complex models involving a large array of kinetic and stoichiometric coefficients. Activated Sludge Model No. 1 (ASM1) should be regarded as the pioneering effort in this respect, providing a giant improvement in the mechanistic understanding of carbon and nitrogen removal (Henze *et al*., 1987). It was soon modified for endogenous decay (Orhon and Artan, 1994). Recently, Activated Sludge Model No. 3 (ASM3) was proposed adopting endogenous decay and advocating biochemical storage as the primary mechanism of substrate utilization (Gujer *et al*., 2000).

It should be noted that the merit of the new models mainly depends on the accuracy and reliability of the information they reflect on the biochemical mechanisms involved. This information must be experimentally determined. Respirometric methods have served extensively in the experimental assessment of kinetic and stochiometric coefficients associated with ASM1 (Ekama *et al*., 1986; Spanjers and Vanrolegghem, 1995; Sözen *et al*., 1998).

ASM3 introduced, together with the concept of biochemical storage, a new set of kinetic and stoichiometric coefficients, totally different from ASM1, with practically no experimental information on applicable values. Only some default levels were suggested with the model. Recent studies on the application of ASM3 have been limited to special cases in terms of parameter estimation (Koch *et al*., 2000). The trend however is to use, as illustrated in this study, the same respirometric procedures for the assessment of ASM3 parameters. This approach has been successfully applied for the determination of the storage yield coefficient, Y_{STO} under aerobic conditions (Karahan Gül *et al*., 2001).

It is also important to determine the difference in the rate of major biochemical processes described in ASM3 under aerobic and anoxic conditions. ASM3 interprets this difference using a single correction factor,η_D. The major objective of this study was to

Table 1 A simplified ASM3 matrix for aerobic and anoxic processes

j ↓	Component i→ Process expressed as→	1 S_S COD	2 S_O COD	3 S_{NO} N	4 X_P COD	5 X_H COD	6 X_{STO} COD	Process rate equation, ρ_j, all $\rho_j \geq 0$
1	Aerobic storage of COD	−1	$-(1-Y_{STO})$				Y_{STO}	$k_{STO}\frac{S_S}{K_S+S_S}X_H\frac{S_O}{K_O+S_O}$
2	Anoxic storage of COD	−1		$-\frac{1-Y_{STOD}}{2.86}$			Y_{STO}	$k_{STO}\eta_D\frac{S_S}{K_S+S_S}X_H\frac{S_{NO}}{K_{NO}+S_{NO}}$
3	Aerobic growth		$-\frac{1-Y_H}{Y_H}$			1	$\frac{1}{Y_H}$	$\mu_H\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}X_H\frac{S_O}{K_O+S_O}$
4	Anoxic growth			$-\frac{1-Y_{HD}}{2.86Y_{HD}}$		1	$\frac{1}{Y_{HD}}$	$\mu_H\eta_D\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}X_H\frac{S_{NO}}{K_{NO}+S_{NO}}$
5	Aerobic endogenous respiration		$-(1-f_{EX})$		f_{EX}	−1		$b_H X_H\frac{S_O}{K_O+S_O}$
6	Anoxic endogenous respiration			$-\frac{1-f_{EX}}{2.86}$	f_{EX}	−1		$b_{HD}X_H\frac{S_{NO}}{K_{NO}+S_{NO}}$
7	Aerobic respiration of X_{STO}		−1				−1	$b_{STO}X_{STO}\frac{S_O}{K_O+S_O}$
8	Anoxic respiration of X_{STO}			$-\frac{1}{2.86}$			−1	$b_{STOD}X_{STO}\frac{S_{NO}}{K_{NO}+S_{NO}}$

illustrate the merit of respirometry in the experimental evaluation of ASM3 under aerobic and anoxic conditions. In this context, oxygen and nitrogen uptake rate measurements (OUR, NUR) were carried out in batch reactors for the estimation of significant kinetic and stoichiometric parameters involved in ASM3 structure.

