

The SHARON[®]-Anammox[®] process for treatment of ammonium rich wastewater

U. van Dongen, M.S.M. Jetten* and M.C.M. van Loosdrecht**

Department of Biotechnology, Faculty of Applied Sciences, Delft Univ. of Technology, Julianalaan 67, NL 2828 BC Delft (E-mail: *M.C.M.vanLoosdrecht@TNW.TUdelft.NL*)

* Present address: Dept. of Microbiology, Faculty of Science, Univ. of Nijmegen, NL 6525 ED Nijmegen, the Netherlands

** Corresponding author

Abstract The treatment of ammonium rich wastewater, like sludge digester effluent, can be significantly improved when new biotechnological processes are introduced. In this paper, the combination of a partial nitrification process (SHARON[®]) and anoxic ammonium oxidation (Anammox[®]) process for the treatment of ammonia rich influents is evaluated. Herein the combined process has been studied with sludge recycle liquor from the WWTP Rotterdam-Dokhaven. The SHARON process was operated stably for more than 2 years in a 10 l CSTR under continuous aeration with a HRT of 1 day. The ammonia in the sludge liquor was converted by 53% to nitrite only. During the test period no formation of nitrate was observed. The effluent of the SHARON process was ideally suited as influent for the Anammox reactor. The Anammox process was operated as a granular sludge SBR-process. More than 80% of the ammonia was converted into dinitrogen gas at a load of 1.2 kgN/m³ per day. Planctomycete-like bacteria dominated the mixed community of the Anammox reactor, and only a small percentage of the population consisted of aerobic ammonium-oxidizing bacteria. This showed that the ammonium-oxidizers in the effluent of the SHARON process did not accumulate in the SBR. The test period showed that the combined SHARON-Anammox system can work stably over long periods and the process is ready for full-scale implementation.

Keywords Partial nitrification; nitrite; aerobic and anaerobic ammonium oxidation; sludge liquor; SHARON; Anammox

Introduction

Ammonia is one of the most important components in wastewater which has to be removed before effluents can be discharged. This is mostly achieved by complete oxidation to nitrate, and subsequent reduction of the nitrate to dinitrogen gas under anoxic conditions at the expense of COD. The introduction of oxygen (air) into wastewater for the oxidation of ammonium requires a large amount of energy. Furthermore, the amount of COD present in the wastewater is often limited, making the purchase of COD in the form of methanol necessary. Due to the long sludge age required for nitrification, large reactors (area requirement) are needed. Some of these limitations might be circumvented by application of two recently developed new biotechnological processes: partial nitrification of ammonia to nitrite by fast growing nitrifiers, and denitrification of nitrite to dinitrogen gas using ammonia as electron donor. In this way nitrogen is removed with a minimum of COD and energy.

A nitrogen removal process with minimal energy and COD use

In Figure 1 a basic flow scheme of the proposed SHARON-Anammox concept, already partially implemented at the WWTP Dokhaven, Rotterdam, the Netherlands, is depicted. The sludge recycle water contains typically 15% of the total plant load with only 1% of the hydraulic load. The ammonia (1–1.5 gNH₄-N/l) in the sludge liquor is removed by applying a partial oxidation of ammonium to nitrite, whereafter the nitrite is denitrified with ammonium as electron donor. The two systems essential for these processes have recently been

