Granular biomass capable of partial nitritation and anammox

S. E. Vlaeminck, L. F. F. Cloetens, M. Carballa, N. Boon and W. Verstraete

ABSTRACT

A novel and efficient way of removing nitrogen from wastewater poor in biodegradable organic carbon, is the combination of partial nitritation and anoxic ammonium oxidation (anammox), as in the one-stage oxygen-limited autotrophic nitrification/denitrification (OLAND) process. Since anoxic ammonium-oxidizing bacteria grow very slowly, maximum biomass retention in the reactor is required. In this study, a lab-scale sequencing batch reactor (SBR) was used to develop granular, rapidly settling biomass. With SBR cycles of one hour and a minimum biomass settling velocity of 0.7 m/h, OLAND granules were formed in 1.5 months and the nitrogen removal rate increased from 50 to 450 mg N L^{-1} d^{-1} in 2 months. The granules had a mean diameter of 1.8 mm and their aerobic and anoxic ammonium-oxidizing activities were well equilibrated to perform the OLAND reaction. Fluorescent *in-situ* hybridization (FISH) demonstrated the presence of both β-proteobacterial aerobic ammonium oxidizers and planctomycetes (among which anoxic ammonium oxidizers) in the granules. The presented results show the applicability of rapidly settling granular biomass for one-stage partial nitritation and anammox.

Key words | anammox, CANON, granulation, nitritation, OLAND, SBR

S. E. Vlaeminck
L. F. F. Cloetens
M. Carballa
N. Boon
W. Verstraete
Laboratory of Microbial Ecology and Technology (LabMET),
Ghent University,
Coupure Links 653,
Ghent 9000,
Belgium
E-mail: *Siegfried.Vlaeminck@UGent.be*;
LievenC@Gmail.com;
Marta.Carballa@UGent.be;
Nico.Boon@UGent.be;
Willy.Verstraete@UGent.be

INTRODUCTION

In the last years, the technology of one-stage nitrogen removal via partial nitritation and anammox has developed rapidly, as demonstrated by the construction of several pilot and full scale reactors tabulated by Cema *et al.* (2006) and van der Star *et al.* (2007). One of these processes is oxygen-limited autotrophic nitrification/denitrification (OLAND), with aerobic ammonium-oxidizing bacteria (AerAOB) oxidizing ammonium to nitrite, and anoxic ammonium-oxidizing bacteria (AnAOB) oxidizing the residual ammonium with nitrite into dinitrogen gas and some nitrate (Pynaert *et al.* 2003). The overall OLAND stoichiometry is the following (Vlaeminck *et al.* 2007*a*):

$$NH_4^+ + 0.85O_2 \rightarrow 0.44N_2 + 0.11NO_3^- + 1.08H^+ + 1.44H_2O \quad (1)$$

One of the bottlenecks in all AnAOB applications, is the slow bacterial growth (Strous *et al.* 1998). To prevent washout of AnAOB, the choice of a good biomass retention mechanism is an important aspect in the design of OLAND-type reactors. One way of avoiding biomass loss is a biofilm-based setup, such as a rotating contactor (Pynaert *et al.* 2003), a moving bed reactor (Cema *et al.* 2006), or a fixed bed reactor (Furukawa *et al.* 2006). In suspended growth systems, other measures are taken to prevent biomass loss. In a chemostat, a biomass settling device in the outflow tube can retain biomass (Third *et al.* 2005), whereas a three-phase separator can ensure biomass retention in a air-lift reactor (Sliekers *et al.* 2003). For a sequencing batch reactor (SBR), high biomass retention is obtained from a low minimum biomass settling velocity which is the ratio between the settling time and the vertical distance from the water surface to the effluent discharge point (Liu *et al.* 2005). Reported minimum biomass settling velocities for OLAND-type SBRs are about 0.3 m/h (Third *et al.* 2001; Sliekers *et al.* 2002; Wett 2006).

doi: 10.2166/wst.2008.731

In suspended growth systems, biomass aggregate morphology has an influence on the settling properties. The morphology of aggregated AerAOB and AnAOB however is mostly undiscussed or vague in literature. In most studies, mainly floccular biomass was formed (Third *et al.* 2001; Sliekers *et al.* 2002; Sliekers *et al.* 2003; Third *et al.* 2005). To the best of our knowledge, granular biomass was only mentioned by Nielsen *et al.* (2005). In view of the high biomass retention of granules, their good settling capacities and their compact structure allowing for high biomass concentrations and thus high volumetric loading rates, the goal of this study was to develop granules in an OLAND SBR.

