

# Biological treatment of ammonium-rich wastewater by partial nitrification and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant

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## Abstract

In wastewater treatment plants with anaerobic sludge digestion, 15–20% of the nitrogen load is recirculated to the main stream with the return liquors from dewatering. Separate treatment of this ammonium-rich digester supernatant would significantly reduce the nitrogen load of the activated sludge system. Some years ago, a novel biological process was discovered in which ammonium is converted to nitrogen gas under anoxic conditions with nitrite as the electron acceptor (anaerobic ammonium oxidation, anammox). Compared to conventional nitrification and denitrification, the aeration and carbon-source demand is reduced by over 50 and 100%, respectively. The combination of partial nitrification to produce nitrite in a first step and subsequent anaerobic ammonium oxidation in a second reactor was successfully tested on a pilot scale (3.6 m<sup>3</sup>) for over half a year. This report focuses on the feasibility of nitrogen removal from digester effluents from two different wastewater treatment plants (WWTPs) with the combined partial nitrification/anammox process. Nitrification was performed in a continuously stirred tank reactor ( $V = 2.0 \text{ m}^3$ ) without sludge retention. Some 58% of the ammonium in the supernatant was converted to nitrite. At 30 °C the maximum dilution rate  $D_x$  was 0.85 d<sup>-1</sup>, resulting in nitrite production of 0.35 kg NO<sub>2</sub>-N m<sub>reactor</sub><sup>-3</sup> d<sup>-1</sup>. The nitrate production was marginal. The anaerobic ammonium oxidation was carried out in a sequencing batch reactor (SBR,  $V = 1.6 \text{ m}^3$ ) with a nitrogen elimination rate of 2.4 kg N m<sub>reactor</sub><sup>-3</sup> d<sup>-1</sup> during the nitrite-containing periods of the SBR cycle. Over 90% of the inlet nitrogen load to the anammox reactor was removed and the sludge production was negligible. The nitrification efficiency of the first reactor limited the overall maximum rate of nitrogen elimination.

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**Keywords:** Anaerobic ammonium oxidation; Anammox; Nitrification; Nitrite; Nitrogen removal

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## 1. Introduction

### 1.1. Nitrogen elimination in WWTPs

In wastewater treatment plants with anaerobic sludge digestion, 15–20% of the inlet nitrogen load is recycled with the return liquors from sludge dewatering. Separate treatment of this digester supernatant, containing 600–1000 g NH<sub>4</sub>-N m<sup>-3</sup>, would significantly reduce the nitrogen load of the main stream and improve nitrogen elimination. Chemical elimination of ammonium with magnesium–ammonium–phosphate (MAP) precipitation or with air stripping is feasible but much more expensive than classical nitrification and denitrification with addition of an organic carbon source (Siegrist, 1996). In the late 1990s, Hellinga et al. (1998) presented the SHARON process for nitrogen elimination from concentrated waste streams. At relatively high temperatures (35 °C) and without sludge retention, nitrite oxidation was permanently prevented and denitrification with nitrite could begin. As a result, 25% of the oxygen and 40% of the carbon demand can be saved compared with complete nitrification/denitrification but an external electron donor for denitrification such as methanol as well as an effective aeration system are still necessary.

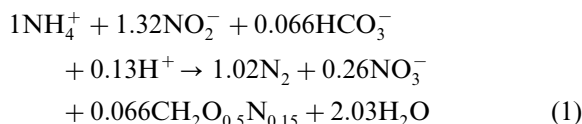
So far, only aerobic processes have been discussed for ammonium oxidation. A novel biological process was also discovered in the nineties in which ammonium is converted to nitrogen gas under anoxic conditions with nitrite as the electron acceptor (Mulder et al., 1995). Because ammonium is oxidised in the absence of oxygen, this novel process has been named anaerobic ammonium oxidation (anammox). This autotrophic process allows over 50% of the oxygen to be saved and no organic carbon source is needed (Fig. 1). In addition, the biomass yield is very low so that little sludge is produced.

### 1.2. Anaerobic ammonium oxidation (anammox)

Up to the mid-1990s, oxidation of ammonium had been known to proceed only under aerobic conditions but unaccounted high nitrogen losses were increasingly observed. Under oxygen-limited

conditions, a considerable part of the nitrogen load was eliminated at the leachate treatment plant of the landfill in Mechernich (Hippen et al., 1997). Extended nitrogen loss of up to 70% was also observed in a nitrifying rotating biological contactor in Koelliken treating ammonium-rich leachate from a hazardous waste landfill (Siegrist et al., 1998). Heterotrophic denitrification was excluded due to COD removal in previous processes. It was shown by using batch experiments, mathematical modelling and microbial analysis that the nitrite produced in the aerobic biofilm layer close to the surface diffuses into the deeper anoxic layer of the biofilm where it reacts with the remaining ammonium to form nitrogen gas (Koch et al., 2000).

