A new pH-based procedure to model toxic effects on nitrifiers in activated sludge

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Abstract: A combination of a titration experiment and a biokinetic parameter estimation procedure is proposed as an experimental tool to study the kinetics of NH_4^+-oxidizing bacteria in activated sludge. The method was used to quantify the effect of low concentrations of a toxic compound on the maximum substrate removal capacity and the substrate affinity constant (K_{NH}) of NH_4^+-oxidizing bacteria in activated sludge samples. Experiments in the presence of increasing concentrations of a toxic compound (CN^-, 3,5-DCP, Cu^{2+} and phenol) were performed with nitrifying activated sludge samples obtained from two full-scale wastewater treatment plants. The repeatability of the proposed procedure was found to be sufficient to deduce trends in the behavior of the NH_4^+-N-oxidizing bacteria based on one series of experiments with increasing toxicant concentrations. The experimental results showed that the two sludge samples reacted completely differently in the presence of a certain concentration of the same toxic compound. For phenol, the shape of the titration curves did not correspond any longer to a simple Monod model. In this case, titration curves could be described by a model including both nitrification inhibition by phenol and degradation of the phenol by heterotrophic bacteria.

Keywords: inhibition; nitrification; parameter estimation; titration experiment

NOTATION

B	Raw titration data (base pulses)
B'	Titration data (meq dm^{-3} reactor)
$BPPR$	Background proton production rate (meq dm^{-3} min^{-1})
CV	Coefficient of variation
dH^+/dt	Proton production rate (meq dm^{-3} min^{-1})
dNH_4/dt	Ammonium removal rate (mg N dm^{-3} min^{-1})
f_{BA}	Fraction of autotrophic biomass
f_{BH}	Fraction of heterotrophic biomass
i_{XB}	Fraction of N in biomass (mg N mg^{-1} COD)
K_i	Inhibition constant of phenol for the NH_4^+ oxidation process (mg dm^{-3})
K_{NH}	Monod half-saturation coefficient for NH_4^+-N (mg N dm^{-3})
K_{Ph}	Monod half-saturation constant for phenol degradation (mg dm^{-3})
N	Base normality (meq dm^{-3})
Q_b	Base flux (dm^3 (base pulse)$^{-1}$)
S_{NH}	Ammonium concentration (mg N dm^{-3})
$S_{NH}(0)$	Initial ammonium concentration (mg N dm^{-3})
S_{Ph}	Phenol concentration (mg dm^{-3})
SS	Suspended solids
SSE	Sum of squared errors
V	Volume reactor vessel (dm^3)
WWTP	Wastewater treatment plant
X	Biomass concentration (mg COD dm^{-3})
X_{BA}	Autotrophic biomass concentration (mg COD dm^{-3})
Y_A	Yield coefficient for autotrophic biomass (mg COD mg^{-1} N nitrified)
Y_H	Yield coefficient for heterotrophic biomass (mg COD biomass mg^{-1} phenol)
μ_A	Maximum specific growth rate for autotrophic biomass (min^{-1})
$(\mu_A{*}X_{BA})/Y_A$	Maximum nitrification capacity of the activated sludge (mg N dm^{-3} h^{-1})
μ_{MPh}	Maximum specific growth rate for heterotrophs degrading phenol (min^{-1})

1 INTRODUCTION

Nitrogen removal in wastewater treatment plants is normally obtained through a biological reaction sequence of nitrification (aerobic conditions) and

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denitrification (anoxic conditions). From these processes, nitrification is generally accepted to be the slowest step, determining the overall reaction rate.[1] It was also shown that nitrification reaction rates are more sensitive to temperature variations and inhibitory effects due to toxic compounds compared with heterotrophs.[2–4] Nitrification is in fact a two-step process: NH_4^+-oxidizing bacteria oxidize NH_4^+ to NO_2^-, which is subsequently oxidized to NO_3^- by NO_2^--oxidizing bacteria. More detailed studies revealed that NH_4^+-oxidizing bacteria are often most rapidly inhibited when toxic compounds enter the treatment plant.[3,5,6] Therefore it could be important to have a simple method that specifically allows the inhibitory effects of toxic compounds for the first nitrification step, to be quantified.

Toxicity tests with activated sludge to which pure toxic compounds or toxic wastewater are added are performed regularly. Results of such tests (eg information about a decrease of the nitrification rate in the presence of a toxic wastewater) can be extrapolated to full-scale treatment plants to improve the treatment plant process design and the overall nitrogen removal efficiency.[7] In case a chemical analysis has been done on a toxic wastewater, laboratory toxicity tests with the pure toxic compound or with wastewater spiked with the toxic compound can be applied to verify if the toxic effect is only due to the presence of the compound that was identified.[8]

Several techniques are currently applied to study nitrification in activated sludge. The most obvious, but also one of the most labor-intensive methods consists of monitoring substrate consumption (NH_4^+-N) or product formation ($NO_2^- + NO_3^-$-N) rates.[3,9–11] Alternatively, respirometric measurements have shown their usefulness for nitrification monitoring purposes.[4,12–16] However, respirometry only allows the oxygen uptake of both nitrification steps to be studied separately under very specific conditions, eg when selective nitrification inhibitors are used to inhibit one of the nitrification steps or when an appropriate mixture of NH_4^+-N and NO_2^--N is dosed at the start of the experiment.[17] Methods using specific nitrification inhibitors are not environmentally friendly due to additional use of chemicals, while the design of an appropriate mixture of NH_4^+-N and NO_2^--N requires extra laboratory work.

