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ON-LINE ESTIMATION OF *NITROSOMONAS* KINETIC PARAMETERS IN ACTIVATED SLUDGE SAMPLES USING TITRATION IN-SENSOR-EXPERIMENTS

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Abstract—A method is presented that uses H^+ production of the first nitrification step as an alternative to oxygen consumption to characterise activated sludge nitrification kinetics. The method consists of a titration in-sensor-experiment with nitrifying sludge combined with a parameter estimation procedure. Based on a cumulative base addition curve, it is possible to estimate the volumetric nitrification capacity of the mixed liquor ($\mu_A \times X_{BA}/Y_A$, in (mg-N/litre)/h) and the Monod half-saturation coefficient K_{NH} for the *Nitrosomonas* bacteria in the sludge. It is shown with lab tests and on-line in-sensor-experiments on a pilot activated sludge plant for N removal that the proposed combination of a titration experiment and a parameter estimation procedure can also be used to estimate nitrifiable N concentrations present in a mixed liquor sample. © 1998 Elsevier Science Ltd. All rights reserved

Key words—activated sludge, ammonium, estimation, kinetics, nitrification. *Nitrosomonas*, on-line, titration experiment

INTRODUCTION

Biological nitrogen (N) removal in activated sludge processes is conventionally obtained using a reactor configuration which guarantees a sequence of aerobic and anoxic process conditions. Aerobic conditions are necessary to obtain nitrification, while anoxic conditions are essential to support denitrification. Nitrification involves the aerobic conversion of NH_4^+ to NO_3^- by two groups of autotrophic bacteria. During this oxidation process, *Nitrosomonas* bacteria first convert the NH_4^+ into NO_2^-, which is subsequently oxidised to NO_3^- by *Nitrobacter* bacteria.

To achieve a better control and understanding of biological N removal processes, on-line information is needed not only about the NH_4^+-N and NO_3^--N concentrations but also about the biokinetic characteristics of the sludge (Vanrolleghem and Coen, 1995). This has led to the development of methods which allow the extraction of kinetic parameters from data provided by, for example, a substrate depletion experiment.

In this research field, the concept "in-sensor-experiment" covers a group of experimental devices consisting of a down-scaled bioreactor in which a simple and robust sensor element (DO, pH, ORP, . . .) is used to monitor the biological response of the biomass on a substrate addition (Vanrolleghem and Coen, 1995). The in-sensor-experiment concept was originally developed for respirometric experiments with activated sludge sampled from a full-scale plant. Respirometry was preferred above substrate specific monitoring methods because respirometry is generally applicable, easy to automate and sensitive to rather small substrate concentrations. Therefore, manifold respirometric methods have been used to characterise heterotrophic and nitrifying biomass in activated sludge samples (Kappeler and Gujer, 1992; Kroiss *et al.*, 1992; Drtil *et al.*, 1993; Nowak and Svardal, 1993; Vanrolleghem and Verstraete, 1993; Spanjers and Vanrolleghem, 1995; Kong *et al.*, 1996; Surmacz-Gorska *et al.*, 1996).

As an alternative, it has been realised that measuring the pH is useful in monitoring biological N removal processes (Ramadori *et al.*, 1980; Al-Ghusain *et al.*, 1994; Chang and Hao, 1996). The advantage of pH measurement is that it can be used under both aerobic and anoxic conditions, because nitrification as well as denitrification will influence the pH of the mixed liquor by the destruction and the formation of alkalinity respectively (EPA, 1993).

The pH effect of nitrification and denitrification reactions has potential as input to the control of biological N removal in alternating aerobic–anoxic systems. Control algorithms have been proposed based on the bending points in the pH profile obtained from a pH probe located in the process tank

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itself (Al-Ghusain *et al.*, 1994; Chang and Hao, 1996). In doing so, the pH profile is used in a comparable way to an oxidation-reduction potential (ORP) profile, in which the reaction endpoint is detected as a so-called knee in the profile (Sasaki *et al.*, 1993; Wareham *et al.*, 1993; Al-Ghusain *et al.*, 1994; Demuynck *et al.*, 1994; Vanrolleghem and Coen, 1995). The pH effect of nitrification and denitrification has been used also as the basic principle to construct sensors to monitor nitrification and denitrification in activated sludge (Ramadori *et al.*, 1980; Aivasidis *et al.*, 1992; Massone *et al.*, 1995, 1996; Vanderhasselt, 1995; Gernaey *et al.*, 1997).

The scope of this paper is limited to the nitrification process. It is shown how an existing parameter estimation procedure, which was originally developed to estimate kinetic parameters from respirometric in-sensor-experiments, can be used to estimate kinetic parameters of nitrification on the basis of information provided by a titration in-sensor-experiment using a nitrifying activated sludge sample.

MATERIALS AND METHODS

Data were collected using a titration technique which was developed earlier for nitrification monitoring in activated sludge (Massone *et al.*, 1995, 1996; Gernaey *et al.*, 1997). Data were derived from two different series of measurements: off-line titration experiments and on-line experiments on a pilot activated sludge plant for N removal.

Off-line titration experiments

For each experiment, an activated sludge sample in the endogenous respiration phase was transferred to the reactor vessel of a titration unit (Fig. 1). This vessel was continuously stirred, aerated and temperature-controlled (25°C). In a first phase the pH of the mixed liquor sample was increased to the pH setpoint (empirically determined: pH setpoint = pH mixed liquor sample + 0.4 pH units). Once the pH setpoint was reached, base was only dosed to the mixed liquor when the pH became lower than the pH setpoint minus a ΔpH interval. Titration experiments with a pH controller which keeps the sludge sample at a fixed pH setpoint were preferred over a measuring principle in which only pH profiles are recorded. A pH profile can not be used for, for example, the calculation of stoichiometry-based nitrification rates in the sludge, because a pH decrease can not be related to a precise number of protons formed. Indeed, the buffer capacity of the mixed liquor ((meq/litre)/pH unit) is changing when the pH is varying, due to the presence of different acid/base buffer systems which have a pH-dependent buffer intensity (Stumm and Morgan, 1981). Problems with a pH-dependent buffer capacity are avoided by working at a fixed pH. The pH controller continuously neutralises the protons formed during nitrification by the addition of NaOH. Moreover, a different alkalinity of different sludge samples could not influence the measurements.

