# The impact of influent total ammonium nitrogen concentration on nitrite-oxidizing bacteria inhibition in moving bed biofilm reactor

Vojtech Kouba, Michael Catrysse, Hana Stryjova, Ivana Jonatova, Eveline I. P. Volcke, Pavel Svehla and Jan Bartacek

## ABSTRACT

The application of nitrification–denitrification over nitrite (nitritation–denitritation) with municipal (i.e. diluted and cold (or low-temperature)) wastewater can substantially improve the energy balance of municipal wastewater treatment plants. For the accumulation of nitrite, it is crucial to inhibit nitrite-oxidizing bacteria (NOB) with simultaneous proliferation of ammonium-oxidizing bacteria (AOB). The present study describes the effect of the influent total ammonium nitrogen (TAN) concentration on AOB and NOB activity in two moving bed biofilm reactors operated as sequencing batch reactors (SBR) at 15 °C (SBR I) and 21 °C (SBR II). The reactors were fed with diluted reject water containing 600, 300, 150 and 75 mg TAN $L^{-1}$. The only factor limiting NOB activity in these reactors was the high concentrations of free ammonia and/or free nitrous acid (FNA) during the SBR cycles. Nitrite accumulation was observed with influents containing 600, 300 and 150 mg TAN $L^{-1}$ in SBR I and 600 and 300 in SBR II. Once nitrate production established in the reactors, the increase of influent TAN concentration up to the original 600 mg TAN $L^{-1}$ did not limit NOB activity. This was due to the massive development of NOB clusters throughout the biofilm that were able to cope with faster formation of FNA. The results of the fluorescence *in situ* hybridization analysis preliminarily showed the stratification of bacteria in the biofilm.

**Key words** | ammonium-oxidizing bacteria, biological wastewater treatment, nitritation, nitrite-oxidizing bacteria, sequencing batch reactor

**Vojtech Kouba**
**Hana Stryjova**
**Jan Bartacek** (corresponding author)
Department of Water Technology and Environmental Engineering,
Institute of Chemical Technology Prague,
Technická 5, 166 28 Prague 6,
Czech Republic
E-mail: *jan.bartacek@vscht.cz*

**Michael Catrysse**
**Eveline I. P. Volcke**
Department of Biosystems Engineering,
Ghent University,
Coupure links 653, 9000 Gent,
Belgium

**Ivana Jonatova**
Department of Environmental Engineering,
Faculty of Chemical and Food Technology,
Institute of Chemical and Environmental Engineering,
Slovak University of Technology,
Radlinského 9, 812 37 Bratislava,
Slovakia

**Pavel Svehla**
Department of Agro-Environmental Chemistry and Plant Nutrition,
Czech University of Life Sciences Prague,
Kamycka 129, 165 21 Prague 6,
Czech Republic

## INTRODUCTION

Biological nitrogen removal by nitritation–denitritation is a sustainable method for increasing energy self-sufficiency of modern wastewater treatment plants (WWTPs) when applied to wastewater streams with low carbon-to-nitrogen ratio (De Clippeleir *et al.* 2011). In comparison to a conventional nitrification–denitrification process, nitrogen removal via nitrite allows savings of up to 25% of oxygen consumption, 40% of electron donor requirements in the anoxic stage, lower sludge production and 1.5 to 2 times higher nitrite denitrification rates in comparison to nitrate denitrification (Beccari *et al.* 1983; Turk & Mavinic 1987; van Kempen *et al.* 2001; Pollice *et al.* 2002).

The successful applications of nitritation-denitritation have so far been focused mostly on streams with high nitrogen concentration, such as reject water, piggery manure, landfill leachate or specific industrial wastewaters (Ganigué *et al.* 2012). Side-stream treatment of reject water accounts for only 15 to 30% of total nitrogen load of a WWTP (Müller *et al.* 1998). Applying optimized nitritation–denitritation to the main stream of municipal wastewater should result in significantly higher energy savings. Even more relevant to the optimization of energy requirements of the wastewater treatment process is placement of nitritation after either direct anaerobic treatment of wastewater or advanced primary clarification (Hendrickx *et al.* 2012). Thus, there is a need for exploration of nitritation sustainability for low wastewater temperatures of 10–20 °C and low influent ammonium concentrations of 20–100 mg $L^{-1}$.