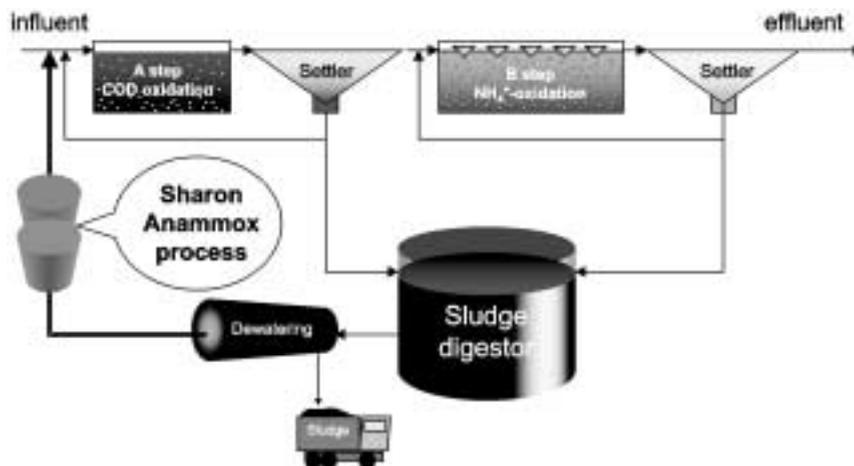


Figure 1 Implementation of the SHARON-Anammox process at the WWTP Rotterdam-Dokhaven

developed in our department: The SHARON[®] and Anammox[®] process (van Loosdrecht and Jetten 1998). In this way the oxygen requirement for nitrogen removal is reduced by 60%, no COD is needed, the sludge production is marginalized, and the net CO₂ emissions strongly reduced.

Ammonium oxidation without biomass retention

The SHARON process (Hellinga *et al.*, 1997, 1999) is operated without any biomass retention. This means that the sludge age (SRT) equals the hydraulic retention time (HRT). In such a system the effluent concentration is only dependent on the growth rate ($1/SRT$) of the bacteria involved, and independent of the influent concentration. During operation of the SHARON process at temperatures above 25°C, fast growing ammonium oxidisers are selected. However, these organisms have a low affinity for ammonium (affinity constant 20–40 mgNH₄-N/l). In practice this will lead, by application in a chemostat, to effluents with relatively high ammonium concentrations (50–100 mg N/l). Therefore the SHARON process is most suited to treat wastewater with a high ammonium concentration (>500 mg N/l), where the effluent quality is not critical.

The SHARON process on sludge digestion effluents is performed at 30–40°C in a chemostat without any biomass retention, therefore the dilution rate can be set at such a rate that ammonium oxidizers grow fast enough to stay in the reactor, while the nitrite oxidizers are washed out. The SHARON process has been operated in the laboratory (2 l reactor) on digestion effluent for more than 2 years. It was directly scaled-up to full scale (1800 m³) where it is working according to expectation (Mulder *et al.*, 2001).

The mixed microbial community present in the SHARON biomass was investigated with molecular ecological techniques (Logemann *et al.*, 1998). Total DNA was extracted from biomass samples and used for PCR amplification with universal bacterial primers. The PCR product was used to construct a gene library. Analysis of the clones revealed that the dominant clone (69%) was highly similar to *Nitrosomonas eutropha*. This was qualitatively and quantitatively confirmed by two independent microscopic methods. The presence of about 50–70% ammonia oxidizing bacteria was demonstrated using a 16 S rRNA targeted fluorescent oligonucleotide probe (NEU653) specific for *Nitrosomonas* species. *Nitrosomonas eutropha* has been described in the literature as a fast growing nitrifier able to grow at high ammonium and nitrite concentrations.

The SHARON process producing an ammonium-nitrite mixture

When the SHARON reactor is used to provide the feed for the Anammox process only 50% of the ammonium needs to be converted to nitrite:



This reaction stoichiometry implies that no extra addition of base is necessary, since sludge liquor resulting from anaerobic digestion will generally contain enough alkalinity (in the form of bicarbonate) to compensate for the acid production if only 50% of the ammonium is oxidized. The possibility to produce a 50:50 mixture of ammonium and nitrite with the Sharon process has been evaluated extensively in a laboratory system with sludge liquor from the WWTP Rotterdam as influent. The results (Figure 1, Table 1) showed that indeed a stable conversion was possible. The ammonium was oxidized by 53% to nitrite at 1.2 kg N load per m³ per day, without any need of pH control. The ammonium oxidizing bacteria tolerated high concentrations of nitrite (>0.5 g NO₂-N/l at pH 7).

