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Applications of Anammox based processes to treat anaerobic digester supernatant at room temperature

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ABSTRACT

The supernatant of an anaerobic digester was treated at 20 °C in two systems. The first one is a two units configuration, conformed by two sequencing batch reactors (SBR), carrying out partial nitrification and Anammox processes, respectively. Partial nitrification was achieved by granular biomass with a mean diameter of 3 mm, operating at a dissolved oxygen concentration of 2.7 mg/L. The combined system allowed the removal of nitrogen loading rates around 0.08 g N/(L d).

Afterwards, Anammox biomass was spontaneously developed in the inner core of the nitrifying granules of the SBR and therefore, partial nitrification and Anammox process were carried out in a single unit. Once the stable CANON process was established, a mean nitrogen removal rate of 0.8 g N/(L d) was registered. The settling velocities of the granules ranged from 70 to 150 m/h with sludge volumetric index values lower than 50 mL/g VSS during the whole operation.

1. Introduction

The combination of a partial nitrification followed by the Anammox process, is recommended to remove ammonia from wastewater without biodegradable carbon source. The Anammox process consists of the anaerobic oxidation of ammonia (van de Graaf et al., 1996) using nitrite as electron acceptor according to the stoichiometry described by Strous et al. (1999) (Eq. (1)):

$$NH_4^+ + 1.3NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2H_2O \quad (1)$$

In general it is not common to find effluents with the required composition to be treated via the Anammox process. For this reason several alternatives have been studied to obtain an influent with these characteristics. To reach this objective, half of the ammonium fed has to be converted into nitrite by the ammonium oxidizing bacteria (AOB) and therefore, the oxidation of nitrite to nitrate carried out by nitrite oxidizing bacteria (NOB) has to be avoided. The AOB and NOB are two phylogenetically unrelated groups whose different growth rates and the way this growth rates are affected by parameters like temperature, pH, dissolved oxygen (DO), etc. can be used to outcompete NOB and to uncouple both reaction rates.

Nitrite accumulations have been reported in several systems, for biomass growing in suspension (Blackburne et al., 2008), in biofilm (Garrido et al., 1997) or in aggregates/granules (Kim and Seo, 2006). Several strategies have been used to reach partial nitrification:

(1) Increasing free ammonia concentration working at high pH values and limiting the growth of NOB due to their higher sensitivity to free ammonia inhibition than AOB (Anthonisen et al., 1976).
(2) Decreasing the dissolved oxygen concentration due to the lower oxygen affinity of the NOB compared to AOB (Wiesmann, 1994).
(3) Operating at temperatures above 25 °C since the maximum specific growth rate of the AOB will be higher than that of NOB at these conditions. In fact this is the basis of the SHARON technology which consists of a continuous stirring tank reactor operated a hydraulic retention time of around 1 d and 30 °C to favor the growth of AOB and the washout of the NOB (Hellinga et al., 1998).

In order to apply the partial nitrification and the Anammox processes it is important to take into account that they can be performed in two different units (Sharon–Anammox processes) or in a single one, called by different names, CANON: Completely Autotrophic Nitrogen removal Over Nitrite process (Sliekers et al., 2002), OLAND: Oxygen-Limited Autotrophic Nitrification–Denitrification (Pynaert et al., 2004) or aerobic/anoxic deammonification (Helmer et al., 2001). Under oxygen-limited conditions a co-culture

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of aerobic and anaerobic ammonium oxidizing bacteria can be established in a single unit in a CANON system. In those systems, NOB compete for oxygen with the aerobic AOB and for the nitrite with Anammox bacteria, and thus its growth (and subsequent nitrate production) is prevented. Another reason to maintain low oxygen concentrations is that Anammox bacteria are reversibly inhibited by dissolved oxygen concentration higher than 0.5% of air saturation (Strous et al., 1997).

The main disadvantage of these processes relies on the low growth rate of AOB and Anammox bacteria. To enhance the performance of reactors involving slow growing bacteria, high sludge retention times are mandatory and therefore the attachment of bacteria on a carrier material to develop biofilms or the self-aggregation concept in granules are being applied. Aerobic granulation presents the advantage of excellent settleability of the granules, high biomass retention and high resistance to toxic compounds.

The aim of this work was to test the feasibility of the application of the Anammox process at lower temperatures using both, one and two units configurations. In the two units configuration the partial nitrification process was carried out in a nitrifying granular SBR fed with anaerobic digester supernatant. Once an adequate nitrite/ammonium ratio was obtained, the effluent of the granular SBR was fed to an Anammox SBR and the nitrogen removal process was studied. In the case of the single unit configuration, the previous granular SBR developed Anammox biomass which grew in the inner core of the nitrifying granules and provoked a change in the autotrophic nitrogen removal from a two reactors configuration to one reactor strategy. The maximal nitrogen removal rate achievable was evaluated in this case.

