



# RESPIROMETRY AS A TOOL FOR RAPID CHARACTERIZATION OF WASTEWATER AND ACTIVATED SLUDGE

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## ABSTRACT

A procedure is presented to estimate biokinetic parameters for heterotrophic and autotrophic process models and to estimate wastewater characteristics in the context of the Activated Sludge Models No. 1 and No. 2. The procedure is based on respirometric measurements at low substrate to biomass ratio ( $S/X$ ). The addition of nitrification inhibitor is avoided by applying a calibrated nitrification model to the respiration rate data resulting from both heterotrophic and autotrophic degradation. Furthermore, a new procedure is developed for simultaneous assessment of decay coefficients for heterotrophic and autotrophic biomass. The results show that, for a given wastewater/sludge combination  $S/X$  can be crucial in obtaining reliable parameter estimates; at a very low ratio not all parameters could be identified. A higher ratio caused problems because of nitrification inhibition.

## KEYWORDS

Activated sludge, characterization, hydrolysis, inhibition, kinetics, modelling, nitrification, respirometry.

## INTRODUCTION

Advanced mathematical models summarise recent knowledge of the activated sludge wastewater treatment process. The models are useful for design and operational assistance of the activated sludge process. However, their practical applicability is limited by the availability of information on activated sludge and wastewater characteristics. Therefore, considerable effort is being directed to the development of methods for the determination of these characteristics (Henze and Gujer, 1992). The publication of the more complicated Activated Sludge Model No. 2 (Gujer *et al.*, 1995) will generate an even greater need for good and rapid methods.

Batch measurements are generally considered valuable for determination of wastewater and sludge characteristics (Chudoba *et al.*, 1992; Henze, 1992; Kappeler and Gujer, 1992; Spanjers and Keesman, 1994; Vanrolleghem *et al.*, 1994). Continuous experiments are mainly suitable for determination of stoichiometric coefficients. However, since steady state must be attained, they require a long measuring period. Chudoba *et al.* (1992) demonstrate that the most important factor in batch experiments is the ratio of the initial substrate concentration to the initial biomass concentration  $S/X$ . High  $S/X$  results in substantial biomass growth and

shift in biomass population so that the obtained kinetic parameters are no longer representative for the original biomass (Novák *et al.*, 1994). For studies with the aim of obtaining kinetic parameters it is therefore necessary to work at low  $S/X$ . The consequence of measuring at low  $S/X$  is that, due to the excess of biomass, the substrate is degraded in the short term.

Respirometry provides good means to obtain kinetic parameters and stoichiometric coefficients (Kappeler and Gujer, 1992). Within the context of the IAWQ-model it is also applied to determine the biodegradable substrate of wastewater (Henze, 1992). A problem associated with short-term measurements (low  $S/X$ ) using respirometric principles may be that these principles do not allow sufficiently high measuring frequencies. Most respirometric techniques for short-term batch experiments use cyclic operation: aeration is stopped, the respiration rate is calculated from the decline of DO concentration with time and the sludge is reaerated for a new cycle. This procedure requires a minimum measuring interval of about 7–10 min (e.g. Ekama *et al.* 1986; Kappeler and Gujer, 1992; Watts and Garber, 1993). However, such a measuring frequency is too low to permit accurate determination of small affinity parameters (Spanjers and Keesman, 1994; Vanrollegheem and Coen, 1995) in respirometric experiments, especially with low  $S/X$  (high degradation rate).

This paper presents a rapid procedure based on respirometric experiments, at low  $S/X$  ratio, to estimate as many as possible biokinetic parameters for heterotrophic and autotrophic process models and to estimate wastewater characteristics, all in the context of the Activated Sludge Models No. 1 and No. 2. Moreover, we have tried to avoid addition of nitrification inhibitor in the proposed procedure. With this procedure the parameters are obtained by identifying models of the relevant processes. A new procedure was developed for simultaneous assessment of decay for heterotrophic and autotrophic biomass. The respirometric principle employed allows a higher measuring frequency than usually obtained with a cyclic respiration meter.

## PROPOSED PROCEDURE

### Model

In this paper we consider a model (Table 1), which is, in certain aspects, a simplified and, in other aspects, an extended version of the original model proposed by Henze *et al.* (1987). The following modifications, made to be able to explain our experimental findings (see below), are introduced.

- Dissolved oxygen is not limiting and, consequently, denitrification is not incorporated.
- Slowly biodegradable material ( $X_S$ ) is replaced with rapidly hydrolysable material ( $X_R$ ).
- Hydrolysis is modelled as first-order kinetics (Sollfrank and Gujer, 1991).
- Decay is directly associated with endogenous oxygen consumption (Sollfrank and Gujer, 1991).
- Readily biodegradable substrate is split up into two fractions, one of which is produced by hydrolysis. The same yield applies for both fractions.
- Ammonification is, as hydrolysis, not dependent on biomass concentration.

