Influence of temperature and pH on the kinetics of the Sharon nitritation process

Stijn WH Van Hulle,[1,2]* Eveline IP Volcke,[1] Josefa López Teruel,[1] Brecht Donckels,[1] Mark CM van Loosdrecht[3] and Peter A Vanrolleghem[1,4]

[1]Ghent University, Gent, Belgium
[2]Hogeschool West-Vlaanderen, Kortrijk, Belgium
[3]Delft University of Technology, Delft, The Netherlands
[4]Université Laval, Québec, Canada

Abstract: The SHARON (Single reactor High activity Ammonia Removal Over Nitrite) process is an innovative process that improves the sustainability of wastewater treatment, especially when combined with an Anammox process. It aims at ammonium oxidation to nitrite only, while preventing further nitrate formation. In order to optimize this process by means of modelling and simulation, parameters of the biological processes have to be assessed. Batch tests with SHARON sludge clearly showed that ammonia rather than ammonium is the actual substrate and nitrous acid rather than nitrite is the actual inhibitor of the ammonium oxidation in the SHARON process. From these batch tests the ammonia affinity constant, the nitrous acid inhibition constant and the oxygen affinity constant were determined to be 0.75 mgNH_3-N L^{-1}, 2.04 mgHNO_2-N L^{-1} and 0.94 mgO_2 L^{-1}. The influence of pH and temperature on the oxygen uptake rate of SHARON biomass was determined, indicating the existence of a pH interval between 6.5 and 8 and a temperature interval from 35 to 45 °C where the biomass activity is maximal. The kinetic parameters of the SHARON process were determined based on batch experiments. These parameters can now be implemented in a simulation model for further optimization of the SHARON process.

Keywords: SHARON; partial nitritation; ammonia; affinity constant; nitrous acid; inhibition

LIST OF SYMBOLS

b	OUR (oxygen uptake rate) temperature dependency parameter
c	OUR temperature dependency parameter
DO	dissolved oxygen concentration [mgO_2 L^{-1}]
K_{e,NH_4^+}	acidity constant of the ammonium/ammonia equilibrium
K_{e,HNO_2}	acidity constant of the nitrite/nitrous acid equilibrium
K_{I,HNO_2}^{NH}	inhibition constant for nitrous acid of ammonium oxidizers [mgHNO_2-N L^{-1}]
$K_{NH_3}^{NH}$	saturation constant for ammonia of ammonium oxidizers [mgNH_3-N L^{-1}]
$K_{O_2}^{NH}$	saturation constant for oxygen of ammonium oxidizers [mgO_2 L^{-1}]
K_{pH}	OUR pH dependency parameter
pH_{opt}	OUR pH dependency parameter
S_{NH3}	ammonia nitrogen concentration [mgNH_3-N L^{-1}]
S_{HNO2}	nitrous acid nitrogen concentration [mgHNO_2-N L^{-1}]
S_{TAN}	total ammonium nitrogen concentration [mgTAN-N L^{-1}]
S_{TNO2}	total nitrite nitrogen concentration [mgTNO_2-N L^{-1}]
S_{O2}	oxygen concentration [mgO_2 L^{-1}]
OUR	oxygen uptake rate [mg O_2 L^{-1} d^{-1}]
T_{min}	OUR temperature dependency parameter
T_{max}	OUR temperature dependency parameter
TAN	total ammonia nitrogen [mgTAN-N L^{-1}]
TNO_2	total nitrite nitrogen [mgTNO_2-N L^{-1}]
X_{NH}	ammonium oxidizers [mgCOD L^{-1}]
Y_{NH}	autotrophic yield of ammonium oxidizers [mgCOD mgN^{-1}]
$\mu_{\max}^{NH}$	maximum growth rate of ammonium oxidizers [d^{-1}]
θ	Arrhenius constant

INTRODUCTION

SHARON–Anammox process

With the discovery of the Anammox process over 10 years ago[1] a new path could be taken towards the sustainable removal of nitrogen from wastewater. In this Anammox process ammonium and nitrite are combined on an equimolar basis to produce nitrogen gas, although also some nitrate is produced. The Anammox process requires a partial nitritation step in which half of the influent ammonium concentration is oxidized to nitrite without further conversion to nitrate. As such, a suitable influent for the Anammox reactor is produced. An example of such a partial

* Correspondence to: Stijn WH Van Hulle, PIH, Departement of Industrial Engineering and Technology, Hogeschool West-Vlaanderen, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium
E-mail: Stijn.Van.Hulle@Howest.be
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nitritation process is the SHARON process[2] (Single reactor High activity Ammonia Removal Over Nitrite) in which stable nitrite formation at high temperature (35 °C) and neutral pH is established by washing out the nitrite oxidizers, which grow more slowly than the ammonium oxidizers under these conditions. The combination of this Anammox process with a partial nitrification process has great potential since there is no longer any need for external carbon addition, sludge production is very low, and oxygen input and aeration energy requirements are reduced.[3]

Very interesting and useful tools to further optimize the SHARON process are modelling and simulation environments such as WEST®,[4] or Matlab (The Mathworks Inc., www.mathworks.com). With such a simulation tool a large number of virtual experiments can be conducted in order to investigate the behaviour of the combined system under different operating conditions. In this way, time and money can be saved. However, in order to have a correct representation of reality by these simulations, correct kinetic equations describing the biological processes have to be put forward. Furthermore the parameters in these equations have to be assessed.

SHARON kinetics

Biochemical experiments carried out over more than half a century on different cultures clearly indicated that kinetics are influenced by many physico-chemical and biological environmental factors among which the most important are substrate concentration, product concentration, pH, temperature, dissolved oxygen and various inhibitors such as salts.[5] The specific growth rate is then commonly expressed by the multiplication of individual terms, e.g. Monod-type expressions each of them referring to one of the influencing factors.

