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ACTIVATED SLUDGE MONITORING WITH COMBINED RESPIROMETRIC–TITRIMETRIC MEASUREMENTS

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Abstract—A short review of different respirometric methods is presented, and advantages and disadvantages of different principles are discussed. In this study a combined respirometric–titrimetric set-up was applied to monitor the degradation processes during batch experiments with activated sludge. The respirometer consists of an open aerated vessel and a closed non-aerated respiration chamber. It is operated with two oxygen probes resulting in two sources of information on the oxygen uptake rate; both collected at a high frequency. The respirometer is combined with a titrimetric unit that keeps the pH of the activated sludge sample at a constant value through the addition of acid and/or base. The cumulative amount of added acid and base serves as a complementary information source on the degradation processes. Interpretation of respirometric data resulting from validation experiments (additions of acetate and urea as ammonium source) showed that the set-up provided reliable data. Data interpretation was approached in two ways: (1) via a basic calculation procedure, in which the oxygen uptake rates were obtained by an oxygen mass balance over the respiration chamber, and (2) via a model-based procedure in which substrate transport was included for a more accurate data interpretation. Simulation examples showed that the presence of substrate transport in the model may be crucial for a correct data interpretation, since experimental conditions (e.g. low flow rate) and/or the biodegradation kinetic parameters (e.g. high K_S) may otherwise lead to data interpretation errors. Earlier studies already pointed out that titrimetric data can be related to nitrification, and this was also confirmed in this study. However, in addition, it was shown here for experiments with acetate that the amount of acid dosed was clearly related to the amount of acetate degraded. This indicates that the titrimetric data can be used to study the carbon source degradation. For the titrimetric data in this study, a model-based analysis was however only applied for the nitrification process. For an experiment with ammonium, it was illustrated that the estimation of biodegradation kinetics on a combined respirometric–titrimetric data set significantly improves confidence intervals of the parameters compared to the parameter estimation based on respirometric or titrimetric data separately.

Key words—activated sludge, confidence interval, nitrification, oxygen uptake rate, parameter estimation, respirometry, titrimetry

NOMENCLATURE

ASM1	activated sludge model N°1
b	biomass decay coefficient, 1/min
BOD_{st}	short-term biochemical oxygen demand, mg/l
BOD_{st}(basic)	BOD_{st} resulting from basic calculation method, mg/l
BOD_{st}(model)	BOD_{st} resulting from model-based data interpretation, mg/l
COD	chemical oxygen demand, mg/l
DO	dissolved oxygen, mg/l
f_{BA}	fraction of autotrophic biomass in activated sludge
f_{BH}	fraction of heterotrophic biomass in activated sludge
H_p	cumulative amount of protons formed or consumed during biodegradation, meq/l
i_{XB}	fraction of nitrogen in biomass, g N/g biomass COD
K_La	oxygen transfer coefficient, 1/min
K_S	heterotrophic half-saturation substrate concentration, mg COD/l
K_{SA1}	half-saturation substrate concentration for first nitrification step, mg N/l
K_{SA2}	half-saturation substrate concentration for second nitrification step, mg N/l
NH_4-N	ammonium nitrogen
Q_{in}	flow rate of liquid entering the system, l/min
r_{HpB}	background proton production rate, meq/l min
r_O	oxygen uptake rate, mg/l·min
$r_{O,1}$	oxygen uptake rate in aeration vessel, mg/l·min
$r_{O,2}$	oxygen uptake rate in respiration chamber, mg/l·min

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$r_{O,end}$	endogenous oxygen uptake rate, mg/l·min
$r_{O,ex}$	exogenous oxygen uptake rate, mg/l·min
R.S.D.	relative standard deviation (%)
S_S	readily biodegradable substrate concentration, mg COD/l
$S_{S,1}$	readily biodegradable substrate concentration in aeration vessel, mg COD/l
$S_{S,1(0)}$	initial readily biodegradable substrate concentration in aeration vessel, mg COD/l
$S_{S,2}$	readily biodegradable substrate concentration in respiration vessel, mg COD/l
S_{ALK}	alkalinity concentration, meq/l
S_{NH}	ammonium concentration, mg N/l
$S_{NH,1}$	ammonium concentration in aeration vessel, mg N/l
$S_{NH,1(0)}$	initial ammonium concentration in aeration vessel, mg N/l
$S_{NH,2}$	ammonium concentration in respiration chamber, mg N/l
S_{NO_2}	nitrite concentration, mg N/l
$S_{NO_2,1}$	nitrite concentration in aeration vessel, mg N/l
$S_{NO_2,2}$	nitrite concentration in respiration chamber, mg N/l
S_{NO_3}	nitrate concentration, mg N/l
S_O	dissolved oxygen concentration in the liquid phase, mg/l
S_O°	saturated dissolved oxygen concentration, mg/l
$S_{O,1}$	dissolved oxygen concentration in the aeration vessel, mg/l
$S_{O,2}$	dissolved oxygen concentration in the respiration chamber, mg/l
$S_{O,eq}$	equilibrium dissolved oxygen concentration, mg/l
$S_{O,in}$	dissolved oxygen concentration in the liquid entering the system, mg/l
S_O/X_O	initial substrate concentration to initial biomass concentration ratio
t	time, min
V	liquid volume of the respirometer, l
V_1	liquid volume of the aeration vessel, l
V_2	liquid volume of the respiration chamber, l
X	biomass concentration, g COD/l
X_{BA}	autotrophic biomass concentration, g COD/l
X_{BH}	heterotrophic biomass concentration, g COD/l
Y_A	autotrophic biomass yield, g COD/g N oxidised
Y_{A1}	autotrophic biomass yield of the first nitrification step, g COD/g N oxidised
Y_{A2}	autotrophic biomass yield of the second nitrification step, g COD/g N oxidised
Y_H	heterotrophic biomass yield, g COD biomass/g COD oxidised
μ_{max}	maximum specific growth rate, 1/min
μ_{maxH}	maximum specific growth rate for the heterotrophic biomass, 1/min
μ_{maxA1}	maximum specific growth rate for the first nitrification step, 1/min
μ_{maxA2}	maximum specific growth rate for the second nitrification step, 1/min

INTRODUCTION

In this paper respirometric and titrimetric techniques are reviewed. The advantages and disadvantages of different respirometric and titrimetric approaches are discussed with respect to their applicability for wastewater and sludge kinetics characterisation. In addition recent applications of a combined titrimetric–respirometric measuring principle for activated sludge are presented shortly.

Respirometry

Respirometry is the measurement and interpretation of the respiration rate of activated sludge, and is defined as the amount of oxygen per unit of volume and time that is consumed by the microorganisms in activated sludge. It is a frequently used tool for the characterisation of wastewater and activated sludge kinetics. The resulting data can, for example be applied in the frame of modelling and control of the aerobic parts of the activated sludge process (Henze *et al.*, 1987; Spanjers *et al.*, 1998; Vanrolleghem *et al.*, 1999). Several respirometric principles were developed in the past, and one can classify them into a number of basic measurement principles depending on two criteria: (1) The phase where oxygen is measured (gas or liquid), and (2) The flow regime of both gas and liquid phase, which can be either flowing or static (Spanjers *et al.*, 1998).