### Identifiable parameters

It can be shown that, from respiration rate only, not all parameters can be uniquely estimated (Dochain *et al.*, 1995). The parameters or parameter combinations that can be identified from the proposed procedure are summarized in Table 2. To obtain these parameters or parameter combinations,  $i_{XB}$  must be assumed. We have used a value of  $0.086 \text{ gN g}^{-1}\text{COD}$  (Henze *et al.*, 1987). Clearly it is only possible to obtain e.g.  $\mu_{mH}$  if  $X_{BH}$  and  $Y_H$  are assessable.

### Estimation from experiment with $S/X_{200}$

In this procedure we propose an experiment with very low  $S/X$ , typically  $1/200$  on COD basis. This ratio will be denoted  $S/X_{200}$  here. There are indications that part of the hydrolysable material may be suspended (Henze, 1992). Therefore, for comparison, filtered wastewater is also investigated.

TABLE 1. Process Kinetics and Stoichiometry for Carbon Oxidation and Nitrification

component →	i	1	2	3	4	5	6	7	8	9	10	process rate
j	process ↓	S <sub>S1</sub>	S <sub>S2</sub>	X <sub>R</sub>	X <sub>BH</sub>	X <sub>BA</sub>	X <sub>I</sub>	S <sub>O</sub>	S <sub>N</sub>	S <sub>ND</sub>	X <sub>ND</sub>	
1	growth heterotrophs on S <sub>S1</sub>	$-\frac{1}{Y_H}$			1			$-\frac{1-Y_H}{Y_H}$	$-i_{XB}$			$\mu_{mH1} \frac{S_{S1}}{K_{S1} + S_{S1}} X_{BH}$
2	growth heterotrophs on S <sub>S2</sub>		$-\frac{1}{Y_H}$		1			$-\frac{1-Y_H}{Y_H}$	$-i_{XB}$			$\mu_{mH2} \frac{S_{S2}}{K_{S2} + S_{S2}} X_{BH}$
3	growth autotrophs					1		$-\frac{4.57-Y_A}{Y_A}$	$-i_{XB} - \frac{1}{Y_A}$			$\mu_{mA} \frac{S_N}{K_N + S_N} X_{BA}$
4	decay heterotrophs				-1		f <sub>I</sub>	-(1-f <sub>I</sub> )				b <sub>H</sub> X <sub>BH</sub>
5	decay autotrophs					-1	f <sub>I</sub>	-(1-f <sub>I</sub> )				b <sub>A</sub> X <sub>BA</sub>
6	rapid hydrolysis organics	1		-1								k <sub>R</sub> X <sub>R</sub>
7	ammonification								1	-1		k <sub>ND</sub> S <sub>ND</sub>
8	hydrolysis organic nitrogen									1	-1	$\rho_n(X_{ND}/X_R) = k_R X_{ND}$

Procedure:

1. Add a known amount of ammonium to activated sludge in the endogenous state and record the respiration rate until the endogenous respiration rate is reached again.
2. By using data from step 1 and the model for nitrification (Table 1, process 3), estimate parameter combinations for the autotrophs (Table 2).
3. Add a known volume of raw wastewater to the same activated sludge so that the S/X ratio is about 1/200 (on COD basis) and record the respiration rate until the endogenous rate is reached.
4. By using the model for both heterotrophic degradation and nitrification (Table 1, processes 1, 2, 3 and 6) with nitrification parameters obtained from step 2, estimate parameter combinations for the heterotrophs and the wastewater characteristics (see Table 2).
5. Repeat steps 3 and 4 with filtered wastewater to determine which parts of S<sub>S</sub> and X<sub>R</sub> are particulate.

TABLE 2. Identifiable Parameter Combinations. S<sub>S</sub> Applies to S<sub>S1</sub> and S<sub>S2</sub>.

Wastewater characteristics	Sludge characteristics (biokinetics)
(1-Y <sub>H</sub> )S <sub>S</sub>	$\mu_{mH} X_{BH} (1-Y_H) / Y_H$
(1-Y <sub>H</sub> )X <sub>R</sub>	$\mu_{mA} X_{BA} (4.57-Y_A) / Y_A$
(4.57-Y <sub>A</sub> )S <sub>NH</sub>	(1-Y <sub>H</sub> )K <sub>S</sub>
(4.57-Y <sub>A</sub> )S <sub>ND</sub>	(1-Y <sub>A</sub> )K <sub>N</sub>
(4.57-Y <sub>A</sub> )X <sub>ND</sub>	k <sub>R</sub>
	k <sub>ND</sub>
	Y <sub>A</sub>

### Estimation from experiment with $S/X_{20}$

Under conditions with  $S/X_{200}$  it may be difficult to determine the contribution from hydrolysis of organic nitrogen and ammonification. Under such conditions hydrolysis may be swamped by the endogenous process. Therefore we propose an experiment with higher – but still low –  $S/X$ , typically  $1/20$  on COD basis. This ratio will be denoted  $S/X_{20}$ . Because of increased model complexity, an additional measurement with suppressed nitrification is required to distinguish between respiration of nitrogen from hydrolysis/ammonification and respiration of organic carbon from hydrolysis.