However, besides oxygen consumption nitrification also results in proton formation, as can be concluded from the general nitrification reaction equation (eqn (1)).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \qquad (1)$$

This pH effect of the nitrification reaction has been applied already in the past to control nitrogen removal processes in alternating activated sludge systems, based on data collected with a pH probe in the process tank.[18,19] Based on this principle several titrimetric sensors were developed in the past for nitrification monitoring in activated sludge.[20–24] These sensors quantify the amount of protons formed during nitrification. A titration method is advantageous for a toxicity test with nitrifying activated sludge because only the NH_4^+-oxidizing activity is measured, as can be concluded from eqns (2) and (3).[1]

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- \qquad (2)$$
(NH_4^+-oxidizing bacteria)

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad (3)$$
(NO_2^--oxidizing bacteria)

The experimental approach proposed in this paper will be different from titration experiments that have been presented elsewhere.[21–23] The method presented here uses batch experiments following pulse substrate additions, while Aivasidis *et al*[23] measured nitrification rates in a flow-through reactor by continuously neutralizing the protons formed during nitrification. In this case the presence of a toxic compound in the wastewater, which was continuously supplied to the reactor, could be detected as a decrease of the base addition rate to the reactor vessel. A feature of the method proposed in this paper is that nitrification completely comes to an end within a reasonable time (20–40 min for the reference experiments). By doing so, the whole range of substrate concentrations between 0 and 2.5 mg NH_4^+-N dm^{-3} is covered as oxidation of the NH_4^+-N proceeds. This allows estimation of both the maximum nitrification capacity and the substrate affinity constant (K_{NH}) by processing the titration data with a non-linear parameter estimation procedure according to the model of eqns (5) and (6) (see below). A similar approach has already been developed to study the effects of toxic compounds on activated sludge based on respirometric experiments.[16] Such a substrate depletion experiment is advantageous because biokinetic information can be obtained from a single experiment. Other methods (where reactions do not come to an endpoint) need one experiment for each substrate concentration in order to obtain the same information about the biological process under study.[12,13]

Ramadori *et al*[22] proposed an experimental set-up that was similar to the titration unit used for the experiments described in this paper. However, the system of Ramadori *et al*[22] was more complicated, including, for example, an aeration system with pure oxygen that was controlled based on the data collected with an additional oxygen probe. The titration unit used for our experiments (Fig 1) is aerated with ambient air. Thus far, tests with different sludge samples using this approach were very satisfying, and did not justify the need to install an aeration system with pure oxygen.[24]

The experimental approach that will be used here is more realistic compared with the experiments of Beccari *et al*[21] where rather high NH_4^+-N concentrations were used (70–900 mg NH_4^+-N dm^{-3}), to

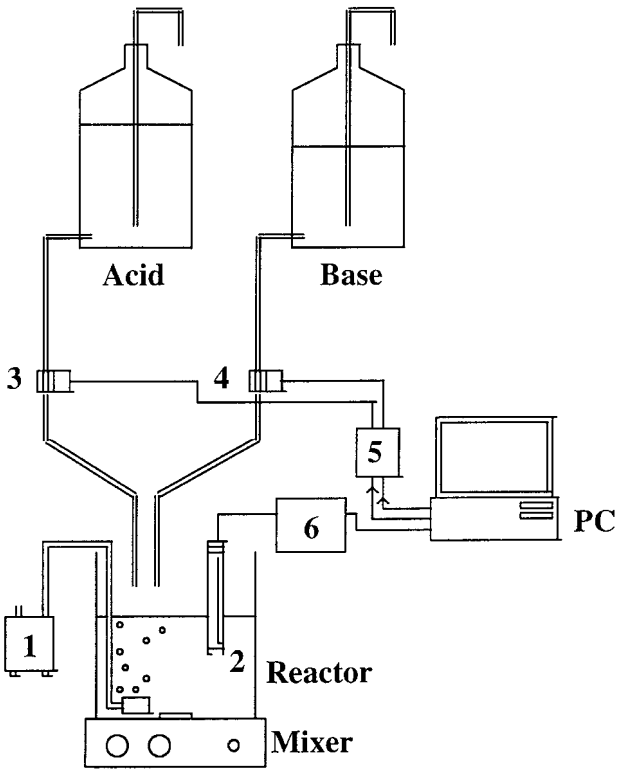

Figure 1. Titration unit used for the experiments with nitrifying activated sludge. The PC, connected to a pH electrode by a 4–20 mA transmitter, controls the dosage of base to the sludge sample. 1 = Aeration pump; 2 = pH electrode; 3, 4 = 24 V DC electromagnetic valves; 5 = 24 V DC supply; 6 = 4–20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

guarantee a zero order nitrification rate. For the experiments described here, low NH_4^+-N concentrations were applied (2.5 mg NH_4^+-N dm^{-3}) to simulate actual conditions of a properly operating activated sludge plant and to avoid substrate inhibition of the nitrifiers caused by a too high NH_4^+-N concentration.