For most practical applications oxygen measurements are performed in the liquid phase. Hence, respirometric methods described in the sequel of this introduction will be limited to respirometers where oxygen is measured in the liquid phase using a dissolved oxygen electrode. The respiration rate is calculated by making a general mass balance for oxygen over the liquid phase (equation (1)). The equation includes, in that order, a transport term, an aeration term and a term describing the oxygen uptake rate (r_O) by the microorganisms. However, depending on the design of the respirometer the transport and the aeration term may not be needed as will be illustrated below.

$$\frac{dS_O}{dt} = \frac{Q_{in}}{V}(S_{O,in} - S_O) + K_L a(S_O^\circ - S_O) - r_O \quad (1)$$

Static gas–static liquid

A static gas–static liquid respirometer is typically operated by monitoring the decline in dissolved oxygen concentration S_O with time in a closed vessel after a short aerated phase (Vernimmen *et al.*, 1967; Cech *et al.*, 1984; Kappeler and Gujer, 1992; Kristensen *et al.*, 1992; Kroiss *et al.*, 1992; Drtil *et al.*, 1993; Ubay Çokgör *et al.*, 1998). In this type of respirometer calculating the mass balance of equation (1) becomes very simple because the transport and aeration terms can be omitted, resulting in equation (2).

$$\frac{dS_O}{dt} = -r_O \quad (2)$$

A completely closed respiration chamber with no headspace is needed because no aeration of the activated sludge sample (e.g. through surface aera-

tion or air bubbles in the liquid phase) may take place during the experiment. In case an open respiration chamber is used surface aeration may influence the measured data, and in that case equation (3) (see below) applies for a correct data interpretation. However, the contribution of the surface aeration is typically neglected (Farkas, 1969; Takamatsu *et al.*, 1982; Randall *et al.*, 1991). Alternatively one can try to limit the oxygen transfer through the liquid–air interface of the open vessel, for example by covering the surface with small plastic balls (Wentzel *et al.*, 1995), or through the use of a narrow respiration chamber that has about the same diameter as the dissolved oxygen electrode (Gernaey *et al.*, 1997b).

Because of the absence of aeration, and thereby danger of oxygen limitation, application of this type of respirometer is limited, especially for the determination of sludge kinetics and wastewater characteristics. Typically, experiments with these set-ups are carried out with high substrate concentrations and low biomass concentrations (high S_O/X_O ratio) to avoid the limitation of oxygen. However, the sludge behaviour when subjected to a very high S_O/X_O ratio may not be representative for the full-scale system (Novák *et al.*, 1994). Furthermore, experiments with high S_O/X_O ratio will most often only allow a determination of maximum growth rate and not of the half-saturation substrate concentration (K_S) since the substrate concentration may never drop to values near the value of K_S. Alternatively, oxygen limitation can be avoided by a regular reaeration of the sample (Suschka and Ferreira, 1986; Watts and Garber, 1993). This will allow higher sludge concentrations and thereby more realistic S_O/X_O ratios. Another way to solve the oxygen limitation problem is to oversaturate the activated sludge sample with pure oxygen or increased air pressure and in that way achieve a higher initial S_O concentration (Ellis *et al.*, 1996). The disadvantage may however be that the micro-organisms are exposed to S_O levels different from their natural environment. A closed respiration vessel is also very important in this case to avoid the transfer of oxygen from the oversaturated liquid phase to the gas phase. It should be added here that the respirometric methodology of Ellis *et al.* (1996) is not based on equation (2). In their approach the cumulative oxygen consumption (expressed in mg/l as function of time) is calculated as the difference between the initial and actual oxygen concentration. Finally, static gas–static liquid respirometers have been developed where the activated sludge in the closed vessel is replaced when the S_O concentration drops below a certain minimum, or when the retention time in the vessel exceeds a pre-set maximum (Dircks *et al.*, 1999).

The r_O sampling frequency is often rather low in these different kinds of static gas–static liquid respirometers since one will typically obtain one r_O value per aeration-measurement cycle. A low r_O sampling frequency is a disadvantage especially when the data are to be used to estimate activated sludge kinetic parameters (Vanrolleghem and Spanjers, 1998).

Flowing gas–static liquid

Flowing gas–static liquid respirometers are continuously aerated and have the advantage that higher sludge concentrations can be used, because there is a continuous input of oxygen to avoid oxygen limitation (Blok, 1974; Farkas, 1981; Ros *et al.*, 1988; Vanrolleghem *et al.*, 1990, 1994). A higher sludge concentration will typically allow a shortening of the experiment. The transport term of equation (1) is not needed and the mass balance over the liquid phase becomes

$$\frac{dS_O}{dt} = K_L a(S_O^o - S_O) - r_O \quad (3)$$

It should be noted that the oxygen dynamics might not be visible in case if the oxygen transfer coefficient $K_L a$ is too high. Moreover, too high aeration intensity may increase the risk of measurement noise. It is thus important to optimise the aeration in the respirometer in such a way that a reliable r_O value can be obtained. In general, the oxygen uptake rate (r_O) may be considered to consist of two components (Spanjers, 1993). The exogenous oxygen uptake rate ($r_{O,ex}$), that is the immediate oxygen uptake needed to degrade a substrate, and the endogenous oxygen uptake rate ($r_{O,end}$). The $r_{O,ex}$ is zero when no substrate is present, and in that case the oxygen concentration in the flowing gas–static liquid respirometer reaches a steady-state concentration $S_{O,eq}$ representing the equilibrium between oxygen transfer and endogenous respiration. Therefore, equation (3) can be transformed into equation (4) (Vanrolleghem *et al.*, 1994), under the assumption that $r_{O,end}$ is constant, which is a reasonable assumption for short-term experiments.

$$\frac{dS_O}{dt} = K_L a(S_{O,eq} - S_O) - r_{O,ex} \quad (4)$$

By applying equation (4) attention can be focused on the substrate degradation-induced respiration ($r_{O,ex}$) only. A flowing gas–static liquid respirometer allows to record r_O data with a higher frequency compared to most static gas–static liquid respirometers (e.g. one measurement every 10 s as in Vanrolleghem *et al.*, 1994). According to equation (4), $r_{O,ex}$ can be calculated from S_O data measured during the substrate degradation when the values of dS_O/dt, $S_{O,eq}$ and $K_L a$ are known. The factor dS_O/dt is the slope of the S_O curve, and is typically obtained by a moving data window regression on the S_O data. The $S_{O,eq}$ can be obtained easily from the respirogram as the S_O concentration measured during the endogenous respiration phase. For the oxygen transfer coefficient $K_L a$ several methods can be used to obtain its value (ASCE, 1996). The $K_L a$ can for example be obtained from a separate reaeration experiment with

sludge in endogenous state (Bandyopadhyay *et al.*, 1967). Alternatively, the K_La value can be estimated from a reaeration curve obtained after addition of a known readily biodegradable substrate to an activated sludge sample (Vanrolleghem, 1994).

Static gas–flowing liquid

In static gas–flowing liquid respirometers the S_O concentration at both the inlet and the outlet of a closed respiration chamber is measured (Spanjers, 1993). Aerated sludge is pumped continuously through the respiration chamber. The r_O is calculated by making an oxygen mass balance over the respiration chamber using the inlet ($S_{O,in}$) and outlet (S_O) dissolved oxygen concentration and the residence time (V/Q_{in}) in the chamber (equation (5)).

$$\frac{dS_O}{dt} = \frac{Q_{in}}{V}(S_{O,in} - S_O) - r_O \quad (5)$$

Knowledge of K_La is not necessary, which gives this type of respirometer an advantage in the study of more complex substrates such as wastewater for which K_La estimation may be problematic. The residence time (V/Q_{in}) is assumed to be known in this approach, and should be properly chosen to avoid oxygen limitation in the respiration chamber. A disadvantage is that a relatively small difference ($S_{O,in}-S_O$) must be calculated from the signals of two different dissolved oxygen probes, indicating that drift of the electrodes may cause erroneous r_O data. To deal with this, the S_O concentrations in inlet and outlet of the respiration chamber are measured by the same dissolved oxygen probe in the static gas–flowing liquid respirometer of Spanjers (1993). This was achieved by regular switching of the flow direction in the respiration vessel (e.g. once every minute). However, the frequent flow direction switch means that the response time of the electrode itself becomes important for the data interpretation (Spanjers and Olsson, 1992). Moreover, it is the cause of a lower measurement frequency of r_O (Vanrolleghem and Spanjers, 1998), typically one data point per minute (Spanjers, 1993). As an alternative to this respirometric principle, where the residence time in the respiration chamber is kept constant, Kalte (1990) proposed a respirometer in which the flow rate is varied to maintain a constant ($S_{O,in}-S_O$) set-point in order to asses the short-term biochemical oxygen demand (BOD_{st}).