Conceptual approach

A simplified matrix representation of ASM3 for aerobic and anoxic conditions is depicted in Table 1. As the table shows, storage is defined for both aerobic and anoxic conditions involving aerobic and anoxic stoichiometric coefficients, Y_{STO} and Y_{STOD} respectively. Growth under anoxic conditions is reduced by a correction factor, η_D. Endogenous respiration and respiration of storage products processes are also specified for aerobic and anoxic conditions.

Respirometric batch tests have been conveniently used to evaluate different activated sludge models and thus, respirometric responses obtained from batch tests can be evaluated to estimate ASM3 parameters, as shown in Figures 1(a) and (b).

Figure 1(a) depicts the interpretation of an OUR curve according to ASM3. The mechanisms participating in oxygen utilisation are storage, growth, endogenous decay and respiration of storage products. The aerobic rate expressions and the associated stoichiometric coefficients defined in the model are given in Table 1.

The initial phase of the curve is endogenous decay, where oxygen utilisation is given by Eq. (1):

$$OUR_{Dec.phase}\left(\frac{dS_O}{dt}\right)_{Dec.phase} = \left(\frac{dS_O}{dt}\right)_{Dec.} + \left(\frac{dS_O}{dt}\right)_{Resp.X_{STO}} \quad (1)$$

Assuming that the concentration of the storage products is much less than that of heterotrophic biomass, the first phase of the OUR curve can be successively used to obtain an estimate value for active biomass concentration, X_H, for an accepted value of aerobic heterotrophic decay rate, b_H, and the inert biomass fraction, f_{EX}, as shown in Eq. (2) (Marais and Ekama, 1976; Dold *et al*., 1980; Dold and Marais, 1986):

$$OUR_{Dec.phase}\left(\frac{dS_O}{dt}\right)_{Dec.phase} = (1 - f_{EX}) \cdot b_H X_H \quad (2)$$

The second phase of the OUR curve is generated after the addition of exogenous substrate and is associated mainly with storage, where the other three processes are also active. The rate of storage, k_{STO}, can be estimated from the initial OUR value after substrate addition, since the storage process has its maximum rate at this point and this OUR level is defined

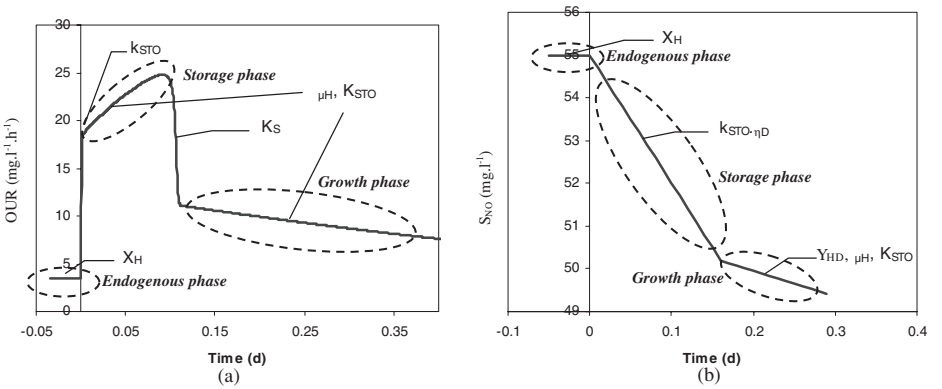

Figure 1 Estimated parameters of ASM3 using (a) aerobic and (b) anoxic respirometric curves

directly by $k_{STO}.X_H$, provided that the stoichiometry of storage is well defined. Storage phase OUR response is defined by Eq. (3):

$$OURS_{to.phase} = \left(\frac{dS_O}{dt}\right)_{Sto.phase} = \left(\frac{dS_O}{dt}\right)_{Sto.} + \left(\frac{dS_O}{dt}\right)_{Gro.} + \left(\frac{dS_O}{dt}\right)_{Dec.} + \left(\frac{dS_O}{dt}\right)_{Resp.X_{STO}} \quad (3)$$

Eqs (1) and (3) predict the difference between the OUR values of the two phases due to the oxygen utilisation by storage and growth, however with the previously accepted assumption of relatively low concentration of storage products ($X_{STO} << X_H$) at the start of feeding (t = 0), the contribution of the growth process is negligible. The maximum rate of storage, k_{STO}, can be estimated by expression (4) when there is no substrate limitation. The value of storage yield can be determined by the estimation of amount of oxygen utilised for storage from experimental OUR data (Karahan-Gül *et al.*, 2001).