METHODS

Lab-scale reactor set-up

The OLAND SBR consisted of a cylindrical vessel with an internal diameter of 14 cm and a working volume of 1.87 L. The reactor was inoculated with OLAND biomass harvested from a lab-scale rotating contactor described by Pynaert *et al.* (2003), at an initial biomass concentration of 1.6 g VSS/L. Tap water-based influent consisted of $(NH_4)_2SO_4$ at an initial concentration of 0.1 mg N/L, 0.308 g KH_2PO_4/L, and 2 mL/L of a trace elements solution (Kuai & Verstraete 1998) amended with final concentrations of 0.050 mg Ni/L, 0.050 mg Se/L and 0.002 mg B/L. To provide buffering capacity, 1 mole of $NaHCO_3$ was added per mole of nitrogen. If necessary, the latter ratio was increased temporarily to ensure that the reactor pH did not drop below 7.4. The reactor temperature was controlled at 35 ± 2°C. The reactor dissolved oxygen (DO) concentration was controlled, either automatically (Consort R305 controller with DO probe SZ10T), or manually by daily adjustment of aeration intervals and air flow rate. Reactor mixing was with a magnetic stirrer.

The phase durations of the one-hour reaction cycle are schematized in Figure 1. Each cycle, 0.37 L (less than 10% deviation) of synthetic medium was fed to the reactor with a peristaltic influent pump. The reactor was mixed and DO controlled, both during the feeding and the reaction phase. Subsequently, the biomass was allowed to settle for two minutes, so that the minimum biomass settling velocity was 0.73 m/h. Finally, a peristaltic effluent pump removed the supernatant. The reactor was operated with a fixed residual volume of 1.50 L, and therefore with a volumetric exchange ratio of 20% per cycle. At one cycle per hour, the hydraulic residence time was 5.0 h.

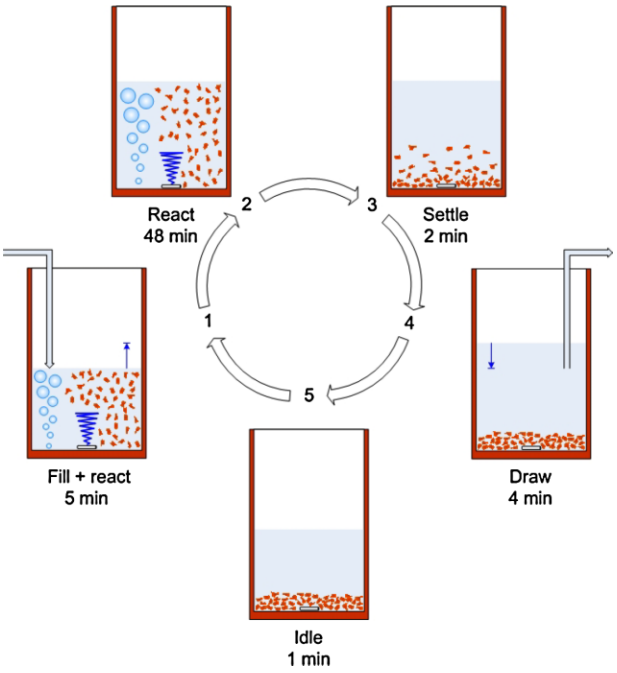

Figure 1 | Schematic representation of the OLAND SBR cycle.