Procedure:

1. Add a known amount of ammonium to activated sludge in the endogenous state and record the respiration rate until the endogenous respiration rate is measured.
2. By using data from step 1 and the model for nitrification (Table 1, process 3), estimate parameter combinations for the autotrophs (Table 2).
3. Add a known volume of raw wastewater to the same activated sludge so that the  $S/X$  ratio is about  $1/20$  (on COD basis) and record the respiration rate until the endogenous rate is reached.
4. Add nitrification inhibitor and repeat step 3.
5. By using the data from step 4 and the model for heterotrophic degradation (Table 1, process 1, 2 and 6), estimate parameters of the heterotrophs (Table 2).
6. Calculate a new dataset of respiration rates by subtracting data of step 4 from those of step 3.
7. Using data from step 6 and the model for hydrolysis of organic nitrogen, ammonification and nitrification (Table 1, process 3, 7 and 8), estimate concomitant parameters (Table 2).
8. Repeat previous steps (1–7) with filtered wastewater to determine which parts of  $S_S$ ,  $X_R$ ,  $S_{ND}$  and  $X_{ND}$  are particulate.

### Estimation of decay coefficients

In the traditional method (Ekama *et al.*, 1986) the decay coefficient for heterotrophic biomass is determined by measuring the respiration rate of endogenous sludge, in the presence of nitrification inhibitor, over a period of several days. The decay coefficient is calculated from the slope of a plot of the natural logarithm of respiration rate versus time. The obtained coefficient differs from the one in the Activated Sludge Model No. 1.

In this paper we propose an alternative method for the simultaneous determination of the traditional decay coefficients of heterotrophs and autotrophs. The method is based on the measurement of the respiration rate obtained after addition of an optimal mixture of acetate and ammonium (Vanrolleghem and Coen, 1995). By applying the model for heterotrophic and autotrophic degradation, the parameter combinations involving  $\mu_{mH}$ ,  $\mu_{mA}$ ,  $X_{BH}$  and  $X_{BA}$  can be estimated. This is repeated over a period of several days. If yield and maximum growth rate are constant, the value of the parameter combinations will only depend on the active biomass concentration. Then the decrease of this value will be governed by the decay coefficient of the biomass. It can be shown that the logarithm of the respective parameter combinations as a function of time generates a straight line with slopes equal to  $b_H$  and  $b_A$ , respectively.

## MATERIALS AND METHODS

The measuring unit consisted of a continuous respiration meter (RA-1000, Manotherm, The Netherlands) connected to a laboratory-scale aeration tank, the total system having a content of about 2 l of activated sludge. The respiration meter was set to a measuring interval of one minute. Temperature and pH were kept constant at  $20 \pm 1^\circ\text{C}$  and  $7.5 \pm 0.1$ , respectively.

Sludge and wastewater were sampled from a nitrifying activated sludge plant treating municipal and hospital sewage (Maria Middelaers plant, Belgium). Part of the wastewater was centrifuged during 10 min at 25000 rpm and then filtered over a  $0.45 \mu\text{m}$  membrane (S&S BA 85). Analysis for COD, Kjeldahl-N and

ammonium has yielded the following values:  $817 \text{ g m}^{-3}$ ,  $47.4 \text{ gN m}^{-3}$  and  $33.0 \text{ gN m}^{-3}$ , respectively, for raw wastewater and  $359 \text{ g m}^{-3}$ ,  $38.3 \text{ gN m}^{-3}$  and  $31.8 \text{ gN m}^{-3}$ , respectively, for filtered wastewater. Standard solutions were prepared containing  $828 \text{ mg N per l}$  (as ammonium chloride) and  $7640 \text{ mg COD}$  (as sodium acetate) per l, respectively.

The batch experiments were commenced by transferring 1-2 l of sludge to the measuring unit and starting the respiration meter. After the endogenous respiration rate was measured, a sample was added and the rate was recorded. When the endogenous respiration rate was reached again and observed to be constant for at least 10 minutes a new sample was added. If required, nitrification was suppressed with  $10 \text{ mg allylthiourea (ATU) per l}$  activated sludge. When after an addition the total volume was increased significantly, the sludge was decanted to maintain approximately the same activated sludge concentration. At the end of the batch experiment sludge was sampled for analysis of ML(V)SS.

