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Integration of anammox into the aerobic granular sludge process for main stream wastewater treatment at ambient temperatures

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ARTICLE INFO

Article history:
Received 25 May 2011
Received in revised form
10 October 2011
Accepted 17 October 2011
Available online 6 November 2011

Keywords:
Anammox
Granular sludge
AOB
Acetate
Ambient temperature

ABSTRACT

Anaerobic ammonium oxidation, nitrification and removal of COD was studied at ambient temperature (18 °C ± 3) in an anoxic/aerobic granular sludge reactor during 390 days. The reactor was operated in a sequencing fed batch mode and was fed with acetate and ammonium containing medium with a COD/N ratio of 0.5 [g COD/gN]. During influent addition, the medium was mixed with recycled effluent which contained nitrate in order to allow acetate oxidation and nitrate reduction by anammox bacteria. In the remainder of the operational cycle the reactor was aerated and controlled at a dissolved oxygen concentration of 1.5 mg O_2/l in order to establish simultaneous nitritation and Anammox. Fluorescent in-situ hybridization (FISH) revealed that the dominant Anammox bacterial population shifted toward Candidatus "Brocadia fulgida" which is known to be capable of organotrophic nitrate reduction. The reactor achieved stable volumetric removal rates of 900 [g N_2–N/m^3/day] and 600 [g COD/m^3/day]. During the total experimental period Anammox bacteria remained dominant and the sludge production was 5 fold lower than what was expected by heterotrophic growth suggesting that consumed acetate was not used by heterotrophs. These observations show that Anammox bacteria can effectively compete for COD at ambient temperatures and can remove effectively nitrate with a limited amount of acetate. This study indicates a potential successful route toward application of Anammox in granular sludge reactors on municipal wastewater with a limited amount of COD.

1. Introduction

Anaerobic ammonium oxidizing bacteria (Anammox) are capable of autotrophic ammonium oxidation with nitrite as electron acceptor (Strous et al., 1999). After the discovery of Anammox by Mulder in 1985, Anammox bacteria have successfully been implemented in full scale wastewater treatment systems to treat ammonium rich wastewater cost effectively (Abma et al., 2010; Sliekers et al., 2003; van der Star et al., 2007; Wett, 2007). Currently Anammox is applied at mesophilic temperatures and on wastewater containing high concentrations of ammonium. In order to supply Anammox with nitrite for the oxidation of ammonium two different systems are proposed. Nitrite can be either produced in a separated tank by partial nitrification and in turn be fed into an non-aerated Anammox reactor (Sharon-Anammox) or produced in an oxygen limited one stage system (CANON) (Sliekers et al., 2003; van Dongen et al., 2001). In the latter

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doi:10.1016/j.watres.2011.10.034

system ammonium oxidizing bacteria (AOB) and Anammox grow together in one granule in which AOBs are located on the outer oxygen penetrated shell, where they oxidize ammonium to nitrite. Anammox can grow in the oxygen shielded inner core were ammonium and nitrite are available (Hao et al., 2005). Currently the nitritation/anammox processes are applied predominantly for treatment of sludge digester rejection water and effluent from industrial anaerobic wastewater treatment that both do not contain any or only limited amounts of organic carbon. If Anammox can be applied at lower temperatures and nitrogen concentrations, its application potential could be extended to municipal sewage treatment (Jetten et al., 1997).

In order to implement Anammox in sewage treatment, pre-removal of COD is normally required. Heterotrophic growth results in a decrease of the SRT and a very high SRT is essential for successful cultivation of Anammox at ambient temperatures. Given the low yield and growth rate of Anammox bacteria, heterotrophic growth should be minimized, to maintain a high fraction of Anammox bacteria in the sludge. Nitrate produced either by Anammox or nitrite oxidizing bacteria would have to be removed by nitrate reduction processes. Pre-removal of organic carbon can be established after the A-stage in an A/B process, after physio-chemical pretreatment, or after anaerobic digestion (Jetten et al., 1997; Joss et al., 2009; Kartal et al., 2010; Wett, 2007). Simultaneous partial nitrification, anammox and denitrification has been reported for treating wastewater with an approximate COD/N ratio of 0.5 [g COD/gN] at temperatures of 30–36 °C under constantly aerated conditions (Chen et al., 2009; Lan et al., 2011; Xu et al., 2010). In these studies the COD was removed by regular heterotrophic bacteria.