2. Methods

2.1. Reactors description

2.1.1. Granular SBR

A SBR with a working volume of 1.5 L was used. Dimensions of the unit were: height of 465 mm and inner diameter of 85 mm, the height to the diameter ratio being 5.5. The exchange volume was fixed at 50%. A set of two peristaltic pumps was used to introduce the feeding solution (on top of the reactor) and to discharge the effluent (at medium height in the column reactor), respectively. The hydraulic retention time was fixed at 0.25 d. A programmable logic controller Siemens model S7-224CPU controlled the actuations of the pumps and valves, and regulated the different periods of the operational cycle. The duration of the operational cycles was of 3 h distributed according to the scheme described in Fig. 1.

A thermostated bath was installed to control the temperature at 20 ± 1 °C. Air was supplied to the bottom of the reactor by using an air pump to promote the transfer of oxygen into the bulk liquid and to reach a suitable mixing. The concentration of DO in the liquid phase was regulated by changing the ratio of fresh air to recycled air injected in the reactor.

The nitrifying granular biomass operated in this reactor was obtained from heterotrophic aerobic granules by the stepwise decrease of the COD/N ratio in the influent (Mosquera-Corral et al., 2005). The concentration of biomass inside the system at the beginning of this study was of 5 g VSS/L with an average diameter of the granules around 2.8 mm. The reactor was carrying out partial nitrification with a DO concentration around 2 mg/L treating a synthetic wastewater with a specific ammonia oxidation rate of 0.12 g N/(g VSS d).

2.1.2. Anammox reactor

A SBR with an effective volume of 1 L was used to carry out the Anammox process. The SBR was provided with a thermostatic jacket to keep the temperature at 20 ± 1 °C. The pH value was not controlled and remained around 7.5. Complete mixture inside the reactor was achieved with a mechanical stirrer at 100 rpm. Norprene tubing and connections were used to prevent the diffusion of oxygen into the system. The SBR was operated in cycles of 6 h (Fig. 1) according to Dapena-Mora et al. (2004) and controlled with a programmable logic controller system (CPU224, Siemens). The exchange volume was of 25% and the hydraulic retention time was fixed at 1 d. The effluent from the nitrifying granular SBR was collected and stored in a cold room (4 °C) prior to feed the Anammox reactor.

The used Anammox biomass was developed at 30 °C with synthetic wastewater (Dapena-Mora et al., 2004). The reactor contained a biomass concentration of 1.5 g VSS/L with an Anammox specific activity of 0.28 g N/(g VSS d) at 30 °C.

2.2. Operational strategy

2.2.1. Granular SBR

The nitrifying granular reactor was fed with the supernatant of an anaerobic sludge digester of the WWTP of Lugo (Spain) which was collected every month in 20 L containers and stored in a cold room (4 °C). The composition of the supernatant was: pH 7.5–8.3; NH_4^+ 400–700 mg N/L; total inorganic carbon 300–505 mg C/L and total organic carbon 20–50 mg C/L. Prior to the feeding of the supernatant, the reactor was carrying out the partial nitrification treating a synthetic influent (data not shown). The reactor operation was divided into different periods (Table 1). From period I to IV, the raw wastewater was diluted in a proportion 1:1 with tap

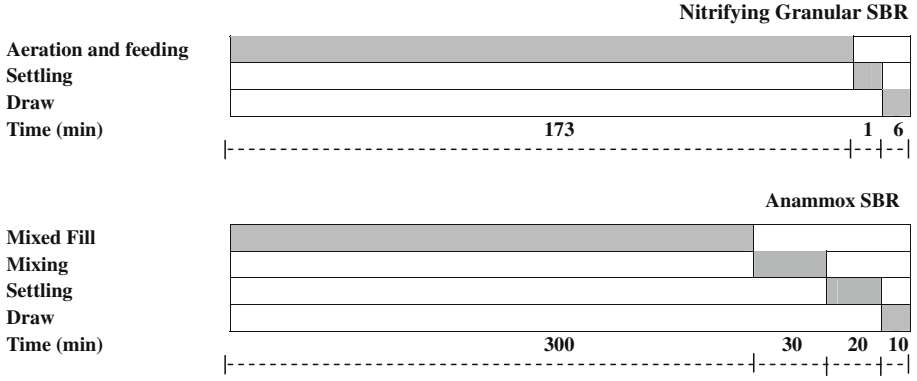

Fig. 1. Distribution of the operational cycles of the nitrifying granular and the Anammox SBRs.