To obtain the respiration rate due to substrate oxidation, the endogenous respiration rate was subtracted from the measured rate. The endogenous rate was found from linear interpolation between the rate measured before the addition and the rate at the end of the respirogram. The obtained time series were converted into datasets for model identification. For model identification the direction set optimization technique was used (Brent, 1973). It was verified that the residuals were well behaved, having a mean near zero and an approximately constant variance.

## RESULTS

### S/X<sub>200</sub>

Figure 1 shows the respiration rate measured after the addition of ammonium along with the best model fit curves. The parameter estimates are presented in Table 3. An explanation for the delay in the respiration rate on day 2 is that the sludge had been aerated without feed one day longer. This may also explain the higher yield (Table 3) as compared with day 1.

Figure 2 shows the respirogram for the addition of wastewater. For verification, similar measurements in the presence of ATU are also shown. By using the calibrated nitrification model, parameters of the heterotrophic degradation were obtained from the respiration data of the wastewater (Table 4). Four models for heterotrophic degradation were evaluated (indicated by the components involved):

- $S_S$ : This model showed a bad fit (Fig. 2, dotted line).
- $S_S, X_R$ : Fits only to the raw wastewater data (Fig. 2, dashed line).
- $S_S, X_R, X_S$  (as proposed by Sollfrank and Gujer, 1991): bad model fit (not shown).
- $S_{S1}, S_{S2}$ : best fit (Fig. 2, full line).

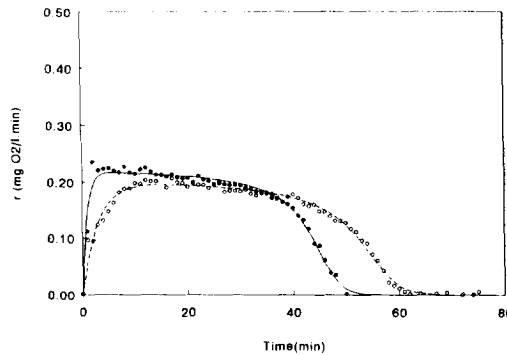


Fig. 1. Respiration rate after addition of  $3.31 \text{ mg ammonium-N}$  to  $1.4 \text{ l}$  activated sludge. Filled markers: day 1, open markers: day 2.

It is concluded, and it was ascertained with structure characterization methods (Vanrolleghem *et al.*, 1994), that the model with two readily biodegradable substrates fits best to the data. The respirogram of the filtered wastewater shows a shorter shoulder as compared with the raw wastewater (Fig. 2), indicating that a part of  $S_{S2}$  was retained on the filter.

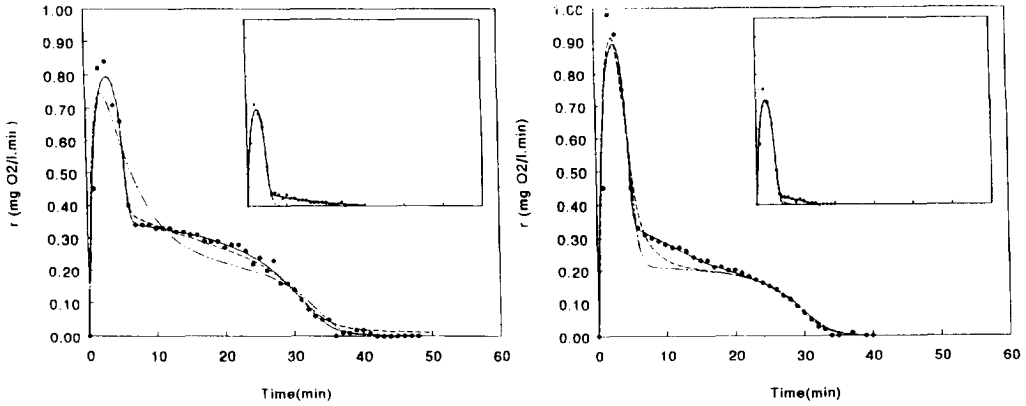


Fig. 2. Respiration rate after addition of 70 ml raw wastewater (left) and filtered wastewater (right) to 1.5 l activated sludge. Insertions: ATU added. Points: measurements, full line: best model fit, other lines: alternative models (see text).