This process was originally discovered in a denitrifying fluidised-bed reactor treating effluent from a methanogenic reactor (van de Graaf et al., 1990; Mulder et al., 1995) but the existence of anaerobic ammonium oxidation was already predicted over 2 decades ago (Broda, 1977) on the basis of thermo-dynamic calculations. In another fluidised-bed reactor on a laboratory scale fed with sludge digestion effluent from a domestic WWTP and a synthetic nitrite solution, 82% ammonium and 99% nitrite-removal efficiency was achieved (Strous et al., 1997). The maximum nitrogen removal capacity in this reactor was 1.5 kg N m<sup>-3</sup> d<sup>-1</sup>. Some important physiological parameters such as the maximum specific rate of ammonium consumption (45 ± 5 nmol mg<sub>protein</sub><sup>-1</sup> min<sup>-1</sup>), the maximum specific growth rate (0.0027 h<sup>-1</sup>, doubling time 11 days) and the biomass yield (0.066 ± 0.01 mol C mol<sup>-1</sup> NH<sub>4</sub>-N) are mentioned in Strous et al. (1998). This report gives the stoichiometry of anaerobic ammonium oxidation as:



assuming that nitrate is produced from nitrite to generate reducing equivalents for CO<sub>2</sub> fixation (van de Graaf et al., 1996). Using supernatant as an influent, ammonium is present in abundance but nitrite must first be produced up to a nitrite/

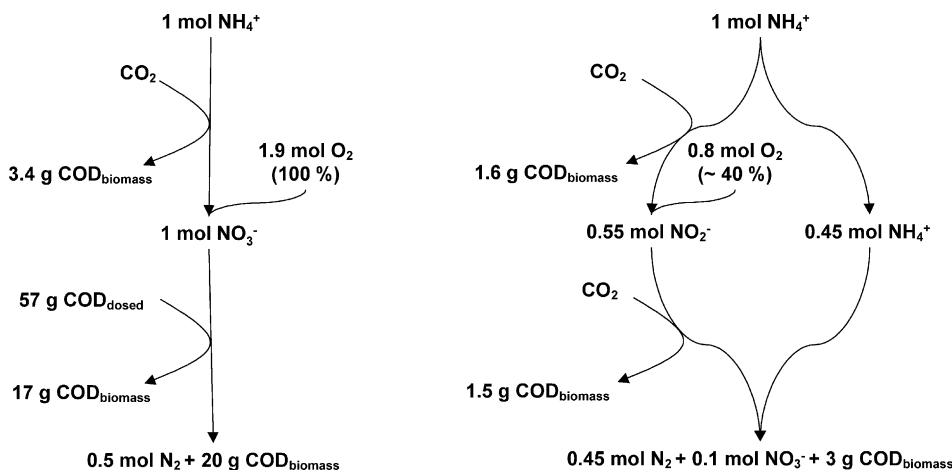


Fig. 1. Comparison of oxygen and COD consumption of classical nitrification/denitrification (left) and partial nitrification/anammox (right). Remarkably, more sludge is produced by the conventional process (yield of heterotrophic denitrification:  $0.3 \text{ g COD}_{\text{biomass}} \text{ g}^{-1} \text{ COD}_{\text{dosed}}$ ).

ammonium ratio of about 1.3, as shown in Eq. (1). Stable partial nitrification of the supernatant is thus essential for the subsequent anammox step.

### 1.3. Partial nitrification combined with anaerobic ammonium oxidation

Stable partial nitrification in a first aerobic reactor can be combined with anaerobic ammonium oxidation in a second tank to ensure total nitrogen removal throughout an autotrophic process (Jetten et al., 1997). Two aspects are essential in the nitrification step. In the first place the nitrite oxidisers must be continuously suppressed, and secondly the nitrite/ammonium ratio produced must be about 1.3 (Eq. (1)). If too much nitrite is produced, additional supernatant can be added directly to the anammox reactor to satisfy the stoichiometry. Because nitrite can completely inhibit the anammox process at concentrations higher than  $100 \text{ g NO}_2\text{-N m}^{-3}$  (Strous et al., 1999), ammonium should be added in slight excess with respect to Eq. (1). Not all the process conditions which suppress nitrite oxidation are currently understood. At temperatures above  $30 \text{ }^\circ\text{C}$  the ammonium oxidisers grow faster than the nitrite oxidisers so that nitrite oxidation can be prevented by carefully controlling the HRT (Hel-

linga et al., 1998). Total inhibition of the nitrite oxidisers by  $1 \text{ g NH}_2\text{OH m}^{-3}$  (hydroxylamine, an intermediate of the nitrosomonas monooxygenase reaction) is reported in Stüven et al. (1992). Free ammonia begins to inhibit nitrosomonas between 10 and  $150 \text{ g NH}_3 \text{ m}^{-3}$ , whereas nitrobacter activity is already seriously reduced between 0.1 and  $10 \text{ g NH}_3 \text{ m}^{-3}$  (Anthonisen et al., 1976).

The production of the appropriate nitrite/ammonium mixture depends on the alkalinity/ammonium ratio in the influent. For oxidation of ammonium to nitrite, two proton equivalents are produced per mol of ammonium converted. Supernatant contains about 1.2 times more bicarbonate than ammonium on a molar basis, so about 60% of the ammonium will be oxidised to nitrite, resulting in a nitrite/ammonium ratio of about 1.5. So no extra base needs be added in the nitrification step. In the second reactor, the nitrite produced together with the remaining ammonium as an electron donor is converted to nitrogen gas according to Eq. (1).

With this reactor configuration, the two processes do not take place in the same reactor (in contrast to fixed-bed reactors or rotating biological contactors) and can be controlled separately. This publication focuses on the potential of nitrogen removal from sludge digester supernatant

on a pilot scale by applying partial nitrification combined with anaerobic ammonium oxidation in two different reactors.

