Comparison and combination of titrimetric and respirometric techniques to estimate nitrification kinetics parameters

Elena Ficara, Andrea Musumeci and Alberto Rozzi*
DIIAR, Department of Hydraulic Environmental Engineering and Survey – Environmental Section
Politecnico di Milano, Piazza L. da Vinci 32, 20133 Milan, Italy

Abstract

A respirometric technique (open respirometry) and a titrimetric technique (pH-stat) were compared to monitor the first nitrification step (ammonia oxidation to nitrite) by providing a titrimetric biosensor (ANITA), previously developed to measure ammonia oxidation kinetics, with an oxygen electrode. Then, a new procedure for the simultaneous estimation of kinetic constants related to both ammonia and nitrite oxidisers which couples both open respiration and titration, is presented. This procedure allows for the complete characterisation of the nitrifying biomass kinetics with simple and short (1 to 1.5 h) tests without any analytical substrate determination. All data handling was implemented on Excel data sheets in order to be able to follow data interpolation step by step instead of using software packages which automatically perform data processing.

Introduction

In order to increase the performance and reliability of biological wastewater treatment plants, effective and fast on-line monitoring is required to promptly identify malfunctioning factors and to upgrade the control level of the overall biodegradation process (Nielsen and Onnerth, 1994). New control technologies, such as biosensors, which enable us to obtain more complete information than conventional physico-chemical sensors, such as DO (dissolved oxygen), pH, ORP meters etc., are being developed in order to attain this goal.

Nitrifying bacteria are considered to be, among the aerobic microbial populations, the most sensitive to inhibition and toxicity effects, especially in activated sludge processes (Dutka et al., 1983; Beg and Hassan, 1987; Blum and Speece, 1991; Kroiss et al., 1992). Nitrification requires the simultaneous presence of two bacterial groups which perform two reactions in series: the oxidation of ammonia to nitrite (brought about by ammonia oxidisers, hereafter referred to as AO) and, subsequently, the oxidation of nitrite to nitrate (carried out by nitrite oxidisers, referred to as NO). Usually, nitrite is overlooked as an intermediate reaction product, and the overall nitrification kinetics are more simply described as the direct oxidation of ammonia to nitrate, using the kinetics parameters of the first reaction. This procedure is followed by several authors, e.g. Henze et al. (1995) in defining the IAWQ model No. 2. This approximation is acceptable if, as it occurs in most practical applications, the rate-limiting step is the first reaction, i.e. the oxidation of ammonia is appreciably slower than the oxidation of nitrite, thus making accumulation of nitrite negligible. However, there are cases in which the kinetics related to the second step are slower than those related to ammonia oxidation and appreciable nitrite concentrations build up (Alleman, 1985; Holiencin and Gujer, 1996; Nowak et al., 1995). Environmental conditions which slow down nitrite oxidation rate include: high temperature levels (Randall and Buth, 1984; Nowak et al., 1995), low DO concentrations associated with high pH values (Yang and Alleman, 1992), high free ammonia concentrations (Suthersan and Ganczarczyk, 1986; Balmelle et al., 1992) and the presence of specific inhibitors to nitrite oxidisers (Hynes and Knowles, 1993). On account of the very low nitrites concentration in final effluents imposed by national regulations (e.g. 0.6 $mg \cdot \ell^{-1}$ NO_2-N in Italy, 1 $mg \cdot \ell^{-1}$ NO_2-N in Switzerland and Austria), it is crucial to detect incoming conditions unfavourable to nitrite oxidation. A model describing the two steps separately was proposed by Nowak et al. (1994). In order to calibrate this model it is necessary to evaluate the kinetic parameters of the two oxidation reactions but, according to the authors' knowledge, this separate determination is seldom carried out.

Biosensors for the estimation of the kinetics of autotrophic bacteria may be classified into two main categories: respirometers and titrimetric systems. Respirometers allow for the measurement of kinetic parameters of heterotrophic and autotrophic populations by processing DO concentration data. These instruments may be further subdivided into closed and open respirometers. Among the former, the NITROX respirometer (Surmacz-Gorska et al. 1995, 1996; Gernaey et al., 1997) was proposed to evaluate the maximum nitrification rate based on the difference between the oxygen uptake rate (OUR) due to the overall heterotrophic and autotrophic populations and the OUR obtained after selective inhibition of ammonia (AO) or nitrite (NO) oxidisers through addition of specific inhibitors (allylthiourea or sodium chlorate respectively). Through this method, the separate determination of the activity related to the two groups of nitrifying bacteria is attained (see also Nowak et al. 1994; Andrews et al., 1980). However, the inhibition step in this procedure requires biomass inactivation and, therefore, a new sludge sample for each test, which may be a drawback in laboratory-scale investigations. Moreover, this method uses inhibitors whose specificity has been questioned (Hynes and Knowles, 1983). Vanrolleghem and Verstraete (1993) have proposed the evaluation of the kinetic constants of both heterotrophic and autotrophic populations in a single test by simultaneously dosing carbonaceous and nitrogenous substrates in the RODTOX apparatus which is an open respirometer (Vandebroek, 1986; Vanrolleghem et al., 1990). From the resulting oxygen profile, which depends on the oxygen diffusing through aeration into the mixed liquor and on

* To whom all correspondence should be addressed.
☎0039–02–23996407; fax 0039–02–23996499;
e-mail: rozzi@amb1.amb.polimi.it
Received 29 July 1999; accepted in revised form 23 February 2000.

the oxygen consumed by microbial respiration (endogenous and exogenous respiration), the OUR profile is derived (the respirograph). A proper calibration of the mixed substrate injection allows for the identification of a first part of the respirograph where the OUR is due to both heterotrophic and autrophic activity and a second part, after the consumption of all carbonaceous substrates, related to the autotrophic population only. Kong et al. (1996) set forth a similar procedure which allows for the automatic estimation of the effects of an inhibitor on the kinetic constants of heterotrophic and nitrifying populations, but this procedure does not allow for the evaluation of the kinetics of AO and NO oxidising bacteria separately.