Table 1
Operational periods in the granular SBR.

Period	Days	DO (mg/L)	Influent dilution (%)	Configuration
I	1–64	2.2 ± 0.4	50	Two units
II[a]	65–120	2.7 ± 0.3	50	Two units
III	121–243	2.7 ± 0.5	50	One unit
IV	244–287	3.5 ± 0.5	50	One unit
V	288–360	3.5 ± 4.6	50–0	One unit
VI	361–400	2.8 ± 0.5	0	One unit

[a] During this period, the effluent of the granular SBR was fed to the Anammox reactor.

water. After a first period of adaptation to the supernatant (period I), the effluent of the granular SBR (period II) was fed to the Anammox reactor.

Once the Anammox developed in the core of the granules (period III), the DO was increased in order to favor the nitrogen removal. Afterwards (period V), the dilution ratio of the supernatant was decreased until raw sludge liquor was used as the feeding to the reactor. The DO concentrations and the nitrogen loading rate (NLR) were increased to study the maximal nitrogen removal rate achievable in the system and the effect of the free ammonia on NOB. Finally, during period VI, the DO concentration was decreased to avoid nitrite oxidation. The mean molar ratio alkalinity/NH_4^+ during the whole operation period was 1.0 ± 0.2.

2.2.2. Anammox SBR

The strategy followed was similar to the one reported by Dosta et al., 2008. The temperature of the Anammox reactor was initially 30 °C, and it was stepwise decreased down to 20 °C to allow the acclimatization of the biomass. The composition of the synthetic media was, in mg/L: NH_4^+–N: 130–140; NO_2^-–N: 160–170; $KHCO_3$: 1.25; $CaCl_2$: 1.41; KH_2PO_4: 50; $MgSO_4$: 58.6; $FeSO_4{\cdot}7H_2O$: 9.08; EDTA: 6.25 and 1.25 mL/L of a trace solution (described by van de Graaf et al. (1996)). The nitrite to ammonium ratio on molar basis of this wastewater was 1.3 mol/mol, the stoichiometric one. Once the temperature of the reactor was decreased to 20 °C and the operation stabilized, the effluent of the granular SBR was fed to the reactor being the NH_4^+/NO_2^- molar ratio of 1.0.

2.3. Calculations

2.3.1. Nitrogen removal rates

Ammonia and nitrite oxidation rates (AOR and NOR, respectively) and nitrogen removal rate by Anammox bacteria (ANR) of the nitrifying granular reactor were estimated as g N/(L d) based on nitrogen balances and the stoichiometry of the Anammox process:

$$\Delta N = (NH_4^+\text{—}N_{inf}) - ((NH_4^+\text{—}N_{eff}) + (NO_2^-\text{—}N_{eff}) + (NO_3^-\text{—}N_{eff})) \quad (2)$$

$$AOR = \frac{(NH_4^+\text{—}N_{inf}) - (NH_4^+\text{—}N_{eff}) - \frac{\Delta N}{2.04}}{HRT} \quad (3)$$

$$NOR = \frac{(NO_3^-\text{—}N_{eff}) - \frac{0.26\cdot\Delta N}{2.04}}{HRT} \quad (4)$$

$$ANR = \frac{\Delta N}{HRT} \quad (5)$$

being NH_4^+–N_{inf} the ammonium concentration in the influent (mg N/L) and NH_4^+–N_{eff}, NO_2^-–N_{eff}, NO_3^-–N_{eff} the ammonium, nitrite and nitrate concentrations in the effluent (mg N/L), respectively.

2.3.2. Total surface of the granules

The total surface of the granules (S (m^2)) was calculated as follows:

$$S = \frac{V\cdot X_R\cdot 4\cdot\pi\cdot R^2}{\frac{4}{3}\cdot\pi\cdot R^3}\cdot\rho_{granule} \quad (6)$$

being V the reactor volume (L), X_R the biomass concentration in the reactor (g VSS/L), $\rho_{granule}$ the granules density (g VSS/$L_{granule}$) and R the mean radius of the granule (m).