For ammonium oxidation to nitrite, Anthonisen *et al*.[6] formulated the hypothesis that ammonia (NH_3) rather than ammonium (NH_4^+) is the actual substrate and that at higher concentrations ammonia becomes inhibiting. This hypothesis has been verified in this study, while the corresponding parameter values for a partial nitritation SHARON reactor have been determined. Nitrous acid (HNO_2) inhibition, not discussed by Anthonisen *et al*.,[6] has also been investigated. In general Monod type expressions are used for the influence of ammonia and nitrous acid.

The influence of temperature on biological activity is most often modelled by an Arrhenius-type of equation:

$$\mu(T) = \mu(T_r)e^{\theta(T-T_r)} \tag{1}$$

where $\mu(T)$ is the maximum specific growth rate μ at the actual temperature T, T_r is the reference temperature (often taken as 20 °C) and θ is the Arrhenius constant. The Arrhenius constant for autotrophs can be calculated with the activation energy (E_{act}) of the autotrophic biomass:[7]

$$\theta = \frac{E_{act}}{(R\,293(T+273))} \tag{2}$$

where R is the universal gas constant (8.31 J mol^{-1} K^{-1}). As the activation energies of aerobic ammonium oxidation ranges in the literature from 60 to 72 kJ mol^{-1},[8–11] the value for θ lies in the range 0.085 to 0.1. This equation, however, does not take the decrease of activity at temperatures above 40 °C into account. Therefore equations such as the Hinshelwood model

$$\mu = k_1\, e^{-\frac{E_1}{R(T\ +273)}} - k_2 e^{-\frac{E_2}{R(T\ +273)}} \tag{3}$$

and the modified Rathowsky model[12]

$$\mu = [b(T - T_{\min})]^2\{1 - e^{c(T-T_{\max})}\} \tag{4}$$

were put forward.

The Hinshelwood model is based on the fundamental Arrhenius model, E_1 and E_2 are the activation energies of the reaction and the high-temperature denaturation respectively, but the parameters are strongly correlated and are therefore very difficult to estimate. The modified Rathowsky model has no biological basis but was shown to be the most suitable to describe the specific growth rate as a function of temperature.[12] The parameters $T_{\min}$ and $T_{\max}$ are the minimum and maximum temperature at which growth is observed. The parameters b and c are two parameters without biological basis.

The effect of pH on biological activity is normally less pronounced than the effect of temperature because the cell is reasonably well able to regulate its internal hydrogen ion concentration in the face of adverse external concentrations, though the maintenance energy required to do this is obviously affected. In addition, the pH of the external medium has an important effect on the structure and permeability of the cell membrane.[13] Typically bell-shaped functions as given in Eqns (5)[5] and (6)[14] are used to model this pH dependency:

$$\mu = \mu^{\max}\frac{1}{1 + 10^{pK_1 - pH} + 10^{pH - pK_2}} \tag{5}$$

$$\mu = \mu^{\max}\frac{K_{pH}}{K_{pH} - 1 + 10^{|pH_{opt} - pH|}} \tag{6}$$

In this contribution Eqn (6) was chosen, because the data obtained showed a better fit to this equation. Based on the above considerations, the following expression for the growth rate of ammonium oxidizers is proposed:

$$\begin{aligned}\mu^{NH} = \mu^{NH}_{\max}&\frac{S_{NH_3}}{S_{NH_3} + K^{NH}_{NH_3}}\frac{K^{NH}_{I,NH_3}}{S_{NH_3} + K^{NH}_{I,NH_3}}\\ &\times \frac{K^{NH}_{I,HNO_2}}{S_{HNO_2} + K^{NH}_{I,HNO_2}}\frac{S_{O_2}}{S_{O_2} + K^{NH}_{O_2}}\\ &\times \frac{K_{pH}}{K_{pH} - 1 + 10^{|pH_{opt} - pH|}}\\ &\times [b(T - T_{\min})]^2\{1 - e^{c(T-T_{\max})}\}\end{aligned} \tag{7}$$

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To allow the determination of the ammonia affinity constant ($K_{NH_3}^{NH}$), the ammonia inhibition constant (K_{I,NH_3}^{NH}) and the nitrous acid inhibition constant (K_{I,HNO_2}^{NH}), batch experiments with SHARON sludge were carried out at two different temperatures (25 and 35 °C) and three different values of pH (6.5, 7 and 7.5).

The oxygen affinity constant ($K_{O_2}^{NH}$) was determined with a similar experiment. Further the maximum growth rate $\mu_{\max}^{NH}$ and the influence of temperature and pH on the maximum oxygen uptake rate (OUR) were assessed.

MATERIALS AND METHODS

SHARON reactor for sludge sampling

Sludge for the experiments was sampled from a lab-scale SHARON reactor.[15] The reactor is a 2 L continuously stirred tank reactor (CSTR) without biomass retention. The synthetic influent is pumped with a peristaltic pump from the 5 L influent vessel to the reactor. The reactor is aerated through a pumice stone using air from a compressor (1 bar overpressure). The temperature of the reactor is controlled at 35 °C. In the reactor the dissolved oxygen (DO) and pH are measured. Data logging is performed using Labview® software (National Instruments, Austin, TX, USA).

