Development of Anammox Reactor Equipped with a Degassing Membrane to Improve Biomass Retention

Kosuke Matsunaga* Tomonori Kindaichi*, Noriatsu Ozaki*, Akiyoshi Ohashi*, Yoshihito Nakahara**, and Manabu Sasakawa**

* Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima 739-8527, Japan
(E-mail: *m106324@hiroshima-u.ac.jp*; *tomokin@hiroshima-u.ac.jp*; *d103229 @hiroshima-u.ac.jp*; *ojaki@hiroshima-u.ac.jp*; *ecoakiyo@hiroshima-u.ac.jp)*
** Aqua Development Center, Mitsubishi Rayon Co., Ltd., 4-1-2 Ushikawadori, Toyohashi, 440-8601, Japan

Abstract
In up-flow anammox reactors, one of the contributing factors of biomass wash-out is the adherence of nitrogen gas produced by anammox reaction to biomass. In this study, we operated an up-flow anammox reactor equipped with a degassing membrane to minimize the biomass wash-out from the reactor by separating the produced gas from the biomass. In addition, both the effect of degassing on the anammox reactor performance and the durability of the membrane submerged in the anammox reactor were investigated. The results show that the use of the degassing membrane in the anammox reactor could 1) Improve the biomass retention ability (by separating the produced gas from the biomass), and 2) Increase the component ratio of anammox bacteria in the reactor. In addition, degassing could reduce the N_2O emission produced in the reactor (for the gas selectivity of the degassing membrane). No membrane fouling was observed even after two months of operation without washing, indicating an advantage to the use of the degassing membrane.
Keywords
Anaerobic ammonium oxidation (anammox), degassing membrane, biomass retention

INTRODUCTION

The anaerobic ammonium oxidation (anammox) process is a novel and promising alternative in which ammonium is oxidized to nitrogen gas using nitrite as the electron accepter (Mulder *et al*., 1995). This process has advantages such as lower oxygen demanded and no requirement for external carbon sources, so the anammox process would allow the reduction of costs compared to the traditional nitrogen treatment process. However, the anammox bacteria grow so slowly, with doubling times of weeks in many ecosystems (Strous *et al*., 1998, 1999a, b), thus the application of anammox needs a long start-up time. Moreover, an additional problem is caused by the loss of a fraction of the sludge washed out with the effluent despite minimizing the wash-out of biomass from the reactor, and this becomes critical when slow-growing bacteria are used. For this reason, in order to avoid biomass wash-out with the effluent, an efficient system or operation strategy is required. In order to improve the biomass retention and the stability process, a sequencing batch reactor (SBR) was successfully used to grow anammox biomass (Strous *et al*., 1997, 1999; Dapena-Mora *et al*., 2004a). These reactors were operated with an additional mechanical stirring in order to improve the biomass retention and prevent the entrapment of nitrogen bubbles, therefore increasing the stability of the process. Other systems that have been used with success include a reactor containing non-woven media for biomass immobilization (Furukawa *et al*., 2003) and an up-flow system seed with anaerobic granular sludge (Imajo *et al*., 2004). However, a fraction of the generated biomass is inevitably washed out with the effluent in all these systems. Therefore, other approaches for improving biomass retention are required. In up-flow anammox reactors, one of the contributing factors of biomass wash-out is the adherence of nitrogen gas produced by anammox reaction to biomass. We hypothesized that the retention of anammox biomass in the reactor may be improved when the produced gas is separated from the biomass. Then, we focused on the degassing membrane to achieve separation of the produced gas from the biomass. As an example of the application of the degassing membrane to the biological treatment process, Bandara *et al*. (2011) operated a degassing membrane reactor, using degasification to recover the residual dissolved

methane in the effluent of an anaerobic wastewater treatment reactor.

In this study, we operated an up-flow anammox reactor equipped with a degassing membrane to minimize the biomass wash-out from the reactor by separating the produced gas from the biomass. In addition, the effects of degassing on the anammox reactor performance and the durability of the membrane submerged in the anammox reactor were investigated.