Few experimental procedures related to titrimetric biosensors are available in literature, mainly because of their more recent development compared with respirometers. They are based on the measurement of acidifying or alkalising biological reactions by titrating with a neutralising solution at constant pH. The first applications of titrimetric biosensors (Ramadori et al., 1980; Aivasidis et al., 1992; Massone et al., 1995; 1998) refer to the determination of the ammonia oxidation rate by AO bacteria. Titration biosensors were also applied for the evaluation of the effect of inhibitors on ammonia oxidisers (Maffei, 1995; Gernaey, 1997). On-line applications of the titrimetric biosensor ANITA are reported by Massone and Rozzi (1997) and Gernaey et al. (1998). These authors applied the biosensor to measure ammonium concentrations in the aeration basin and to estimate the nitrification capacity of the activated sludge, the former on wastewater treatment plant processing industrial wastewater, the latter on a 150 ℓ pilot plant. Both these experiences showed a satisfactory relationship between biosensor estimations and laboratory analyses.

In this paper, a comparison between titrimetric and respirometric techniques to measure reaction rates of AO bacteria is first set forth and thereafter a procedure to couple open respirometry to titration is described in order to determine kinetic parameters for both the AO and NO populations. From the stoichiometry of the two reactions:

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + NO_2^- + H_2O \quad (1)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (2)$$

it may be observed that the biological activity of ammonia oxidisers implies a production of acidity and a consumption of oxygen, while the activity of nitrite oxidisers only brings about oxygen consumption. Therefore, it is possible to derive the kinetic constants for AO populations either by titration or by DO measurements and the kinetics related to NO bacteria by titration plus DO measurements, as indicated below. Earlier attempts to compare titration and respirometry were performed by Devisscher (1997) and Ficara et al. (1998).

Materials and methods

The modified biosensor ANITA-DO

The titration biosensor used in this research is named ANITA (Ammonium NITrification Analyser) and manufactured by Austep (Milan, Italy). The layout of the instrument with those parts which were modified for this experimentation are indicated in Fig. 1. The system controls the pH of the nitrifying sludge contained into the reaction vessel by adding base (NaOH 0.05 M) to neutralise the protons produced by the biological process. Therefore, it works as a pH-stat reactor. Taking into account the stoichiometric relationship (see Eq. (1)) between ammonium oxidation and acidity

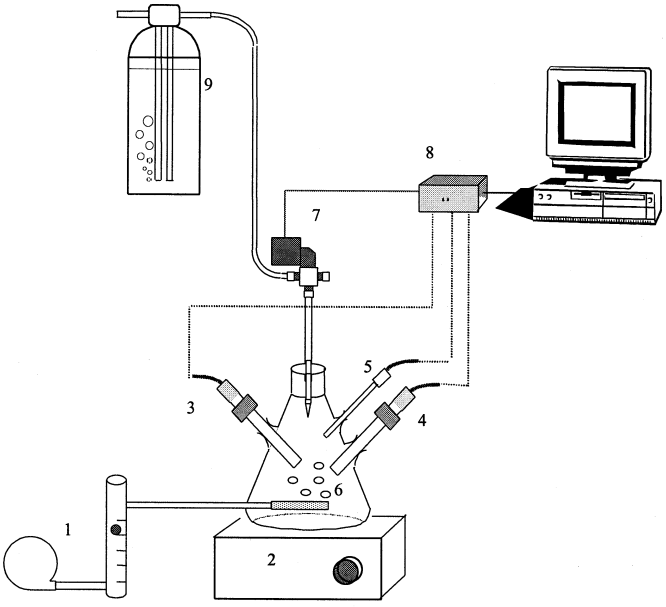

Figure 1

ANITA-DO Layout: 1 – aerator and flow meter (*); 2 – magnetic stirrer; 3 – pH-meter; 4 – oxygen electrode (*); 5 – temperature probe; 6 – flask (*); 7 – electrovalve; 8 – signal amplifier and actuator; 9 – Mariotte bottle: (modified from Massone, 1997). (*): parts which have been modified for this experimentation.

production (and thus the corresponding base addition by the titration system), the following relation is obtained:

$$\frac{d[NH_4]}{dt} = \frac{14}{2}\frac{d[H^+]}{dt} = -\frac{14}{2}\frac{d[OH^-]}{dt} \quad (3)$$

where $[NH_4]$ is expressed in $mgN \cdot \ell^{-1}$ and $[H^+]$ and $[OH^-]$ are expressed in mM. Eq. (3) enables us to calculate $[NH_4]$ and NH_4 oxidation rate as a function of time from the recorded titration data, see Massone et al. (1995) and (1998) for more details.

For experimentation purposes, the ANITA biosensor was modified as follows:

(i) The beaker used as the reaction vessel was substituted with a thermostated Erlenmeyer flask.
(ii) A DO probe (Endress and Hauser mod. OM8, Germany) was inserted into the reaction vessel and connected through a pre-amplifier to the data acquisition card which interfaces the instrument hardware to the controlling PC.
(iii) A flow meter was incorporated in the aeration line.

Modification (i) was needed to avoid interfering oxygen transfer from the atmosphere into the nitrifying sludge during re-aeration experiments for $K_l a$ (oxygen mass transfer coefficient) estimation. Modification (ii) enabled on-line DO concentrations data to be recorded. These determinations were used in comparison and in combination with titration data. Finally, modification (iii) allowed for a precise control of the aeration flow during open respirometry experiments. The simultaneous monitoring of DO and titrant addition was thus achieved, while temperature and air-flow rate, which affect the DO concentration in the mixed liquor, were controlled and kept constant.

Nitrifying sludge

The nitrifying sludge used during this investigation was drawn from a batch-fed reactor where an enriched culture of nitrifiers (volume of 8 ℓ) was grown from an activated sludge inoculum sampled in a domestic sewage treatment plant. The concentrated feed was added four times per day by a peristaltic pump. The volumetric loads were: 100 $mg \cdot \ell^{-1} \cdot d^{-1}$ NH_4-N, 250 $mg \cdot \ell^{-1} \cdot d^{-1}$ COD and 800 $mg \cdot \ell^{-1} \cdot d^{-1}$ $NaHCO_3$). The latter, together with the alkalinity of the tap water, balanced the protons produced by ammonia oxidation. The carbonaceous substrate was provided as powdered skimmed whey. Once a day the sludge was settled until about 3 ℓ of concentrated sludge were obtained. Then, 5 ℓ of supernatant were replaced by the same volume of tap water. Therefore, the culture hydraulic retention time was 1.6 d. The average sludge concentration of the enriched culture was about 1.1 $g \cdot \ell^{-1}$ VSS, and the pH was between 8.1 and 8.5. The enrichment culture was kept at room temperature (20 to 25°C). Activity tests were carried out using 1 ℓ of culture sludge settled to a concentration of about 3 $g \cdot \ell^{-1}$ VSS.