$$\Delta OUR_{(t=0)} = \Delta\left(\frac{dS_O}{dt}\right)_{(t=0)} = (1 - Y_{STO}) \cdot k_{STO} \cdot X_H \quad (4)$$

The maximum heterotrophic growth rate, μ_H, can be estimated from the slope of the storage (second) phase of the OUR curve, as shown in the figure, since the slope is determined by the growth process, thus by μ_H and the half saturation coefficient for storage products, K_{STO}, when the growth yield, Y_H, is known. Although the substrate half saturation coefficient, K_S, also has considerable effect on this slope and the maximum attainable value of OUR, K_S can be directly and almost independently estimated by the sharpness and inclination of the drop in the OUR. The growth (third) phase is governed by three processes, since the storage process stops after the depletion of readily biodegradable substrate, as given in Eq. (5). The slope of the third phase, namely the growth phase, is given by the μ_H and K_{STO} couple, thus, the values of μ_H and K_{STO} can be estimated via model simulations by using both the storage (second) and growth (third) phases of the OUR curve.

$$OUR_{Gro.phase} = \left(\frac{dS_O}{dt}\right)_{Gro.phase} = \left(\frac{dS_O}{dt}\right)_{Gro.} + \left(\frac{dS_O}{dt}\right)_{Dec.} + \left(\frac{dS_O}{dt}\right)_{Resp.X_{STO}} \quad (5)$$

In the same manner, a nitrate utilisation rate (NUR) profile can be evaluated according to ASM3 as shown in Figure 1(b). The rate for the first phase is associated with endogenous respiration and the respiration of storage products, the latter neglected due to the comparably less amount of storage products. Thus, the active initial heterotrophic biomass concentration can be determined by:

$$NUR_{Dec.phase} = \left(\frac{dS_{NO}}{dt}\right)_{Dec.} = \frac{(1 - f_{EX})}{2.86} b_{HD} X_H \quad (6)$$

Literature values can be assumed for the heterotrophic anoxic decay coefficient, b_{HD} and the fraction of inert endogenous matter, f_{EX} (Gujer *et al.*, 2000).

The storage phase emerges after the addition of exogenous substrate. The rate of this phase is determined by the four anoxic processes namely, storage, growth, endogenous decay and respiration of storage products:

$$NUR_{Sto.phase} = \left(\frac{dS_{NO}}{dt}\right)_{Sto.phase} = \left(\frac{dS_{NO}}{dt}\right)_{Sto.} + \left(\frac{dS_{NO}}{dt}\right)_{Gro.} + \left(\frac{dS_{NO}}{dt}\right)_{Dec.} + \left(\frac{dS_{NO}}{dt}\right)_{Resp.X_{STO}} \quad (7)$$

The third phase in Figure (1b) is the growth phase and the total rate is due to the processes of growth, endogenous respiration and respiration of storage products:

$$NUR_{Gro.phase} = \left(\frac{dS_{NO}}{dt}\right)_{Gro.phase} = \left(\frac{dS_{NO}}{dt}\right)_{Gro.} + \left(\frac{dS_{NO}}{dt}\right)_{Dec.} + \left(\frac{dS_{NO}}{dt}\right)_{Resp.X_{STO}} \quad (8)$$

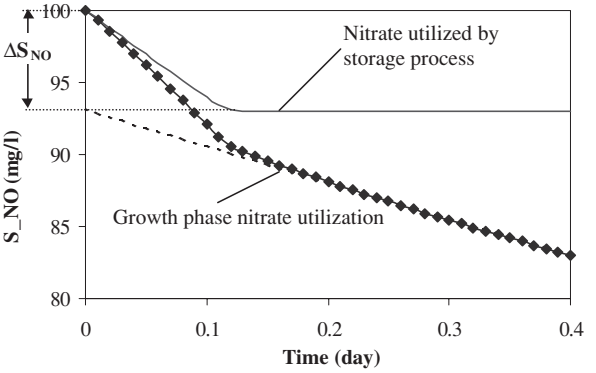

Figure 2 Graphical representation of the calculation of S_{NO} utilized for storage

Thus, the difference between the rates of storage and the growth phases, i.e. denoted as ΔS_{NO} on the y-intercept as depicted in Figure 2, defines the amount of nitrate utilised for storage.

