Nitrogen removal rates at a technical-scale pilot plant with the one-stage partial nitritation/Anammox process

G. Cema*,, B. Szatkowska*, E. Plaza*, J. Trela* and J. Surmacz-Górska****

*Department of Land and Water Resources Engineering, Royal Institute of Technology (KTH), 100 44 Stockholm, Sweden (E-mail: *grzegorz@kth.se*; *beata@kth.se*; *elap@kth.se*; *trela@kth.se*)

** Environmental Biotechnology Department, Silesian University of Technology, 44 100 Gliwice, Poland (E-mail: *joanna.s.gorska@polsl.pl*)

Water Science & Technology **Vol 54 No 8 pp 209–217** © IWA Publishing 2006

Abstract Traditional nitrification/denitrification is not suitable for nitrogen removal when wastewater contains high concentrations of ammonium nitrogen and low concentrations of biodegradable carbon. Recently, a deammonification process was developed and proposed as a new technology for treatment of such streams. This process relies on a stable interaction between aerobic bacteria *Nitrosomonas,* that accomplish partial nitritation and anaerobic bacteria *Planctomycetales*, which conduct the Anammox reaction. Simultaneous performance of these two processes can lead to a complete autotrophic nitrogen removal in one single reactor. The experiments where nitrogen was removed in one reactor were performed at a technical-scale moving-bed pilot plant, filled with Kaldnes rings and supplied with supernatant after dewatering of digested sludge. It was found that a nitrogen removal rate obtained at the pilot plant was 1.9 $g\,m^{-2}d^{-1}$. Parallel to the pilot plant run, a series of batch tests were carried out under anoxic and aerobic conditions. Within the batch tests, where the pilot plant's conditions were simulated, removal rates reached up to 3 $g\,N\,m^{-2}d^{-1}$. Moreover, the batch tests with inhibition of *Nitrosomonas* showed that only the Anammox bacteria (not anoxic removal by *Nitrosomonas*) are responsible for nitrogen removal.

Keywords Anammox; CANON; deammonification; digested supernatant; nitrogen removal

Introduction

A nitrogen concentration is a significant factor indicating the quality of treated wastewater. To remove ammonium nitrogen from wastewater using Anammox bacteria, a proper nitrite-to-ammonium ratio is needed. Nitrite can be produced according to reaction 1, by aerobic autotrophic ammonia oxidizers.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad (1)$$

$$NH_4^+ + 1.32NO_2^- \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \qquad (2)$$

Usually, ammonia oxidation is performed under oxic conditions in the first stage, while Anammox performs under anoxic in the second one (reaction 2). Such a two-step system has been investigated by many research groups from Sweden (Trela *et al.*, 2004; Cema *et al.*, 2005; Gut *et al.*, 2006), Switzerland (Fux *et al.*, 2002) and the Netherlands (Van Dongen *et al.*, 2001).

Both types of bacteria, nitrifiers and Anammox, can also co-exist in one reactor due to oxygen and oxygen free-zones within the biofilm depth (Third *et al.*, 2001; Sliekers *et al.*, 2003). German researchers called such a single-stage conversion of ammonium into molecular nitrogen – deammonification (Hippen *et al.*, 1997). In the Netherlands, system where ammonia is partially oxidized under oxygen-limited conditions to nitrite and next nitrite together with remaining ammonia is converted to dinitrogen gas by Anammox bacteria was named CANON (Completely Autotrophic Nitrogen removal Over Nitrite).

doi: 10.2166/wst.2006.816

The combination of the two processes – partial nitritation and Anammox- in one reactor can be expressed by the following reaction:

$$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.3H_2O + 1.4H^+ \qquad (3)$$

Simultaneous nitritation and Anammox processes were observed and studied in different types of reactors under different conditions. The summary of different studies with a one–stage autotrophic process for nitrogen removal is shown in Table 1.

The deammonification process has the potential to be more cost-efficient than nitrification/denitrification commonly used for nitrogen removal, nowadays. Oxygen and aeration energy are largely reduced and no external carbon source is required. The process can be applied for treatment of ammonium-rich wastewaters such as supernatant and landfill leachate. However, performed experiments presented in Table 1 and Swedish experiences with one-stage partial nitritation/Anammox process indicated that the main problem is a start-up of second Anammox step. It can take even half a year. Moreover, a proper oxygen condition in a bulk liquid was found as the most important factor affecting the process.