Reagents

A 0.05 M standardised NaOH solution was used as titrant. As substrates, a standard solution of NH_4Cl (1$g \cdot \ell^{-1}$ NH_4-N) pH corrected to 7.9 by NaOH 1M addition (this pH value being close to the sludge equilibrium pH under endogenous conditions) and a standard solution of $NaNO_2$ (1 $g \cdot \ell^{-1}$ NO_2-N) were used. All chemicals were reagent grade and were obtained from commercial sources.

Test procedure and data processing

The 1 ℓ nitrifying sludge sample drawn from the enriched culture was transferred into the biosensor reaction flask, aerated (about 150 ℓ air ℓ^{-1} mixed liquor h^{-1} resulting in an oxygen mass transfer coefficient K_la of 0.33 ± 0.06 min^{-1}) and thermostated until endogenous equilibrium conditions were reached. Equilibrium is considered to be achieved when oscillations keep within 1/10 of the unit for temperature (°C) and DO ($mg \cdot \ell^{-1}$) and within 1/100 of the unit for pH. This preliminary operation was usually completed within 1 h. The nitrogenous substrate was then added, i.e. ammonium (3 to 15 $mg \cdot \ell^{-1}$ NH_4-N) and/or nitrite (3 to 7 $mg \cdot \ell^{-1}$ NO_2-N). Every 10 s the ANITA-DO biosensor recorded the following parameters: volume of titrant, in-solution temperature, DO and pH values. During the test, the latter was kept within the range of pH setpoint (the aerated mixed liquor equilibrium pH) ± 0.02 pH units. Tests were considered to be completed when the DO concentration returned to the initial equilibrium value. Other experimental conditions were: temperature in the range 28.6÷30.4°C and equilibrium pH in the range 8.12÷8.5. The pH and temperature plots vs. time were not directly used within the calculation procedure, but they were used to detect malfunctions.

In the calculation procedure the following assumptions were made:

(i) The yield coefficient for nitrifying bacteria was neglected.
(ii) Ammonium uptake due to heterotrophic biomass was neglected.
(iii) No significant ammonium stripping took place within the test period (tests were performed at pH<8.5, pK_a at 25°C for NH_3 is 9.26, Sawyer et al., 1994).
(iv) The substrate (ammonium and nitrite) was completely oxidised within the test period (confirmed by substrate analytical measurements at the end of the tests).
(v) The DO and pH influence on kinetics was neglected (DO>> $K_{S,DO}$ for both AO and NO bacteria; pH within the optimal range).

Assumption (i) may be considered reasonable because of the very low nitrogen uptake due to biomass synthesis by autotrophic bacteria ($Y \approx 0.02$ gN_{cell} g^{-1} $N_{removed}$). Moreover, heterotrophic biomass was kept in endogenous conditions, thus its nitrogen uptake was also negligible, this fact justifying assumption (ii). The error introduced by these assumptions is of the order of 2%. The values of the overall titration error e_s (the difference between the ammonium added to the sludge sample and the ammonium estimated from titration data) in these tests was 0.03 ± 0.08 (n=17), which is quite acceptable.

Calculations were based on the kinetic and mass balance equations which describe the biological oxidation of ammonia and nitrite within the system. The latter is closed with regard to the liquid flow and open with regard to the gas flow. The following equations model the system:

• **Nitrification dynamics**. Both nitrification oxidation steps follow a Michaelis-Menten equation:

$$-\frac{d[NH_4]}{dt} = V_{max,AO}\frac{[NH_4]}{[NH_4] + K_{s,AO}} \quad (4)$$

$$\frac{d[NO_3]}{dt} = V_{max,NO}\frac{[NO_2]}{[NO_2] + K_{s,NO}} \quad (5)$$

where:

$[NH_4]$, $[NO_2]$, $[NO_3]$ are ammonium, nitrite and nitrate concentrations (expressed as $mg \cdot \ell^{-1}$ N);

$V_{max,AO}$ and $V_{max,NO}$ ($mgN \cdot g^{-1}VSS \cdot h^{-1}$) are the maximum substrate oxidation rates for AO and NO bacteria;

$K_{s,AO}$ and $K_{s,NO}$ ($mg \cdot \ell^{-1}$ N) are the half saturation constants for AO and NO bacteria.

• **Nitrogen mass balance**. During the test, nitrogen is oxidised from ammonium to nitrite and then to nitrate:

$$\frac{d[NO_2]}{dt} = -\frac{d[NH_4]}{dt} - \frac{d[NO_3]}{dt} \quad (6)$$

• **Dissolved oxygen dynamics**. DO concentration depends on air bubbling aeration and on oxygen consumption due to bacteria respiration:

$$\frac{d[DO]}{dt} = K_la([DO]_e - [DO]) - OUR_{AO} - OUR_{NO} \quad (7)$$

$$OUR_{AO} = -3.42\frac{d[NH_4]}{dt} \quad (8)$$

$$OUR_{NO} = 1.14\frac{d[NO_3]}{dt} \quad (9)$$

where:

$[DO]$ ($mg \cdot \ell^{-1}$) is the DO concentration,

$[DO]_e$ ($mg \cdot \ell^{-1}$) is the DO equilibrium concentration in endogenous conditions;

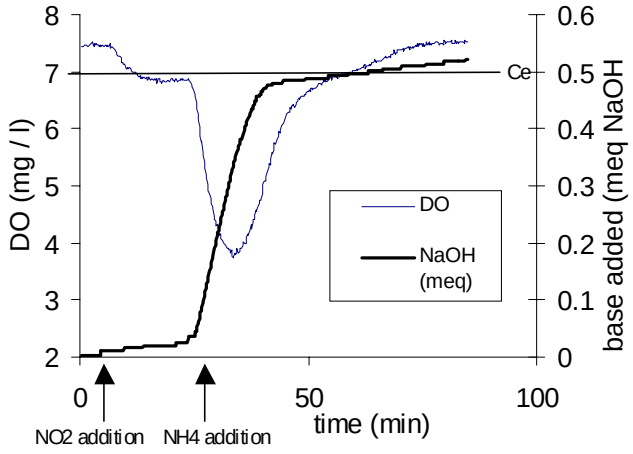

Figure 2
Typical profiles for titration and DO data to compare TI and OR procedure.