Hybrid respirometer

As a compromise, taking the good and leaving out the bad elements of the different existing respirometers, the theoretical concept of a hybrid respirometric measurement principle was proposed (Vanrolleghem and Spanjers, 1998). In the hybrid respirometer, the principles of a flowing gas–static liquid and a static gas–flowing liquid respirometer are combined. The respirometer consists of an open aerated vessel and a closed non-aerated respiration chamber, and is equipped with two dissolved oxygen probes (Fig. 3). Sludge is continuously pumped between the aeration vessel and the respiration chamber. The advantages of the different respirometer principles are combined (Table 1): Use of two dissolved oxygen probes results in the high r_O data collection frequency of the flowing gas–static liquid respirometer. At the same time r_O can be calculated with a similar procedure as the static gas–flowing liquid respirometer because a mass balance for oxygen over the closed respiration chamber (equation (6)) avoids the need to estimate K_La values. In addition, a second mass balance for oxygen can be made over the aeration vessel as a second source of r_O data on condition that the K_La value can be estimated (equation (7)).

$$\frac{dS_{O,2}}{dt} = \frac{Q_{in}}{V_2}(S_{O,1} - S_{O,2}) - r_{O,2} \quad (6)$$

$$\frac{dS_{O,1}}{dt} = \frac{Q_{in}}{V_1}(S_{O,2} - S_{O,1}) + K_La(S_O^o - S_{O,1}) - r_{O,1} \quad (7)$$

Vanrolleghem and Spanjers (1998) proposed different implementations of the hybrid respirometer principle by changing the position of the dissolved oxygen probes in the set-up. In this study the configuration with the dissolved oxygen electrodes placed in an aeration vessel and in a respiration chamber was applied. For further information on the development and the evaluation of the hybrid respirometer the reader is referred to Petersen (2000). This respirometric principle has already been described earlier (Clarke *et al.,* 1978; Hissett *et al.*, 1982; Sollfrank and

Table 1. Comparison of advantages and disadvantages of different respirometric principles

Respirometer type	Static gas–static liquid	Flowing gas–static liquid	Static gas–flowing liquid	Hybrid respirometer
Advantages	Easy to operate	High r_O measurement frequency	No K_La needed	No K_La needed High r_O measurement frequency
Disadvantages	Danger for oxygen limitation Low r_O measurement frequency	K_La estimation needed	Low r_O measurement frequency	Two dissolved oxygen probes

Gujer, 1990). Both Clarke *et al.* (1978) and Hissett *et al.* (1982) applied a rather simple data interpretation method based only on the difference between the two measured S_O concentrations. Sollfrank and Gujer (1990) connected a respiration chamber to the aeration basin of an activated sludge pilot plant. They used the system to measure r_O of the biomass and the oxygen transfer coefficient in the aeration basin. However, in none of these cases a model-based interpretation of the data has been carried out.

Titrimetry

Besides respirometry, titration experiments can also yield information about the biological nitrogen removal processes in activated sludge (Ramadori *et al.*, 1980; Bogaert *et al.*, 1997; Gernaey *et al.*, 1997a). Indeed, the pH value of a biological system responds to microbial reactions and the evolution of the pH of a system often provides a good indication of some of the ongoing biological reactions. For activated sludge wastewater treatment plants the processes that mostly influence the pH of the liquid phase are: (1) nitrification which causes a pH decrease due to proton production (Ramadori *et al.*, 1980; Gernaey *et al.*, 1997a), (2) denitrification which causes a pH increase due to proton consumption (Bogaert *et al.*, 1997), (3) degradation of organic matter which affects pH due to (a) the uptake of the carbon source through the cell wall of the bacteria, (b) the release of CO_2 resulting from respiration processes in the liquid phase, (c) the uptake of ammonium for growth (San and Stephanopoulos, 1984; Iversen *et al.*, 1994; Siano, 1995 among others), (4) stripping of CO_2 due to aeration.

The pH effects observed in a liquid medium can be related to the biological process rates and kinetics. However, one difficulty encountered with the observation of pH changes is the variable buffer capacity of the liquid medium due to the presence of several acid–base buffer systems with pH depending buffer capacity (Stumm and Morgan, 1981). The pH variation of the liquid medium during biological reactions is thus difficult to convert into a precise number of protons that is released or consumed. The problems caused by the pH depending buffer capacity of the liquid medium can be avoided by controlling the pH of a liquid medium at a constant pH setpoint through the addition of acid and/or base. In that case, monitoring the acid and/or base consumption rate, needed to keep the pH constant, provides the rate of proton formation or consumption due to biological reactions.

Model-based analysis of titration data can be used to estimate parameters of the reactions. This approach has been used in fermentation (Iversen *et al.*, 1994), and specifically for wastewater treatment, a model-based analysis of titration data has already been successfully applied to the nitrification process (Gernaey *et al.*, 1998).

The main goal of this paper is to demonstrate and validate the methodology of combined respirometric and titrimetric experiments. The validation is performed with the addition of simple substrates, such as acetate and urea as ammonium source, to an activated sludge sample. Both a basic spread-sheet calculation and a model-based data interpretation will be applied for the interpretation of the data. Kinetic parameters will be estimated both on separate and combined data sets to investigate the parameter accuracy.

MATERIALS AND METHODS

Set-up

A schematic overview of the different components of the set-up is shown in Fig. 1. The set-up consists of an aeration vessel ($V = 2$ l) and a respiration chamber ($V = 0.5$ l). The respiration chamber is completely closed and does not contain air. Magnetic stirrers with adjustable speed mix the contents of both vessels. A peristaltic pump with adjustable speed (pump 1 in Fig. 1) is used to continuously pump the activated sludge in and around the set-up. A cooling system (Lauda WK1400) is used to control the temperature in the aeration vessel. The aeration vessel as well as the respiration chamber are equipped with a dissolved oxygen electrode (Ingold/Mettler Toledo, Inpro 6400). The dissolved oxygen probes are connected to a transmitter (Knick 73 O_2 for the aeration vessel and Knick Stratos 2401 Oxy for the respiration chamber). The 4–20 mA signals from the transmitters are logged by a PC equipped with the Labview software package (National Instruments) and a combined A/D I/O card (National Instruments, AT-MIO-16XE-50). In this set-up the calibration of the dissolved oxygen electrodes is done in two steps while aerated tap water is pumped through the set-up. First, the electrode in the aeration vessel is calibrated, and second the electrode in the respiration vessel is calibrated to give identical readings as the electrode in the aeration vessel.

The pH controller is installed in the aeration vessel. The pH in the aeration vessel is measured with a Mettler Toledo HA 405-DXK-S8/120 Xerolyte pH electrode connected to a Knick 73 pH transmitter. The 4–20 mA signal is logged with the same Labview software. pH control was also implemented in the Labview. The pH was controlled within a narrow pH setpoint $\pm \Delta$pH region, as described by Gernaey *et al.* (1997a). Only the base dosage system is

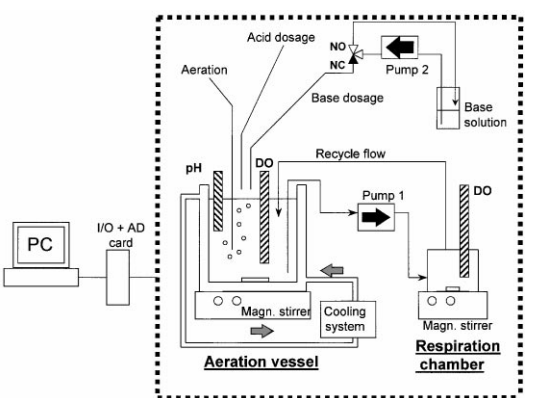

Fig. 1. Overview of the components of the combined respirometric–titrimetric set-up that was used to collect experimental data.

shown in Fig. 1 to avoid the scheme to be overloaded. The pH setpoint was typically chosen between 7.5 and 8.3, and a ΔpH value of 0.03 pH units was used. When the pH was out of the pH setpoint ± ΔpH region, dosage of acid (0.05 N) or base (0.05 N) was done by opening an electromagnetic pinch valve for a short period (typically 1.5 s = 1 pulse). Acid and base solutions were continuously pumped around by a peristaltic pump (pump 2 in Fig. 1) to keep a constant liquid pressure in the tubes and thus a constant dosage rate. When the valves are closed the acid and base flows are recycled to the storage vessels. Opening a valve diverts the acid or base flow to the aeration vessel. Calibration of the dosage system was done by collecting the volume of acid or base dosed during 50 subsequent pulses (average dosage = 3.32 ± 0.013 ml/50 pulses for 19 calibrations). The cumulative amount of acid and base dosed during an experiment were logged with the Labview software package.

