EVALUATION OF THE FACTORS INFLUENCING THE SPECIFIC ANAMMOX ACTIVITY (SAA) USING SURFACE MODELLING

A. Malovanyy**, E. Plaza* and J. Trela*

* Department of Land and Water Resource Engineering, Royal Institute of Technology, SE-100 44 Stockholm, Sweden (E-mail: elap@kth.se; trela@kth.se)
** Department of Ecology and Environment Protection, Lviv National Polytechnic University, 79013 Bandery 12, Lviv, Ukraine (E-mail: malovanyy@gmail.com)

Abstract The study on relationship between performance of the Anammox process and total nitrogen, temperature and the free ammonia (FA)/free nitrous acid (FNA) ratio was made. During the study the method of determination of the specific Anammox activity (SAA) was used with measurements of the nitrogen gas pressure, as well as the computer program MODDE v.7.0. The program investigated cumulative effects of the factors. The results have proven that activity of Anammox bacteria increases with the increase of temperature within the range of 15 to 37.5 °C. The increase of activity for temperatures of 22.5-30 °C was also observed with growth of total nitrogen (TN) up to 500 mg/L. Decrease of the ratio FA/FNA from 30 to 0.3 led to an exponential growth of SAA. The systems with TN of 700 mg/L and FA/FNA as low as 0.003 showed decrease of SAA. Regarding a cumulative effect, it was found that temperatures had the influence on the TN values that can be tolerated and that SAA grows faster with the temperature increase when the concentration of TN is higher in the system.

Key words: Activity; Anammox; Inhibition; Nitrogen; Response surface modelling

INTRODUCTION

The novel biological process of anaerobic ammonium oxidation (Anammox), studied nowadays by many researchers (Mulder et al., 1995; Strous et al., 1999; Hao et al., 2002; Fux et al., 2004; Güven et al., 2004; Gut, 2006; Dapena-Mora et al., 2007; Jung et al., 2007; Dosta et al., 2008; Fernández et al., 2008; Isaka et al., 2008) gave possibility to develop systems of efficient fully autotrophic removal of ammonium from highly concentrated wastewater streams.

The first step of ammonium removal using this new technology is a transformation of nearly half of ammonium into nitrite using well-known nitritation process:

$$NH_3 + 1.5O_2 \xrightarrow{Nitrosomonas} NO_2^- + H_2O + H^+ \qquad (1)$$

The second step is the Anammox process, where the rest of ammonia is oxidized into nitrogen gas using nitrite, produced in the first step, as an electron acceptor:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad (2)$$

These two steps can be run sequentially (two-step deammonification process) or simultaneously (one-step deammonification). In case of a one-step process dissolved oxygen (DO) in the reactor is kept on proper levels to ensure satisfactory conditions for running the both processes. A nitritation process needs high oxygen values, while the Anammox reaction takes place in an oxygen free environment. In the biofilm systems the nitritation process occurs in the outer layer of biofilm and the Anammox process in the inner layer of biofilm.

The process of fully autotrophic removal of ammonium has been studied in pilot installations (Gut, 2006; Fernández et al., 2008) and applied in industrial ones (Läckeby Water Group, 2007; van der Star 2007).

Identification of the parameters that have influence on performance of the industrial process is an important task that allows operating it at minimal cost and maximum efficiency.

Generally, all the factors that influence performance of the Anammox process as well as any other biological processes can be divided into three groups:

- Inhibition factors
- Substrate availability
- Temperature

The main parameters having inhibitory effects on the Anammox activity are DO and organic compounds of low molecular size (Güven et al., 2004; Isaka et al., 2008). Also, nitrate, sulfide, phosphate and acetate were identified as inhibitors of the Anammox process both in lower or higher concentrations (Strous et al., 1999; Dapena-Mora et al., 2007). Mechanical stress was also discovered as an inhibitory factor (Arrojo et al., 2006).

Ammonium and nitrite are the main substrates for Anammox bacteria, so increasing availability of this substrate should increase bacterial activity because of increased diffusion both into biofilm and through a bacteria cell membrane. At the same time higher concentrations of ammonium and nitrite were identified as inhibitors of the Anammox bacteria activity (Strous et al., 1999; Dapena-Mora et al., 2007). However the two studies identified different ammonium and nitrite concentrations that inhibited activity of Anammox bacteria. Studies on SAA made by Dapena-Mora et al. (2007) showed a 50% decrease in activity when concentrations of ammonium and nitrite nitrogen rose up to 770 and 350 mg/L respectively. On the other hand, Strous et al. (1999) observed no inhibition by ammonium at concentrations up to 980 mg NH_4-N/L and a total loss of activity at a nitrite concentration of 98 mg NO_2-N/L. Fux et al (2004) also reported serious inhibition of Anammox activity when the concentration of nitrite stayed at 30-50 mg NO_2-N/L during six days.

