Microbial Biotechnology (2012) **5**(3), 425–432 doi:10.1111/j.1751-7915.2012.00336.x

Stable performance of non-aerated two-stage partial nitritation/anammox (PANAM) with minimal process control

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Summary

Partial nitritation/anammox (PANAM) technologies have rapidly developed over the last decade, but still considerable amounts of energy are required for active aeration. In this study, a non-aerated two-stage PANAM process was investigated. In the first-stage upflow fixed-film bioreactor, nitratation could not be prevented at ammonium loading rates up to 186 mg N l^{-1} d^{-1} and low influent dissolved oxygen (0.1 mg O_2 l^{-1}). Yet, increasing the loading rate to 416 and 747 mg N l^{-1} d^{-1} by decreasing the hydraulic retention time to 8 and 5 h, respectively, resulted in partial nitritation with the desired nitrite to ammonium nitrogen ratio for the subsequent anammox stage (0.71–1.05). The second-stage anammox reactor was established with a synthetic feeding based on ammonium and nitrite. After establishing anammox at low biomass content (0.5 g VSS l^{-1}), the anammox influent was switched to partial nitritation effluent at a loading rate of 71 mg N l^{-1} d^{-1}, of which 78% was removed at the stoichiometrically expected nitrite to ammonium consumption ratios (1.19) and nitrate production to ammonium consumption ratio (0.24). The combined PANAM reactors were operated for 3 months at a stable performance. Overall, PANAM appeals economically, saving about 50% of the energy costs, as well as technically, given straightforward operational principles.

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Introduction

Anoxic ammonium oxidation or anammox was discovered about 15 years ago, opening up a new dimension in the treatment of nitrogenous wastewaters (Vlaeminck, *et al.*, 2009). Anammox is carried out by a distinct clade of chemolithoautotrophic Planctomycetes, which thrive on the oxidation of ammonium with nitrite, producing mainly dinitrogen gas and some nitrate (Eq. 1) (Strous *et al.*, 1998):

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad \text{(Eq. 1)}$$

With preceding partial nitritation, anammox has considerable economical and ecological potential for ammonium removal from wastewaters with a low biodegradable carbon to nitrogen ratio, saving 30–40% of the overall costs (Fux and Siegrist, 2004). Several anammox-based processes have been developed at larger scale, in which partial nitritation and anammox are either executed in the same reactor stage (Abma *et al.*, 2010; Jeanningros *et al.*, 2010), or in separate stages (van der Star *et al.*, 2007; Desloover *et al.*, 2011). For any type of partial nitritation/anammox (PANAM) system it is important to minimize the aerobic oxidation of nitrite to nitrate, i.e. nitratation executed by nitrite oxidizing bacteria. Control options include high free ammonia levels (Anthonisen *et al.*, 1976), low dissolved oxygen (DO) levels (Bernet *et al.*, 2001), a short sludge retention time at high temperature (Hellinga *et al.*, 1998) and the addition of sulfide (Erguder *et al.*, 2008). To suppress nitratation in one-stage systems, the DO level is the most obvious control parameter, whereas sludge retention time and free ammonia offer mainly an added control possibility for two-stage systems.

In view of sustainable wastewater treatment, minimum energy consumption is desirable (Verstraete and Vlaeminck, 2011), and active aeration constitutes about 90% of the energy use in PANAM processes (Fux and Siegrist, 2004). Although active aeration allows to control bulk DO

levels and hence partial nitritation adequately, technologies relying on passive aeration such as rotating biological contactors consume up to three times less energy (Mathure and Patwardhan, 2005), but strict control of the DO level is less evident. Besides, energy-friendly and hence more cost-effective, technologies without active aeration can be techno-economically viable for developing countries, in case control requirements can be minimal and performance can be stable and robust. Considering these energy and complexity aspects, an energy-friendly technology was elaborated in this study using partial nitritation and anammox in two reactor stages. Although this entails higher investments costs than one stage, it facilitates nitratation suppression, prevents anammox inhibition by nitrite and lower the risk of denitrifiers outcompeting anammox bacteria, given the lower level of organics (Lackner *et al*., 2008).

The development of a partial nitritation biofilm in a fixed-film bioreactor (FFB) was previously tested using the combined addition of sodium sulphite and cobalt chloride to the influent (Bagchi *et al*., 2009). Although this effectively decreased the influent DO level to about 1 mg O_2 l^{-1}, and supposedly played a role in successful nitratation suppression, in the long term this strategy led to nitritation inhibition. The first aim of this study was to re-establish nitritation in the FFB without adding chemicals. Additionally, previous operation of the FFB was focused on obtaining complete nitritation, aiming at maximum nitrite to ammonium nitrogen ratios in the effluent. However, in view of coupling the FFB to an anammox reactor, the effluent nitrite to ammonium ratio should be approximately equimolar (Eq. 1). Therefore, the second aim of this study was to obtain stable partial nitritation. For anammox, a continuous stirred-tank reactor (CSTR) was previously established using nitrite supplemented ammoniacal influent (Bagchi *et al*., 2010). The final aim of this study was to feed the anammox CSTR with effluent from the partial nitritation FFB using minimal control mechanisms to obtain two-stage non-aerated PANAM.

Results

Re-establishing partial nitritation

To re-establish nitrification, the experimental setup with the acclimatized biomass of the previous study was fed at a low volumetric loading rate (81 mg N l^{-1} d^{-1}) in period 1. This was increased in a stepwise manner over periods 2 and 3 by increasing the influent ammonium concentration, establishing nitrification of the majority of the fed ammonium, i.e. the oxidized ammonium ended up as nitrate in the effluent (Table 1; Fig. 1). The feed DO was 4.4–4.5 mg O_2 l^{-1} in periods 1–3 (Table 1).