In this paper, a titrimetric method is used to study acute inhibitory effects of toxic compounds on NH_4^+-oxidizing bacteria in activated sludge. Data resulting from the titration experiments were processed using a biokinetic parameter estimation procedure. This allowed conclusions to be drawn about the short-term effects of toxic compounds on the maximum substrate removal capacity and the substrate affinity constant (K_{NH}) of NH_4^+-oxidizing bacteria.

2 MATERIALS AND METHODS

2.1 Data collection

Experiments were performed with nitrifying activated sludge in the endogenous respiration phase, using a titration technique developed to monitor nitrification in activated sludge.[17,20,24] Basically this titration technique quantifies the protons formed during nitrification using a pH controller, which will continuously compensate the protons produced by adding equivalent amounts of base to a sludge sample.

Sludge was sampled from two different plants: a municipal wastewater treatment plant (WWTP) (Zwalm, Belgium; 2–3 g dm^{-3} suspended solids (SS)) and a plant treating the wastewater of a hospital (Middelares, Gent, Belgium; 4–5 g dm^{-3} SS). Sludge concentrations were determined according to standard methods.[25] Sludge was sampled and stored at 4 °C for a maximum of 2 weeks. The day before an experiment was performed, an aliquot of sludge was taken from the refrigerator and aerated overnight at room temperature. At the beginning of this acclimation phase, about 10 mg dm^{-3} NH_4^+-N was added to the sludge to activate the nitrifying bacteria. The validity of this procedure is shown in section 2.3.

For each titration experiment, an activated sludge sample in the endogenous respiration phase was transferred to the reactor vessel ($V = 4\,dm^3$) of a titration unit (Fig 1), which was continuously stirred and aerated ($Q_{air} = 60\,dm^3\,h^{-1}$). Experiments were all performed at a temperature of 25 ± 1 °C. The sludge concentration applied during the experiments was identical to the sludge concentration in the full-scale plant. The pH of the activated sludge was increased to the pH setpoint at the beginning of each titration experiment (empirically determined: pH setpoint = pH activated sludge sample + 0.3 pH units). When the pH setpoint was reached, base was only dosed to the activated sludge when the pH dropped below the pH setpoint minus a ΔpH interval. Typically, experiments were conducted at a pH setpoint $\pm$ ΔpH interval of 8.00 ± 0.03. This is well within the pH region where the highest nitrification rates were reported.[1] Base was added by opening an electromagnetic valve, connected to a mariotte bottle containing a 0.1 mol dm^{-3} NaOH solution, for 1.5 s (= 1 base pulse). This procedure was repeated until the pH in the titration vessel returned to within the pH setpoint $\pm$ ΔpH interval. The computer, controlling the titration unit, stored the actual pH in the reactor vessel every 10 s as well as the total number of base pulses dosed so far during the titration experiment. The flow per base pulse (Q_b) was checked at the end of each titration experiment by measuring the volume of base corresponding to 50 subsequent base pulses. Before applying a non-linear parameter estimation procedure, raw titration data were converted into meq dm^{-3} units, according to eqn (4).

$$B' = \frac{B \times Q_b \times N}{V} \tag{4}$$

Two organic compounds (3,5-dichlorophenol and phenol) and two inorganic compounds (Cu^{2+} as $CuSO_4.5H_2O$; CN^- as KCN) have been used as test chemicals. A 1 g N dm^{-3} NH_4Cl stock solution was used as NH_4^+-N source for all the experiments. A fresh stock solution was prepared every week and stored at 4 °C. All chemicals were of analytical grade.

In all experiments described in this paper, a known amount of a toxic compound was added to the activated sludge sample (4 dm^3) a few minutes after the pH setpoint was reached. After a contact time of 5 min, 10 mg NH_4^+-N was added to the activated sludge sample (initial concentration $S_{NH}(0) = 2.5$ mg N dm^{-3}). A typical titration curve recorded

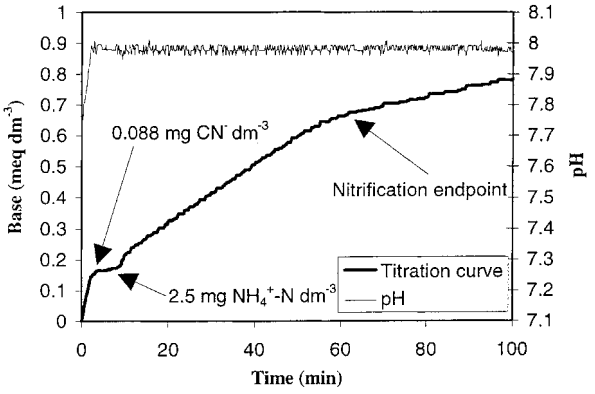

Figure 2. Typical titration curve obtained during a titration experiment with an activated sludge sample. Experimental conditions: pH=8.00±0.03, $V=4\,dm^3$, $S_{NH}(0)=2.5\,mg\ NH_4^+\text{-}N\,dm^{-3}$, $0.1\,mol\,dm^{-3}$ NaOH, base flux=$7.3\times10^{-3}\,dm^3$ (50 base pulses)$^{-1}$, $X=2.66\,g\,dm^{-3}$.

