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

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Abstract
The SHARON process is an innovative nitrogen removal process that improves the sustainability of waste water treatment, especially when combined with an Anammox process. In order to further 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, 2.04 mgHNO_2-N/l and 0.94 mgO_2/l. 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 oxygen uptake rate is optimal.

Keywords
SHARON, partial nitritation, ammonia, affinity constant, nitrous acid, inhibition

INTRODUCTION

Biological nitrogen removal from wastewater with high nitrogen contents can become a major cost factor, in particular when the wastewater contains only small amounts of biologically degradable carbon compounds (Seyfried *et al.*, 2001). Conventionally nitrogen removal from wastewater is achieved using nitrification/denitrification. In such systems, nitrifying bacteria oxidize ammonium to nitrate under oxic conditions, and nitrate is subsequently or simultaneously reduced to dinitrogen gas, under anoxic conditions. Recently however, innovative processes for nitrogen removal have been developed, for example the combined SHARON-Anammox process (van Dongen *et al.*, 2001). These processes significantly improve nitrogen removal and are based on the combination of partial nitritation, where ammonium is partially oxidized to only nitrite, and anaerobic ammonium oxidation (Anammox), a process in which ammonium and nitrite are combined to form nitrogen gas. This combined completely autotrophic process has great potential since there is no longer need for external carbon addition, sludge production is very low, and oxygen input and aeration energy requirements are largely reduced (Jetten *et al.*, 1997). In the SHARON (Single reactor High activity Ammonia Removal Over Nitrite) process, nitritation of ammonium to nitrite is established by working at high temperature (above

25°C) and maintaining an appropriate sludge retention time (SRT) of 1 to 1.5 days, so that ammonium oxidizers are maintained in the reactor, while nitrite oxidizers are washed out and further nitrification of nitrite to nitrate is prevented. In order to produce an Anammox suitable influent, with a 1:1 nitrite:ammonium ratio approximately, only half of the ammonium should be oxidized. In case the SHARON influent contains ammonium and bicarbonate on an equimolar basis, the protons produced during conversion of half of the ammonium are balanced 'exactly' via carbon dioxide stripping. For the high-concentrated streams to which the SHARON process is typically applied, the protons produced during ammonium conversion over 50% would cause a significant pH drop, preventing further nitrification (van Dongen *et al.*, 2001).

Very interesting and useful tools to further optimize the SHARON process are modelling and simulation environments such as WEST® (Vanhooren *et al*., 2003). 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. However, in order to have a correct representation of reality by these simulations, parameters of the biological processes have to be assessed.

Anthonisen *et al.* (1976) formulated the hypothesis that ammonia rather than ammonium is the actual substrate and that at higher concentrations ammonia becomes inhibiting. Nitrous acid inhibition, not discussed by Anthonisen *et al.* (1976), was also investigated in this study. Hence, the following expression for the growth rate of ammonium oxidizers is proposed:

$$\mu = \mu^{\max} \frac{C_{NH_3}}{C_{NH_3} + K_{NH_3}} \frac{K_{I,NH_3}}{C_{NH_3} + K_{NH_3}} \frac{K_{I,HNO_2}}{C_{HNO_2} + K_{I,HNO_2}} \frac{C_{O_2}}{C_{O_2} + K_{O_2}}$$

In this contribution the hypothesis of Anthonisen *et al.* (1976) was tested in separate batch experiments with SHARON sludge at two different temperatures (25 and 35°C) and three different pH's (6.5, 7 and 7.5). These batch experiments also allowed the determination of the ammonium affinity constant (K_{NH3}) and the nitrous acid inhibition constant ($K_{I,HNO2.}$). The oxygen affinity constant (K_{O2}) was determined with a similar experiment. Further the maximum growth rate μ^{max} and the influence of temperature and pH on the maximum oxygen uptake rate were determined.

MATERIALS AND METHODS

SHARON reactor

A lab-scale SHARON reactor was constructed in the BIOMATH lab. The reactor is a 2 litre continuously stirred tank reactor (CSTR) without biomass retention. The synthetic influent is pumped with a peristaltic pump from the 5 litre 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 to be 35°C. In the reactor the dissolved oxygen (DO) and pH are measured. The pH is controlled through the Labview® software (National Instruments, www.ni.com) by means of acid (HCl) or base (NaOH) addition. Data logging is also performed with the Labview® software. Further details concerning start-up and operation of the SHARON reactor are described by Van Hulle *et al.* (2003).