Typically, experiments were conducted at a pH setpoint ± ΔpH interval of 8.00 ± 0.03. Base was added by opening for 1.5 s (= 1 base pulse) an electromagnetic valve connected to a mariotte bottle contaning a 0.1 M NaOH stock solution. Opening the valve for 1.5 s was repeated until the pH in the titration vessel returned within the pH setpoint ± ΔpH interval. Every 10 s, the computer controlling the titration unit stored the actual pH in the reactor vessel and the total number of base pulses dosed so far during the titration experiment. The flow per base pulse was checked regularly (two times per day, between two experiments) by measuring the volume of base corresponding to 50 subsequent base pulses. Raw titration data (B) were converted into meq/litre units (B') according to

$$B' = \frac{B \times Ob \times N}{V} \quad (1)$$

where B represents the raw titration data (base pulses), B' represents the titration data (meq/litre reactor), N is the base normality (meq/ml), Qb is the Base flux (ml/base pulse) and V is the volume of the reactor vessel (litres).

On-line in-sensor-experiments

The titration unit was installed on an activated sludge pilot plant for nutrient removal (V = 150 litres; sludge residence time = 3–4 days). The pilot plant was a pre-denitrification system with an anoxic zone of 30 litres and an aerobic zone divided into three compartments. The first two aerobic compartments had a volume of 30 litres each; the volume of the third compartment was 60 litres. To simulate a diurnal loading pattern, the influent flow to the pilot plant corresponded to a sine wave with an average flow of 8 litres/h and an amplitude of 1.8 litres/h. The pilot plant was fed with a synthetic wastewater (mainly based on skimmed milkpowder and NH_4Cl), whose concentration was varied in phase with the flow. The influent N (NH_4^+-N and organic N) concentration varied between 73 and 108 mg-N/litre, with an average of 91 mg-N/litre, resulting in a load variation between 453 and 1058 mg-N/h with an average of 759 mg-N/h. This corresponded to an average volumetric N loading of 6.3 (mg-N/litre)/h (calculated on the volume of the aerobic compartment). The average COD load to the plant was 7.4 g-COD/h (C/N ≈ 10). The COD load varied in phase with the N load variations. At regular times, sludge was sampled from the pilot plant and analysed

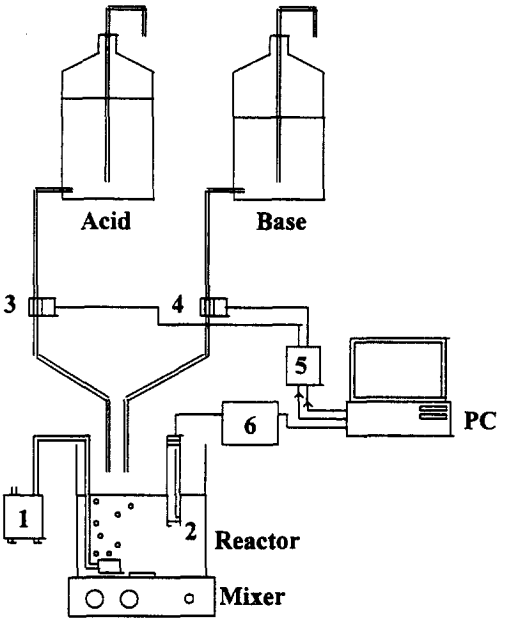

Fig. 1. Titration unit used for the experiments with nitrifying activated sludge. The PC, connected to a pH electrode by a 4–20-mA transmitter, controls the dosage of base to the sludge sample. (1) Aeration pump; (2) pH electrode; (3, 4) 24-V DC electromagnetic valves; (5) 24-V DC supply; (6) 4–20-mA transmitter.