The key aspect for achieving accumulation of nitrite is the washout of nitrite-oxidizing bacteria (NOB) and

doi: 10.2166/wst.2013.757

proliferation of ammonium-oxidizing bacteria (AOB) due to their different sensitivities to free ammonia (FA, $NH_3$) and free nitrous acid (FNA, $HNO_2$) concentrations, temperature, pH or dissolved oxygen concentration (Van Hulle *et al.* 2010). Selective inhibition of NOB of nitrifying suspension sludge by FA and FNA was previously reported to be successful in combination with pH adjustment, achieving FA and FNA concentrations of 1.5–10 mg $L^{-1}$ and 0.011 mg $L^{-1}$ respectively (Vadivelu *et al.* 2006). Ceased growth of *Nitrobacter* was reported under FA concentrations higher than 6 mg $NH_3$-N, with 25% decrease of activity (Vadivelu *et al.* 2007). Nitrite accumulation of up to 80–90% was previously achieved for biofilm systems operated under specific inhibitory concentrations greater than 1.5 mg $NH_3$-N per gram of biomass determined as volatile attached solids (Villaverde *et al.* 1997). However, adaptation of NOB to high FA levels can be challenging for long-term sustainability of nitritation (Villaverde *et al.* 2000).

The present study describes oxidation of total ammonium nitrogen (TAN) by the nitritation process applied to two moving bed biofilm reactors (MBBR) operated as sequencing batch reactors (SBR) at 15 °C (I) and 21 °C (II). The reactors were fed with diluted reject water simulating effluent from anaerobic treatment of municipal wastewater. The aim of this study was to evaluate the impact of influent TAN concentration on both AOB and NOB. The specific objectives of the study were as follows: (i) determination of the lowest influent TAN concentration selectively inhibiting the growth of NOB in biofilm; (ii) determination of the reversibility of influent TAN concentration effect; and (iii) description of the temperature effect on nitritation stability. MBBR was chosen for its stability and for its ability to retain even slow-growing biomass (including NOB). As a consequence, the only limiting factor for NOB activity was the high concentration of FNA and/or FA.

## MATERIALS AND METHODS

### Influent wastewater

Reject water from thermophilic anaerobic sludge digestion was adopted as the main substrate with corresponding dilution. The most important parameters of the raw wastewater were 1.25 g TAN $L^{-1}$, 12.5 mg N-$NO_2^-$ $L^{-1}$, 11 mg N-$NO_3^-$ $L^{-1}$, 2.3 g $COD_{total}$ $L^{-1}$, 1.1 g CODsoluble $L^{-1}$, 3.6 g TS $L^{-1}$ and 1.15 g TSS $L^{-1}$. The influent TAN concentrations used in this study were about 600, 300, 150 and 75 mg $L^{-1}$ with corresponding hydraulic retention times of 3, 1.5, 0.75 and 0.375 days to keep the TAN loading rate constant.

### Reactors set-up

Two biofilm reactors operated as SBR with working volumes of 0.78 L (SBR I) and 3 L (SBR II) were used in the study. SBR I and SBR II were operated at 15 ± 1 and 21 ± 1 °C, respectively. The air supply was provided by air diffusers placed at the bottom of the reactors with the dissolved oxygen concentration kept above 3 mg $L^{-1}$ in order to exclude NOB inhibition by low dissolved oxygen concentration. The value of pH was measured continuously. The reactor operation was fully automatic. The cycle consisted of a filling phase followed by aerated reaction phase. The length of the reaction phase was adjusted according to the influent TAN concentration in order to sustain a constant TAN loading. Before the decanting, a 5 min settling phase occurred.

The influent TAN concentration was stepwise reduced for both reactors from 600 mg $L^{-1}$ to 300 mg $L^{-1}$ and 150 mg $L^{-1}$, and for the reactor operated at 15 °C even to 75 mg $L^{-1}$, while preserving constant TAN loading rate (NLR) of 0.2 kg TAN $m^{-3}$ $d^{-1}$.

### Reactor start-up and seed sludge

The start-up of each reactor was performed by adding biomass-free polyvinyl alcohol (PVA) pellets. Manufactured by company LentiKat's, the pellets are lenticular-shaped (diameter 4 mm, thickness 0.2 mm) with porous structure.

Subsequently, SBR I was inoculated by nitrifying sludge from a municipal WWTP and SBR II was inoculated by the decanted effluent of SBR I (day 145).