during one of the experiments is shown in Fig 2. For the experiment presented in Fig 2, the pH of the sludge was first increased to the pH setpoint (8.00±0.03). At $t=3.5\,min$, $0.088\,mg\,CN^-\,dm^{-3}$ were dosed to the reactor vessel. Ammonium was added at $t=8.5\,min$. The base addition rate immediately increased after the NH_4^+-N addition, due to a small pH decreasing effect caused by adding the NH_4Cl solution. After having compensated this pH effect (less than 1 min after the NH_4^+-N addition), base was added at a higher rate than before the NH_4^+-N addition, to compensate for the protons produced during nitrification of the NH_4^+-N (see eqn (1). When nitrification was finished (after about 60 min in Fig 2), base still had to be added, but at a significantly lower rate. In the following sections, the slope of this last part of the titration curve will be called the background proton production rate (BPPR). The BPPR is assumed to be constant during the short period of an experiment. As was explained elsewhere,[26] the BPPR results from different processes such as CO_2 stripping from the mixed liquor due to aeration, CO_2 production due to endogenous respiration of the heterotrophic activated sludge bacteria, and CO_2 consumption from the nitrifiers.

2.2 Data processing: non-linear parameter estimation procedure

A non-linear parameter estimation algorithm was used to estimate nitrification kinetic parameters,[27] by fitting a model to the data provided by each titration experiment. Minimization of the sum of squared errors (SSE) was used as the fit criterion. The titration data were described by a Monod biodegradation model and an additional factor describing the background proton production rate (BPPR) (eqns (5) and (6)).[26]

$$\frac{dH^+}{dt}=\frac{2+Y_A\times i_{XB}}{14\times Y_A}\times\mu_A\times\frac{S_{NH}}{K_{NH}+S_{NH}}\times X_{BA}+BPPR \quad (5)$$

$$\frac{dS_{NH}}{dt}=-\left(\frac{1}{Y_A}+i_{XB}\right)\times\mu_A\times X_{BA}\times\frac{S_{NH}}{K_{NH}+S_{NH}} \quad (6)$$

For each titration curve, the part of the curve recorded during the nitrification phase following the addition of NH_4^+-N was used as input data to the parameter estimation procedure. The estimation procedure was performed automatically with MOSIFIT (MOdel SImulator and FITter, available on request), a software package which has already been used successfully for the estimation of kinetic parameters based on respirometric data.[4,16,28,29] Similarly to respirometric data,[29] three parameter combinations are identifiable based on the data provided by each titration experiment:

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

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

The concentration of autotrophic biomass in the sludge (X_{BA}) was not determined. The effects of toxic compounds on the maximum nitrification capacity ($\mu_A\times X_{BA}/Y_A$) and the Monod half-saturation constant (K_{NH}) could be quantified by assuming $Y_A=0.24\,mg$ COD mg^{-1} N nitrified and $i_{XB}=0.086\,mg\,N\,mg^{-1}$ COD.[30] Precise knowledge of Y_A and i_{XB} is not really necessary because the factor $Y_A\times i_{XB}$ will only have a minor influence on the value of the factor $2+Y_A\times i_{XB}$ ($2+0.24\times0.086=2.021$). On the basis of that it is realistic to calculate the maximum nitrification capacity ($\mu_A\times X_{BA}/Y_A$) and K_{NH} from the estimated combinations of parameters.

2.3 Preliminary tests

A test was carried out to confirm that the storage of the sludge at 4 °C and the acclimation procedure did not influence the activity of the NH_4^+-oxidizing bacteria in the sludge. Ten reference titration experiments (addition of $2.5\,mg\,N\,dm^{-3}$ to an activated sludge sample in the endogenous respiration phase) were performed in a 2-week period with aliquots of sludge obtained from the same sludge sample (WWTP Zwalm). Kinetic parameters were estimated for each data set. Results are summarized in Table 1. A maximum nitrification capacity of $18.27\pm2.05\,mg\,N\,dm^{-3}\,h^{-1}$ and a Monod half-saturation coefficient of $0.14\pm0.04\,mg\,N\,dm^{-3}$ were found. An analysis of variance was done on the 10 parameter sets. Parameter values obtained on different days were not significantly different ($\alpha=0.05$), indicating that storage at 4 °C and the subsequent acclimation procedure did not influence the nitrification capacity and the substrate affinity of the nitrifying bacteria in the sludge.

Low concentrations of a toxic compound are sufficient to inhibit nitrification in activated sludge. Figure 3 contains some data sets recorded for sludge sampled from WWTP Zwalm in the presence of increasing concentrations of CN^-. The first data set is a reference experiment, performed by just adding $2.5\,mg\ NH_4^+\text{-}N\,dm^{-3}$ to an activated sludge sample in the endogenous respiration phase. Nitrification was

Table 1. Results of a repeatability test with a sludge sample obtained from WWTP Zwalm. For each experiment, 2.5 mg NH_4^+-N was added to a sludge sample in the endogenous respiration phase and nitrification kinetic parameters were estimated

Time (days after sampling)	*Maximum nitrification capacity ($mg N dm^{-3} h^{-1}$)*	*K_{NH} ($mg N dm^{-3}$)*
1	16.60	0.15
	14.89	0.14
3	21.60	0.17
	18.12	0.13
	17.53	0.16
10	18.75	0.08
	16.81	0.12
	21.26	0.21
11	19.03	0.09
	18.09	0.17
Average	18.27	0.14
Standard Deviation	2.05	0.04

finished after 20 min. A low concentration of CN^- ($0.038 mg CN^- dm^{-3}$) already caused inhibition of nitrification, as it took about 30 min to oxidize the 2.5 mg NH_4^+-N dm^{-3} in this experiment. The third data set was recorded in the presence of $0.138 mg CN^- dm^{-3}$. Nitrification proceeded much more slowly, while the shape of the titration curve was also different compared with the reference experiment. For the reference experiment, the slope of the titration curve remained constant for almost the complete nitrification phase. For the experiment in the presence of $0.138 mg CN^- dm^{-3}$, the slope of the titration curve decreased continuously during the nitrification phase.