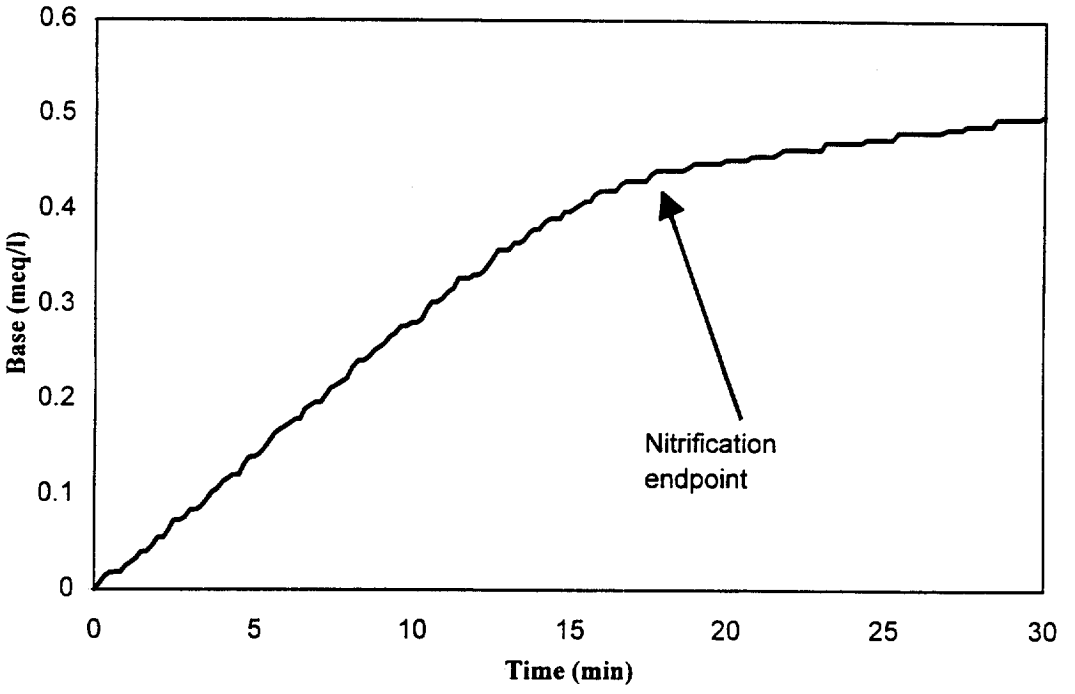

Fig. 2. Typical cumulative base addition curve obtained during a titration experiment with a mixed liquor sample. Experimental conditions: $T = 25°C$, pH = 8.20 ± 0.03, $V = 4$ litres, $S_{NH}(0) = 2.5$ mg-NH_4^+-N/litre, 0.1 M NaOH, base flux = 7.3 ml per 50 base pulses.

for its suspended solids (SS) content according to *Standard Methods* (APHA, 1992).

During the titration experiments, an on-line NH_4^+-N analyser (Minworth Systems, Sutton Coldfield, UK) was installed at the effluent line of the pilot plant. Location at the effluent was chosen to avoid the need for an ultrafiltration unit which would have been required if mixed liquor NH_4^+-N concentrations were to be monitored. The on-line analyser was operated without any problem, because the effluent contained little or no sludge flocs due to the good performance of the pilot-plant clarifier ($V = 10$ litres). Data from the on-line NH_4^+-N analyser were stored as the average of the concentrations measured during 1 h.

The reactor vessel of the titration unit was not temperature-controlled, in order to maintain the same temperature in the measuring vessel as in the pilot plant. Every 2 h, an activated sludge sample was taken from the last aerobic compartment of the activated sludge pilot plant. Immediately after sampling (before the pH setpoint was reached), 1.33 ± 0.05 mg-NH_4^+-N/litre were dosed to the sludge sample. Addition of NH_4^+-N allowed to estimate kinetic parameters of the nitrifiers, even when no NH_4^+-N was present in the original mixed liquor sample.

RESULTS

Off-line experiments

Figure 2 shows a typical cumulative base addition curve obtained during an off-line titration experiment in which 10 mg-NH_4^+-N were dosed to 4 litres of activated sludge at $t = 0$. Two different phases can be distinguished following the addition of the substrate. In a first phase, the sludge is actively nitrifying and base was dosed at a rather constant rate (0.028 (meq/litre)/min). After about 15 min, the slope of the cumulative base addition curve decreased rapidly, indicating that almost all NH_4^+-N was nitrified. When nitrification was completed (after about 20 min in Fig. 2), about 0.45 meq-base/litre had been dosed to the sludge. However, Fig. 2 also shows that base continued to be added to the sludge sample, albeit at a much lower rate compared to the nitrification phase. In the sequel, this second slope will be called the background proton production rate (BPPR).

Parameter estimation method

The stoichiometry of the nitrification reaction (equation 2) was obtained based on data of Henze *et al.* (1987), assuming that carbon for the formation of new nitrifying biomass was supplied as CO_2. Units used in equation (2) are those proposed by Henze *et al.* (1987), except for the alkalinity destruction due to nitrification, which is expressed here as formation of protons. Equation (3) is the same reaction equation, calculated for the default parameter set of activated sludge model No 1 and for 1 mg COD biomass formed, but expressed in molar units where appropriate. To obtain the conversion rates, the stoichiometry is employed in a reaction kinetic equation, typically a Monod-based reaction kinetics. The titration data could be described by a Monod biodegradation model and a model describing the BPPR (equations 4 and 5).

$$\frac{1 + Y_A \times i_{XB}}{Y_A} S_{NH} + \frac{4.57 - Y_A}{Y_A} S_0 + i_{XC} S_C \rightarrow 1 X_{BA} + \frac{1}{Y_A} S_{NO} + \alpha H_2O + \frac{2 + Y_A \times i_{XB}}{14 \times Y_A} H^+ \quad (2)$$

$$0.304 S_{NH} + 0.564 S_0 + 0.031 S_C \rightarrow 0.0062 X_{BA} + 0.298 S_{NO} + 0.285 H_2O + 0.601 H^+ \quad (3)$$

where H^+ is the protons formed during nitrification (meq/litre), i_{XB} is the fraction of N in the biomass (g-N/g-COD), i_{XC} is the fraction of C in the biomass (mmol/g-COD), S_C is the CO_2-C for the formation of autotrophic biomass (mmol-C/litre), S_{NH} is the ammonium concentration (mg-N/litre), S_{NO} is the nitrate concentration (mg-N/litre), S_0 is the oxygen consumed during nitrification (mg/litre), X_{BA} is the autotrophic biomass concentration (mg-COD/litre), Y_A is the yield coefficient for autotrophic biomass (g-COD/g-N nitrified), α is a stoichiometric constant.

$$\frac{dH^+}{dt} = \frac{2 + Y_A \times i_{XB}}{14 \times Y_A} \times \mu_A \times \frac{S_{NH}}{K_{NH} + S_{NH}} \times X_{BA} + BPPR \quad (4)$$

$$\frac{dNH_4}{dt} = -\left(\frac{1}{Y_A} + i_{XB}\right) \times \mu_A \times X_{BA} \times \frac{S_{NH}}{K_{NH} + S_{NH}} \quad (5)$$

where BPPR is the background proton production rate ((meq/litre)/min), dH^+/dt is the proton production rate ((meq/litre)/min), dNH_4/dt is the ammonium removal rate ((mg-N/litre)/min), K_{NH} is the Monod half-saturation coefficient for NH_4^+-N (mg-N/litre), μ_A is the maximum specific growth rate for autotrophic biomass (1/min).