### Microscopic analysis

AOB and NOB attached to the pellets were identified by fluorescence *in situ* hybridization (FISH). At first, fixation of pellets by 4% paraformaldehyde was performed with subsequent hybridization by complemental probes specific to 16S rRNA sequences of detected organisms. The methodology developed by Amann (1995) was adopted and adjusted to the immobilized samples used in this study .

### Analytical methods

Regular sampling of the bulk liquid, influent and effluent, from the reactors was carried out to determine the TAN as

a sum of $N\text{-}NH_3$ and $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, COD and volatile suspended solids concentration according to *Standard Methods* (2005). Temperature and pH value were determined by a WTW inolab pH 730 probe. Dissolved oxygen concentration was measured by WTW Oximeter 'oxi 340'.

The concentrations of FA and FNA (as the main suspected NOB inhibitors) were calculated, respectively, according to Abeling & Seyfried (1992) and Furukawa *et al.* (2009):

$$c_{FA} = \frac{17}{14} \frac{c_{Namon} \cdot 10^{pH}}{\exp\left(\frac{6334}{(273 + ^\circ C)}\right) + 10^{pH}} \quad (1)$$

$$c_{FNA} = \frac{47}{14} \frac{c_{N-NO_2^-}}{\exp\left(\frac{-2300}{(273 + ^\circ C)}\right) \cdot 10^{pH}} \quad (2)$$

## RESULTS AND DISCUSSION

### Inhibition of NOB by varying influent TAN concentration

Assuming constant influent pH, alkalinity:TAN ratio and NLR as used in this study, the influent TAN concentration is the only variable governing FA concentration at the beginning and subsequently FNA concentration at the end of each SBR cycle. Lower influent TAN concentration translates to lower FA and FNA concentration during the cycle. Therefore, a threshold influent TAN concentration should exist, preventing proliferation of NOB in the biofilm.

The effluent nitrogen concentrations of SBR I and SBR II are shown in Figures 1 and 2, respectively. For influent TAN concentration 600 mg $L^{-1}$, stable partial nitrification was obtained in both reactors. Very low $N\text{-}NO_3^-$ concentrations (maximally up to 15% of effluent $N\text{-}NO_x^-$) indicate the presence of a certain amount of NOB. The semi-continuous cycle with the pH value swings facilitated inhibition of NOB at the beginning of the cycle by FA and subsequently by FNA because of the AOB generating $N\text{-}NO_2^-$ and decreasing pH value (Figure 3). This caused the exceeding of previously reported inhibition limits for suspended biomass, i.e. 1.5–10 mg $L^{-1}$ of FA and 0.011 mg $L^{-1}$ of FNA, in the whole cycle, resulting in successful NOB inhibition of NOB activity (Anthonisen *et al.* 1976). The highest pH values obtained (around 8.0) are not limiting for NOB activity (Grunditz & Dalhammar 2001; Jiménez *et al.* 2011), leaving FA and FNA as the only significant NOB inhibitors.

The influent TAN concentration 300 mg $L^{-1}$ caused an increase of effluent $N\text{-}NO_3^-$ concentrations in SBR II without any similar change in SBR I. The subsequent decrease of influent TAN concentration in SBR II to 150 mg $L^{-1}$ resulted in rapid increase of NOB activity, indicated by the conversion of all nitrite to nitrate. Since inhibition levels of FA and FNA were still exceeded for the whole cycle of influent TAN concentration 300 mg $L^{-1}$ (Figure 3), acclimation of NOB on FA is the suspected cause (Villaverde *et al.* 2000). Increase of NOB activity in SBR I was observed only after influent TAN concentration was decreased to 75 mg $L^{-1}$.

