MODEL-BASED INTERPRETATION OF TITRIMETRIC DATA TO ESTIMATE AEROBIC CARBON SOURCE DEGRADATION KINETICS

Krist Gernaey[1,2], Britta Petersen[1,2] and Peter A. Vanrolleghem[1]

[1] BIOMATH Department, Ghent University, Coupure Links 653, B-9000 Gent, Belgium
E-mail: Peter.Vanrolleghem@rug.ac.be
[2] Dept. of Civil, Geological and Mining Engineering, École Polytechnique de Montréal

Abstract: Combined respirometric – titrimetric data are presented, resulting from aerobic batch degradation experiments in activated sludge with acetate and dextrose as a substrate. A model including substrate uptake, CO_2 production and NH_3 uptake for biomass growth is proposed to describe the titrimetric data. Combining respirometric and titrimetric data is more informative compared to the separate data sets, since an extra parameter combination including the biomass yield becomes structurally identifiable. The parameter values estimated on respirometric and titrimetric data proved that titrimetric data are a valuable alternative to respirometry to obtain carbon source biodegradation kinetics, with potential application under anoxic conditions too.

Keywords: Biotechnology, Environmental engineering, Estimation, Identifiability, Sensors

1. INTRODUCTION

The process studied is the aerobic degradation of a $C_xH_yO_z$ type carbon source by a mixed population of bacteria (activated sludge). Respirometry, the measurement and interpretation of the respiration rate of activated sludge, is often applied as a tool to characterize aerobic degradation processes in activated sludge (Henze *et al.*, 1987; Spanjers *et al.*, 1998). However, respirometry has its limitations. It can e.g. not be used to characterize biodegradation kinetics under anoxic conditions (presence of nitrate as electron acceptor instead of oxygen). For nitrogen removal in wastewater treatment it is often desirable to also characterize biomass degradation kinetics under anoxic conditions (where the last step in nitrogen removal, denitrification, occurs). A measurement technique that could be applied under aerobic and anoxic conditions could be a valuable tool to characterize carbon source degradation processes in activated sludge.

Under anoxic conditions it appeared that the pH of the activated sludge will change because protons are consumed during anoxic degradation of a carbon source (Bogaert *et al.*, 1997). For the nitrification process, i.e. the aerobic conversion of ammonium to nitrate by activated sludge, it has been shown that titrimetric experiments, where the proton production due to nitrification is measured, can yield information about biodegradation kinetics (Gernaey *et al.*, 1998), similar to respirometry. 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 the ongoing biological reactions. Generally, under aerobic conditions the processes that mostly influence the pH value of the liquid phase in activated sludge are: 1) Nitrification, which causes a pH decrease due to proton production, 2) 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 NH_3 for growth, 3) 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. Such problems can be avoided by controlling the pH of the liquid medium at a constant pH setpoint through 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 related to biological reactions.

This study evaluates the application of titrimetry to extract aerobic carbon source biodegradation kinetics. This evaluation is the first step in the development of a method that could be applicable under both anoxic and aerobic conditions to study carbon source degradation processes. A combined respirometric - titrimetric set-up was used to collect data during aerobic degradation of two different carbon sources (acetate and dextrose). Based on an existing model for interpretation of respirometric data, a model is presented that links the degradation of a $C_xH_yO_z$ type carbon source to the proton consumption or production observed during the aerobic biodegradation process. The structural identifiability of the new model was studied. Finally, the respirometric and titrimetric data were used to estimate kinetic biodegradation parameters, and parameter values resulting from the estimations are compared.

2. METHODOLOGY

Data were collected using a combined respirometric – titrimetric set-up (Fig. I), which is described in more detail in Gernaey *et al.* (2001). The set-up consists of an aeration vessel (V_1 = 2 l) and a respiration chamber (V_2 = 0.5 l). The respiration chamber is closed and is not aerated. The contents of both vessels is mixed, and a pump is used to continuously pump the activated sludge between both vessels (flow rate Q_{in}). The aeration vessel and the respiration chamber are both equipped with a dissolved oxygen electrode (Ingold/Mettler Toledo, Inpro 6400). The pH in the aeration vessel is measured with a Mettler Toledo HA 405-DXK-S8/120 Xerolyte pH electrode, and the pH was controlled within a narrow pH setpoint ± ΔpH region through addition of acid or base. The pH setpoint was typically chosen between 7.5 and 8.5, and a ΔpH value of 0.03 pH units was used. Dosage of small amounts of acid (0.05 N) or base (0.05 N) for pH control was done by opening an electromagnetic pinch valve for a short period (typically 1.5 s = 1 pulse) to adjust the pH. The cumulative amounts of acid and base dosed during an experiment were monitored.