As well as the rate of chemical reactions, the rate of biological transformation is dependent on temperature. Generally, increase of temperature increases both the rate of chemical and biological processes. The only limitation is that increase of temperature to high levels can be harmful for biological organisms.

The influence of temperature on biological processes as well as on chemical ones can be expressed as activation energy. Hao et al. (2002) used a modified Arrhenius equation (3) to model the performance of the CANON process with temperature, as one of the variables:

$$SAA_T = SAA_{293} \cdot e^{-\frac{E_{act} \cdot (293 - T)}{R \cdot 293 \cdot T}} \qquad (3)$$

Where SAA_T – specific Anammox activity at the temperature T;

SAA_{293} – specific Anammox activity at the temperature 293 K;

E_{act} – activation energy of Anammox reaction, J;

R – ideal gas constant, equals 8.32 J/(mol K).

Here, it is worth mentioning that specific Anammox activity (SAA) is defined as transformation of nitrogen from different forms into nitrogen gas, during 1 day on the biofilm area of 1 m^2, referring to bacteria attached to carriers or per gram of volatile suspended solids, when granular or activated sludge is used.

Activation energy can be calculated from the equation (3) and was used as an identification of temperature influence on Anammox as well as other biological processes.

The influence of temperature on the rate of the Anammox process was studied earlier by several authors and its increase up to the levels of 40 °C led to increase of Anammox rate in most studies.

The further increase of temperature up to 45 °C had a negative effect on process activity (Dosta et al., 2008). However, Dalsgaard and Thamdrup (2002) and Rysgaard et al. (2004) who worked on establishing a temperature dependence for Anammox bacteria in marine sediments, reported the highest activity at the temperature of 15 °C and loss of activity at the temperatures higher than 30 °C.

Bator (2006) studied the performance of the CANON process as a function of temperature, incoming ammonium and DO with the use of MODDE program, which gave possibility to study cumulative effects of several parameters on response of interest (Anammox activity in this case).

All the researchers who studied ammonium and nitrite inhibition of the Anammox process mentioned concentrations of ammonium and nitrite nitrogen in mg/L or mmol, when referring to inhibition of Anammox activity. However Anthonisen et al. (1976) who studied nitritation and nitratation concluded that unionized free ammonia (FA) and free nitric acid (FNA) rather then total ammonia or nitrite ion inhibit nitrification. The same can be valid for the Anammox process. Also, there were found no studies that would analyzed the activity of anaerobic oxidizing bacteria in a multivariable system that includes a FA/FNA ratio, as one of the parameters.

Based on the above considerations there is a need for more studies on separate and cumulative effects of different parameters on the Anammox process activity and performance. Temperature, total nitrogen (TN) and the FA/FNA ratio were chosen as the three parameters that may have strong influence on the Anammox process.

MATERIALS AND METHODS

Anammox culture

Kaldnes biofilm carriers collected from a one-stage deammonification reactor were used during the experiments. Kaldnes carriers had the diameter of 10 mm and height of 7 mm and were made of polyethylene. A specific area of the carriers was 500 m^2/m^3 , once they have been completely packed.

Determination of SAA using gas pressure measurements

The methodology used for determination of SAA using gas pressure measurements is well known and is adapted from the works of Dapena-Mora (2007). The methodology is based on the fact that with transformation of different nitrogen forms into nitrogen gas the pressure inside a close gas volume should increase proportionally to the amount of nitrogen, converted to nitrogen gas.

The tests were performed in glass bottles of total volume of 38 ml with a gas phase of 13 ml. To keep pH at the same level during the tests, a phosphate buffer (0.14 g/L KH_2PO_4 and 0.75 g/L K_2HPO_4) was added to a substrate. pH of newly prepared buffer was around 7.8 and it was modified to a desired level by addition of small quantities of HCl or NaOH.

In every test 15 Kaldnes carriers with attached biomass were used (the number of carriers was the maximum possible to fit inside the volume of 25 ml). The free volume of the bottles was deaerated using a compressed nitrogen gas line. Required ammonium and nitrite concentrations in the bottles were obtained by addition of concentrated ammonium and nitrite solutions.

The bottles were kept in a thermostatically controlled water bath (model Messgeräte Werk Landa MS/2) and periodically removed from it to measure a pressure increase inside the bottles. The pressure was measured with a Centrepoint Electronics pressure transducer, capable of measuring an overpressure in the range from 0 to 5 psi.

Using the ideal gas law, the pressure measurements were transformed into the nitrogen removal from the liquid by Anammox bacteria and the values that represented stable removal of nitrogen were used to calculate the average nitrogen removal rate with further transformation into SAA.