During the period in which the experiments were performed the reactor was operated without pH control, at an influent total ammonia nitrogen (TAN) concentration of 500, 1000 or 2000 mgTAN-N L^{-1}, at a hydraulic residence time (HRT) of 1.54 d and a total ammonia nitrogen over total inorganic carbon ratio (TAN:TIC) of 1:1. Reactor performance is expressed in Fig. 1, where the influent TAN concentration and the effluent TAN, TNO_2 and nitrate concentration are depicted. Effluent concentrations are expressed as percentages of total effluent nitrogen concentration for easy comparison between both operating modes (different influent concentrations). At the time of the experiments the reactor operation was very stable, producing an effluent that consisted, on average, of 56% TNO_2, 43.5% TAN and 0.5% nitrate. This low nitrate concentration indicates that the oxygen uptake activity measured during the batch experiments will be attributed to ammonium oxidizer activity only. This was further evidenced by the semi-quantitative determination of the nitrate concentration with test strips after each experiment. This semi-quantitative determination revealed that nitrate was present in none of the experiments. As such OUR can be linked to ammonium oxidizer activity only.

For a determination of the maximum specific growth rate (see below), a stable period of 24 days was selected during which the HRT was 1.54 days, the influent TIC:TAN ratio was 1:1, the influent concentration was 2000 mgTAN-N L^{-1} and the reactor temperature was 35 °C. The average pH was 6.83. The average effluent TAN, TNO_2, NO_3^-, NH_3, HNO_2 and DO concentrations in this period were 1064 mgTAN-N L^{-1}, 1202 mgTNO_2-N L^{-1}, 8 mgNO_3^--N L^{-1}, 10.24 mgNH_3-N L^{-1}, 0.37 mgHNO_2-N L^{-1} and 6.07 mgO_2 L^{-1} respectively. The sum of the effluent concentrations is about 10% higher than the influent concentrations because of evaporation, as discussed by Van Hulle *et al*.[15]

Batch experiments for estimation of affinity and inhibition constants

General methodology

Respirometric batch experiments[16] with the SHARON sludge were performed to assess the ammonia affinity constant, the nitrous acid inhibition constant, the oxygen affinity constant and the influence of pH and temperature on the maximum specific growth rate. Figure 2 displays a schematic representation of the experimental set-up.

In the experimental set-up, OUR was measured, and was assumed to be proportional to the maximum specific growth rate. If a constant biomass yield is assumed then the OUR is proportional to the maximum specific growth rate according to:

$$OUR_{NH} = \frac{3.43 - Y_{NH}}{Y_{NH}} \mu_{NH} X_{NH} \qquad (8)$$

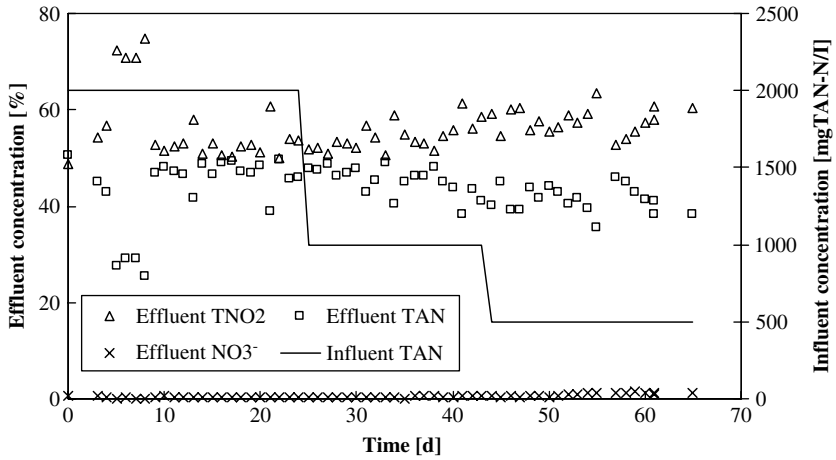

Figure 1. Lab-scale SHARON influent TAN concentration (–) and effluent TAN (□), TNO_2 (△) and nitrate (x) concentration during the experimental period.

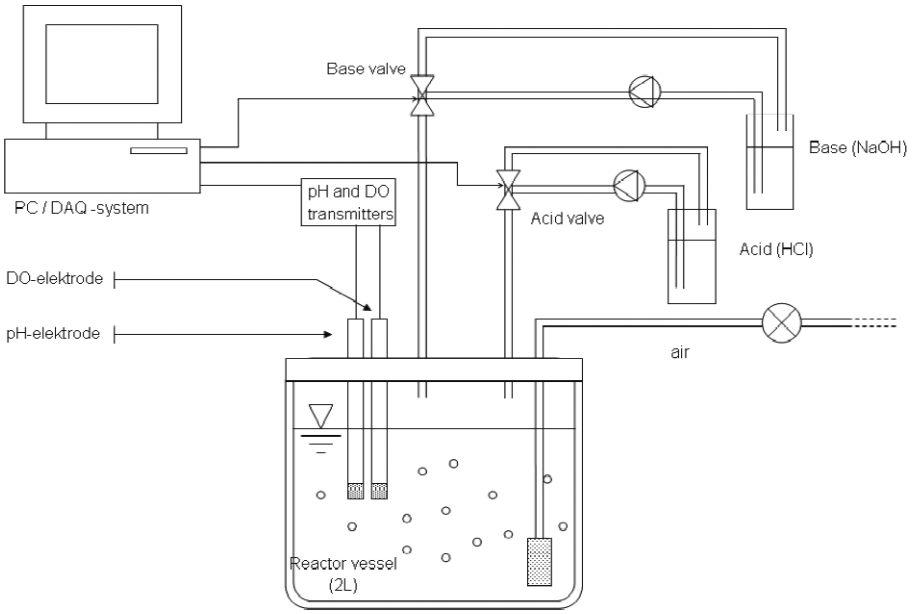

Figure 2. Schematic representation of the experimental set-up used for determining the influence of temperature and pH on the kinetics of the SHARON nitritation process.

