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ESTIMATING (COMBINATIONS OF) ACTIVATED SLUDGE MODEL NO. 1 PARAMETERS AND COMPONENTS BY RESPIROMETRY

Peter A. Vanrolleghem*, Henri Spanjers**, Britta Petersen*,***, Philippe Ginestet† and Imre Takacs‡

** BIOMATH Department, University Gent, Coupure Links 653, B-9000 Gent, Belgium*
*** Wageningen Agricultural University, P.O. Box 8129, NL-6700 EV Wageningen, The Netherlands*
**** EPAS nv, Technologiepark 3, B-9052 Zwijnaarde, Belgium*
† CIRSEE-Lyonnaise des Eaux, 38 rue du president Wilson, F-78230 Le Pecq, France
‡ Hydromantis Inc., Suite 302, 1685 Main St W., Hamilton, Ontario, L8S 1G5, Canada

ABSTRACT

The paper presents a concise overview of respirometric experiments for the calibration of ASM1. First, the popularity of respirometry is explained by its historical impact and its sensitivity and robustness. The body of the text consists of a systematic overview of existing methods for assessment of nearly all ASM1: (i) component concentrations in sludge and waste water; and (ii) biokinetic and stoichiometric parameters. Real-life examples illustrate the methods. A difference is made between direct methods that use explicit calculations and optimisation methods that require numerical optimisation algorithms. It is stressed that the latter approach is especially useful to extract multiple parameters and component concentrations from single respirometric experiments. Finally, the importance of reflecting on the translation of lab-scale respirometric results to a full-scale model is stressed. © 1999 IAWQ Published by Elsevier Science Ltd. All rights reserved

KEYWORDS

Activated sludge; calibration; modelling; optimal experimental design; respirometry.

INTRODUCTION

The use of dynamic models in activated sludge processes has become more and more widespread. The Activated Sludge Model No. 1 (ASM1) presented by the IAWQ Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment Processes (Henze *et al.*, 1987) is generally accepted as state-of-the-art and is used for simulation of waste water treatment plants in many studies. However, the biological nature of waste water treatment processes (WWTP) implies that the characteristics of the process be determined over and over again according to the local situation.

Setting up the plant configuration in a model and characterising the influent and sludge are called "model calibration". To calibrate a WWTP model, information-rich lab-scale experimental data is used, and data

obtained from traditional and dedicated measuring campaigns at the plant under study (Coen *et al.*, 1998). The purpose of such experiments is to determine the numerical values of model parameters related to:

1. The hydraulics (e.g. number of tanks in series; De Clercq *et al.*, 1998).
2. Sludge settling properties (e.g. Vesilind settling parameters, Ekama *et al.*, 1997).
3. Biodegradation (stoichiometric and kinetic coefficients).
4. Concentrations of the waste water components.

In this paper attention is only given to the latter two aspects. The question obviously rises why respirometry has become such a popular tool in model calibration. The following reasons come to mind:

1. There is a history of scientific development in which respirometry has been very supporting.
2. A main goal of WWT is to reduce the (biological) oxygen demand of waste water.
3. ASM1 was primarily developed to yield a good description of sludge production and consumption patterns of electron acceptors (nitrate and oxygen respiration) in treatment plants.
4. Respirometry is a very sensitive method to study bioprocesses as oxygen concentration changes in the order of ten parts-per-billion can be monitored at high frequencies in non-pretreated samples.

Respirometry is the measurement and interpretation of the biological oxygen consumption rate under well defined experimental conditions (Spanjers *et al.*, 1998). Respiration rate is usually measured with respirometers. These are based on some technique for measuring the rate at which biomass takes up dissolved oxygen from the liquid. This can be done by using a restricted number of measuring principles (Spanjers *et al.*, 1998). The review in this paper does not adhere to a particular measuring principle. We just assume that the measurements are done with the optimal measuring principle.

Before the relation is established between respiration rate and the parameters and components of ASM1, it may be useful to explain this model for the heterotrophic process (Table 1). In the mass balance of the heterotrophic organisms X_{BH} (c. 5) the production of X_{BH} by aerobic growth (r. 1) is counteracted by the loss of X_{BH} by heterotrophic decay (r. 4). In this decay process component X_{BH} (c. 5) is converted to component X_S (c. 4). This production of X_S is counteracted by the loss of X_S by hydrolysis (r. 7), leading to production of component S_S (c. 2). S_S is used for heterotrophic growth (r. 1) where it is converted to component X_{BH} (c. 5) at the expense of component oxygen S_O (c. 8), i.e. respiration. A similar reasoning can be made for the processes involving the nitrogen components (S_{NH}, S_{ND} and X_{ND}) and autotrophic (nitrifying) organisms (X_{BA}).

The total respiration rate of biomass in contact with waste water is according to the ASM1:

$$r_{tot} = \frac{1 - Y_H}{Y_H} \cdot X_{BH} \cdot \mu_H + \frac{4.57 - Y_A}{Y_A} \cdot X_{BA} \cdot \mu_A \qquad (1)$$

where the specific growth rates μ_H and μ_A are functions of S_S and S_{NH}, respectively (Table 1). The concentrations of S_S and S_{NH}, in turn, depend on the rates at which X_S, S_{ND} and X_{ND} are degraded (see Table 1).

It is clear that the whole "wheel-work" summarised in Table 1 eventually acts on the mass balance of oxygen. In summary, the oxygen mass balance is affected by:

1. Rates of processes, which are in turn dependent on:
   a) kinetics (process rate ρ)
   b) stoichiometry (ϖ)
   c) component concentrations
2. Transport terms, which are in turn dependent on the concentrations.

Table 1. Kinetic and stoichiometric realtionship for COD removal, nitrification and denitrification (Henze *et al.*, 1987)

Component i → / j ↓ Process	1 S_I	2 S_S	3 X_I	4 X_S	5 $X_{B,H}$	6 $X_{B,A}$	7 X_P	8 S_O	9 S_{NO}	10 S_{NH}	11 S_{ND}	12 X_{ND}	13 S_{ALK}	Process rate ρ_j $ML^{-3}T^{-1}$
1 Aerobic hetero-trophic growth		$-\frac{1}{Y_H}$			1			$1-\frac{1}{Y_H}$		$-i_{XB}$			$-\frac{i_{XB}}{14}$	$\mu_H X_{B,H}$
2 Anoxic hetero-trophic growth		$-\frac{1}{Y_H}$			1				$-\frac{1-Y_H}{2.86Y_H}$	$-i_{XB}$			$\frac{1-Y_H}{2.86\cdot 14\cdot Y_H}-\frac{i_{XB}}{14}$	$\mu^{\circ}_H X_{B,H}$
3 Aerobic auto-trophic growth						1		$1-\frac{4.57}{Y_A}$	$\frac{1}{Y_A}$	$\frac{1}{Y_A}-i_{XB}$			$-\frac{2}{14\cdot Y_A}-\frac{i_{XB}}{14}$	$\mu_A X_{B,A}$
4 Het. decay				$1-f_P$	-1		f_P					$i_{XB}-f_P i_{XP}$		$b_H X_{B,H}$
5 Aut. Decay				$1-f_P$		-1	f_P					$i_{XB}-f_P i_{XP}$		$b_A X_{B,A}$
6 Ammonification										1	-1		1/14	$k_a S_{ND} X_{B,H}$
7 Hydrolysis		1		-1										$k_h X_S$
8 Hydrolysis of N											1	-1		$k_h X_{ND}$
Observed conversion rates $ML^{-3}T^{-1}$	$r_i = \sum_j r_{ij} = \sum_j \nu_{ij}\rho_j$													
Stoichiometric parameters (see text)	Nomenclature, see text All units in ML^{-3} (COD or N, depending on variable)													Kinetic parameters (see text)