MATERIALS AND METHODS

Degassing membrane reactor

Two types of anammox reactors each equipped with degassing membrane modules, —a degassing reactor, and a control reactor—, were operated to compare the reactor and degassing performances in this study (Fig. 1). The membrane module involved three-layer hollow fiber membrane (MHF, Mitsubishi Rayon Co., Ltd., Tokyo, Japan) with a total membrane area of 1.766 m^2. To determine the effect of degassing, one membrane module was continuously degassed by using a vacuum pump (DAP-6D, ULVAC Inc. Chigasaki, Japan) at 0.09 MPa during the reactor operation. The reactor volume was 1340 cm^3 (diameter, 8.9 cm; height 40 cm). The hydraulic retention time (HRT) was 5.1 h. Temperature was maintained at 37 °C. The anammox biomass was obtained from a fixed-bed biofilm column reactor, which was developed previously (Tsushima *et al.*, 2007), and then 1.37 g (dry weight) of granular anammox biomass was directly inoculated into the rectors. The synthetic nutrient medium consisted of $(NH_4)_2SO_4$ (35.7-214.3 mM), $NaNO_2$ (71.4-428.6 mM), $KHCO_3$ (5.0 mM), KH_2PO_4 (0.2 mM), $MgSO_4 \cdot 7H_2O$ (1.2 mM), $CaCl_2 \cdot 2H_2O$ (1.4 mM), and 1.0 ml/l of trace elements solution I and II (van de Graaf *et al.*, 1996).

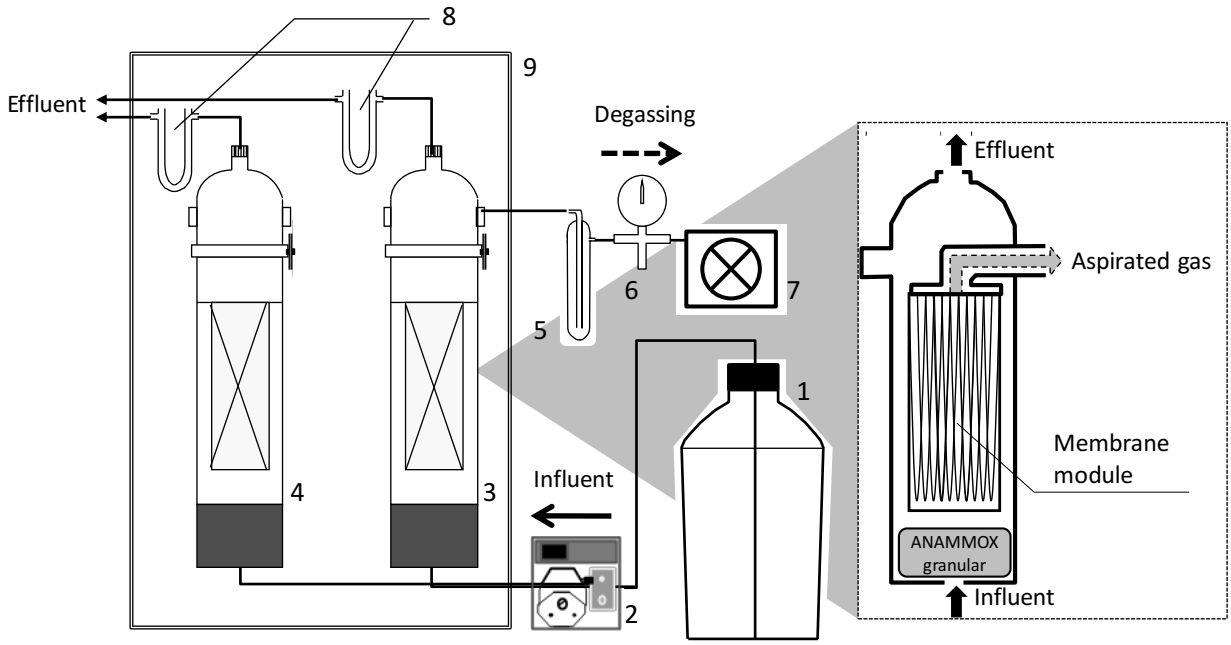

Fig. 1. Scheme of the reactors. The numbers represent the feed vessel (1), influent pump (2), degassing reactor (3), control reactor (4), cold trap (5), pressure meter (6), vacuum pump (7), U-trap (8), and incubator (9).