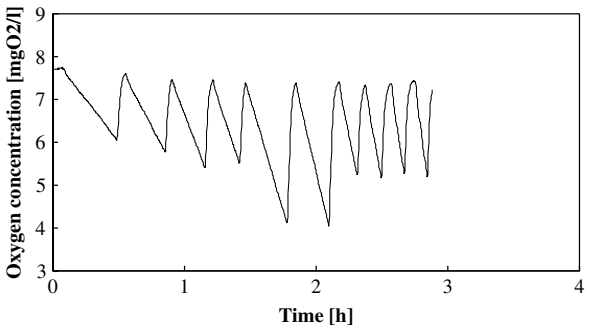

Figure 3. Raw data of a typical experiment conducted for the determination of the SHARON kinetics.

This hypothesis of constant biomass yield is not always valid, certainly when maintenance effects start playing a role.

Oxygen uptake rates were determined by turning off the aeration in the reactor and recording the drop in DO concentration. The slope of this DO concentration versus time plot equals the OUR (i.e. $OUR = \mathrm{d}DO/\mathrm{d}t$). Linking this OUR to the conditions in the reactor (temperature, pH, ammonium and nitrite concentration, ...) gives information on the kinetics of the SHARON process. Raw data from a typical experiment is depicted in Fig. 3.

Aeration through the headspace was always smaller than 5% of the OUR. Hence, the error introduced by this aeration in the DO balance used for the OUR determination can be assumed negligible.

Before each experiment the sludge was washed with softened water to ensure that no ammonium or nitrite was present at the beginning of the experiment. The same softened water was used for influent preparation of the SHARON reactor to ensure a similar osmotic pressure during the experiments as during normal sludge conditions.

Ammonia affinity constant and inhibition constant

Batch tests at two different temperatures (25 and 35 °C) and three different values of pH (6.5, 7 and 7.5) were performed for the determination of $K_{NH_3}^{NH}$. In every batch test sequential additions of $(NH_4)_2SO_4$ were carried out leading to an accumulated amount of TAN as depicted in Table 1. After each addition the OUR was determined. $(NH_4)_2SO_4$ was used as ammonium source in the experiments as $(NH_4)_2SO_4$ is also used as substrate in the synthetic influent. Before and after every oxygen drop a sample was taken for TAN (total ammonia nitrogen, ammonium + ammonia) analysis. In this way, OUR can be linked to TAN concentration.

A similar experiment to determine ammonia inhibition was conducted at 35 °C and pH 8. This last experiment was performed 3 months after the other experiments, but with sludge from the same reactor.

Nitrous acid inhibition constant

Similar batch tests as for the determination of $K_{NH_3}^{NH}$ were conducted to determine the nitrous acid inhibition constant. Before the experiment, an excess of 1000 mgTAN-N L^{-1} was added to the reactor to exclude substrate limitation. In every batch test sequential additions of KNO_2 were carried out (Table 1) and after each addition the OUR was determined. Before and after every oxygen drop a sample was taken for subsequent TNO_2 (total nitrite nitrogen, nitrous acid + nitrite) analysis.

Oxygen affinity constant

Again batch tests at two different temperatures (25 and 35 °C) and three different values of pH (6.5, 7 and 7.5) were performed for the determination of $K_{O_2}^{NH}$. In every batch test an excess of 1000 mgTAN-N L^{-1}

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Table 1. Applied cumulative substrate concentration, pH and temperature profiles for the corresponding kinetic experiments performed in this study. Each column of the table is independent and not related to numbers on the same row

	Ammonia affinity constant	Ammonia inhibition constant	Nitrous acid inhibition constant	Influence of pH	Influence of *T*
Experiment	Accumulated TAN concentration [mgTAN-N L^{-1}]	Accumulated TAN concentration [mgTAN-N L^{-1}]	Accumulated TNO2 concentration [mgTNO2-N L^{-1}]	pH value	*T* [°C]
	10	100	100	7	15
	25	250	300	6.75	20
	50	500	500	7.25	25
	75	1000	700	6.5	30
	100	2000	900	7.5	35
	200	3000	1100	6.25	40
	300	4000	1300	7.75	45
	500	5000	1500	6	50
	1000	6000	1700	8	
	2000	7000	1900	5.75	
		8000	2000	8.25	
		9000		5.5	
		10 000		8.5	
		20 000		5.25	
				8.75	
				5	
				9	

was added. Aeration was turned off. The drop in DO concentration was recorded until the concentration reached 0.1 $mgO_2\,L^{-1}$. Plotting the time derivative of the DO concentration versus the concentration itself yields a Monod curve expressing oxygen limitation of the OUR.

Maximum OUR

To exclude substrate limitation, an excess of substrate (1000 mgTAN-N L^{-1}) was first added to the reactor. The pH was varied between 5 and 9 in steps of 0.25 (Table 1) at temperatures of 25 °C and 35 °C. For every pH value the maximum OUR was determined twice and was linked to pH.

A similar experiment was conducted for the temperature dependency. This time six different temperature setpoints (15, 20, 25, 30, 35 and 40 °C) were applied, keeping pH constant at 7. The temperatures in the experiment were applied in an increasing order because of practical considerations.

Chemical analysis

Concentrations of TAN and TNO_2 were analyzed after proper dilution using spectrophotometric methods (Dr Lange GmbH, Germany) according to Standard Methods.[17] Every sample was analyzed twice. The absence of TAN and TNO_2 at the beginning and NO_3^- at the end of the experiment was checked semi-quantitatively with test strips (Merckoquant, www.vwr.com). The dissolved oxygen was measured by Ingold (Mettler Toledo) Clarck type oxygen electrode. The pH was measured with a glass electrode.