Experimental work

Activated sludge was sampled at the combined municipal–industrial wastewater treatment plant of Zele (operated by Aquafin NV, Aartselaar, Belgium) and transported to the lab. At the start of an experiment the set-up was filled with 2.5 l of activated sludge. The activated sludge was aerated until the endogenous respiration phase was reached. During the experiments small substrate pulses (e.g. 10 ml) of acetate (10 g COD/l), ammonium (1 g N/l) and urea (1 g N/l) stock solutions were dosed to the activated sludge.

BASIC AND MODEL-BASED DATA INTERPRETATION

Data derived from each experiment were interpreted using both a spreadsheet program (Excel) and a model-based data interpretation method. The modelling work was done with the WEST™ software tool (Hemmis NV, Kortrijk, Belgium).

Basic data interpretation

The dissolved oxygen and titration data were processed using a spreadsheet program. The $r_{O,2}$ values were calculated by making a mass balance over the respiration vessel using the available S_O data of the aeration vessel ($S_{O,1}$) and the respiration chamber ($S_{O,2}$), as described in equation (6). The S_O measurements were corrected for the electrode response time, according to Spanjers and Olsson (1992), and $dS_{O,2}/dt$ was simply calculated with a moving window regression (over three data points). The BOD_{st} for each substrate addition was obtained as the area under the $r_{O,2}$ curve. The value of $r_{O,end}$ was subtracted from $r_{O,2}$ in this last calculation step. The titration data was interpreted by extrapolating the different slopes of the titration curves to obtain the amounts of base or acid needed to compensate the production or consumption during substrate degradation, as described by Gernaey *et al.* (1997a). Note that the titrimetric data are collected for the total volume of the set-up (values in meq) and converted to the unit meq/l via the division by the total reactor volume.

Model-based data interpretation

Respirometric data (the $r_{O,2}$ values that were calculated in the basic data interpretation step) were modelled by applying the model structure that is summarised in Table 2. The model in Table 2 is based on ASM1 (Henze *et al.*, 1987), with some modifications:

- Nitrification was modelled as a two-step process. In the first nitrification step ammonium (S_{NH}) is oxidised to nitrite (S_{NO_2}), which is subsequently oxidised to nitrate (S_{NO_3}) in the second nitrification step.
- Incorporation of S_{NH} into new biomass was neglected for the second nitrification step. It has been estimated that the error introduced by this assumption is less than 0.5%.
- Biomass decay was included in the model as endogenous respiration, and was assumed to be constant during the short duration of each experiment.
- The active biomass was lumped into one fraction X, instead of subdividing it into a separate fraction of nitrifiers ($X_{BA} = f_{BA}X$) and heterotrophs ($X_{BH} = f_{BH}X$) as proposed by Henze *et al.* (1987). The main reason for this is that biomass fractionation is difficult to perform for activated sludge, and on the other hand the main interest is

Table 2. Model used for interpretation of the respirometric and titrimeric data. Processes 1–5 were necessary to model respirometric data. For titrimetric data, processes 2 and 6 were used to model nitrification experiments

Process	Component							
	1 X	2 S	3 S_O	4 S_{NH}	5 S_{NO_2}	6 S_{NO_3}	7 H_p	Process rate
1. Heterotrophic growth on S_S	1	$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{Y_H}$	$-i_{XB}$			$\frac{i_{XB}}{14}$	$\mu_{maxH}\frac{S_S}{K_S+S_S}X$
2. Nitrification step 1, S_{NH} oxidation	1		$-\frac{3.43-Y_{A1}}{Y_{A1}}$	$-\frac{1}{Y_{A1}}-i_{XB}$	$\frac{1}{Y_{A1}}$		$\frac{i_{XB}}{14}+\frac{1}{7Y_{A1}}$	$\mu_{maxA1}\frac{S_{NH}}{K_{SA1}+S_{NH}}X$
3. Nitrification step 2, S_{NO_2} oxidation	1		$-\frac{1.14-Y_{A2}}{Y_{A2}}$		$-\frac{1}{Y_{A2}}$	$\frac{1}{Y_{A2}}$		$\mu_{maxA2}\frac{S_{NO_2}}{K_{SA2}+S_{NO_2}}X$
4. Endogenous respiration	−1		−1					bX
5. Aeration			1					$K_La(S_O^\circ - S_O)$
6. CO_2 stripping							1	r_{HpB}

usually focused on the maximum substrate removal rate of the total sludge (e.g. $X\mu_{maxH}/Y_H$ for the heterotrophs).

- In this study, a detailed model for the titrimetric data is applied only for the nitrification process (see Table 2). In the model protons (H_p) replace the ASM1 S_{ALK} component, which means that the signs of the stoichiometric factors in the H_p column are the opposite of the signs that appear in the S_{ALK} column in the ASM1 matrix (Henze *et al.*, 1987). In the model S_{NH} oxidation and uptake of S_{NH} for biomass growth during nitrification will produce H_p. A constant background H_p production is included in the titrimetric model to take CO_2 stripping into account, as was introduced by Gernaey *et al.* (1998). The effect of CO_2 stripping is assumed to be constant during the short duration of each experiment (Gernaey *et al.*, 1998). For heterotrophic growth, the standard ASM1 conversion term is included in the H_p column of Table 2 (uptake of S_{NH} to be incorporated into new biomass produces H_p). However, as stated above heterotrophic substrate (here acetate) degradation is not modelled in this study. For more details on this topic the reader is referred to Gernaey *et al.* (2000a, b).
- In the model the oxygen mass balances for the aeration vessel and the respiration chamber were described by equations (6) and (7). Furthermore, the model includes terms to describe (i) the first-order dissolved oxygen probe dynamics (Spanjers and Olsson, 1992) and (ii) the biological start-up phenomena, that are typically observed in batch experiments, before the oxygen uptake rate has reached its maximum value. This start-up phase, which typically lasts for 0.5–2 min, was assumed to be the time needed by a cell to take up fresh substrate and oxidise it, and can be described with a simple first-order equation (Vanrolleghem *et al.*, 1998). Whether $r_{O,1}$ and $r_{O,2}$ are identical will depend on the experimental design. This will be illustrated and explained below for a better understanding of the experimental results and their interpretation.

Besides substrate degradation and endogenous respiration (Table 2), substrate transport was included in the model, similar to the mass balances for oxygen (equations (6) and (7)). As an example the mass balance over the respiration chamber for the biodegradable substrate is given in equation (8).

$$\frac{dS_{S,2}}{dt} = \frac{Q_{in}}{V_2}(S_{S,1} - S_{S,2}) - \frac{\mu_{maxH} X}{Y_H} \cdot \frac{S_{S,2}}{K_S + S_{S,2}} \quad (8)$$

In this paper the model-based interpretation is applied in three steps:

1. Model-based evaluation of the behaviour of the respirometer
2. Model-based interpretation and evaluation of the experimental data to validate the respirometric method.
3. Model-based interpretation of the combined respirometric–titrimetric data including the calculation of confidence intervals.

RESULTS

Model-based evaluation of the behaviour of the respirometer

At the start of an experiment substrate is added in the aeration vessel, and not to the respiration chamber as stated above. Hence, the substrate concentration in the respiration chamber must build up from zero through substrate supply from the aerated vessel via the liquid flow. Obviously, the oxygen uptake rate in the two vessels ($r_{O,1}$ and $r_{O,2}$, respectively) is only equal when the substrate concentration is identical in both vessels.

Figures 2 and 3 show simulation results for an addition of biodegradable substrate to the aeration vessel at time zero. In Fig. 2 the results are given for two simulations obtained with different liquid flow rates (0.185 and 0.046 l/min). In the high flow rate experiment the initial substrate concentration in the respiration vessel ($S_{S,2}$) is zero, and it takes a little while before the substrate is properly mixed and the concentrations in the two vessels become identical (see Fig. 2(B)). Thus, initially $r_{O,1}$ and $r_{O,2}$ are not identical. However, as can be observed in Fig. 2(A) this difference is negligible at a flow rate of 0.185 l/min. In contrast, a flow rate of 0.046 l/min results in a situation where the substrate concentration never reaches the same value in the two vessels (Fig. 2(D)). As a consequence the values of $r_{O,1}$ and $r_{O,2}$ remain different during the whole experiment (Fig. 2(C)).