With nitrification established, the transition to partial nitritation was attempted through low influent DO levels. In periods 4 and 5, the influent was purged with nitrogen gas to decrease the DO down to 1.0 and 0.1 mg O_2 l^{-1} respectively. However, despite of a 3 months operation at the lowest attainable DO value, nitrite did not start to accumulate, nor did the ammonium removal efficiency decrease (Table 1; Fig. 1).

The ongoing nitratation at low influent DO levels indicated that the amount of available oxygen in the FFB was still sufficient to fully oxidize the majority of the loaded ammonium. Therefore, the volumetric nitrogen load was increased over the periods 6–8 by decreasing the hydraulic retention time (HRT) in a stepwise manner from 17.6 h down to 5.0 h, while maintaining the influent DO at 0.1 mg O_2 l^{-1} through N2 purging during all subsequent periods of operation. This strategy was successful in achieving nitrite accumulation. Already following the first increased loading (period 6), nitrite effluent levels increased, yet nitrate were still higher (Fig. 1), with nitrite representing 29% of the oxidized effluent nitrogen (Table 1). A further increase of the loading in period 7 yielded a further increase of the nitrite accumulation efficiency to 61%, and an effluent

Table 1. Operational strategy and performance of the partial nitritation upflow fixed-bed reactor.

			Influent				Effluent		
Period	Action	Days	NH_4^+ (mg N l^{-1})	B_V (mg NH_4^+-N l^{-1} d^{-1})	DO (mg O_2 l^{-1})	HRT (h)	NO_2^-/(NO_2^- + NO_3^-) (–)	NO_2^-/NH_4^+ (–)	FA (mg N l^{-1})
1	$NH_4^+\uparrow \rightarrow B_V\uparrow$	1–29	59 ± 21	81 ± 29	4.5 ± 0.2	17.6	0.04 ± 0.09	0.94 ± 1.16	0.2 ± 0.2
2		30–53	108 ± 8	147 ± 10	4.4 ± 0.3	17.6	0.01 ± 0.01	0.03 ± 0.03	0.7 ± 0.7
3		54–94	136 ± 12	186 ± 16	4.5 ± 0.1	17.6	0.01 ± 0.01	0.01 ± 0.02	2.8 ± 0.5
4	Influent DO↓	95–116	137 ± 5	187 ± 7	1.0 ± 0.1	17.6	0.01 ± 0.05	0.03 ± 0.04	2.1 ± 0.8
5		117–207	136 ± 13	186 ± 18	0.1 ± 0.3	17.6	0.03 ± 0.05	0.11 ± 0.33	1.9 ± 0.9
6	HRT↓ → $B_V\uparrow$	208–219	149 ± 16	264 ± 29	0.1 ± 0.1	13.5	0.29 ± 0.06	0.50 ± 0.22	2.9 ± 0.7
7		220–240	139 ± 10	416 ± 31	0.1 ± 0.2	8.0	0.61 ± 0.19	0.90 ± 0.26	3.3 ± 0.4
8		241–280	155 ± 18	742 ± 87	0.1 ± 0.2	5.0	0.97 ± 0.07	0.71 ± 0.23	6.2 ± 1.3
9	HRT↑ → $B_V\downarrow$	281–333	139 ± 21	417 ± 63	0.1 ± 0.2	8.0	0.90 ± 0.16	1.05 ± 0.40	5.0 ± 1.3

In periods 8 and 9, the reactor's effluent was fed to the anammox reactor.
B_V, volumetric loading rate; FA, free ammonia.