Table 1. The ranges of TN, FA/FNA and T used in two sets of tests.

	1st set of tests			2nd set of tests		
	TN (mg/L)	FA/FNA	T (°C)	TN (mg/L)	FA/FNA	T (°C)
Min	100	0.3	15	300	0.003	22.5
Middle	300	30	22.5	500	0.3	30
Max	500	3000	30	700	30	37.5

Application of the MODDE program to build a model of dependence of SAA from different parameters

To plan the experiments on evaluation of influence of temperature and substrate availability on SAA, the MODDE ver. 7.0 program, developed by Umetrics AB, was selected. This program gives an opportunity to determine the dependence of several responses of the system on different factors (up to 20) using the minimum number of experiments.

The work can be divided into three parts.

First, the factors together with their ranges that are to be analyzed are submitted to the program. The program suggests the number of tests with different values of factors to be identified.

After running the tests the values of response for all the tests are submitted to the program and the model is fitted to the experimental data. Multiple Linear Regression (MLR) method was used for fitting the model to data in this work.

The third phase is the use of the model. After the model was created program can calculate response for every point within the range of investigation together with the uncertainty limits and build two, three and four dimensional graphs in order to help investigate the influence of factors on the response.

Selection of factors influencing SAA using surface modelling

The ranges of investigation for two tests are specified in Table 1. It was decided to use the values of a FA/FNA parameter in a logarithmic scale in order to have wider range of values for

Table 2. List of parameters of the first set of experiments

Name of experiment	TN (mg/L)	FA/FNA	T (°C)	pH	$C(NH_4\text{-}N)$ (mg/L)	$C(NO_2\text{-}N)$ (mg/L)	SAA ($gN{\cdot}m^{-2}{\cdot}d^{-1}$)
N1	100	0.3	15	6.26	50	50	1.06
N2	100	0.3	30	5.93	50	50	2.74
N3	500	0.3	15	6.26	250	250	1.14
N4	500	0.3	30	5.93	250	250	5.15
N5	100	3000	15	8.27	50	50	0.71
N6	100	3000	30	7.95	50	50	2.57
N7	500	3000	15	8.27	250	250	0.71
N8	500	3000	30	7.95	250	250	3.99
N9	300	30	15	7.26	150	150	0.87
N10	300	30	30	6.93	150	150	3.92
N11	100	30	22.5	7.09	50	50	1.33
N12	500	30	22.5	7.09	250	250	2.28
N13	300	0.3	22.5	6.09	150	150	2.63
N14	300	3000	22.5	8.10	150	150	1.74
N15	300	30	22.5	7.09	150	150	2.05
N16	300	30	22.5	7.09	150	150	2.08

investigation. The first set of tests did not show the maximum value of SAA and it was decided to perform the second set of tests and shift the ranges to increase of SAA.

For the first set of experiments CCF model with two central points was used (16 experiments). Experimental work for the second set consisted of 17 experiments. Fully Factorial model with two central points was used in order to make use of that data of the first set of experiments that lay in the range of the second set. Values for NH_4-N, NO_2-N and pH were chosen so, that TN and a FA/FNA ratio would be the ones needed for experiment. FA and FNA concentrations were calculated using formulas (4) and (5) respectively, which were for the first time obtained and described by Anthonisen et al. (1976).

$$FA = \frac{10^{pH}}{e^{\frac{6344}{T}} + 10^{pH}} \cdot C_{NH_4-N} \tag{4}$$

$$FNA = \frac{1}{e^{\frac{-2300}{T}} \cdot 10^{pH}} \cdot C_{NO_2-N} \tag{5}$$

RESULTS AND DISCUSSION

The list of the experiments of the first and the second sets, together with the values of T, TN, FA/FNA, pH, NH_4-N and NO_2-N that were used in experiments and determined SAA are presented in Table 2 and 3, respectively. The data that was taken from the first set of experiments into the second one is highlighted as grey.

After fitting a model with data from the first set of tests using the MLR method the system identified experiment N2 as an outlier and it was excluded from the analyzed data. The models of

Table 3. List of parameters of the second set of experiments.