The anoxic storage process shown in Table 1 can be expressed in terms of substrate utilisation as follows:

$$\left(\frac{dS_{NO}}{dt}\right)_{Sto.} = \frac{(1 - Y_{STOD})}{2.86}\frac{dS_S}{dt} \tag{9}$$

Rearranging and integrating Eq. (9) with respect to time:

$$2.86\Delta S_{NO} = (1 - Y_{STOD})\Delta S_S \tag{10}$$

Thus, Y_{STOD} can be calculated by for a known amount of S_S by Eq. (11):

$$Y_{STOD} = 1 - \frac{2.86\Delta S_{NO}}{\Delta S_S} \tag{11}$$

The rate expression for storage phase is governed by the storage rate and can be written as given in Table 1:

$$NUR_{Sto.phase} = \left(\frac{dS_{NO}}{dt}\right)_{Sto.} = \frac{(1 - Y_{STOD})}{2.86} k_{STO}\eta_D \frac{S_S}{K_S + S_S} X_H \tag{12}$$

The $S_S/(K_S+S_S)$ term can be neglected for a readily biodegradable substrate expected to have a low half saturation constant, K_S, value. Then, expression (12) can be reduced to:

$$NUR_{Sto.phase} = \left(\frac{dS_{NO}}{dt}\right)_{Sto.} = \frac{(1 - Y_{STOD})}{2.86} k_{STO}\eta_D X_H \tag{13}$$

Substituting the value of the calculated Y_{STOD}, it would be possible to calculate the product of the storage rate constant, k_{STO} and the anoxic reduction factor, η_D in Eq. (13).

Similar to the aerobic case, the anoxic heterotrophic yield coefficient, Y_{HD}, μ_H and K_{STO} can be estimated from the slope of the growth phase by the aid of model simulation. Substrate affinity coefficient, K_S can be adjusted by the inclination and the smoothness of the transition from the second phase to the third phase.

Materials and methods

Aerobic batch experiments

Aerated batch reactors of 2 l volume were used for the aerobic respirometric batch tests.

The tests were carried out in completely mixed, aerated batch reactors, seeded with active biomass taken from a fill and draw reactor of 5 l volume acclimated to acetate and operated at a sludge age of 20 days. The tests were started with the biomass seeding alone, for the assessment of the OUR level associated with the endogenous phase, for a minimum period of 45 minutes. Aerobic reactors were provided with buffer and mineral solutions. The final volume was made up with distilled water. Acetic acid solutions were prepared with 100% glacial acetic acid and were added to the biomass in the reactor to reach the final concentrations of 164, 187, 274 and 548 mg COD.l^{-1} in order to attain F/M ratios of 0.22, 0.25, 0.29 and 0.64 mg COD (mg VSS)$^{-1}$, respectively. The OUR response was monitored for 2–3 hours with a Manotherm RA-1000 continuous respirometer with PC connection.

Anoxic batch experiments

Anoxic 1 l batch reactors were set up to monitor the nitrate utilisation rate. Biomass was inoculated from fill and draw reactors acclimated to acetate, operated under 24-h aerobic/24-h anoxic conditions with a sludge age of 20 days. Nitrate was added externally in adequate amounts. The reactors were provided with buffer and mineral solutions. The final volume in the reactors was adjusted to 1 *l* using tap water. Acetate was added as the external substrate. Initial concentrations of acetate in the anoxic reactors were 50, 100, 120 and 175 mg COD.l^{-1}. The corresponding F/M values for acetate were between 0.14–0.16 mg COD (mg VSS)$^{-1}$. 100% glacial acetic acid was used as the acetate source. The anoxic reactors were equipped with magnetic stirrers and rubber stoppers with piping for sampling, nitrogen supply and outflow. Nitrogen gas was continuously purged to avoid air intrusion. Samples were withdrawn from the reactors every 5–10 minutes. Nitrite and nitrate-nitrogen analyses were performed on filtered samples, using a CHEMLAB autoanalyser operated in accordance with the hydrazine reduction method as defined in *Standard Methods* (1998).