A non-linear parameter estimation algorithm (Brent, 1973) was used to estimate kinetic nitrification parameters by fitting the model presented in equations (4) and (5) to the data provided by each titration experiment. Minimisation of the sum of squared errors (SSE) was used as the fit criterion. The estimation procedure was performed automatically with MOSIFIT (MOdel SImulator and FITter, available on request), a software package that has already shown its usefulness for the estimation of kinetic parameters based on respirometric data (Vanrolleghem and Verstraete, 1993; Spanjers and Vanrolleghem, 1995; Kong *et al.*, 1996).

When estimating parameters for a single Monod model from respirometric data, three parameter combinations are structurally identifiable (Vanrolleghem *et al.*, 1995): $[(1 - Y_i) \times \mu_{maxi} \times X/Y_i]$, $[(1 - Y_i) \times K_i]$ and $[(1 - Y_i) \times S_i(0)]$. The identifiability problem for parameter estimations from titration data is comparable. Based on titration data, the following three combinations of parameters are identifiable from perfect data:

$$\frac{2 + Y_A \times i_{XB}}{14} \times \frac{\mu_A \times X_{BA}}{Y_A}$$

$$\frac{2 + Y_A \times i_{XB}}{14} \times K_{NH}$$

$$\frac{2 + Y_A \times i_{XB}}{14} \times S_{NH}(0)$$

where $(\mu_A \times X_{BA})/Y_A$ is the nitrification capacity of the mixed liquor sample ((mg-N/litre)/h) and $S_{NH}(0)$ is the initial substrate concentration (mg-N/litre).

The fraction of autotrophic biomass X_{BA} was not determined. Calculation of $(\mu_A \times X_{BA})/Y_A$, K_{NH} and $S_{NH}(0)$ was done assuming $Y_A = 0.24$ and $i_{XB} = 0.086$. In fact, exact knowledge of the values for Y_A and i_{XB} is not really necessary, as this will only have a minor influence on the factor $2 + Y_A \times i_{XB}$ (e.g. $= 2 + 0.24 \times 0.086 = 2.021$). This indicates that it is realistic to calculate $(\mu_A \times X_{BA})/Y_A$, K_{NH} and $S_{NH}(0)$ from the estimated combinations of parameters. Theoretically, it should be possible to determine the exact value of the factor $2 + Y_A \times i_{XB}$ by performing a titration experiment with a known concentration of NH_4^+-N. However, this approach is not realistic as the influence of the factor $Y_A \times i_{XB}$ will be of the same order of magnitude as the error on the measurements. Moreover, other reactions (hydrolysis, heterotrophic activity) will probably overshadow the contribution of N going into new autotrophic cells.

A simulated NH_4^+-N profile and corresponding cumulative base addition curve with residuals

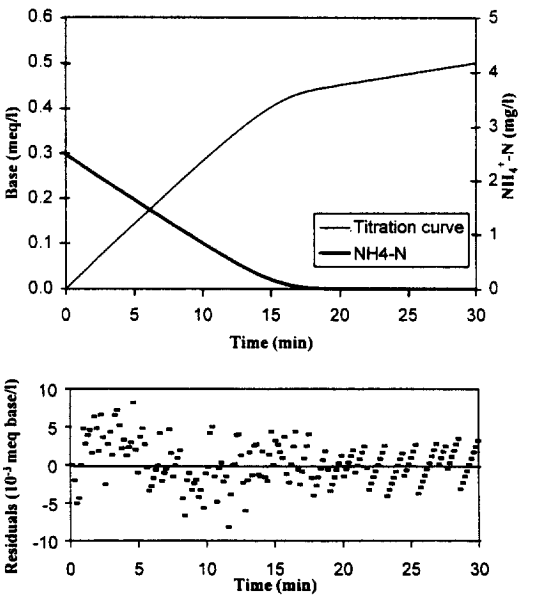

Fig. 3. Simulated NH_4^+-N concentration profile and cumulative base addition curve obtained with the kinetic nitrification parameters estimated from the base addition curve of Fig. 2 $[(\mu_A \times X_{BA}/Y_A) = 10.67$ (mg-N/litre)/h; $K_{NH} = 0.16$ mg-NH_4^+-N/litre; $S_{NH}(0) = 2.46$ mg-NH_4^+-N/litre; BPPR = 0.284 (meq/litre)/h]. Residuals are given as well.

Table 1. Results of the off-line repeatability tests for activated sludge sampled from two different wastewater treatment plants (sludge was only sampled once for each treatment plant, stored at 4°C and tested on different days; all tests were performed at 25°C)

Date	$(\mu_A \times X_{BA})/Y_A$ ((mg-N/litre)/h)	K_{NH} (mg-N/litre)	BPPR ((meq/litre)/h)	$S_{NH}(0)$ (mg-N/litre)
	WWTP1 Zwalm	*SS = 2.66 g/litre*	*VSS = 1.88 g/litre*	
16-07-96	16.60	0.15	0.288	2.49
16-07-96	14.89	0.14	0.289	2.55
18-07-96	21.60	0.17	0.190	2.38
18-07-96	18.12	0.13	0.218	2.38
18-07-96	17.53	0.16	0.275	2.28
25-07-96	18.75	0.08	0.117	2.29
25-07-96	16.81	0.12	0.123	2.41
25-07-96	21.26	0.21	0.127	2.38
26-07-96	19.03	0.09	0.190	2.47
26-07-96	18.09	0.17	0.161	2.40
Average	18.27	0.14	0.198	2.40
St. Dev.	2.05	0.04	0.068	0.08
C.V.	11.21	26.55	34.24	3.49
	WWTP 2 Hospital	*SS = 4.78 g/litre*	*VSS = 3.65 g/litre*	
2-08-96	5.37	0.06	0.413	2.45
2-08-96	6.46	0.23	0.294	2.72
2-08-96	6.47	0.15	0.438	2.32
3-08-96	6.88	0.16	0.388	2.63
Average	6.30	0.15	0.383	2.53
St. Dev.	0.65	0.07	0.063	0.18
C.V.	10.33	48.13	16.39	7.13

resulting from the estimation procedure are shown in Fig. 3. The simulation results of Fig. 3 were obtained by using the parameter values estimated from the base addition curve of Fig. 2 in the model described by equations (4) and (5). Residuals were independent (except for the BPPR-tail, where the stepwise addition of base resulted in dependence of the residuals), had a constant variance and their distribution corresponded to a normal distribution. This indicated a good data quality and model fit and the adequateness of a least-squares objective function (Robinson, 1985).