Recently, it was reported that certain Anammox species have the capacity to oxidize volatile fatty acids with nitrate as electron acceptor, while forming ammonium with nitrite as intermediate (Gueven et al., 2005; Kartal et al., 2007a, 2007b). Anammox does not incorporate the fatty acids into biomass, but completely converts them into CO_2 (Kartal et al., 2007a). Why Anammox remains growing autotrophically while oxidizing acetate is not well understood but the low biomass yield associated with autotrophic growth is beneficial for wastewater treatment since sludge production is minimized. When COD oxidation with nitrate can be catalyzed by Anammox, nitrate produced by Anammox bacteria (or nitrite oxidizing bacteria) can be removed resulting in a lower nitrogen effluent concentration. Previous research in anoxic reactors showed that heterotrophs will win the competition for nitrate if the COD/N ratio exceeds 1 (Gueven et al., 2005).

If oxygen and acetate are available at the same time Anammox bacteria do not only need to compete with NOBs for nitrite but also general heterotrophs will get an advantage over Anammox since Anammox is inhibited by oxygen (Strous et al., 1997). A better strategy would be therefore to proceed a nitritation/anammox period with an anoxic COD oxidation period. This allows treating wastewater with easy degradable soluble compounds such as acetate to promote the oxidation of acetate with nitrate by Anammox bacteria. In this study we aimed to explore the possibility to convert COD (in the form of acetate) and ammonium at a COD/N ratio of 0.5 by Anammox and AOB in a granular sludge reactor at ambient temperature (18 °C ± 3). Hereto we operated a granular sludge SBR. During one fourth of the cycle time the ammonium and acetate were mixed with reactor effluent containing nitrate from the previous cycle. In this way nitrate reduction can be catalyzed anoxically by Anammox with acetate. During the remaining three-fourth of the cycle the reactor was aerated for ammonium removal by nitritation and Anammox.

2. Material and methods

2.1. Long term operation

A lab-scale anoxic/aerobic bubble column reactor with a total volume of 2.9 L was run for 390 days at ambient temperature (18 ± 3 °C) in sequencing fed batch mode. The reactor was inoculated with granular Anammox sludge from the Rotterdam Dokhaven Anammox reactor. The reactor was operated in two phases (Fig. 3). In phase I (day 0–260), the reactor was fed with medium containg ammonium and nitrite (115 mg NO_2–N/l ($NaNO_2$), 190 mg NH_4–N/l (NH_4Cl)) and low concentrations of COD (25–90 mg COD/l (C_2H_3OONa)) to establish

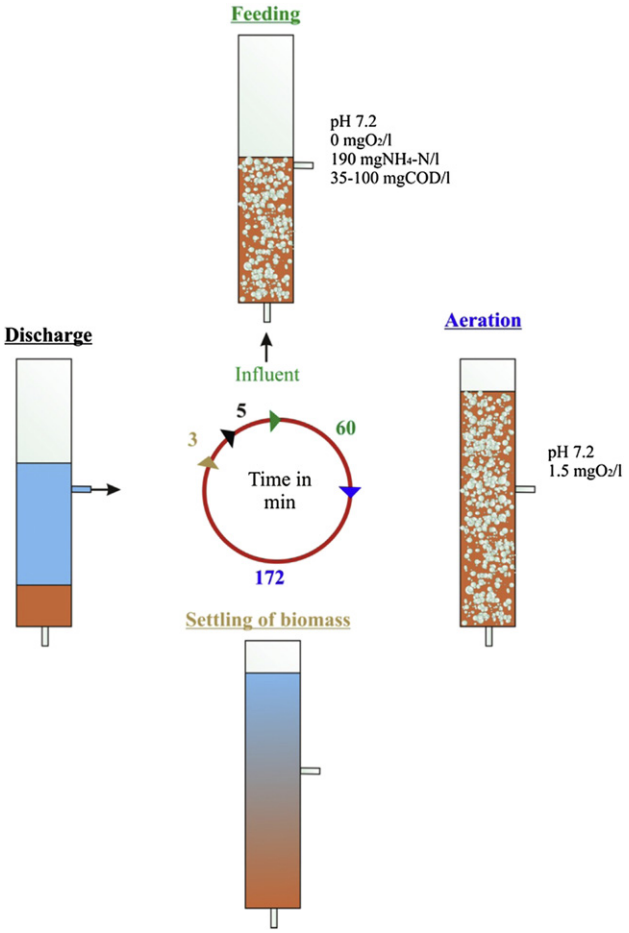

Fig. 1 – Shows the cycle operation in experimental set-up of 1) mixed anaerobic fed batch period with nitrate from aerobic period and ammonium and COD in the influent (60 min) 2) aerobic period (172 min) 3) settling period (3 min) 4) discharge period (5 min).