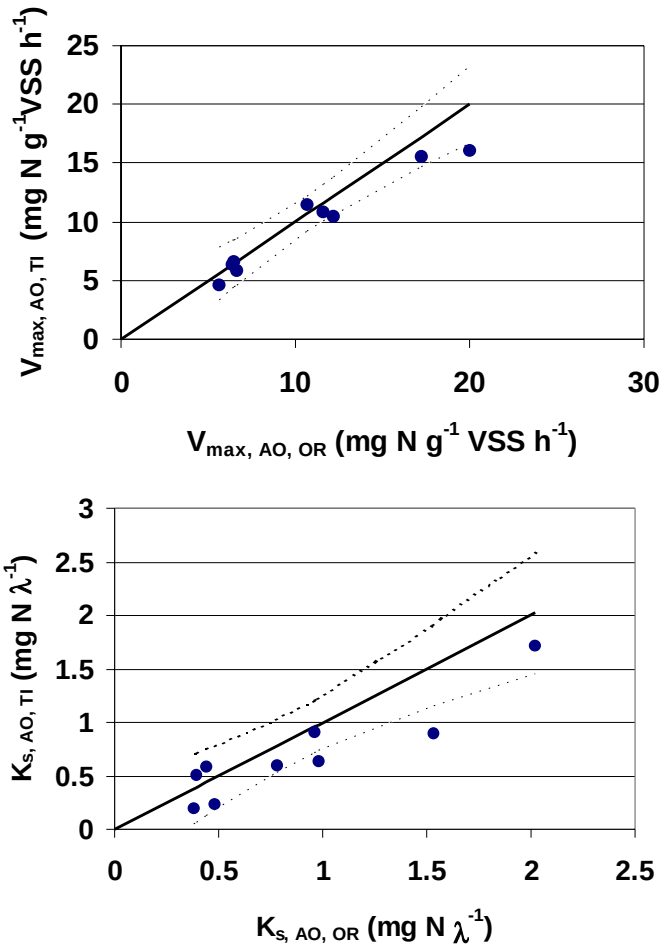

Figure 3
Comparison between AO-bacteria kinetics parameters estimated by TI and OR. V_{max} values (top graph); K_s values (bottom graph). The line represents the perfect correlation, dashed lines represent the 95% confidence intervals.

OUR_{NO} and OUR_{AO} ($mg \cdot \ell^{-1} \cdot min^{-1}$) are the OURs for NO and AO bacteria; and
3.42 and 1.14 are the stoichiometric conversion factors on mass basis between nitrogen oxidation and oxygen consumption for AO and NO conversions (see Eqs. (1) and (2)).

• **Oxygen mass balance**. The amount of oxygen consumed because of nitrogen oxidation (NOD) by AO and NO bacteria can be experimentally estimated from the respirograph area (Vanrolleghem and Verstraete, 1993) as:

$$NOD_t = \int OURdt = K_la \int([DO]_e - [DO]_t)dt + ([DO]_e - [DO]_t) \quad (10)$$

or calculated from a mass balance:

$$NOD_t = ([NH_4]_0 - [NH_4]_t)*4.56 + ([NO_2]_0 - [NO_2]_t)*1.14 \quad (11)$$

where:
NOD_t ($mg \cdot \ell^{-1}$) is the nitrogen oxygen demand at time t;
$[NH_4]_t$, $[NH_4]_0$, $[NO_2]_t$ and $[NO_2]_0$ are ammonium and nitrite concentrations ($mg \cdot \ell^{-1}$ N) at time t and at the beginning of the test respectively; and
the factor 4.56 = 1.14+3.42 takes into account the oxidation of nitrite produced from ammonium oxidation.

At the end of the test, i.e. at time tf, ammonium and nitrite have been completely oxidised, therefore $NOD_t = NOD_f = [NH_4]_0*4.56 - [NO_2]_0*1.14$. Substituting t = tf in Eq.(10), K_la can be calculated by the following equation:

$$K_la = \frac{[NH_4]_0*4.56 + [NO_2]_0*1.14}{\int_0^{tf} OURdt} \quad (12)$$

Data measured by the ANITA-DO biosensor were:

- volume of added titrant vs. time;
- [DO] vs. time.

Substituting $d[NH_4]/dt$ from Eq. (3) in Eq. (8), the OUR_{AO} was obtained and by substituting the latter in Eq. (7), the OUR_{NO} was calculated. Moreover, Eq. (9) enabled us to determine nitrate formation rate, while from Eq. (11), nitrite concentration was estimated. Therefore, oxidation rates could be correlated to substrate concentration for AO and NO micro-organisms. Finally, through minimum square error estimation, kinetics parameters for both micro-organisms were calculated. It was chosen to perform all data processing on Excel data sheets instead of using dedicated software packages which automatically perform data interpolation, in order to evaluate data processing step by step.

Results and discussion

Comparison of titration (TI) and open respirometry (OR) for ammonia oxidisers

The aim of this series of tests was to compare the Michaelis-Menten kinetic parameters estimated by titration and open respirometry. As already mentioned, ammonia oxidation (Eq. (1)) consumes oxygen and releases acidity. Therefore, both titration at constant pH and respirometry may be used to monitor the ammonia oxidation rate. In order to avoid the interference of nitrite oxidation by NO bacteria which simultaneously takes place using a balanced nitrifying sludge, a non-limiting concentration of nitrite was kept in solution