TABLE 3. Estimates of Parameter Combinations for Nitrification (Fig. 1)

	$X$ (gVSS l <sup>-1</sup> )	$\mu_{mA}X_{BA}(4.57-Y_A)/Y_A$ (gO <sub>2</sub> m <sup>3</sup> min <sup>-1</sup> )	$(4.57-Y_A)K_N$ (gO <sub>2</sub> m <sup>-3</sup> )	$Y_A$ (gCOD g <sup>-1</sup> N)
day 1	2.40	0.23	0.43	0.36
day 2	2.34	0.21	0.54	0.56

TABLE 4. Estimates of Parameter Combinations for Heterotrophic Degradation

ratio	wastewater	ATU	$X$ (gVSS l <sup>-1</sup> )	$\mu_{mH1}X_{BH}(1-Y_H)/Y_H$ (gO <sub>2</sub> m <sup>3</sup> min <sup>-1</sup> )	$(1-Y_H)K_{S1}$ (gO <sub>2</sub> m <sup>-3</sup> )	$k_R$ (min <sup>-1</sup> )	$\mu_{mH2}X_{BH}(1-Y_H)/Y_H$ (gO <sub>2</sub> m <sup>3</sup> min <sup>-1</sup> )	$(1-Y_H)K_{S2}$ (gO <sub>2</sub> m <sup>-3</sup> )
$S/X_{200}$	raw	-	2.52	0.57	0.17		0.16	0.75
		+	2.32	0.62	0.20		0.20	0.61
	filtered	-	2.41	0.73	0.20		0.18	0.58
		+	2.20	0.68	0.22		0.06	0.80
$S/X_{20}$	raw	-	1.55	0.47	0.07	0.04	0.48	1.16
		+	1.25	0.33	0.10	0.01	0.38	0.96
	filtered	-	1.55	0.36	0.39		1.33	6.85
		+	1.25	0.34	0.06		0.55	1.54

TABLE 5. Wastewater Characteristics

ratio	wastewater	ATU	$S_{S1}(1-Y_H)$ (gCOD m <sup>-3</sup> )	$X_R(1-Y_H)$ (gCOD m <sup>-3</sup> )	$S_{S2}(1-Y_H)$ (gCOD m <sup>-3</sup> )	$S_N(4.57-Y_A)$ (gN m <sup>-3</sup> )
S/X <sub>200</sub>	raw	-	43.4		60.8	143.4
		+	45.5		24.6	
	filtered	-	48.0		31.2	137.1
		+	47.7		11.0	
S/X <sub>20</sub>	raw	-	2.8	50.9	25.8	
		+	30.6	7.5	15.9	
	filtered	-	35.4		8.6	
		+	19.2		12.1	

Table 4 shows that there is, for both raw and filtered wastewater, a good agreement between the parameters obtained from the proposed procedure and those from the traditional method with the addition of ATU. It should, however, be noted that the values of parameter combination with  $\mu_{mH}$  depend on  $X_{BH}$  which varies between the different experiments as is indicated by X. The wastewater concentration of  $S_{S2}$  is lower, for both raw and filtered water, when the measurements in the presence of ATU are considered (Table 5). The wastewater ammonium concentration,  $S_N$ , can be calculated from the parameter combination  $S_N(4.57-Y_A)$  found from the model fit (Table 5) by using the estimated  $Y_A$  (Table 3). The obtained values, 34.1 gN m<sup>-3</sup> (raw) and 32.6 gN m<sup>-3</sup> (filtered) are in good agreement with the ammonium concentration found by analysis: 33.0 and 31.8 gN m<sup>-3</sup>, respectively.

### S/X<sub>20</sub>

At the applied ratio the wastewater appeared to be inhibitory to nitrification, which is confirmed by results from earlier investigation with this wastewater-sludge combination (Kong, 1994). However, the response on the addition of ammonia shows that, prior to the addition of wastewater, nitrification capacity was still intact (Fig. 1 and Table 3, day 2). The following interpretation of the data leads to the conclusion that nitrification was inhibited: Incorporation of nitrification cannot contribute to a reasonable fit of the model to the data of both raw and filtered wastewater, suggesting that nitrification was absent during these measurements. Another indication for nitrification inhibition is that the total amount of oxygen used is too small to cover the oxidation of both organic matter and nitrogen: this amount should have been proportional to that obtained from the measurements with S/X<sub>200</sub>. We must conclude that the measurements with S/X<sub>20</sub> (Fig. 3) in combination with the S/X<sub>20</sub> in the presence of ATU (not shown) cannot be used for determination of  $S_{ND}$ ,  $X_{ND}$  and  $k_{ND}$ .

If we assume that the respiration data (Fig. 3) represent only carbon oxidation, we can apply the model describing heterotrophic degradation of rapidly hydrolysable matter and two readily biodegradable substrates. Like the experiment with S/X<sub>200</sub> this model gives the best fit. Figure 3 shows that for both raw and filtered wastewater a good fit is obtained. Parameter values and wastewater characteristics are presented in Table 4 and 5, respectively, which also show the estimates from the measurements in the presence of ATU. It appeared that, for the filtered water, incorporating  $X_R$  does not improve the goodness of fit. Apparently, in the wastewater studied, the soluble  $X_R$  is only a small fraction of the total  $X_R$ . As with S/X<sub>200</sub> we observed that part of  $S_{S2}$  is retained on the filter.