Whether $r_{O,1}$ is equal to $r_{O,2}$ will also depend on the value of the half-saturation concentration K_S for the particular substrate under study. In the simulations given in Fig. 2(A) and (B) the K_S value was set low to 0.5 mg COD/l, and as noticed above $r_{O,1}$ and $r_{O,2}$ were similar. However, a higher K_S value of 10 mg COD/l (Fig. 3(A) and (B)) resulted in an initial difference between $r_{O,1}$ and $r_{O,2}$ even at the high flow rate. A flow rate higher than 0.185 l/min would result in a smaller initial difference between $r_{O,1}$ and $r_{O,2}$ whereas a lower flow rate would increase the difference again.

It is important to remember that r_O values calculated with the basic method are based on the S_O mass balance over the respiration vessel only (equation (6)). The calculation thus yields $r_{O,2}$ values. This means that the BOD_{st} calculated based on the area under this $r_{O,2}$ profile may underestimate the real value depending on the experimental conditions and the biodegradation kinetics (e.g. K_S), as shown with the simulation examples. Besides an

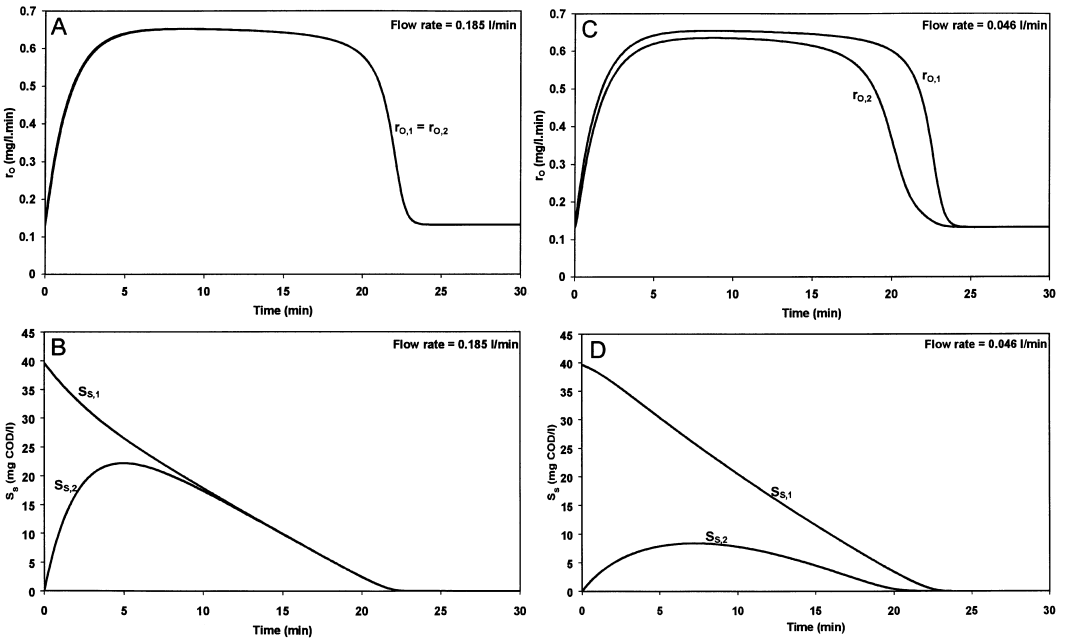

Fig. 2. Results of simulations with the respirometer model for an addition of readily biodegradable substrate to the aeration vessel at time zero. $S_{S,1}$ at time zero is 39.6 mg COD/l, $K_S = 0.5$ mg COD/l, $(\mu_{maxH}X) = 1.084$ g/l min: (A) $r_{O,1}$ and $r_{O,2}$ as a function of time for a liquid flow rate of 0.185 l/min; (B) $S_{S,1}$ and $S_{S,2}$ as a function of time for a liquid flow rate of 0.185 l/min; (C) $r_{O,1}$ and $r_{O,2}$ as a function of time for a liquid flow rate of 0.046 l/min; (D) $S_{S,1}$ and $S_{S,2}$ as a function of time for a liquid flow rate of 0.046 l/min.

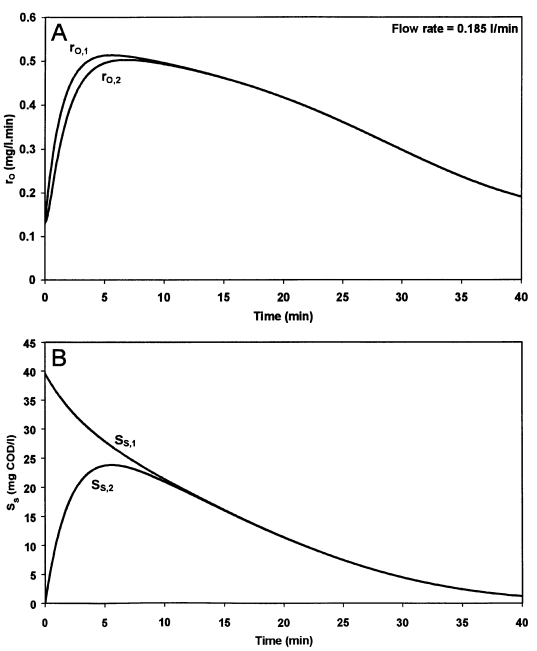

Fig. 3. Results of simulations with the respirometer model for an addition of readily biodegradable substrate to the aeration vessel at time zero. $S_{S,1}$ at time zero is 39.6 mg COD/l, $K_S = 10.0$ mg COD/l, $(\mu_{maxH}X) = 1.084$ g/l min. (A) $r_{O,1}$ and $r_{O,2}$ as a function of time for a liquid flow rate of 0.185 l/min; (B) $S_{S,1}$ and $S_{S,2}$ as a function of time for a liquid flow rate of 0.185 l/min.

underestimation of BOD_{st} there may also be a risk to underestimate μ_{max} (see Fig. 2(C)), and to overestimate K_S values (Fig. 2(C), tail of $r_{O,2}$ profile).

It should be noticed that the initial phase in the $r_{O,1}$ profile, before $r_{O,1}$ has reached a maximum, can only be due to biological start-up phenomena (Vanrolleghem *et al.*,1998). The initial phase of the $r_{O,2}$ profile, on the contrary, is due to a combination of biological start-up and substrate mixing phenomena, as described above. Thus, for a complete and accurate analysis of the respirometric data derived from the hybrid respirometer a model-based data interpretation, as will be illustrated below, may be needed.

Basic interpretation of experimental data

A typical raw data set recorded from an acetate dosage is shown in Fig. 4(A). At time zero 100 mg of acetate COD was added to the aeration vessel of the respirometer. The corresponding $r_{O,2}$ profile in Fig. 4(B) is rather typical, with a steep decrease from maximum r_O to endogenous $r_{O,end}$ as soon as the substrate is completely degraded (Vanrolleghem *et al.*, 1995). The pH controller also shows a clear response (Fig. 4(A)). During acetate degradation dosage of acid is needed to keep the pH of the mixed liquor at the pH setpoint (8.25 in this case). This is clearly related to the acetate degradation since acid dosage and $r_{O,2}$ drop at the same moment ($t = 22$ min). From $t = 22$ min on, acid dosage

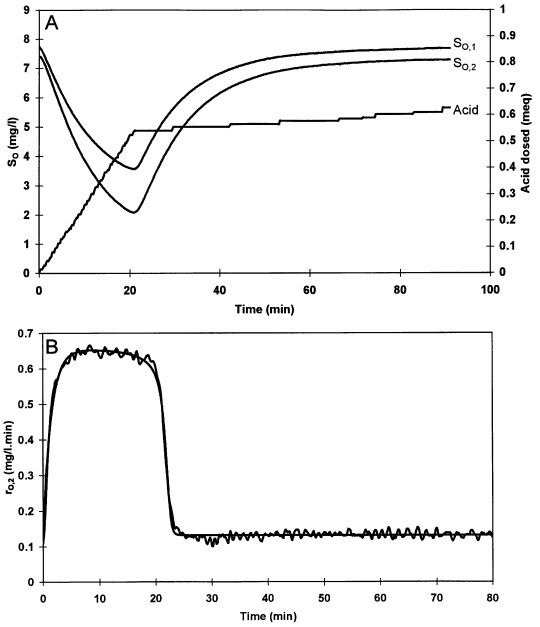

Fig. 4. Response of the combined respirometric–titrimetric sensor following a 100 mg COD acetate addition to the aeration vessel at $t = 0$. (A) S_O and titrimetric data; (B) $r_{O,2}$ values calculated based on the S_O data using a mass balance for S_O over the respiration vessel. The smooth line represents the model fit to the $r_{O,2}$ data.