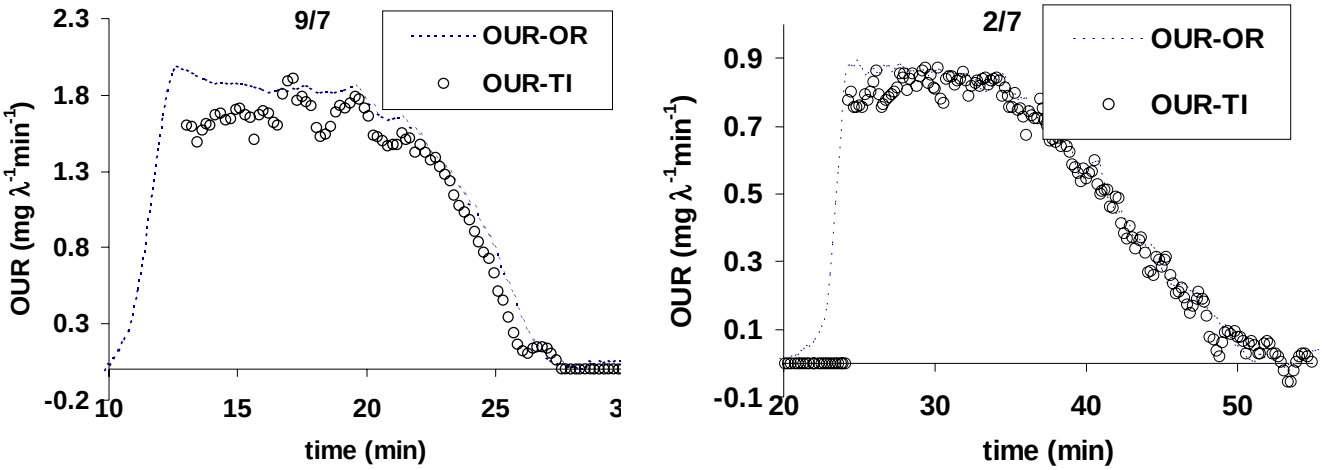

Figure 4
Two types of OUR profiles. TI-based (dashed line) and OR-based (circles) OUR profiles.

during the test, thus ensuring a constant (maximum) respiration rate for nitrite oxidisers. The oxygen consumption term due to NO bacteria was then considered as an endogenous respiration and its contribution accounted as a decrease in DO_e in Eq. (7) ($K_la*\Delta[DO]_e = OUR_{NO}$). The resulting OUR was, therefore, only due to ammonia oxidisers. A typical data set for titration and oxygen measurements in this kind of tests is plotted in Fig. 2. These data were processed, according to the procedure described in **Test procedure and data processing**, to calculate the maximum oxidation rate and the affinity constant K_S. Nine tests were performed within 20 d on the same nitrifying sludge and results are summarised in Fig. 3. As shown in this figure, the V_{max} for ammonium oxidisers did not keep constant during the test period but values from 5 to 18 mg NH_4-N·g^{-1}VSS ·h^{-1} were measured. These variations were due, on the one hand, to selective biomass growth in the nitrifying enriched culture and, on the other hand, to an inhibitory effect due to an occasional increase in the chlorine concentration in the tap water used to dilute the substrate. Ammonium oxidation rates were generally higher then those which apply to activated sludge (typically in the range of 1.5 to 5 mg NH_4-N·g^{-1}VSS ·h^{-1}, Andreottola et al., 1990). However, this was due to the carbon to nitrogen ratio in the culture feed which was appreciably lower than in most activated sludge influents (C/N = 2.5 and from 5 to 10 respectively).

In Fig. 3 a good correlation between TI and OR results can be observed with the exception of a couple of tests. However, it may be observed that OR seems to give slightly higher values for both V_{max} and K_s. An explanation for the difference between respiration and titration estimates may be found by carefully examining the OUR profiles calculated from the oxygen concentration data and from the titration data. In Fig. 4, results related to two tests are plotted. For the test dated 2 July, TI-based and OR-based OUR profiles compare very well; on the other hand the 9 July graph shows that the OR-based profile is significantly higher in the beginning of the test while the difference between the two OUR profiles decreases later on. A clear explanation for these discrepancies was not found. It may be related to the response of the DO probe to a sharp DO transient or to a biological effect such as a time lag between the oxygen consumption and the acidity production. A shift between oxygen consumption and CO_2 production was observed by Devisscher et al. (1998) for aerobic oxidation of ethanol and acetate. However, it is not clear why this phenomenon was not observed in all tests.

The Sign hypotheses test was performed on the two sets of V_{max} and K_s values in order to establish whether the two procedures (TI and OR) give comparable parameter estimates or whether the OR-based estimates should be considered to be significantly higher than TI-based ones. This test is a non-parametric test and, therefore, it is suitable for small data sets whose probability distribution is unknown (Helsen and Hirsch, 1992), as the ones described here. The test results showed that the OR procedure and the TI procedure are statistically equivalent for AO-bacteria kinetics evaluation with a significance level of 5%.

Determination of nitrite oxidisers kinetic parameters by a combined titrimetric and respirometic (T+R) procedure

A series of titration and open respirometry tests were performed in order to investigate the use of combined titration and open respirometry data for the simultaneous determination of AO and NO bacteria kinetics. It is well known (see Reactions 1 and 2) that AO bacterial activity affects both pH and DO of the solution, while the activity of NO bacteria results only in a DO decrease. Therefore, titration data were used to estimate AO bacteria activity and the latter was subtracted from the total nitrifying activity in order to obtain the NO bacteria respiration contribution. Parallel experiments were performed either according to this procedure (labelled hereafter as T+R procedure) or following the open respirometry procedure (Farkas, 1981; Vanrolleghem and Verstraete, 1993) i.e. by adding a certain amount of sodium nitrite (5 to 7 mg·ℓ^{-1} NO_2-N) to the nitrifying sludge and using data of the OUR profile. In T+R experiments either ammonium (3 to 12 mg·ℓ^{-1} NH_4-N) or ammonium and nitrite (3 to 15 mg·ℓ^{-1} NH_4-N and 3 to 9 mg·ℓ^{-1} NO_2-N) were used as substrates. In Figs. 5 and 6 typical OUR profiles obtained in these experiments are shown. In Table 1 the kinetic parameters estimated by the T+R procedure and by the classical OR procedure are summarised. The reported values compare well with those reported by Chudoba et al. (1985). These authors used an activated sludge culture fed on a synthetic wastewater whose composition was similar to the one of the nitrifying enriched culture (C/N of 3.4) and they found a maximum nitrite oxidation rate of 6.8 ± 2.4 mg NO_2-N·g^{-1}VSS·h^{-1} and a K_s of 0.76 ± 0.44 mg·ℓ^{-1} NO_2-N. As far as repeatability is concerned, Tab. 1 shows that the K_s estimate is generally affected by higher variations than the V_{max} estimate as previously found by other authors (Grady et al., 1989; Brouwer and Klapwijk, 1995; Kong et al. 1996).