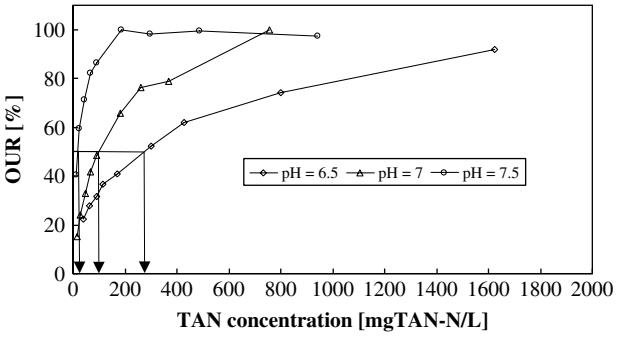

Figure 4. Process kinetics expressed as a percentage of OUR_{max} and TAN concentration obtained at 35 °C. These curves show that for each experiment a different affinity constant would be obtained if expressed in terms of the TAN concentration.

Parameter estimation

Parameter estimation was performed with the WEST® modelling and simulation software.[4]

RESULTS AND DISCUSSION

Ammonia affinity and inhibition constant

Figure 4 summarizes the OUR values measured at different TAN concentrations, for different pH values at 35 °C. These three Monod curves are expressed in percentages, referring to the highest OUR value for each experiment to enable comparison between the different experiments. It can be seen from Fig. 4 that for each experiment a different affinity constant would be obtained if expressed in terms of the TAN concentration. A higher pH results in a lower affinity constant for TAN: the TAN concentration at which the OUR reaches half of its maximum value is then lower.

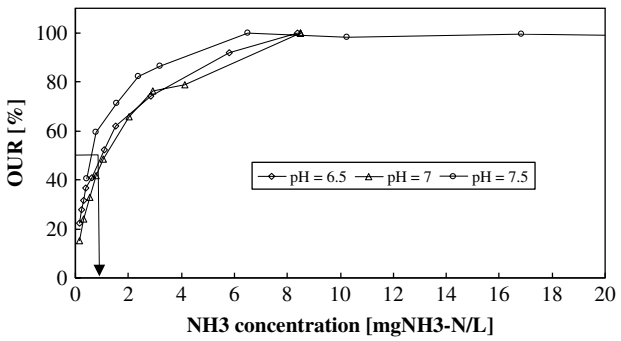

Figure 5. Process kinetics expressed as a percentage of OUR_{max} and NH_3 concentration obtained at 35 °C. This data shows that Monod curves coincide if expressed in NH_3 concentration.

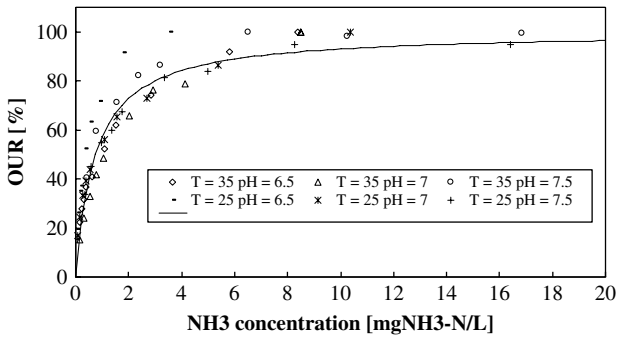

Figure 6. Process kinetics expressed as a percentage of OUR_{max} and NH_3 concentration, showing the coincidence of different experimental results if expressed in terms of NH_3 concentration.

In order to test Anthonisen's hypothesis that the uncharged ammonia is the actual substrate for the ammonium oxidizers, the Monod curves of the three experiments aree expressed in terms of NH_3 concentration in Fig. 5. From

$$TAN = NH_3 + NH_4^+ \tag{9}$$

and

$$K_{e,NH_4^+} = \frac{NH_3 \cdot H^+}{NH_4^+} = 1.13\ 10^{-9} \text{ at } 35\,^\circ\text{C} \tag{10}$$

the fraction of total ammonium present in the form of uncharged ammonia (NH_3) is given by

$$S_{NH_3} = \frac{S_{TAN}}{1 + \dfrac{10^{-pH}}{K_{e,NH_4^+}}} \tag{11}$$

Figure 5 shows that the Monod curves now coincide: the ammonia affinity constant, reflecting the concentration of uncharged ammonia at which the OUR reaches half of its maximum value, remains almost constant for varying pH.

The same experiment was conducted at 25 °C. In order to compare experimental results at the two temperatures (25 °C and 35 °C) two temperature (T in K) dependencies for the equilibrium constant (Eqn (12)[6] and Eqn (13)[18]) were used. Both dependencies yield the same result.

$$K_{e,NH_4^+} = e^{\frac{-6344}{T\ +273}} \tag{12}$$

$$K_{e,NH_4^+} = 10^{-\left(\frac{2835.8}{T+273} - 0.6322 + 0.00123(T\ +273)\right)} \tag{13}$$

In Fig. 6 all collected experimental data (at two temperatures) are given as a function of the NH_3 concentration. This NH_3 concentration was calculated with the temperature dependent equilibrium constant and the measured TAN concentration using Eqn (10).

All data clearly overlap, indicating that NH_3 rather than NH_4^+ is the actual substrate. The affinity constant for ammonia can be considered to be independent of pH and temperature and was determined to be $0.75 \pm 0.052\,mgNH_3\text{-}N\,L^{-1}$. All experimental data except the experiment at 25 °C and pH 6.5 (because of experimental problems) were used for this parameter estimation.