For temperatures 15 and 21 °C, the threshold influent TAN concentrations observed to be effectively inhibiting NOB growth were 150 and 600 mg $L^{-1}$, respectively. The lower temperature in SBR I resulted in decreasing the FA concentration by 36% while increasing the FNA

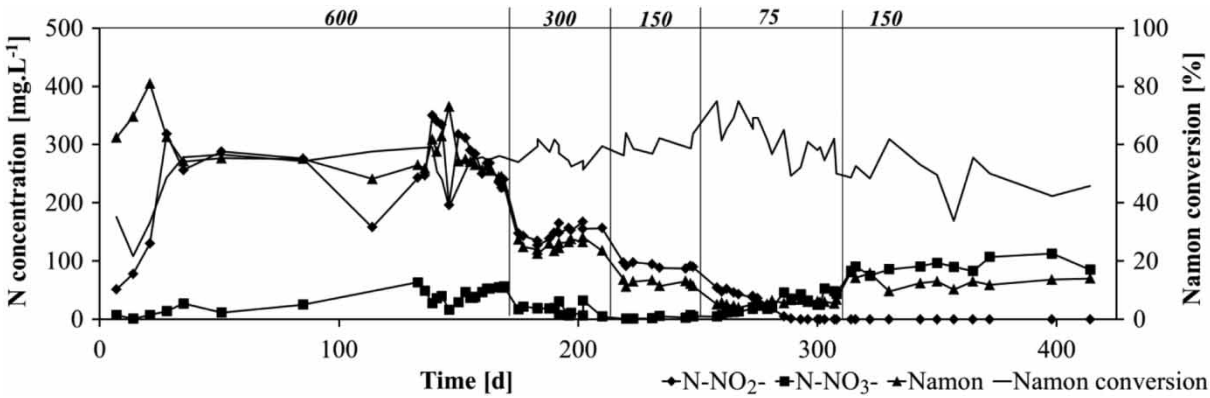


**Figure 1** | Effluent concentrations of TAN, $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ and TAN conversion for SBR I operated at 15 °C and influent concentrations of 600, 300, 150 and 75 mg $L^{-1}$ and TAN loading rate of 0.2 kg $m^{-3}$ $d^{-1}$.

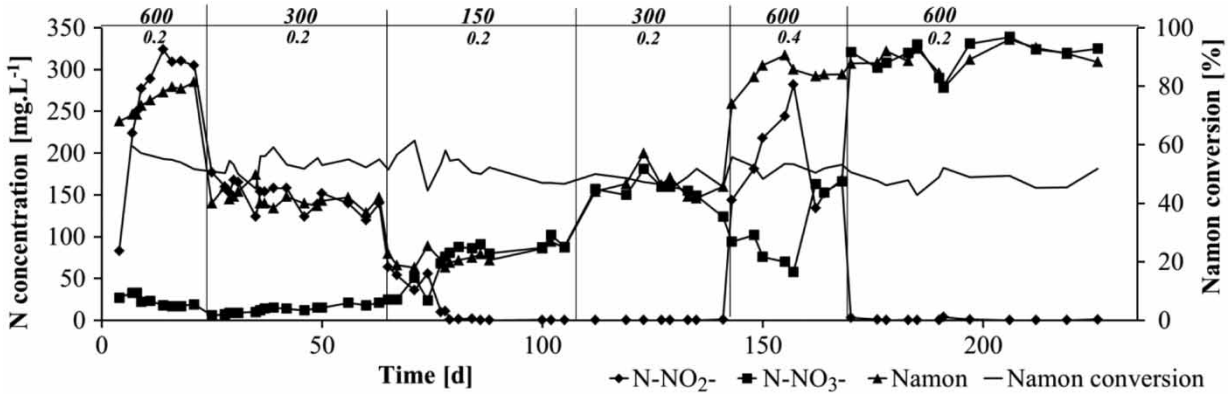


**Figure 2** | Effluent concentrations of TAN, $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ and TAN conversion for SBR II operated at 21 ± 1 °C and influent concentrations of 600, 300 and 150 mg $L^{-1}$ and TAN loading rate of 0.2 and 0.4 kg $m^{3-}$ $d^{-1}$.