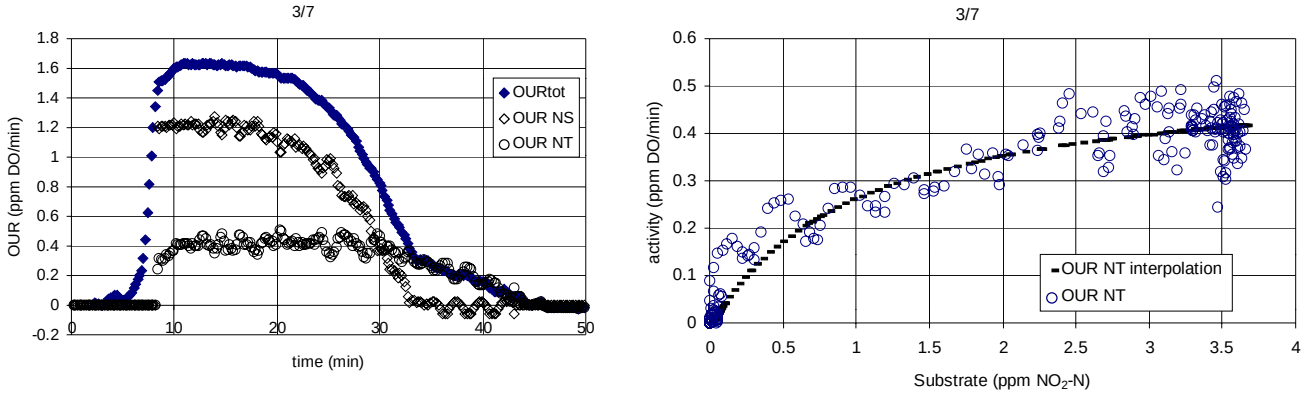

Figure 5

Typical OUR profiles for the T+R procedure with ammonium (15 mg·ℓ$^{-1}$ NH_4-N) and nitrite (3 mg·ℓ$^{-1}$ NO_2-N) as substrates (left graph) and relative Michaelis-Menten kinetic interpolation for NO micro-organisms (right graph)

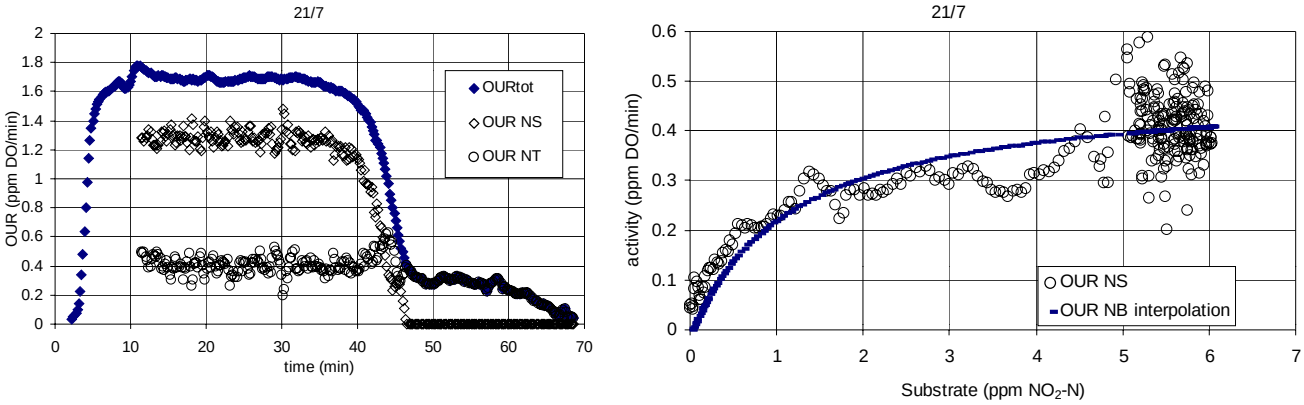

Figure 6

Typical OUR profiles in the T+R procedure with ammonium (8 mg·ℓ$^{-1}$ NH_4-N) as only substrate (left graph) and relative Michaelis-Menten kinetic interpolation for NO micro-organisms (right graph)

TABLE 1

ESTIMATES OF NO MICRO-ORGANISM KINETICS PARAMETERS FOR THE COMBINED T+R PROCEDURE AND FOR THE OR PROCEDURE. $V_{max\text{-}NO}$ (mg NO_2-N·g^{-1}VSS·h^{-1}), $K_{s\text{-}NO}$ (mg·ℓ$^{-1}$ NO_2-N)

	OR		T+R. Only NH_4 as substrate		T+R. NH_4 & NO_2 as substrate		T+R. All data (*)	
	$V_{max\text{-}NO}$	$K_{S\text{-}NO}$	$V_{max\text{-}NO}$	$K_{S\text{-}NO}$	$V_{max\text{-}NO}$	$K_{S\text{-}NO}$	$V_{max\text{-}NO}$	$K_{S\text{-}NO}$
Mean	5.7	0.5	6.8	0.6	9.0	1.5	7.9	1.1
Standard deviation	0.88	0.25	3.02	0.63	1.50	0.37	2.54	0.66
CV	0.15	0.51	0.45	0.98	0.17	0.25	0.32	0.60
Number of observations	11	11	8	8	9	9	17	17

(*) refers to all the data obtained with the T+R procedure (either with only ammonium or with nitrite plus ammonium as substrate).

It may be remarked that the T+R procedure on average gave higher values for V_{max} and K_s than the OR procedure both with only ammonium or with ammonium and nitrite as substrates. As expected, differences for the two procedures were much larger for K_s values than for the V_{max} ones. However, a better agreement between the OR and T+R procedures seemed to be obtained when only ammonium was used as substrate.