Repeatability

Some tests were performed to check for the repeatability of the proposed kinetic characterisation procedure. Activated sludge was sampled at two treatment plants and stored at 4°C. Tests with different aliquots of the same sludge sample were performed on different days. For each test, 10 mg NH_4^+-N were initially added to 4 litres of activated sludge in endogenous state. Finally, the biokinetic parameter estimation procedure was applied to the resulting data. Results are summarised in Table 1. A relatively low coefficient of variation (C.V.) was obtained for the nitrification capacity (11%). For K_{NH} the C.V. was considerably higher (26.55%). Repeatability of the proposed procedure was good for $S_{NH}(0)$ determinations (C.V. = 3.49%). Also, the estimated $S_{NH}(0)$ value of 2.40 mg-NH_4^+-N/litre was close to 2.5 mg-NH_4^+-N/litre, the NH_4^+-N concentration initially added to the sludge. For the BPPR factor, a high C.V. of 34.67% was found. An analysis of variance was done on the 10 parameter sets obtained for the first sludge sample. Only BPPR values obtained on different days were significantly different ($\alpha = 0.05$). For the second sludge sample, about the same pattern could be observed. Again, estimated $S_{NH}(0)$ values (2.53 ± 0.18 mg-N/litre; $n = 4$) were very close to the added 2.5 mg-NH_4^+-N/litre.

On-line experiments

The shape of the cumulative base addition curves obtained in the on-line set-up (Fig. 4) was different compared to the off-line experiments. For the on-line experiments NH_4^+-N was already present at the beginning of a titration experiment (either from the mixed liquor itself or from the intentional addition of 1.33 mg-N/litre). Consequently, nitrification was going on while the pH was increased to the setpoint during a first experimental phase. Hence, one should realise that only a minor part of the amount of base added during this first phase was needed to compensate for the protons formed by the nitrifying activity of the sludge sample. In this phase the main fraction of base was required to increase the pH. As a result, the data to be used as input for the proposed parameter estimation procedure were obtained by subtracting the amount of base necessary to reach the pH setpoint. This amount corresponds to the intercept with the Y-axis obtained by linearly (i.e. assumption of constant nitrification rate) backtracking that part of the cumulative base addition curve recorded immediately after reaching the pH setpoint (Fig. 4).

Figure 5 shows SS concentrations measured in the pilot plant and nitrification capacity ($\mu_A \times X_{BA}/Y_A$) and K_{NH} values estimated from the titration in-sensor-experiments. Two different periods can be distinguished. During the first 2 weeks, the nitrification capacity varied around 4.5 (mg-N/litre)/h. This situation suddenly changed around day 14. For the

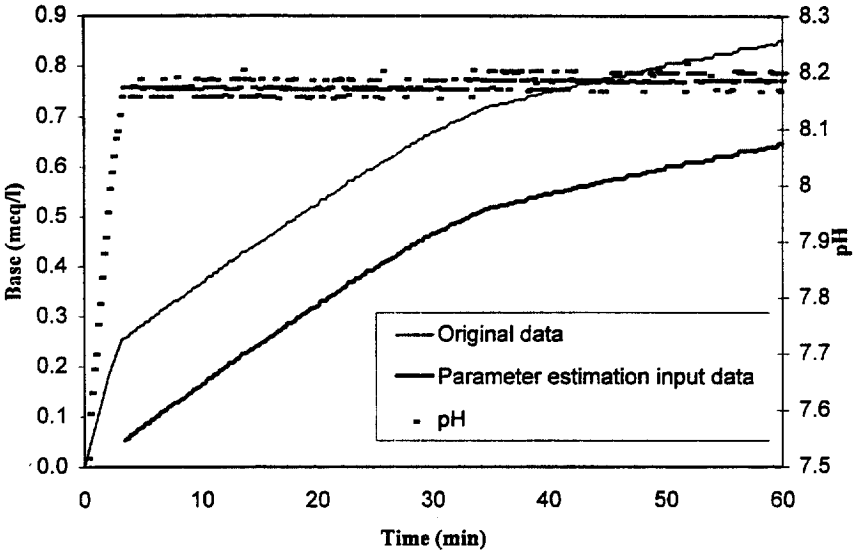

Fig. 4. Typical cumulative base addition curve recorded during the on-line experiments. During a first phase, the pH of the sludge is increased to the pH setpoint (8.2 ± 0.03). The data used in the parameter estimation procedure are obtained by subtracting the amount of base necessary to reach the pH setpoint (0.203 meq/litre for this example) from the original cumulative base addition curve.

second period (day 14–18), the nitrification capacity was considerably lower (3 (mg-N/litre)/h). The decrease of the nitrification capacity coincided with a slight decrease of the SS concentration. Estimated K_{NH} values were almost always lower than 0.5 mg-NH_4^+-N/litre.