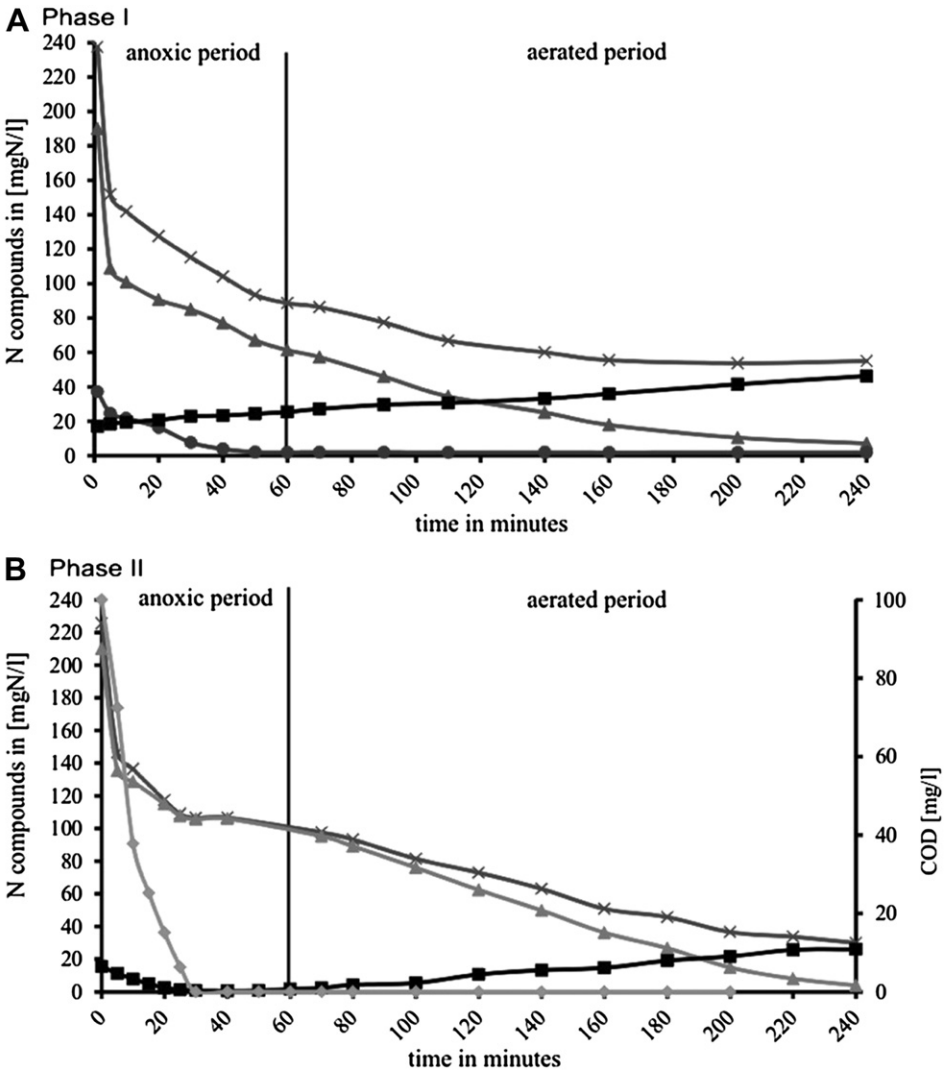

Fig. 2 – Nitrogen compounds and COD (in form of acetate) during one cycle of operation in A) phase I and B) phase II. Graph displays nitrate (■), nitrite (●) ammonium (▲) total nitrogen (×) and acetate (◆) profiles over time. Note that opposed to normal cycle operation all media was fed at once into reactor in order to follow trends over time during anoxic period.

a stable system for combined nitritation and Anammox and organic carbon removal. During phase I the COD/N ratio was kept at 0.1 until day 170 after which it was raised gradually by decreasing nitrite and increasing COD in the influent until a ratio of 0.4 (day 260). In phase II the medium consisted of a nitrite free and acetate rich medium (100 mg COD/l) reaching a COD/N ratio of 0.5 (phase II; day 260–390). The mineral medium consisted of 0.2 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM KCl, 2 mM $NaHCO_3$, 0.2 mM K_2HPO_4, 0.1 mM KH_2PO_4. 'Visniac and Santer' solution was used to provide trace elements (Visniak and Santer, 1957). The pH was maintained at 7.2 ± 0.2 during the aerobic period by dosage of hydrochloric acid and sodium hydroxide. The dissolved oxygen (DO) concentration was controlled at 1.5 mg O_2/l. The DO was set by recirculating the off-gas and blending with fresh air. In this way the DO could be regulated while maintaing a constant superficial air velocity of 2 l/min (Mosquera-Corral et al., 2005). Samples were taken on a weekly basis and analyzed for N-compounds by the use of standard test kits (Hach-Lange). Sludge bed height remained constant over time (±1 cm). Biomass production was monitored over time by catching effluent from one cycle and determining dry weight and ash content.