Name of experiment	TN (mg/L)	FA/FNA	T (°C)	pH	C(NH_4-N) (mg/L)	C(NO_2-N) (mg/L)	SAA ($gN \cdot m^{-2} \cdot d^{-1}$)
N1	300	0.003	22.5	6.09	150	150	2.66
N3	300	0.003	37.5	5.26	30	270	7.54
N5	500	0.003	30	5.53	30	470	4.48
N7	700	0.003	22.5	5.77	30	670	1.24
N9	700	0.003	37.5	5.46	30	670	7.49
N10	300	30	30	6.93	150	150	2.63
N11	300	0.3	30	6.28	50	250	5.38
N13	500	0.3	22.5	6.39	100	400	1.97
N14	500	0.3	30	5.93	250	250	5.15
N15	500	0.3	37.5	5.97	150	350	7.83
N17	700	0.3	30	6.61	30	670	4.34
N19	300	30	22.5	7.09	150	150	2.06
N20	300	30	30	6.93	150	150	3.92
N21	300	30	37.5	6.79	150	150	5.77
N22	500	30	22.5	7.09	250	250	2.28
N23	500	30	30	6.93	250	250	4.19
N24	500	30	37.5	6.97	150	350	7.15
N25	700	30	22.5	7.77	30	670	1.68
N27	700	30	37.5	7.47	30	670	4.94
N28	500	0.3	30	5.93	250	250	5.23
N29	500	0.3	30	5.93	250	250	4.54

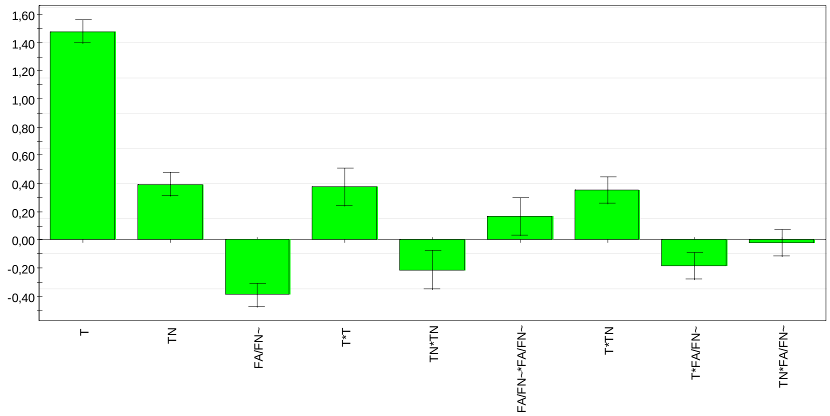

Fig. 1. Scaled and Centered coefficients for the first set of experiments

the first and the second sets could explain 99% and 96% of variation of response and predict 98% and 79% of it respectively, which mean that the models fitted well to the data, though the accuracy of the second model was lower. In order to evaluate which factors influence SAA the Coefficient Plots were displayed (Fig. 1 and 2).

According to the Coefficient Plots (Fig. 1 and 2) temperature shows the highest influence on the process. Two other factors used – TN and FA/FNA – also has influence on SAA but to a lower extent. The two Coefficient Plots show different influence of TN and FA/FNA in the different ranges of their values. From the Coefficient Plot of the first set (Fig. 1) it is seen that in general increase of temperature and total nitrogen has a positive effect on SAA and increase of FA/FNA leads to decrease of SAA and that the average influence of TN and FA/FNA on the absolute value of SAA is the same. However, the influence of TN and FA/FNA in the ranges used in the second set (Fig. 2) is evaluated in another way. First of all, due to a higher error of prediction, the error bars of the factors TN and FA/FNA are wider, so the upper limits are close to the zero line. Second, as it will be seen later, these factors do not have the same influence on SAA in the different ranges of values.

The fact that the temperature has the biggest influence on SAA can also be seen from one of the graphs of modelled response surface (Fig. 3). To determine the separate effect of temperature on