The $S_{NH}(0)$ values estimated from the same on-line titration experiments were compared with on-line effluent NH_4^+-N measurements (Fig. 6). During periods that no NH_4^+-N was measured in the effluent, estimated $S_{NH}(0)$ values were close to the amount of 1.33 ± 0.05 mg-NH_4^+-N/litre intentionally dosed to the sludge sample at the beginning of each titration. Every time the effluent concentration measured with the on-line NH_4^+-N analyser increased, the $S_{NH}(0)$ value estimated from the titration data also increased. In some cases (e.g. on day 12) the NH_4^+-N concentration in the sludge was so high that the 2 h reserved for each titration experiment proved to be insufficient to obtain complete nitrification. For such datasets, no $S_{NH}(0)$ value could be estimated, but one could conclude that the NH_4^+-N concentration for the titration experiment was higher than $[(2 + Y_A \times i_{XB})/14] \times [B\text{-}B_{sp}\text{-BPPR} \times t]$, where B is the total amount

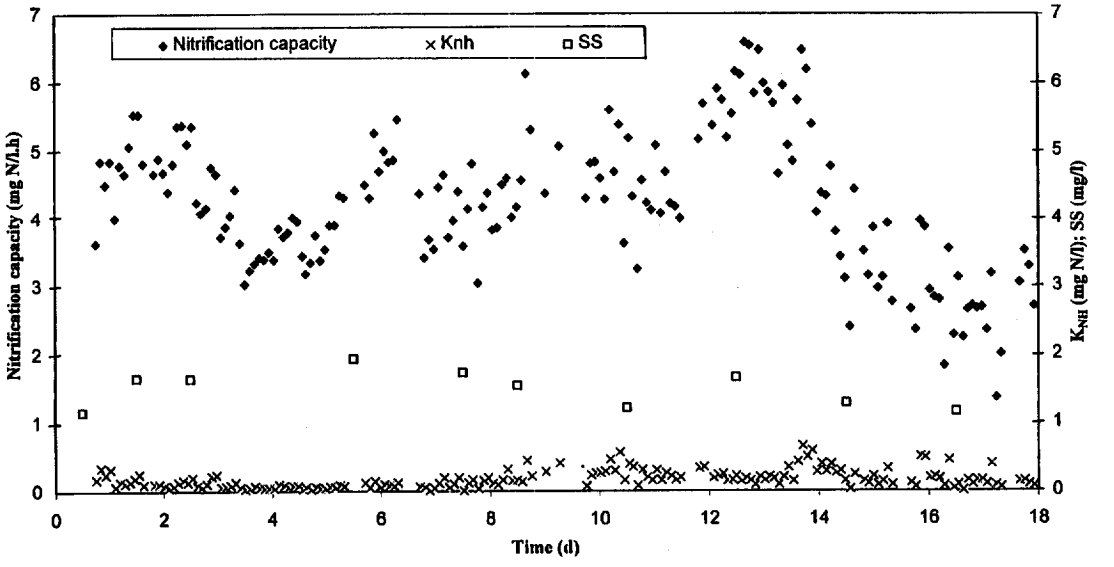

Fig. 5. Nitrification capacity $(\mu_A \times X_{BA})/Y_A$ and K_{NH} values estimated based on three weeks of titration data collected on a pilot activated sludge plant for nutrient removal. SS values of the sludge in the pilot plant are also shown.

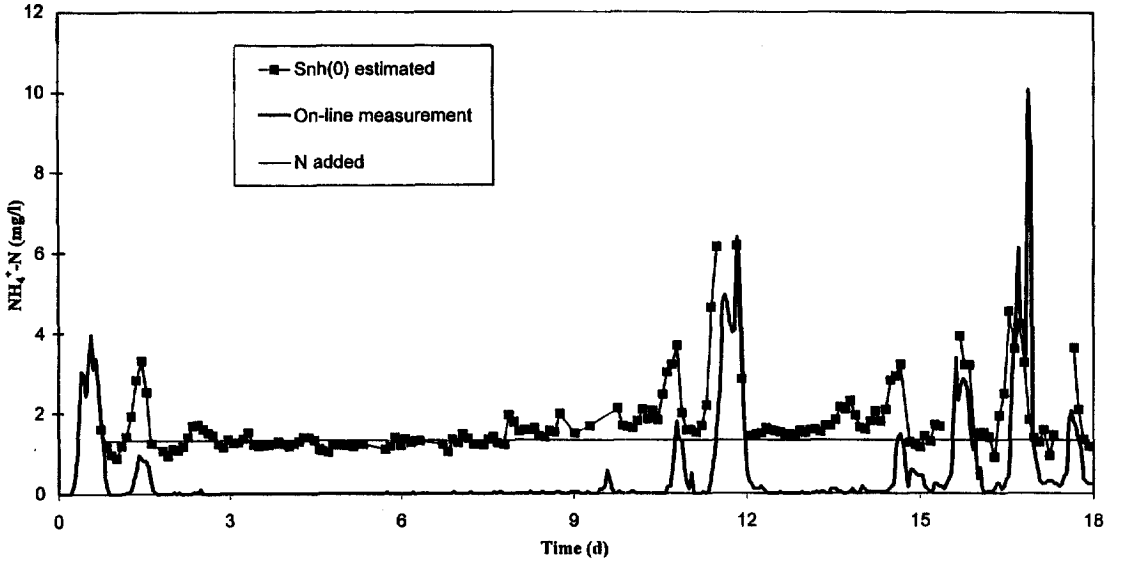

Fig. 6. Comparison between $S_{NH}(0)$ values estimated from titration experiments with mixed liquor and on-line NH_4^+-N measurements on the effluent of the activated sludge pilot plant. The solid line indicates the standard NH_4^+-N concentration intentionally dosed to the activated sludge sample at the beginning of each measurement.

of base added at the end of the titration experiment (meq/litre), B_{sp} is the amount of base needed to increase the pH of the sludge sample to the pH setpoint (meq/litre), BPPR is the background proton production rate ((meq/litre)/min) obtained from the previous titration experiment and t is the total duration of the measurement (min). This explains the gaps in the $S_{NH}(0)$ data (e.g. on day 12).

The correlation matrix for the parameters estimated from on-line titration experiments is presented in Table 2. Correlation between the different parameters was low.