$$\mu_H = \mu_{mH}\left(\frac{S_S}{K_S + S_S}\right)\left(\frac{S_O}{K_{O,H} + S_O}\right)$$

$$\mu^{o}_H = \eta_g \mu_{mH}\left(\frac{S_S}{K_S + S_S}\right)\left(\frac{K_{O,H}}{K_{O,H} + S_O}\right)\left(\frac{S_{NO}}{K_{NO} + S_{NO}}\right)$$

$$\mu_A = \mu_{mA}\left(\frac{S_{NH}}{K_{NH} + S_{NH}}\right)\left(\frac{S_O}{K_{O,A} + S_O}\right)$$

$$k_h = k_{mh}\frac{1}{K_X + \left(X_S/X_{B,H}\right)}\left(\frac{S_O}{K_{O,H} + S_O}\right) + \eta_h\left(\frac{K_{O,H}}{K_{O,H} + S_O}\right)\left(\frac{S_{NO}}{K_{NO} + S_{NO}}\right)$$

Because all these characteristics of a WWTP affect the oxygen mass balance, respiration rate measurements can, the other way round, be used to characterise these processes. Hence, all parameters and components involved in the functional relationships that are (directly and indirectly) connected to the oxygen balance (see arrows in Table 1) can be assessed from respirometry:

1. Kinetic parameters (each process rate row where a horizontal arrow is present)
2. Stoichiometric parameters (each cell where an arrow starts or ends)
3. Component concentrations (each column where an arrow starts)

Obviously, the challenge is to create such experimental conditions that allow reliable determination of these process parameters and component concentrations. From the following, it will become clear that considerable creativity has been displayed to generate such experimental conditions in respirometric set-ups.

There are two approaches for the determination of model parameters and components: *direct methods* focus on specific parameters and components which can directly be evaluated from the measured respiration rates (Ekama *et al.*, 1986; Spanjers and Takacs, 1998), whereas *optimisation methods* use a (more or less simplified) model that is fitted to the measured data (Kappeler and Gujer, 1992; Larrea *et al.*, 1992; Wanner *et al.*, 1992; Spanjers and Vanrolleghem, 1995). In the latter, numerical techniques are used to find parameter values that lead to the smallest deviation between model predicted and measured respiration rates. In the paper the numerical techniques for estimation will not be discussed, but reference is made to the literature (Robinson, 1985; Vanrolleghem and Dochain, 1998).

In the following, an overview is presented of respirometric methods to determine ASM1 (kinetic and stoichiometric) parameters and (wastewater and sludge) component concentrations. The overview was structured according to the components and parameters and not according to the separate respirometric experiments. It must be stressed though that single experiments can result in combinations of parameter and/or component estimates. This is less visible from this overview but is an important aspect as it may reduce the experimental efforts during calibration. It is not possible within the limited space available to extensively discuss the experimental details, nor to discuss the estimation accuracy. Some hints will be given though on important considerations when performing respirometric experiments for ASM1 calibration.

CONCENTRATION OF COMPONENTS IN WASTE WATER

General

Assessment of waste water components is often referred to as waste water characterisation. The procedures for characterisation involve a combination of physico-chemical and biodegradation tests (Roeleveld, 1995). With respirometry, basically only biodegradable components in the waste water can be quantified. This means that only the biodegradable components S_S, X_S, S_{NH}, S_{ND} and X_{NH} are directly relevant for respirometry. Notice that each ASM1 component in real life is composed of a number of different compounds associated with a range of degradation rates (and concentrations). These ranges, however, are small compared to the differences between the degradation rates of the ASM1 components. This observation is, by definition, the basis for the distinction between readily and slowly biodegradable components. In addition to the biodegradable substrates, the concentrations of X_{BH} and X_{BA} in the waste water can be assessed by using respirometry. The physico-chemical methods are necessary to complete the picture of the waste water' s composition, e.g. by quantifying the inert components.

From the "wheel-work" described in Table 1, it is clear that the total respiration rate is affected by the concentrations of all the biodegradable components and that the cumulative oxygen consumption (i.e. the integral of r) is a measure of the amount of components degraded. Notice that because integrals of respiration rates are taken, the measuring frequency and signal-to-noise ratios are not very critical for reliable assessment of the component concentrations. This is in contrast to the kinetic characterisation (see

below) where changes of respiration rates (derivative of r) are carrying the information. This implies a much higher dependency of the parameter accuracy on the quality of the respirometric measurement.

Inherent with respirometric tests for waste water characterisation is the use of biomass: the assessment of waste water components is based on the respirometric response of biomass to waste water. Two important aspects are associated with the use of biomass. The first aspect is the amount of waste water with respect to biomass (S/X ratio, see also next sections) that is used. Second, in the death-regeneration concept adopted in ASM1, new S_S, X_S, S_{NH}, S_{ND} and X_{ND} are continuously generated from decaying biomass. Within this model it is therefore difficult to distinguish between the components originating from the waste water and from the biomass itself. In fact the transition between exogenous respiration and endogenous respiration is gradual. The respirometric test should be organised in such a way that these rates can be distinguished. This is one of the most challenging problems in respirometric characterisation of waste water and sludge in the context of ASM1.

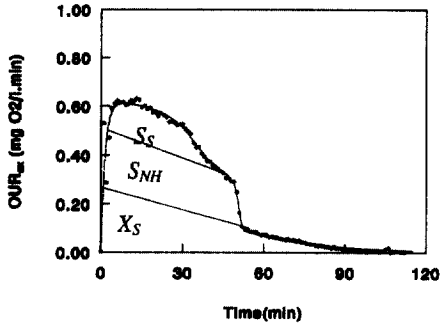

Figure 1. Respiration rate profile obtained after addition of 0.7 l waste water into 1.3 l activated sludge (Spanjers and Vanrolleghem, 1995).

Figure 1 shows a respirogram (i.e. a time course of respiration rates) collected in a batch experiment where at the start waste water is added to endogenous sludge. In Fig. 1 only the oxygen consumption due to substrate oxidation is given, i.e. the endogenous respiration is subtracted from the total respiration rate, leaving the exogenous respiration rate r_{ex}. A typical respirogram shows an initial peak brought about by the oxidation of readily biodegradable matter, followed by one or more shoulders where successively other components continue to be oxidised. The full area under the respirogram represents the total of biodegradable components $(S_S + X_S)/(1-Y_H) + (S_{NH} + S_{ND} + X_{ND})/(4.57 - Y_A)$, as follows from Eq. 1. In Fig. 1 three substrate fractions can be discerned, corresponding to S_S, X_S and S_{NH}.

Readily biodegradable substrate S^S

The readily biodegradable substrate is presumably composed of (simple/low molecular) soluble compounds, such as volatile fatty acids, alcohols, etc. (Henze, 1992). The characteristic of these compounds is that they are degraded rapidly and hence provoke a fast respirometric response.