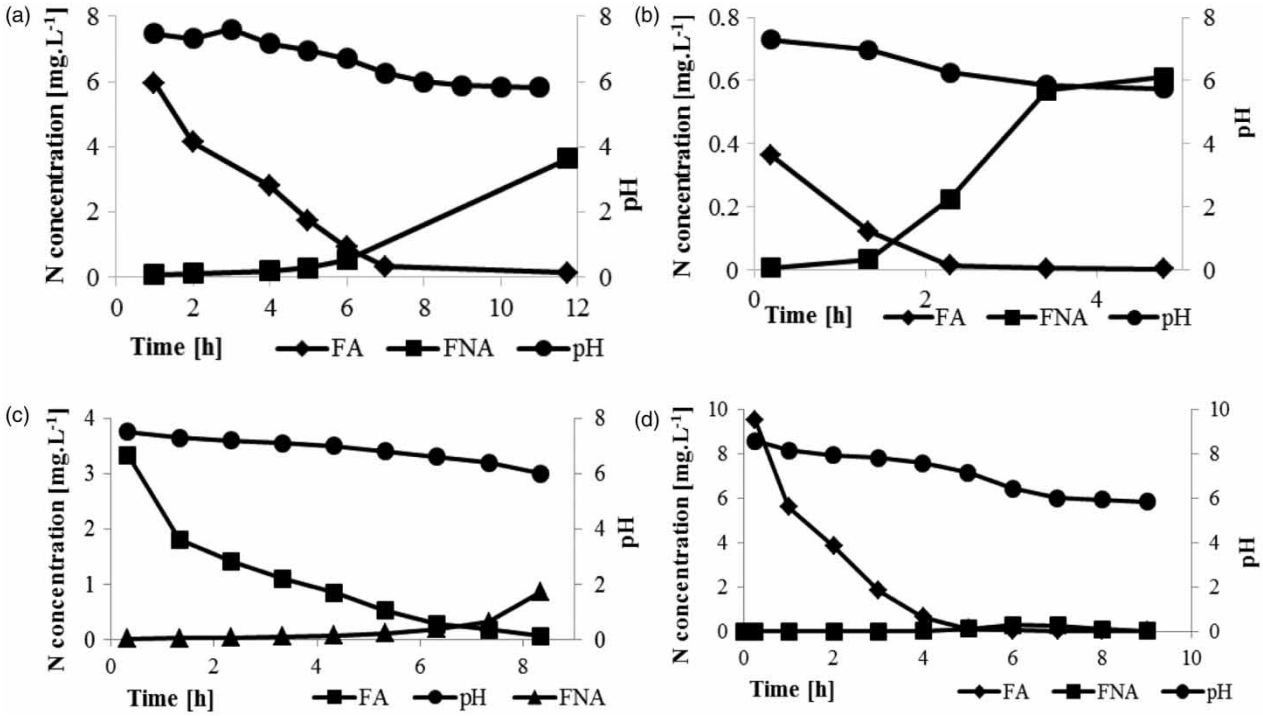


**Figure 3** | The development of FA, FNA and pH during a typical cycle in SBR I (a), (b) and SBR II (c), (d). The influent TAN concentrations were 600 mg $L^{-1}$ (a), 150 mg $L^{-1}$ (b), 300 mg $L^{-1}$ (c) and 150 mg $L^{-1}$ (d).

concentration by 18%. Thus, the conditions induced by lower temperature do not result in a more effective NOB inhibition. The difference between NOB proliferation at different temperatures can be attributed to the lower growth rate under 15 °C. Operation at influent TAN concentration 150 mg $L^{-1}$ and 15 °C would probably eventually result in NOB proliferation.

An important aspect of the study is the irreversibility of the colonization of the biofilm by NOB. It was clearly shown that once NOB are abundant enough to remove all FNA, they cannot be easily eliminated by elevated FA concentrations, at least not within several months. It was shown that even influent TAN concentration as high as 600 mg $L^{-1}$ did not inhibit NOB present in the biofilm.

## Effect of biomass cultivation in biofilm on the inhibition of NOB by FA and FNA

After the establishment of NOB activity in biofilm, the increase of influent TAN concentration did not selectively inhibit NOB. Only oxygen limitation as a result of short-term increase of TAN loading led to the partial recovery of nitritation at day 160 (Figure 2) in SBR II. Intact long-term NOB activity even during highly inhibiting conditions in the medium, as induced by increasing influent TAN concentration to 600 mg $L^{-1}$, was attributed to the cultivation of biomass in biofilm, where NOB could hardly be washed out from the biomass.

It also can be expected that by creating a concentration profile in the biofilm, NOB are exposed to lower concentrations of FA and therefore NOB inhibition should be lower in the biofilm. However, according to the simulation study of Park *et al.* (2010), the inhibiting effect of FNA is even enhanced in biofilms, especially when simultaneous dissolved oxygen (DO) limitation occurs. Thus, if DO limitation is properly used, growing biomass in biofilm can even enhance NOB inhibition.