The one reactor system for nitrogen removal was tested at a technical-scale pilot plant, supplied with the supernatant coming directly from dewatering of digested sludge. The main goal of the investigations was to study the process performance and to estimate nitrogen removal rates in the partial nitritation/Anammox process.

Methodology

Pilot plant

The pilot plant experiment has been run for more than 3 years at the Himmerfjärden Waste Water Treatment Plant (WWTP) in the Stockholm region. It was a two-stage process where partial nitritation and Anammox took place in two separate reactors (see Trela *et al.*, 2004). Moving-bed reactors, each of 2 m^3 volume and filled with Kaldnes rings, were supplied with supernatant coming directly from dewatering of the digested sludge. In March 2005 the pilot plant was modified and an experiment with recirculation of a nitrate-rich Anammox effluent to the nitritation reactor was started. It was intended to denitrify nitrates in the first zone of reactor 1; zones 2 and 3 of the nitritation reactor were connected and in zone 1 the aeration was ceased to obtain oxygen-free conditions for denitrification. After a few weeks, nitrogen loss was observed in reactor 1. It was due to the fact that Anammox bacteria found excellent conditions to develop its bacteria culture in zone 1. Then recirculation was stopped and aeration was switched on again in zone 1 to develop a two-step biofilm layer, an outer layer with oxygen-rich conditions for nitrifiers and an inner layer for the Anammox growth. The studies are based on a four-month experiment, when both simultaneous partial nitritation and the Anammox took place in the same reactor. In the preliminary phase, the hydraulic retention time (HTR) was equal to 1 day. To estimate influence of a nitrogen load increase in the following period HRT was shortened to 16 hours. The process was monitored by analysis of ammonium, nitrite and nitrate nitrogen. Moreover, measurements of pH, dissolved oxygen (DO), conductivity and temperature have been done. The scheme of the pilot-plant configuration is shown in Figure 1.

Batch tests

During the pilot plant operation several batch tests were performed. Each batch test was run in a bottle of 1 litre working capacity, filled up to 50% of its volume with the Kaldnes material taken from the reactor zone 1 (z1) and zone 2 + 3 (z2 + 3) of the

Table 1 Comparison of conditions in a one-stage nitrogen removal process

Reactor type/scale	Application	T [°C]	pH	DO [$g\,m^{-3}$]	Nitrogen load	N rem [%]	References
Moving-bed/pilot-plant	Sludge liquor	28.0	8.0	0.8	4.7 $g\,m^{-2}d^{-1}$	75	Seyfried *et al*., 2002
		29.1	8.1	2.0	4.8 $g\,m^{-2}d^{-1}$	71	Hippen *et al*., 2001
		25.1	8.1	5.9	4.9 $g\,m^{-2}d^{-1}$	10	
SBR/ pilot-plant	Synthetic wastewater	30	7.8	0.2	0.12–0.22 $kg\,N\,m^{-3}d^{-1}$	36–92	Third *et al*., 2001
Gas-lift/pilot-plant	Synthetic wastewater	30	7.5	0.5	3.7 $kg\,N\,d^{-1}$	42	Sliekers *et al*., 2003
RBC/full-scale	Leachate	27–30	8.3	0.7–1	1.5 $g\,m^{-2}d^{-1}$	40–70	Seyfried *et al*., 2002
		13–20	7.3	1–2	2.7 $g\,m^{-2}d^{-1}$	40–70	Hippen *et al*., 2001
		10–28	8.1	0.8–1.2	3.3 $g\,m^{-2}d^{-1}$	40–70	
Moving-bed/pilot-plant	Sludge liquor	27	8–8.5	<1	4–8 $g\,m^{-2}d^{-1}$	60–70	Johansson *et al*., 1998
RBC/full-scale	Leachate	15–20	7.2	1–3	3.7 $g\,m^{-2}d^{-1}$	30–70	Siegrist *et al*., 1998
Moving-bed/ full-scale	Sludge liquor	20–35	no data	0–4	2 $g\,m^{-2}d^{-1}$ designed	80	Rosenwinkel *et al*., 2005