Batch experiments

Respirometric batch experiments (Spanjers *et al.*, 1996) with the SHARON sludge were performed to asses the ammonia affinity constant, the nitrous acid inhibition constant, the oxygen affinity constant and the influence of pH and temperature on the maximum oxygen uptake rate. 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 oxygen uptake rate (OUR = dDO/dt). Aeration through the headspace was always smaller than 5% of the OUR.
Before each experiment the sludge was washed to ensure that no nitrite or ammonium was present at the beginning of the experiment. Also it was verified after each experiment that no nitrate was formed, in order to link the oxygen uptake rate to ammonium oxidizer activity only.

Ammonia affinity and inhibition constant. Batch tests at two different temperatures (25 and 35°C) and three different pH's (6.5, 7 and 7.5) were performed for the determination of K_{NH3}. In every batch test sequential additions of $(NH_4)_2SO_4$ were carried out and after each addition the OUR was determined. Before and after every oxygen drop a sample was taken for TAN (ammonium + ammonia) analysis using spectrophotometric methods (Dr Lange GmbH, Germany). This way the OUR can be linked to the 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.

Nitrous acid inhibition constant. Similar batch tests as for the determination of K_{HNO2} were conducted to determine the nitrous acid inhibition constant. Before the experiment, an excess of 1000 mg NH_4^+-N/l was added to the reactor to exclude substrate limitation. In every batch test sequential additions of KNO_2 were carried out and after each addition the OUR was determined. Before and after every oxygen drop a sample was taken for later TNO2 (nitrous acid + nitrite) analysis, also using spectrophotometric methods. This way the OUR can be linked to the TNO2 concentration.

Oxygen affinity constant. Again batch tests at two different temperatures (25 and 35°C) and three different pH's (6.5, 7 and 7.5) were performed for the determination of K_{O2}. In every batch test an excess of 1000 mg NH_4^+-N/l was added. Aeration was turned off. The drop in DO concentration was recorded until the concentration reached 0.1 mgO_2/l. Plotting the time derivative of the DO concentration versus the concentration itself yields a Monod curve expressing oxygen limitation.

Maximum oxygen uptake rate (OUR). An excess of substrate to exclude substrate limitation was first added to the reactor. Starting from pH 7 the pH was varied between 5 and 9.5 in steps of 0.25 at a temperature 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 6 different temperature setpoints (15, 20, 25, 30, 35 and 40°C) were applied, while keeping pH constant at 7.

Parameter estimation

Parameter estimation was performed with the WEST® modelling and simulation software (Vanhooren *et al*., 2003).

RESULTS AND DISCUSSION

Ammonia affinity constant.

Figure 1 summarizes the OUR values measured at different TAN concentration, for different pH values at 35°C. These Monod curves are expressed in %, with the highest OUR value in each experiment as reference, and TAN concentration. The Monod curves are expressed in % to enable comparison between the different experiments. It can be seen from Figure 1 that for each experiment a different affinity constant would be obtained if the constant would be expressed in terms of the TAN concentration. A higher pH results in a lower affinity constant for total ammonium: the total ammonium concentration at which the OUR reaches half of its maximum value is then lower.