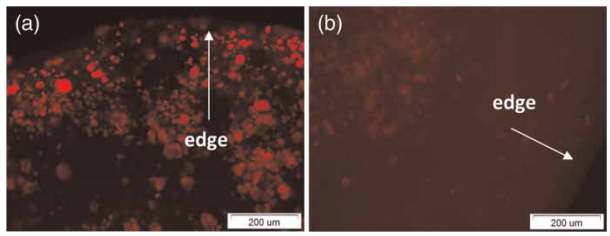


**Figure 4** | FISH analysis for SBR I. (a) AOB probes NSO190 + NSO1225 displaying various sized clusters and single cells; (b) *Nitrobacter* probe NIT3 showing single cells situated in the inner parts of biofilm (day 280). The white arrows indicate the edge of the PVA pellets.

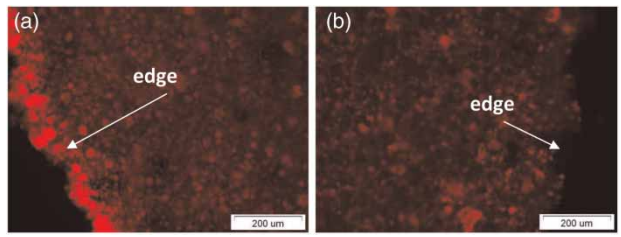


**Figure 5** | FISH analysis for SBR II of (a) AOB by probes NSO190 + NSO1225 displaying large clusters and single cells, and (b) *Nitrobacter* by probe NIT3 showing clusters and single cells situated in the inner parts of biofilm (day 134).

## TAN conversion

The aim of this experiment was to explore the sustainability of nitritation at different low influent TAN concentrations without other operational strategies. Together with substrate of relatively low total inorganic carbon content, these conditions implied relatively limited TAN conversion. Depletion of neutralization capacity during the nitritation resulted in pH decrease at the end, constraining further TAN oxidation. Thus, TAN conversion for both reactors throughout the experiment was about 54%. When the prevalent oxidized form of nitrogen is $NO_2^-$, subsequent treatment by anammox (anaerobic ammonium oxidation) is possible (van de Graaf *et al.* 1996). Nitritation and anammox in two subsequent reactors is favourable because of absence of dissolved oxygen contact with anammox bacteria (Li *et al.* 2011). Full-scale application of a single-stage nitritation and anammox has also been demonstrated (Abma *et al.* 2010; Jeanningros *et al.* 2010). Nevertheless, the overall TAN conversion can be increased by pH value manipulation if denitritation is preferred over anammox (Jeníček *et al.* 2004).

## Fluorescence *in situ* hybridization

FISH analysis was performed in order to determine the presence and relative amount of NOB and AOB in the biofilm grown on the PVA pellets. Figure 4 shows significant AOB prevalence in biofilm from SBR I. Figure 5 displays relatively equal prevalence of both AOB and NOB in biofilm of SBR II. The outer part of biofilm from SBR II seems to be dominated by AOB, with most of the NOB growing in the inner parts of biofilm.

Hypothetically, AOB growth is favoured on the edges of biofilm because of a lower affinity constant for oxygen than that of NOB and high initial FA content in the medium. Stratification of bacteria allows for proliferation of NOB inside the biofilm and subsequent cooperation of AOB and NOB resulting in complete nitrification and resistance to NOB-inhibiting conditions in the medium.

Even though the FISH analysis supports the hypothesis of bacteria stratification, the confirmation would require quantitative analysis. Additional FISH analysis in different phases of the experiment could have given better information of AOB and NOB evolution.

Variations in sampling caused different results of FISH analysis of biofilm from SBR I and SBR II. Clusters of NOB in SBR II are the result of a long-term NOB activity before the sampling. Occurrence of only a small number of NOB in SBR I biofilm is caused by their proliferation only 20 days before the sampling. Higher numbers of NOB in biofilm of SBR II can be attributed to higher temperature.

### Better process control for nitritation sustainability

The key to long-term nitritation in biofilm is in effective process start-up and subsequent practical operation strategy. The experiment clearly showed the impossibility of sustainable nitritation in biofilm at conditions approaching those of domestic wastewater after anaerobic pretreatment without dynamic control strategy. In order to achieve realistic process conditions, lower wastewater temperatures unlike those used in recent papers must be evaluated (Gu *et al.* 2012; Kwak *et al.* 2012; Lee *et al.* 2013). Also, addition of external substances like hydroxylamine does not seem viable from a long-term point of view (Xu *et al.* 2012). Further research will be focused on establishment of dissolved oxygen control for low temperature wastewater. Recently, one of the most successful strategies for NOB inhibition based on manipulating DO/TAN ratio was demonstrated by Bartrolí *et al*. (2010).