The ammonium/nitrite ratio in the effluent of the SHARON process can be sensitively influenced by changing the reactor pH between 6.5 and 7.5. In this manner the exact ratio for full nitrogen removal in the Anammox process can be obtained. During the experimental period several successful tests were performed (phase 3 and 5) to evaluate the possibility of using the pH as a control method for setting the desired ammonium/nitrite ratio in the

Table 1 Conversion in the SHARON reactor during the test period. The influent was the centrate of the digested sludge centrifuges at the WWTP Rotterdam-Dokhaven (HRT = SRT = 1 day)

Parameter	Unit	Steady state operation	Total period (240 d)
Influent NH ₄ -N	kg/m ³	1.18±0.14	1.17±0.25
Influent NO _x	kg/m ³	0	0
Effluent NH ₄ -N	kg/m ³	0.55±0.10	0.60±0.20
Effluent NO ₂ -N	kg/m ³	0.60±0.10	0.55±0.20
Effluent NO ₃ -N	kg/m ³	0	0
pH		6.7±0.3	6.8±1.2
NH ₄ -N conversion	%	53	49
N-conversion	kg/m ³ /d	0.63±0.10	0.52±0.20

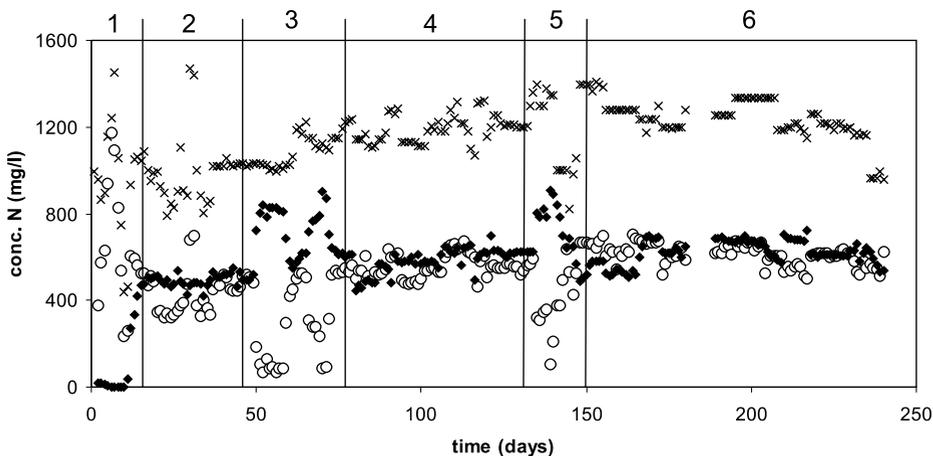


Figure 2 Ammonium conversion in the SHARON reactor with continuous operation. HRT and SRT were both one day. Period 1: start up period, Period 2,4 and 6 steady state operation without pH control, Period 3 and 5 test period to evaluate effect of reactor pH on conversion. (x: NH₄-N in; o: NH₄-N out; •: NO₂-N out)

effluent. For this control the principle of a chemostat system is used: at a constant dilution rate the effluent substrate concentration will be constant. It has been shown that NH_3 rather than NH_4^+ is the active substrate (Hellenga *et al.*, 1999). If the pH is increased, a constant ammonia level means a lower ammonium level. i.e. by increasing the pH the amount of ammonium in the effluent decreases rapidly. It was shown in period 3 and 5 that indeed a slight change in pH already leads to a large change in the effluent ammonium/nitrite ratio. Without control already a total conversion of >90% can be obtained, it is therefore questionable whether the extra removal by a pH control is economically worthwhile.