2.3.3. Maximum specific Anammox activity tests

The maximum specific Anammox activity was determined following the method described by Dapena-Mora et al., 2007. It consisted on the measurement of the overpressure generated in closed vials by the produced nitrogen gas along time using a differential pressure transducer with a measurement range of 0–5 ψ, linearity 0.5% of full-scale, from Centerpoint Electronics. The Anammox sludge was washed with phosphate buffer which provided the desired pH of 7.8. Then, 24 mL of the mixed liquor were placed in 38 mL vials. These vials were introduced in an incubator shaker (New Brunswick Scientific, C24 Incubator; Edison, USA) which operated at 150 rpm and with temperature control. After 30 min to acclimate the biomass inside the vials to the operating conditions of the test, 0.5 mL of the ammonium sulphate solution and 0.5 mL of the sodium nitrite solution were injected into the vials to reach the initial concentrations of 70 mg NH_4^+–N/L and 70 mg NO_2^-–N/L. The reaction was followed by measuring the pressure increase along the time, which is related to the nitrogen production according to Eq. (7). In this equation, n is the number of moles of nitrogen produced per unit of time (moles N/d), V_G is the volume of the gas phase (L), R is the ideal gas constant (atm L/(mol K)), T is the temperature (K) and α is the slope of the pressure increase inside the vial versus time (atm/d).

$$n = \alpha\frac{V_G}{R\cdot T} \quad (7)$$

$$SAA = n\frac{M}{V_L\cdot X} \quad (8)$$

Then, the maximum specific Anammox activity expressed in g N/(g VSS d) is assessed according to Eq. (8), where M is the molecular weight of N_2 (g N/mol), X is the biomass concentration inside the vial (g VSS/L) and V_L is the volume of the liquid phase (L).

2.3.4. Free ammonia and free nitrous acid concentrations

The concentrations of free ammonia and free nitrous acid were calculated (Eqs. (9) and (10)) according to the equilibrium between the ionized and unionized species as proposed by Anthonisen et al. (1976).

$$C_{NH_3} = \frac{C_{NH_4^+}}{\frac{e^{\frac{6334}{273+T}}}{10^{pH}} + 1} \quad (9)$$

$$C_{HNO_2} = \frac{C_{NO_2^-}}{10^{pH}e^{\frac{-2300}{273+T}}} \quad (10)$$

being C_{NH4}, C_{NH3}, C_{NO2} and C_{HNO2} the concentrations of ammonium, free ammonia, nitrite and free nitrous acid (mg N/L), T the temperature (°C) and pH the reactor pH.

2.4. Analytical methods

The pH and the concentrations of DO, ammonia, volatile suspended solids (VSS), inorganic suspended solids, total suspended

solids and sludge volumetric index were determined according to the Standard Methods (APHA–AWWA–WPCF, 1998). Nitrite and nitrate concentrations were determined by capillary electrophoresis. Concentrations of total organic carbon and inorganic carbon were measured with a Shimadzu analyser (TOC-5000). The morphology and size distribution of the granules were measured regularly by using an image analysis procedure with a stereomicroscope (Stemi 2000-C, Zeiss) provided with a digital camera (Coolsnap, Roper Scientific Photometrics). For the digital image analysis the programme Image Pro Plus was used.

In order to identify bacterial populations of AOB, NOB and Anammox bacteria, granules from the reactor were collected, kept in their aggregated form or disaggregated, and fixed according to Amann (1995) with 4% paraformaldehyde solution. Entire granules were embedded in OCT reagent (Tissue-Tek; Miles, Ind.) prior to their cryosectioning at −35 °C. Slides with a thickness of 14 μm were cut at −16 °C, and these single sections were placed on the surface of poly-L-lysine coated microscopic slides. Hybridization was performed at 46 °C for 90 min adjusting formamide concentrations at the percentages shown in Table 2 (Details on oligonucleotide probes are available at probeBase (Loy et al., 2007)). The used probes for in situ hybridization were 5′ labelled with the dyes FLUOS or Cy3 or Cy5. A TCS-SP2 confocal laser scanning microscope (Leica, Germany), equipped with a HeNe laser for detection of Cy3 and one Ar ion laser for detection of FLUOS, was used with the sliced samples.

3. Results and discussion

3.1. Nitrogen removal in the two units configuration

Regarding the removal of nitrogen compounds during period I fluctuations of the ammonia oxidation efficiency were observed (Fig. 2a) attributed mainly to the change in the feeding composition caused by the introduction of the anaerobic supernatant. By adjusting the DO concentration during period II (Fig. 2b), stable partial nitrification was reached between days 60 and 120 with a NO_2^-/NH_4^+ molar ratio of 1.1 ± 0.2; the concentrations of both ammonium and nitrite in the effluent being around 140 mg N/L. The ammonia oxidation rate maintained a stable value of nitrite production around 0.6 g NO_2^-–N/(L d) during this period whereas nitrite oxidation was neglected.