## 2. Materials and methods

### 2.1. Operation of the pilot plant

The flow scheme of the experimental system is shown in Fig. 2. The composition of the supernatant obtained from the activated sludge digestion facilities of the Werdhoelzli WWTP (Zurich) for the first 161 days and the Au WWTP (St. Gallen) for another 30 days is given in Table 1. The supernatant from the Werdhoelzli WWTP was taken from a little buffer tank (about 2 m<sup>3</sup>) and was therefore up to 10 °C cooler than in the sludge dewatering facilities. The sludge digester effluent from St. Gallen was brought to Zurich once a week and stored in a 12 m<sup>3</sup> tank.

Partial nitrification was carried out in an aerated and continuously stirred tank reactor (sludge residence time equal to hydraulic residence time, total volume 2.5 m<sup>3</sup>, height 2.5 m, maximum liquid volume 2.1 m<sup>3</sup>, adjustable by a level control). The reactor was inoculated with 1 m<sup>3</sup> of activated sludge (approx. 10 kg TSS m<sup>-3</sup>) from the Werdhoelzli WWTP at  $t=0$  and filled with secondary effluent up to a volume of  $V=2$  m<sup>3</sup>. No pH

adjustment occurred in the nitrification reactor. Anaerobic ammonium oxidation took place in a sequencing batch reactor (total volume 2.5 m<sup>3</sup>, height 2.5 m, maximum liquid volume 2.1 m<sup>3</sup>, adjustable by a level control). The anammox reactor was inoculated with excess sludge (about 1000 g TSS) collected at the effluent cloth filter system of the Koelliken WWTP at  $t=15$  days. This sludge contained anammox organisms closely related to the anammox strain of *Candidatus Kuenenia Stuttgartiensis* (Egli et al., 2001). To accelerate the start-up period, the reactor was inoculated again with excess sludge (1700 g TSS) at  $t=26$  days. The surface of this anammox reactor (1 m<sup>2</sup>) was covered with polypropylene balls (diameter: 25 mm) to reduce the oxygen input. However, this precaution is not essential as the start-up of another anammox reactor without covering was also successful. There were obviously already enough ammonium oxidisers in the inoculum to use up the oxygen imported by stirring. The oxygen concentration in the reactor always remained below detection levels.

The nitrite produced together with the remaining ammonium, including the nitrification biomass from the first reactor, is used as an influent for the subsequent anaerobic ammonium oxidation. Depending on the exchange volume (0.3–9.5% per cycle), the filling occurred in the first 2–10 min during the pre-set reaction time (1.5–2 h). The

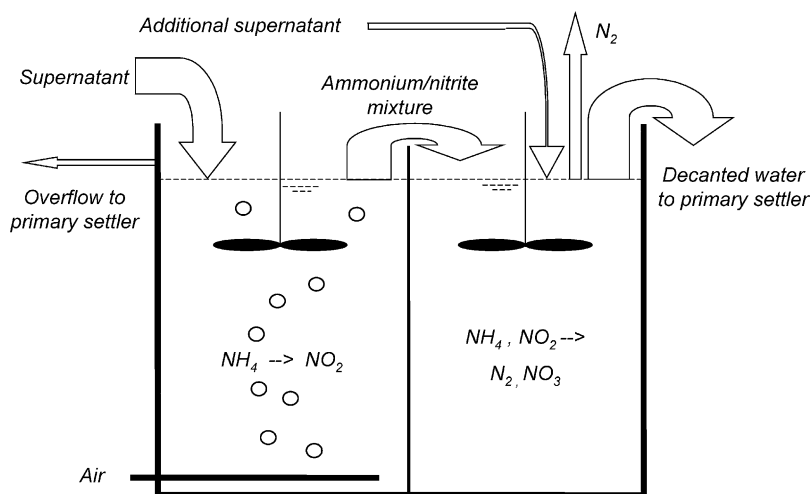


Fig. 2. Reactor configuration for partial nitrification (left) and anaerobic ammonium oxidation (right).

Table 1  
Composition of the two supernatants (grab samples) from the Werdhoelzli WWTP (Zurich) and the Au WWTP (St. Gallen)

Supernatant	Unit	Werdhoelzli (Zurich)		Au (St. Gallen)	
		Mean $\pm$ S.D.	Measurements	Mean $\pm$ S.D.	Measurements
TSS	g TSS m <sup>-3</sup>	344 $\pm$ 112	15	384 $\pm$ 137	11
NH <sub>4</sub>	g N m <sup>-3</sup>	657 $\pm$ 56	50	619 $\pm$ 21	15
NO <sub>2</sub>	g N m <sup>-3</sup>	0.4 $\pm$ 0.7	46	4.7 $\pm$ 6.2	15
NO <sub>3</sub>	g N m <sup>-3</sup>	0.2 $\pm$ 0.7	44	0.2 $\pm$ 0.2	14
PO <sub>4</sub>	g P m <sup>-3</sup>	7.3 $\pm$ 5.0	46	0.6 $\pm$ 0.8	6
Alkalinity	mol m <sup>-3</sup>	56.7 $\pm$ 6.5	30	53.1 $\pm$ 2.5	15
pH	–	7.78 $\pm$ 0.07	45	7.37 $\pm$ 0.15	14
Temp	°C	26.4 $\pm$ 1.3	5	28.0 $\pm$ 2.5	4
Alkalinity/NH <sub>4</sub>	–	1.20 $\pm$ 0.09	30	1.20 $\pm$ 0.04	15