At the start of an experiment the set-up was filled with 2.5 liters of activated sludge. The activated sludge was aerated until the endogenous respiration phase was reached (typically 12 hours or more). During the experiments small substrate pulses (e.g. 10 ml) of concentrated stock solutions of known carbon sources (e.g. acetate 10 g COD/l, dextrose 10 g COD/l) were dosed in the aeration vessel.

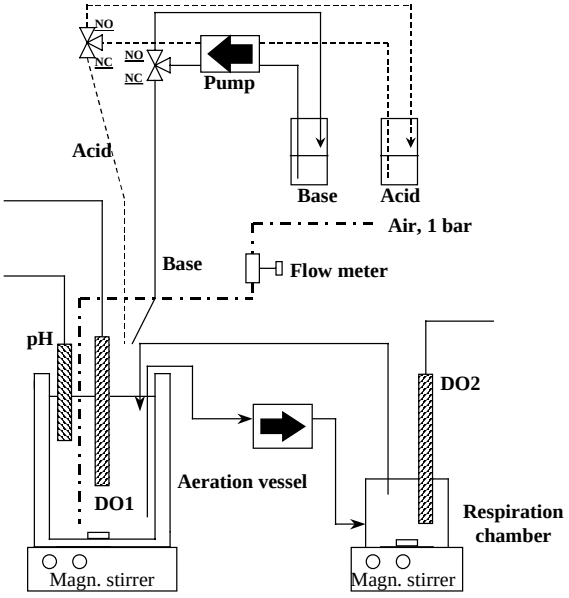

Figure I. Scheme of the experimental set-up

3. RESULTS AND DISCUSSION

3.1. Data set

A typical data set is given in Fig. II. At t = 0, a 0.781 mmol acetate (50 mg COD) substrate pulse was added to the aeration vessel of the set-up. The dissolved oxygen concentration in the aeration vessel ($S_{O,1}$) and the respiration chamber ($S_{O,2}$) decreases immediately, and acid is added to compensate for a net proton consumption of the activated sludge (to maintain a constant pH). The substrate is degraded at t = 10 minutes. From then on, $S_{O,1}$ and $S_{O,2}$ increase again due to oxygen input via the continuous aeration in the aeration vessel.

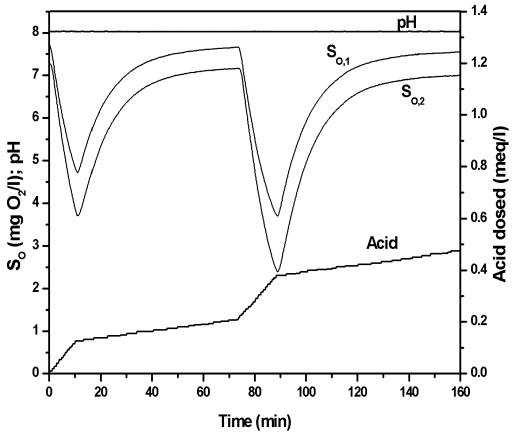

Figure II. Typical data series, in this case for the addition of 0.781 mmol acetate (t = 0) and 1.172 mmol acetate (t = 74 min)

When the acetate degradation is finished, the acid dosage rate drops back to a background proton production rate (BPPR), governed by CO_2 stripping and CO_2 production by the biomass due to endogenous respiration. The BPPR was assumed to

be constant for the short duration of each experiment, an assumption that is supported by the data of Fig. II (the slope of the acid addition curve is identical before and after the addition of the second substrate pulse). A second substrate pulse of 1.172 mmol acetate (75 mg COD) was added to the aeration vessel at t = 74 min (Fig. II), and the same phenomena as described for the first substrate addition can again be observed. Similar data sets were obtained for dextrose.