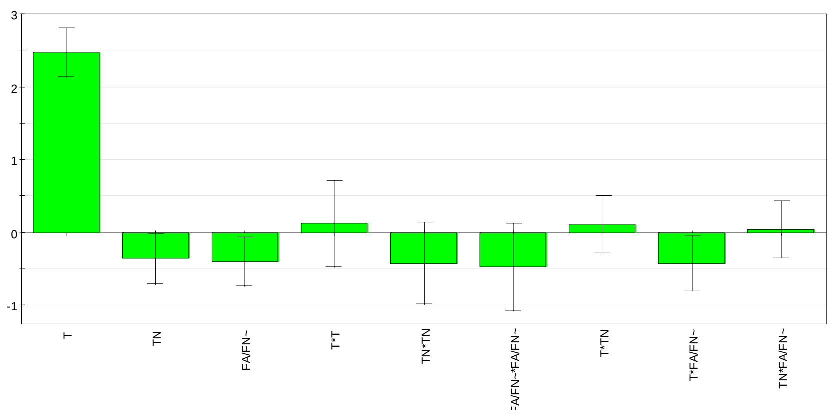

Fig. 2. Scaled and Centered coefficients for the first set of experiments

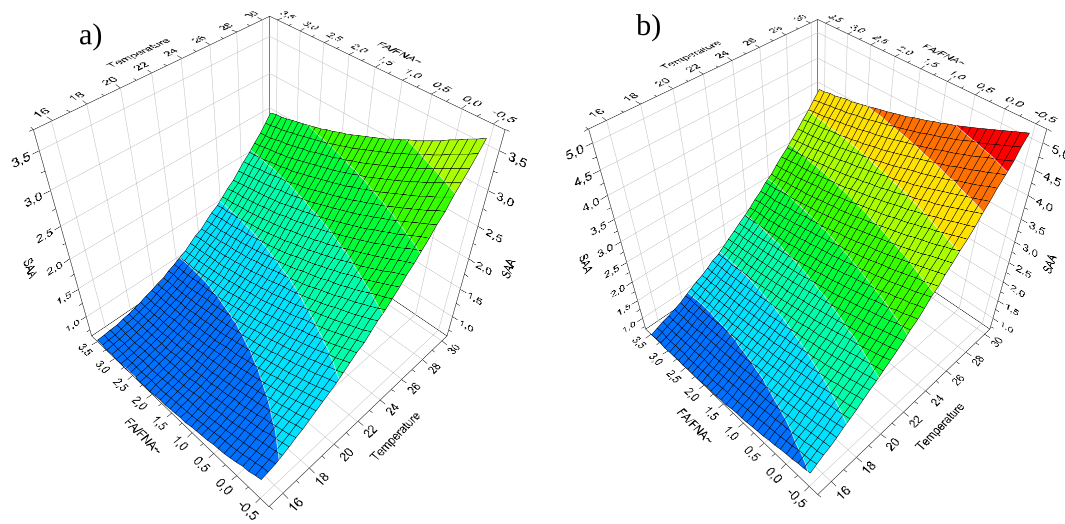

Fig. 3. Dependence of SAA from temperature and FA/FNA value at the fixed levels of TN: a)TN=100 mg/L; b)TN=500 mg/L.

SAA the graphs of a relative increase of SAA with increase of temperature were build where TN and a FA/FNA ratio were fixed at lower, central and higher levels of the ranges used for the first set (Fig. 4 a-f). The lowest SAA of every series was taken as the base.

Temperature has different influence on SAA depending on TN and a FA/FNA ratio value (Fig. 4 a-f). The highest relative increase of SAA is observed with high TN values. An increase in temperature from 15 to 30 °C can result in an increase of SAA by 251-484%, which corresponds to the average increase of SAA by 8.7-12.5% per 1 °C. For the presented temperature influence curves (Fig. 4 a-f) activation energies were calculated using formula (3) and they ranged from 67 to 86 kJ/mol.

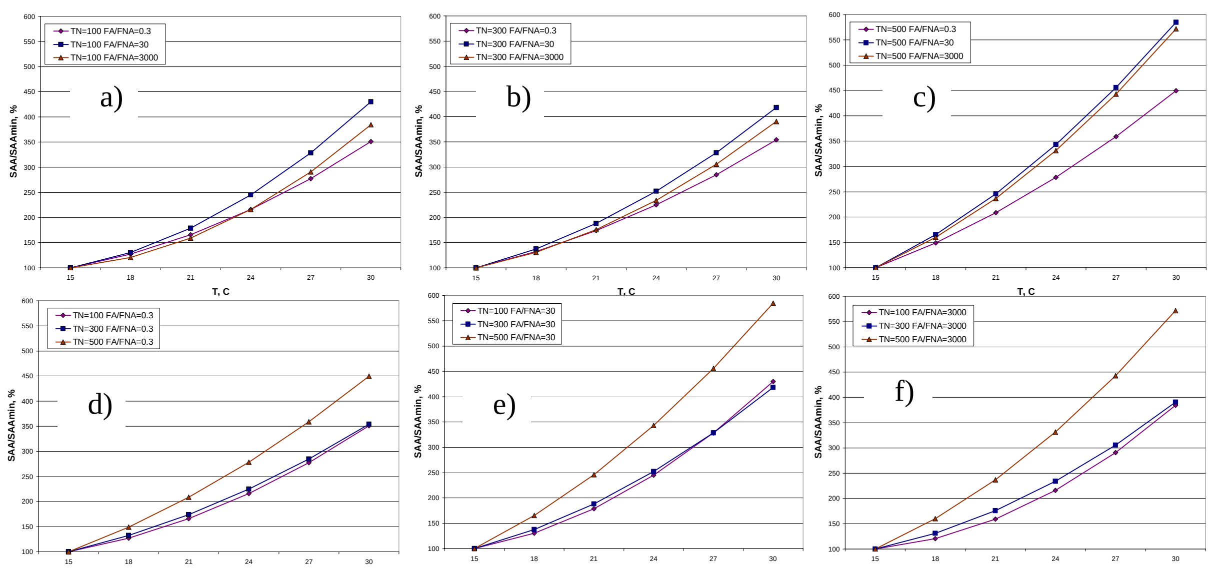

Fig. 4. Relative increase of SAA depending on temperature for different TN and FA-FNA values: a)TN=100 mg/L; b)TN=300 mg/L; c)TN=500 mg/L; d)FA/FNA=0,3; e)FA/FNA=30; f)FA/FNA=3000