The Anammox process

The Anammox process is a process in which under anoxic conditions nitrite is converted to dinitrogen gas with ammonium as electron donor:



The bacteria catalyzing the Anammox reaction are autotrophic, this means that nitrite can be converted to dinitrogen gas without the use of COD or the addition of external methanol (Jetten *et al.*, 1998). The Anammox process has been discovered in a pilot plant installation of Gist-brocades (Mulder *et al.*, 1992, 1995). The biological nature of the process could be demonstrated since the Anammox activity was inactivated by either gamma irradiation, heating of the pilot plant sludge or incubation with various inhibitors (Jetten *et al.*, 1998). The cells were reversibly inhibited by oxygen at concentrations as low as 0.5% air saturation (Strous *et al.*, 1997a, Jetten *et al.*, 1998). Furthermore it was observed that nitrite was the preferred electron acceptor for the process.

The bacteria responsible for the process have been enriched in a sequencing batch reactor on a synthetic medium containing ammonium, nitrite and bicarbonate (Strous *et al.*, 1998, 1999b). The growth rate (doubling time 11 days) and growth yield (0.11 gVSS/g $\text{NH}_4\text{-N}$) of the organisms is very low. A clear advantage of the Anammox process is therefore a low sludge production. However, systems with an efficient biomass retention such as the used SBR system will be necessary to keep all the Anammox biomass in the reactor and long start-up times will be required to grow enough biomass. The high maximum specific nitrogen consumption rate (0.82 gN/gVSS.day), the very high affinity for ammonia and nitrite ($K_s < 0.1$ mg N/l) and the granular growth allowing efficient biomass retention, makes the design of very compact installations possible.

Previous studies have shown that some *Nitrosomonas* species were also capable of ammonium oxidation with nitrite as the electron acceptor. Under anoxic or oxygen-limiting conditions the reaction rate was less than 0.08 gN/gVSS.day (Bock *et al.*, 1995; Jetten *et al.*, 1999; Kuai, Verstraete, 1998; Schmidt, Bock, 1997; Schmidt, Bock, 1998; Zart, Bock, 1998). The Anammox activity of our cultures was much higher than this rate. Furthermore our cultures were dominated 70% or more by one morphotypical microorganism. It was shown to have three properties in common with members of the Order *Planctomycetales*: cell division by budding, internal cell compartmentalization, the presence of crateriform structures in the cell wall, and the presence of unusual lipids in the membranes (Strous *et al.*, 1999a). Based on the 16S RNA analysis the Candidatus name *Brocadia Anammoxidans* has been proposed as the responsible organism for the Anammox process.

Recently substantial N-losses (Table 2) have been reported for several wastewater treatment systems (Helmer and Kunst, 1998; Hippen *et al.*, 1996; Siegrist *et al.*, 1998, Schmid *et al.*, 2000). With a very high nitrogen load and limited air supply, a substantial amount of ammonia was lost as gaseous nitrogen compounds. In such systems conditions might pre-

Table 2 Reports on Anammox like activity and presence of planctomycete like Anammox bacteria

System	Influent	Conditions	FISH Neu/Amx	Reference
RBC	Wastewater	O ₂ -limited	+/+	Siegrist <i>et al.</i> 1998
RBC	Leachate	O ₂ -limited	+/+	Hippen <i>et al.</i> 1996 Helmer 1998
Trickling Filter	NH ₄ -medium	O ₂ -limited	+/+	Schmid <i>et al.</i> 2000
Packed Bed	NH ₄ -medium	Anoxic	-/+	Ashbolt pers. comm.
Fluid Bed	NH ₄ -medium	Anoxic	-/+	Jetten <i>et al.</i> 1998
SBR	NH ₄ -medium	Anoxic	-/+	Strous <i>et al.</i> 1998
SBR	Sludge liquor	Anoxic	-/+	This paper

vail in which both nitrifiers and anaerobic ammonia-oxidizing bacteria could co-exist (Schmid *et al.*, 2000). With the aid of specific FISH probes it was established that Anammox-like bacteria are present in substantial amounts in these processes. Only in the microaerophilic reactors were substantial conventional nitrifiers found. These observations indicate that anaerobic ammonium oxidation might be widespread in nature and can be obtained from many different sources.