For the raw wastewater, kinetic parameters are in agreement, irrespective of the presence of ATU, which is another evidence for nitrification inhibition due to the wastewater. In contrast to the raw wastewater, the parameters of the filtered water show some inconsistency, i.e. ATU appears to influence the estimates for wastewater characteristics (also S/X<sub>20</sub>): the total carbonaceous oxygen demand (sum of  $S_{S1}$ ,  $S_{S2}$  and  $X_R$ ) is 26-33% lower in the presence of ATU (Table 5). A comparison of kinetic parameters and wastewater characteristics obtained without ATU reveals significant differences between raw and filtered water,

suggesting that the inhibition by wastewater depends on the presence of suspended matter. Parameters from measurements with ATU show better agreement between raw and filtered water.

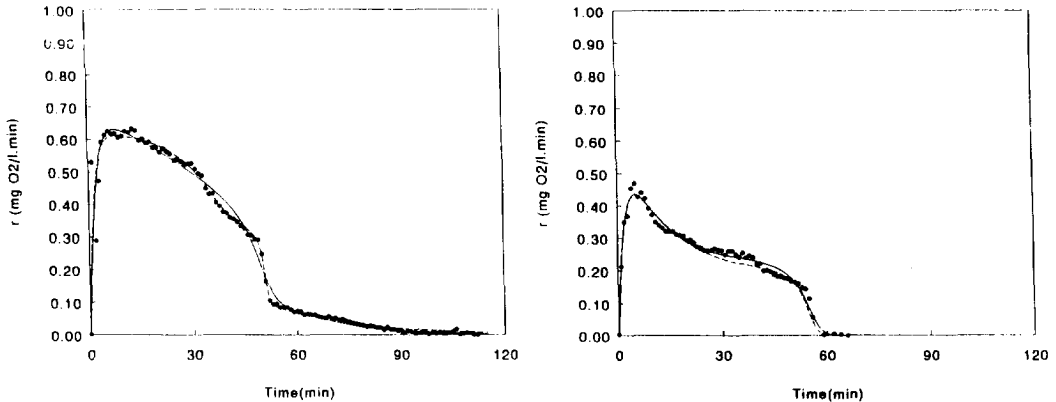


Fig. 3. Respiration rate after addition of 700 ml raw wastewater (left) and filtered wastewater (right) to 1.3 l of activated sludge. Points: measurements. Full lines: best-fit model including  $S_{S1}$ ,  $S_{S2}$  and  $X_R$  (left) or  $S_{S1}$ ,  $S_{S2}$  (right). Dashed lines: model including only  $S_S$  and  $X_R$ .

### Decay coefficients

The model for carbon oxidation and nitrification was applied to respiration data from acetate/ammonium addition (Fig. 4) to estimate parameter combinations involving  $\mu_{mH}$  and  $\mu_{mA}$ . Subsequently,  $b_H$  and  $b_A$  were estimated:  $0.04 \text{ d}^{-1}$  and  $0.11 \text{ d}^{-1}$ , respectively. These are in good agreement with the values reported in the literature (Sollfrank and Gujer, 1991; Lesouef *et al.*, 1992). Here, the traditional decay coefficients are denoted. For use in the Activated Sludge Model No. 1, they should be converted as indicated by Henze *et al.* (1987). In this paper only two respirograms were used (Fig. 4), so the estimated coefficients are not very reliable. At least three respirograms should be recorded.

A rough guess for  $b_H$  may be obtained directly from one endogenous respiration rate measurement and MLVSS ( $X$ ). Since the endogenous rate (that of the nitrifiers is assumed negligible) is equal to  $(1-f_1)b_H X_H$  or  $(1-f_1)b_H(X_H/X)X$  (Table 1),  $b_H$  can be calculated if  $f_1$  and the active fraction ( $X_H/X$ ) are known. The latter is, for instance, based on a previous estimate of  $b_H$  from the method described above.

## DISCUSSION

The aim of the investigation was to develop a rapid procedure based on respirometric measurements, at low  $S/X$ , to estimate as many as possible biokinetic parameters for heterotrophs and autotrophs, and to estimate wastewater characteristics in the context of the Activated Sludge Models. The procedure has been shown to be suitable for the identification of combinations of important parameters. For the estimation of the separate parameters additional information is required, e.g.  $Y_H$  should be known.

The advantages of avoiding addition of ATU are: the measurements are less time consuming, activated sludge can be reused and there is no risk for inhibition of heterotrophic processes or adaptation of nitrification. When measurements in the presence of ATU are used, the possible effect of ATU on the heterotrophic process should be considered.