3.2. Data interpretation

A detailed description of the interpretation of the respirometric data can be found in Gernaey *et al.* (2001). Only the basic principles will be repeated here. A mass balance for oxygen (S_O) can be made over the aeration vessel and the respiration chamber (Eq. 1–2). The subscripts 1 and 2 in these equations stand for the aeration vessel and the respiration chamber, respectively. Similar subscripts will be used further on to indicate the concentration of biodegradable substrate in the aeration vessel ($S_{S,1}$) and the respiration chamber ($S_{S,2}$), for the biomass (X_1, X_2), and for the oxygen uptake rate ($r_{O,1}$, $r_{O,2}$).

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

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

For substrate degradation processes one is mainly interested in the oxygen uptake rate of the biomass (r_O). The mass balance for S_O over the aeration vessel (Eq. 1) includes, besides the oxygen uptake rate and a transport term for oxygen, an aeration term that requires the additional estimation of the oxygen transfer coefficient K_La and knowledge of the saturation oxygen concentration S_O^o. The main motivation for operating this rather complicated experimental set-up for these experiments, is that r_O can also be obtained from the S_O mass balance over the respiration chamber without the need of a K_La value (Eq. 2). The available S_O data of the aeration vessel ($S_{O,1}$) and the respiration chamber ($S_{O,2}$) are used to this purpose. 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.

Fig. IIIA contains a S_O data set obtained by adding 1.563 mmol acetate (100 mg COD) to the aeration vessel of the set-up at t = 0. Fig. IIIB shows the resulting $r_{O,2}$ data that were obtained from the S_O data by applying the mass balance of Eq. 2. The calculated $r_{O,2}$ value is 0.64 mg O_2/l.min during substrate degradation, and decreases to 0.12 mg O_2/l.min when the endogenous respiration phase is reached (t = 22 min in Fig. IIIB).

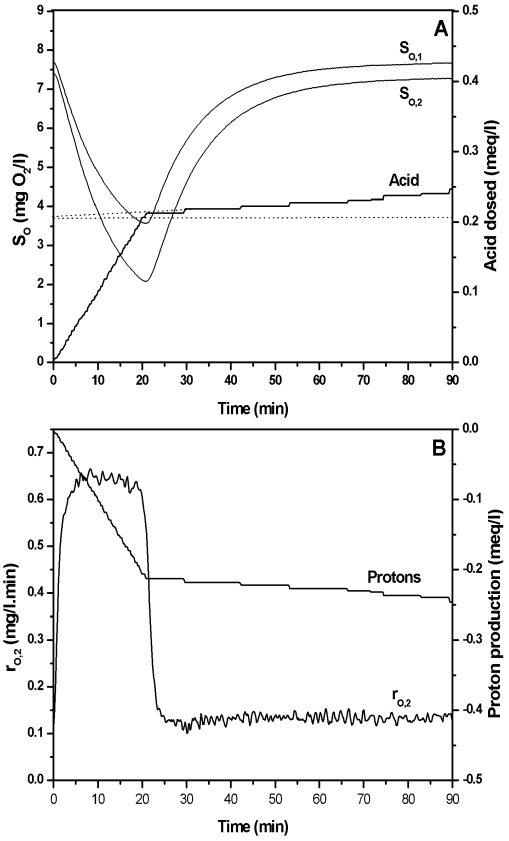

Figure III. A. Data obtained following the addition of 1.563 mmol acetate (100 mg COD) to 2.54 l of activated sludge; B. Resulting $r_{O,2}$ and proton production data

The titrimetric data set obtained from the same substrate addition experiment is shown in Fig. IIIA. Interpretation of these data is relatively easy since one can extrapolate the different slopes of the titration curves to t = 0 (the dotted line in Fig. IIIA), and thus obtain the amount of acid or base dosed during the substrate degradation. For the example of Fig. IIIA the substrate degradation caused an extra acid addition of about 0.21 meq/l. Acid and base addition data were subsequently converted into proton production data, where acid addition indicates a proton consumption, and base addition corresponds to proton production. Fig. IIIB contains an example of proton production data for the experimental acetate data of Fig. IIIA.