Feasibility study

In a recent feasibility study (Strous *et al.*, 1997a) the removal of ammonium from sludge digester effluents was investigated with the Anammox process. The results of this study showed that compounds in the digester effluent did not negatively affect the Anammox sludge. The pH (7.0–8.5) and temperature (30–37°C) optimum for the process were well within the range of the values expected for digester effluents. Experiments with a laboratory scale (2-litre) fluidized bed reactor showed that the Anammox sludge was capable of removing ammonium and nitrite efficiently from the sludge digester effluent. The nitrogen load of the Anammox fluidized bed reactor could be increased from 0.2 kg N_{tot}/m³d to 2.6 kg N_{tot}/m³d. Due to the nitrite limitation, the maximum capacity was not reached. During experiments with synthetic wastewater, values of 5.1 kg N_{tot}/m³d have been obtained (Jetten *et al.* 1998).

The combination of the Anammox process and a partial nitrification (SHARON) process has been successfully tested using sludge digester effluent. The SHARON reactor was operated without pH control with a total nitrogen load of about 1.2 kg N/m³ per day. The ammonium present in the sludge digester effluent was converted for 53% without pH control (Table 1). In this way an ammonium-nitrite mixture suitable for the Anammox process was generated. The effluent of the SHARON reactor was used as influent for the Anammox sequencing batch reactor. In the nitrite limited Anammox reactor all nitrite was removed, the surplus ammonium remained. During the test period the nitrogen load was 0.75 kg N per m³ per day (Table 3). The activity reached values as high as 0.8 kg N per kg dry weight per day.

One of the critical aspects of the feasibility study was the possible influence of biomass (ammonium oxidizers and bacteria in the sludge liquor) in the influent of the Anammox process on the Anammox process. Even a slight accumulation of the sludge from the influent in the Anammox reactor could negatively influence the Anammox process. The net production of Anammox cells is low and accumulation of the influent biomass would dilute the Anammox biomass significantly. FISH analysis showed that the majority of the bacteria in the Anammox reactor were of the Anammox type, only a small amount of nitrifiers originating from the SHARON process could be detected. Moreover the amount of ammonium oxidizing bacteria in the Anammox effluent and influent were comparable. This indicated that the washed out biomass from the SHARON system (which operates without biomass

Table 3 Conversion in a granular sludge SBR Anammox reactor fed with a nitrified effluent from a SHARON reactor (Table 1)

Parameter	Unit	Steady state operation
Test period	day	110
Influent NH ₄ -N	kg/m ³	0.55±0.10
Influent NO ₂ -N	kg/m ³	0.60±0.10
NH ₄ -N conversion	kg/m ³ /d	0.35±0.08
NO ₂ -N conversion	kg/m ³ /d	0.36±0.01
Effluent NO ₂ -N	kg/m ³	0
Volumetric conv.	kg N _{tot} /m ³ /d	0.75±0.20
Sludge conversion	kg N _{tot} /kg SS/d	0.18±0.03

retention) has no negative effect on the Anammox process when it is performed in a granular sludge reactor.

Presently the full-scale implementation of the combined SHARON-Anammox process is evaluated. To this purpose a full process design and an economical evaluation were made for the treatment of the sludge liquor at the WWTP Rotterdam Dokhaven. The process design is given in Table 4. Three cases were evaluated since the sludge management can have considerable effects on the flow and concentration of the centrate water. Direct digestion of the excess sludge leads to an ammonium content of 500 mgN/l. Concentrating the sludge by thickening or centrifuge before digestion gives a much higher ammonium concentration and lower flow. Processes without sludge retention (SHARON) are mainly dimensioned on the hydraulic retention time, the SHARON reactor size is therefore strongly influenced by a more concentrated influent. Biofilm processes are largely dimensioned on the actual load and will not be influenced by the influent concentration. The retention time is here the variable parameter. Since in biofilm reactors the biofilm area is largely determining the conversion capacity, granular sludge type processes (e.g. Granular sludge SBR, UASB or Internal Circulation (IC) reactors) lead to a much smaller reactor size.