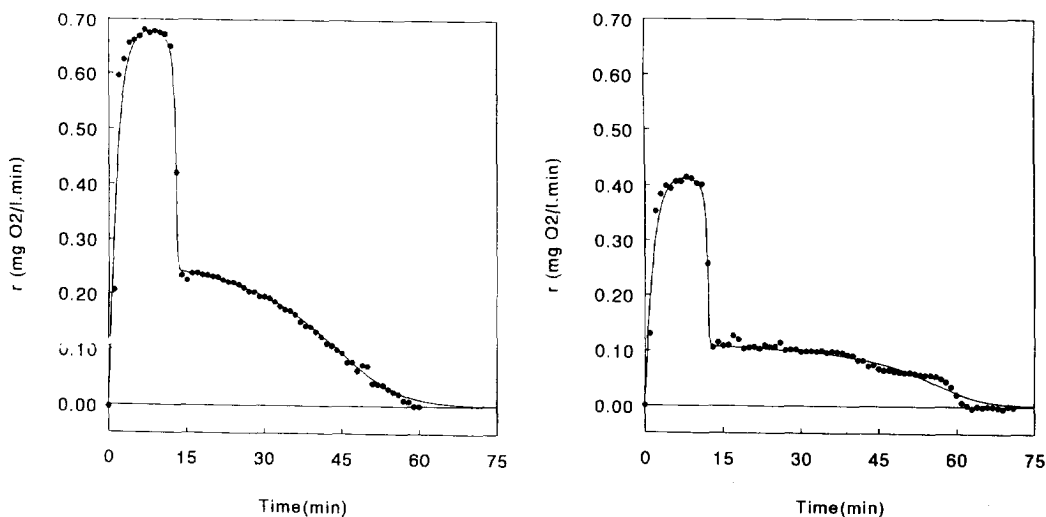


Fig. 4. Respirograms obtained after addition of acetate/ammonium mixture. Left: 1st day (38.2 mgCOD and 4.14 mgN to 2 l), right: 7th day (19.1 mgCOD and 2.07 mgN to 1.7 l).

The investigation showed that the  $S/X$  ratio can be crucial. The information content of the  $S/X_{200}$  measurements was insufficient to obtain reliable estimates of  $k_R$  and  $X_R$ . This may be improved by increasing the measuring frequency (Vanrolleghem and Spanjers, 1994). Another possibility would be to increase the  $S/X$  ratio. However, our  $S/X_{20}$  measurements showed that this may lead to unintentional inhibition of nitrification. This phenomenon may be typical for the sludge/water combination used.

We showed that models including  $S_S$  and  $X_R$  only or  $S_S$ ,  $X_R$  and  $X_S$  as proposed by Sollfrank and Gujer (1991) did not fit our data well, and a second readily biodegradable substrate had to be included. This fraction appeared to be partly retained by filtration, which indicates that  $S$  and  $X$  should not be defined in terms of solubility, but merely in terms of kinetic properties. Note that, since only short-term experiments were done, slowly hydrolysable material cannot be estimated from the data presented here.

Further investigation should be directed to the evaluation of statistically significant parameter estimates by conducting repeat experiments. These estimates should be compared with estimates obtained from other methods. Other sludge/wastewater combinations should be tried, taking into account that wastewater and sludge must be sampled from the same plant (Vanrolleghem and Spanjers, 1994).

## CONCLUSIONS

The proposed procedure has been shown to be suitable for the identification of combinations of biokinetic parameters and wastewater characteristics. The only unknowns remaining are  $Y_H$ , the active fractions of two populations of biomass,  $X_S$  and  $i_{XB}$ .

It can be incorrect to mix a relative large amount of wastewater with sludge, e.g. to compensate for low respiration measuring frequency, because of possible inhibitory effects on nitrification.

## NOMENCLATURE

$b$	decay coefficient ( $\text{day}^{-1}$ )	$S_s$	concentration readily degradable substrate ( $\text{gCODm}^{-3}$ )
$f_i$	fraction of inert particulate matter	$X$	total particulate matter concentration ( $\text{gVSS m}^{-3}$ )
$i_{\text{XB}}$	mass N per mass COD in biomass ( $\text{gN g}^{-1}\text{COD}$ )	$X_B$	concentration biomass ( $\text{gCOD m}^{-3}$ )
$K_N$	half-saturation parameter for autotrophic biomass ( $\text{gNH}_4\text{-N m}^{-3}$ )	$X_I$	concentration inert organic matter ( $\text{gCOD m}^{-3}$ )
$k_{\text{ND}}$	ammonification rate ( $\text{day}^{-1}$ )	$X_{\text{ND}}$	concentration particulate organic nitrogen ( $\text{gN m}^{-3}$ )
$k_R$	rate of rapid hydrolysis ( $\text{day}^{-1}$ )	$X_R$	concentration rapidly hydrolysable matter ( $\text{gCODm}^{-3}$ )
$K_S$	half-saturation parameter for heterotrophic biomass ( $\text{gCOD m}^{-3}$ )	$X_S$	concentration slowly hydrolysable matter ( $\text{gCOD m}^{-3}$ )
$S_N$	ammonium concentration ( $\text{gN m}^{-3}$ )	$Y$	yield coefficient ( $\text{gCOD g}^{-1}\text{COD}$ )
$S_{\text{ND}}$	concentration soluble organic nitrogen ( $\text{gN m}^{-3}$ )	$\mu_m$	maximum specific growth rate ( $\text{day}^{-1}$ )
$S_O$	dissolved oxygen concentration ( $\text{gO}_2 \text{m}^{-3}$ )	1 or 2	index relative to substrates $S_{S1}$ or $S_{S2}$
		A or H	index relative to autotrophic or heterotrophic biomass