The repeatability of the proposed method was tested for CN^- as toxic compound, using sludge from WWTP Zwalm. A series of titration experiments was repeated three times with aliquots of sludge obtained from the same sludge sample. Nitrification kinetic parameters were estimated for each titration curve. Results are summarized in Fig 4. For the repeatability test with CN^- ($n = 3$), the coefficient of variation (CV) varied between 6 ($0.063 mg CN^- dm^{-3}$) and 30% ($0.138 mg CN^- dm^{-3}$) for the maximum nitrification capacity values resulting from the estimation procedure. The CV for K_{NH} values varied between 10 ($0.063 mg CN^- dm^{-3}$) and 66% ($0.088 mg CN^- dm^{-3}$). Based on the satisfying results presented in Fig 4, it was decided to perform subsequent titration experiments for only one concentration series of the remaining toxic compounds 3,5-DCP, Cu^{2+} and phenol. The repeatability of the method was sufficient to deduce trends in the behavior of the maximum nitrification capacity and the substrate affinity of the nitrifying biomass from a single series of experiments with increasing concentrations of a toxic compound.

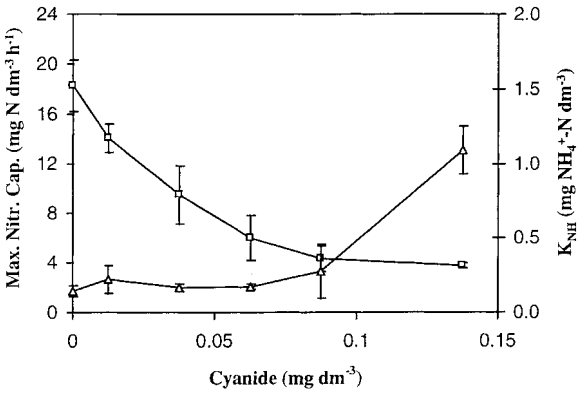

Figure 4. Results of the repeatability test ($n = 3$) with CN^- for activated sludge of WWTP Zwalm (□ = maximum nitrification capacity; △ = K_{NH}).

3 RESULTS

For the sludge sampled from WWTP Zwalm (Fig 4), increasing CN^- concentrations obviously resulted in a decrease of the maximum nitrification capacity and an increase of the K_{NH} value (for concentrations higher than $0.1 mg CN^- dm^{-3}$), as discussed above. For activated sludge sampled from the hospital WWTP (Fig 5) a decrease of the maximum nitrification capacity was again observed for increasing CN^- concentrations. Contrary to the activated sludge sample from WWTP Zwalm, all K_{NH} values were

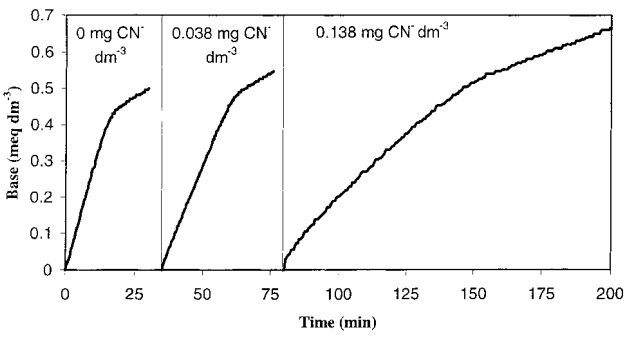

Figure 3. Example of data sets recorded for sludge sampled from WWTP Zwalm ($X = 2.66 g dm^{-3}$) in the presence of increasing concentrations of CN^-. For each data set the CN^- concentration is indicated. Increasing concentrations of CN^- result in a decrease of the nitrification rate.

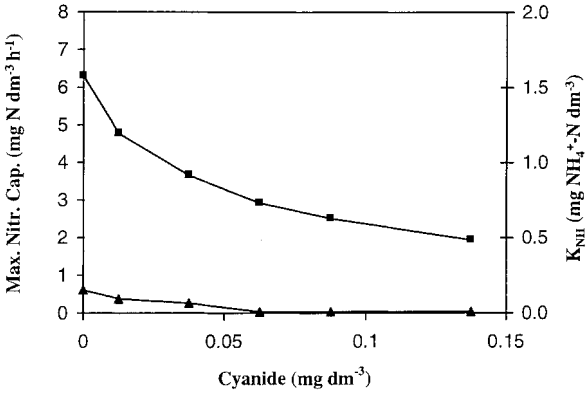

Figure 5. Results of toxicity tests with CN^- for activated sludge from a hospital WWTP (■ = maximum nitrification capacity; ▲ = K_{NH}).