mixing was then stopped and the biomass settled for 10 min before the supernatant was decanted by a floating pump a few centimetres below the surface. A fraction of the raw digester supernatant was added continuously to the second stage to prevent nitrite accumulation. This supply was regulated manually about every second day (between 0 and 10% of the flow from the nitrification reactor) aiming to attain an ammonium effluent concentration of 10–50 g NH<sub>4</sub>-N m<sup>-3</sup>. The pH in the reactor was controlled at 7.52  $\pm$  0.07 by adding a 2 M HCl solution or by CO<sub>2</sub> sparging. The temperature was kept constant in both reactors with the aid of heat exchangers.

## 2.2. Analytical procedures

The ammonium and nitrite were measured colorimetrically after filtration with a flow injection analyser (ASIA, Ismatec AG, CH-Glattbrugg). The nitrate was analysed on an ionchromatograph with a Dionex DX300 (Dionex Corporation, Sunnyvale, CA) and the chromatograms were processed with the AI450 Model 1 software from the Dionex Corporation. The alkalinity was determined by titrating the sample with hydrochloric acid to pH 4.3 using a Mettler–Toledo titrator with an autosampler (Mettler Toledo, Greifensee, Switzerland). The chemical oxygen demand (COD) measurement was based on digestion with potassium dichromate in concentrated sulphuric acid for 2 h at 150 °C. The

test tubes used were manufactured by the Hach Company (Loveland, CO).

The total suspended solids (TSS) of the supernatant, the nitrification biomass and the effluent of the anammox reactors were quantified by filtering the sample through a pre-dried Whatman GF/F glassfibre filter (effective pore width 0.7  $\mu$ m). The filter was dried at 105 °C and weighed in a desiccator after cooling. The anammox biomass was first centrifuged and then flushed on a Schleicher and Schuell paper filter (black ribbon, 591/1, Schleicher & Schuell GmbH, pore width 12–25  $\mu$ m).

## 3. Results

### 3.1. Partial nitrification with supernatant from the Werdhoelzli WWTP, Zurich

The digester supernatant was added continuously, starting with a dilution rate of 0.1 d<sup>-1</sup> (Fig. 3). Until  $t = 49$  days, the nitrogen load was increased in steps and then kept constant for over 1 month at a dilution rate of  $D_x = 1/\text{HRT} = 0.62$  d<sup>-1</sup>. Up to  $D_x = 0.85$  d<sup>-1</sup>, the nitrite/ammonium ratio was around 1.4. A further increase of the influent flow resulted in a rapid decrease of the nitrite concentration, indicating that the ammonium oxidisers were washed out somewhere between  $D_x = 0.85$ – $0.95$  d<sup>-1</sup> (29.8  $\pm$  0.2 °C).

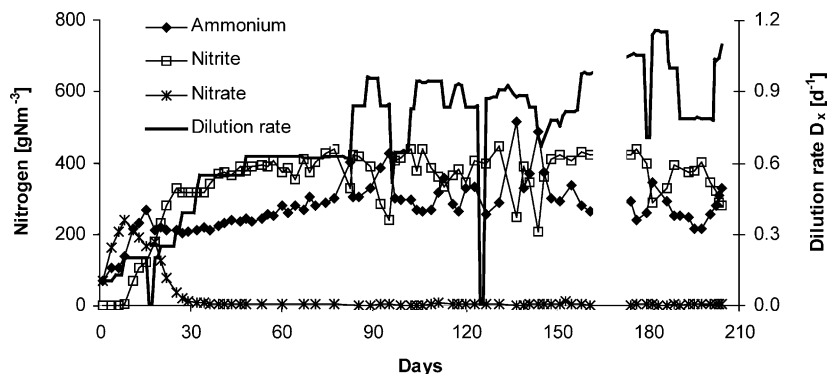


Fig. 3. Soluble nitrogen compounds in the effluent of the nitrification reactor and the dilution rate. Supernatant from the Werdhoelzli WWTP was used for the first 165 days, digester effluent from the Au WWTP for the last 30 days. The reactor was still operated between  $t = 161$  days and  $t = 174$  days but no samples were taken. The low dilution rates at  $t = 16$  days and  $t = 126$  days were caused by a breakdown of the influent pump and aeration failures occurred on days 82, 95, 137 and 144.

The nitrate concentration reached its maximum at  $t = 8$  days ( $240 \text{ g NO}_3\text{-N m}^{-3}$ ) and decreased rapidly thereafter (Fig. 3). At  $t = 29$  days it was already below  $20 \text{ g NO}_3\text{-N m}^{-3}$ . A favourable nitrite/ammonium mixture for the anammox process was already obtained at  $t = 25$  days.