2.2. *Cycle operation*

The reactor was operated in a sequencing fed batch mode and the different periods are displayed in Fig. 1. During the mixed anoxic feeding period (60 min) nitrate produced during the previous cycle was mixed with medium containing acetate and ammonium. After the feeding period an aerated period for partial nitrification was introduced lasting 172 min, followed by a settling period (3 min), and an effluent withdrawal period (5 min). During the effluent removal period half of the reactor liquid volume (1.5 l) was discharged and half (1.4 l) remained in the system.

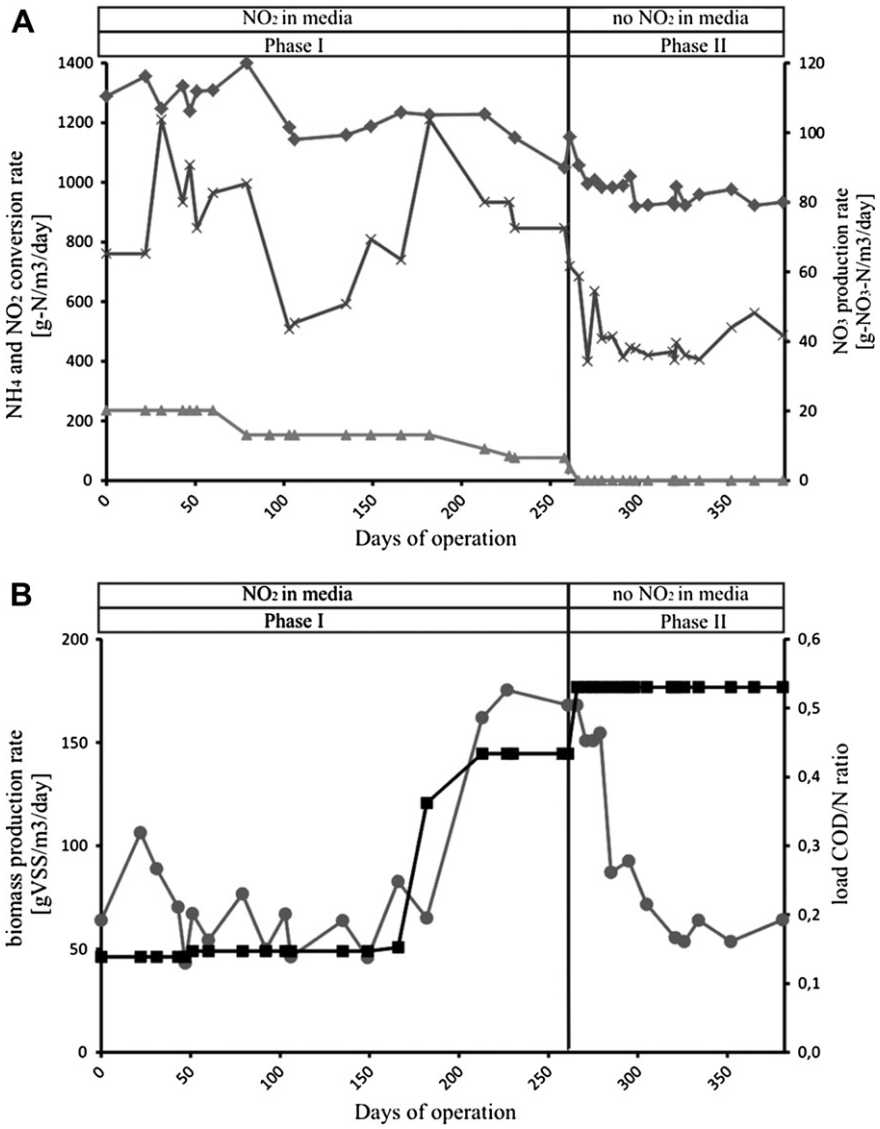

Fig. 3 – A) Volumetric conversion rates of ammonium (◆) and nitrite (▲) as well as production of nitrate (×) B) biomass production rate (●) and the COD/N ratio (■) over time.

The reactor performance during one cycle of operation was analyzed for phase I (nitrite in the feed) and phase II (no nitrite in the feed) (Fig. 2). Samples were taken every 10–20 min to measure ammonium, nitrite and nitrate by means of flow injection analysis (Quick Chem8500, Lachat instruments). Acetate was measured by using a High Performance Liquid Chromatography (HPLC).