Aerobic and anoxic batch activity tests

Aerobic and anoxic ammonium conversion of the different morphological SBR biomass types was tested in batch. Granular SBR biomass was retained by a 1.0 mm pore size sieve, while the residual fraction of the biomass, retained by a 0.1 mm sieve, was defined as floccular SBR biomass. Prior to the activity tests, biomass was centrifuged (5 min at 5000 × *g*) and washed with phosphate buffer (100 mg P/L; pH 8) twice to remove residual dissolved reactor compounds. Aerobic and anoxic tests were described more in detail by Vlaeminck *et al.* (2007*b*). In short, biomass was incubated in a shaking Erlenmeyer with ammonium as substrate for the aerobic activity tests. For the anoxic tests, biomass incubation occurred in a gas-tight anoxic serum flask with ammonium and nitrite as substrates. During the incubation, liquid samples were taken over time for ammonium, nitrite and nitrate analysis. Since few SBR biomass was available, no replica were included in the

activity tests of floccular and granular biomass. However, the inoculum was tested in triplicate and the ammonium oxidation rates of the replicas differed less than 10% from the average oxidation rate, justifying the results from the singular test on floccular and granular SBR biomass.

Fluorescent *in-situ* hybridization (FISH)

FISH was used to investigate the presence of β-proteobacterial AerAOB and Planctomycetales (among which AnAOB, Strous *et al.* 1999*b*) in granular and floccular biomass. Biomass samples were treated as described by Vlaeminck *et al.* (2007*b*). The following oligonucleotide probes were used: (i) Cy3 labelled Nso1225, at 35% formamide (FA), targeting β-proteobacterial AerAOB (Mobarry *et al.* 1996), (ii) Cy3 labelled Pla46, at 30% FA, targeting Planctomycetales (Neef *et al.* 1998) and (iii) a 1:1:1 mixture of FLUO labelled EUB338I, EUB338II and EUB338III, at 30 or 35% FA depending on the complementary probe, targeting all bacteria (Amann *et al.* 1990; Daims *et al.* 1999).

Granule diameter

ImageJ software was used to calculate the area of 50 biomass granules on images obtained on a Leica/Wild Heerbrugg M8 stereomicroscope. The average diameter was determined as a circle-equivalent diameter.

Analytical methods

Ammonium was determined colorimetrically with Nessler reagent according to standard methods (Greenberg *et al.* 1992). Both nitrite and nitrate were determined using a Metrohm 761 Compact Ion Chromatograph equipped with a conductivity detector. The operational parameters were as follows: column Metrosep A Supp 5–150; guard column Metrosep A Supp 4/5 Guard; eluent 1.0 mM $NaHCO_3$, 3.2 mM Na_2CO_3, 5 vol% acetone; flow 0.7 mL/min; sample loop 20 μL. The total and volatile suspended solids (TSS and VSS) content of the biomass were determined by drying and weighing following standard methods (Greenberg *et al.* 1992). pH was determined potentiometrically with a portable Consort C532 pH meter. DO concentration and water temperature were measured with a portable Endress-Hauser COM381 DO meter.

RESULTS AND DISCUSSION

OLAND SBR performance

The reactor pH was kept above 7.4 and did never exceed 7.8. According to changes in the aeration of the reactor, the SBR performance was subdivided into seven operational periods (Figure 2).

At the initial setpoint of 0.85 mg O_2/L, increasingly more ammonium was converted into nitrite and nitrate (Figure 2), but the nitrogen removal rate remained fairly constant. Since oxygen inhibits the AnAOB reversibly (Strous *et al.* 1997), the DO setpoint was lowered to 0.4 mg O_2/L in period II, to limit the oxygen penetration depth in the biomass aggregates. As a result, the AnAOB reaction increased, as follows from the sharp increase of the nitrogen removal rate in period II.

A lower oxygen supply limiting the increase in removal rate in period III, derived from rapid drifting of the probe. Since this could not be overcome by daily calibration and maintenance, the automatic DO control was replaced by a manual control mechanism.

From period IV on, the initial oxygen sensitivity of the AnAOB disappeared: higher DO setpoints (0.8 to 1.1 mg O_2/L) were not inhibitory for the nitrogen removal and thus the AnAOB reaction. On the contrary, the higher DO setpoints resulted in increasing nitrogen removal rates in periods IV and VI.

In periods V and VII, the oxygen supply was decreased (lower setpoint/anoxic phase) to lower the nitrite effluent concentrations in order to prevent nitrite inhibition of AnAOB (Strous *et al.* 1999*a*). This measure did not reduce the nitrite level however, probably because of the development of floccular biomass with a high nitrite-producing activity, as is discussed more in detail below.