The reactor was not covered for the whole experiment. Until  $t = 28$  days, only a low-powered temperature controller (0.3 kW) was in operation. Consequently the temperature increased rather slowly up to  $26 \text{ }^\circ\text{C}$ , also under the influence of the warm supernatant ( $24\text{--}28 \text{ }^\circ\text{C}$ ) and the reaction enthalpy of the biological process. After  $t = 28$  days, a 2-kW heater kept the temperature at  $30.4 \pm 0.3 \text{ }^\circ\text{C}$ . Although the time required to reach an appropriate nitrite/ammonium mixture in this experiment was then almost a month, the nitrite oxidisers could be washed out even at  $24 \text{ }^\circ\text{C}$ . In order to maintain the temperature in a full-scale nitrification reactor as high as possible, it is advisable to take the supernatant directly from the sludge dewatering facilities. The start-up period is reduced significantly at  $30 \text{ }^\circ\text{C}$ .

No nitrogen loss occurred in the nitrification reactor. The sum of the dissolved nitrogen compounds in the effluent was even a little higher (on average less than 3%) compared with the inlet ammonium concentration. This small deviation could be caused by evaporation in the reactor

because dry air is used for aeration and the air was not recycled into the reactor. The concentration of the suspended solids decreased rapidly during the first few days of the experiment. Under stable operation, the TSS concentration in the nitrification reactor ( $270 \pm 70 \text{ g TSS m}^{-3}$ ) was even lower than in the sludge liquor ( $344 \pm 112 \text{ g TSS m}^{-3}$ ), which indicates a substantial biological degradation of the particulate inlet COD. The pH was an excellent parameter for monitoring nitrification in order to keep the nitrite/ammonium ratio close to 1.3 in the effluent. When the supernatant described in Table 1 was used with a molar alkalinity/ammonium ratio of 1.2, the effluent was optimally suited for the anammox process when the pH was close to 7 (Fig. 4). As long as the nitrification was operated below the maximum dilution rate for the ammonium oxidisers, stable operation was achieved for nearly half a year.

### 3.2. Partial nitrification with supernatant from the Au WWTP, St. Gallen

At  $t = 174$  days (Fig. 3) the pilot plant was fed with digester supernatant from the Au WWTP for another 30 days (same reactor configuration). The reactor temperature was  $28.7 \pm 0.2 \text{ }^\circ\text{C}$  and the average oxygen concentration remained at  $4.5 \text{ g O}_2 \text{ m}^{-3}$  until  $t = 201$  days and then decreased to

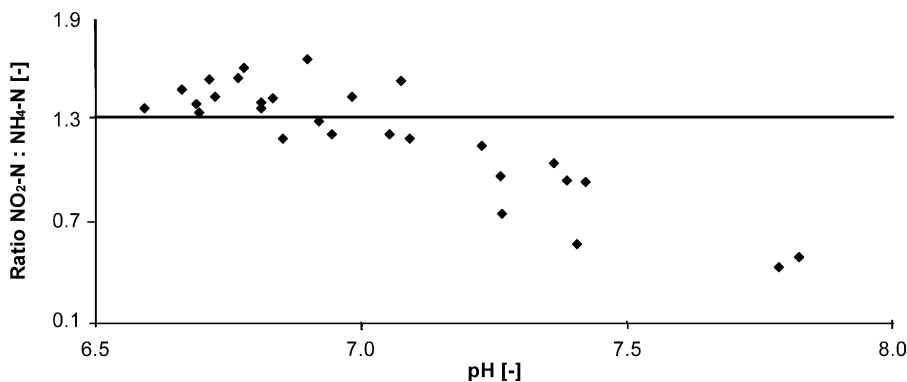


Fig. 4. Ratio of nitrite to ammonium and pH in the nitrification reactor. The bold line indicates the optimal nitrite/ammonium ratio for the anammox reactor according to Eq. (1). The lower the pH the more of the supernatant alkalinity is used up by the nitrification.

$2.7 \text{ g O}_2 \text{ m}^{-3}$ . Between  $t = 181$  and  $186$  days, the dilution rate  $D_x$  was higher than  $1 \text{ d}^{-1}$  causing the nitrite/ammonium ratio to drop below 1.3 in the effluent of the nitrification reactor and therefore also producing high ammonium losses in the effluent of the anammox reactor (Fig. 5). The same effect was also observed after  $t = 201$  days when the lower oxygen concentration reduced the nitrite production. Because the air flow measurements in these experiments were rather inaccurate, it is impossible to characterise the influence of air flow on nitrification. No major difference was observed to the supernatant of the Werdhoelzli WWTP.

### 3.3. Anaerobic ammonium oxidation using supernatant from the Werdhoelzli WWTP

Nitrogen was initially supplied between 50 and  $100 \text{ g N m}_{\text{reactor}}^{-3} \text{ d}^{-1}$  and the influent flow was gradually increased as long as the effluent nitrite concentration was close to zero. Additional supernatant was continuously added by a separate pump, resulting in an ammonium surplus in the effluent of the anammox reactor ( $38 \pm 47 \text{ g NH}_4\text{-N m}^{-3}$ ). The temperature was kept constant at  $31.1 \pm 0.7 \text{ }^\circ\text{C}$ . At  $t = 53$  days (nitrogen load  $270 \text{ g N m}_{\text{reactor}}^{-3} \text{ d}^{-1}$ ), the nitrite concentration in the reactor increased to  $60 \text{ g NO}_2\text{-N m}^{-3}$  over the