3.3. Model

A biokinetic model for interpretation of the respirometric data is available (Gernaey *et al.*, 2001). Expressions for the substrate removal rate (rS_S), the biomass growth rate (rX) and the oxygen uptake rate (r_O) are summarized in Eq. 3–5. In Eq. 3–5 μ_{maxH} is the maximum specific growth rate, K_S is the Monod half-saturation constant, Y_H is the biomass yield and b is the decay coefficient. Note that all concentrations are expressed in Chemical Oxygen Demand (COD).

$$rS_S = -\frac{\mu_{max\,H} \cdot X}{Y_H} \cdot \frac{S_S}{K_S + S_S} \quad (3)$$

$$rX = \mu_{max\,H} \cdot X \cdot \frac{S_S}{K_S + S_S} - b \cdot X \quad (4)$$

$$r_O = \frac{(1 - Y_H)}{Y_H} \cdot \mu_{max\,H} \cdot X \cdot \frac{S_S}{K_S + S_S} + b \cdot X \quad (5)$$

Besides substrate degradation and endogenous respiration, substrate transport was included in the model, similar to the mass balances for oxygen (Eq. 1–2). As an example the mass balance over the respiration chamber for the biodegradable substrate is given in Eq. 6.

$$\frac{dS_{S,2}}{dt} = \frac{Q_{in}}{V_2} \cdot (S_{S,1} - S_{S,2}) - \frac{\mu_{max\,H} \cdot X_2}{Y_H} \cdot \frac{S_{S,2}}{K_S + S_{S,2}} \quad (6)$$

Finally, biological start-up phenomena, that are typically observed in batch experiments before the oxygen uptake rate has reached its maximum value, were described with a simple first order term that multiplies the growth rate (Vanrolleghem *et al.*, 1998). For parameter estimations on combined respirometric - titrimetric datasets it was necessary to allow a different first order time constant to adequately fit to the respirometric and titrimetric data. Typically the start-up term will only be of influence during the first few minutes of each experiment (e.g. from t = 0 to t = 5 min. in Fig. IV).

Generally, it will be assumed for the titrimetric data that substrate uptake, CO_2 production and NH_3 uptake for biomass growth are the main processes that will influence the proton equilibrium in the liquid phase during degradation of a $C_xH_yO_z$ substrate compound. It is assumed that the substrate is taken up through the cell wall in its undissociated form. A weak acid HA present in the liquid phase in its dissociated form A^- will thus pass the cell wall together with a proton. This proton will be measured through addition of acid (to maintain the pH setpoint) when applying the titrimetric measurement technique. The fraction m of a substrate (monoprotic acid) present in the dissociated form A^- can be expressed as a function of the pH of the liquid phase and the pKa of the acid (Eq. 7).

$$m = \frac{[A^-]}{[HA] + [A^-]} = \frac{10^{-pKa}}{10^{-pH} + 10^{-pKa}} \quad (7)$$

All carbon that is respired by the cells is converted into CO_2. For the model substrate $C_xH_yO_z$, x mmol CO_2 will be produced per mmol substrate that is completely respired. The number n of protons released per molecule of CO_2 produced will depend on the pH of the liquid phase, and can be obtained using Eq. 8 (Iversen *et al.*, 1994). In Eq. 8 pK1CO2 and pK2CO2 represent the negative logarithm of the first and the second acid dissociation constant of H_2CO_3.

$$n = \frac{2 \cdot 10^{2 \cdot pH} + 10^{(pH + pK2CO2)}}{10^{2 \cdot pH} + 10^{(pH + pK2CO2)} + 10^{(pK1CO2 + pK2CO2)}} \quad (8)$$

Biomass will grow during substrate degradation and incorporate a nitrogen fraction i_{XB} in the new biomass that is produced. Similar to the Activated Sludge Model No. 1 (Henze *et al.*, 1987), it is assumed that nitrogen incorporated into new biomass is taken up from the mixed liquor as NH_3. Since NH_3 is in equilibrium with NH_4^+ in the mixed liquor, a pH depending fraction p of protons will be released in the mixed liquor per mole of NH_3 taken up to form new biomass (Eq. 9). In Eq. 9 pKNH4 represents the pKa of NH_4^+.