The standard batch test for determination of S_S (Ekama *et al.*, 1986, and many others) involves the addition of a waste water sample to endogenous sludge, and monitoring the respiration rate until it returns to the endogenous level. The concentration of readily biodegradable substrate initially present in the mixture of biomass and waste water can be calculated as follows:

$$S_S(0) = \frac{1}{1-Y_H}\left(\int_0^{t_{fin}} r_{ex}\,dt\right) \tag{2}$$

The concentration of S_S in the waste water is then easily calculated by taking into account the dilution. The end point t_{fin} of the integration interval is the time instant where S_S is completely oxidised and where the exogenous respiration rate for S_S becomes zero. The integral can easily be obtained by determining the area under the curve, for instance by using a spreadsheet program, also known as the graphical method. An alternative consists of solving the mass balance equations with a numerical integrator to predict the exogenous respiration rates for S_S in such batch experiments. Depending on the initial value $S_S(0)$ given to the integration algorithm, the simulation will result in a different respirogram. One can therefore search for the $S_S(0)$ value that gives the best "fit" to the measured data. This is the optimisation approach mentioned above. For this simple application it may be a bit overdone, but for more complex estimation tasks (see below), the approach becomes more straightforward than direct calculation methods.

Notice that knowledge of the heterotrophic yield coefficient Y_H is needed for the calculation of S_S from respiration rates. This stoichiometric coefficient is always involved when oxygen consumption is converted to substrate equivalents (see also next sections). The batch test described above is also used to assess other ASM1 components and, likewise, kinetic parameters. This explains its popularity in calibration procedures.

Another batch test (Wentzel *et al.*, 1995) consists of monitoring the respiration rate of unsettled sewage without seed for a relatively long period (approximately 20 hours). A respirogram similar to the one depicted in Fig. 7 is obtained. S_S is calculated from the respiration rates observed between the start of the test up to the precipitous drop (due to depletion of S_S), with correction for the increasing endogenous respiration due to the increase of biomass during the test. In addition to Y_H, knowledge of the net growth rate is required, which can be obtained from the same test.

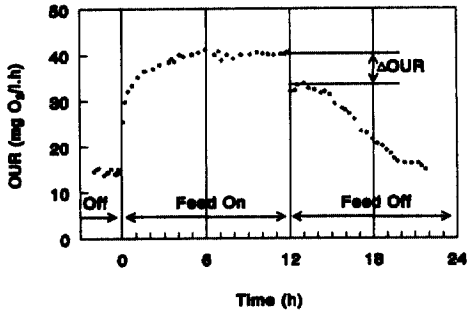

Figure 2. Respiration rates (OUR) obtained with the experimental set-up of Ekama *et al.* (1986).

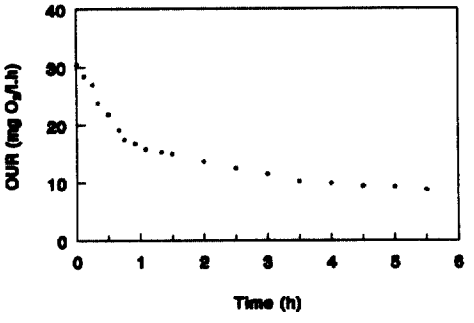

Figure 3. Respiration rates (OUR) obtained according to Kappeler and Gujer (1992) for estimation of X_S and the hydrolysis kinetics.

Ekama *et al.* (1986) presented a method that involves monitoring of respiration rate in a completely mixed reactor operated under a daily cyclic square-wave feed (see also further use of such data for characterising hydrolysis, section on k_h, K_x). It is hypothesised that the sudden drop in respiration rate to a lower level,

observed upon termination of the feed (Fig. 2), corresponds uniquely to the S_S that has entered via the influent. Hence, the concentration readily biodegradable substrate in the waste water can be calculated as:

$$S_S = \frac{V_{react}}{Q_{ww}} \frac{\Delta r_{tot}}{1 - Y_H} \tag{3}$$

Finally, another continuous flow method proposed by Witteborg *et al.* (1996) is based on the measurement of respiration rate under different waste water loading conditions applied to a continuously operated respirometer. The waste water S_S is calculated by numerically solving a set of mass balances pertaining to different loading conditions of the respirometer.

Slowly biodegradable substrate X_S

It is presumed that slowly biodegradable substrate X_S, sometimes also defined as particulate material, is composed of (high-molecular) compounds ranging from soluble to colloidal and particulate (Henze, 1992). The common feature of these compounds is that they cannot pass the cell membrane and undergo hydrolysis to low-molecular compounds (S_S) which are subsequently assimilated and oxidised. Because the rate of hydrolysis is lower than the oxidation rate of S_S, the respirometric response on X_S is slower.

In a batch test an exponentially decreasing "tail" can frequently be observed in respirograms after the initial S_S peak (Fig. 1). In Fig. 3, this tailing starts after approximately 0.75 hour. The waste water concentration of X_S can be assessed in a similar way as above, Eq. 2 (Kappeler and Gujer, 1992). Simultaneously occurring oxidation processes such as nitrification might interfere and complicate the identification of respiration rate governed by hydrolysis. In that case a nitrification inhibitor may be used to facilitate the assessment of X_S (Spanjers and Vanrolleghem, 1995). Alternatively, if the data of such respirometric batch tests are used in combination with mathematical curve fitting techniques to match the response of the model to the data, the nitrification part can rather easily be extracted from the respirogram (Spanjers and Vanrolleghem, 1995).

Heterotrophic biomass X_{BH}

Some waste waters can contain significant concentrations of heterotrophic biomass (Henze, 1992), so there is a need to quantify this component. A batch test has been described (Kappeler and Gujer, 1992; Wentzel *et al.*, 1995) to assess X_{BH} from the respirometric response of raw waste water without seed. The calculation requires Y_H and two parameters that can be assessed from the same data: maximum specific growth rate μ_{mH} and decay coefficient b_H. Respirograms look like the one presented in Fig. 7. The procedure basically backtracks the amount of heterotrophic biomass originally present in the waste water by comparing the original respiration rate with the respiration rate after significant (hence, well quantifiable) growth of X_{BH}.

Autotrophic biomass X_{BA}

So far, the authors are not aware of procedures in which the autotrophic biomass concentration in waste water is assessed. However, it could be imagined that a similar procedure as the one developed for X_{BH} is applicable, i.e. evaluate the respiration rate for nitrification r_{nit} of the autotrophs present in the waste water and compare it to the respiration rate of a culture with known autotrophic biomass concentration X_{BA}, e.g. after significant growth.

Ammonium S_{NH}

Waste water ammonium concentration can be assessed by using conventional analytical techniques. However, respirometry also offers the possibility to deduce S_{NH} from batch measurements in a similar way as S_S and X_S (provided the test is done with nitrifying activated sludge). As follows from Table 1, the nitrifier yield coefficient Y_A is needed to convert the oxygen consumption for nitrification to nitrogen concentration by division by $(4.57\text{-}Y_A)$ However, the value of S_{NH} is not very sensitive to Y_A as Y_A is small compared to 4.57. Notice that ammonia is also used for assimilation (i.e. incorporation into new biomass)

which may be a considerable fraction of the nitrogen (tens of percent) in case a large amount ($COD^{Degraded}$) of COD is biodegraded. The nitrogen that can be nitrified can be approximated by

$$N^{Nitr} = S_{NH} - i_{XB} Y_H COD^{Degraded} \tag{4}$$

in which i_{XB} is the nitrogen content of newly formed biomass. From this equation one can easily deduce the original nitrogen concentration when $COD^{Degraded}$, and the stoichiometric parameters i_{XB} and Y_H are given. Fitting a model in which carbon and nitrogen oxidation are included to the respirometric data will automatically take this correction into account (Spanjers and Vanrolleghem, 1995; Brouwer *et al.*, 1998).