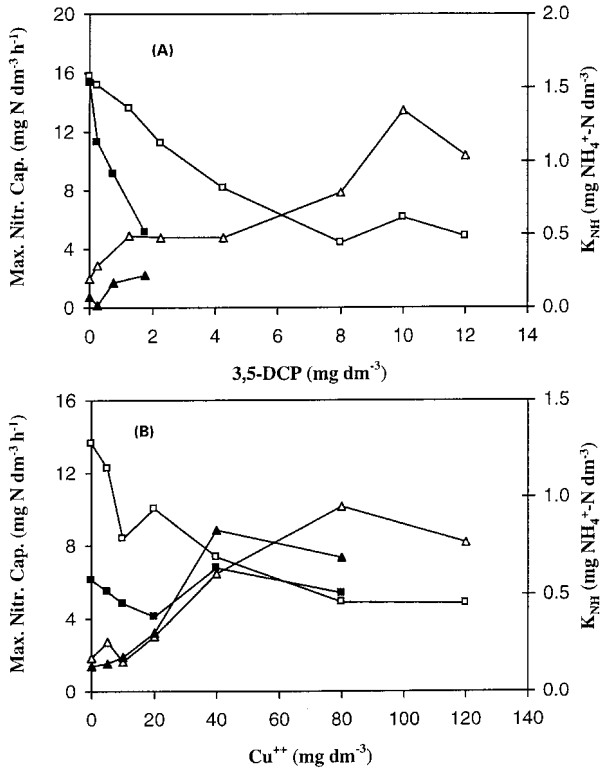

Figure 6. (A): Results of toxicity tests with 3,5-DCP; (B): Results of toxicity tests with Cu^{2+} (activated sludge WWTP Zwalm: □ = maximum nitrification capacity, △ = K_{NH}; activated sludge hospital WWTP: ■ = maximum nitrification capacity, ▲ = K_{NH}).

lower than 0.2 mg NH_4^+-N dm^{-3} and increasing CN^- concentrations did not result in an increase of the K_{NH} value.

For tests with 3,5-DCP, identical trends could be observed for both sludge samples (Fig 6(A)). An increasing concentration of 3,5-DCP resulted in a decrease in the maximum nitrification capacity and an increase in the K_{NH} value. However, the sensitivity of both sludge samples to inhibition with 3,5-DCP was different. For activated sludge sampled from WWTP Zwalm, nitrification continued up to 3,5-DCP concentrations of 12 mg dm^{-3}, while the maximum nitrification capacity of sludge sampled from the hospital WWTP already showed a decrease of more than 60% for a 3,5-DCP concentration of only 2 mg dm^{-3}.

Results for Cu^{2+} are summarized in Fig 6(B). For both activated sludge samples, K_{NH} values tended to increase with increasing Cu^{2+} concentrations. The maximum nitrification capacity of activated sludge from the hospital WWTP was not influenced by the Cu^{2+} additions, whereas the maximum nitrification capacity of activated sludge obtained from WWTP Zwalm decreased in the presence of increasing Cu^{2+} concentrations.

A typical titration curve recorded in the presence of phenol is shown in Fig 7, for sludge of the hospital WWTP. Phenol was added at $t = 0$ (2 mg dm^{-3}), while NH_4^+-N (2.5 mg dm^{-3}) was added at $t = 5$ min. The nitrifying bacteria in the activated sludge sample

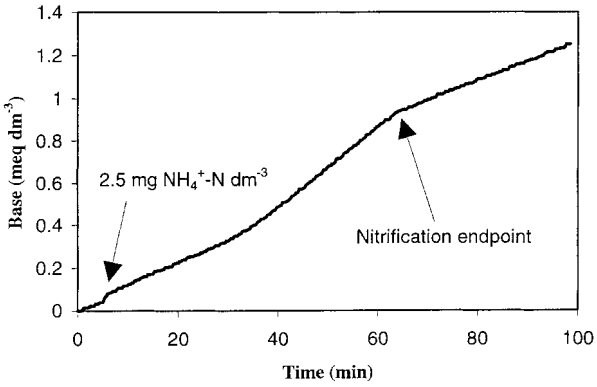

Figure 7. Titration curve recorded for activated sludge of the hospital WWTP in the presence of 2 mg dm^{-3} phenol. Phenol has been added at $t = 0$, NH_4^+-N was dosed at $t = 5$ min.

showed little or no response immediately after the addition of NH_4^+-N, as the slope of the titration curve did not change significantly after the addition of NH_4^+-N. However, after about 30 min, the slope of the titration curve increased, most probably indicating an increase in the nitrification rate. Nitrification seemed to be completely finished after 65 min. Similar data sets were recorded for sludge from WWTP Zwalm.

4 DISCUSSION

For the repeatability test with CN^- (Fig 4), the highest coefficient of variation (CV) was observed for K_{NH} values. The same has been observed for respirometric experiments combined with a similar parameter estimation procedure.[16] This observation is typical for this type of experiment due to the limited amount of information which is available in the low concentration range (the concentration range providing information to estimate K_{NH} values).[29] A higher reliability of estimated parameter values from titration data can be obtained by an improved experimental design, eg by dosing an additional small substrate pulse when nitrification is finished. In this way, much more information would be available in the low concentration range, which would especially benefit the K_{NH} estimation. By applying such an optimized experimental design, improvements in parameter estimation accuracy with a factor of 2 were mentioned.[29]