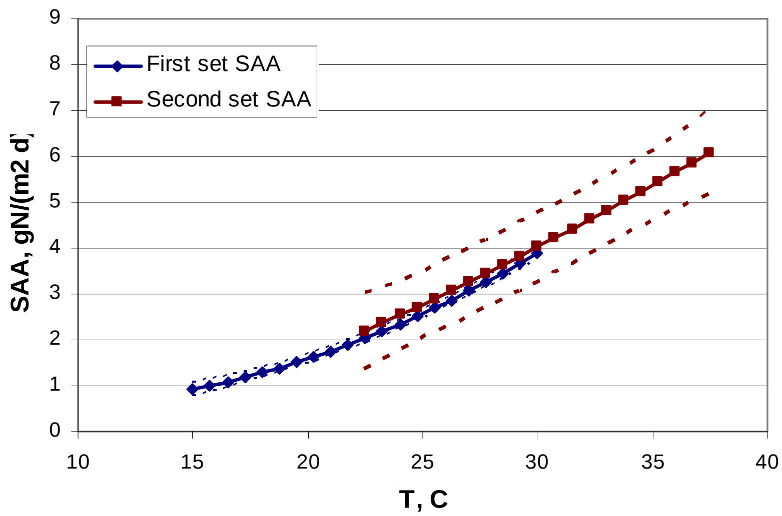

Fig. 5. SAA as a function of temperature where TN=300 mg/L, FA/FNA=30. Dashed lines identify 95% confidence interval.

To compare the temperature influence on SAA for both sets, the graphs of temperature dependence were built on the same chart for TN of 300 mg/l and a FA/FNA ratio of 30 (Fig. 5). They show that the data from both tests are consistent and SAA continues to increase almost linearly with the increase of temperature up to 37.5°C. Investigation of higher temperatures is considered to be unreasonable because temperatures of supernatant and landfill leachate never reach higher values. As long as it is not reasonable to heat incoming wastewater because of high energy needs, it is important to maintain other parameters that influence SAA on the optimum levels.

The influence of TN was identified to be different for different ranges of temperatures. For the temperature of 15°C SAA reaches the maximum when TN is in the range of 300-350 mg/L, while for the temperature of 22.5 °C the maximum SAA is reached when TN is in the range of 450-500 mg/L and for the temperature 30 °C even higher TN is tolerated (Fig. 6). It can be concluded that inhibition by total nitrogen is strongly dependent on the temperature and it moves to the higher

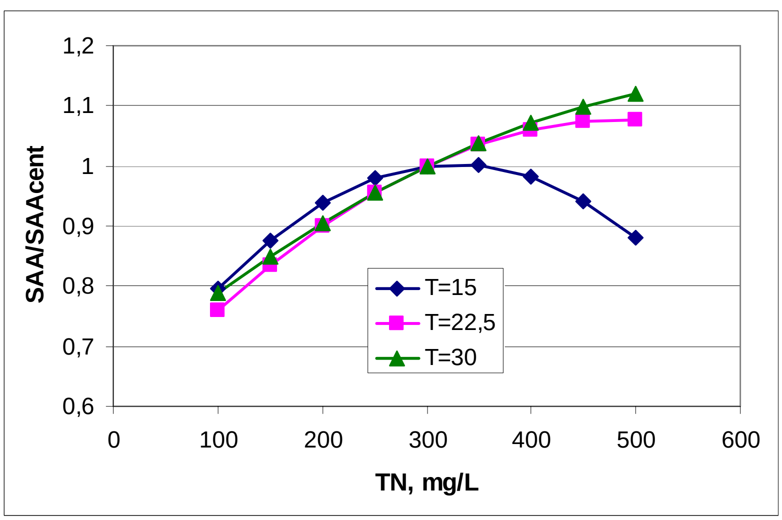

Fig. 6. Dependence of SAA on TN with different temperature values. Central point is taken as a base.

levels of TN with the temperature increase. Further temperature increase does not allow increasing of TN to even higher values.

From Figure 3 it is also seen that in the first set with the decrease of a FA/FNA ratio SAA increases in most cases. However, when it drops lower than 0.1-1 SAA starts to decrease (Fig. 7).