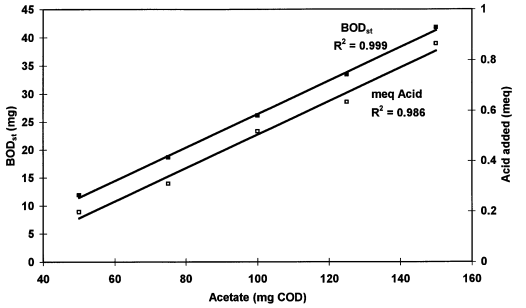

Fig. 5. BOD_{st} (calculated based on the area under the $r_{O,2}$ profiles) and total amount of acid dosed during degradation as a function of the initial amount of acetate added.

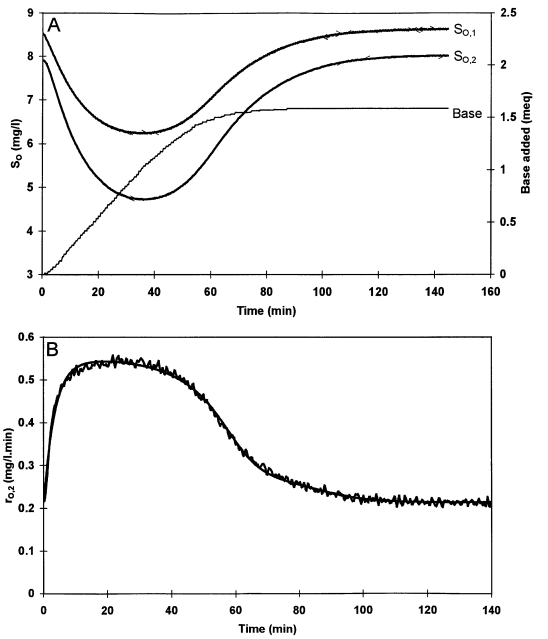

Fig. 6. Response of the combined respirometric–titrimetric sensor following an 11 mg N urea addition to the aeration vessel at $t = 0$. (A) S_O and titrimetric data; (B) $r_{O,2}$ values calculated based on the S_O data using a mass balance for S_O over the respiration vessel. The smooth line represents the model fit to the $r_{O,2}$ data.

dropped to the background dosage rate that was also observed before acetate addition. The background acid dosage rate is due to CO_2 stripping and is assumed to be constant during the experiment (Gernaey *et al.*, 1997a, 1998).

A linear relationship between the amount of substrate added and BOD_{st} is usually observed (Spanjers, 1993). The slope of this curve represents the oxygen demand per unit of COD or N, and allows the calculation of the biomass yield (via $BOD_{st} = (1-Y_H)COD$ or $BOD_{st} = (4.57-Y_A)S_{NH}$). The BOD_{st} values were calculated as the area under the $r_{O,2}$ curve. A linear increase of BOD_{st} was indeed observed when increasing amounts of acetate were added to the respirometer (Fig. 5). The maximum average yield for this data series is 0.74 with a standard deviation of 0.016. This yield is slightly higher but still comparable to the range of Y_H values (0.24–0.72) mentioned in literature for aerobic acetate degradation (Brands *et al.*, 1994; Liebeskind *et al.*, 1996; Xu and Hasselblad, 1996; Dircks *et al.*, 1999). Furthermore, a linear increase of the amount of acid (in meq) added during the degradation was observed as a function of the initial amount of acetate added (Fig. 5).

A data set collected from an addition of 11 mg urea NH_4-N in the aeration vessel of the respirometer is shown in Fig. 6(A), and the corresponding $r_{O,2}$ profile is given in Fig. 6(B). The $r_{O,2}$ profile has a tail indicating that the second nitrification step (oxidation of S_{NO_2} to S_{NO_3}) is slower compared to the first step. To verify the respirometric method the linearity between calculated BOD_{st} values and NH_4-N concentrations added is illustrated in Fig. 7. The slope $(4.57 - Y_A)$ of this curve is typically expected to be 4.33 g O_2/g NH_4-N for nitrification (Henze *et al.*, 1987). The slope of the curve in Fig. 7 is 4.44 ± 0.16 g O_2/g NH_4-N (95% confidence interval) which is slightly higher (2.5%) than expected, but acceptable considering that 4.33 lies within the confidence boundaries.

During nitrification dosage of base is needed to compensate for the proton production in the first nitrification step, as already observed in earlier literature (Ramadori *et al.*, 1980). For a series of NH_4-N additions to activated sludge, an average recovery of 1.01 with a standard deviation of 0.05 has

been obtained from titration data. Calculations were done assuming a production of 2 protons per NH_4-N oxidised, according to the method described by Gernaey *et al.* (1997a). This confirms the stoichiometric conversion factor of 2 protons/mg NH_4-N, in accordance with earlier observations (Massone *et al.*, 1995; Gernaey *et al.*, 1997a).

Model-based interpretation and evaluation of experimental data

Examples of the model fit on $r_{O,2}$ data obtained for an acetate and an urea addition are given in Figs 4(B) and 6(B) together with the respective $r_{O,2}$ data. To illustrate the two-step nitrification model further, the estimated evolution of the S_{NH} concentration in both reactors and the build up of S_{NO_2} are shown in Fig. 8.

The theoretically identifiable parameter combinations of a respirometric model describing the degradation of a single carbon substrate with Monod kinetics have been shown to be $(1 - Y_H)\mu_{maxH}X_{BH}/Y_H, (1 - Y_H)K_S$ and $(1 - Y_H)S_S(0)$, assuming that only $r_{O,ex}$ data are available, (Dochain *et al.*, 1995). For the two-step nitrification model assuming no growth, identifiable parameter combinations are obtained from Petersen *et al.* (2000), see Table 4.

The values of the parameter combinations estimated on acetate and urea $r_{O,2}$ profiles are given in Tables 3 and 4. In both cases the reproducibility of the estimates is good. Observed coefficient of variation (C.V.) values are low for μ_{max} related parameter combinations. In the literature C.V. values of 15.0% (Kong *et al.*, 1996) and 10.3% (Gernaey *et al.*, 1998) are mentioned from a similar estimation procedure with respirometric and titrimetric data, respectively. The C.V. values calculated for the K_S related parameter combinations are higher compared to C.V. values of μ_{max} related parameter combinations. This has been observed before in estimation of kinetic parameters based on respirometric data (Kong *et al.*, 1996), titrimetric data (Gernaey *et al.*, 1998) or substrate concentration data measured during a substrate depletion experiment (Robinson and Tiedje, 1982; Simkins and Alexander, 1985). The reason for the generally higher C.V. values for the K_S related parameter combinations compared to the

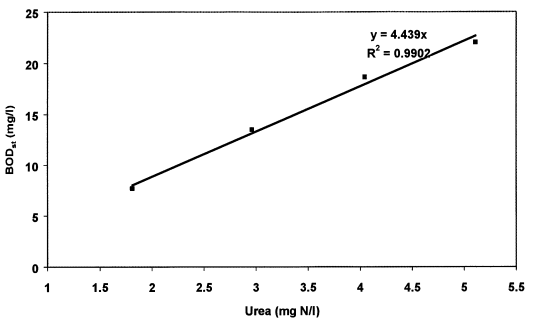

Fig. 7. BOD_{st} (calculated based on the area under the $r_{O,2}$ profiles) as a function of the initial urea concentration in the respirometer (expressed as mg N/l) .