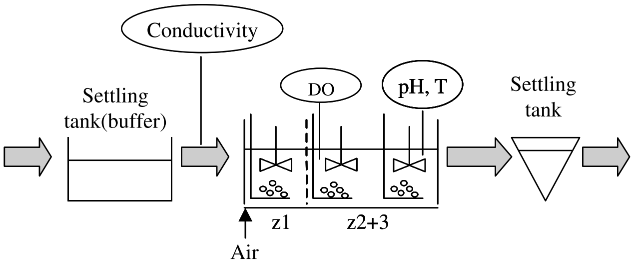

Figure 1 Flow sheet of a pilot-plant with one-stage partial nitritation/Anammox process

continuously working pilot plant. An effective specific biofilm surface of $0.25\,m^2$ per $1\,dm^3$ of a reactor bottle was provided. Afterwards, the liquid taken from the corresponding reactor's zones was poured over Kaldnes material. Then the bottles were placed in a water bath to keep the temperature constant. In two bottles, with medium from z1 and z2 + 3, anoxic conditions were maintained to estimate the Anammox reaction rates. In this test, a sodium nitrite solution at concentration of $20\,mg\,NO_2\text{-}N\,ml\,L^{-1}$ was used to provide a required nitrite concentration. The other two bottles with the same medium (z1 and z2 + 3) performed under aerobic conditions to simulate the pilot plant conditions. The test bottles were equipped with magnetic stirrers to ensure suitable movement of Kaldnes carriers. Parallel to the sampling, measurements of pH, DO, conductivity and temperature were performed. Additionally, few batch tests with medium taken from z1, were conducted to evaluate the Anammox bacteria activity. Allylthiourea (ATU) was added (concentration in the batch reactor $5\,g\,m^{-3}$) to inhibit nitrifiers and to find out the reaction rates of a pure Anammox culture. The concentration of the suspended solids and volatile suspended solids were measured at the beginning of the tests. The free ammonia concentrations were calculated according to (Anthonisen *et al.*, 1976).

Results and discussion

Pilot-plant

During the four-month operation period, the influent ammonium nitrogen concentration remained within the range of $350\text{–}720\,g\,NH_4\text{-}N\,m^{-3}$. During the first 3 months (period 1) the HRT was 1 day. During that period nitrogen removal rates varied from 0.6 to $1.7\,g\,N\,m^{-2}d^{-1}$ (58–88% of nitrogen reduction). In October (period 2) the HRT was reduced to 16 hours to evaluate how the systems responded to a higher nitrogen load; the new loads varied from 3.1 to $3.7\,g\,N\,m^{-2}d^{-1}$. At the beginning of the period 2 (with a reduced HTR) a decrease in nitrogen removal rates was noticed. Then, a gradual increase in nitrogen removal was observed up to the maximum value of $1.9\,g\,N\,m^{-2}d^{-1}$ (see Figure 2).

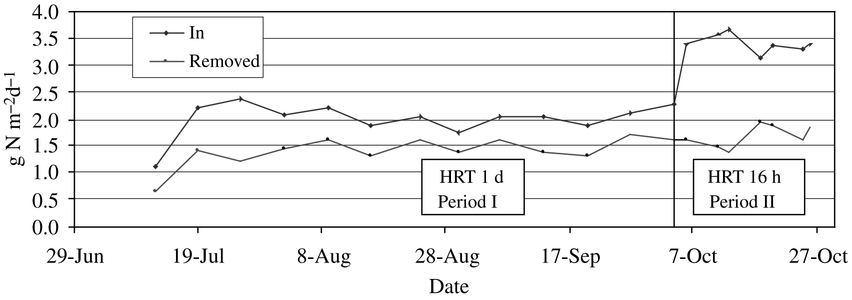

Figure 2 Nitrogen load in the influent and effluent from the pilot plant

The process was performed at a dissolved oxygen concentration of 1.6 g $O_2\,m^{-3}$ (the average bulk value for the range of 0.7–3 g $O_2\,m^{-3}$). The process temperature was close to a natural temperature of supernatant coming from centrifuges (26.2 °C, on average).