During period II, the effluent of the nitrifying granular SBR was fed into the Anammox SBR reactor at a NLR of 0.28 g N/(L d). During the acclimation process the Anammox reactor was fed with the synthetic media (Fig. 3) and the temperature was stepwise decreased from 30 to 20 °C, reaching the latter on day 20. The estimated SAA decreased from 0.28 to 0.13 g N/(g VSS d) due to the temperature decrease. On day 33 of operation of the Anammox reactor, the synthetic wastewater was switched to the effluent of the granular SBR (Period II in this unit). The mean efficiency of the Anammox reactor in terms of nitrogen removal decreased from

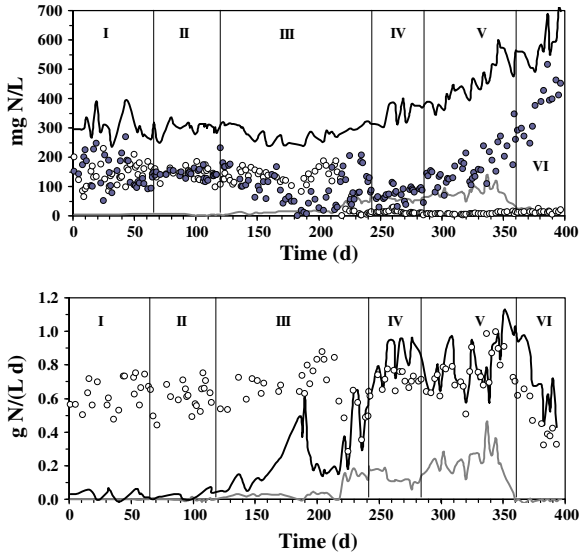

Fig. 2. (a) Concentrations of nitrogen compounds in the influent mg NH_4^+–N/L (—), and in the effluent mg NH_4^+–N/L (○), mg NO_2^-–N/L (●), mg NO_3^-–N/L (—). (b) Ammonia oxidation rate (○), nitrite oxidation rate (—) and, nitrogen removal rate (—).

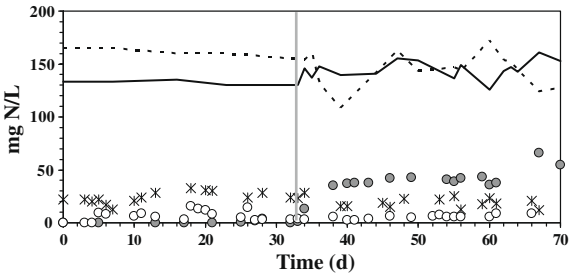

Fig. 3. Concentrations of nitrogen compounds in the influent: mg NH_4^+–N/L (—) and mg NO_2^-–N/L (- - -); and in the effluent mg NH_4^+–N/L (●), mg NO_2^-–N/L (○) and mg NO_3^-–N/L (*).

80% to 69% due to the operation under nitrite limitation. The global nitrogen removal efficiency of the two units configuration corresponded to a ANR of 0.08 g N/(L d).

3.2. Nitrogen removal in the one unit configuration

From day 120 on, growing nitrogen losses were registered in the granular SBR with a simultaneous change in the colour of the biomass to reddish. After an exponential increase in the nitrogen removal rate during period III (day 190, Fig. 2b), a problem with the feeding pump caused an important decrease in the ANR down to 0.2 g N/(L d). One month latter, the system recovered its effi-

Table 2
Targeted organisms and the corresponding formamide (*F*) percentages for the used oligonucleotide probes.

Probe	Probe sequence (5'→3')	% *F*	Targeted organisms
EUB338I	GCT GCC TCC CGT AGG AGT	20	Bacteria domain
Amx820	AAA ACC CCT CTA CTT AGT GCC C	40	Anaerobic ammonium-oxidizing bacteria *Candidatus* "*Brocardia anammoxidans*" and *Candidatus* "*Kuenenia stuttgartiensis*"
NSO190	CGA TCC CCT GCT TTT CTC C	55	Betaproteobacterial ammonia-oxidizing bacteria
NEU653	CCC CTC TGC TGC ACT CTA	40[a]	Most of the halophilic and halotolerant *Nitrosomonas* spp.
Ntspa712	CGC CTT CGC CAC CGG CCT TCC	50[a]	Most members of the phylum *Nitrospirae*
Nit3	CCT GTG CTC CAT GCT CCG	40[a]	*Nitrobacter* spp.