Based on equations (4) and (5), FA and FNA depend on concentrations of ammonium and nitrite correspondingly together with the value of pH. Increase of pH leads to higher FA/FNA ratios when the concentrations of ammonium and nitrite are kept constant. That means that in order to reach low FA/FNA ratios there should be as little ammonium as possible, as much nitrite as possible and low pH. This conclusion is valid only for the Anammox process. In a one-stage deammonification system conditions have to be suitable for running both Anammox and nitritation. In a one-stage deammonification system, FA/FNA ratios in the range of 0.1-1 are usually not accessible. First of all, ammonium removal in a one-stage deammonification process is usually limited by nitrification (Szatkowska 2007). That means that nitritation runs slower than the Anammox process and most of nitrite that is produced is assimilated immediately. In order to increase the concentration of nitrite in the system, the nitrification rate needs to be increased. This can be done by increasing DO in the reactor. As long as Anammox is inhibited by DO, an increase of DO will lead to a decrease of SAA. Finding the proper balance between nitritation and an Anammox rate (so that the proportion of nitrite to ammonium is highest but concentration of nitrite is not too high to cause inhibition) is important for the high overall deammonification efficiency.

pH is important parameter not only for Anammox but also for nitritation. Higher pH is needed for nitritation mainly because of alkalinity requirements. During a nitritation process pH decreases due to alkalinity consumption. Keeping an effluent TN concentration at the minimum level results in the maximum drop of pH, which in turn is preferable for the Anammox process.

When a two-stage deammonification system is used**,** low FA/FNA ratios can be reached if nitrite, produced in a preceding nitritation reactor (usually the SHARON process is used to avoid nitratation) and ammonium in supernatant are dosed to the Anammox reactor so that the desired ratio between ammonium and nitrite is reached. pH can also be lowered because alkalinity requirement for the Anammox process is extremely low, if compared to nitrification.

In order to achieve low FA/FNA ratios and high TN the combinations of concentrations and pH were used that led to rise of FNA concentrations to the levels that had been reported as inhibiting to

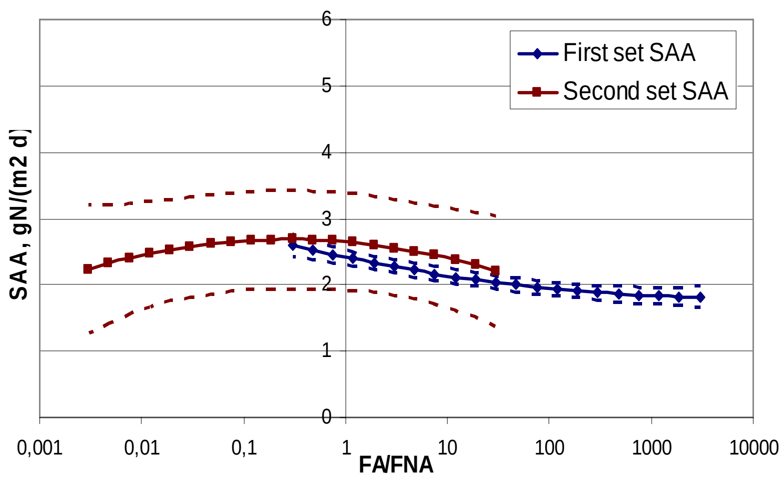

Fig. 7. SAA as a function of FA/FNA when TN=300 mg/L, T=22.5°C. Dashed lines identify 95% confidence interval.

the Anammox process (Jung et al. 2007). However, no inhibition was observed both in the first set of experiments where FNA of 0.58 mg N/L was used in some experiments and in the second one, where the maximum FNA of 3.83 mg N/L was used. The minimum pH of 5.26 was used and no inhibition was observed as well. The explanation for absence of inhibition at such high FNA concentrations and so low pH can be that the outer biofilm layer with nitrification bacteria protects Anammox bacteria in the inner biofilm layer from such severe environmental conditions. So long-term experiments in pilot plants have to be made, in order to investigate inhibition effects of FNA and pH. Such information may be useful in design and operation of full-scale plants.