For the reference experiments (no toxic compound present), K_{NH} values varying between 0.09 and 0.21 mg NH_4^+-N dm^{-3} were obtained for sludge from WWTP Zwalm, while K_{NH} values varied from 0.07 to 0.15 mg NH_4^+-N dm^{-3} for sludge from the hospital WWTP. Compared with literature data for K_{NH} values of NH_4^+-oxidizing bacteria, it can be concluded that the obtained values are at the lower end of values reported in the literature (K_{NH} = 0.06–5.6 mg NH_4^+-N dm^{-3}).[31] K_{NH} values of 0.53–0.88 mg NH_4^+-N dm^{-3} were reported by Kong *et al*,[16] for sludge from the hospital WWTP, which is considerably higher than the values reported in this study for sludge

sampled from the same plant (0.07–0.15 mg NH_4^+-N dm^{-3}). One reason for the observed differences could be that nitrification kinetic parameters of the sludge have indeed changed in the 2-year period between the two measurements. However, the observed differences between both measuring methods could be explained also because values reported by Kong *et al*,[16] were estimated from respirometric data, meaning that activities of both NH_4^+- and NO_2^--oxidizing bacteria were included, probably lumping their biokinetic characteristics in the overall nitrification kinetics. The titration method on the other hand yields data which are only valid for the NH_4^+ oxidizers. Higher K_{NH} values were found for NO_2^--oxidizing bacteria (0.8–1.1 mg N dm^{-3}) compared with NH_4^+-oxidizing bacteria (0.4–0.8 mg N dm^{-3}),[13] confirming the differences between the results of Kong *et al*[16] and the results reported here.

The high sensitivity of nitrifying bacteria for CN^- (Figs 4 and 5) is consistent with literature data, where CN^- concentrations of less than 0.5 mg dm^{-3} were reported to give 50% or more inhibition of nitrification.[6,7,16,21,32] For 3,5-DCP experiments with hospital WWTP sludge, the 3,5-DCP concentration range used and the observed inhibitory effects were comparable to the effects reported elsewhere for respirometric nitrification inhibition tests with sludge sampled from the same plant.[16]

For Cu^{2+}, concentrations up to 120 mg dm^{-3} were used for the tests (Fig 6(B)). The low sensitivity of the NH_4^+-oxidizing bacteria for Cu^{2+} is consistent with literature data,[6,16] and can be explained by the adsorption of Cu^{2+} on the activated sludge matrix, where it is no longer available for the bacteria.[33] Indeed, for a pure *Nitrosomonas europaea* culture 75% inhibition of the activity was already observed in the presence of 4 mg Cu^{2+} dm^{-3}, while a Cu^{2+} concentration of about 200 mg Cu^{2+} dm^{-3} was needed to obtain 75% inhibition of the activity of NH_4^+-oxidizing bacteria in activated sludge.[6]

The two sludge samples showed different inhibition patterns for the same concentrations of 3,5-DCP (Fig 6(A)) and Cu^{2+} (Fig 6(B)). This could be explained by a different composition of the wastewater entering both treatment plants. Indeed, it has been known for some time that activated sludge bacteria have an adaptive capacity which allows them to survive in the presence of certain concentrations of toxic compounds after an adaptation period.[6] Closely related to this adaptation phenomenon, a better reproducibility was reported for interlaboratory nitrification inhibition tests with the same toxic wastewater when all laboratories used nitrifying sludge obtained from the same plant than when different sludges were used.[34]

Titration curves recorded for phenol no longer corresponded to a simple Monod model (Fig 7). The shape of the titration curve of Fig 7, recorded in the presence of 2 mg phenol dm^{-3}, could be explained by interpreting the data according to the findings of Benmoussa *et al*,[35] who measured phenol and NH_4^+-N concentrations during experiments with a nitrifying activated sludge. They found that phenol was degraded in a first phase, while nitrification only started when phenol was degraded to a concentration level low enough to support nitrification. Interpreting the data of Fig 7 in a similar way, phenol degradation by heterotrophic bacteria is initiated immediately after the addition of phenol to the activated sludge sample (at $t = 0$ min). However, the phenol concentration in the activated sludge seems too high to support nitrification at the moment of NH_4^+-N addition (at $t = 5$ min in Fig 7). Nitrification finally speeds up after about 30 min, as indicated by the increasing slope of the titration curve, when phenol has been degraded to a sufficiently low concentration. During this nitrification start-up phase, the nitrification rate gradually increases until the maximum nitrification capacity is reached.

We tried to describe the titration curve of Fig 7 with a model including both the inhibition of nitrification by phenol and the degradation of phenol by the heterotrophic activated sludge bacteria. The model, including a factor for noncompetitive inhibition of the NH_4^+ oxidation process,[36] is presented in eqns (7)–(9).

$$\frac{dH^+}{dt} = \frac{2 + Y_A \times i_{XB}}{14 \times Y_A} \times \mu_A \times f_{BA} \times X \times \frac{S_{NH}}{K_{NH} + S_{NH}} \times \frac{K_i}{K_i + S_{Ph}} + BPPR \quad (7)$$

$$\frac{dS_{Ph}}{dt} = -\frac{\mu_{MPh} \times f_{BH} \times X}{Y_H} \times \frac{S_{Ph}}{K_{Ph} + S_{Ph}} \quad (8)$$

$$\frac{dS_{NH}}{dt} = -\left(\frac{1}{Y_A} + i_{XB}\right) \times \mu_A \times f_{BA} \times X \times \frac{S_{NH}}{K_{NH} + S_{NH}} \times \frac{K_i}{K_i + S_{Ph}} - i_{XB} \times \mu_{MPh} \times f_{BH} \times X \times \frac{S_{Ph}}{K_{Ph} + S_{Ph}} \quad (9)$$