Organic nitrogen S_{ND} and slowly biodegradable organic nitrogen X_{ND}

Probably because the ammonification and hydrolysis rates of organic nitrogen compounds are relatively fast, little attention has been devoted so far to the establishment of respirometric techniques for S_{ND} and X_{ND} quantification. In batch tests, these compounds are typically converted to S_{NH} before the S_{NH} that was originally present in the waste water is removed by nitrification. Therefore, S_{ND} and X_{ND} are not directly observable in such tests. Still, for some waste waters the ammonification and hydrolysis steps may be considerably slower and quantification of the component concentrations may be required. In such cases, one can imagine a procedure in which the nitrification respiration rate r_{nit} is monitored and interpreted in terms of ammonification and hydrolysis, similar to the way the respiration resulting from COD degradation is interpreted in terms of the hydrolysis process. Subsequently, the amounts of nitrogen containing substrates could be assessed by taking the integral of r_{nit} for the corresponding fractions and dividing these by (4.57-Y_A), the stoichiometric coefficient corresponding to nitrification. In case simultaneous COD-removal is taking place, correction should again be made for nitrogen assimilated into new heterotrophic biomass (see above).

CONCENTRATION OF COMPONENTS IN SLUDGE

In this section special attention is only paid to the assessment of the slower varying sludge characteristics. Knowing the initial value of the concentrations of soluble components (e.g. ammonia) is not really essential because it has little impact on typical simulation results with a calibrated model. Hence, the concentrations of the following particulate, slowly varying components must be assessed: X_{BH}, X_{BA}, X_I ($+X_P$) and X_S. Only three concentrations must be assessed since the sum of the concentrations is equal to the particulate COD (X_{tot}) of the sludge that can easily be measured by using traditional analysis:

$$X_{tot} = X_I + (X_P) + X_S + X_{BH} + X_{BA} \tag{5}$$

Below some fast and direct methods for assessing sludge components are summarised. Notice that the particulate nitrogen components are not considered here as their concentrations are assumed to be low.

Concentration of heterotrophic organisms X_{BH}

One can show that the concentration of heterotrophs in a continuous system in steady state is equal to:

$$X_{BH} = Y_H \cdot \frac{\theta_X}{\theta_H} \cdot \frac{COD^{Degraded}}{1 + b_H \theta_X} \tag{6}$$

where θ_X is the sludge age, θ_H is the hydraulic retention time, $COD^{Degraded}$ the total amount of COD) removed (taken over a sufficiently long period, e.g. one sludge age), b_H the decay rate coefficient and Y_H the yield coefficient. Respirometric methods to determine parameters b_H and Y_H are discussed below, while a respirometric evaluation of $COD^{Degraded}$ can be performed with the respirometric measurements of biodegradable COD fractions (S_S, X_S) presented above.

As an alternative, Lokkegaard Bjerre *et al.* (1995) used the method of Kappeler and Gujer (1992) to determine the concentration of heterotrophs in the mixed liquor. Recently, this method was thoroughly evaluated by Ubisi *et al.* (1997).

Concentration of autotrophic organisms X_{BA}

In much the same way, the concentration of nitrifying organisms in the activated sludge can be evaluated by means of a mass balance for the autotrophs (over a sufficiently long time) (Dupont and Sinkjaer, 1994):

$$X_{BA} = Y_A \cdot \frac{\theta_X}{\theta_H} \cdot \frac{f^{Aerobic} N^{Nitrified}}{1 + b_A . \theta_X} \tag{7}$$

where f^{Aerobic} is the aerobic fraction of the reactor; $N^{Nitrified}$ the amount of nitrogen nitrified; b_A the autotrophic decay rate coefficient and Y_A the autotrophic yield coefficient. The methods to determine parameters b_A and Y_A are discussed in the next paragraph, while $N^{nitrified}$ can be quantified using respirometry-based nitrifiable nitrogen evaluation methods given above.

Inert fraction X_p

For determination of the inert fraction an evaluation of the mass balance of inerts in steady state can also be made. When the autotrophic fraction can be neglected, the following equation is obtained:

$$X_P = \frac{\theta_X}{\theta_H} X_{i,\text{inf}} + f_P b_H X_H \theta_X \tag{8}$$

In most cases, the concentration of inert particulates in the influent ($X_{I,inf}$) should indeed be taken into account. Respirometry is involved in calculating this fraction via f_p and b_H (see below).

Concentration of particulate substrate X_S

For determination of the slowly biodegradable substrate one can use the batch experimental methods for determining the X_S concentration in waste water where a waste water sample is added to endogenously respiring sludge (previous section). However, when X_S is to be assessed in a fresh sludge sample taken from the treatment plant under study, it will not be as easy to assess the endogenous respiration rate. This may complicate the interpretation of the respirometric data.

As an alternative method, one can apply the general procedure for estimation of sludge composition (Henze *et al.*, 1987) by performing a steady state analysis, e.g. by performing long term simulation with the model under constant (average) inputs.

STOICHIOMETRIC PARAMETERS

By definition, estimation of stoichiometric parameters requires the measurement of two conversion rates. One of these rates may be the respiration rate. Theoretically, for ASM1 the following stoichiometric parameters can be evaluated using respirometry: Y_H, Y_A, i_{XB} and f_p, though attempts are reported only for the former two.

Heterotrophic yield coefficient Y_H

This parameter influences not only the estimation of sludge production and oxygen demand but also has an impact on the value of other parameters whose determination requires a value for Y_H (see below). Hence, an accurate value for Y_H is of great importance. Sollfrank and Gujer (1991) and Brands *et al.* (1994) determine Y_H using respirometry. An amount of waste water COD is added to a batch reactor with activated sludge and

the respiration rate for substrate oxidation (r_{ex}) is measured. The following equation is then applied to evaluate the yield coefficient:

$$Y_H = \frac{COD^{Degraded} - \int r_{ex}(t)dt}{COD^{Degraded}} \tag{9}$$

Knowledge of the inert substrate fraction S_I and the soluble COD of the sample is necessary for the calculation of $COD^{Degraded}$. Brands *et al.* (1994) circumvent the S_I-problem by using a completely biodegradable substrate (Na-acetate) instead of waste water. Hence, the $COD^{Degraded}$ is known exactly. From the authors' experience it seems that this approach is not without risk. First, the choice of acetate is rather arbitrary and there is quite some evidence that the yield coefficient for acetate differs from the substrates in the influent of the WWTP. Second, due to the experimental conditions in the batch reactor, it is possible that acetate is stored in the cell. In this case the oxygen demand only represents the needs for transport of the substrate and incorporation in storage material of the cell and not the complete conversion into new cells.

Autotrophic yield coefficient Y_A

Although a value of 0.24 g biomass-COD per g nitrified nitrogen is established as a good value for Y_A, it is possible to estimate the actual autotrophic yield coefficient from a respirometric batch experiment in which a pulse of ammonium is added to a nitrifying activated sludge sample (for an example, see Fig. 9). Care has to be taken that in the mean time there is no net growth of heterotrophs (incorporating a part of the supplied ammonium) (Spanjers and Vanrolleghem, 1995). The cumulative respiration rate for nitrification r_{nit} (= total respiration rate minus endogenous respiration rate) enables the calculation of the yield coefficient Y_A:

$$Y_A = \frac{4.57\, S_{NH} - \int r_{nit}(t)dt}{S_{NH}} \tag{10}$$

A value of the nitrified nitrogen ($N^{nitrified}$) is necessary and can be obtained from nitrate measurements or an ammonium measurement corrected for incorporation into the biomass (Eq. 4).