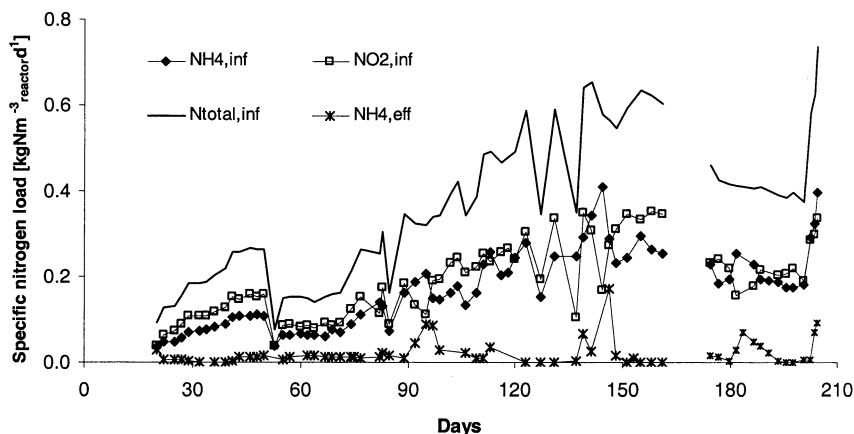


Fig. 5. Ammonium and nitrite in the influent ('inf') and ammonium in the effluent ('eff') of the anammox reactor.  $N_{\text{total,inf}}$  is the sum of  $\text{NH}_{4,\text{inf}}$  and  $\text{NO}_{2,\text{inf}}$ . The nitrate load in the in- and effluent as well as the nitrite in the effluent were rather low and are therefore not shown.

weekend for no apparent reason. The anammox activity was strongly inhibited by this nitrite surplus and the influent nitrogen load had to be reduced to nearly 50% during the following 2 weeks (Fig. 5). Strous et al. (1999) reported a complete loss of anammox activity when the nitrite concentration remained above 5 mM (70 g NO<sub>2</sub>-N m<sup>-3</sup>) for a longer period (12 h). In this experiment, the anammox biomass recovered slowly and the nitrogen load was gradually increased to 650 g N m<sub>reactor</sub><sup>-3</sup> d<sup>-1</sup> at *t* = 139 days (with short load reductions due to pumping failures at *t* = 85, 127 and 137 days). About 85% of the nitrification effluent was finally treated in the anammox process. The cycle length of the anammox SBR was about 120 min with a 90-min reaction time after subtracting the settling and decantation times. At *t* = 134 days, the added nitrite was degraded within 20 min of a cycle which leads to more than a 4-fold overcapacity of the anammox compared with the nitrification reactor of identical volume.

The total nitrogen elimination was 92 ± 7% and depended strongly on the nitrite/ammonium ratio in the inlet of the anammox reactor. A nitrite surplus in the effluent of the nitrification reactor can be balanced by adding raw digester supernatant directly to the anammox reactor. However, a nitrite/ammonium ratio below 1.3 in the influent (caused by aeration problems in the nitrification reactor, for example) will result in an ammonium surplus in the effluent of the anammox reactor (Fig. 5). According to Eq. (1), there should be significant nitrate production, which would lower the nitrogen elimination efficiency. The average ammonium elimination rate with reference to the total influent flow to the anammox reactor was 270 g NH<sub>4</sub>-N m<sup>-3</sup>. The expected nitrate concentration in the effluent should therefore be 26% of the ammonium inflow or 70 g NO<sub>3</sub>-N m<sup>-3</sup> plus the nitrate already produced in the nitrification reactor (4 g NO<sub>3</sub>-N m<sup>-3</sup>). However, only 18 g NO<sub>3</sub>-N m<sup>-3</sup> were detected. It is therefore likely that about 56 g NO<sub>3</sub>-N m<sup>-3</sup> was denitrified by heterotrophs in the anammox reactor (see next section). The TSS concentration increased from 2.9 kg TSS m<sup>-3</sup> at *t* = 90 days to 7.8 kg TSS m<sup>-3</sup> at *t* = 151 days (Fig. 6) and remained more or less

constant until the end of the experiments because there was no further increase in the specific nitrogen load. The solid concentration never rose above 8.3 kg TSS m<sup>-3</sup> in our experiments, but even higher concentrations are possible as long as the sedimentation is good. Well-settling granules (flocs) of a brocade colour accumulated in the course of the experiment.

#### 3.4. Anaerobic ammonium oxidation using digester supernatant from the Au WWTP

The results of anaerobic ammonium oxidation using supernatant from the Au WWTP are also shown in Fig. 5. The total nitrogen elimination rate was 95%, depending mainly on the nitrite/ammonium ratio in the reactor inlet.

On *t* = 204 days about 40 grab samples were taken from the anammox reactor during nearly two SBR cycles to detect the concentration profile of the soluble nitrogen compounds. The total cycle length was 130 min with a mixed *reaction* period of 100 min (including *filling* in the first 20 min), followed by *sedimentation* (10 min) and *decantation* (20 min). As can be seen from Fig. 7, the nitrite is depleted after 40 min and there is no anammox activity during the remaining mixing period (60 min) until the settling period starts. The reactor should therefore either be filled at shorter intervals or the exchange volume could be enhanced. Both approaches were not possible since the capacity of the preceding nitrification step limited the overall maximum rate of nitrogen elimination.

Considering only the mixed cycle period without nitrite limitations of these two cycles, high nitrogen removal rates of up to 2.4 kg N m<sup>-3</sup> d<sup>-1</sup> were obtained (Fig. 7). In this experiment, the average ammonium and nitrite degradation and the nitrate production yielded a ratio of 1:1.38:0.32 and is similar to the stoichiometric ratio given in Eq. (1) (1:1.32:0.26).