Overall, a competitive maximum removal rate of 450 mg N L^{-1} d^{-1} was reached. Most reported maximum removal rates of one-stage partial nitritation and anammox in suspended growth systems vary from 50 to 110 mg N L^{-1} d^{-1} (Third *et al.* 2001; Sliekers *et al.* 2002; Li *et al.* 2004; Third *et al.* 2005). Only Wett (2006) and Sliekers

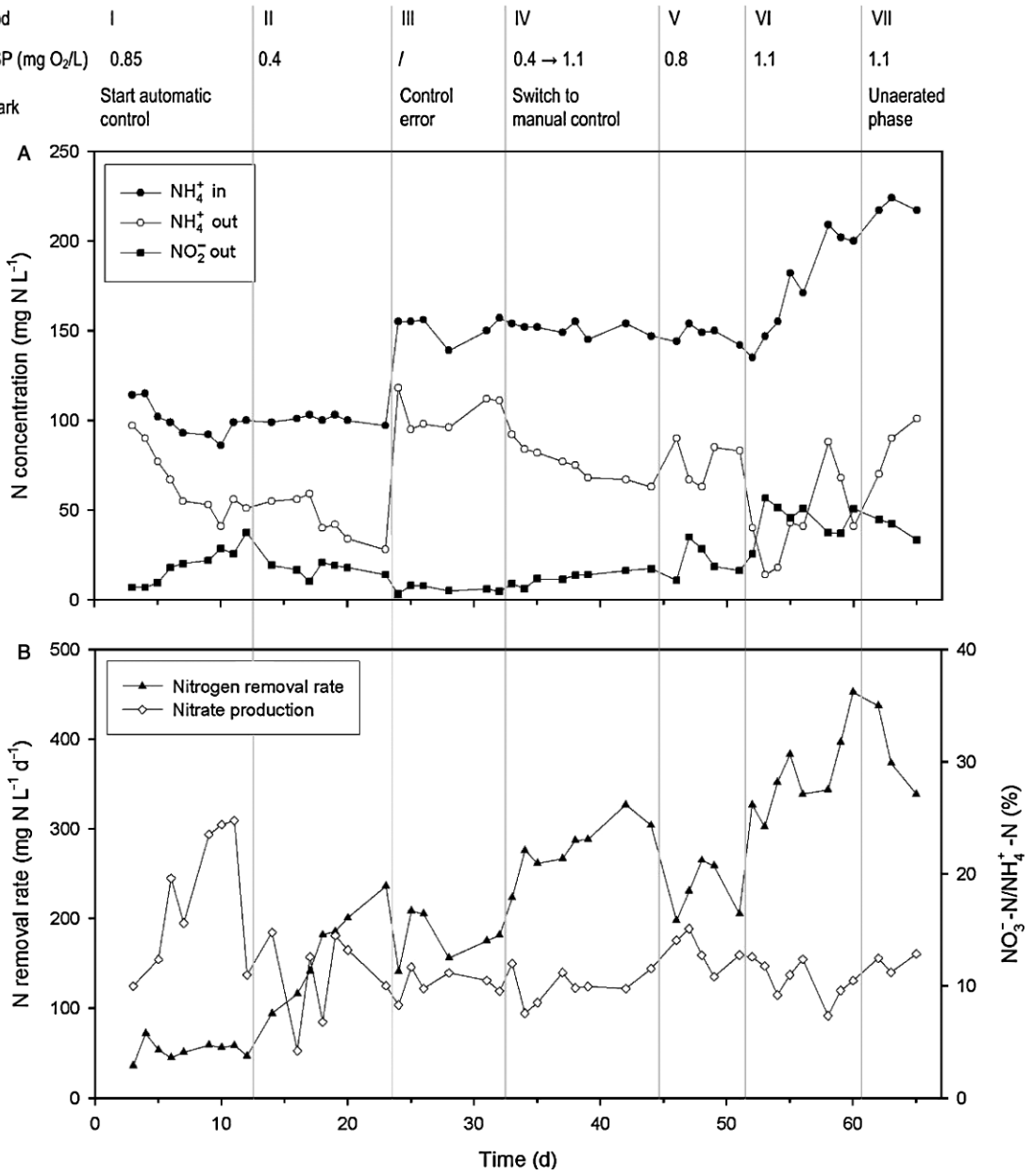

Figure 2 | Performance of the OLAND SBR, subdivided in seven operational periods, with indication of DO setpoints (SP) and remarks on the DO control mechanism. In period III, probe drifting resulted in a lower DO concentration compared to period II. In period VII, eight minutes without aeration were introduced at the end of the reaction phase. (*A*) Ammonium concentration in influent and effluent, and nitrite concentration in effluent. (*B*) Nitrogen removal rate and production of nitrate as a percentage of the ammonium removal.

et al. (2003) report considerably higher removals of 600 and 1,500 mg N L^{-1} d^{-1} respectively.

When fitting an exponential curve to the increase of the removal rate over the full operational period ($R^2 = 79\%$), a doubling time of 21 days could be calculated. This value lies within the reported range of AnAOB doubling times of 14 to 21 days (Strous & Jetten 2004). Since oxygen was limiting the increase in removal rate in some periods and since some wash-out of the biomass with the supernatant occurred, it is likely that the real doubling time of the OLAND biomass was less than 21 days.

Biomass characterization

The OLAND inoculum aggregates derived from a 5 mm thick biofilm and had no distinct or uniform morphology. During SBR operation, the morphology of the inoculum gradually changed and from period V, two different forms of biomass could be distinguished (Figure 3).