If net heterotrophic growth occurs (e.g. when acetate is added for simultaneous characterisation of the heterotrophs, Vanrolleghem and Verstraete, 1993), r_{nit} is obtained by subtraction of endogenous and heterotrophic respiration rate from the total rate. In the model based approach of Spanjers and Vanrolleghem (1995) these corrections are done implicitly by fitting ASM1 to the respirometric data.

Nitrogen content of the biomass i_{XB}

Obviously, the most likely method for evaluation of i_{XB} would consist of a nitrogen analysis of biomass. However, one can imagine (albeit maybe not very realistically) that nitrogen incorporation into biomass can be assessed using two respirometric experiments in which different amounts of COD are degraded, the difference being denoted as $\Delta COD^{Degraded}$. The reduction in the oxygen consumption for nitrification ($\Delta\int r_{nit}dt$) that can be observed for the higher COD loading allows to calculate i_{XB} (development of Eq. 4).

$$i_{XB} = \frac{Y_H}{4.57 - Y_A} \frac{\Delta COD^{Degraded}}{\Delta \int r_{nit}(t)dt} \tag{11}$$

Inert particulate fraction of the biomass f_p

Decay of biomass results in a fraction being transformed into inert particulate products. Typically 20% of the biomass consists of inert material (Henze *et al.*, 1987). This inert biological fraction is called f'_p. The model f_p can be calculated starting from the biological f'_p with the following implicit equation:

$$f_P' = \frac{f_P}{1 - Y_H(1 - f_P)} \tag{12}$$

If the studied activated sludge has a yield coefficient (estimated for instance by using respirometry) deviating from the one reported in literature, the f_p-value must be adapted for this. Keesman *et al.* (1998) showed that, theoretically, the value of f_p can be estimated directly from a batch test in which only the evolution of the respiration rate and MLVSS are monitored over a sufficiently long time.

Nitrogen content of the inert biomass-COD i_{xp}

Although theoretically possible, as illustrated with the arrows in Table 1, no respirometric method has been reported to estimate this parameter.

KINETIC PARAMETERS

Heterotrophic decay coefficient b_H

The death-regeneration concept, adopted in the ASM1, implies that the classical methods for determination of the decay of biomass cannot be used directly. The parameter of the one concept have to be translated to the parameter of the other concept, similarly to f_p (see Eq. 12), leading to the model decay coefficient:

$$b_H = \frac{b_H'}{1 - Y_H(1 - f_P)} \tag{13}$$

Here too, the stoichiometric parameters Y_H and f_p are necessary for estimation of b_H.

The respirometric method for determination of b_H described by Henze *et al.* (1987) is the protocol proposed by Marais and Ekama (1976). Sludge is removed from a completely mixed reactor and put into an aerated and non-fed batch reactor. The (endogenous) respiration rate is measured at many times t_k over a period of several days. The plot of the logarithm of the respiration rate rend (Sollfrank and Gujer, 1991):

$$r_{end} = (1 - f_P') \cdot b_H' \cdot X_{BH} \tag{14}$$

versus time visualises the exponential decrease of the biomass as a straight line with slope b'_H.

Spanjers and Vanrolleghem (1995) base their estimation of b_H on the fact that the respiration rate for substrate oxidation is also proportional to the heterotrophic biomass concentration:

$$r_{ex} = \mu_{mH} \cdot \frac{1 - Y_H}{Y_H} \cdot \frac{S_S}{K_S + S_S} \cdot \frac{S_O}{K_{O,H} + S_O} \cdot X_{BH} \tag{15}$$

If a sufficiently high amount of oxygen S_O and substrate S_S is present, r_{ex} is not substrate limited and is proportional to X_{BH}. Consequently, the decay of the heterotrophic biomass can be determined by: (i) taking a sludge sample from the aerated and non-fed batch reactor at times t_k; (ii) adding sufficient substrate; and (iii) measuring the maximum respiration rate. Assuming that Y_H and μ_{mH} remain constant during incubation, plotting the logarithm of $r_{ex}(t_k)$ versus time allows the determination of b'_H as the slope of the linear regression.

Vanrolleghem *et al.* (1992) described a fast method for estimation of b'_H using only one measurement of the endogenous respiration (in absence of nitrification) in a batch reactor. By means of Eq. 13 describing endogenous respiration, b_H can be calculated on condition that f_p and X_{BH} are known.

Autotrophic decay rate coefficient b_A

Notice that the death-regeneration concept is not used for the autotrophic biomass. Hence, the determination of the decay rate coefficient can be performed by using the classical method: the autotrophic decay is estimated from a batch experiment with addition of ammonium at different times. The decrease in the maximum r_{nit} is the result of the decrease of the autotrophic biomass concentration X_{BA}, on condition that sufficient ammonium and oxygen are supplied in the batch experiments. The r_{nit} is modelled by:

$$r_{nit} = \mu_{mA} \cdot \frac{4.57 - Y_A}{Y_A} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \cdot \frac{S_O}{K_{O,A} + S_O} \cdot X_{BA} \tag{16}$$

Logarithmic transformation of the maximum r_{nit} and linear regression of these measurements versus time provides the autotrophic decay rate coefficient as the slope of the regression.

Vanrolleghem and Verstraete (1993) proposed an experimental design which enables simultaneous measurement of both heterotrophic and autotrophic maximum respiration rates. A mixture of ammonium and carbon source (e.g. acetate) is added to endogenous sludge. The maximum respiration rates for carbon oxidation and nitrification can be derived from the respirograms (e.g. Fig. 4). Hence, if such experimental data are collected at different times t_k, they can be used for the simultaneous determination of the heterotrophic and autotrophic decay rate coefficient. Spanjers and Vanrolleghem (1995) applied this method and Fig. 4 shows the r_{ex}-data for two respirometric tests performed after one and seven days of sludge incubation.

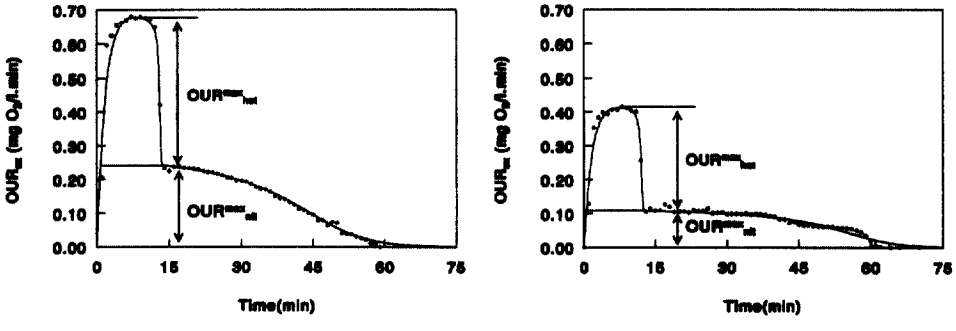

Figure 4. Respirograms obtained after injection of a C/N mixture for the simultaneous determination of b_H and b_A according to the procedure of Spanjers and Vanrolleghem (1995). Left after 1 day incubation, right after 7 days.

Nowak *et al.* (1994) pointed to the fact that the release of nitrogen due to decay of heterotrophic biomass may result in some growth of nitrifying organisms. Therefore they proposed the incubation of the sludge under anoxic conditions to prevent their growth. A daily sludge sample was removed from the anoxic reactor and (after aeration) the maximum r_{nit} was determined. This experiment gave an anoxic decay rate coefficient b_A which had a 50% lower value than the b_A determined under aerobic incubation. Note, however, that this may be an intrinsic reduction in decay rate as a result of the different environmental conditions.