2.3. *Microscopic characterization of granules*

Granules were taken for microscopic analysis in order to assess their morphology and microbiological composition. Slicing and FISH was accomplished by the method proposed by (Winkler et al., 2011b) (Fig. 4) to see the spatial distribution of bacteria as a function of depth within the granule. FISH was performed for determination of general Anammox bacteria (Cy3) general bacteria Eub (Cy5) and Candidatus "Brocadia fulgida" (Fluos). For AOB a mix of two probes was prepared and labeled with (Cy5). Probe sequences are listed in Table 1.

2.4. *Biomass yields*

The estimated community composition was based on produced biomass per consumed acetate ($Y_{x/HAC}$) and ammonium (Y_{X/NH_4^+}), respectively (Table 2). It was assumed that all acetate which was fed into the reactor (100 mg COD/l) would be metabolized by either Anammox or heterotrophic bacteria. In case of Anammox being the only active bacteria half of the consumed ammonium was supposed to be used for partial nitrification (AOB) and the other half by anaerobic ammonium oxidation. Since Anammox does not incorporate acetate into biomass no growth on acetate for Anammox was assumed. For the conversion from COD to VSS a factor of 1.4 was used (Scherer et al., 1983).

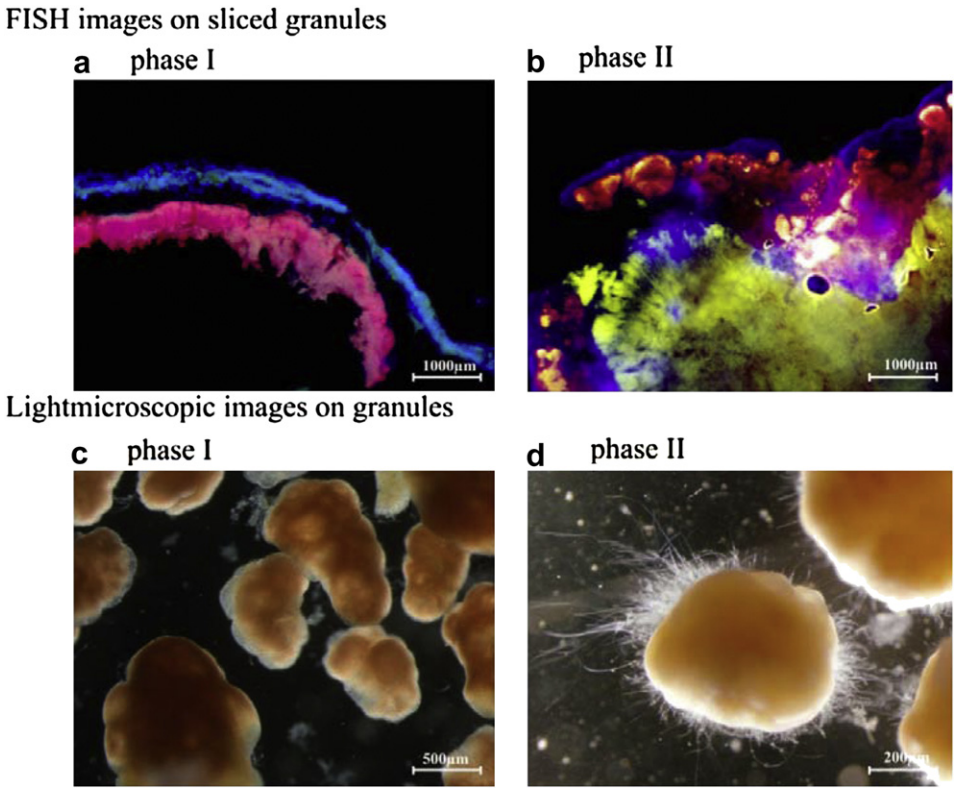

Fig. 4 – Microbial images from phase I (a,c) and II (b,d) a) FISH on sliced granules with general anammox bacteria (red), Candidatus "Brocadia fulgida" (green) and general AOB (blue) b) FISH on sliced granules with general anammox bacteria (red), Candidatus "Brocadia fulgida" (green) and general Eub (blue) c, d) Light microscopic images of granules in phase I (c) and II (d). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. *Long term operation*

The reactor was operated in two phases over a time period of 390 days. The volumetric conversion rates are displayed in Fig. 3 and are based on the difference between the total soluble nitrogen compounds in the influent and effluent of the reactor. In phase I when the reactor influent contained nitrite (115 mg NO_2–N/l) and small amounts of acetate (25 mg COD/l) the nitrogen removal rate reached 1200 [g N_2–N/m^3/day] and gradually decreased upon decreasing the nitrite and increasing the COD concentration in the medium (Fig. 3A). Phase I started with a low COD/N ratio of 0.1 and reached a ratio of 0.5 in phase II. In phase II no nitrite was supplied and the influent COD concentration was kept constant at 100 mg COD/l reaching an average volumetric N conversion rate of 900 [g N_2–N/m^3/day] as well as a COD removal rate of 600 [g COD/m^3/day] (Fig. 3A). The measured nitrate in the effluent during phase I was 71 ± 16 [mg NO_3/m^3/day] (Fig. 3A day phase I (day0–260)), and decreased when acetate was increased

Table 1 – Oligonucleotide probes and primers target microorganisms, and references used in this study.