Results of the parameter estimation procedure with phenol, according to the model given in eqns (7)–(9), are illustrated in Fig 8. Residuals are all very close to 0, indicating that the proposed model is able to describe the titration data very well. According to the model, most of the phenol is degraded during the first 25 min of the experiment, while little or no NH_4^+-N is removed at the same time. Nitrification speeds up after 25 min, when phenol is almost completely degraded. For the data set of Fig 7, a maximum nitrification capacity of 5.11 mg N dm^{-3} h^{-1}, K_{NH} = 0.11 mg N dm^{-3} and K_i = 0.022 mg phenol dm^{-3} resulted from the estimation procedure, while for phenol degradation a maximum phenol removal rate of 6.82 mg phenol dm^{-3} h^{-1} and a K_{Ph} value of 0.17 mg phenol dm^{-3} were obtained. Parameters were estimated assuming all phenol (2 mg dm^{-3}) was still present at the moment of the NH_4^+-N addition to the sludge, resulting in an overestimation of the maximum phenol degradation rate. In fact, part of the phenol was already degraded at $t = 0$ because phenol was added to

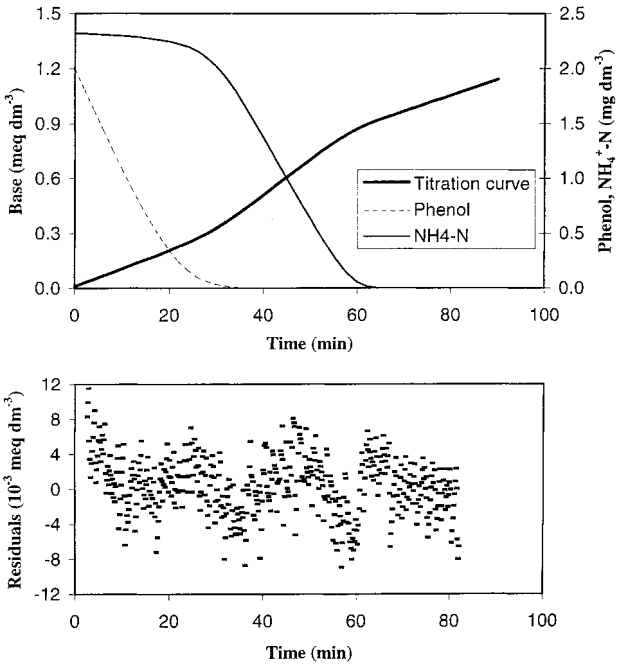

Figure 8. Simulated phenol and NH_4^+-N profile obtained with kinetic parameters estimated based on the data of Fig 8, according to the model described in eqns (7)–(9). The simulated titration curve and residuals are given as well. For more explanation, see text.

the sludge 5 min before the NH_4^+-N addition, as illustrated in Fig 7. However, it should be clear from Figs 7 and 8 that the proposed model (eqns (7)–(9)), including the degradation of a toxic compound, is able to describe titration data recorded for phenol as a readily biodegradable toxic compound. However, it might be clear that more experimental work is needed to understand these phenol inhibition phenomena on nitrification better.

For practical applications, the presented method seems suitable for studying the impact of shock-loads of specific toxic compounds on nitrification in activated sludge wastewater treatment plants. Moreover, application of the method is not limited to pure chemicals only, but can be applied also to the study of the effects of a toxic wastewater on the performance of NH_4^+-oxidizing bacteria in activated sludge. This could be done by dosing a volume of wastewater suspected to contain toxic compounds to a nitrifying activated sludge sample in the endogenous respiration phase. For biodegradable toxic compounds it could be useful to repeat the test for different contact times, as was shown for phenol, because the inhibitory effect of the toxic compound can disappear within a few hours due to degradation of the toxic compound by heterotrophic bacteria. It is important for the treatment plant operator to know if nitrification inhibition caused by a toxic shock-load is reversible or not. Based on the results of the experiments the model could be used to calculate which load of a biodegradable toxic compound or a wastewater can enter an activated sludge system before NH_4^+-N concentrations in the effluent of the treatment plant will exceed effluent standards.

5 CONCLUSIONS

A combination of a titrimetric method and a non-linear parameter estimation procedure was used to study the kinetics of NH_4^+-oxidizing bacteria in activated sludge. Based on a single titration experiment, the maximum nitrification capacity $(\mu_A \times X_{BA})/Y_A$ and the Monod half-saturation constant K_{NH} can be estimated. The method was applied to quantify short-term effects of low concentrations of toxic compounds on the biokinetic parameters of NH_4^+-oxidizing bacteria. The repeatability of the method was sufficient to deduce trends in the behavior of the NH_4^+ oxidizers based on one series of titration experiments with increasing concentrations of a toxic compound. It is concluded from the experimental data that identical concentrations of the same toxic compound can have a different effect on NH_4^+-oxidizing bacteria present in activated sludge samples obtained from different treatment plants. For phenol a more complicated model including phenol degradation and inhibition of nitrification by phenol is needed to describe the titration data.

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