In the experiments, pH was kept in the range of 7.0–8.0, suitable for biological activity. COD measurements were performed as described in the method ISO6060 (1986). Testing of the experimental data was performed by model simulation, using the AQUASIM® computer program developed by the Swiss Federal Institute for Environmental Science and Technology (Reichert *et al.*, 1998).

Results and discussion

Oxygen utilisation

OUR measurements obtained for four sets of aerobic experiments were used to estimate the main stoichiometric and kinetic parameters of ASM3 as explained in the previous sections and the results are presented in Table 2. The endogenous phase OUR data were used to calculate the amount of active heterotrophic biomass, according to Eq. (2). The second phase, namely the storage phase, was used to estimate the storage yield according to a predefined procedure (Karahan-Gül *et al.*, 2001) and the rate of storage, k_{STO}, was determined using Eq. (4) for different substrate concentrations and F/M ratios. The obtained storage rates (13 ± 2.60 d^{-1}) were much higher than the default value of ASM3, however they were in agreement with that of Krishna and van Loosdrecht (1999), where k_{STO} for acetate was reported as 10 d^{-1}.

Model simulations were performed for the estimation of μ_H, K_{STO} and K_S with the growth yield, Y_H, assumed as the default value of ASM3 in order to decrease the degrees of freedom of the model. The maximum value of OUR and the inclination of the OUR drop at the end of the second phase was used to evaluate the K_S value by model simulations. Model parameters like b_H, b_{STO} and f_{EX} were assumed as the default values due to their relatively low sensitivities for batch respirometric tests.

The second and the third phases were modeled to calculate the μ_H-K_{STO} couple and the

Table 2 Aerobic kinetic and stoichiometric coefficients of ASM3 obtained for acetate

		Set 1	Set 2	Set 3	Set 4
S_{S1}	mgCOD.l^{-1}	164	187	274	548
F/M	gCOD.g^{-1}VSS	0.22	0.25	0.29	0.64
b_H	d^{-1}	0.20	0.20	0.20	0.20
b_{STO}	d^{-1}	0.20	0.20	0.20	0.20
f_{EX}		0.20	0.20	0.20	0.20
K_S	mgCOD.l^{-1}	3	2	3	2
K_{STO}		1	1	1	1
k_{STO}	d^{-1}	16	12	10	14
μ_H (2nd phase)	d^{-1}	5	6	3	4
μ_H (3rd phase)	d^{-1}	2	2.5	1.6	2
Y_H	gCOD.g^{-1}COD	0.63	0.63	0.63	0.63
Y_{STO}	gCOD.g^{-1}COD	0.77	0.75	0.80	0.80

best fits were obtained for a K_{STO} default value of 1 gCOD.g^{-1}COD for all experimental runs. However, the simulation results of 4 sets of aerobic respirometric data indicated that it was not quite possible to obtain ASM3 calibration by using a single μ_H value. Two different μ_H values were used during simulation studies for fitting the first and second OUR plateaus. The higher μ_H value fitted the first plateau while the value of the second plateau was appreciably higher than the experimental plateau. In the same way, the lower μ_H value gave better fit to the second plateau value while the value of the first plateau stayed remarkably lower than the experimental data. Apparently two different growth rates were needed in order to simulate OUR data in the storage and growth phases as shown in Figure 3.

Nitrate utilisation

Nitrate utilisation data obtained from four sets of anoxic runs were used as input for model simulation using the AQUASIM computer programme modified for ASM3 structure. For modelling purposes, the initial active heterotrophic biomass concentration, X_H, was calculated from the aerobic endogenous decay phase data using expression (2).