Table 2. Resulting TAN affinity constants expressed in mgTAN-N L^{-1}, indicating the wide range of values obtained at different pH and temperatures

	pH		
	6.5	7	7.5
T[°C]			
25	420.1	133.4	42.7
35	210.8	67.2	21.7

In Table 2 the resulting affinity constants for the different experiments ($T = 25$ and 35 °C, pH = 6.5, 7, 7.5) expressed in mgTAN-N L^{-1} are given. Large differences exist between the affinity constants. Independent of the experiment it can be seen that the ammonium affinity constant is high compared to values found in the literature for normal nitrifying sludge (0.06–27.5 mgTAN L^{-1};[19] $0.034\,mgNH_3\text{–}N\,L^{-1}$ at 20 °C[20]), although Suzuki *et al.*[21] found a fairly high affinity constant of $0.32\,mgNH_3\text{–}N\,L^{-1}$ between 6.5 and 8.5 in cell-free extracts of *Nitrosomonas europaea*. A possible explanation is that the SHARON organisms are exposed to high ammonia concentrations and, as such, are not selected for their substrate affinity.

A similar high affinity constant was found by Hellinga *et al.*[22] for their SHARON reactor ($K_{NH_3}^{NH} = 0.47\,mgNH_3\text{–}N\,L^{-1}$ at 35 °C and pH 7) and Hunik *et al.*[23] for a pure culture of *Nitrosomonas europea* ($K_{NH_3}^{NH} = 0.3\,mgNH_3\text{–}N\,L^{-1}$ at 35 °C and pH 7). Wyffels *et al.*[24] used an ammonia affinity constant of $0.85\,mgNH_3\text{–}N\,L^{-1}$ to simulate a partial nitritation OLAND reactor. The value for this ammonia affinity constant was calculated based on model-based evaluation of reactor performance.

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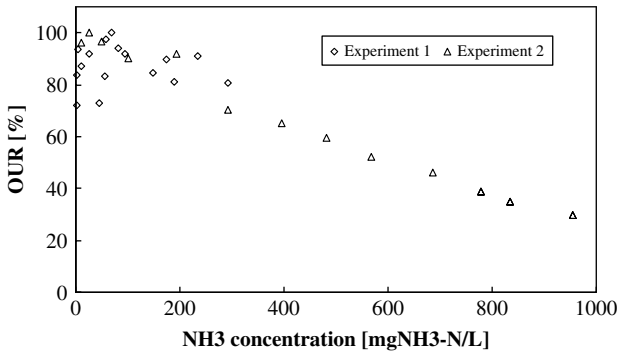

Figure 7. Process kinetics expressed as a percentage of OUR_{max} and NH_3 concentration (35 °C and pH = 8) showing inhibition of the nitritation kinetics at high NH_3 concentrations.

Ammonia inhibition was detected only in an experiment at pH 8 at concentrations above 300 $mgNH_3\text{-}N\,L^{-1}$ as can be seen from Fig. 7. The high ammonia concentration present in the lab-scale SHARON reactor from which the sludge was sampled might explain this absence of inhibition. The SHARON organisms are adapted to high ammonia concentrations.

This is in contrast with the findings of Groeneweg *et al.*[25] who observed TAN inhibition at pH 8 at concentrations above 100 $mgTAN\text{-}N\,L^{-1}$ or 10 $mgNH_3\text{-}N\,L^{-1}$.

Hellinga *et al.*[21] performed a similar experiment at 40 °C and pH 7 and found no inhibition until concentrations of 6000 $mgNH_4\text{-}N\,L^{-1}$ or 93 $mgNH_3\text{-}N\,L^{-1}$. The decrease of OUR in this experiment can probably be attributed to salinity effects.[26,27]

Inhibition of ammonia was therefore not considered further in this study aiming at optimizing the SHARON reactor for the treatment of, for example, digester effluent, which has a typical influent TAN concentration of 1 $gTAN\text{-}N\,L^{-1}$. The Monod term dealing with ammonia inhibition was therefore omitted from the kinetic expression.

Nitrous acid inhibition constant

The inhibition by TNO_2 at two different pH-values and two different temperatures is given in Fig. 8. The curves are again expressed in TNO_2 concentration and percentage relative to the highest OUR at the given temperature and pH. Clearly, the TNO_2 inhibition coefficient is different for the different cases but the temperature dependency is not significant.

Results for the six different experiments are again summarized in one figure (Fig. 9) by expressing the Monod curves in terms of HNO_2, using

$$S_{HNO_2} = \frac{S_{TNO_2}}{1 + \dfrac{K_{e,HNO_2}}{10^{-pH}}} \tag{14}$$

where K_{e,HNO_2} is the acidity constant of the nitrite/nitrous acid equilibrium ($HNO_2 \leftrightarrow NO_2^- + H^+$). For this equilibrium constant a temperature (T in K) dependency was used as given by[5]

$$K_{e,HNO_2} = e^{\dfrac{-2300}{T + 273}} \tag{15}$$

From Fig. 9 it is clear that HNO_2 is the real inhibitor since all curves now coincide, although less pronounced than for the affinity constant. This HNO_2 inhibition was not found by Anthonisen *et al.*[6]

Note that the inhibition curve is only determined up to 60% inhibition. This is because the experiments were stopped at 2000 $mgTNO_2\text{-}N\,L^{-1}$, which is in practice the upper level for TNO_2 concentrations in a SHARON reactor treating digester effluent. Also,