Based on the process a cost estimate was made. Herein it was assumed the installation would have to be built on a new site. The costs should be seen as indicative since absolute values can be very site specific. These costs can be compared to similar calculations for other processes that have been tested on pilot plant scale for N-removal from sludge digestion liquors (STOWA, 1995). For a SHARON process with denitrification with methanol this gave an estimation of $f2-3$ /kgN removed. In this comparison it turns out that the costs for methanol and aeration for conventional denitrification balance the extra investments for a second Anammox reactor. Other biological techniques (e.g. biofilm and membrane

Table 4 Dimensions of a full scale Sharon-Anammox process for three different cases

Reactor	Parameter	Unit	Case 1	Case 2	Case 3
General	N-Load	kg N/day	1,200	1,200	1,200
	NH ₄ -N Concentration	kg N/m ³	500	1,200	2,000
	Influent flow	m ³ /day	2,400	1,000	600
Sharon-reactor	Volume	m ³	3,120	1,300	780
	Oxygen Demand	kg O ₂ /day	2,181	2,181	2,181
	Air Demand	Nm ³ /day	56,000	56,000	56,000
Moving bed	Volume	m ³	450	450	450
	Anammox-reactor	HRT	4.5	11	18
Granular Sludge	Volume	m ³	75	75	75
	Anammox-reactor	HRT	0.75	1.8	3

^aCalculated assuming an oxygen consumption of 15 g/Nm³/m_{reactor}

Table 5 Cost estimation for the SHARON Anammox process for the three cases mentioned in Table 4

Parameter	Unit	Case 1	Case 2	Case 3
N-load	kg N/day	1,200	1,200	1,200
Flow	M ³ /day	2,400	1,000	600
Concentration	kg/m ³	500	1,200	2,000
Investments	kf	4,983	3,997	3,603
Depreciation	kf/year	528	433	393
Maintenance	kf/year	101	90	83
Personel	kf/year	24	24	24
Total D-M-P	kf/year	653	547	500
Electricity	kf/year	181	167	163
Total cost	kf/year	834	714	663
Cost per kg N removed	f	2.30	1.97	1.83

processes) have a higher investment cost and higher running costs due to the conversion over nitrate resulting in f 5–10/kg N removed. For the physical/chemical techniques values of f 10–25/kg N removed were estimated. These values can change greatly if e.g. energy is freely or at low cost available. Nevertheless the pretreatment necessary to remove carbonates in the physical processes contributes significantly to the price.

Conclusions

Two new concepts for the removal of nitrogen from wastewater have been developed in which a substantial reduction in the energy and chemical use is achieved. By the use of the combined SHARON-Anammox process, the nitrogen removal will no longer require the input of COD. The combined system can thus be operated independently. This makes it possible to optimize the COD and nitrogen removal separately. The proposed concept has been tested over a prolonged period showing a stable effluent, high ammonium removal without need for process control. Given the positive cost calculation a full-scale implementation can be expected in the near future.