The storage (second) phase emerged after the addition of the readily biodegradable substrate, S_S, namely acetate. The expression representing the storage mechanism (Eq. (13)) involved three unknowns namely, the anoxic storage yield coefficient Y_{STOD}, storage rate constant, k_{STO} and anoxic reduction factor, η_D. Y_{STOD} could be calculated for pre-selected

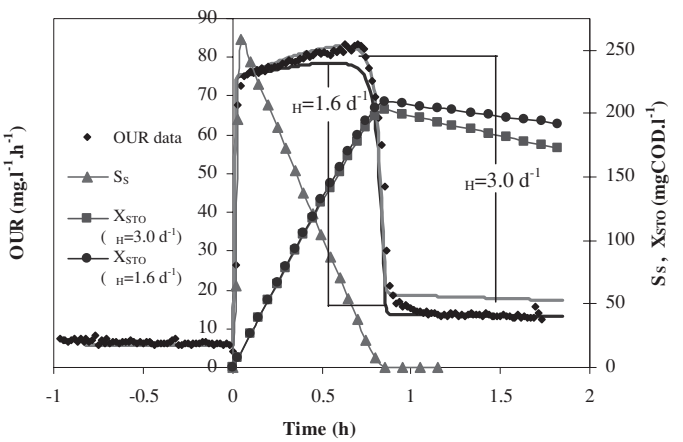

Figure 3 ASM3 simulation results for acetate aerobic run no 3

concentrations of S_{S1} (Table 3), by using Eq. (11). k_{STO} values were adopted as 13 $gCODg^{-1}COD\ d^{-1}$ from the model output of aerobic experimental sets. Thus, η_D was left as the only unknown parameter in expression (13) and could be calculated for each run by using the experimental slope of the storage phase in the S_{NO} profile. The calculated η_D values were between 0.50 and 0.56, with an average of 0.53.

The rate of the third phase of the anoxic electron acceptor utilisation profile was dependent on the growth of heterotrophic biomass on the stored substrate. As also shown in Figure (1b), the major rate determining parameters in this phase were the anoxic heterotrophic yield coefficient, Y_{HD}, the heterotrophic maximum growth rate, μ_H, and the saturation constant for storage products, K_{STO}. μ_H was adopted as the average value, i.e. 4.5 d^{-1}, obtained from the aerobic sets. By substituting the calculated η_D and Y_{STOD} values together with the average k_{STO} value as the model inputs, by simulation, Y_{HD} was obtained with an average of 0.44 $gCODg^{-1}COD$. In contrast to the aerobic respirometric data, model calibration was achieved for the anoxic data for all sets by using a single μ_H value. The effect exerted by μ_H was more pronounced for the storage and growth phases of the OUR vs. time data but the same parameter exerted a significant effect only on the growth phase of the S_{NO} vs. time profile because although OUR is an obtainable parameter for each time unit, it is experimentally not possible to monitor NUR vs. time. Instead, only an average nitrate utilization rate can be calculated for each phase.

The default saturation constant for substrate, K_S value (2 mg $CODl^{-1}$) seemed to provide the right transition from the second to the third phase. The experimental data gave a good fit with the model output using the suggested value of K_{STO} as in ASM3. The remaining kinetic coefficients, namely, the anoxic respiration rate for storage products, b_{STOD}, and nitrate-nitrogen half saturation constant, K_{NO} did not exert significant effects on the S_{NO} profile and were assumed as in ASM3. Table 3 lists the values of the kinetic and stoichiometric coefficients adopted for model evaluation. Figures 4 (a) and (b) show S_{NO}, S_S and X_{STO} vs. time profiles obtained by ASM3 simulation for acetate anoxic runs no 1 and 2.

Conclusions

ASM3 has been proposed for activated sludge systems both for aerobic and anoxic conditions. Introducing storage phenomena has also introduced a number of stoichiometric and kinetic coefficients making the model rather complicated with many degrees of freedom. Although some default values have been given in the model, calibration in terms of kinetic and stoichiometric parameters is still needed for various applications. This study aimed to reduce the complexity brought by the numerous parameters in ASM3 by providing a sys-

Table 3 Kinetic and stoichiometric coefficients used for anoxic acetate experiments