Sampling and analysis

The concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N were determined using ion-exchange chromatography (HPLC 10Avp; Shimadzu Co., Kyoto, Japan). Suspended solids (SS) in effluent were determined according to the Standard Methods (APHA, 1998). The amount of biomass wash-

out (SS) was measured as two different biomass: free biomass and granular biomass. The free biomass was periodically corrected from the effluent. On the other hand, granular biomass was trapped in the U-trap, and was measured at random times (as shown as arrows in Fig. 3). The doubling time of anammox bacteria was estimated based on the amount of biomass before and after the reactor operation. The exhaust gas was collected into the gas bag and the gas volume was measured by using a gas-meter (Shinagawa Co., Tokyo, Japan). The aspirated gas was directly collected in a syringe via the degassing port. To determine gas composition, N_2 and O_2 gas concentrations were measured utilizing a gas chromatograph equipped with a thermal conductivity detector (TCD) (GC-8A, Shimadzu), and the N_2O and CO_2 gases were determined by using a gas chromatograph equipped with an electron capture detector (ECD) (GC-14BPsE, Shimadzu).

Measurement of permeation rate

To evaluate the membrane fouling, permeation tests were conducted before and after the reactor operation. In this test, the permeation rates of five different gases (O_2, N_2, CO_2, N_2O, and CH_4) were measured separately to confirm the gas selectivity of the membrane. The permeation rate was calculated according to the following equation:

$$P = \frac{V}{A \cdot p \cdot t}$$

where, V is the permeated gas volume (m^3), A is the total membrane area (1.766 m^2), p is the suction pressure (0.09 MPa), and t is the suction time (h).

FISH analysis

A FISH analysis was performed according to the procedure described by Amann *et al*. (1995) and Okabe *et al*. (1999). The 16S rRNA-targeted oligonucleotide probes used in this study were EUB338 (Amann *et al*., 1990), EUB338-II (Daims *et al*., 1999) and EUB338-III (Daims *et al*., 1999), all used in a mixture (EUB338mix) for most bacteria, and Amx820 (Schmid *et al*., 2000) for anammox bacteria. The probes were labeled with fluorescein isothiocyanate (FITC) or tetramethylrhodamine 5-isothiocyanate (TRITC). A LSM5 PASCAL confocal laser scanning microscope (CLSM; Carl Zeiss) equipped with an Ar ion laser (488 nm) and a HeNe laser (543 nm) was used for microscopic observation. Image combining and processing were performed with the standard software package provided by Zeiss, as described previously (Kindaichi *et al*., 2004; Kindaichi *et al*., 2007; Kindaichi *et al*., 2011). For quantitative determination of the microbial composition in the reactor, the surface fractions of the Amx820 probe-hybridized cell area and the EUB338mix probe-hybridized cells (total biomass) were determined after simultaneous *in situ* hybridization. The average surface fraction was determined from at least 10 randomly-chosen CLSM projection images obtained from each of the duplicate anammox biomass samples by using image analysis software provided by Zeiss. The fraction was statistically compared using a Student's t-test.

RESULS AND DISCUSSION

Reactor performance

The simultaneous removal of NH_4^+ and NO_2^- was observed shortly after the inoculation, suggesting that an anammox reaction was occurring in both the reactors. From Day 12, effluent was collected into a tank and SS was determined to evaluate the biomass concentration in effluent (i.e., biomass wash-out) from the reactors. The degassing was started in the degassing reactor on Day 24. After the degassing, the nitrogen removal rates in both the reactors were almost the same (Fig. 2). The

amounts of biomass in the effluent of both the reactors were clearly different after degassing started (Fig. 3). Generally, when an anammox reactor is operated with a high nitrogen loading rate, some produced gas attaches to the anammox biomass and then the floating biomass tends to overflow and is discharged from the reactor. On the other hand, when an anammox reactor is operated using the degassing process, the produced gas is separated from the biomass, suggesting that the degassing reduces the overflow of biomass from the reactor. The amount of biomass wash-out during the degassing period in the degassing reactor and the control reactor were 1.55 g and 2.22 g, respectively. These values are corresponding to 113% and 162% of inoculated biomass (1.37 g), and we found that the degassing reactor could reduce approximately 30% of biomass wash-out compared with the control reactor in this study. However, it is not clear why nitrogen removal rate in the degassing reactor was lower than that of the control reactor at present. It is possible that specific anammox activity was different between the degassing and the control reactor. The doubling time was estimated to be 11.8 days for both of the reactors. This doubling time estimated in this study was similar to the value of 11 days reported by Strous *et al*. (1998).