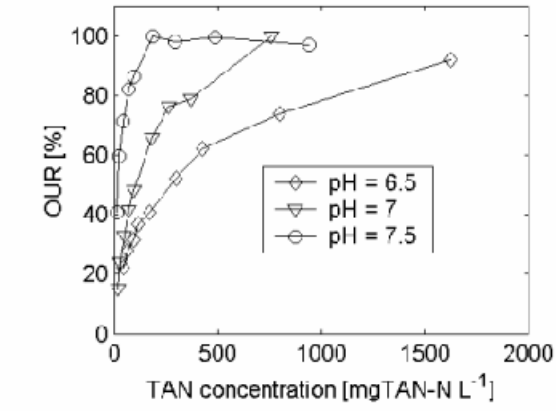

Figure 1 Monod curves expressed in % and TAN concentration obtained at 35°C

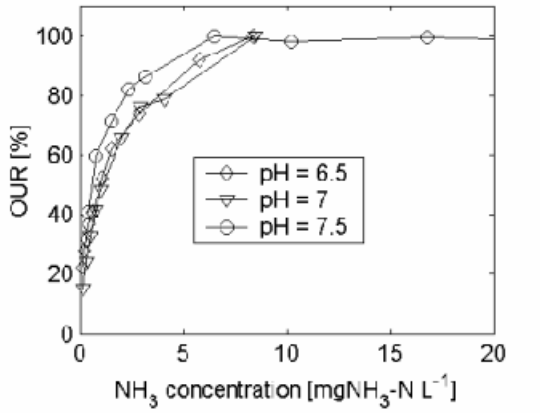

Figure 2 Monod curves expressed in % and NH_3 concentration obtained at 35°C

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 were expressed in terms of NH_3 concentration in Figure 2. From TAN = NH_3 + NH_4^+ and $K_e^{NH} = \frac{NH_3 \cdot H^+}{NH_4^+} = 1.13\,10^{-9}$ at 35°C the fraction of total ammonium present in the form of uncharged ammonia (NH_3) is calculated as $C_{NH_3} = C_{TAN} \Big/ 1 + \frac{10^{-pH}}{K_e^{NH}}$.

Figure 2 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.

For the equilibrium constant two temperature (T in K) dependencies proposed by Anthonisen *et al.* (1976): $K_e^{NH} = e^{\frac{-6344}{T}}$ and Helgeson (1967): $K_e^{NH} = 10^{-\left(\frac{2835.8}{T} - 0.6322 + 0.00123T\right)}$ were used to compare experimental results at two temperatures. Both dependencies yielded the same result. In Figure 3 all collected experimental data (of 2 temperatures) are given as function of the NH_3 concentration. All data clearly overlap, indicating that NH_3 rather than NH_4^+ is the actual substrate. The affinity constant for ammonia can be considered as independent of pH and temperature and was determined to be 0.75 ± 0.052 mgNH_3-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. A similar high affinity constant (K_{NH3} = 0.47 mg NH_3–N L^{-1} at 35°C and pH 7) was found by Hellinga *et al*. (1999) for their SHARON reactor. This affinity constant for ammonia substrate is very high compared to most literature values (e.g. K_{NH3} = 0.034 mg NH_3–N L^{-1} at 20°C, Wiesmann, 1994), most likely due to the fact that the organisms were exposed to high ammonium concentrations and as such were not selected for their substrate affinity. This high ammonium concentration exposure might also explain why ammonium inhibition was only detected in an experiment at pH 8 at concentrations above 300 mg NH_3 –N L^{-1} as can be seen from Figure 4. Inhibition of ammonia was therefore not considered further in this study that deals with treating digester effluent. The Monod term dealing with ammonia inhibition was therefore omitted from the kinetic expression. Hellinga *et al*. (1999) performed a similar experiment at 40°C and pH 7 and found no inhibition until concentrations of 6000 mg NH_4–N L^{-1} or 93 mg NH_3–N L^{-1}. Note that the decrease of OUR in this experiment may also be attributed to salinity effects.

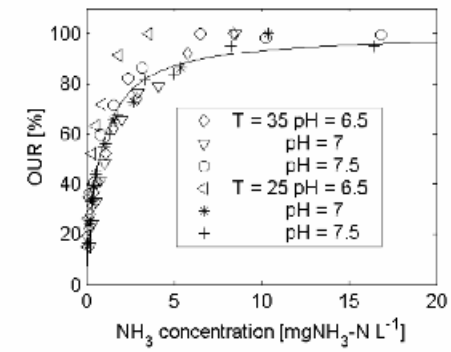

Figure 3 Monod curves expressed in % and NH_3 concentration

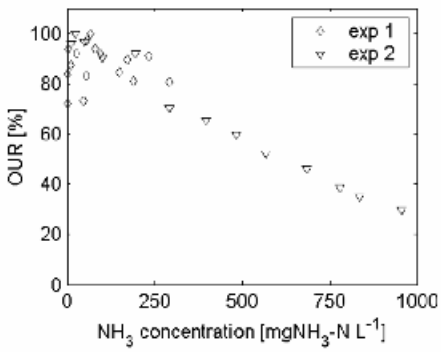

Figure 4 Monod curves expressed in % and NH_3 concentration (35°C and pH=8)

Nitrous acid inhibition constant

The inhibition by nitrite at two different pH and two different temperatures is given in Figures 5a and b. The curves are again expressed in TNO_2 concentration and % relative to the highest OUR at the given temperature and pH. Clearly, the nitrite inhibition coefficient is different for the different cases but the temperature dependency is not significant.