As long as nitrite was available during the SBR cycle, the pH increased continually. It can be easily controlled between 7.5 and 8 by adding hydrochloric acid or CO<sub>2</sub> gas. When all the nitrite in the reactor was depleted, the pH remained constant. If the pH is monitored on-line, the frequency of these



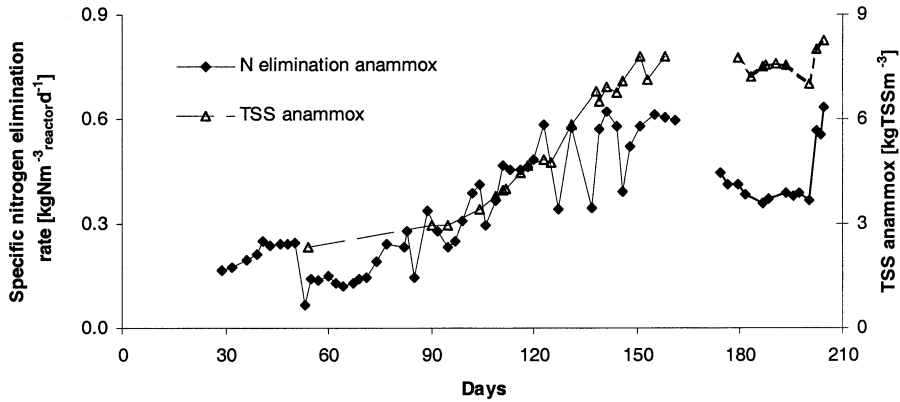


Fig. 6. Increase of the volume-specific nitrogen elimination rate and of the total concentration of suspended solids in the anammox reactor.

acid additions and the length of the constant periods tell the operator whether he can increase the nitrogen load to the anammox reactor or not.

3.5. Sludge production in the combined nitrification/anammox process

Nitrification and anammox are both autotrophic processes so that low sludge production can be

expected. During the last 30 days (supernatant Au WWTP), no significant TSS accumulation was observed in the anammox SBR (Fig. 6). Table 2 shows the total suspended-solids concentration of the supernatant and of the effluents of both reactors for this period. All concentrations were in the same range. A fraction of the inlet solids was consequently hydrolysed and degraded aerobically in the nitrification reactor as well as anoxically in the

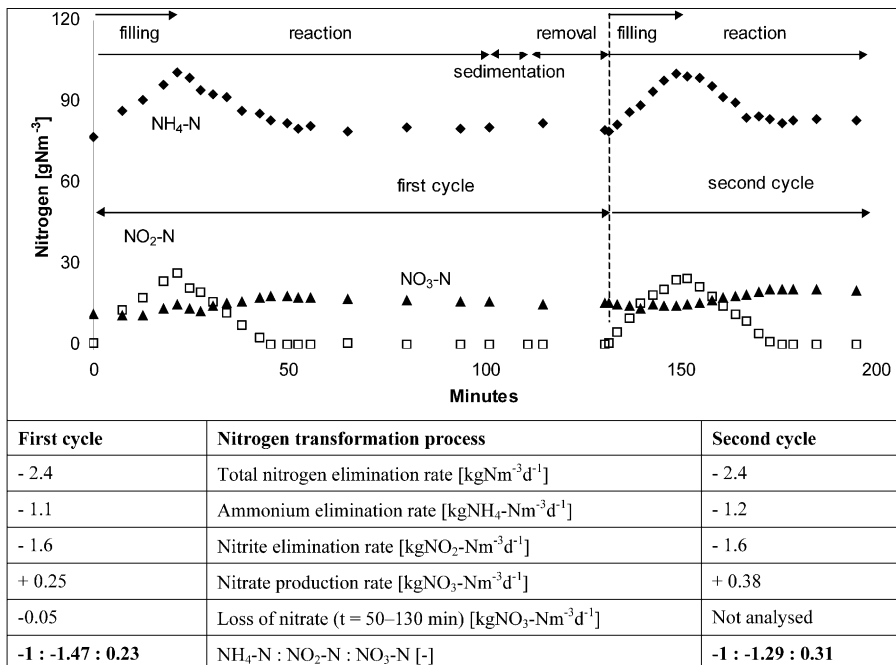


Fig. 7. Concentration profiles of soluble nitrogen compounds and degradation rates in the anammox reactor.

Table 2

Total concentration of suspended solids in the supernatant (Au WWTP) and the effluents of both reactors. When a CSTR is used for nitrification, the TSS concentrations in the effluent and reactor are equal. No excess sludge was withdrawn from the anammox SBR

TSS ( $\text{g m}^{-3}$ )	Mean $\pm$ S.D.	Measurements
Supernatant	384 $\pm$ 137	11
Effluent nitrification	307 $\pm$ 77	8
Effluent anammox	354 $\pm$ 204	8

anammox stage. This assumption is also supported by the nitrate denitrification in the anammox reactor. According to Fig. 7, all the nitrite was used up after 40 minutes and the ammonium stayed constant for the remaining period of the cycle. Therefore no anaerobic ammonium oxidation occurred between  $t = 50$  min and  $t = 130$  min, and the nitrate loss must be due to conventional heterotrophic denitrification. This also explains the low nitrate concentration in the effluent of the anammox process.