A first form of biomass was rather floccular, slowly settling and brownish, with an aggregate size between 0.1 and 1.0 mm. These flocs made up about 43% of the reactor's VSS content retained on a 0.1 mm sieve (day 54). The larger VSS fraction consisted of granular biomass, which was more reddish and had an average particle diameter of 1.8 ± 0.3 mm. This biomass settled instantaneously as soon as the aeration and mixing stopped. The VSS/TSS ratios of floccular and granular biomass were 86 and 76% respectively, indicating that the granules had a higher anorganic content than the flocs.

Floccular and granular biomass was harvested on day 65 and examined for their aerobic and anoxic ammonium-oxidizing activity (Figure 4). Compared to the inoculum, floccular biomass was specialized in aerobic ammonium oxidation (426 mg N g^{-1} VSS d^{-1}), and granular biomass was specialized in anoxic ammonium oxidation (133 mg N g^{-1} VSS d^{-1}). Similarly but without discussing the biomass morphology (flocs/granules), Nielsen *et al.* (2005) reported that OLAND-type aggregates smaller than 500 μm were specialized in aerobic ammonium oxidation, and that aggregates larger than 500 μm were specialized in anoxic ammonium oxidation. Our results further show that the aerobic activity of the floccular biomass was more than ten times higher than the anoxic activity. It is therefore likely that the undesired nitrite accumulation in the reactor

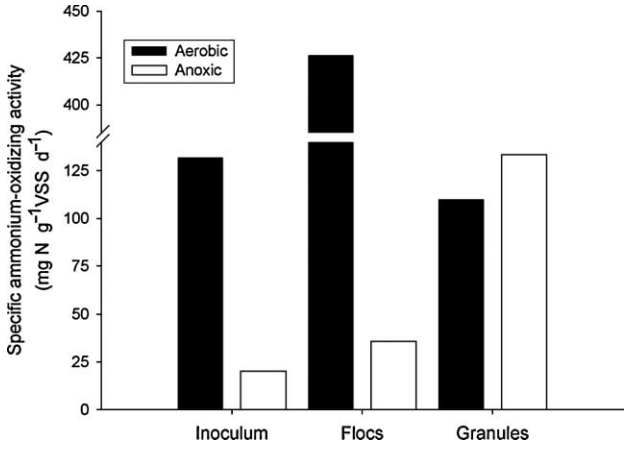

Figure 4 | Specific aerobic and anoxic ammonium-oxidizing activity of the SBR inoculum, flocs and granules on day 65 ($n = 1$). Note the break in the ordinate.

was caused by the floccular biomass. Interestingly, the aerobic and anoxic activity of granular biomass were in the same order of magnitude, suggesting that the granular biomass composition was well equilibrated to perform the OLAND reaction without excessive nitrite production.