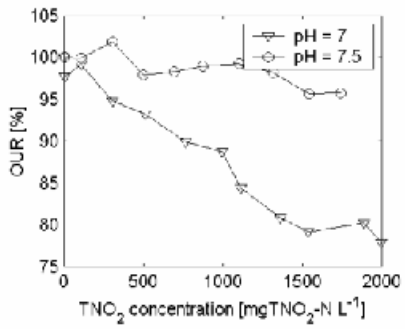

Figure 5a Curves expressed in % and TNO_2 concentration obtained at 35°C

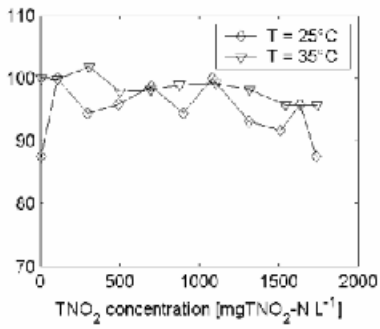

Figure 5b Curves expressed in % and TNO_2 concentration obtained at pH 7.5

Results for the six different experiments are again summarized in one Figure (Figure 6) by expressing the Monod curves in terms of HNO_2: $C_{HNO_2} = C_{TNO_2} \Big/ 1 + \frac{K_e^{NO}}{10^{-pH}}$,

where K_e^{NO} 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 proposed by Anthonisen *et al.* (1976), $K_e^{NO} = e^{\frac{-2300}{T}}$. From Figure 5 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.* (1976). Note that the inhibition curve is only determined until 60 % inhibition. This is because the experiments were stopped at 2000 mgTNO_2-N/l, which is in practice the upper level for nitrite concentrations in a SHARON reactor treating digester effluent. Also from Figure 5 $K_{I,HNO2}$ could be determined to be 2.04 ± 0.017 mgHNO_2-N L^{-1}. This time all 6 experiments were included for parameter estimation. The value is tenfold higher than the one determined by Hellinga *et al.* (1999) (0.203 mgHNO_2-N L^{-1} at pH 7 and T=35°C), indicating high nitrous acid resistance, possibly because our system has run at higher concentrations resulting in adaptation of the biomass.

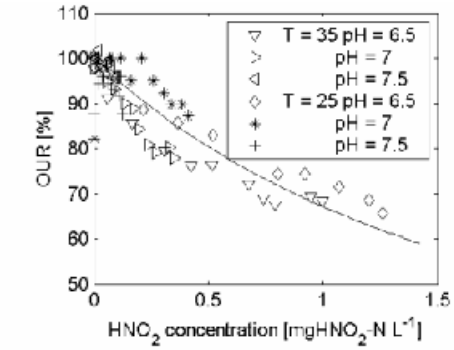

Figure 6 Monod curves expressed in % and HNO_2 concentration

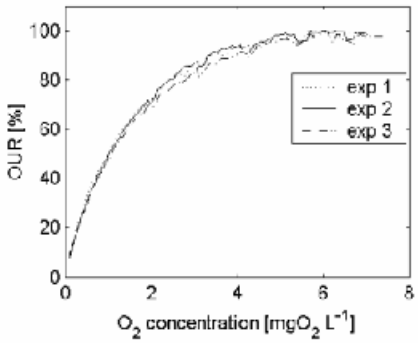

Figure 7 Monod curves expressed in % and O_2 concentration for K_{O2} determination

Oxygen affinity constant.

No real influence of pH and/or temperature on K_{O2} was noticed in the different experiments. The average K_{O2} was determined to be 0.94 ± 0.091 mgO_2 L^{-1}. This value is well in the range of values found in literature for activated sludge nitrifiers. As an example, the results from three experiments at pH 7 and 25°C are depicted in Figure 7.