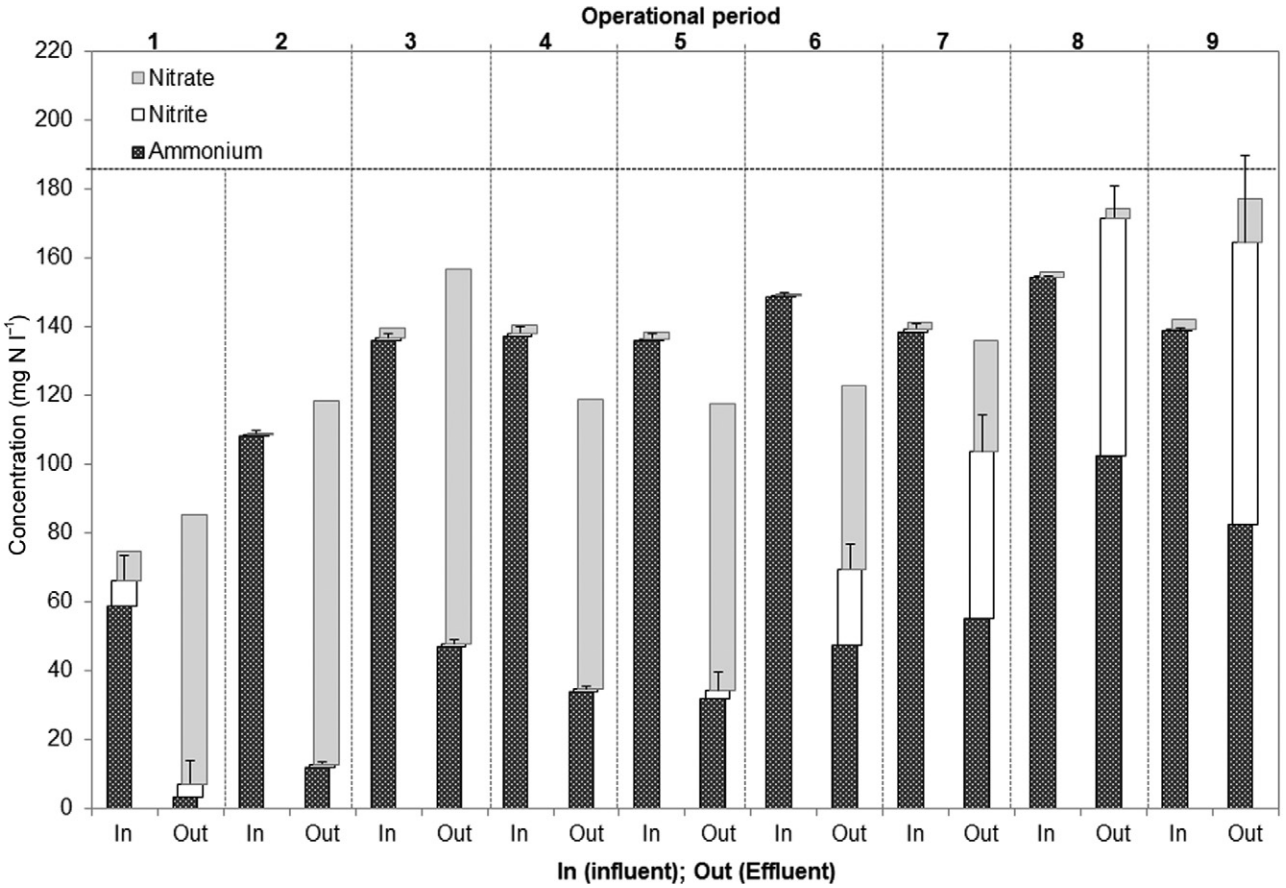

Fig. 1. Influent and effluent nitrogen concentrations (average ± standard deviation) of the upflow fixed-bed bioreactor for partial nitritation. In periods 8 and 9, the reactor's effluent was fed to the anammox reactor. For details on the operational periods, please refer to Table 1.

nitrite to ammonium ratio of 0.90 (Fig. 1; Table 1). Because the latter is in the range of the desirable influent for anammox (Eq. 1), the partial nitritation reactor was coupled to the anammox reactor at the end of period 7.

Combined two-stage operation of PANAM

The anammox CSTR was already optimized separately for achieving a stable process performance (Bagchi *et al.*, 2010). Before feeding the partial nitritation effluent to the anammox reactor, the anammox was stably operated for 1 month, fed with ammonium at 200 ± 7 mg N l^{-1} and a nitrite to ammonium influent ratio of 1.25, at a volumetric nitrogen and ammonium loading rate of 240 ± 20 and 100 ± 3 mg N l^{-1} d^{-1}, respectively, removing 77 ± 2 and 90 ± 3% of the nitrogen and ammonium respectively. From partial nitritation period 8 on, the partial nitritation FFB was coupled to the anammox CSTR. The increased loading resulted in minimal nitratation with an average nitrite accumulation efficiency of 97% (Table 1; Fig. 1). It seemed, however, that the ammonium loading rate at 742 mg N l^{-1} d^{-1} was too high compared to the oxygen entry, as the nitrite to ammonium effluent ratio dropped to 0.71. In order to prevent that this ratio would become too low to achieve high nitrogen removal efficiencies in the anammox step, the loading rate of the partial nitritation was decreased to 417 mg N l^{-1} d^{-1} in period 9. Although this loading rate was the same as in period 7, performance of the partial nitritation was superior. High FA concentration (5.0 ± 1.3 mg N l^{-1}) during this period may had selectively inhibited NOB and resulted into higher nitrite accumulation efficiency (90%) and more suitable nitrite to ammonium ratio (1.05) (Table 1).

The performance of combined PANAM was very stable, as can be seen from the readily constant nitrogen levels in the intermediate stream (FFB effluent = CSTR influent) and anammox effluent (Fig. 2). The anammox step completely removed nitrite while 24% of ammonium remained unutilized by anammox, yielding residual ammonium concentrations at 18 ± 7 mg N l^{-1}. The molar ratio of nitrite to ammonium consumption was 1.19 while nitrate production to ammonium consumption ratio in the final effluent was 0.24. The total nitrogen content of the anammox effluent was 32 ± 6 mg N l^{-1}, resulting in overall nitrogen removal efficiencies of 78%, both for the anammox process separately as for combined PANAM, because