[a] Used with an equimolar amount of corresponding unlabeled competitor oligonucleotide probe.

ciency and an ANR of 0.6 g N/(L d) was achieved at the end of this period. Applied ALR to the system was increased from 1.2 to 1.5 g N/(L d) during period IV. Nitrite was almost not detected in the effluent but ammonia concentrations between 30 and 120 mg NH_4^+–N/L still remained in the effluent. In spite of the increase of the DO up to 3.5 mg/L during this period the ammonia oxidation was the limited step of the CANON process. This result agrees with the results reported by Sliekers et al. (2003) working with the CANON process in an airlift reactor. An ANR of 1.0 g N/(L d) was achieved and the nitrogen removal percentage was around 60%.

During period V ALR applied and DO concentration were progressively increased up to 2.2 g N/(L d) and 4.6 mg/L, respectively. Both AOR and ANR remained in similar values to those obtained during period IV and only an increase of the NOR from 0.1 to 0.3 g NO_3^-–N/(L d) was observed. This fact caused that ammonia concentration in the effluent increased significantly. Finally, DO concentration was restored to 2.8 mg/L in order to avoid nitrite oxidation. This action completely stopped nitrate production but also caused a strong decrease of the AOR to 0.4 g N/(L d)

3.3. Biomass characteristics

3.3.1. Physical properties

The biomass concentration in the granular SBR reactor ranged during the whole operational period between 5 and 8 g VSS/L (Fig. 4). During the partial nitrification period (I and II), the biomass concentration was maintained around 5 g VSS/L. This value increased once the CANON process developed (from day 120 on) presumably due to the growth of Anammox biomass inside the granules (Periods III to VI), reaching a maximum concentration of 8 g VSS/L which remained stable after day 300 of operation. A similar trend was registered in the evolution of the solids concentration in the effluent increasing from a mean value of 40 mg VSS/L during the partial nitrification to values up to 80 mg VSS/L during the CANON operation. The mean sludge retention time during the whole operational period remained at values close to 30 d.

A slight increase in the mean diameter during the reactor operation from 2.3 to 3.2 mm was registered also associated to the development of Anammox biomass inside the granules. These values are higher than those reported from nitrifying granular systems (Tsuneda et al., 2003; Kim and Seo, 2006) and similar to those reported for aerobic granules grown on carbon sources (de Kreuk et al., 2005). The sludge volumetric index value was kept always under 50 mL/g VSS and the settling velocity of the granular sludge ranged from 70 to 150 m/h during the whole operation, maintaining, therefore excellent settling properties.

Biomass concentration inside the Anammox SBR did not change significantly and was around 1.5 g VSS/L during the whole operational period while the solids concentration in the effluent was around 11 mg VSS/L, corresponding to a sludge retention time of 90 d. The Anammox biomass was grown as granules with an average diameter of 1 mm.

3.3.2. Identification of bacteria populations by FISH

By the application of the FISH technique to a sample collected from the granular SBR during period II (day 100), bacteria belonging to the genus *Nitrosomonas* were identified as the dominant AOB population in the samples. No positive results were obtained when the probes Nit3 and Nitspa712 were applied indicating the absence of nitrite oxidizing bacteria.

In order to confirm the results obtained applying mass balances during period VI a sample of granules was collected on day 380. The granules were sliced and pictures after applying the FISH technique were taken to confirm the presence of AOB and Anammox bacteria. Once more bacteria belonging to the genus *Nitrosomonas* were identified as the dominant AOB population in the samples and they were located in the outermost layers of the granules. Anammox bacteria gave positive results of Amx820 probes, indicating the presence of *Candidatus Brocardia* anammoxidans and/or *Candidatus Kuenenia stuttgartiensis* located in more internal layers of the granules, where oxygen is not present due to its consumption by AOB.

This configuration in layers could explain the robustness of this process. As it was already reported by Pynaert et al., 2004, the autotrophic nitrogen removal in one stage can be developed in a rotating disk contactor or in a granular SBR which confer to the Anammox process high resistance to temperature, pH or oxygen changes. This effect can be caused by the protection provided by the oxic layer with a high density of *Nitrosomonas* hindering and lowering the variations registered in the bulk liquid.

3.4. Two units or one unit configuration systems for autotrophic nitrogen removal at low temperatures?

The SHARON process is one the technologies proposed to achieve the partial nitrification previously to the application of the Anammox process and it was successfully used at full-scale to treat the effluent from sludge digesters (van Dongen et al., 2001; van der Star et al., 2007). However when the temperature of the treated effluent is lower than 24 °C the maximal growth rate of AOB turns lower than that of NOB and ammonia is fully oxidized into nitrate (Fux et al., 2002). Therefore, to achieve partial nitrification at temperatures lower than 24 °C other strategies, such as inhibition of NOB by NH_3 or HNO_2 (Kim and Seo, 2006) or operation at low DO concentrations (Blackburne et al., 2008), should be applied.