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References

- Bock, E., Schmidt, I., Stuvén, R. and Zart, D. (1995). Nitrogen loss caused by denitrifying *Nitrosomonas* cells using ammonium or hydrogen as electron acceptor. *Arch. Microbiol.* **163**, 16–20.
- Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M. and Heijnen, J.J. (1998). The SHARON process: an innovative method for nitrogen removal from ammonium-rich waste water. *Wat. Sci. Tech.* **37**(9), 135–142.
- Hellinga, C., van Loosdrecht, M.C.M. and Heijnen, J.J. (1999). Model based design of a novel process for nitrogen removal from concentrated flows. *Math. Comp. Modell. Dyn. Sys.* **5**, 1–13.
- Helmer, C. and Kunst, S. (1998). Simultaneous nitrification/denitrification in an aerobic biofilm system. *Wat. Sci. Tech.* **37**(4–5), 183–187.
- Hippen, A., Rosenwinkel, K.H., Baumgarten, G. and Seyfried, C.F. (1996). Aerobic deammonification: a new experience in the treatment of wastewaters. *Wat. Sci. Tech.* **35**(10), 111–120.
- Jetten, M.S.M., Horn, S.J. and van Loosdrecht, M.C.M. (1997). Towards a more sustainable municipal wastewater treatment system. *Wat. Sci. Tech.* **35**(9), 171–180.

- Jetten, M.S.M., Strous, M., van de Pas-Schoonen, K.T., Schalk, J., van Dongen, L., van de Graaf, A.A., Logemann, S., Muyzer, G., van Loosdrecht, M.C.M. and Kuenen, J.G. (1998). The anaerobic oxidation of ammonium. *FEMS Microbiol. Reviews* **22**, 421–437.
- Logemann, S., Schantl, J., Bijvank, S., van Loosdrecht, M.C.M., Kuenen, J.G. and Jetten, M.S.M. (1998). Molecular microbial diversity in a nitrifying reactor system without sludge retention. *FEMS Microbiol Ecol* **27**, 239–249.
- Mulder, A. (1992). Anoxic Ammonium Oxidation US patent 427849(5078884) United States patent.
- 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 Microbiol Ecol.* **16**, 177–83.
- Mulder, J.W., Van Loosdrecht, M.C.M., Hellinga, C. and Van Kempen, R. (2001). Full scale application of the SHARON process for treatment of rejection water of digested sludge dewatering. *Wat. Sci. Tech.*, **43**(11), 127–134.
- Siegrist, H., Reithaar, S. and Lais, P. (1998). Nitrogen loss in a nitrifying rotating contractor treating ammonium rich leachate without organic carbon. *Wat. Sci. Tech.* **37**(4–5), 589–591.
- Schmid, M., Twachtmann, U., Klein, M., Strous, M., Juretschko, S., Jetten, M., Metzger, J., Schleifer, K.H. and Wagner, M. (2000). Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. *Sys. Appl Microbiol.* **23**, 93–106.
- Stowa (1995). Treatment of nitrogen rich return flows of sewage treatment plants (in Dutch). STOWA report 95–08, Utrecht The Netherlands.
- Strous, M., van Gerven, E., Ping, Z., Kuenen, J.G. and Jetten, M.S.M. (1997a). Ammonium removal from concentrated waste streams with the Anaerobic Ammonium Oxidation (Anammox) process in different reactor configurations. *Wat. Res.* **31**, 1955–1962.
- Strous, M., van Gerven, E., Kuenen, J.G. and Jetten, M.S.M. (1997b). Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge. *Appl. Environ. Microbiol.* **63**, 2446–2448.
- Strous, M., Heijnen, J.J., Kuenen, J.G. and Jetten, M.S.M. (1998). The sequencing batch reactor as a powerful tool to study very slowly growing micro-organisms. *Appl. Microbiol. Biotechnol.* **50**, 589–596.
- Strous, M., Fuerst, J., Kramer, E., Logemann, S., Muyzer, G., van de Pas, K., Webb, R., Kuene, J. and Jetten, M. (1999a). Missing lithotroph identified as new planctomycete. *Nature* **400**, 446–449.
- Strous, M., Kuenen, J.G. and Jetten, M.S.M. (1999b). Key physiology of anaerobic ammonium oxidation. *Appl. Environ. Microbiol.* **65**, 3248–3250.
- van Loosdrecht, M.C.M. and Jetten, M.S.M. (1998). Microbiological conversions in nitrogen removal. *Wat. Sci. Tech.* **38**(1), 1–7.