#### 4. Discussion

Nitrification was performed in a continuously stirred tank reactor without sludge retention inoculated with normal activated sludge from the Werdhoelzli WWTP. Even at 24 °C it was possible to outperform the nitrite oxidisers and an appropriate nitrite/ammonium mixture for the anammox process was reached within a month. The volume of the nitrification reactor is determined by the reactor temperature, the ammonium concentration in the digester effluent and the growth rate of the ammonium oxidisers. The maximum dilution rate of the ammonium oxidisers ( $D_{x,\text{max}} = 0.85 \text{ d}^{-1}$  to  $0.95 \text{ d}^{-1}$  at 30.0 °C) limited the nitrite production rate to  $0.35 \text{ kg NO}_2\text{-N m}_{\text{reactor}}^{-3} \text{ d}^{-1}$  or  $1.2 \text{ kg NO}_2\text{-N kg}^{-1} \text{ TSS d}^{-1}$ , respectively. This is in accordance with the observations of van Dongen et al. (2001), who reported stable nitrification for over 2 years in a 10 l CSTR with  $D_x = 1 \text{ d}^{-1}$  at temperatures above 30 °C. Because their reactor

was operated at elevated temperatures and the supernatant was more concentrated ( $1.18 \text{ kg NH}_4\text{-N m}^{-3}$ ), the nitrite production rate achieved was as high as  $0.63 \text{ kg NO}_2\text{-N m}_{\text{reactor}}^{-3} \text{ d}^{-1}$ . van Kempen et al. (2001) reported the stable operation of a full-scale SHARON process with denitrification mainly via nitrite at an aerobic retention time of 1.5 d ( $D_x = 0.67 \text{ d}^{-1}$ ) at 35 °C. As long as the process is not run at the limit of the maximum dilution rate of the ammonium oxidisers, stable nitrification was achieved in our experiments, which ran for almost half a year.

Anaerobic ammonium oxidation was carried out in a sequencing batch reactor (SBR) with suspended biomass inoculated with excess sludge from the Koelliken WWTP. Taking into account only the nitrite-containing periods, total nitrogen removal rates of up to  $2.4 \text{ kg N m}^{-3} \text{ d}^{-1}$  or  $0.3 \text{ kg N kg}^{-1} \text{ TSS d}^{-1}$  were obtained at 30 °C. However, the overall nitrogen elimination rate was only about  $0.60 \pm 0.04 \text{ kg N m}^{-3} \text{ d}^{-1}$  due to limitation of the inlet nitrogen load from the nitrification reactor. This is somewhat lower than the  $0.75 \text{ kg N m}^{-3} \text{ d}^{-1}$  or  $0.18 \text{ kg N kg}^{-1} \text{ TSS d}^{-1}$  respectively reported in van Dongen et al. (2001) during a test period of 110 days in a granular-sludge SBR. Helmer et al. (2001) achieved a nitrogen elimination rate of  $1.5 \text{ kg N m}^{-3} \text{ d}^{-1}$  at 28 °C in an anoxic batch test with a moving bed, and the same maximum nitrogen conversion capacity was obtained in a fluidised-bed reactor fed with sludge digestion effluent (Strous et al., 1997). Therefore, provided that the nitrification capacity is high enough, an overall nitrogen elimination rate of  $1 \text{ kg N m}^{-3} \text{ d}^{-1}$  in the anammox reactor is feasible, resulting in a hydraulic dilution rate of  $1\text{--}1.5 \text{ d}^{-1}$ . On average, over 90% of the nitrogen load was eliminated in the anammox reactor with the correct nitrite/ammonium ratio of 1.3 in the influent. The overall sludge production was negligible and a substantial amount of the produced nitrate was denitrified by heterotrophs in the anammox reactor.

The specific growth rate of the anammox biomass can hardly be determined by the TSS concentration in the reactor because the solids accumulation from the digester effluent and the nitrification reactor are unknown. In addition, the

anammox biomass retention efficiency was not measured. However, on the basis of the increase in the specific nitrogen elimination rate between  $t = 90$  days to 151 days (Fig. 6) and an estimated biomass retention efficiency of 95%, the observed specific growth rate is  $0.024 \text{ d}^{-1}$ , corresponding to a doubling time of 29 days. This value is far below the maximum specific growth rate of  $0.065 \text{ d}^{-1}$  (doubling time: 11 days) reported by Strous et al. (1998). By using the anammox biomass of our pilot plant ( $1.5 \text{ m}^3$ ) as an inoculum for the first full-scale installation (about  $500 \text{ m}^3$ ) and assuming an average doubling time of 29 days, we would obtain a start-up period of about 8 months.

Partial nitrification combined with anaerobic ammonium oxidation is feasible and has been thoroughly tested on a pilot plant with digester supernatant. The proposed compact reactor combination (total HRT = 2 d) can be easily implemented within the infrastructure of existing WWTPs. Further research with a nitrification SBR would allow the hydraulic dilution rate and the nitrite production to be increased. Any nitrified supernatant not needed for the anammox reactor can be directly discharged to the primary settler of the WWTP, where the remaining nitrite is denitrified with the degradable COD of the raw wastewater. This step has the advantage of allowing successful partial nitrification if the anammox reactor is not working optimally.

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