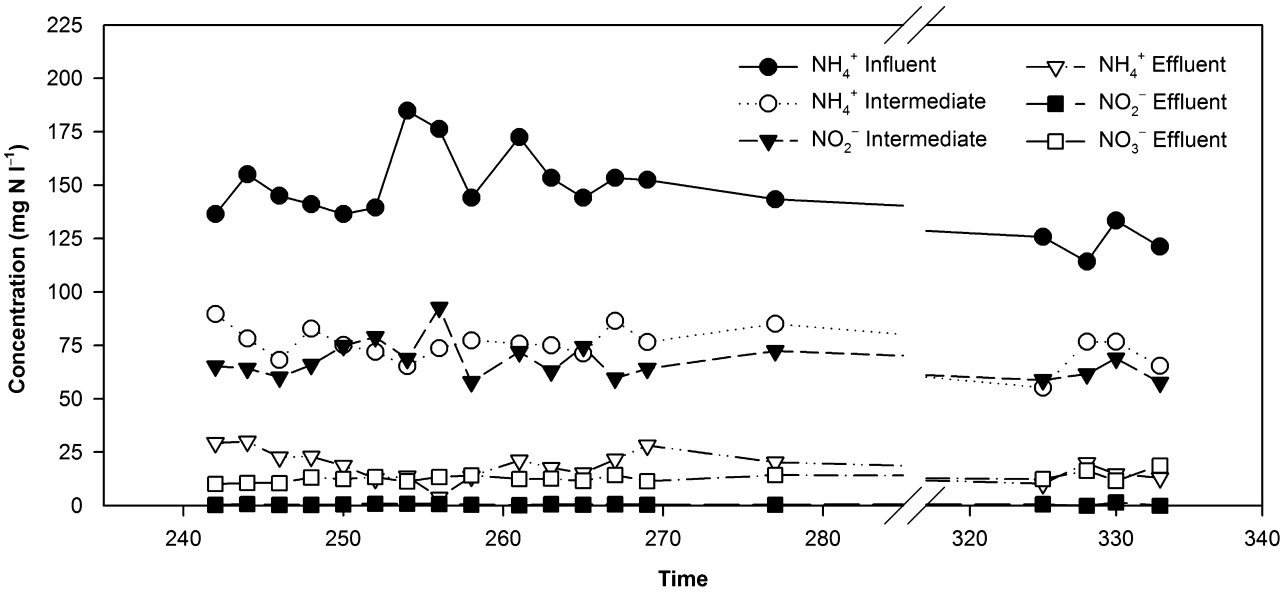

Fig. 2. Evolution of the nitrogen concentrations for the combined PANAM process. 'Influent' refers to the partial nitritation influent, 'Intermediate' to the partial nitritation effluent, i.e. the anammox influent, and 'Effluent' to anammox effluent. For the days 278–324, no analytical data are available.

practically no nitrogen was removed during partial nitritation (Table 2).

Discussion

Nitratation inhibition strategy

The initial aim of this work was to re-establish nitritation without addition of oxygen scavenging chemicals. In period 1–3, no nitrogen was purged to decrease DO in the influent. The feed DO (4.4–4.5 mg O_2 l^{-1}) was able to re-establish complete nitrification. Because no active aeration was provided to maintain the DO level in the reactor, the internal DO was likely much lower than the influent DO. Such DO levels have always favoured nitratation, as observed in our previous study (Bagchi *et al.*, 2009; Biswas *et al.*, 2011). It was expected that lowering

Table 2. Operational parameters and performance of the partial nitritation reactor (periods 8 and 9) and anammox steps in the combined PANAM process.

Parameter	Partial nitritation	Anammox	PANAM
Influent flow rate (l d^{-1})	2.33[a] 1.46[b]	1.38	NA
Reactor volume (l)	0.49[c]	2.75	3.24
Hydraulic retention time (h)	5[a] 8[b]	48	53[a] 56[b]
pH (–)	7.9 ± 0.2	7.7 ± 0.2	NA
Dissolved oxygen level (mg O_2 l^{-1})	0.1 ± 0.2[d]	0.1 ± 0.1	NA
Temperature (°C)	30 ± 4	31 ± 3	NA
Ammonium loading rate (mg N l^{-1} d^{-1})	742 ± 87[a] 440 ± 65[b]	38 ± 4	NA
Ammonium removal efficiency (%)	48 ± 8	76 ± 8	87 ± 5
Nitrogen loading rate (mg N l^{-1} d^{-1})	749 ± 89[a] 451 ± 69[b]	71 ± 6	NA
Nitrogen removal efficiency (%)	3 ± 11	78 ± 4	78 ± 5
NO_2^- consumed/NH_4^+ consumed (–)	NA	1.19 ± 0.15	NA
NO_3^- produced/NH_4^+ consumed (–)	–0.02 ± 0.06	0.24 ± 0.05	0.09 ± 0.04
Biomass concentration (g VSS l^{-1})	5.19	0.51	NA

a. For the operational period 8.
b. For the operational period 9.
c. Void volume; the carrier material occupied 0.51 l.
d. in the influent tank.
NA, not applicable.

DO will result in nitritation, but nitratation could not be inhibited at the lowest attainable DO value of 0.1 mg O_2 l^{-1} over a period of 3 month. This is contradictory to other work where nitritation was achieved at DO level below 1 mg O_2 l^{-1} (Bernet *et al.*, 2001; Chuang *et al.*, 2007). The relative loading of oxygen per NH_4^+-N during this period was 0.004 mg O mg N^{-1}, which was much lower than reported stoichiometric value of 1.9 mg O mg N^{-1} for partial nitritation (Vázquez-Padín *et al.*, 2009). Maybe the long-term operation of FFB under low DO condition has selectively enriched NOB population adapted to low DO concentration. It was recently found that lower DO concentrations (< 1.0 mg O_2 l^{-1}) selectively stimulate *Nitrospira* spp. in contrast to *Nitrobacter* spp. (Huang *et al.*, 2010). Furthermore, Park and Noguera (2008) found enduring *Nitrospira* spp. activity at 0.1–0.2 mg O_2 l^{-1}.