DISCUSSION

Off-line experiments

The combination of a titration experiment and the parameter estimation procedure allows the estimation of nitrification kinetics based on one experiment of about 30 min. This is possible because the experiment results, in fact, in a series of nitrification rates at variable NH_4^+-N concentrations so that the rate as Monod function of S_{NH} can be determined. This is an advantage compared to some other methods where a separate experiment is to be conducted for each substrate concentration (Drtil *et al.*, 1993; Nowak and Svardal, 1993). Basically, the titration experiments are very comparable to respirometric experiments as presented by Vanrolleghem and Verstraete (1993) and Kong *et al.* (1996).

In contrast to the respirometric data traditionally used for biokinetic characterisation (Vanrolleghem and Verstraete, 1993; Kong *et al.*, 1996), no K_La mass transfer coefficient estimation is needed when estimating kinetic parameters from titration data. It was found that for the titration experiments, the K_La of the reactor vessel of the titration unit only influences the BPPR of the cumulative base addition curve. This can be explained as follows: when aerating the mixed liquor sample in the reactor vessel of the titration unit, the mixed liquor will normally tend to an equilibrium pH which is determined by the alkalinity and the CO_2 content of the sludge sample (Stumm and Morgan, 1981). The BPPR is caused by the fact that the pH setpoint of the pH controller is higher than the bicarbonate system equilibrium pH of the sludge sample in the reactor vessel of the titration unit. Even when nitrification is finished, some base addition is still necessary to keep the pH of the sludge at the pH setpoint, because the mixed liquor will continuously tend to the lower equilibrium pH. During the experiments, an increase of the BPPR was indeed observed when the difference between the pH setpoint and the equilibrium pH increased. A more

Table 2. Correlation matrix for parameters obtained from on-line titration experiments (174 datasets)

	$(\mu_A \times X_{BA})/Y_A$	K_{NH}	$S_{NH}(0)$	BPPR
$(\mu_A \times X_{BA})/Y_A$	1			
K_{NH}	0.4546	1		
$S_{NH}(0)$	−0.0367	0.2464	1	
BPPR	−0.1481	0.0797	0.2170	1

Table 3. Literature data for K_{NH} values of nitrifying bacteria (data for *Nitrosomonas* bacteria only are indicated with an asterisk)

Reference	Experimental design	Numerical technique	K_{NH} (mg-N/litre)
Sharma and Ahlert (1977)	Not indicated	—	0.06–5.6*
Henze *et al.* (1987)	Not indicated	—	1.0
Tchobanoglous and Burton (1991)	Not indicated	—	0.2–2.0*
Drtil *et al.* (1993)	Respirometry	Regression for linearized Monod equation	0.28–0.61*
Nowak and Svardal (1993)	Respirometry	Non-linear regression	0.4–0.8*
Kong *et al.* (1996)	Respirometry	Non-linear parameter estimation	0.88 ± 0.13
Hellinga *et al.* (1996)	Respirometry	Non-linear parameter estimation	3.13–196
The present work	Titration	Non-linear parameter estimation	0.06–0.23*

efficient aeration in the reactor vessel will result in more CO_2 stripping from the sludge sample, which will in turn result in a higher equilibrium pH of the sludge sample and a lower BPPR.

Repeatability

Results of parameter estimations performed with datasets collected during lab experiments are presented in Table 1. The observed C.V. values of 11.21 and 10.33% for $(\mu_A \times X_{BA}/Y_A)$ are comparable with the C.V. values of 15.0% mentioned for values of a μ_A-related parameter combination obtained for autotrophic bacteria using a similar estimation procedure with respirometric data (Kong *et al.*, 1996). In the literature, C.V. values varying between 1.4 and 128.9% are mentioned when estimating μ_{max} values using non-linear parameter estimation techniques (Robinson and Tiedje, 1982; Simkins and Alexander, 1984, 1985).

The C.V. calculated for the K_{NH} values estimated from titration data is considerably higher than the C.V. for $(\mu_A \times X_{BA}/Y_A)$. Similar phenomena were already observed when estimating kinetic parameters based on respirometric data (Kong *et al.*, 1996) or substrate concentrations measured during a substrate depletion experiment (Robinson and Tiedje, 1982; Simkins and Alexander, 1984, 1985). The higher parameter estimation error for K_{NH} is intrinsic to this type of batch experiments, but could be alleviated by improved experimental design such that more data are obtained related to the conversion at low substrate concentration. This approach could be similar in nature to the respirometric batch experiments in which the reliability for parameter estimation was improved by adding a small additional substrate pulse at the end of the experiment (Vanrolleghem *et al.*, 1995). It can be expected that similar improvements can be obtained when estimating parameters using titration data.

For both sludge samples used in the lab experiments, K_{NH} values around 0.15 mg-N/litre were found. The K_{NH} values obtained with this method are only related to the *Nitrosomonas* bacteria, corresponding to the well-established stoichiometric equations for the first and the second nitrification step (EPA, 1993):

$$NH_4^+ + 1.5\,O_2 \rightarrow 2\,H^+ + H_2O + NO_2^- \qquad \textit{Nitrosomonas} \quad (6)$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \qquad \textit{Nitrobacter} \quad (7)$$

when compared to K_{NH} values found in the literature (Table 3), it can be concluded that the obtained K_{NH} values are in the lower end of K_{NH} values reported for *Nitrosomonas* bacteria in the literature. However, K_{NH_3} is in fact a better representation of reality instead of the affinity constant K_{NH} (for NH_4^+-N), because NH_3 is the real substrate for *Nitrosomonas* bacteria (Groeneweg *et al.*, 1994; Hellinga *et al.*, 1996). The concentration of NH_3 in the sludge sample is determined by the pH dependent equilibrium reaction $NH_4^+ \leftrightarrow NH_3 + H^+$. For a constant total $(NH_4^+ + NH_3)$-N concentration, the NH_3 concentration will increase by increasing the pH of the sludge sample, as is done at the beginning of a titration experiment (typically 0.4 pH units). This indicates that K_{NH} values resulting from the estimation procedure are not completely representative for reality, but K_{NH} values could be recalculated to the original pH value. The pH dependence of the K_{NH} values could in fact be investigated by performing experiments at different pH with the experimental set-up proposed in this paper.