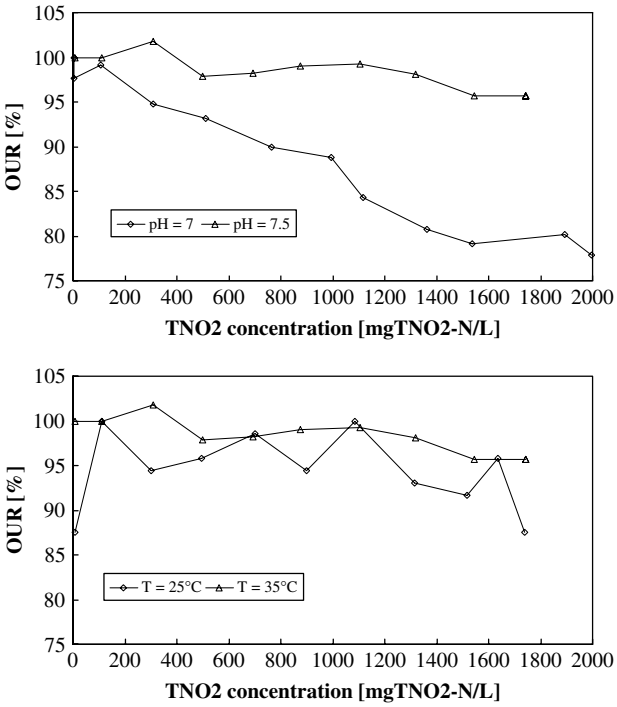

Figure 8. Top: Process kinetics expressed as a percentage of OUR_{max} and TNO_2 concentration obtained at 35 °C. Bottom: Process kinetics expressed as a percentage of OUR_{max} and TNO_2 concentration obtained at pH 7.5. These curves show that for each experiment a different inhibition constant would be obtained if expressed in terms of the TNO_2 concentration.

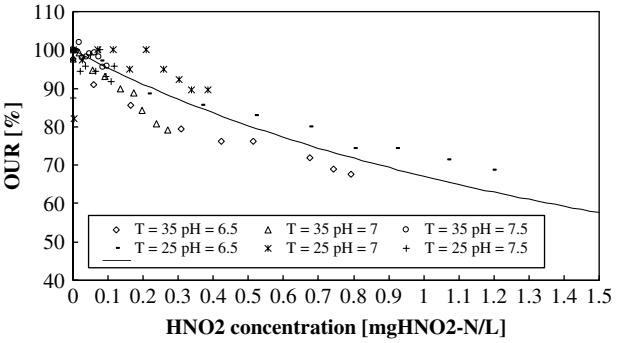

Figure 9. Process kinetics expressed in percent and HNO_2 concentration, showing the coincidence of different experimental results if expressed in terms of HNO_2 concentration.

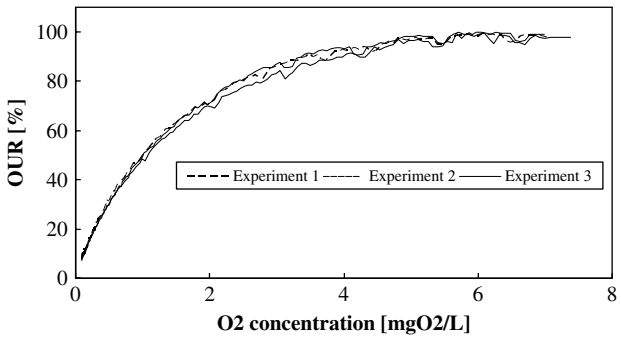

Figure 10. Process kinetics expressed in percent and O_2 concentration for $K_{O_2,NH}$ determination.

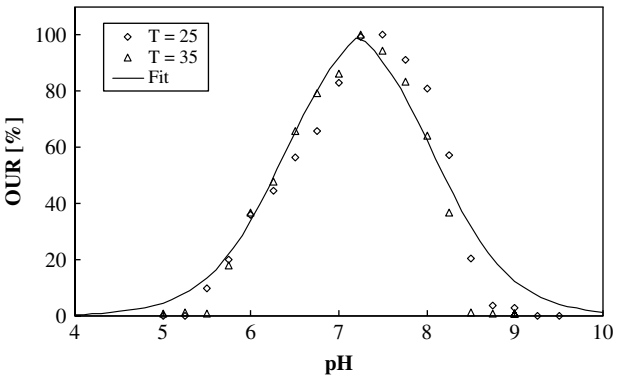

Figure 11. Influence of pH at 25 °C and 35 °C on the OUR.

from Fig. 9 K_{I,HNO_2} could be determined to be $2.04 \pm 0.017\,\text{mgHNO}_2\text{-N}\,\text{L}^{-1}$. This time all six experiments could be included for parameter estimation. The value is tenfold higher than the one determined by Hellinga *et al.*[22] ($0.203\,\text{mgHNO}_2\text{-N}\,\text{L}^{-1}$ at pH 7 and T = 35 °C), indicating high nitrous acid resistance, possibly because the system has run at higher concentrations resulting in adaptation of the biomass.

Oxygen affinity constant

No real influence of pH and/or temperature on $K_{O_2}^{NH}$ was noticed in the different experiments in which the OUR evolution as a function of a lowering oxygen concentration was observed. The average $K_{O_2}^{NH}$ was determined to be $0.94 \pm 0.091\,\text{mg}_{O_2}\,\text{L}^{-1}$. This value is well within the range of values found in the literature for activated sludge nitrifiers in general and ammonium oxidizers in particular ($0.16-2\,\text{mg}_{O_2}\,\text{L}^{-1}$, as recently summarized by Guisasola *et al.*[28]). As an example, the results from three experiments at pH 7 and 25 °C are depicted in Fig. 10.

Maximum specific growth rate

The maximum specific growth rate ($\mu_{\max}^{NH}$) was determined from the parameters estimated above in dedicated batch experiments, as well as from steady state data from the continuous SHARON reactor over a 24 day period.[15] Inserting the 24 daily measurements one by one in the following well-known chemostat equation

$$D = \frac{1}{HRT} = \mu^{NH} = \mu_{\max}^{NH} \frac{C_{NH_3}}{C_{NH_3} + K_{NH_3}^{NH}} \frac{K_{I,HNO_2}^{NH}}{C_{HNO_2} + K_{I,HNO_2}^{NH}} \times \frac{C_{O_2}}{C_{O_2} + K_{O_2}^{NH}} \quad (16)$$

gives $\mu_{\max}^{NH} = 1.0 \pm 0.2\,\text{d}^{-1}$.