The development of nitrite-oxidizing bacteria (NOB) in the SBR was negligible at the chosen DO setpoints. Indeed, no nitrate was produced in the aerobic batch tests by the granules and the flocs (data not shown). Further, the observed nitrate production in the reactor was 11 ± 2% per ammonium removed from period III on (Figure 2). This value corresponds well with the expected nitrate production deriving from the AnAOB metabolism, i.e. 11% per removed ammonium (Equation 1). It is likely that the lower oxygen affinity constant of AerAOB (Laanbroek & Gerards 1993) allowed the AerAOB to outcompete the NOB at the chosen DO setpoints (0.4 − 1.1 mg O_2/L).

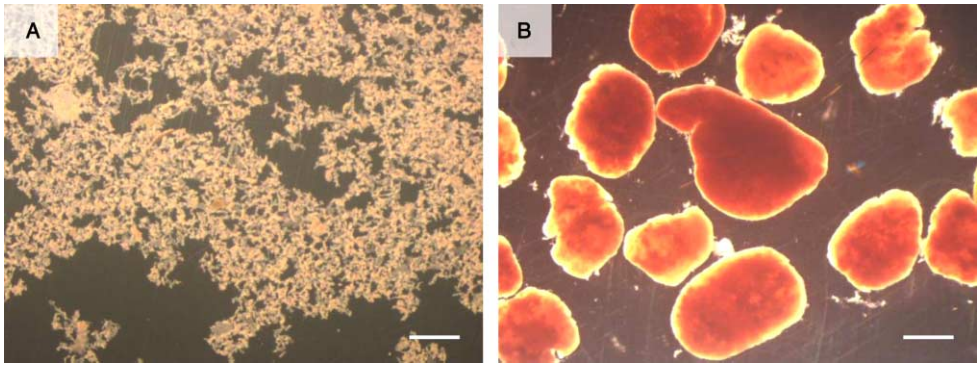

Figure 3 | Morphological differences in the OLAND SBR biomass on day 65. (A) Floccular biomass (B) Granular biomass. Scale bar = 1.0 mm.

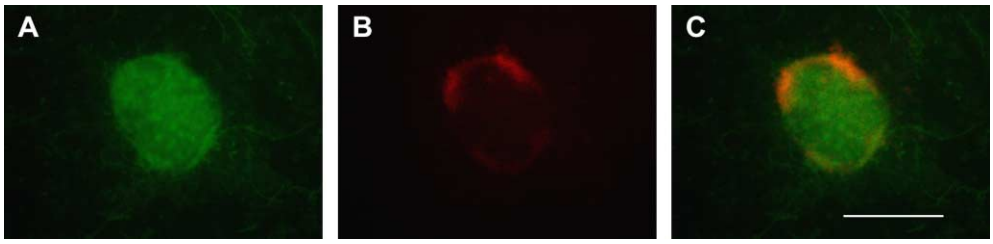

Figure 5 | FISH picture of a small OLAND SBR biomass floc. (*A*) All bacteria stained with the EUB probe mixture (green) (*B*) β-proteobacterial AerAOB hybridized with the Nso1225 probe (red) (*C*) Merged picture of (*A*) and (*B*). Scale bar = 50 μm.

FISH revealed the presence of both β-proteobacterial AerAOB and planctomycetes in SBR flocs and granules (data not shown). A FISH picture of a small SBR floc is shown in Figure 5, with AerAOB present in the outer 10 μm layer of the floc, similar to the FISH results of Nielsen *et al.* (2005). It should be noted that the spherical form of the floc in Figure 5 was exceptional and that most flocs had no distinct morphology.

Overall, OLAND granules had good settling and nitrogen conversion capacities. Another advantage of granules is that they can shield the AnAOB from oxygen exposure. Initially, low DO concentrations (0.4 mg O_2/L) were required to achieve an increase in the AnAOB activity in the reactor. With the development of granules from period IV on however, higher DO concentrations (1.1 mg O_2/L) were not inhibitory for an increase in the AnAOB activity. Based on the measured specific aerobic ammonium oxidation activity, an oxygen penetration depth of 120 μm was calculated in the OLAND granules at 1.1 mg O_2/L, assuming spherical granules of 1.8 mm diameter and zero-order substrate uptake (Perez *et al.* 2005). Since the smallest granule diameter was 1.3 mm, all granules had an internal anoxic zone. As such, oxygen-consuming AerAOB in the outer layer of a granule were able to create central anoxic zones for oxygen-sensitive AnAOB.