A strategy of increasing ammonia loading by gradual decrease of HRT established nitritation in FFB. Likely, the increased volumetric loading rate was the main cause to achieve partial nitritation in the given FFB configuration, because it increased the relative supply ratio of nitrogen to oxygen to the biofilm. Similarly, an increased nitrogen loading rate (decreased HRT) led to a lower ammonium removal efficiency in a partial nitritation FFB (Liang *et al.*, 2011). Nogueira and Melo (2006) showed that increased substrate concentration selectively inhibited *Nitrospira*, a dominat NOB in nitrifying reactors. Additionally, the decreased HRT might have played a role in suppressing nitratation. In nitrifying biofilms, nitrite oxidizing bacteria are often located beneath a layer of ammonia oxidizing bacteria (Gieseke *et al.*, 2003; Vlaeminck *et al.*, 2010). Hence, nitrite and oxygen need some time to diffuse to the nitrite oxidizing bacteria. At a lower HRT, the contact time between the reactor liquid and biofilm was shorter, and might have restricted some diffusion and hence nitratation (Ahn *et al.*, 2008).

The increased free ammonia (FA) levels from 2.9–3.3 to 6.2–5.0 mg N l^{-1} in periods 6–7 and 8–9, respectively (Table 1), could have played an additional role in restricting nitrate production. Although nitratation suppression should already have occurred at lower FA levels (0.1–0.8 mg N l^{-1}; Anthonisen *et al.*, 1976), higher levels of 3–6 mg N l^{-1} have also been described (Vadivelu *et al.*, 2007; Vlaeminck *et al.*, 2009). Some studies express a specific inhibitory FA concentration for nitratation. At the biomass concentration of 5.19 g VSS l^{-1}, specific FA levels were 0.6–0.6 and 1.2–1.0 mg N g^{-1} VSS for periods 6–7 and 8–9 respectively. These values are also in the order of the typical nitratation inhibition threshold (0.2–1.5 mg N g^{-1} VSS; Fdz-Polanco *et al.*, 1994; Villaverde *et al.*, 1997; 2000), indicating that FA likely played a role in preventing nitrate formation in the FFB.

PANAM process performance

The two-stage PANAM was stably operated for more than 3 months with overall nitrogen removal efficiencies of 78%. The decrease in volumetric nitrogen loading rate in the second step Anammox CSTR did not affect the process performance. The molar ratio of nitrite to ammonium consumption (1.19) was similar to the value of 1.31 obtained previously from separate operation of the anammox CSTR (Bagchi *et al.*, 2010), and near the expected value of 1.32 (Eq. 1). The nitrate production to ammonium consumption ratio (0.24) in the final effluent also matched the near expected value of 0.26 (Eq. 1). Both nitrite consumption and nitrate production ratios indicate that the anammox unit was operating under nitrite-limiting conditions and also that the contribution of other nitrogen converting processes such as denitrification was negligible. It supports previous observations that nitrite-limiting conditions guarantee a more stable anammox process operation (Wyffels *et al.*, 2004).

Under independent operation, the highest nitrogen removal rates of 216 mg N l^{-1} d^{-1} were achieved in anammox CSTR reactor (Bagchi *et al.*, 2010). However, due to limitation of flow and nitrogen concentration (nitrogen loading rates) from FFB reactor, the anammox CSTR was sub-normally operated. The nitrogen removal rates of the anammox step can be further increased by higher anammox biomass loading rates, obtained by either using smaller second stage compared with first stage or by using higher anammox biomass concentrations. But under the given circumstances of reactor operation, no sufficient flow rates of partial nitritation effluent were available to increase the nitrogen loading rate, which was rather low (71 mg N l^{-1} d^{-1}). Given the low biomass content in the CSTR (0.51 g VSS l^{-1}), specific anoxic ammonium oxidation rates of 34 mg N g^{-1} VSS d^{-1} were in the expected order of magnitude for flocs (Vlaeminck *et al.*, 2010; Desloover *et al.*, 2011).

Straightforward operation of PANAM

In practice the purging of the partial nitritation influent would probably not be necessary, because this seemed not crucial in achieving partial nitritation, and because typical wastewaters for autotrophic nitrogen removal include digestates and landfill leachate (Paredes *et al.*, 2007), which are anaerobic streams. The main advantages between PANAM and other two-stage processes are the energy-friendly and non-complicated way of operation. Although two-stage process entails higher investments costs than one stage, a two-stage process allows attuning and optimizing the main reactions individually. Separating nitritation and anaerobic ammonium oxidation in two stages allows flexibility in operation and

easier process control as both the stages can be controlled independently.

In the FFB, stable partial nitritation could be achieved energy-friendly and cost-effectively, with minimal pumping energy (only influent, no recirculation) and no aeration energy. If one conceptually considers the FFB treatment of wastewater at the nitrogen concentrations and loading rates from this study, i.e. 0.15 kg N m^{-3} and 0.4 kg N m^{-3} d^{-1}, at an example load of 450 kg N d^{-1}, in the order of a potato-processing company (Desloover *et al*., 2011), as such a FFB reactor volume of 1125 m^3 is required, and will be fed at 125 m^3 h^{-1}. If one aims at an upflow velocity of 2 m h^{-1}, the reactor would have a height of 9 m, given the packed volume of 50%. The energy consumption of the FFB would derive from an influent pump, which has to pump the liquid from an influent reservoir upwards through the reactor. With a pump submerged for 0.5 m in the reservoir, a pump efficiency of 60% and negligible head losses in the reactor due to the low Reynolds number (4391), the electrical energy requirement is calculated at 5.4 kW, or 0.30 kWh kg^{-1} N treated. For the anammox, a loading rate of 1 kg N m^{-3} d^{-1} should be feasible in practice, which would yield in this example an anammox reactor of 450 m^3. At an electricity consumption of 0.01 kW m^{-3} reactor, the mixing of the anammox stage would require 0.24 kWh kg^{-1} N. As such, PANAM is estimated to consume 0.54 kWh kg^{-1} N. In contrast, in the case of autotrophic nitrogen removal with blower aeration, an electricity consumption of 1.2 kWh kg^{-1} N has been reported (Wett *et al*., 2010). Hence, PANAM could save about 50% of the energy costs.