Maximum specific heterotrophic growth rate μ_{mH} and half-saturation constant K_s

The increase of the growth rate with increasing S_S concentration is depicted in Fig. 5. The maximum specific growth rate μ_{mH} and the half-saturation constant K_s determine this Monod-type evolution and need to be estimated. Cech *et al.* (1984) described a method in which a number of measurements is performed each of

which add one point to this $\{\mu_H, S_S\}$ plot. In this procedure experiments are performed with addition of different amounts of waste water (substrate) to endogenous sludge, allowing achievement of various exogenous respiration rates up to the maximum rate. The specific growth rate, μ_H, for each experiment is:

$$\mu_H = \frac{Y_H}{1-Y_H} \cdot \frac{r_{ex}}{X_{BH}} \tag{17}$$

Knowledge of the yield coefficient and the concentration of heterotrophs is essential for the determination of μ_H. Provided the concentration of substrate in the waste water is known, the parameters μ_{mH} and K_s can then be estimated by fitting the Monod model:

$$\mu_H = \mu_{mH} \cdot \frac{S_S}{K_S + S_S} \tag{18}$$

to the constructed $\{\mu_H, S_S\}$ data set.

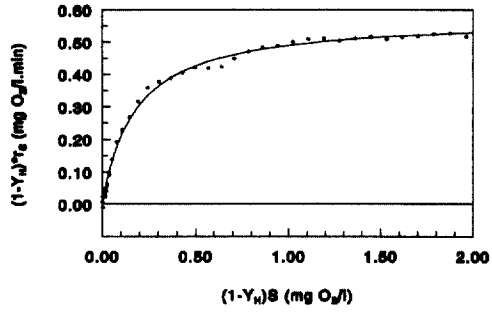

Figure 5. r_{ex} versus substrate plot enables the estimation of the parameters for growth.

The method described above is time consuming and the experimental effort is high. A more efficient experimental design is presented by Kong *et al.* (1994). It is based on the fact that added readily biodegradable substrate is converted by the sludge, so that in a batch set-up each substrate concentration lower than the initial concentration appears in the mixed liquor as the experiment proceeds (Fig. 6). Provided the initial substrate concentration is sufficiently high, one experiment can suffice to determine the heterotrophic growth kinetics. Indeed, after integration of the r_{ex} data set and given Y_H, the evolution of S_S is given by:

$$S_S(t_k) = S_S(0) - \frac{1}{1-Y_H}\left(\int_0^{tk} r_{ex}\,dt\right) \tag{19}$$

At this point a plot of $r_{ex}(t_k)$ vs. $S_S(t_k)$ can be made as illustrated in Fig. 5 that is the result of replotting the data of Fig. 6. After calculation of the growth rates μ_H from the r_{ex} data and estimates for Y_H and X_{BH} (Eq. 17), μ_{mH} and K_S can be estimated from Eq. 18. Note that it is not necessary to convert the $\{r_{ex}(t_k), t_k\}$ data to a $\{S_S, r_{ex}\}$ data set because a direct fit of the mathematical model to the $\{r_{ex}(t_k), t_k\}$ data will result in the same parameters (Sollfrank and Gujer 1991; Spanjers and Keesman, 1994, Vanrolleghem and Keesman, 1996).

A batch experiment with high waste water/sludge ratio was proposed by Kappeler and Gujer (1992), that also enables estimation of μ_{mH} and K_S from a single experiment. Figure 7 shows a respirogram obtained with such an experiment. Assessment of μ_{mH} can be done without knowledge of Y_H due to the fact that the exponential growth can be measured directly in the presence of excess substrate. A plot of the logarithm of

the remeasurements versus time has the slope (μ_{mH}-b_H). Provided b_H is known, calculation of μ_{mH} is possible. Attention has to be paid to the fact that the high substrate/biomass ratio in this experimental set-up (S_{to}/X_{to} ratio equals about 4/1) gives rise to significant growth of the biomass during the experiment. This means that the observed kinetic characteristics are no longer representative for the microbial population, but only for the organisms that become dominant during the experiment. Novak *et al.* (1994) gave practical evidence for this hypothesis by evaluating results from experiments with different S_{to}/X_{to} ratios. A 2.5 times higher specific growth rate was obtained at high S_{to}/X_{to} ratio.

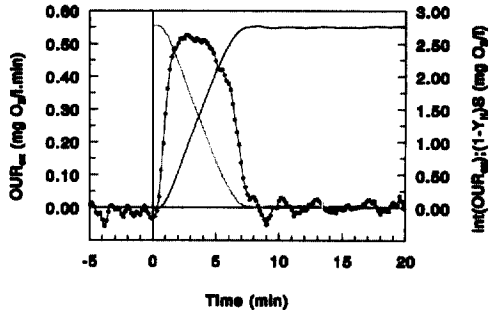

Figure 6. r_{ex} (symbols), cumulative oxygen uptake (increasing line) and substrate concentration (decreasing line) in batch experiment.

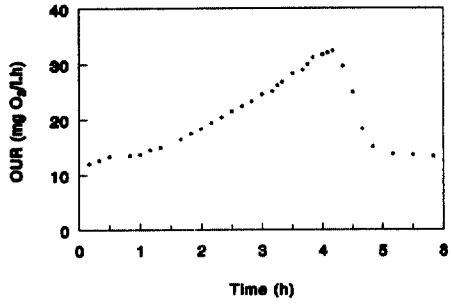

Figure 7. Respiration rates measured in a batch nitrification model is used to "eliminate" the nitrification experiment for estimation of μ_{mH} and K_S.

With this in mind Spanjers and Vanrolleghem (1995) presented experiments with a much lower substrate biomass ratio. Due to the necessity to have sufficiently high substrate concentrations in the respiration vessel to attain saturation of the respiration rate, the experiment has to be performed with a high biomass concentration to achieve a low S_{to}/X_{to}-ratio. Obviously, this results in short experiments. Figure 8 shows a typical respirogram from a so-called S_{to}/X_{to} =1/200 experiment. First, the oxygen consumption is governed by simultaneous carbon oxidation and nitrification. The peak in r_{ex} is due to the oxidation of S_S. After some time only nitrification and oxidation of substrates released by hydrolysis occur. Spanjers and Vanrolleghem (1995) showed that, provided Y_H and X_{BH} are known, it is possible to estimate the kinetic parameters μ_{mH} and K_s by fitting the mathematical model to the respirometric data.

Experiments in the presence of a nitrification inhibitor ATU were performed to quantify the contribution of nitrification to the respiration rate. This is shown in the insert of Fig. 8. It is reported that ATU may have negative effects on the heterotrophic biomass.

Another approach circumventing ATU-addition consists of the following two-step procedure. First, the nitrification process is kinetically characterised (μ_{mA}, K_{NH}) using the methods described below. In a second step, this calibrated (after Kappeler and Gujer, 1992). oxygen consumption In an experiment with waste water (e.g. Fig. 8). The amount of nitrogen in the waste water sample can be estimated at the same time as it

determines the length of the nitrification shoulder. Spanjers and Vanrolleghem (1995) demonstrated that the ATU- and model-based elimination of the nitrification respiration rate lead to similar values for the biokinetic parameters and waste water characteristics.