Liu *et al.* (2005) state that a high minimum biomass settling velocity is the major selection pressure responsible for SBR granulation, so that only rapidly settling granules can stay in the reactor. In most SBR granulation studies with activated or nitrifying sludge, biomass settling velocities are at least 4.5 m/h (Beun *et al.* 1999; Kim & Seo 2006; Kishida *et al.* 2006). In this study however, granulation occurred at a relatively low minimum settling velocity of 0.7 m/h. It seems therefore that a very high selection pressure was not required for the formation of OLAND granules. It is currently under research if the application of higher selective settling velocities can remove the undesirable floccular biomass and prevent growth of new flocs.

CONCLUSIONS

- With a minimum biomass settling velocity of 0.7 m/h, granular OLAND biomass was successfully developed in an SBR configuration after 1.5 months. In a period of 2 months, the nitrogen removal rate increased from about 50 to 450 mg N L^{-1} d^{-1}, which is a competitive rate for OLAND-type reactors.
- A higher minimum biomass settling velocity might have prevented the formation of floccular biomass, specialized in aerobic ammonium oxidation and probably causing nitrite accumulation in the reactor.
- Granular OLAND biomass had an average diameter of 1.8 mm and the aerobic and anoxic ammonium-oxidizing activities were well equilibrated to perform the OLAND reaction without excessive nitrite production. The presence of both β-proteobacterial AerAOB and planctomycetes was demonstrated by FISH.

ACKNOWLEDGEMENTS

This research was funded by a PhD grant (Aspirant) for Siegfried E. Vlaeminck from the Fund of Scientific Research-Flanders (Fonds voor Wetenschappelijk Onderzoek (FWO) Vlaanderen), and by a postdoctoral grant for Marta Carballa from the Spanish Ministry of Education and Science (EX2006-0963). The authors gratefully thank Jeroen Bursens for assistance with the reactor design,

Winnok De Vos for assistance with the microscopy and image processing, and Kim Windey, Peter De Schryver, Tuba Hande Ergüder and Lieven Wittebolle for the helpful discussions.

REFERENCES

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

Beun, J. J., Hendriks, A., Van Loosdrecht, M. C. M., Morgenroth, E., Wilderer, P. A. & Heijnen, J. J. 1999 Aerobic granulation in a sequencing batch reactor. *Water Res.* **33**, 2283–2290.

Cema, G., Szatkowska, B., Plaza, E., Trela, J. & Surmacz-Gorska, J. 2006 Nitrogen removal rates at a technical-scale pilot plant with the one-stage partial nitritation/anammox process. *Water Sci. Technol.* **54**(8), 209–217.

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.

Furukawa, K., Lieu, P. K., Tokitoh, H. & Fujii, T. 2006 Development of single-stage nitrogen removal using anammox and partial nitritation (SNAP) and its treatment performances. *Water Sci. Technol.* **53**(6), 83–90.

Greenberg, A. E., Clesceri, L. S. & Eaton, A. D. 1992 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC.

Kim, D. J. & Seo, D. 2006 Selective enrichment and granulation of ammonia oxidizers in a sequencing batch airlift reactor. *Process Biochem.* **41**, 1055–1062.

Kishida, N., Kim, J., Tsuneda, S. & Sudo, R. 2006 Anaerobic/oxic/anoxic granular sludge process as an effective nutrient removal process utilizing denitrifying polyphosphate-accumulating organisms. *Water Res.* **40**, 2303–2310.

Kuai, L. P. & Verstraete, W. 1998 Ammonium removal by the oxygen-limited autotrophic nitrification-denitrification system. *Appl. Environ. Microbiol.* **64**, 4500–4506.