Besides the economical benefit, the PANAM process control mechanisms did not involve sophisticated procedures. Partial nitritation control consisted of finding an optimal volumetric loading rate (416–742 mg N l^{-1} d^{-1}) and HRT (5–8 h), yielding excellent effluent properties to feed the anammox process. Operation of the anammox step only comprised delicate mixing and no sludge wasting. Further investigation is needed to test the applicability of PANAM for treatment of real industrial effluents under field conditions.

Experimental procedures

Synthetic wastewater

Synthetic wastewater was freshly prepared on a daily basis, based on Milli-Q water (Millipore, Billerica, USA) supplemented with (in g l^{-1}) $NaHCO_3$ 1.25, KH_2PO_4 0.025, $CaCl_2{\cdot}2H_2O$ 0.3, $MgSO_4{\cdot}7H_2O$ 0.2, $FeSO_4$ 0.00625, EDTA 0.00625. Additionally, 25 ml l^{-1} of a trace element solution, containing (in g l^{-1}) EDTA 15, $ZnSO_4{\cdot}7H_2O$ 0.43, $CoCl_2{\cdot}6H_2O$ 0.24, $MnCl_2{\cdot}4H_2O$ 0.99, $CuSO_4{\cdot}5H_2O$ 0.25, $NaMoO_4{\cdot}2H_2O$ 0.22, H_3BO_4 0.014. The ammonium source in the wastewater was $(NH_4)_2SO_4$ and the influent concentrations for the partial nitritation reactor were varied during the experiment (Table 1). Note that the molar HCO_3^- to N ratio was always higher than 1, ensuring sufficient buffering capacity for the proton production from partial nitritation. For the anammox reactor, nitrite was added as $NaNO_2$ to the influent at a No_2^-N/NH_4^+-N ratio of 1.3 ± 0.1, until the influent was replaced with the partial nitritation effluent.

Partial nitritation FFB

The FFB was previously described in detail (Bagchi *et al*., 2009). The 1.2 l reactor had a working volume of 1 l, of which 0.51 l was occupied by uniformly sized pieces of refractory bricks (0.2–0.3 cm diameter) as support media for biofilm growth. Hence, the liquid volume in the reactor was 0.49 l. A peristaltic pump was employed for feeding, and the flow rate (0.66–2.33 l d^{-1}) was calculated from the desired volumetric loading rates and hydraulic retention times (HRT) according to the operational period (Table 1). The reactor was operated in an upflow mode, without recirculation. The effluent was collected in an effluent collection tank. All vessels were made of glass and were covered to minimize oxygen entry. The reactor was operated at ambient temperatures (30 ± 4°C). Until 94th day, the DO concentration in the synthetic feed was 4.5 ± 0.2 mg O_2 l^{-1}. From day 96 onwards to the end of the study, it was reduced to the required concentration in the feed tank through N_2 gas purging (Table 1).

Anammox CSTR

The CSTR was previously described in detail (Bagchi *et al*., 2010) and the experimental setup of combined PANAM process is shown in Fig. 3. The 3.3 l reactor had a working volume of 2.75 l. The feed was introduced using a peristaltic pump at a constant flow rate (1.38 l d^{-1}). The reactor content was stirred continuously with a bladed impeller at low speed (30 ± 5 r.p.m.). A 0.57 l settler was provided before the effluent collection tank and all biomass was recycled manually from the settler to the reactor. All vessels were made of glass and were covered to minimize oxygen entry. Once stable partial nitritation was obtained in the FFB, the feed for the anammox CSTR was manually transferred from the outlet tank of the partial nitritation reactor. Care was taken during manual transfer to maintain anoxic conditions. The reactor was operated at ambient temperatures (31 ± 3°C).

Analytical methods

Ammonium was measured colourimetrically according to standard methods (Greenberg *et al*., 1992). Free ammonia values were calculated using measured temperature, pH and ammonium values, according to Anthonisen and colleagues (1976). Nitrate and nitrite were analysed by ion chromatography using a carbonate/bicarbonate eluent and conductivity detection (833 IC Liquid handling unit, 818 IC Pump, 820 IC Separation Centre with IonPac A 250A Column, 819 IC Detector and 830 IC Interface Metrohm, Herisau, Switzerland). DO, temperature and pH were measured with Multiparameter PCD 650 (Eutech Instruments, Singapore). Volatile suspended solids (VSS) were determined at the end of the