The pH value of incoming supernatant was equal to 7.83. During the first part of the experiment (HRT = 1 day) a drop of the pH value by about 0.3 was observed. It was due to the H^+ ions compensation (partial nitritation–production, Anammox-consumption). After HRT reduction (HRT = 16 h) an increase in pH value was noticed. Usually, during ammonium oxidation in the nitritation stage almost a complete consumption of alkalinity took place that resulted in a pH decrease. Insignificant changes in pH during simultaneous ammonium oxidation and Anammox were covered with a lower consumption of alkalinity, if compared to the partial nitritation.

An increase in the influent load resulted in a lower bacteria activity in zone 1 and a higher activity of zones 2 + 3. The difference in zone's activity and lower consumptions of alkalinity can be clearly seen from conductivity changes. It was proved that partial nitritation/Anammox process could be monitored by conductivity measurements (Szatkowska *et al.*, 2005). Both nitrogen removal and alkalinity consumption patterns reflected in conductivity variations. As it can be seen from Figure 3 a smaller drop between incoming (in) and outcoming (z2 + 3) conductivity corresponded to a lower consumption of alkalinity during the shortened HRT. A larger difference in conductivity between zone 1 and zone 2 + 3 indicates a greater capability to remove nitrogen in zone 2 + 3.

Batch tests

The main purpose of batch tests was to investigate the influence of nitrogen load increase to the reactor on the nitrogen removal rates and bacteria activity. The examples of nitrogen conversions in both anoxic and aerobic conditions during batch tests are presented in Figure 4A and 4B.

Under anoxic conditions simultaneous removal of ammonium and nitrite with only a small production of nitrate was observed (Figure 4A). This was in agreement with the Anammox reaction (see reaction 2) where for one mole of ammonium 1.3 mole of nitrite is required. Different nitrogen forms were observed under aerobic conditions where only ammonium utilisation and insignificant nitrate production were observed (Figure 4B). It was due to nitrite nitrogen production by nitrifiers and its simultaneous consumption by the Anammox bacteria.

The results of batch tests from z1 (period 1) showed an increase in inorganic nitrogen removal rates. They rose from 1.7 to 4.1 g N $m^{-2}d^{-1}$ and from 2.2 to 3.0 g N $m^{-2}d^{-1}$, for anoxic and aerobic conditions, respectively. The tests results are shown in Figure 5A. During this time the influent nitrogen load to the reactor was stable with the average value equal to 2.0 ± 0.2 g N $m^{-2}d^{-1}$ (Figure 2).