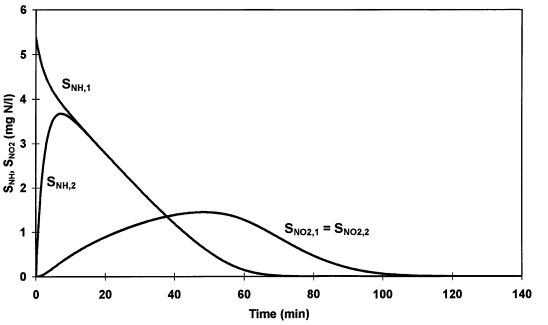

Fig. 8. Results of a simulation with the respirometer model for an addition of urea to the aeration vessel at time zero. $S_{NH,1}$ at time zero is 5.30 mg N/l, $K_{SA1} = 0.43$ mg N/l, $(\mu_{maxA1}X) = 0.318$ mg/l min, $K_{SA2} = 0.62$ mg N/l, $(\mu_{maxA2}X) = 0.090$ mg/l min, liquid flow rate = 0.18 l/min.

Table 3. Values of parameter combinations resulting from estimations on acetate $r_{O,2}$ profiles. The last 3 columns give a comparison between BOD_{st} values resulting from the model-based and basic data interpretation procedure, respectively

Parameter combinations → mg COD ↓ (Exp. No.)	μ_{maxH}[a]	K_S[b]	BOD_{st} (model)[c]	BOD_{st} (basic)	% deviation[d]
50 (1)	0.489	0.177	4.895	4.743	−3.1
75 (2)	0.506	0.160	7.560	7.388	−2.3
100 (3)	0.534	0.163	10.342	10.273	−0.7
125 (4)	0.558	0.155	13.066	13.017	−0.4
150 (5)	0.579	0.189	15.421	16.073	+4.2
Average	0.533	0.169	Correlation	0.999	
C.V. (%)	6.9	8.2			

[a] $\frac{1 - Y_H}{Y_H}\mu_{max}X$.

[b] $(1-Y_H)K_S$.

[c] $\frac{V_1}{V_1 + V_2}(1 - Y_H)S_1(0)$.

[d] $\frac{BOD_{st}(model) - BOD_{st}(basic)}{BOD_{st}(model)}100\%$.

Table 4. Values of parameter combinations for μ_{maxA1}, μ_{maxA2}, K_{SA1}, and K_{SA2} resulting from estimations on urea $r_{O,2}$ profiles. The last 3 columns give a comparison between BOD_{st} values resulting from the model-based and basic data interpretation procedure, respectively

Parameter combinations → mg NH_4-N↓ (Exp. No.)	μ_{maxA1}[a]	μ_{maxA2}[b]	K_{SA1}[c]	K_{SA2}[d]	BOD_{st} (model)[e]	BOD_{st} (basic)	% deviation[f]
5 (4)	0.334	0.098	1.872	1.285	8.106	7.712	−4.9
8 (1)	0.322	0.113	1.427	1.170	13.677	13.517	−1.2
11 (2)	0.318	0.090	1.398	0.666	18.467	18.651	+1.0
14 (3)	0.304	0.103	1.277	0.993	22.072	22.033	−0.2
Average	0.319	0.101	1.493	1.028	Correlation	0.999	
C.V. (%)	3.8	9.4	17.4	26.2			

[a] $\frac{3.43 - Y_{A1}}{Y_{A1}}\mu_{maxA1}X$.

[b] $\frac{1.14 - Y_{A2}}{Y_{A2}}\mu_{maxA2}X$.

[c] $(3.43 - Y_{A1})K_{SA1}$.

[d] $(1.14 - Y_{A2})K_{SA2}$.

[e] $\frac{V_1}{V_1 + V_2}((3.43 - Y_{A1}) + (1.14 - Y_{A2}))S_{NH,1}(0)$.

[f] $\frac{BOD_{st}(model) - BOD_{st}(basic)}{BOD_{st}(model)}100\%$.

μ_{max} related parameter combinations is probably that the confidence intervals on the estimated K_S values are larger than the confidence intervals on μ_{max} (Petersen *et al.*, 2000). Note also that the C.V. for the parameters of the second nitrification step is higher than for the first nitrification step. This is related to the lower amount of available data points related to the second step (i.e. the tail in the r_O profile) and thereby a poorer practical parameter identifiability.

It is also important to compare the calculated BOD_{st} (basic interpretation of $r_{O,2}$ values) with estimated BOD_{st} values. Estimated BOD_{st} is defined as $(1 - Y_H)S_{S,1}(0)$ for acetate and as $((3.43 - Y_{A1}) + (1.14 - Y_{A2}))S_{NH,1}(0)$ for nitrification. Note that the estimated substrate concentration is the initial substrate concentration in the aeration vessel at time zero and that therefore the estimated BOD_{st} values have to be multiplied with $V_1/(V_1 + V_2)$ to enable a comparision with the calculated BOD_{st}. When the calculated BOD_{st} values were regressed against the estimated BOD_{st} the slopes were close to 1, both in the case of urea and acetate and, furthermore, the correlation coefficients were close to 1 (0.99). This indicates that the experimental conditions applied in the experiments described in this study were such that the BOD_{st} values calculated as the area under the $r_{O,2}$ profile, obtained from a mass balance over the respiration vessel only, were sufficiently accurate.

Model-based interpretation of combined respirometric–titrimetric data

The two-step nitrification model was applied to describe and estimate parameters for an experiment with addition of ammonium. Parameters were estimated on $r_{O,2}$ and H_p data separately as well as on the combined $r_{O,2}$ and H_p data set. The data and the model fit resulting from parameter estimation on the combined data set are illustrated in Fig. 9. The resulting values of the parameters μ_{maxA1} and K_{SA1} together with their 95% confidence interval are given in Table 5. However, details on the confidence

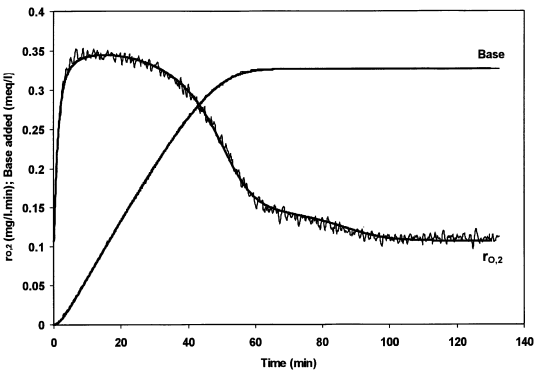

Fig. 9. Model fit to $r_{O,2}$ and base addition data collected in an experiment with addition of 7.5 mg NH_4-N to the respiration vessel at $t = 0$. Parameter combinations were estimated on the combined respirometric–titrimetric data.

Table 5. Results of parameter estimations on $r_{O,2}$ and H_p data separately and on combined data for an addition of NH_4-N to activated sludge. R.S.D. = relative standard deviation

Data source	Estimated parameter	
	μ_{maxA1} (min^{-1})	K_{SA1} (mg NH_4-N/l)
$r_{O,2}$ data	3.27E-06	0.299
H_p data	3.11E-06	0.253
$r_{O,2} + H_p$ data	3.26E-06	0.295
95% confidence intervals (R.S.D. %)	μ_{maxA1}	K_{SA1}
$r_{O,2}$ data	1.19	5.88
H_p data	0.84	5.66
$r_{O,2} + H_p$ data	0.64	3.70

calculations are beyond the scope of this paper but are described and discussed in Petersen *et al.* (2000), where this example is further developed. From Table 5 it becomes clear that the confidence intervals on μ_{maxA1} and K_{SA1} are slightly improved when based on H_p data compared with the parameter estimation based on $r_{O,2}$ data. More importantly, it appears that the confidence intervals improve significantly when $r_{O,2}$ and H_p data are combined for parameter estimations. The confidence on μ_{maxA1} and K_{SA1} improves 46 and 37%, respectively, compared with the estimation on only $r_{O,2}$, and compared with estimation on H_p the confidence improvements are 24 and 35% on μ_{maxA1} and K_{SA1}, respectively.