Probes	Sequence (from '5 to '3)	Specificity	Reference
Amx 368	CCTTTCGGGCATTGCGAA	All Anammox bacteria	(Schmid et al., 2003)
Bfu613	GGATGCCGTTCTTCCGTTAAGCGG	*Candidatus Brocadia fulgida*	(Kartal et al., 2008)
EUB 338	GCTGCCTCCCGTAGGAGT	Most bacteria	(Amann et al., 1990)
EUB 338 III	GCTGCCACCCGTAGGTGT	Verrucomicrobiales	(Daims et al., 1999)
NSO190	CGATCCCCTGCTTTTCTCC	All AOB	(Mobarry et al., 1997)
NSO1225	CGCCATTGTATTACGTGTGA	All AOB	(Mobarry et al., 1997)

Probes for Anammox fulgida were tagged with the fluorescent dye Fluos (green) general Anammox with Cy3 (red) and Eubs as well as AOBs with Cy5 (blue). For analysis probes of one target group were mixed.

Table 2 – Biomass yields for anammox, AOB and heterotrophic bacteria and the corresponding biomass concentration and relative community composition according to consumed substrate (100 mg COD/l; 150 mg NH_4–N/l) in reactor during one day of operation in phase II.

	$Y_{Cx/Hac}$	Y_{Cx/NH_4^+}	g VSS/m^3/day	Community composition [%]		Reference for yields
Anammox	–	0.07	30	32[a]	7[b]	(van der Star, 2008)
AOB	–	0.13	63	68[a]	15[b]	(Blackburne et al., 2007)
Heterotrophs	0.4	–	336	–	78[b]	(Beun et al., 2001)

a Calculations based on assumption that all acetate was converted by anammox.
b Calculations based on assumption that all acetate was converted by heterotrophic bacteria.

down to 40 ± 5 [mg NO_3/m^3/day] (Fig. 3A day phase II (day 260–380)). The biomass production in phase I was 61 ± 14 g VSS/m3/day when a COD/N ratio of 0.1 was applied and 126 ± 49 g VSS/m^3/day when the COD/N ratio was elevated until 0.5. During phase II when COD/N ratio was kept constant at 0.5 the biomass production decreased from initial values of approximately 150 g VSS/m^3/day to values around 55 g VSS/m^3/day.

3.2. *Reactor performance during one cycle of operation*

A cycle measurement was conducted and changes in nitrogen concentrations during one cycle of operation during phase I (influent COD/N ratio 0.1) and phase II (influent COD/N ratio 0.5) are depicted in Fig. 2. Note that for a cycle measurement influent was fed at once into the reactor at t = 0 min to be able to measure concentration profiles over time. During normal operation the mixed feeding period lasted 60 min. In phase I, when nitrite was present during anoxic feeding (0–60 min) nitrite and ammonium decreased while nitrate was formed as is can be expected to common Anammox stoichiometry. During the aerobic period (60–232 min) ammonium was oxidized by AOB and Anammox. Nitrate formation was due to Anammox activity as well the oxidation of nitrite by a small proportion of NOB, this gave a nitrate accumulation of 42 mg NO_3–N/l nitrate at the end of the cycle in phase I. In the phase II, acetate removal occurred simultaneously with nitrate removal until complete depletion of electron acceptor and donor (0–60 min) (Fig. 2B). During the aeration period (60–232 min) reactor performance in phase II (Fig. 2B) was similar to the aerated period observed in phase I (Fig. 2A) with the difference that nitrate accumulation was lowered from average values of 42 (phase I) to average values 20 mg NO_3–N/l (phase II). Ammonium measurements in the liquid are not completely indicative for the conversions since significant ammonium adsorption occurred (Bassin et al., 2011). Upon feeding ammonium is adsorbed to the granular sludge, while during conversion it gradually desorbs again (maintaining an adsorption equilibrium) during conversion.