After the two-month operation, the rate of membrane permeability was determined and compared to the rate prior to the reactor's operation. No differences in membrane permeability or selectivity before or after the rector operation were observed, indicating that membrane fouling did not occur during the reactor operation (Table 1). This would be an advantage to the use of a degassing membrane.

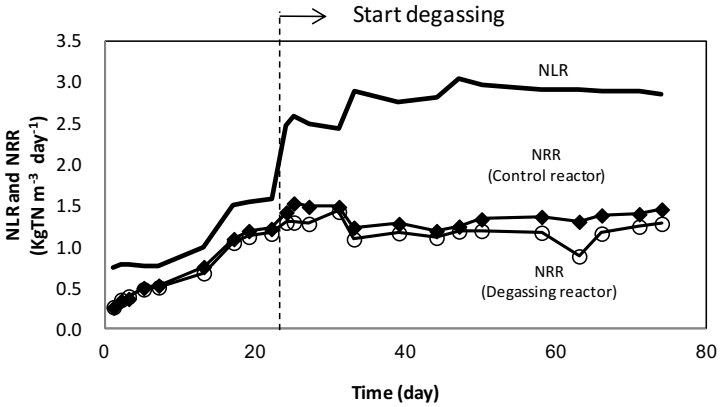

Fig. 2. Profile of nitrogen loading rate (NLR) and nitrogen removal rate (NRR).

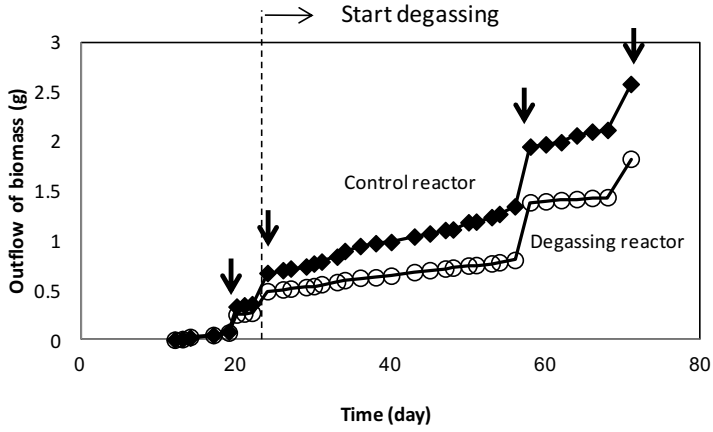

Fig. 3. The amount of cumulative wash-out biomass. Arrows indicate measurement points of granules wash-out.

Table 1. Comparison of the component ratio of anammox bacteria.

	Degassing reactor	Control reactor
Before operation	77.8(±8.7)%	78.9(±8.6)%
After operation	87.6(±8.6)%	82.1(±7.9)%

Table 2. Comparison of the rates of membrane permeability between before and after operation.

	Rate of membrane permeability (m^3 m^{-2} h^{-1} MPa^{-1})									
	O_2		N_2		CO_2		N_2O		CH_4	
Before operation	0.10	(2.3)[a]	0.04	(1.0)	1.11	(26.0)	1.24	(29.1)	0.15	(3.5)
After operation	0.24	(2.8)	0.09	(1.0)	2.13	(24.5)	2.08	(23.9)	0.25	(2.8)

[a] Values in parentheses are the separation factors (each permeability rate divided by the permeability rate of N_2 gas) which indicate the membrane selectivity.