Maximum oxygen uptake rate-μ^{max}

The maximum specific growth rate (μ^{max}) was determined from the parameters estimated above, as well as steady state data of the continuous SHARON reactor over a 24 days period where the hydraulic retention time (HRT) was 1.6 days, the influent concentration was 2000 mgNH_4^+-N L^{-1} and the influent bicarbonate:ammonium ratio was 1:1 (Van Hulle *et al.*, 2003). The average effluent NH_3, HNO_2 and DO concentrations in this period were 10.24 mgNH_3-N L^{-1}, 0.37 mgHNO_2-N L^{-1} and 6.07 mgO_2/L respectively. The average pH was 6.8. Inserting the 24 daily measurements one by one in the well known chemostat

equation: $D = \frac{1}{HRT} = \mu = \mu^{\max} \frac{C_{NH_3}}{C_{NH_3} + K_{NH_3}} \frac{K_{HNO_2}}{C_{HNO_2} + K_{I,HNO_2}} \frac{C_{O_2}}{C_{O_2} + K_{O_2}}$ allowed the determination of a μ^{max} of 1.0 ± 0.2 d^{-1}. This is a value quite lower than normally found in literature (e.g. 1.5 d^{-1} at 35°C and pH 7, Hellinga *et al.*, 1999), possibly because pH has a direct effect on the specific growth rate, which is not taken into account in the above mentioned equations.
In order to investigate the influence of pH at two different temperatures (25°C and 35°C) the oxygen uptake rate of the SHARON organisms at varying pH was measured (Figure 8a). Again curves are expressed in % relative to the highest OUR for the given temperature. This OUR was fitted to the following equation: $OUR[\%] = 100 \frac{K_{pH}}{K_{pH} - 1 + 10^{|pH_{opt} - pH|}}$ (Henze *et al.*, 1995), 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 oxygen uptake rate. So, at an optimal pH of 7.23 the μ^{max} would be 1.25 d^{-1} compared to 1.0 d^{-1} at pH 6.8. Figure 8a 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 Figure 8b the influence of temperature on the oxygen uptake rate of the SHARON organisms determined in two independent batch tests at pH 7 is depicted. This OUR was fitted to the modified Ratkowsky model (Zwietering *et al.*, 1991) (T in °C): $OUR = 100\left[b\left(T - T_{\min}\right)\right]^2 \left\{1 - e^{c\left(T - T_{\max}\right)}\right\}$, with b = 0.045 ± 0.002, c = 0.0459 ± 0.0068, T_{min} = 10.12 ± 0.76 and T_{max} = 56.06 ± 1.0. It is clear that temperatures between 35°C to 45°C are beneficial for the SHARON process. However, only short-term temperature effects were investigated here. Long term exposure to temperatures above 40 °C is expected to lead to deactivation.

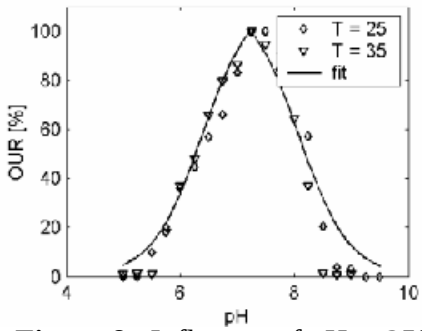

Figure 8a Influence of pH at 25°C and 35°C on the oxygen uptake rate

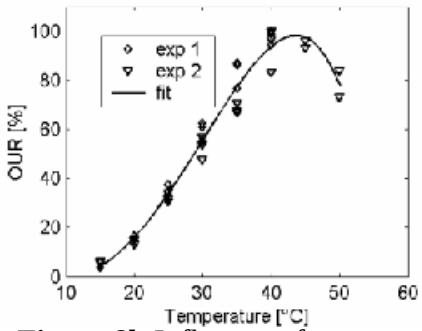

Figure 8b Influence of temperature at pH 7 on the oxygen uptake rate

CONCLUSIONS

Batch experiments at two different temperatures (25 and 35°C) and three different pH's (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 could be determined to be 0.75 $mgNH_3$-N L^{-1}, and was found not to depend on pH nor temperature. In contrast with the findings of Anthonisen *et al.* (1976), only ammonia inhibition of ammonium oxidisers at high concentrations was detected. This can be attributed to adaptation of the SHARON process to high ammonia concentrations.

Further, similar 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-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}.
The influence of pH and temperature on the maximum oxygen uptake rate 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 were the oxygen uptake rate is optimal.
The parameter values determined in this study will be implemented in a simulation model for further optimization of the SHARON process.

Acknowledgements

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