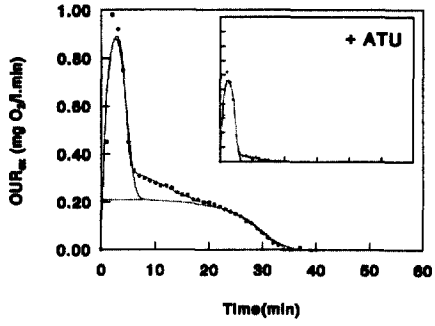

Figure 8. Respiration rate after injection of 70 ml raw waste water to 1.5 l activated sludge. Insertion: similar experiment but after addition of ATU.

Maximum specific autotrophic growth rate μ_{mA} and half-saturation constant K_{NH}

Nowak *et al.* (1994) applied the method of Cech *et al.* (1984) for determination of the heterotrophic growth kinetics to evaluate nitrification kinetics as well. For different ammonium concentrations the specific respiration rates for nitrification are determined. The specific growth rate, calculated from the respiration rate after division by $(4.57\text{-}Y_A)/Y_A$ and the nitrifying biomass concentration X_{BA}, can be evaluated as a function of the ammonium concentration (similarly as in Fig. 5) giving estimates for the half-saturation constant K_{NH} and the specific growth rate μ_{mA}. Drtil *et al.* (1993) proposed a respirometric protocol with less experimental effort for the determination of the growth parameters of a sludge with low nitrifying activity.

Experimental data as presented in Fig. 9 allow estimation of Y_A, μ_{mA} and K_{NH} by direct fitting of the model for respiration by nitrifying biomass to the data set. However, the value for, μ_{mA} only can be found when the concentration of autotrophic biomass is known (Spanjers and Vanrolleghem, 1995).

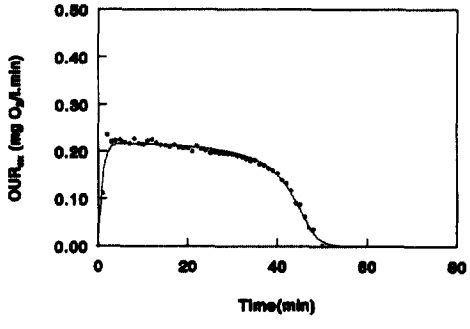

Figure 9. Respirogram obtained after injection of 3.31 mg NH_4-N in 1.4 l activated sludge.

Hydrolysis constants k_h, K_x

The only experimental protocol - as far as is known to the authors - enabling determination of both parameters of the hydrolysis is the "cyclic square wave feed" experiment proposed by Ekama *et al.* (1986). Respiration rates are measured in a completely mixed pilot reactor operated under a daily cyclic square wave feeding pattern (12 h with feed; 12 h without feed). Typically, a profile as shown in Fig. 2 is obtained. The sudden drop in the respiration rate after termination of the feeding can be used to estimate the S_S-

concentration in the influent. To determine the parameters of hydrolysis the data obtained after the *r*-drop are important. If *r* remains constant on a plateau value (as is noticed in Fig. 2 between 12 and 15 h), this is related to the fact that the hydrolysis proceeds at maximum rate and the biomass is saturated with hydrolysable products ($X_S/X_{BH} >> K_x$). As such, these data contain the information to assess the value of k_h on condition that the heterotrophic biomass concentration X_{BH} and the yield coefficient Y_H are known. With decreasing X_S the rate of hydrolysis also decreases and the respiration rate is dependent on the value for K_x, allowing its estimation. Estimation of the parameters is best by means of model optimisation (Henze *et al.*, 1987).

In many cases the dependency of the rate of hydrolysis on the heterotrophic biomass concentration may be neglected and a first order hydrolysis is obtained (Sollfrank and Gujer, 1991). This implies that $X_S/X_{BH} << K_x$. The first order coefficient k_h/K_x is derived by semilog transformation or by non-linear parameter estimation.

Sollfrank and Gujer (1991) proposed a method to determine the first order hydrolysis constant using respiration rates measured by dosage of wastewater to a continuous flow pilot reactor. To simplify the estimation, they suggested to plot the respiration rate versus the residual amount of substrate. In this plot one is able to isolate a linear part from which the hydrolysis constant k_h/K_x is deduced (provided Y_H is known).

For estimation of the hydrolysis constant k_h/K_x Kappeler and Gujer (1992) performed a batch-wise experiment with an initial COD based substrate/biomass ratio which was 10 times higher than for the experiment for determination of the maximum growth (S_{to}/X_{to} = 1/2). Figure 3 shows the respiration data of such an experiment. Once the readily biodegradable substrate S_S is removed (in Fig. 3 after 0.75 h) the further decrease of the respiration rate is determined by hydrolysis of X_S. As a consequence, *r*-measurements enables estimatation of the hydrolysis rate constant. The authors advise to do this exercise at different biomass concentrations to check for a possible dependency of the rate of hydrolysis on the biomass concentration.

Spanjers and Vanrolleghem (1995) preferred an experiment with low S_{to}/X_{to} and a model based approach for the estimation of k_h/K_x. Moreover, they tried to avoid the use of a nitrification inhibitor for elimination of the nitrification respiration rate (Fig. 8). After degradation of the readily biodegradable S_S in the first 7 minutes, only the hydrolysis "tail" remains (with nitrification "eliminated" with ATU or a nitrification model). By means of the model for hydrolysis and heterotrophic growth the hydrolysis rate constant can be found. An example of a data set that could be interpreted in this way is given in Fig. 1.

Parameters of "switching functions" $K_{O,H}$, $K_{O,A}$

Kappeler and Gujer (1992) determined the respiration rate in function of different oxygen concentrations in the respiration chamber of their respirometer. According to these authors the concentration of readily biodegradable substrate S_S needs to exceed a minimal concentration in order to have an accurate determination of $K_{O,H}$ The same technique can be used for $K_{O,A}$ with ammonia as substrate.

Ammonification rate constant k_a

So far, no respirometric method has been reported for the determination of the ammonification rate. However, it is theoretically possible (see Table 1) to assess this parameter from the evolution of the oxygen consumption for nitrification resulting from ammonified nitrogen.

Correction factors for anoxic growth and hydrolysis η_g, η_h

For determination of the anoxic correction factors, the ratio of nitrate to oxygen utilisation rates under anoxic and aerobic conditions must be calculated. For η_g an experiment has to be performed in the presence of an excess of S_S. The correction factor for anoxic hydrolysis η_h is calculated from an experiment in which growth is limited by hydrolysis. The correction factors are given by (Kristensen *et al.*, 1992):

$$\eta_g = 2.86\frac{r_{NO,g}}{r_{O,g}} \quad ; \quad \eta_h = 2.86\frac{r_{NO,h}}{r_{O,h}} \tag{20}$$

Information-rich data e.g. nitrate, ammonium, oxygen and biomass measurements in alternating systems (Carstensen *et al.*, 1995), enable estimation of correction factors from data of the full scale installation only.

DISCUSSION

The review has been structured along the estimation of: (i) concentrations of the different components in waste water and sludge; and (ii) stoichiometric and kinetic parameter values. Another approach could have been followed as well consisting of structuring the overview according to the different respirometric experiments used in model calibration. In such a structure, it would have been more obvious that efficient experiments are gradually being designed for simultaneous estimation of ASM1 biokinetic parameters and component concentrations or combinations of these. It can be envisaged that in the future efficient experimental designs will be created in which a maximum of information is gathered at a minimum of experimental efforts.