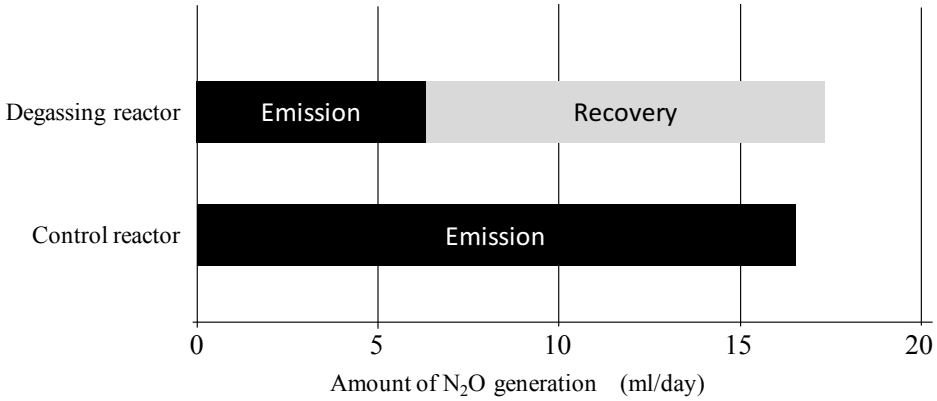

Fig. 4. Gas volume and composition of collected gas on Day 70.

Component ratio of anammox bacteria

At the start of the operation, the percentages of anammox bacteria in the degassing reactor and in the control reactor were 77.8% and 78.8%, respectively (Table 1). Two months later, the percentage in the control reactor was 82.1%, and in the degassing reactor was 87.6%. A statistical analysis using an independent t-test verified that the difference in the values before and after the reactor operation in the degassing reactor was statistically significant ($P<0.05$). On the other hand, with respect to the control reactor, there was no significant difference in the values before and after the reactor operations. As a result, the percentage of anammox bacteria increased. This result indicates that the use of the degassing membrane enhances the anammox bacterial population in granules and/or biofilms.

Gas composition

Gas samples were collected from gas bags and the degassing port of the degassing reactor on Day 70. The exhaust gas volume in the control reactor was 1.65 l/day, whereas the gas volume in the degassing reactor was 0.79 l/day, and thus, the gas recovery efficiency using the degassing was 48%. Concerning the components of the aspirated gas, N_2 was 81.2%, O_2 was 8.1%, CO_2 was 9.1%, and N_2O was 1.5%. The main component of both the exhaust and the aspirated gases was nitrogen gas in both of the reactors. Also, the exhaust gases contained N_2O (0.8% in the degassing reactor, 1.0 % in the control reactor), which is known as a powerful greenhouse gas. The N_2O conversion ratio per loaded nitrogen was 1.7% in the degassing reactor and 2.1% in the control reactor. These values are

approximately twenty times higher than that of previous study reported by Okabe et al. (2011). However, the microbial community composition of anammox bacteria reported by Okabe et al. (2011) was more than 90%, which is higher than that of this study (Table 1). One possible explanation is that coexisting denitrifying bacteria might be responsible for the N_2O production. Therefore the N_2O conversion ratio per loaded nitrogen might be high in this study. On the other hand, it is noteworthy that the N_2O concentration in the aspirated gas was two times higher than the concentration in the exhaust gas. This result could be attributed to the high N_2O selectivity of the membrane module, as shown in Table 2. This shows that the use of the degassing membrane condensed the N_2O concentration in the aspirated gas, bringing about the reduction of N_2O emission produced in the reactor.

In order to evaluate the inhibitory effect of N_2O gas, the amounts of N_2O gas emissions and the recovery were investigated. As shown in Fig. 4, 63% of N_2O gas produced in the reactor was recovered by degassing under the operating condition in this study. Although the gas recovery efficiency in this study was 52%, if it can be improved, the recovery rate of N_2O gas also can be improved.

CONCLUSIONS

The use of the degassing membrane in the anammox reactor successfully improved biomass retention ability (by separating the produced gas from the biomass) and increased the component ratio of anammox bacteria in the reactor. This result indicates that the use of the degassing membrane may be suitable for the start-up of an anammox reactor. In addition, degassing could reduce the N_2O emission produced in the reactor (for the gas selectivity of the degassing membrane). No membrane fouling was observed even after two months of operation without washing, indicating an advantage to the use of a degassing membrane.

ACKNOWLEDGMENTS

This study was conducted at the Analysis Center of Life Science, Hiroshima University.

REFERENCES

APHA-AWWA-WEF. 1998 Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, DC, USA.

Amann, R. I. 1995 In situ identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. pp. 1–15. In A. D. L. Akkerman, J. D. van Elsas, and F. J. de Bruijn (ed.), Molecular microbial ecology manual. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R. & Stahl, D. A. 1990 Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl. Environ. Microbiol*. **56**, 1919–1925.