CONCLUSIONS

- The results of investigation have showed that the temperature has the highest effect on SAA, from the factors that had been investigated. An increase of temperature from 15 to 30 °C can result in an SAA increase by 250-480%, which corresponds to the average SAA increase by 8.7-12.5% per 1 °C. Activation energy of the Anammox process was calculated and ranged from 67 to 86 kJ/mol This agrees with the values of 70 kJ/mol obtained by Strous et al. (1999) and are close to 63 kJ/mol obtained by Dosta et al. (2008).
- The optimum level of total nitrogen TN depends on the temperature and FA/FNA ratios but lies in the range of 400-500 mg/l. However, such high levels of TN are not feasible to maintain in industrial applications. Keeping TN inside the continuous working reactor at such levels means that the reactor outflow will also have high nitrogen concentrations, while the goal of application of the Anammox process is to reduce the level of TN in wastewater as much as possible. The results also show that the lower is designed TN concentration in the reactor effluent – the longer hydraulic retention time in the reactor should be.
- The most favourable value of the FA/FN ratio lays in the range from 0.1 to 1. However, this value can not be kept in a one-stage deammonification reactor because it would inhibit the nitritation process.
- Response surface modelling applied for the evaluation of the Anammox process provides useful knowledge about the optimum process parameters.

REFERENCES

Anthonisen A.C., Loehr R.C., Prakasam T.B.S. and Srinath E.G. (1976). Inhibition of nitrification by ammonia and nitrous acid. *Journal WPCF* **48**(5), 835-852.

Arrojo B., Mosquera-Corral A., Campos J.L. and Mendez R. (2006). Effects of mechanical stress on Anammox granules in a sequencing batch reactor (SBR). *Journal of Biotechnology* **123**, 453–463.

Bator E. (2006). *Research on optimization of CANON process using MODDE software.* Master thesis, Land and Resources Engineering Department, Royal Institute of Technology, Stockholm, Sweden.

Dalsgaard T. and Thamdrup B. (2002). Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. *Applied Environmental Microbiology* **68**(8), 3802–3808.

Dapena-Mora A., Fernández I., Campos J.L., Mosquera-Corral A., Mendez R. and Jetten M.S.M. (2007). Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. *Enzyme and Microbial Technology* **40**, 859–865.

Dosta J., Fernández I., Vázquez-Padín J. R., Mosquera-Corral A., Campos J. L., Mata-Álvarez J. and Méndez R. (2008). Short- and long-term effects of temperature on the Anammox process. *Journal of Hazardous Materials*, **154**(1-3), 688-693.

Fernández I., Vázquez-Padin J.R., Mosquera-Corral A., Campos J.L. and Mendez R. (2008). Biofilm and granular systems to improve Anammox biomass retention. *Biochemical Engineering Journal* **42**, 308–313.

Fux C., Marchesi V., Brunner I., Siegrist H. (2004). Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors. *Water Science Technology* **49** (11/12), 77–82.

Gut L. (2006). *Assessment of a partial nitritation/anammox system for nitrogen removal.* PhD Thesis, Land and Resources Engineering Department, Royal Institute of Technology, Stockholm, Sweden.

Güven D., Dapena-Mora A., Kartal B., Schmid M.C., Maas B., Pas-Schoonen K., Sozen S., Mendez R., Camp H.J.M, Jetten M.S.M., Strous M. and Schmidt I. (2004). Propionate Oxidation by and Methanol Inhibition of Anaerobic Ammonium-Oxidizing Bacteria. *Applied and Environmental Microbiology* **71**(2), 1066–1071.

Hao X., Heijnen J.J. and Loosdrecht M.C.M. (2002). Model-based evaluation of temperature and inflow variations on a partial nitrification-ANAMMOX biofilm process. *Water Research* **36**, 4839-4849.

Isaka K., Suwa Y., Kimura Y., Yamagishi T., Sumino T. and Tsuneda S. (2008). Anaerobic ammonium oxidation (anammox) irreversibly inhibited by methanol. *Applied Microbiology and Biotechnology* **81**, 379–385.

Jung J.Y., Kang S.H., Chung Y.C. and Ahn D.H. (2007). Factors affecting the activity of anammox bacteria during start up in the continuous culture reactor. *Water Science Technology* **55**(1/2), 459-468.

Läckeby Water Group. (2007). *Nitrogen removal process for high-concentrated wastewater streams.* http://www.lackebywatergroup.se/inc/pdf/en_LWG_DEAMoon_0709.pdf (accessed 17 April 2009).

Mulder A, van de Graaf A.A., Robertson L.A. and Kuenen J.G. (1995). Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS *Microbiology and Ecology* **16**: 177-183.

Rysgaard S., Glud R.N., Risgaard-Petersen N. and Dalsgaard T. (2004). Denitrification and Anammox activity in Arctic marine sediments. *Limnology and Oceanografy* **49**(5), 1493–1502.

Strous M., Kuenen J. G. and Jetten M.S.M. (1999). Key physiology of anaerobic ammonium oxidation. *Applied Environmental Microbiology* **65**, 3248-3250.

Szatkowska B. (2007). *Performance and control of biofilm systems with partial nitritation and Anamox for supernatant treatment.* PhD Thesis, Land and Resources Engineering Department, Royal Institute of Technology, Stockholm, Sweden.