## CONCLUSION

This paper shows that influent TAN concentrations as high as 300 and 75 mg $L^{-1}$ do not inhibit NOB activity in biofilm reactors operated at 21 and 15 °C, respectively. Concentrations of 600 and 150 mg TAN $L^{-1}$ sustained NOB inhibition for more than 30 days.

This paper demonstrates that once NOB colonize the biofilm, their inhibition is alleviated and influent TAN concentration as high as 600 mg $L^{-1}$ cannot establish NOB inhibition.

## ACKNOWLEDGEMENTS

This research was funded by European Commission grant ERG-2010-268417 'Biofilms in Bioreactors for Advanced Nitrogen Removal from Wastewater (BioNIT)' and by MSM 6046137308. The support of the foundation 'Nadání Josefa, Marie a Zdeňky Hlávkových', 'Nadace Sophia' and Department of Water Technology and Environmental Engineering at ICT Prague is greatly acknowledged. Also the authors gratefully acknowledge the support given by the wide internal grant agency of CULS Prague – CIGA, project numbers 20122022 and 20132012.

## REFERENCES

Abeling, U. & Seyfried, C. F. 1992 Anaerobic-aerobic treatment of high strength ammonium wastewater – nitrogen removal via nitrite. *Water Science and Technology* **26** (5–6), 1007–1015.

Abma, W. R., Driessen, W., Haarhuis, R. & van Loosdrecht, M. 2010 Upgrading of sewage treatment plant by sustainable and cost-effective separate treatment of industrial wastewater. *Water Science and Technology* **61**, 1715–1722.

Amann, R. 1995 *In Situ Identification of Microorganisms by Whole Cell Hybridization with rRNA-targeted Nucleic Acid Probes*. Kluwer Academic Publishers, Dordrecht.

Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S. & Srinath, E. G. 1976 Inhibition of nitrification by ammonia and nitrous acid. *Journal Water Pollution Control Federation* **48** (5), 835–852.

Bartrolí, A., Pérez, J. & Carrera, J. 2010 Applying ratio control in a continuous granular reactor to achieve full nitritation under stable operating conditions. *Environmental Science and Technology* **44** (23), 8930–8935.

Beccari, M., Marani, E., Ramadori, R. & Tandoi, V. 1983 Kinetic of dissimilatory nitrate and nitrite reduction in suspended growth culture. *Journal Water Pollution Control Federation* **55**, 58–64.

De Clippeleir, H., Yan, X., Verstraete, W. & Vlaeminck, S. E. 2011 OLAND is feasible to treat sewage-like nitrogen concentrations at low hydraulic residence times. *Applied Microbiology and Biotechnology* **90** (4), 1537–1545.

Furukawa, K., Inatomi, Y., Qiao, S., Quan, L., Yamamoto, T., Isaka, K. & Sumino, T. 2009 Innovative treatment system for digester liquor using anammox process. *Bioresource Technology* **100** (22), 5437–5443.

Ganigué, R., Volcke, E. I. P., Puig, S., Balaguer, M. D. & Colprim, J. 2012 Impact of influent characteristics on a partial nitritation SBR treating high nitrogen loaded wastewater. *Bioresource Technology* **111**, 62–69.

Grunditz, C. & Dalhammar, G. 2001 Development of nitrification inhibition assays using pure cultures of nitrosomonas and nitrobacter. *Water Research* **35** (2), 433–440.

Gu, S., Wang, S., Yang, Q., Yang, P. & Peng, Y. 2012 Start up partial nitrification at low temperature with a real-time control strategy based on blower frequency and pH. *Bioresource Technology* **112**, 34–41.

Hendrickx, T. L. G., Wang, Y., Kampman, C., Zeeman, G., Temmink, H. & Buisman, C. J. N. 2012 Autotrophic nitrogen removal from low strength waste water at low temperature. *Water Research* **46** (7), 2187–2193.

Jeanningros, Y., Kaldate, A., Verstraete, W. & Graveleau, L. 2010 Fast start-up of a pilot-scale deammonification sequencing batch reactor from an activated sludge inoculum. *Water Science and Technology* **61**, 1393–1400.