Results from the granular SBR indicated that nitrite was not oxidized into nitrate until day 215 (Fig. 2b) in spite of the concentration of free ammonia inside the system remained close to zero (Fig. 5) while the highest rate of nitrite oxidation was registered during period V when free ammonia concentrations were between 5 and 10 mg NH_3–N/L, values that would inhibit completely NOB activity according to Anthonisen et al. (1976). Therefore, there is not a direct correlation between the presence of free ammonia

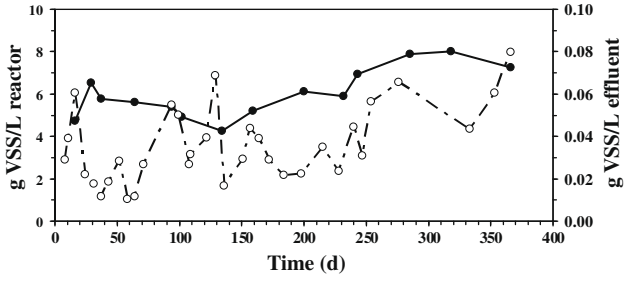

Fig. 4. Concentrations of biomass in the reactor (●) and in the effluent (○).

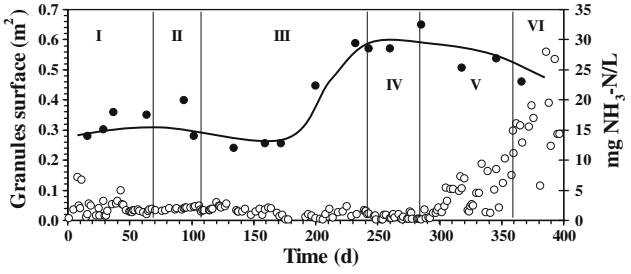

Fig. 5. Concentrations of free ammonia (○) and total surface of the granules with trend line (●).

and the accumulation of nitrite in the system. The appearance of the activity of the NOB occurred simultaneously with an increase of the total surface of the granules due to both factors, the increase of the diameter of the granules and biomass concentration at the end of period III (Fig. 5). This fact could indicate that partial nitrification was due to DO limitation by the external mass transfer resistance which provoked that the DO concentration on the surface of the granule was lower than that in the bulk liquid. This mass transfer resistance together with the internal one would explain that partial nitrification in granular and biofilm systems was observed at higher DO concentrations than in the case of activated sludge systems (Wyffels et al., 2004).

Mass transfer resistance caused that oxygen could only penetrate around 100–400 μm into the granule (Tsuneda et al., 2003; Chiu et al., 2007) meaning that the majority of the volume of the granule was not active and, therefore, its specific aerobic activity was lower in comparison with the activated sludge systems. This drawback is compensated by the large biomass amounts retained inside the system due to its excellent settling properties. The low biomass concentrations in the effluent of the nitrifying granular reactor would minimize the presence of solids in the influent of the Anammox SBR that could enhance heterotrophic activity compromising the process efficiency (Lackner et al., 2007). Partial nitrification with biomass in suspension using limiting DO concentrations could turn unstable due to the AOB wash out and the NOB growth (Blackburne et al., 2008). In this aspect, the granular system showed a stable value of the AOR during whole operational period and NOB activity could be avoided by decreasing DO concentration of the liquid bulk.

As Anammox microorganisms have a low growth rate, a lot of effort was focused on developing systems with biomass retention capacity in order to shorten the start up period of the process (Fernández et al., 2008) and, for this reason, most of the works were carried out under optimum conditions for the Anammox biomass (pH between 7 and 8 and temperature higher than 30 °C). However, up to now, limited information is available on the efficiency of this process operated under low temperatures (Isaka et al., 2007; Dosta et al., 2008). Isaka et al. (2007) and Dosta et al. (2008) adapted respective Anammox systems by gradually decreasing the temperature and, in both cases, a strong decrease of the nitrogen conversion rate was observed. When a similar strategy was applied in the present work, the biomass specific activity decreased from 0.28 g N/(g VSS d) at 30 °C to 0.13 g N/(g VSS d) at 20 °C. This low specific activity allowed achieving a nitrogen removal rate of only 0.08 g N/(L d) relative to the whole configuration which involved that the Anammox unit was the limiting step of the process. To treat higher NLR, higher biomass concentrations in this unit would be necessary.