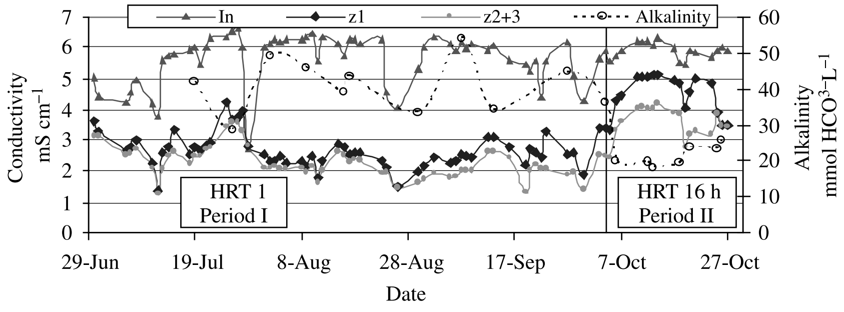

Figure 3 Alkalinity consumption and conductivity changes

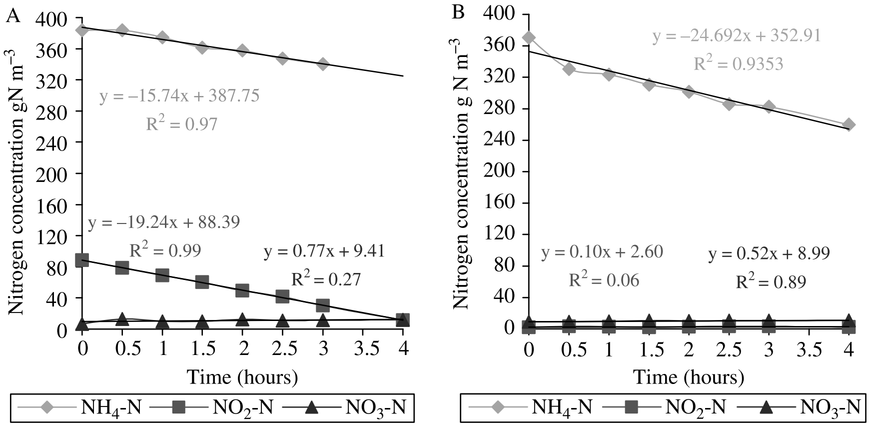

Figure 4 Nitrogen conversions, batch test with medium from z1, period 1: A) anoxic condition; B) aerobic condition

An increase of the influent nitrogen load to the pilot plant resulted in a decrease of nitrogen removal efficiency in period 2. However, the average nitrogen removal rate was higher, if compared to the previous period. It rose from 1.5 g N m^{-2}d^{-1} for period 1 to 1.7 g N m^{-2}d^{-1} for period 2. At the same time, batch tests showed that nitrogen removal rates in zone 1 decreased both in anoxic and aerobic conditions (Figure 5A).

Interesting phenomena were found while comparing the results from batch tests performed for zone 1 and zone 2 + 3 (Figure 5A and B). In period 1, the increase of removal rates during experiments with the medium from zone 2 was observed both in anoxic and aerobic conditions. After HRT reduction, at first the nitrogen removal rates decreased while next the increase of activity was observed in the tests with anoxic conditions (Figure 5B). Analyzing results presented in Figure 5 it can be stated that two phenomena had influence on nitrogen removal rates. The first one was an inhibition of the Anammox bacteria by excess of substrate; the second one was inhibition of nitrifiers by free ammonia concentration. Load to the zone 1 was increasing faster and to the higher values, which resulted in a decrease in reaction rates. The load to the zone 2 + 3 was lower therefore observed inhibition effect was weakened and then adaptation to the new conditions was noticed. Moreover, the high concentrations of free ammonia caused nitrifiers inhibition. Concentration of free ammonia in z 2 + 3 was lower than in z1. Nitrifiers ability to oxidize ammonium was higher in z 2 + 3. As a result, the nitrogen removal rate in the zone 2 + 3 was higher than in z1 under aerobic conditions on the 11th day of October.

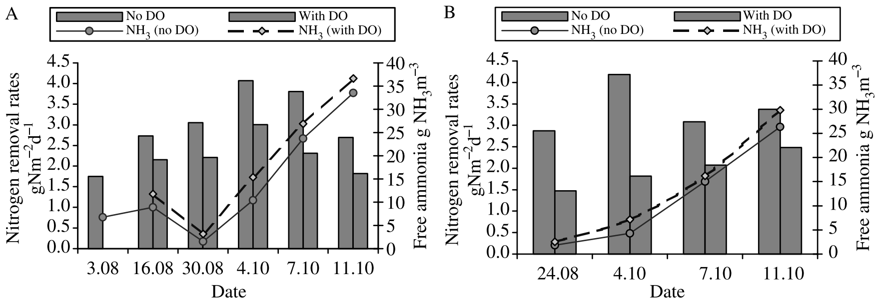

Figure 5 Nitrogen removal rates in anoxic and aerobic conditions: A) zone 1 B) zone 2

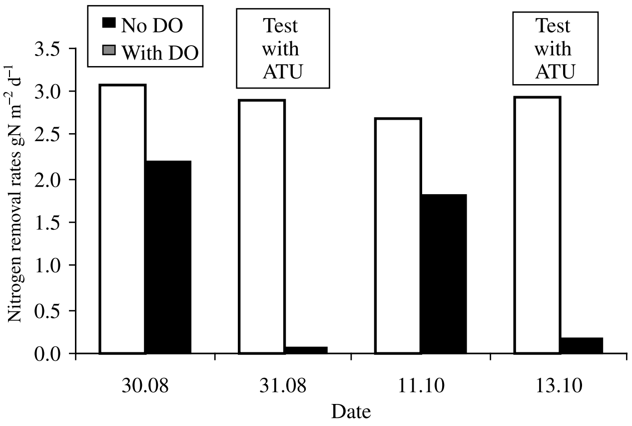

Figure 6 Results of the batch tests without and with allylthiourea (zone 1)