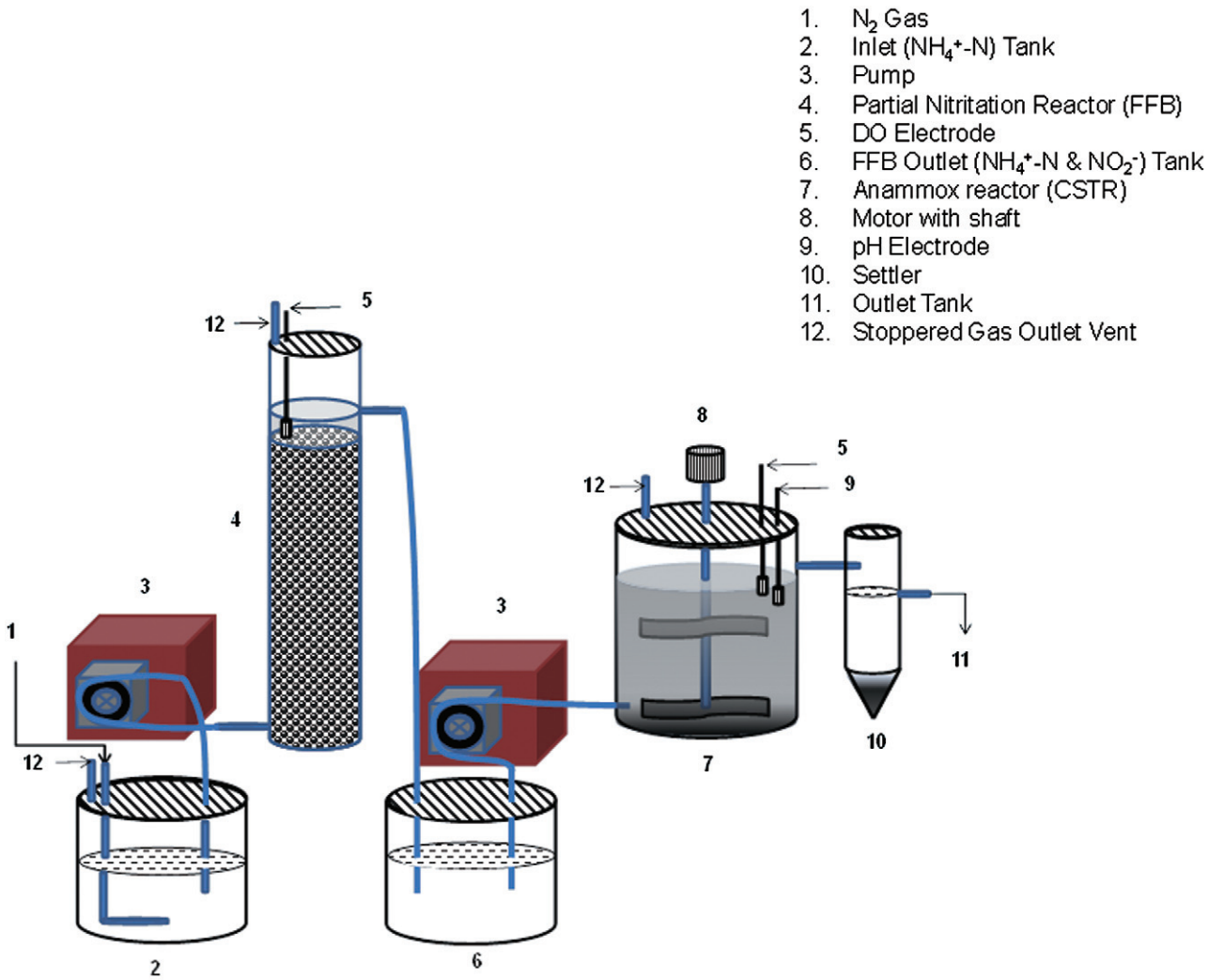

Fig. 3. Schematic diagram of experimental setup of two-stage PANAM process.

experiment, with weighing, drying and ashing, as described in standard methods (Greenberg *et al.*, 1992).

Acknowledgements

The financial support provided by Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi for this project is gratefully acknowledged. S.B. was supported by Senior Research Fellowship from Council for Scientific and Industrial Research (CSIR), India and S.E.V. was supported by a postdoctoral fellowship from the Research Foundation Flanders (FWO-Vlaanderen), Belgium. The authors are thankful to Dr S.R. Wate, Director, NEERI, Nagpur, India for his guidance.

References

Abma, W.R., Driessen, W., Haarhuis, R., and van Loosdrecht, M.C.M. (2010) Upgrading of sewage treatment plant by sustainable and cost-effective separate treatment of industrial wastewater. *Water Sci Technol* **61:** 1715–1722.

Ahn, J.H., Yu, R., and Chandran, K. (2008) Distinctive microbial ecology and biokinetics of autotrophic ammonia and nitrite oxidation in a partial nitrification bioreactor. *Biotechnol Bioeng* **100:** 1078–1087.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., and Srinath, E.G. (1976) Inhibition of nitrification by ammonia and nitrous acid. *J Water Pollut Control Fed* **48:** 835–852.

Bagchi, S., Biswas, R., Roychoudhury, K., and Nandy, T. (2009) Stable partial nitrification in an up-flow fixed-bed bioreactor under an oxygen-limiting environment. *Environ Eng Sci* **26:** 1309–1318.

Bagchi, S., Biswas, R., and Nandy, T. (2010) Start-up and stabilization of an Anammox process from a non-acclimatized sludge in CSTR. *J Ind Microbiol Biotechnol* **37:** 943–952.

Bernet, N., Dangcong, P., Delgenes, J.P., and Moletta, R. (2001) Nitrification at low oxygen concentration in biofilm reactor. *J Environ Eng* **127:** 266–271.

Biswas, R., Bagchi, S., Bihariya, P., Das, A., and Nandy, T. (2011) Stability and microbial community structure of a partial nitrifying fixed-film bioreactor in long run. *Bioresour Technol* **102:** 2487–2494.

Chuang, H.P., Ohashi, A., Imachi, H., Tandukar, M., and Harada, H. (2007) Effective partial nitrification to nitrite by down-flow hanging sponge reactor under limited oxygen condition. *Water Res* **41:** 295–302.