Jeníček, P., Švehla, P., Zábranská, J. & Dohányos, M. 2004 Factors affecting nitrogen removal by nitritation/denitritation. *Water Science and Technology* **49** (5–6), 73–79.

Jiménez, E., Giménez, J. B., Ruano, M. V., Ferrer, J. & Serralta, J. 2011 Effect of pH and nitrite concentration on nitrite oxidation rate. *Bioresource Technology* **102** (19), 8741–8747.

Kwak, W., McCarty, P. L., Bae, J., Huang, Y.-T. & Lee, P.-H. 2012 Efficient single-stage autotrophic nitrogen removal with dilute wastewater through oxygen supply control. *Bioresource Technology* **123**, 400–405.

Lee, P.-H., Cotter, S. F., Reyes Prieri, S. C., Attalage, D. & Sung, S. 2013 pH-gradient real-time aeration control for nitritation community selection in a non-porous hollow fiber membrane biofilm reactor (MBfR) with dilute wastewater. *Chemosphere* **90** (8), 2320–2325.

Li, X., Xiao, Y., Liao, D., Zheng, W., Yi, T., Yang, Q. & Zeng, G. 2011 Granulation of simultaneous partial nitrification and anammox biomass in one single SBR system. *Applied Biochemistry and Biotechnology* **163** (8), 1053–1065.

Müller, J., Lehne, G., Schwedes, J., Battenberg, S., Näveke, R., Kopp, J., Dichtl, N., Scheminski, A., Krull, R. & Hempel, D. C. 1998 Disintegration of sewage sludges and influence on anaerobic digestion. *Water Science and Technology* **38** (8–9), 425–433.

Park, S., Bae, W. & Rittmann, B. E. 2010 Multi-species nitrifying biofilm model (MSNBM) including free ammonia and free nitrous acid inhibition and oxygen limitation. *Biotechnology and Bioengineering* **105** (6), 1115–1130.

Pollice, A., Tandoi, V. & Lestingi, C. 2002 Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. *Water Research* **36** (10), 2541–2546.

*Standard Methods for the Examination of Water & Wastewater* 2005 American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Turk, O. & Mavinic, D. S. 1987 Benefits of using selective inhibition to remove nitrogen from highly nitrogenous wastes. *Environmental Technology Letters* **8**, 419–426.

Vadivelu, V. M., Yuan, Z., Fux, C. & Keller, J. 2006 The inhibitory effects of free nitrous acid on the energy generation and growth processes of an enriched Nitrobacter culture. *Environmental Science & Technology* **40** (14), 4442–4448.

Vadivelu, V. M., Keller, J. & Yuan, Z. 2007 Effect of free ammonia on the respiration and growth processes of an enriched *Nitrobacter* culture. *Water Research* **41** (4), 826–834.

van de Graaf, A. A., de Bruijn, P., Robertson, L. A., Jetten, M. S. M. & Kuenen, J. G. 1996 Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology-UK* **142**, 2187–2196.

Van Hulle, S. W. H., Vandeweyer, H. J. P., Meesschaert, B. D., Vanrolleghem, P. A., Dejans, P. & Dumoulin, A. 2010 Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. *Chemical Engineering Journal* **162** (1), 1–20.

van Kempen, R., Mulder, J. W. & Van Loosdrecht, M. 2001 Overview: full scale experience of the SHARON process for treatment of rejection water of digested sludge dewatering. *Water Science and Technology* **44**, 145–152.

Villaverde, S., García-Encina, P. A. & Fdz-Polanco, F. 1997 Influence of pH over nitrifying biofilm activity in submerged biofilters. *Water Research* **31** (5), 1180–1186.

Villaverde, S., Fdz-Polanco, F. & García, P. A. 2000 Nitrifying biofilm acclimation to free ammonia in submerged biofilters. Start-up influence. *Water Research* **34** (2), 602–610.

Xu, G., Xu, X., Yang, F., Liu, S. & Gao, Y. 2012 Partial nitrification adjusted by hydroxylamine in aerobic granules under high DO and ambient temperature and subsequent Anammox for low C/N wastewater treatment. *Chemical Engineering Journal* **213**, 338–345.

First received 14 June 2013; accepted in revised form 11 November 2013. Available online 31 December 2013