$$p = \frac{[NH_4^+]}{[NH_4^+] + [NH_3]} = \frac{10^{-pH}}{10^{-pH} + 10^{-pKNH4}} \quad (9)$$

Summarizing, the proton production rate (rHp) related to substrate degradation was described by Eq. 10, where m, n and p are pH depending functions (see Eq. 7–9), x is the number of carbon atoms per molecule (e.g. x is 2 for acetate), C is a factor to convert COD units to mmol (e.g. C is 64 for acetate), and BPPR is the constant background proton production rate mainly governed by CO_2 stripping.

$$rH_p = \left(\frac{m}{C \cdot Y_H} + \frac{n \cdot (1 - Y_H) \cdot x}{C \cdot Y_H} + \frac{p \cdot i_{XB}}{14}\right) \cdot \mu_{max\,H} \cdot X \cdot \frac{S_S}{K_S + S_S} + BPPR \quad (10)$$

3.4. Structural parameter identifiability

The structural identifiability of the model was investigated using the Taylor series expansion technique, and by applying the generalization method of Petersen *et al.* (2000). Both methods gave identical results. When considering that only respirometric data are available, and assuming that biomass growth takes place, the following 4 parameter combinations are structurally identifiable (Eq. 11–14):

$$\mu_{max\,H} \quad (11); \qquad \frac{1 - Y_H}{Y_H} \cdot X \quad (12)$$

$$(1 - Y_H) \cdot K_S \quad (13); \qquad (1 - Y_H) \cdot S_S(0) \quad (14)$$

Compared to Dochain *et al.* (1995), who considered respirometric data but did not include biomass growth in the model, the parameter combination including X and μ_{maxH} could be split up further.

When considering that only titrimetric data are available, and assuming that biomass growth takes place, the following 4 parameter combinations are structurally identifiable:

$$\mu_{max\,H} \quad (15)$$

$$\left(\frac{m}{C \cdot Y_H} + \frac{n \cdot (1 - Y_H) \cdot x}{C \cdot Y_H} + \frac{p \cdot i_{XB}}{14}\right) \cdot X \quad (16)$$

$$(\frac{m}{C}+\frac{n\cdot(1-Y_H)\cdot x}{C}+\frac{p\cdot i_{XB}\cdot Y_H}{14})\cdot K_S \qquad (17)$$

$$(\frac{m}{C}+\frac{n\cdot(1-Y_H)\cdot x}{C}+\frac{p\cdot i_{XB}\cdot Y_H}{14})\cdot S_S(0) \qquad (18)$$

These parameter combinations are similar to the ones obtained for respirometric data, but with other stoichiometric factors. Finally, when considering combined respirometric – titrimetric data, a 5th parameter combination becomes structurally identifiable (Eq. 19), besides the ones shown in Eq. 11–14.

$$\frac{1}{1-Y_H}\cdot(\frac{m}{C}+\frac{n\cdot(1-Y_H)\cdot x}{C}+\frac{p\cdot i_{XB}\cdot Y_H}{14}) \qquad (19)$$

From this last parameter combination the biomass yield Y_H can be obtained, assuming that all the other parameters in Eq. 19 are known or can be measured.

3.5. Results parameter estimations

Simulations and parameter estimations were done using WEST (Hemmis NV, Kortrijk, Belgium). Parameter estimation was done using the Simplex optimization algorithm (Nelder and Mead, 1964). Minimization of the sum of squared errors (SSE) was used as the model fit criterion. The r_O data obtained via Eq. 2 were used for parameter estimation applications. When estimating parameters on separate respirometric or titrimetric data sets, it appeared that only 3 parameter combinations could be identified from the available data, since X and μ_{maxH} could only be practically identified together, in a parameter combination. The same was observed for the combined data sets. The explanation is that the biomass does not grow sufficiently during the short-term batch experiments. X and μ_{maxH} would only be practically identifiable as separate parameters when a considerable growth phase can be observed in the data .