An analysis of variance showed that BPPR values obtained on different days (Table 1) were significantly different ($\alpha = 0.05$). However, BPPR variations due to, for example, a difference in aeration efficiency do not influence the other parameter values resulting from the estimation procedure. A possible reason for the high C.V. observed for BPPR values can be a different initial alkalinity content of sludge samples used on different days. Changes in alkalinity could have been due to biological activity during sludge storage at 4°C. This explanation is supported by the observation that BPPR values are normally comparable for experiments carried out on the same day (Table 1).

On-line in-sensor-experiments

For the on-line experiments, the low correlation found between different parameters (Table 2) indicates a good data quality, which supports further use of this method. Correlation between the different parameters was low, indicating that the estimated parameter values were independent and could be estimated uniquely, which is often problematic for Monod-type kinetics (Vanrolleghem *et al.*, 1995).

The nitrification capacity measured for the sludge (2–6 (mg-N/litre)/h; see Figure 5) was always found to be considerably lower than the N load to the plant (6.3 (mg-N/litre)/h). The amount of NH_4^+-N necessary for the formation of new nitrifying biomass is negligible, as only 2% of the removed NH_4^+-N is used for the formation of new nitrifying biomass during nitrification (equation 5). On the other hand, in the denitrification compartment and also in the first aerobic compartments of the pilot plant, considerable heterotrophic growth takes place and removes an important fraction of the influent N. Daily, about 45 litres of sludge were removed as waste sludge from the last aerobic compartment of the pilot plant. Assuming an average SS concentration of 1.5 g/litre (calculated using SS values of Fig. 5), and a biomass N content of 6% (based on SS values), about $(45 \times 1.5 \times 0.06)/24 = 0.170$ g-N/h is incorporated into heterotrophic biomass, compared to an average N load of 0.759 g-N/h.

Compared to the on-line NH_4^+-N analyser operated on the effluent of the pilot plant, the titration method combined with the parameter estimation procedure has the advantage of yielding additional information about the characteristics of the nitrifying biomass. It was observed during the experiments how the nitrification capacity decreased around day 14 (Fig. 5), and this coincided with an increase of the average NH_4^+-N concentration in the effluent of the pilot plant (Fig. 6). The diurnal effluent NH_4^+-N peak observed during the last 4 days of the experiments can be explained by the diurnal loading pattern of the pilot plant. The K_{NH} values of Fig. 5 were comparable with the literature K_{NH} values of Table 3.

The difference between estimated $S_{NH}(0)$ and the NH_4^+-N concentrations measured with the on-line NH_4^+ analyser should theoretically be around 1.33 mg-NH_4^+-N/litre (Fig. 6), the NH_4^+-N concentration which was intentionally added to the sludge at the beginning of each titration experiment. The results show that this difference is found most evidently during the period when the effluent contained no NH_4^+-N (e.g. between day 2 and 9 in Fig. 6). This confirms the good correspondence found in the off-line experiments between the $S_{NH}(0)$ values estimated from the titration data and the amount of NH_4^+-N added (Table 1).

Each time the effluent contained significant amounts of NH_4^+-N, this was detected by both methods. Sometimes the difference between both measurements was found to be higher than 1.33 mg-NH_4^+-N/litre (e.g. on day 2, Fig. 6). This can be explained by the different location of the two sampling points. Samples for the titration experiments were taken from the last aerobic compartment of the pilot plant, while the on-line NH_4^+ analyser was operated on the effluent of the pilot plant for practical reasons (elimination of an ultrafiltration step). When the sludge still contained some NH_4^+-N some nitrification may have occurred in the decanter ($V = 10$ litres). In addition, mixing effects in the decanter and the 1-h averaging performed on the NH_4^+-N data will also smooth the effluent NH_4^+-N peaks.

Only the nitrifiable N will be measured during a titration experiment. The $S_{NH}(0)$ values as obtained here compare well to the NH_4^+-N concentrations measured on the effluent of the pilot plant, because most of the COD is already removed at the point where sludge is sampled for the titrations (last aerobic compartment of the pilot plant). Little or no NH_4^+-N is incorporated in new biomass during the titration experiments described here. In fact, just like respirometric methods (Spanjers and Vanrolleghem, 1995), the proposed titration method could be used to characterise the nitrifiable influent N load. In this case, N incorporated into new biomass (due to influent COD removal) would not be measured. However, this is no problem for practical applications, because this N fraction will not be nitrified in the full-scale plant either. A bigger problem could be the pH effect caused by ammonification of soluble biodegradable organic N (S_{ND}) present in the influent, because this process will counteract the nitrification pH effect (Sceare *et al.*, 1980; Henze *et al.*, 1987). Similarly the uptake of charged organic substrates (e.g. volatile fatty acids) would affect the measurement. This could be solved by processing the influent titration data with a model including ammonification and uptake of charged organic substrates. Alternatively, an experiment in the presence of allylthiourea could be performed to check for the pH effect of non-nitrifying H^+ production or consumption reactions.

CONCLUSIONS

A titration in-sensor-experiment combined with a parameter estimation algorithm can yield information on the kinetics of the activated sludge nitrification process. Based on the titration data, it is possible to estimate the nitrification capacity of the sludge ($\mu_A \times X_{BA}/Y_A$) and its Monod half-saturation constant K_{NH}. The estimated parameter values are related to the *Nitrosomonas* bacteria only. Besides these kinetic parameters, the method can provide on-line information about the nitrifiable N concentration $S_{NH}(0)$ in the mixed liquor.

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