Desloover, J., De Clippeleir, H., Boeckx, P., Du Laing, G., Colsen, J., Verstraete, W., and Vlaeminck, S.E. (2011) Floc-based sequential partial nitritation and anammox at full scale with contrasting N_2O emissions. *Water Res* **45:** 2811–2821.

Erguder, T.H., Boon, N., Vlaeminck, S.E., and Verstraete, W. (2008) Partial nitrification achieved by pulse sulfide doses in a sequential batch reactor. *Environ Sci Technol* **42:** 8715–8720.

Fdz-Polanco, F., Villaverde, S., and Garcia, P.A. (1994) Temperature effect on nitrifying bacteria activity in biofilters: activation and free ammonia inhibition. *Water Sci Technol* **30:** 121–130.

Fux, C., and Siegrist, H. (2004) Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: environmental and economical considerations. *Water Sci Technol* **50:** 19–26.

Gieseke, A., Bjerrum, L., Wagner, M., and Amann, R. (2003) Structure and activity of multiple nitrifying bacterial populations co-existing in a biofilm. *Environ Microbiol* **5:** 355–369.

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

Hellinga, C., Schellen, A., 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. *Water Sci Technol* **37:** 135–142.

Huang, Z.H., Gedalanga, P.B., Asvapathanagul, P., and Olson, B.H. (2010) Influence of physicochemical and operational parameters on *Nitrobacter* and *Nitrospira* communities in an aerobic activated sludge bioreactor. *Water Res* **44:** 4351–4358.

Jeanningros, Y., Vlaeminck, S.E., Kaldate, A., Verstraete, W., and Graveleau, L. (2010) Fast start-up of a pilot-scale deammonification sequencing batch reactor from an activated sludge inoculum. *Water Sci Technol* **61:** 1393–1400.

Lackner, S., Terada, A., and Smets, B.F. (2008) Heterotrophic activity compromises autotrophic nitrogen removal in membrane-aerated biofilms: results of a modeling study. *Water Res* **42:** 1102–1112.

Liang, Z.W., Han, Z.Y., Yang, S.Y., Liang, X.Q., Du, P., Liu, G.F., and Yang, Y. (2011) A control strategy of partial nitritation in a fixed bed biofilm reactor. *Bioresour Technol* **102:** 710–715.

Mathure, P., and Patwardhan, A. (2005) Comparison of mass transfer efficiency in horizontal rotating packed beds and rotating biological contactors. *J Chem Technol Biotechnol* **80:** 413–419.

Nogueira, R., and Melo, L.F. (2006) Competition between *Nitrospira* spp. and *Nitrobacter* spp. in nitrite-oxidizing bioreactors. *Biotechnol Bioeng* **95:** 169–175.

Paredes, D., Kuschk, P., Mbwette, T.S.A., Stange, F., Muller, R.A., and Koser, H. (2007) New aspects of microbial nitrogen transformations in the context of wastewater treatment – a review. *Eng Life Sci* **7:** 13–25.

Park, H.D., and Noguera, D.R. (2008) *Nitrospira* community composition in nitrifying reactors operated with two different dissolved oxygen levels. *J Microbiol Biotechnol* **18:** 1470–1474.

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

Strous, M., Heijnen, J.J., Kuenen, J.G., and 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.

Vadivelu, V.M., Keller, J., and Yuan, Z. (2007) Free ammonia and free nitrous acid inhibition on the anabolic and catabolic processes of *Nitrosomonas* and *Nitrobacter*. *Water Sci Technol* **56:** 89–97.

Vázquez-Padín, J.R., Pozo, M.J., Jarpa, M., Figueroa, M., Franco, A., Mosquera-Corral, A., *et al*. (2009) Treatment of anaerobic sludge digester effluents by the CANON process in an air pulsing SBR. *J Hazard Mater* **166:** 336–341.

Verstraete, W., and Vlaeminck, S.E. (2011) ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future. *Int J Sust Dev World Ecol* **18:** 253–264.

Villaverde, S., García-Encina, P.A., and Fdz-Polanco, F. (1997) Influence of pH over nitrifying biofilm activity in submerged biofilters. *Water Res* **31:** 1180–1186.

Villaverde, S., Fdz-Polanco, F., and Garcia, P.A. (2000) Nitrifying biofilm acclimation to free ammonia in submerged biofilters. Start-up influence. *Water Res* **34:** 602–610.

Vlaeminck, S.E., Terada, A., Smets, B.F., Van der Linden, D., Boon, N., Verstraete, W., and Carballa, M. (2009) Nitrogen removal from digested black water by one-stage partial nitritation and anammox. *Environ Sci Technol* **43:** 5035–5041.

Vlaeminck, S.E., Terada, A., Smets, B.F., De Clippeleir, H., Schaubroeck, T., Bolca, S., *et al*. (2010) Aggregate size and architecture determine microbial activity balance for one-stage partial nitritation and anammox. *Appl Environ Microbiol* **76:** 900–909.

Wett, B., Nyhuis, G., Hell, M., Takács, I., and Murthy, S. (2010) Development of Enhanced Deammonification Selector. In: WEFTEC 2010. New Orleans, US.

Wyffels, S., Boeckx, P., Pynaert, K., Zhang, D., Van Cleemput, O., Chen, G., and Verstraete, W. (2004) Nitrogen removal from sludge reject water by a two-stage oxygen-limited autotrophic nitrification denitrification process. *Water Sci Technol* **49:** 57–64.