An example of the model fit for acetate and dextrose data is shown in Fig. IV and V respectively. Model fits to the data are good. Note again that acetate degradation resulted in proton consumption, while dextrose degradation resulted in proton production. According to the model (Eq. 7–10) this could be explained because most of the acetate is present in undissociated form in the liquid phase. Uptake of acetate by the bacteria will thus cause an important proton consumption that is larger than the amount of protons released via CO_2 production and NH_3 uptake. Dextrose on the other hand is assumed to be a neutral component, which means that its uptake will not result in a proton consumption. Dextrose degradation therefore results in net proton production due to CO_2 production and NH_3 uptake.

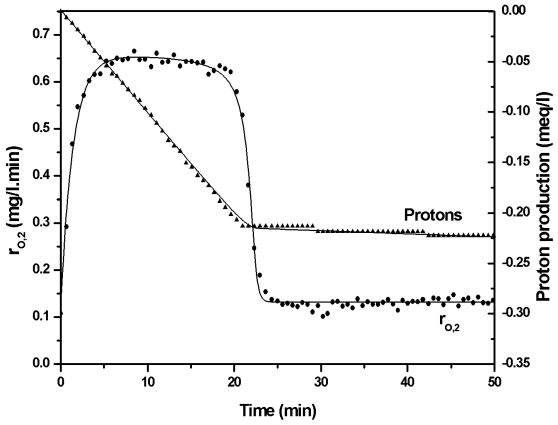

Figure IV. Model fit on combined r_O and Hp data for an acetate experiment (each 3rd datapoint shown)

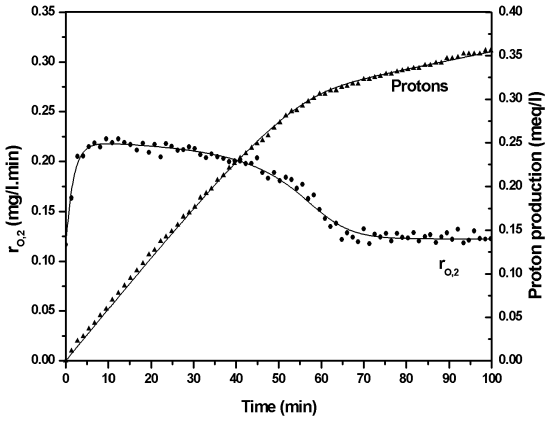

Figure V. Model fit on combined r_O and Hp data for a dextrose experiment (each 6th datapoint shown)

Biokinetic parameters for acetate and dextrose degradation experiments are given in Table 1 and 2. Parameters are given for two representative experiments only, due to lack of space. The initial substrate concentration $S_S(0)$ was known. Consequently, Y_H could be estimated (see Eq. 14) together with $\mu_{maxH}\cdot X$ and K_S. For estimations on titrimetric data both $S_S(0)$ and Y_H were assumed to be known. By making this assumption $\mu_{maxH}\cdot X$, K_S and i_{XB} could be estimated. For estimations on combined data sets Y_H, $\mu_{maxH}\cdot X$, K_S and i_{XB} were estimated, while assuming that $S_S(0)$ was known. The results show that $\mu_{maxH}\cdot X$ estimates on respirometric and titrimetric data are well comparable, both for acetate and dextrose. Differences in estimated K_S values are larger compared to the other parameters. For dextrose, titrimetry-based K_S values are higher than respirometry-based ones. A hypothesis could be that the CO_2 produced during dextrose oxidation is only released slowly, resulting in higher estimated K_S values. For acetate, on the other hand, titrimetry – based K_S values are lower than respirometry-based ones. In this case the observed proton production is mainly due to substrate uptake, and takes place before substrate oxidation. The affinity of the bacteria for substrate uptake is possibly higher than for substrate oxidation. Further research is needed to possibly include such phenomena in the model.

Combined data sets allow the estimation of an extra parameter (Eq. 19), compared to separate respirometric or titrimetric data. Although leading to more complex estimations, it is obviously most optimal to estimate parameters on the combined data sets since the resulting parameter values will thus reflect the information contained in both data sets.