3.3. *Microscopic analyses of granular sludge samples*

During the reactor operation granules of red granulated biomass originating from the full scale Anammox reactor system in Rotterdam (Fig. 4c) developed over time into light-reddish granules (Fig. 4d) indicating a change in community composition. During phase I the Eubacterial microbial population mainly consisted of AOB and Anammox (data not shown) whereas no Candidatus "Brocadia fulgida" was detected (Fig. 4a). Candidatus "Brocadia fulgida" (Fig. 4b) accumulated in the sludge once the nitrite was omitted from the feed during phase II, this organism was neither detected in the seed sludge nor during phase I (Fig. 4a). During phase I typical nitrite-anammox architecture of granular sludge was observed (Vlaeminck et al., 2010), with nitrifiers (here EUB) forming an outside coating of the Anammox granule. During phase II this structure essentially remained but now also a small fraction of heterotrophs got intermixed with the Anammox population (Fig. 4b).

3.4. *Biomass yields*

In order to investigate the conversion of acetate in the reactor we evaluated the biomass production and microbial community composition on calculated biomass production based on two conversion routes. If only Anammox bacteria and AOB would dominate the system and all acetate and ammonium would be consumed by Anammox bacteria and AOB a sludge productivity of approximately 93 mg VSS/m^3/day is expected and hence a system strongly dominated by Anammox bacteria (32%) and AOB (68%). If all acetate was assumed to be oxidized by heterotrophs and all ammonium assumed to be converted by partial nitrification in combination with anaerobic ammonium oxidation the sludge production was calculated to be 429 g VSS/m^3/day. If acetate was fully converted by heterotrophs it can be expected that the community was dominated by heterotrophic bacteria (78%), with only 7% of Anammox bacteria and 15% AOB in the community (Table 2).

4. Discussion

This research showed a possible new application of Anammox for wastewater treatment containing COD and ammonium with a COD/N ratio up to 0.5. It was shown that despite feeding the reactor with acetate, Anammox activity could be maintained and acetate oxidation was combined with anaerobic ammonium oxidation while keeping sludge production low. Removal rates were similar to those reported in system based on one-reactor nitration Anammox processes (Abma et al., 2010; Siegrist et al., 2008; Wett, 2007). Earlier research has shown that Anammox bacteria are capable of using organic acids like propionate and acetate (Kartal et al., 2007a, 2008). These authors demonstrated that Anammox bacteria did not incorporate acetate in biomass leading to low sludge production. Their studies were conducted in flocculent sludge

reactors at a higher temperature and with constant feeding under non-aerated conditions. Our study here showed that Anammox bacteria could outcompete normal heterotrophic denitrifying bacteria for acetate at ambient temperatures. We moreover combined the operation of a SBR similar to aerobic granular sludge systems (Winkler et al., 2011a) with the capability of Anammox to use acetate as a second electron donor for nitrate and nitrite reduction besides ammonium. Excess nitrate from the aerated period was used as electron acceptor to oxidize acetate present in the influent leading to significantly lower nitrate in the effluent in phase II compared to phase I (Figs. 2 and 3).

FISH pictures on sliced granules showed a higher amount of Anammox bacteria as expected from calculations assuming that all acetate is consumed by normal heterotrophic bacteria (Table 2). According to this calculation the microbial population would consist of 78% heterotrophs and only a minor fraction of Anammox bacteria (7%) with a total expected biomass production of 429 g VSS/m^3/day (Table 2). This is 5 fold higher than what is expected for the pure autotrophic Anammox based acetate oxidation (93 g VSS/m^3/day). The measured biomass production remained low during the last 100 days of phase 2 with values of 65 ± 14 g VSS/m^3/day which is close to calculated value of 95 g VSS/m^3/day which assumed acetate oxidation by Anammox bacteria only (Table 2). FISH images showed a significantly higher fraction of Anammox bacteria than what could be expected if heterotrophic denitrification would have outcompeted Anammox bacteria for actetate (Table 2, Fig. 4b). In addition, the color of the biomass remained red after raising the COD concentration to 600 g COD/m^3/day in phase II (Fig. 4cd). A clear shift of the Anammox population toward Candidatus "Brocadia fulgida", known for its capability to use acetate (Kartal et al., 2008), was detected by FISH (Fig. 4a, b).