This value is lower than normally found in the literature (e.g. $1.5\,\text{d}^{-1}$ at 35 °C and pH 7[21]), possibly because pH has a direct effect on the specific growth rate, which is not taken into account in the above equation.

In order to investigate the direct influence of pH at two different temperatures (25 °C and 35 °C) the OUR of the SHARON organisms at varying pH was measured (Fig. 11). The pH experiment was designed in such a way that within the normal pH operational conditions of the SHARON reactor (pH between 6 and 8) the measured pH effect was intrinsic and not influenced by nitrous acid inhibition. At the time of the experiment the influent TAN concentration was $1000\,\text{mgTAN-N}\,\text{L}^{-1}$ and the HRT was 1.54 d, while the average TAN removal was 50%. This means that about $325\,\text{mgTAN-N}\,\text{L}^{-1}$ d is converted into TNO_2. The experiment lasted 3 h, which means that at these conditions a maximum of $40\,\text{mgTNO}_2\text{-N}\,\text{L}^{-1}$ will be formed. A concentration of $40\,\text{mgTNO}_2\text{-N}\,\text{L}^{-1}$ corresponds with $0.089\,\text{mgHNO}_2\text{-N}\,\text{L}^{-1}$ at pH 6 and $0.87\,\text{mgHNO}_2\text{-N}\,\text{L}^{-1}$ at pH 5. This means that for the typical operation range of the SHARON reactor (pH 6–8), the nitrous acid inhibition during the pH influence experiments was limited to a maximum 5–10%, as can be concluded from Fig. 9. Again curves are expressed in percent relative to the highest OUR for the given temperature. This OUR was fitted to

$$OUR\,[\%] = 100 \frac{K_{pH}}{K_{pH} - 1 + 10^{|pH_{opt} - pH|}} \quad (17)$$

with K_{pH} and pH_{opt} estimated to be 8.21 ± 0.87 and 7.23 ± 0.027 respectively. According to this equation a pH of 6.8 would lead to approximately 20% reduction in OUR. So, at an optimal pH of 7.23 the $\mu^{\max}$ would be $1.25\,\text{d}^{-1}$ compared to $1.0\,\text{d}^{-1}$ at pH 6.8. Figure 11 has an important engineering conclusion, i.e. there exists a narrow pH interval between 6.5 and 8 where the growth rate is optimal.

In Fig. 12 the influence of temperature on the OUR of the SHARON organisms determined in two independent batch tests at pH 7 is depicted. This OUR was fitted to the modified Ratkowsky model[12] (T in °C), given by

$$OUR = 100[b(T - T_{\min})]^2 \left\{1 - e^{c(T - T_{\max})}\right\} \quad (18)$$

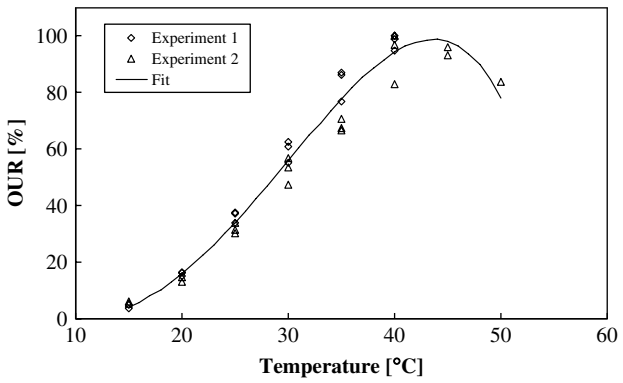

Figure 12. Influence of temperature at pH 7 on the OUR.

with $b = 0.045 \pm 0.002$, $c = 0.0459 \pm 0.0068$, $T_{min} = 10.12 \pm 0.76$ and $T_{max} = 56.06 \pm 1.00$. It is clear that temperatures between 35 °C and 45 °C are optimal for the SHARON process. However, only short-term temperature effects were investigated here. Long-term exposure to a different temperature would lead to adaptation and temperatures above 40 °C would to lead to deactivation.

CONCLUSIONS

In this study the kinetics of the SHARON nitritation process were assessed. Batch experiments at two different temperatures (25 and 35 °C) and three different pH values (6.5, 7 and 7.5) with SHARON sludge clearly confirmed that ammonia rather than ammonium is the actual substrate for ammonium oxidizers. From these experiments the ammonia affinity constant was determined to be $0.75\,mgNH_3\text{-}N\,L^{-1}$, and was found not to depend on pH or temperature. In contrast with the findings of Anthonisen *et al.*,[6] ammonia inhibition of ammonium oxidizers was only detected at high concentrations. This can be attributed to adaptation of the SHARON process to high ammonia concentrations.

Further, similar inhibition-focused experiments have shown that nitrous acid rather than nitrite is the actual inhibitor of the SHARON organisms. For the nitrous acid inhibition coefficient, a value of $2.04\,mgHNO_2\text{-}N\,L^{-1}$ was found, also independent of pH and temperature.

The oxygen affinity constant was determined to be $0.94\,mgO_2\,L^{-1}$, independent of pH and temperature.

The direct influence of pH and temperature on the maximum OUR of SHARON biomass was determined, indicating the existence of a narrow pH and temperature interval between 6.5 and 8 and 35 and 45 °C, respectively, where the OUR is optimal.

The parameter values determined in this study will now be implemented in a simulation model for further optimization of the SHARON process.

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