The obtained results clearly illustrated the differences in removal rates between anoxic (with addition of nitrite) and aerobic (without addition of nitrite) conditions (Figures 4 and 5). Probably it is due to the fact that oxygen is a limiting factor in the biofilm. It determines the reaction rate of nitrogen removal and it is connected with nitrifiers' activity, which produces nitrite nitrogen utilized later by the Anammox bacteria as an electron acceptor. It is also strictly depending on thickness of the biofilm's aerobic layer and diffusion parameters.

Additionally, tests with allylthiourea were carried out in order to more closely examine the nitrogen elimination. ATU is a selective inhibitor of *Nitrosomonas* bacteria at a concentration of 5 g m^{-3} (Surmacz-Górska *et al.*, 1996). The inhibitor was added to prevent influence of *Nitrosomonas* bacteria on the reaction rate, especially because those chemolithotrophic organisms are known as able to denitrify under anoxic condition (Bock *et al.*, 1995). Addition of ATU under aerobic conditions almost stopped nitrogen removal and only a minor part of inorganic nitrogen was removed (Figure 6). On the other hand, the nitrogen removal rates under the anoxic conditions were comparable with the one observed in the tests without ATU (Figure 6); it can indicate that probably only the Anammox bacteria are responsible for nitrogen removal. Moreover, the obtained ratios of nitrite-to-ammonium rate in the anoxic test were 1.39 ± 0.16, 1.29 ± 0.22 and 1.28 ± 0.10, for the tests with medium from zone1, zone 2 + 3 and zone 1 with addition of ATU, respectively. These results are similar to those obtained by Helmer-Madhok *et al.* (2002) (1.37) and to the stoichiometric one (1.32), according to reaction 2.

Conclusions

The experiments showed that the moving-bed reactor with Kaldnes rings as a biofilm carrier could be operated with a stable nitrogen removal rate after an influent load increase. The biofilm with bacterial culture was able to perform two processes simultaneously. Adequate hydraulic retention time combined with proper oxygen conditions and nitrogen loads are essential for high nitrogen removal rates. Increase in a surface nitrogen load to the pilot plant by about 1 g N m^{-2}d^{-1}, after the adaptation period resulted in an increase in nitrogen removal rates; the maximum recorded value was 1.9 g N m^{-2}d^{-1}.

Investigations with batch tests proved that it is possible to obtain even higher nitrogen removal rates. The maximum removal rate amounted to 3 g N m^{-2}d^{-1}. The batch tests indicated that partial nitritation seems to be the bottleneck to reach higher nitrogen removal rates. Additionally, tests with ATU proved that mainly the Anammox bacteria are responsible for nitrogen removal.

The process with partial nitritation and Anammox running parallel can be applied for a separate treatment of supernatant, which would finally lead to the nitrogen load reduction in the WWTP's effluent. However, for better understanding of the balance between biofilm growth, substrate load and influence of such physical parameters as temperature, dissolved oxygen and pH, long- lasting experiments are necessary.

Acknowledgements

Financial support obtained from VA-FORSK, SYVAB and J. Gust. Richert Foundation is acknowledged. The pilot plant was built by PURAC AB and has been operated with co-operation of the Royal Institute of Technology (KTH) and SYVAB. The authors wish to thank Jan Bosander for help with the pilot plant operation and Bengt Hultman for valuable comments on results.

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.

Bock, E., Schmidt, I., Stüven, R. and Zart, D. (1995). Nitrogen loss caused by denitrifying *Nitrosomonas* cells using ammonium or hydrogen as electron donors and nitrite as electron acceptor. *Arch Microbiol.*, **163**, 16–20.

Cema G., Płaza E., Surmacz-Górska J., Trela J. and Miksch, K. (2005). Study on evaluation of kinetic parameters for Anammox process. In: Preprints of the *IWA Specialized Conference "Nutrient Management in Wastewater Treatment Processes and Recycle Streams"*, 19–21 September 2005, Kraków, Poland, 379–388.