It should be considered that the OUR_{NO} data were calculated as the difference between two experimental series of data both of which were affected by a measurement noise. Moreover, the absolute value of OUR_{NO} is generally about ¼ of the value of OUR_{tot}. Therefore, OUR_{NO} data resulting as the difference of OUR_{tot} and OUR_{AO} are characterised by high noise and the corresponding experimental Michaelis-Menten curve generally showed

TABLE 2 K_la Estimation Error (e_{Kla}) For the Examined Procedures			
	OR	T+R (NH_4)	T+R (NO_2+NH_4)
Mean	0.21	0.01	0.22
St. dev.	0.24	0.22	0.14
N observations	11	7	9

high oscillations. Observing the left graph in Fig. 6, one can see that the OUR_{NO} values at high substrate concentrations, which were calculated as the difference between respiration and titration measurements (data between 10 and 40 min in the left graph), are much more scattered than the OUR_{NO} values corresponding to low substrate concentrations which were calculated from DO measurements only, after the complete oxidation of ammonium when $OUR_{tot} = OUR_{NO}$ (data between 45 and 70 min in the left graph).

Finally, a comment on K_la estimation procedure. This parameter is known to be the bottleneck parameter in open respirometry. In this investigation, K_la was estimated, as described before, by Eq. (12) instead of being measured through re-aeration experiments. In this way tests were simplified and their duration reduced. In order to evaluate the reliability of the estimated values, K_la was also obtained by interpolating DO data measured by re-aeration experiments. In Table 2 the K_la estimate error is reported, this error been calculated as:

$$e_{K_la} = (K_la_{\,calculated} - K_la_{\,measured}) / K_la_{\,measured}$$

An average difference of the order of 20% was found between the calculated and the estimated K_la (see mean row in Table 2) except for the T+R (NH_4) procedure. This difference seems quite high and might be related to the delay (1 to 5 h) between the re-aeration experiments and the tests. In the RODTOX procedure, for example, K_la measurements are made immediately before and after each stBOD estimation test. More research is needed to verify data consistency.

Conclusions

An experimental protocol was tested to compare open respirometry and titration at constant pH to monitor the first nitrification step (ammonium oxidation) and determine related kinetic constants. A good correlation between the parameters estimated by the two procedures was generally found.

Moreover, titration and open respirometry were coupled to estimate NO and AO bacteria kinetic parameters simultaneously. The procedure is fast (40 to 70 min) and simple: experimental data are automatically recorded, no substrate analytical analyses are required, data processing can be easily implemented in any *ad hoc* commercially available software. Results are promising: maximum degradation rates V_{max} compare well with data obtained from open respirometry tests and have a low coefficient of variation, while higher dispersion (CV between 24 and 129 %) were observed for K_s estimates.

Acknowledgments

This research has been partly funded by the EU Contract ENV4-CT95-0064 "Integrated Water Recycling and Emission Abatement in the Textile Industry" and by the Italian Ministry of University and Scientific Research (MURST Contract 40% "Trattamento, utilizzazione e monitoraggio di reflui e residui di attività agro-alimentari").

Many thanks are due to the Austep Company of Milan for the loan of the ANITA biosensor used to carry out this research.

Finally, the authors are particularly grateful to Prof. Chris Buckley (University of Natal, South Africa) for the valuable comments.

References

AIVASIDIS A, HOCHSCHERF H, ROTTMANN G, HAGEN T, MERTENS MT, REINERS G and WANDREY C (1992) Neuere Konzepte zur Prozeßüberwachung und Regelung bei der biologischen Stickstoffelimination. *Awt Abwassertechnik.* **5** (43) 48-55 (in German).

ALLEMAN JE (1985) Elevated nitrite occurrence in biological wastewater treatment systems. *Water Sci. Technol.* **17** (2/3) 409-419.

ANDREOTTOLA G, CANZIANI R and COSSU R (1990) *Rimozione Biologica dei Nutrienti Dalle Acque di Scarico.* Published by Istituto per l'Ambiente, Milano (in Italian) p. 59.

ANDREWS JF, S RENSEN PE and GARRETT TM (1980) Control of nitrification in the oxygen activated sludge process. *Prog. Water Technol.* **12** (5) 497-519.

BALMELLE B, NGUYEN KM CAPDEVILLE B, CORNIER JC and DEGUIN A (1992) Study of factors controlling the nitrite build-up in biological processes for water nitrification. *Water. Sci. Technol.* **26** (5-6) 1017-1025.

BEG SA and HASSAN MM (1987) Effects of inhibitors on nitrification in a packed-bed biological flow reactor. *Water Res.* **21** 191.

BLUM DJ and SPEECE RE (1991) A database of chemical toxicity to environmental bacteria and its use in interspecies comparison and correlation. *Res. J. Water Pollut. Control Fed.* **63** (3) 198-207.

BROUWER H and KLAPWIJK A (1995) A method based on respirometry to calculate the aerobic nitrification volume of a nitrifying/denitrifying activated sludge plant. Paper presented at Symposium: "Erfahrungen mit Analysen und Prozeßmeßgeräten in Abwasserreinigungsanlagen" Ostfildern (Nellingen) 27 Nov.

CHUDOBA J, CECH JS and CHUDOBA P (1985) The effect of aeration tank configuration on nitrification kinetics. *JWPCF* **57** (11) 1078-1083.

DEVISSCHER M (1997) pH Effect Sensoren voor de Controle van Biologische Stikstofverwijderingsprocessen in de Waterzuivering. Eng. Thesis, Fac. of Agric. and Appl. Biol. Sci., Univ. Gent, Belgium. (in Dutch).110 pp.

DEVISSCHER M, BOGAERT HM, GERNAEY K and VERSTRAETE W (1998) Use of bubbleless aeration to improve the performance of titrimetric sensors for study of denitrification processes. In: *Proc. of Eur. Conf. on Nitrogen and Phosphorus Removal for Municipal and Industrial Wastewaters.* Narbonne, France, 12-14 Oct. 215–220.

DUTKA BJ, NYHOLM N and PETERSEN J (1983) Comparison of several microbiological toxicity screening tests. *Water Res.* **17**(10) 1363-1368.

FARKAS PA (1981) The use of respirography in biological treatment plant control. *Water Sci. Technol.* **13** 125-131.

FICARA E, TROVATO A, MUSUMECI A and ROZZI A (1998) Determinazione della cinetiche di nitrosazione e nitratazione mediante l'utilizzo combinato di respirometria e titolazione (in Italian). *Ingegneria Ambientale* **28** (9) 428-437.