After day 120, a progressive increase of nitrogen loss was measured in the effluent of the granular SBR indicating the growth of Anammox biomass (and, therefore, the development of the CANON process), in spite of the existence of adverse environmental conditions for these microorganisms: presence of oxygen, high nitrite concentrations and low temperature. The same phenomenon was already reported by Siegrist et al. (1998) in a nitrifying rotating contactor. This is also in accordance to the simulation results of Hao and van Loosdrecht (2004) who stated that the natural development of the CANON process in stable nitrifying biofilms operated at low temperatures is possible. The CANON system was developed both, from an Anammox reactor by inoculating nitrifying biomass or from nitrifying reactor operated at oxygen-imited conditions and inoculated with Anammox biomass (Pynaert et al., 2004; Gong et al., 2007). The second strategy seems to be more suitable because an important decrease of the Anammox activity is observed when the first strategy is applied (Sliekers et al., 2002, 2003).

Generally, CANON systems reported in the literature were operated at 30–35 °C (Sliekers et al., 2003; Pynaert et al., 2004). In the present study the fact that the granular reactor, where the CANON process is performed, was operated at 20 °C, allowed the removal of nitrogen loading rates up to 1.1 g N/(L d) which are in the range of 0.075–1.5 g N/(L d) reported for CANON systems operated at higher temperatures (Sliekers et al., 2002, 2003). The maximum specific Anammox activity of the biomass calculated on the basis of the ANR was 0.12 g N/(g VSS d) which was similar to that obtained during the operation of the Anammox reactor itself. Contrary to the case of the two units configuration, ammonia oxidation was the limiting step of the process in this case.

Different technologies have been used to carry out autotrophic nitrogen removal in one or two units (Table 3). Nitrogen removal rates achieved with both configurations are in the same range when the systems are operated at a temperature close to 30 °C. However, in this study, the use of one single unit allowed the achievement of higher treatment capacities compared to the two units configuration, basically due to the negative effects of low temperatures on Anammox biomass and its necessity of an adaptation period. In general, the use of one single reactor could represent some advantages with respect to the two units configuration such as, lower capital costs and less oxygen consumption by AOB, which prevent possible negative effects on the Anammox bacteria. Nevertheless, the application of the two units configuration would be appropriated when toxic or organic biodegradable compounds

Table 3
Two units or one unit configuration systems for autotrophic nitrogen removal.

Process	Reactor[a]	*T* (°C)	DO (mg/L)	ANR[b] g N/(L·d))	N removal (%)	Ref
2 Units configuration	CSTR + SBR	36	N.A.[c]	0.36	80	van Dongen et al. (2001)
	CSTR + SBR	30	2.8[d]	0.30	90	Fux et al. (2002)
	MBR + MBR	20–30	0.2[d]	0.55	82	Wyffels et al. (2004)
	SBR + SBR	20	2.7[d]	0.08	69	This study
1 Unit configuration	SBR	30	<0.1	0.06	50	Sliekers et al. (2002)
	Airlift	1.8	0.5	1.5	42	Sliekers et al. (2003)
	UGBR	30	ND[e]	0.06	76	Ahn and Choi (2006)
	RBC	14	1.0	0.42	42	Pynaert et al. (2004)
	MBBR	30	1.8	0.36	60	Cema et al. (2006)
	SBR	28	0.3	0.70	86	Wett (2007)
	SBR	20	3.5	1.0	60	This study

[a] MBR = membrane-assisted bioreactor; UGBR = upflow granular sludge bed reactor; MBBR = moving bed biofilm reactor.
[b] ANR for the two units system was calculated on the basis of the total volume of the system.
[c] Data not available.
[d] DO concentration in the reactor carrying out partial nitrification.
[e] The liquid is not aerated in the reactor itself but in an external aeration chamber until reaching 6 mg/L.

are present in the feeding, since these compounds will be degraded in the nitrifying unit avoiding its entrance in the Anammox reactor.

4. Conclusions

AOB and Anammox bacteria are slow growing microorganisms which optimal temperature of operation is around 30 °C. For this reason, Anammox and CANON process were mostly applied to effluents with temperatures higher than 30 °C. In this work, the use of a granular system allowed a good biomass retention achieving high concentrations of AOB and Anammox bacteria in spite of the low temperature of operation and, therefore, a high nitrogen removal rate was still possible under these conditions. The application of this kind of systems would open the possibility to obtain a stable autotrophic nitrogen removal treating effluents with low temperatures.

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