In Table 2 the experiments described above are concisely represented. Attention is drawn to:

(i) the type of reactor set-up (continuous or batch experiment) and the additions performed;
(ii) the requirement for other information collected from other experiments (or assumed);
(iii) major assumptions made during the interpretation of the respirometric data;
(iv) the reference where more information can be found.

Theoretically nearly all parameters and component concentrations can be estimated using respirometry. However, one should bear in mind that the aim of these calibration experiments is to obtain a model that can describe full-scale behaviour. It is well accepted that care should be taken in the transfer of results derived from lab-scale experiments to a model of the full-scale system. The reasons for problems with transferability are on the one hand differences in experimental conditions between lab-scale and full-scale experiments and, on the other hand, differences in the models used.

At the experimental level the lab-scale behaviour may not equal the full-scale behaviour due to, for instance, differences in feeding pattern resulting in other concentration profiles, differences in environmental conditions such as pH, temperature or mixing, or differences in sludge history. The ratio between initial substrate concentration (S_{t0}) and initial biomass concentration (X_{t0}) is considered to be one of the important factors in the design of lab-scale batch experiments in order to get a system response sufficient for interpretation (Vanrolleghem *et al.*, 1995). If relatively little biomass is present then the measured respiration rate may be too small and the test may take too long. If there is much biomass relative to the amount of components, then the respirometric response may be too short for a reliable measurement, or be swamped into the endogenous rate. Of a more basic nature is the observation that the S_{t0}/X_{t0} ratio directly influences the behaviour of the sludge, leading to different characteristics (Chudoba *et al.*, 1992; Grady *et al.*, 1996; Pollard *et al.*, 1998).

At the modelling level the model used to describe the results from lab-scale experiments may be different from the model used to describe the full-scale behaviour. Although not obvious at first sight, the use of a simple model for interpretation of the lab-scale data increases calculation speeds significantly, resulting in, for instance, a faster and more straightforward parameter estimation. Problems arise when the model uses different concepts that may not allow the estimated parameters to be carried from one model to the other, e.g. the death regeneration versus endogenous respiration (Yuan and Stenstrom, 1996).

Thus one may ask why it is necessary to carry out lab-scale experiments at all. The answer to this is simply that full-scale experiments for biological characterisation in most cases are not practicable or not informative enough (Vanrolleghem and Coen, 1995).

Table 2. Overview of respirometric methods for estimation of (combinations of) ASM1 parameters and component concentrations.
(Greyed fields indicate that a respirometric method is either not possible or not relevant)

Sym-bol	Type of experiment	Other information necessary	Assumptions	References
Components in waste water				
S_I				
S_S	B, WW add. B, WW C C (on/off)	Y_H Y_H, μ_{mH}, K_S Y_H Y_H		E86 We95 Wi96 E86
X_I				
X_S	B, WW	Y_H		KG92; SV95
X_{BH}	B, WW	Y_H		KG92; We95
X_{BA}	B, WW	Y_A		this paper
X_P				
S_O				
S_{NO}				
S_{NH}	B, WW	Y_A,$i_{XB}Y_H$ COD^{Deg}		SV95; Br98
S_{ND}	B, WW	Y_A,$i_{XB}Y_H$ COD^{Deg}		this paper
X_{ND}	B, WW	Y_A,$i_{XB}Y_H$ COD^{Deg}		this paper
S_{ALK}				
Components in activated sludge				
X_I				
X_S	B	Y_H		Br98
X_{BH}	C StSt	COD^{Deg}, b_H, Y_H		L95; U97
X_{BA}	C StSt	N^{Nitr}, b_A, Y_A		DS94
X_P	C StSt	X_{BH}, b_H, f_P		this paper
Stoichiometric parameters				
Y_H	B, WW add. B, Ac add.	S_I	 Ac representative of S_S	SG91 Br94
Y_A	B, NH_4 add. B, NH_4+Ac add.	 i_{XB}, Y_H	i_{XB}≅0	SV95 SV95
f_P	B	MLVSS		K98
i_{XB}	B, COD add.	Y_H, Y_A, ΔCOD^{Deg}		this paper
i_{XP}				

Sym-bol	Type of experiment	Other information necessary	Assumptions	References
Kinetic parameters				
μ_{mH}	B, *n* WW adds. B, WW add. B, WW add. B, WW add.	Y_H, X_{BH} Y_H, X_{BH} b_H Y_H, X_{BH}	 μ_H repres. original X_{BH}	C84 K94 KG92 SV95
K_S	B, *n* WW adds. B, WW add. B, WW add. B, WW add.	Y_H, X_{BH} Y_H, X_{BH} b_H Y_H, X_{BH}	 K_S repres. original X_{BH}	C85 K94 KG92 SV95
$K_{O,H}$	B, *n* S_O	S_O	S_S sufficiently high	KG92
K_{NO}				
b_H	B, no add. B, *n* Ac add. B, no add.	f_P, Y_H f_P, Y_H	 Y_H , μ_H constant	ME76; SG91 SV95 V92
μ_{mA}	B, *n* NH_4 adds. B, NH_4 add.	Y_A, X_{BA} Y_A, X_{BA}		C84 D93; SV95
K_{NH}	B, *n* NH_4 adds. B, NH_4 add.	Y_A, Y_A		N94 D93; SV95
$K_{O,A}$	B, *n* S_O	S_{NH}	S_{NH} sufficiently high	KG92
b_A	B, NH_4 add. B, *n* NH_4+Ac add.			H87; N94 SV95
η_g	B, WW add	$r_{NO,g}$		K92
k_a	B, WW add.			this paper
k_h	C (on/off) C, WW add. B, WW add.	Y_H, X_{BH}	max. hydrolysis rate K_X very large K_X very large	E86 SG91 KG92; SV95
K_X	C (on/off)	Y_H, X_{BH}		E86
η_h	B, WW add	$r_{NO,h}$		K92

Ac: acetate
add.: addition
B: respirometry in batch reactor
C: respirometry in continuous system
StSt: steady state
WW: waste water

Br94 Brands *et al.*, 1994
Br98 Brouwer *et al.*, 1998
C84 Cech *et al.*, 1984
D93 Drtil *et al.*, 1993
DS94 Dupont & Sinkjaer, 1994
E86 Ekama *et al.*, 1986
H87 Henze *et al.*, 1987
KG92 Kappeler & Gujer, 1992
K98 Keesman *et al.*, 1998
K94 Kong *et al.*, 1994
K92 Kristensen *et al.*, 1992
L95: Lokkegaard Bjerre *et al.*, 1995
ME76 Marais & Ekama, 1976
N94 Nowak *et al.*, 1994
SG91 Sollfrank & Gujer, 1991
SV95 Spanjers & Vanrolleghem, 1995
U97 Ubisi *et al.*, 1997
V92 Vanrolleghem *et al.*, 1992
We95 Wentzel *et al.*, 1995
Wi96 Witteborg *et al.*, 1996

CONCLUSIONS

The above overview of respirometric experiments for calibration of the Activated Sludge Model No. 1 clearly reflects the creativity shown by researchers involved in this field to estimate parameters and component concentrations from simple and sophisticated experimental designs. The review was structured along the estimation of components and parameters, not revealing the fact that single experiments can yield combinations of parameter values and component concentrations. In the future this minimisation of calibration efforts will be pursued further by design of more comprehensive (respirometric) experiments. The paper has not evaluated the experiments in great detail but has tried to focus attention to the more important limitations of experiments. Special attention was drawn to the potential danger of using lab-scale experimental results in a full-scale model. Clearly, more work is warranted on this aspect.

ACKNOWLEDGEMENT

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