Table 1. Acetate: Results of parameter estimations on respirometric (R), titrimetric (T) and combined respirometric-titrimetric data (R+T). Values in italics were assumed to be known; i_{XB} is not relevant in the model for respirometric data

Exp.	$S_{S,1}(0)$	Data	Y_H	$\mu_{maxH}\cdot X$	K_S	i_{XB}
1	*37.61*	R	0.75	1.48	0.63	-
		T	*0.75*	1.48	0.10	0.081
		R+T	0.75	1.48	0.65	0.078
2	*49.72*	R	0.74	1.50	0.62	-
		T	*0.74*	1.51	0.10	0.055
		R+T	0.74	1.50	0.62	0.051

Table 2. Dextrose: Results of parameter estimations. See Table 1 for explanation of symbols

Exp.	$S_{S,1}(0)$	Data	Y_H	$\mu_{maxH}\cdot X$	K_S	i_{XB}
1	*23.92*	R	0.88	0.61	0.54	-
		T	*0.88*	0.72	3.75	0.042
		R+T	0.89	0.62	1.80	0.041
2	*47.62*	R	0.87	0.71	1.12	-
		T	*0.87*	0.81	6.25	0.046
		R+T	0.87	0.74	3.80	0.043

4. CONCLUSION

A model including substrate uptake, CO_2 production and NH_3 uptake for biomass growth can describe titrimetric data derived from batch carbon source biodegradation experiments.

The combination of titrimetric and respirometric data sets is more information rich compared to the separate data sets.

ACKNOWLEDGEMENTS

Research of Krist Gernaey was financed by a postdoctoral scholarship of the Flemish Institute for the Promotion of Scientific-Technological Research in the Industry (IWT, Brussels) (1998–1999), a scholarship of the Quebec Ministry of Education in the frame of the “Bourses d’excellence” program (2000), and a NATO scholarship (2000).

REFERENCES

Bogaert H., A. Vanderhasselt, K. Gernaey, Z. Yuan, C. Thoeye and W. Verstraete (1997) New sensor based on pH-effect of denitrification process. J. Environ. Eng., **123**, 884 - 891.

Dochain D., P.A. Vanrolleghem and M. Van Daele (1995) Structural identifiability of biokinetic models of activated sludge respiration. Water Res., **29**, 2571 - 2578.

Gernaey K., P.A. Vanrolleghem. and W. Verstraete (1998) On-line estimation of Nitrosomonas kinetic parameters in activated sludge samples using titration in-sensor-experiments. Water Res., **32**, 71 - 80.

Gernaey K., B. Petersen, J.P. Ottoy and P.A. Vanrolleghem (2001) Activated sludge monitoring with combined respirometric-titrimetric measurements. Water Res., **35**, 1280 - 1294.

Henze M., C.P.L.Jr. Grady, W. Gujer, G.v.R. Marais and T. Matsuo (1987) Activated sludge model No. 1. IWA Scientific and technical Report No. 1, London, UK.

Iversen J.J.L., J.K. Thomsen and R.P. Cox (1994) On-line growth measurements in bioreactors by titrating metabolic proton exchange. Appl. Microbiol. Biotechnol., **42**, 256 - 262.

Nelder J.A. and R. Mead (1964) A simplex method for function minimization. Computer J., **7**, 308 - 313.

Petersen B., K. Gernaey and P.A. Vanrolleghem (2000) Improved theoretical identifiability of model parameters by combined respirometric-titrimetric measurements. A generalisation of results. In: Proceedings IMACS 3rd Symposium on Mathematical Modelling, February 2-4, 2000, Vienna University of Technology, Austria. Vol.2, 639 - 642.

Spanjers H. and G. Olsson (1992) Modelling of the dissolved oxygen probe response in the improvement of the performance of a continuous respiration meter. Water Res., **26**, 945 - 954.

Spanjers H., P.A. Vanrolleghem, G. Olsson and P.L. Dold (1998) Respirometry in control of the activated sludge process: Principles. IWA Scientific and Technical Report No. 7. London, UK.

Stumm W. and J.J. Morgan (1981) Aquatic Chemistry, An introduction emphasizing chemical equilibria in natural waters. John Wiley & Sons, New York, 780 p.

Vanrolleghem P.A., K. Gernaey, F. Coen, B. Petersen, B. De Clercq and J.P. Ottoy (1998) Limitations of short-term experiments designed for identification of activated sludge biodegradation models by fast dynamic phenomena. In: Proceedings CAB7. Osaka, Japan, May 31 - June 4 1998, p. 567 - 572.