GERNAEY K (1997) Development of Sensors for On-line Monitoring of Nitrification in Activated Sludge. Ph.D. Thesis, Univ. Gent, Gent, Belgium.

GERNAEY K, BOGAERT H, VANROLLEGHEM P, MASSONE AG, ROZZI A and VERSTRAETE W (1998) A titration technique for on-line nitrification monitoring in activated sludge. *Water Sci. Technol.* **37** (12) 103–110.

GERNAEY K, VERSCHUERE L, LUYTEN L and VERSTRAETE W (1997) Fast and sensitive acute toxicity detection with an enriched nitrifying culture. *Water Environ. Res.* **69** (6) 1163-1169.

GRADY CPL (Jr.), DANG JS, HARVEY DM, JOBBAGY A and WANG XL (1989) Determination of biodegradation kinetics through use of electrolytic respirometry. *Water Sci. Technol.* **21** (8-9) 957-968.

HELSEN DR and HIRSCH RM (1992) *Statistical Methods in Water Resources*. Elsevier Science Publishing Company Inc. 138–141.

HENZE M, GUJER W, MINO T, MATSUO T, WENTZEL MC and MARAIS GvR (1995) *Activated Sludge Model No. 2. IAWQ Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment Processes*. Bourne Press Ltd, Bournemouth, England.

HOLIENCIN A and GUJER W (1996) Production of nitrite in nitrifying sludge systems. *Wasser, Abwasser* **76** (11) 841-846.

HYNES RK and KNOWLES R (1993) Inhibition of chemoautotrophic nitrification by sodium chlorate and sodium chlorite: a reexamination. *Appl. Environ. Microbiol.* (Apr.) 1178-1182.

KONG Z, VANROLLEGHEM P, WILLEMS P and VERSTRAETE W (1996) Simultaneous determination of inhibition kinetics of carbon oxidation and nitrification with a respirometer. *Water Res.* **30** (4) 825-836.

KROISS H, SCHWEIGHOFER P, FREY W and MATSCHE N (1992) Nitrification inhibition – A source identification method for combined municipal and/or industrial wastewater treatment plants. *Water Sci. Technol.* **26** (5/6) 1135-1146.

MAFFEI D (1995) Biosensore per la Misura Dell'attività Nitrificante e Dell'ammoniaca in Soluzione. Eng. Thesis, Politecnico of Milan (in Italian).

MASSONE A G (1997) I Biosensori A titolazione. Ph.D. Thesis, Politecnico di Milano, Italy (in Italian).

MASSONE AG, GERNAEY K, ROZZI A and VERSTRAETE W (1998) Measurement of ammonium concentration and nitrification rate by a new titrometric biosensor. *WEF Res. J. Air and Waste Manag. Assoc.* **70** (3) 343-350.

MASSONE AG, GERNAEY K, ROZZI A, WILLEMS P and VERSTRAETE W (1995) Biosensors for nitrogen control in wastewaters. *Water Sci. Technol.* **34** (1-2) 213-220.

MASSONE AG and ROZZI A (1997) Nitrification and toxicity on line control in an industrial wastewater treatment plant. In: *Proc. Instrumentation Control and Automation*, IAWT Brighton, 6-9 June.

NIELSEN MK and ONNERTH TB (1994) Control of Activated Sludge Plant. State of the art. In: *Proc. of IAWQ Spec. Seminar: Modelling and Control of Activated Sludge Processes*, 22-24 August, Copenhagen, Denmark.

NOWAK O, SCHWEIGHOFER P and SVARDAL K (1994) Nitrification inhibition - A method for the estimation of the actual maximum autotrophic growth rates in activated sludge. *Water Sci. Technol.* **30** (6) 9-19.

NOWAK O, SVARDAL K and SCHWEIGHOFER P (1995) The dynamic behaviour of nitrifying activated sludge systems influenced by inhibiting wastewater compounds. *Water Sci. Technol.* **31** (2) 115-124.

RAMADORI R, ROZZI A and TANDOI W (1980) An automated system for monitoring the kinetics of biological oxidation of ammonia. Tech. Note. *Water Res.* **14** 1555-1557.

RANDALL CW and BUTH D (1984) Nitrite build-up in activated sludge resulting from temperature effects. *J.W.P.C.F.* **56** (9) 1039-1044.

SAWYER CN, MCCARTY PL and PARKIN GF (1994) *Chemistry for Environmental Engineering*. McGraw-Hill International Editions. 114 pp.

SURMACZ-GORSKA J, GERNAEY K, DEMUYNCK C, VANROLLEGHEM P and VERSTRAETE W (1995) Nitrification process control in activated sludge using oxygen uptake rate measurements. *Environ. Technol.* **16** 569-577.

SURMACZ-GORSKA J, GERNAEY K, DEMUYNCK C, VANROLLEGHEM P and VERSTRAETE W (1996) Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements. *Water Res.* **30** (5) 1228-1236.

SUTHERSAN S and GANCZARCZYK JJ (1986) Inhibition of nitrite oxidation during nitrification: Some observations. *Water Pollut. Res. J. Air and Waste Manage. Assoc. Canada* **21** (2) 257-266.

VANDEBROEK R (1986) Study and Development of a Microcomputer Controlled Sensor for the Determination of the Biodegradability and Toxicity of the Wastewaters: The RODTOX. Ph.D. Thesis. State University of Gent, Belgium.

VANROLLEGHEM PA and VERSTRAETE W (1993) Simultaneous biokinetic characterisation of heterotrophic and nitrifying population of activated sludge with an on-line respirographic biosensor. *Water Sci. Technol.* **28** (11-12) 377-387.

VANROLLEGHEM PA, DRIES D and VERSTRAETE W (1990) RODTOX: Biosensor for rapid determination of the biochemical oxygen demand and on-line monitoring of the toxicity of wastewaters. In: *Proc. 5th Eur. Congress on Biotechnol.* Copenhagen, Denmark, 8-13 July. **1** 161-164.

YANG L and ALLEMAN JE (1992) Investigation of batch-wise nitrite build-up by an enriched nitrification culture. *Water Sci. Technol.* **26** (5-6) 997-1005.