Laanbroek, H. J. & Gerards, S. 1993 Competition for limiting amounts of oxygen between nitrosomonas-europaea and nitrobacter-winogradskyi grown in mixed continuous cultures. *Arch. Microbiol.* **159**, 453–459.

Li, X., Zen, G., Rosenwinkel, K. H., Kunst, S., Weichgrebe, D., Cornelius, A. & Yang, Q. 2004 Start up of deammonification process in one single SBR system. *Water Sci. Technol.* **50**(6), 1–8.

Liu, Y., Wang, Z. W., Qin, L., Liu, Y. Q. & Tay, J. H. 2005 Selection pressure-driven aerobic granulation in a sequencing batch reactor. *Appl. Microbiol. Biotechnol.* **67**, 26–32.

Mobarry, B. K., Wagner, M., Urbain, V., Rittmann, B. E. & Stahl, D. A. 1996 Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. *Appl. Environ. Microbiol.* **62**, 2156–2162.

Neef, A., Amann, R., Schlesner, H. & Schleifer, K. H. 1998 Monitoring a widespread bacterial group: *in situ* detection of planctomycetes with 16S rRNA-targeted probes. *Microbiology (UK)* **144**, 3257–3266.

Nielsen, M., Bollmann, A., Sliekers, O., Jetten, M., Schmid, M., Strous, M., Schmidt, I., Larsen, L. H., Nielsen, L. P. & Revsbech, N. P. 2005 Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor. *FEMS Microbiol. Ecol.* **51**, 247–256.

Perez, J., Picioreanu, C. & van Loosdrecht, M. 2005 Modeling biofilm and floc diffusion processes based on analytical solution of reaction-diffusion equations. *Water Res.* **39**, 1311–1323.

Pynaert, K., Smets, B. F., Wyffels, S., Beheydt, D., Siciliano, S. D. & Verstraete, W. 2003 Characterization of an autotrophic nitrogen-removing biofilm from a highly loaded lab-scale rotating biological contactor. *Appl. Environ. Microbiol.* **69**, 3626–3635.

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

Sliekers, A. O., Derwort, N., Gomez, J. L. C., Strous, M., Kuenen, J. G. & Jetten, M. S. M. 2002 Completely autotrophic nitrogen removal over nitrite in one single reactor. *Water Res.* **36**, 2475–2482.

Strous, M., vanGerven, E., Kuenen, J. G. & Jetten, M. 1997 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. & 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., Kuenen, J. G. & Jetten, M. S. M. 1999*a* Key physiology of anaerobic ammonium oxidation. *Appl. Environ. Microbiol.* **65**, 3248–3250.

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. 1999*b* Missing lithotroph identified as new planctomycete. *Nature* **400**, 446–449.

Strous, M. & Jetten, M. S. M. 2004 Anaerobic oxidation of methane and ammonium. *Annu. Rev. Microbiol.* **58**, 99–117.

Third, K. A., Sliekers, A. O., Kuenen, J. G. & 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. *Syst. Appl. Microbiol.* **24**, 588–596.

Third, K. A., Paxman, J., Schmid, M., Strous, M., Jetten, M. S. M. & Cord-Ruwisch, R. 2005 Enrichment of anammox from activated sludge and its application in the CANON process. *Microb. Ecol.* **49**, 236–244.

van der Star, W. R. L., Abma, W. R., Blommers, D., Mulder, J.-W., Tokutomi, T., Strous, M., Picioreanu, C. & van Loosdrecht, M. C. M. 2007 Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research*. **41**(8), 4149–4163.

Vlaeminck, S. E., Dierick, K., Boon, N. & Verstraete, W. 2007*a* Vertical migration of aggregated aerobic and anaerobic ammonium oxidizers enhances oxygen uptake in a stagnant water layer. *Appl. Microbiol. Biotechnol.* **75**, 1455–1461.

Vlaeminck, S. E., Geets, J., Vervaeren, H., Boon, N. & Verstraete, W. 2007*b* Reactivation of aerobic and anaerobic ammonium oxidizers in OLAND biomass after long-term storage. *Appl. Microbiol. Biotechnol.* **74**, 1376–1384.

Wett, B. 2006 Solved upscaling problems for implementing deammonification of rejection water. *Water Sci. Technol.* **53**(12), 121–128.