Earlier studies on Anammox bacteria using granular sludge have shown an increased growth of nitrifiers in smaller granules. This is due to the fact that smaller granules (or flocks) have a larger aerobic volume fraction than larger granules thus favoring the growth of aerobic bacteria (Volcke et al., 2010; Winkler et al., 2011b). Moreover, slow growing organisms (here Anammox bacteria) are expected to grow in the inner part of a biofilm whereas faster growing organisms (here AOB or general heterotrophs) are pushed toward the rim and in turn out of the biofilm (Picireanu et al., 2004). In the granular sludge process flocks and small granules are easier washed-out. This gives a smaller SRT for the population that dominates these smaller granules and flocks (mainly the aerobic organisms and not Anammox bacteria) which is similar to studies as conducted in a nitrifying – denitrifying biofilm airlift suspension reactor (Van Benthum et al., 1997). Visual observation of microbial community composition within a granule confirmed that Anammox bacteria are located in the middle of the granule and a smaller fraction of AOB and heterotrophs are located on the outer shell of the granule (Fig. 4a, b). Therefore also due to erosion the latter organisms will have a shorter retention time. This will lead to a higher SRT for granules and hence to an enrichment of Anammox bacteria in the sludge. Measurements on removal capacities showed a total volumetric nitrogen removal of 900 [g N_2–N/m^3/day] when the COD/N ratio was 0.5 [mg/mg]. Nitrate reduction to ammonium coupled to acetate oxidation proceeds theoretically in a 4.6 mg COD/mg N-ratio which is below the measured ratio (7 ± 0.7) in the system indicating that also a fraction of COD was converted by traditional heterotrophic COD oxidation. Likely the combination of COD removal by anammox bacteria and a selective washout of heterotrophic bacteria led to the high accumulation of anammox bacteria in the studied system.

Previous studies in which Anammox bacteria were exposed to organic acids reported successful operation at low COD/N ratios around 0.5 (Chen et al., 2009; Lan et al., 2011; Xu et al., 2010), an increase in heterotrophic growth at COD/N ratio around 1 (Udert et al., 2008) and a loss in Anammox activity at a constant COD/N ratio above 1 (Gueven et al., 2005). A wastewater stream containing high loads of COD seems to be unsuitable for Anammox bacteria although this likely depends on the actual ammonium load and hence the COD/N ratio. Here we showed an option to increase removal capacity of a one stage nitration Anammox process at ambient temperatures by reducing the excess nitrate from the aeration period by mixing it with the influent containing acetate. Herewith the growth of heterotrophic bacteria can be minimized because excess nitrate can be reduced under non-aerated conditions via the organotrophic pathway of Anammox bacteria. In a continuously aerated reactor heterotrophs would have the availability of a strong electron acceptor and donor (oxygen and acetate), while Anammox bacteria are inhibited by the oxygen (Strous et al., 1997), which would hence give heterotrophs an extra advantage over Anammox bacteria to oxidize acetate.

Current research suggests the usage of Anammox based treatment systems after either an A/B process, after physiochemical pretreatment, or anaerobic digestion (Jetten et al., 1997; Joss et al., 2009; Kartal et al., 2010; Wett, 2007). If after an A-stage or a pretreatment some soluble COD is left and ammonium levels are high then the here presented treatment strategy could be applied. This forms an option to use Anammox in the main stream while obtaining a stable nitrogen removal process at ambient temperatures and low COD/N ratios. The approach chosen here is close to the operational conditions of an aerobic granular sludge system (De Bruin et al., 2004) allowing potential future combination of this novel high rate technology and Anammox processes. The advantage of Anammox bacteria being able to remove COD as well as nitrate makes the implementation of the Anammox technology indeed easier. Nitrate is produced in the regular Anammox conversion due to the coupling of CO_2 reduction for biomass synthesis to oxidation of nitrite to nitrate. Moreover at lower temperatures it might be more difficult to prevent nitrite oxidizing bacteria to grow in the system. This could lead to too high nitrate concentrations in the effluent. Integrating anoxic periods in the sequencing fed batch cycles of aerobic granular sludge reactors would indeed give the option for nitrate removal by organotrophic Anammox bacteria. In the current perspective it is not easy to speculate on potential effluent nitrate levels within Anammox systems but we believe it will not be problematic to reach similar levels of around 60–75% of total nitrogen removal in a municipal treatment plant. The organic composition of wastewater does not only contain acetate and despite the fact that Anammox is

reported to use other C sources such as propionate (Gueven et al., 2005; Kartal et al., 2007b) it remains unclear how competitive Anammox can be in an environment containing a variety of different carbon sources and how sensitive Anammox bacteria are to fluctuations in COD/N ratios.

5. Conclusions

Here we used the acetate oxidizing capacity of Anammox bacteria at ambient temperature conditions and low COD/N ratios in a nitritation/anammox granular sludge system. Nitrate produced by Anammox bacteria or due to the presence of a fraction of nitrite oxidizing bacteria was reduced significantly. Sludge production remained low suggesting that Anammox successfully competed with general heterotrophs for acetate. This is a first step toward the application of Anammox bacteria in the main stream of municipal wastewater treatment processes.

Acknowledgements

This study is partly funded by DHV and STOWA in the framework of the Dutch national Nereda research programme.

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