Fux, C., Boehler, M., Huber, P., Brunner, I. and Siegrist, H. (2002). Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant. *Journal of Biotech.*, **99**, 295–306.

Gut, L., Płaza, E., Trela, J., Hultman, B. and Bosander, J. (2006) Combined partial nitritation/Anammox system for treatment of digester supernatant. *Wat. Sci. Tech.*, **53**(12) 149–159.

Helmer-Madhok, C., Schmid, M., Filipov, E., Gaul, T., Hippen, A., Rosenwinkel, K.-H., Seyfried, C.F., Wagner, M. and Kunst, S. (2002). Deammonification in biofilm systems: population structure and function. *Wat. Sci. Tech.*, **46**(1–2), 223–231.

Hippen, A., Rosenwinkel, K.H., Baumgarten, G. and Seyfried, C.F. (1997). Aerobic deammonification: a new experience in the treatment of wastewaters. *Wat. Sci. Tech.*, **35**(10), 111–120.

Hippen, A., Helmer, C., Kunst, S., Rosenwinkel, K-H. and Seyfried, C.F. (2001). Six years of practical experiences with aerobic/anoxic deammonification in biofilm systems. *Wat. Sci. Tech.*, **44**(2–3), 39–48.

Johansson, P., Nyberg, A., Beier, M., Hippen, A., Seyfried, C.F. and Rosenwinkel, K.-H. (1998). Cost efficient sludge liquor treatment. In: *Joint Polish–Swedish Reports, Report No 3*. Royal Institute of Technology, Stockholm, TRITA-AMI Report 3048, pp. 65–72.

Rosenwinkel, K.H., Cornelius, A. and Ruhrverband, D.T. (2005). Full-scale application of the deammonification process for the treatment of sludge water. In Preprints "*IWA Specialized Conference: Nutrient Management in Wastewater Treatment, Processes and Recycle Streams*", 19–21 September 2005, Kraków, Poland.

Seyfried, C.F., Rosenwinkel, K.H., Hippen, A. (2002). Deammonification: a cost-effective treatment process for nitrogen-rich wastewaters. In: *WEFTEC 2002 Proceedings 75th Annual Conference and Exposition*. McCormick Place, Chicago, Illinois, USA, September 28–October 2, 2002.

Siegrist, H., Reithaar, S., Koch, G. and Lais, P. (1998). Nitrogen loss in a nitrifying rotating contactor treating ammonium-rich wastewater without organic carbon. *Wat. Sci. Tech.*, **38**(8–9), 241–248.

Sliekers, A.O., Third, K.A., Abma, W., Kuenen, J.G. and Jetten, M.S.M. (2003). CANON and Anammox in a gas-lift reactor. *FEMS Microbiol. Lett.*, **218**, 339–344.

Surmacz–Górska, J., Gernaey, K., Demuynck, C., Vanrolleghem, P. and Verstreate, W. (1996). Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements. *Wat. Res.*, **30**(5), 1228–1236.

Szatkowska, B., Płaza, E., Trela, J., Bosander, J. and Hultman, B. (2005). Application of conductivity measurements for monitoring of nitrogen removal in the partial nitritation/Anammox process. In: Preprints of the *IWA Specialized Conference "Nutrient Management in Wastewater Treatment, Processes and Recycle Streams"*, Krakow, Poland, 19–21 September 2005, pp. 717–371.

Third, K.A., Sliekers, A.O., Kuenen, J.G. and Jetten, M.S.M. (2001). The CANON system (Completely Autotrophic Nitrogen-removal Over Nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. *System. Appl. Microbiol.*, **24**, 588–596.

Trela, J., Płaza, E., Szatkowska, B., Hultman, B., Bosander, J. and Dahlberg, A-G. (2004). Pilot–plant experiments with combined nitritation and anaerobic ammonium oxidation (Anammox) in biofilm system. In: Preprints of the "*4th IWA World Water Congress and Exhibition*", Marrakech, 19–24 September 2004.

Van Dongen, L.G.J.M., Jetten, M.S.M. and van Loosdrecht, M.C.M. (2001). *The combined Sharon/Anammox Process, A sustainable method for N-removal from sludge water*, STOWA Report. IWA Publishing, London, UK.