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## REFERENCES

- Brent R.P. (1973) Algorithms for minimization without derivatives. Prentice-Hall, Englewood Cliffs, New Jersey.
- Chudoba P., Capdeville B. and Chudoba J. (1992) Explanation of biological meaning of the  $S_0/X_0$  ratio in batch cultivation. *Wat. Sci. Technol.* **26**(3-4), 743-751.
- Dochain D., Vanrollegheem P.A. and Vandaele M. (1995) Structural identifiability of biokinetic models of activated sludge respirometry. *Water Research* (accepted for publication).
- Ekama G.A., Dold P.L. and Marais G.v.R. (1986) Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Wat. Sci. Technol.* **18**(6), 91-114.
- Gujer W., Henze M., Mino T., Matsuo T., Wentzel M.C. and Marais G.v.R. (1995) The Activated Sludge Model No.2: Biological phosphorus removal. *Wat. Sci. Technol.* (this issue).
- Henze M. (1992) Characterization of wastewater for modelling of activated sludge processes. *Wat. Sci. Technol.* **25**(6), 1-15.
- Henze M. and Gujer W. (eds) (1992) Interactions of wastewater, biomass and reactor configurations in biological treatment plants. *Wat. Sci. Technol.* **25**(6), 320 pp.
- Henze M., Grady C.P.L., Gujer W., Marais G.v.R. and Matsuo T. (1987) Activated Sludge Model No.1. IAWPRC Scientific and Technical Report No.1, IAWPRC, London.
- Kappeler J. and Gujer W. (1992) Estimation of kinetic parameters of heterotrophic biomass under aerobic conditions and characterization of wastewater for activated sludge modelling. *Wat. Sci. Technol.* **25**(6), 125-139.
- Kong Z. (1994) University of Gent. Personal communication.
- Lesouef A., Payraudeau M., Rogalla F. and Kleiber B. (1992) Optimizing nitrogen removal reactor configurations by on-site calibration of the IAWPRC Activated Sludge Model. *Wat. Sci. Technol.* **25**(6), 105-123.
- Novák L., Larrea L. and Wanner J. (1994) Estimation of maximum specific growth rate of heterotrophic and autotrophic biomass: A combined technique of mathematical modelling and batch cultivations. *Wat. Sci. Technol.* **30**(11), 171-180.
- Sollfrank U. and Gujer W. (1991) Characterization of domestic wastewater for mathematical modelling of the activated sludge process. *Wat. Sci. Technol.* **23**(4-6), 1057-1066.
- Spanjers H. and Keesman K. (1994) Identification of wastewater biodegradation kinetics. IEEE-CCA, 24-26 August, Glasgow.
- Vanrollegheem P.A. and Spanjers H. (1994) Comparison of two respirometric principles for the determination of short-term biochemical oxygen demand. In: *Proc 49th Purdue Indust. Waste Conference*, Lewis Publ., Chelsea, Michigan (in press).
- Vanrollegheem P.A. and Coen F. (1995) Optimal design of In-Sensor Experiments for on-line modelling of nitrogen removal processes. *Wat. Sci. Technol.* This issue.
- Vanrollegheem P.A., Kong Z., Rombouts G. and Verstraete W. (1994) On-line respirometric biosensor for the characterization of load and toxicity of wastewater. *J. Chem. Technol. & Biotechnol.* **59**, 321-333.
- Vanrollegheem P.A., Vandaele M., Van Overschee P., Vansteenkiste G.C. (1994) On-line model structure characterization of nonlinear wastewater treatment systems. In: *System Identification*. T. Söderström (ed). Pergamon, Oxford. In press.
- Watts J.B. and Garber W.F. (1993) On line respirometry: A powerful tool for activated sludge plant operation and design. *Wat. Sci. Technol.* **28**(11/12), 389-399.