DISCUSSION

In the results section an experimental set-up with combined respirometric and titrimetric measurements was validated with simple substrates (acetate, and urea as N source). For practical implementations the advantages of the proposed respirometer are the high r_O measuring frequency, and the possibility to calculate $r_{O,2}$ based on a simple oxygen mass balance over the respiration chamber without the need for an estimated K_La value. However, in case the K_La in the aeration vessel is known, a second information source, $r_{O,1}$, is available based on a mass balance for oxygen over the aeration vessel. It has been investigated how this second data source can contribute with respect to the parameter identifiability (Petersen *et al.*, 2000).

The obvious disadvantage of the proposed respirometer is that it involves two dissolved oxygen electrodes with the risk that the measured S_O outputs drift away from each other. With the respirometric approach that was applied in this study, it is important to calibrate and frequently compare the response of the two dissolved oxygen electrodes. It is clear that the reliability of the respirometer output mainly depends on the quality of the dissolved oxygen electrodes used in the set-up.

The second disadvantage of the set-up is that a model-based data interpretation may be needed for a completely correct data interpretation. The necessity for a model-based data interpretation depends on the liquid flow rate applied in the set-up and the K_S of the biodegradation process, as was shown with simulation examples (Figs 2 and 3). However, in case if one is only interested in values of BOD_{st} a sufficiently high flow rate can be applied, and in this way a model-based analysis can be avoided.

The respirometric data benefit from the implementation of the titration method in the hybrid respirometer. It automatically leads to pH control, and consequently, the possible effect of pH changes (e.g. due to proton consumption or production during biological reactions) on r_O data can be excluded. However, the most important advantage of a combination of respirometric and titrimetric measurements is that two independent measurements are obtained simultaneously for the same process. This results in higher information content, and therefore, more accurate determination of wastewater composition and biodegradation kinetics. It was indeed illustrated in this study that the confidence intervals on the estimated parameters improve significantly when combined respirometric and titrimetric data are applied (Table 5). It has also been shown that Y_A became theoretically identifiable by combining the information available from the separate data sets without exact knowledge of the initial substrate concentration (Devisscher, 1997). Mathematical and experimental verification of the improved parameter identifiability with combined respirometric–titrimetric data sets was investigated in more detail in Petersen *et al.* (2000).

A linear increase of the amount of acid (in meq) added during the acetate degradation was observed as a function of the initial amount of acetate added (Fig. 5). Initially it was believed that this could be explained fully by the proton consumption during acetate uptake (Cramer and Knaff, 1991). However, the amount of acid added was lower than 1 meq/mmol of acetate, which is the expected amount assuming that 1 proton is consumed for each mmol of acetate taken up by the cells. Obviously, factors other than substrate uptake affect the proton balance in the mixed liquor. Production of protons due to CO_2 formation and uptake of NH_3 by the cells are processes that will also influence the proton balance (Iversen *et al.*, 1994). The contribution of these processes needs to be investigated in more detail. It should be clear that the ASM1 approach, taking only into account that H_p is produced (or S_{ALK} is consumed) during acetate degradation due to the incorporation of S_{NH} into new biomass, cannot be used to model the titrimetric data that were obtained for acetate. The best illustration is that ASM1 would predict the production of H_p during carbon source degradation, while experimental data show H_p consumption since acid needed to be added to keep the pH of the activated sludge constant during acetate degradation (Fig. 4(A)). The available titration data for acetate have been modelled, and the acetate degradation kinetics were extracted via a parameter estimation procedure (Gernaey *et al.*, 2000a, b). In addition a model-based interpretation of acetate titration profiles was combined with already existing models for interpretation of r_O data. Similar to nitrification (Devisscher, 1997; Petersen, 2000), the full information of a combined respirometric–titrimetric data set allows to extract the heterotrophic biomass yield Y_H immediately from the available data (provided i_{XB} is assumed to be known), i.e. without knowing the initial substrate concentration (Petersen, 2000).

Summarising, the combination of respirometric and titrimetric measurements certainly opens per-

spectives towards a more informative, reliable and accurate characterisation of wastewater and sludge kinetics. Future work will focus on applying the proposed methodology in the study of complex substrates and wastewaters. Yuan *et al.* (1999) recently presented an approach to combine information of titrimetric and respirometric experiments to determine the nitrifiable nitrogen content of wastewater. However, it can be foreseen that titrimetric data can be rather complex to interpret for a wastewater, and it may appear more difficult to generalise results obtained with titrimetry compared to respirometry. During the different degradation processes of wastewater, proton producing as well as proton consuming ones, may affect the pH, contrary to the case where only nitrification occurs. The latter allows a more straightforward interpretation (Massone *et al.*, 1995; Gernaey *et al.*, 1997a, 1998).

Indeed, the results of this paper already illustrate that pH effects of nitrification and acetate uptake and degradation are opposite. When these two substrates are mixed the proton effects of the two processes may simply compensate for each other. An example of combined respirometric–titrimetric data collected after addition of a mixture of acetate and NH_4-N to activated sludge is given in Fig. 10. It is clear from both the S_O and $r_{O,2}$ curves (Fig. 10(A)) that the substrate degradation phase can be divided in two parts. First, both acetate and NH_4-N are oxidised simultaneously, and second only degradation of NH_4-N continues. From the titration data (Fig. 10(B)) it can indeed be seen that the pH effects of both processes (acetate degradation and nitrification) almost compensate for each other, and only a slight amount of acid has to be added. However, as soon as acetate is degraded, base is added to compensate for the protons produced during the nitrification process. Thus, the titrimetric information obtained from this experiment will be more complex to interpret, especially since the information content of the titration profile is low during the phase of simultaneous acetate degradation and nitrification. Finally, there is a bend in the base curve at the nitrification endpoint.

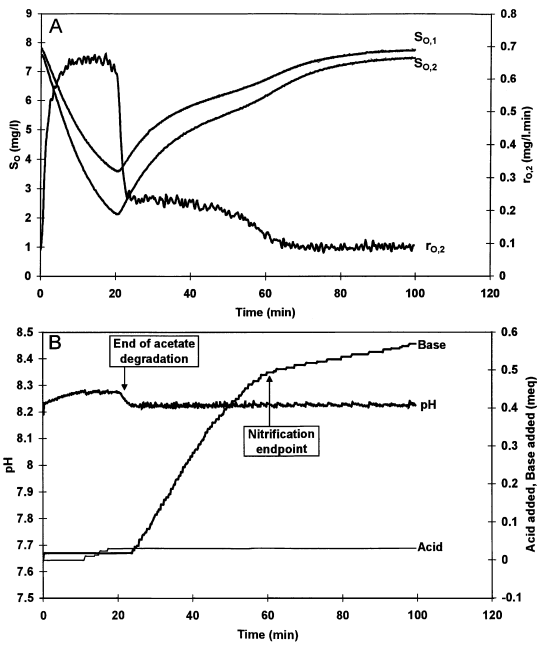

Fig. 10. Example of combined respirometric–titrimetric data obtained after addition of acetate (30 mg COD/l) and ammonium (2 mg N/l) at $t = 0$. (A) respirometric data; (B) titrimetric data (Gernaey *et al.*, 1999).

However, in general when one knows that acetate oxidation consumes protons, while ammonium oxidation produces them, titrimetric data can immediately serve to identify the different shoulders in r_O profiles without knowing the initial composition of the added substrate. Such titrimetric data thus give information on the different processes, and when combined with respirometry it becomes a powerful and very information-rich method for characterisation of biological wastewater treatment processes.

CONCLUSIONS

A respirometric technique was applied that allows high-frequency sampling of respiration rates by making an oxygen mass balance over a non-aerated flow-through respiration chamber without the need for K_La estimation. The respirometer was combined with a titrimetric technique and via validation experiments it was proven that the set-up produced reliable data sets.

Simulations showed that a model-based data interpretation, which takes substrate transport in the respirometer into account, is necessary to exclude that the operating conditions (e.g. flow rate) or the degradation kinetics (e.g. high K_S) which interfere during the interpretation of the respiration rate data.

Consecutive acetate additions to activated sludge showed that titrimetric data can also yield information about carbon source degradation processes.

Applying a combined respirometric–titrimetric measurement approach resulted in improved confidence intervals when the parameter values are obtained from the combined data set compared with the estimations on the separate respirometric or titrimetric data sets.

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