Bandara, W.M.K.R.T.W., Satoh, H., Sasakawa, M., Nakahara, Y., Takahashi, M. & Okabe, S. 2011 Removal of residual dissolved methane gas in an up-flow anaerobic sludge blanket reactor treating low-strength wastewater at low temperature with degassing membrane. *Water Res.* **45**, 3533–3540.

Daims, H., Bruhl, A., Amann, R., Schleifer, K.-H. & Wagner, M. 1999 The domain-specific probe EUB338 is insufficient for the detection of all bacteria: development and evaluation of a more comprehensive probe set. *Syst. Appl. Microbiol.* **22**, 434–444.

Dapena-Mora, A., Van Hulle, S.W.H., Luis, Campos J., Mendez, R., Vanrolleghem, P.A. & Jetten,

M. 2004 Enrichment of anammox biomass from municipal activated sludge: experimental and modelling results. *J. Chem. Technol. Biotechnol.* **79**, 1421–1428.

Furukawa, K., Rouse, J.D., Yoshida, N. & Hatanaka, H. 2003 Mass cultivation of anaerobic ammonium-oxidizing sludge using a novel nonwoven biomass carrier. *J. Chem. Eng. Jpn.* **36**, 1163–1169.

Imajo, U., Tokutomi, T. & Furukawa, K. 2004 Granulation of anammox microorganisms in up-flow reactors. *Water Sci. Technol.* **49**, 155–163.

Kindaichi, T., Ito, T. & Okabe, S. 2004 Ecophysiological interaction between nitrifying bacteria and heterotrophic bacteria in autotrophic nitrifying biofilms as determined by MAR-FISH. *Appl. Environ. Microbiol.* **70**, 1641–1650.

Kindaichi, T., Tsushima, I., Ogasawara, Y., Shimokawa, M., Ozaki, N., Satoh, S. & Okabe, S. 2007 In situ activity and spatial organization of anaerobic ammonium-oxidizing (anammox) bacteria in biofilms. *Appl. Environ. Microbiol.* **73,** 4931–4939.

Kindaichi, T., Awata, T., Suzuki, Y., Tanabe, K., Hatamoto, M., Ozaki, N. & Ohashi, A. 2011 Enrichment using an up-flow column reactor and community structure of marine anammox bacteria from coastal sediment. *Microbes Environ.* **26,** 67–73.

Mulder, A., van de Graaf, A.A., Roberton, L.A. & Kuenen, J.G. 1995 Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiol. Ecol.* **16**, 177–184.

Okabe, S., Satoh, H. & Watanabe, Y. 1999 In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes. *Appl. Environ. Microbiol.* **65**, 3182–3191.

Okabe, S., Oshiki, M., Takahashi, Y. & Satoh, H. 2011 N_2O emission from a partial nitrification-anammox process and identification of a key biological process of N_2O emission from anammox granules. *Water Res.* **45**, 6461–6470.

Schmid, M., Twachtmann, U., Klein, M., Strous, M., Juretschko, S., Jetten, M., Metzger, J.W., Schleifer, K-H. & Wagner, M. 2000 Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. *Syst Appl Microbiol.* **23** 93–106.

Strous, M., Heijnen, J.J., Kuenen, J.G. & Jetten, M.S.M. 1998 The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Appl. Microbiol. Biotechnol.* **50**, 589–596.

Strous, M., Fuerst, J.A., Kramer, E.H.M., Logemann, S., Muyzer, G., Van de Pas-Schoonen, K.T., Webb, R., Kuenen, J.G. & Jetten, M.S.M. 1999a Missing lithotroph identified as new Planctomycete. *Nature* **400**, 446–449.

Strous, M., Kuenen, J.G., & Jetten, M.S.M., 1999b Key physiology of anaerobic ammonium oxidation. *Appl. Environ. Microbiol.* **65**, 3248–3250.

Strous, M., van Gerven, E., Kuenen, J.G. & Jetten, M.S.M. 1997 Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. *Water Res.* **31**, 1955–1962.

Tsushima, I., Ogasawara, Y., Kindaichi, T., Satoh, H. & Okabe, S. 2007 Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors. *Water Res.* **41**, 1623–1634.

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* **142**, 2187–2196.