		Default	Set 1	Set 2	Set 3	Set 4
S_{S1}	$mgCOD.l^{-1}$		50	100	120	175
F/M	$gCOD.g^{-1}VSS$		0.14	0.16	0.13	0.14
b_{HD}	d^{-1}	0.10	0.10	0.10	0.10	0.10
b_{STOD}	d^{-1}	0.10	0.10	0.10	0.10	0.10
η_D		0.60	0.50	0.55	0.56	0.52
f_{EX}		0.2	0.2	0.2	0.2	0.2
K_S	mg $COD\ l^{-1}$	2	2	2	2	2
K_{STO}	$gCODg^{-1}COD$	1	1	1	1	
K_{NO}	$mgNO_3$–$N\ l^{-1}$	0.5	0.5	0.5	0.5	0.5
k_{STO}	$gCODg^{-1}COD\ d^{-1}$	5	13	13	13	13
μ_H	d^{-1}	2	6	6	6	6
Y_{HD}	$gCODg^{-1}COD$	0.54	0.43	0.45	0.41	0.45
Y_{STOD}	$gCODg^{-1}$					

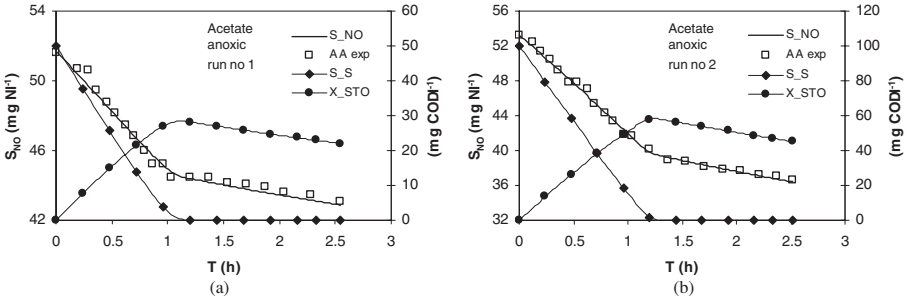

Figure 4 ASM3 simulation results for acetate anoxic runs no 1 (a) and 2 (b)

tematic approach for the estimation of the essential kinetic and stoichiometric parameters via respirometry. Respirometric batch tests with acetate have provided consistent data to be used in parameter determination for both aerobic and anoxic conditions.

The methodology involved the use of aerobic respirometric data for the estimation of aerobic coefficients by model simulation and substituting the obtained aerobic coefficients in the anoxic data to evaluate the anoxic parameters. By respirometric methods, it was possible to calculate the aerobic and anoxic storage yield coefficients independently. The average values of Y_{STOD} and Y_{STO} were 0.66 $gCODg^{-1}COD$ and 0.78 $gCODg^{-1}COD$ respectively, with a ratio of 0.85. The maximum storage rate for acetate was obtained by model simulation. The average k_{STO} rate of 13 $gCODg^{-1}COD\ d^{-1}$ was much higher than the default value of ASM3, however similar results for batch tests have been reported in the literature. The k_{STO} value found to be representative of the aerobic phase was used for calculating the anoxic correction factor η_D by using the rate expression for storage. The calculated η_D range was between 0.50 and 0.56 with an average of 0.53. This coefficient would reduce the aerobic storage rate of 13 $gCODg^{-1}COD\ d^{-1}$ to approximately 7 $gCODg^{-1}COD\ d^{-1}$ under anoxic conditions. The average aerobic heterotrophic growth rate was used in the anoxic simulations to estimate the anoxic heterotrophic growth yield, Y_{HD}. The model simulation outputs yielded an average Y_{HD} value of 0.44 $gCODg^{-1}COD$, which is relatively lower than the suggested value of 0.54 in ASM3. The corresponding Y_{HD}/Y_H ratio was 0.70.

While storage kinetics and stoichiometry could very well be defined, the simulation results of 4 sets of aerobic respirometric data indicated that it was not quite possible to obtain ASM3 calibration by using a single μ_H value. Apparently two different growth rates were needed in order to simulate OUR data in the storage and growth phases and this finding suggests that the concept of growth on storage products adopted in ASM3 needs to be re-evaluated. In this framework, modification of ASM3 considering simultaneous storage and direct growth on readily biodegradable substrate, followed by growth on stored products may be considered.

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