Aerobic granular sludge technology and nitrogen removal for domestic wastewater treatment

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ABSTRACT

This study evaluated aerobic granulation and nitrogen removal via assimilation, nitrification, and denitrification of a system fed with real domestic wastewater. The granulation process was complete after 160 days of operation. The mature granules had an almost spherical structure, an average size of 473.0 μm, and a good settling ability (SVI$_{30}$ of 75.6 mL g^{-1}). Ammonium assimilation for cell growth varied between 3.5 and 64.6% during reactor start-up. After granule formation, assimilation accounted for less than 5% and nitrogen was mainly removed by partial nitrification up to nitrite, followed by denitrification via nitrite. Average efficiencies of 86.6% for nitrification, 59.5% for denitrification, and 60.5% for total nitrogen were obtained in this period. The assimilation ability of the mature granules grown on domestic wastewater was lower than the commonly reported results obtained for synthetic granules.

Key words | aerobic granules, domestic wastewater treatment, nitrogen removal, partial nitrification

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INTRODUCTION

Among the processes that have been proposed for biological nutrient removal from wastewater, aerobic granular sludge (AGS) technology has been successfully applied for nitrogen and/or phosphorus removal (Kishida et al. 2008; Coma et al. 2012). Nitrification and denitrification processes with AGS usually take place simultaneously in a single reactor through the different layers inside the granules. This is possible because the oxygen penetration depth inside the granular sludge is limited even under aerated conditions (Kishida et al. 2008). Moreover, ammonia adsorption and/or assimilation were found to be important phenomena that can occur in AGS reactors fed with synthetic influents (Bassin et al. 2011; Mosquera-Corral et al. 2011).

Investigations using real influents, such as domestic wastewater, have mainly focused on the granulation process and reactor start-up strategies (Ni et al. 2009; Liu et al. 2010; Coma et al. 2012; Wagner & Costa 2013). Until now, information about nitrogen removal, especially ammonia assimilation by granules grown on domestic wastewater, has been very limited. Therefore, these aspects need to be evaluated in order to better understand the mechanisms of nitrogen removal occurring in such systems. In this study, the performance of an AGS reactor was evaluated in terms of nitrogen removal via assimilation, nitrification, and denitrification processes during the formation and maturation of aerobic granules fed with real domestic wastewater.

MATERIAL AND METHODS

Experimental set-up and reactor operation

The AGS-reactor had a working volume of 118.7 L (0.25 m internal diameter and 2.42 m height) and was fed with real domestic wastewater at a volumetric exchange ratio of 59%. Average concentrations of total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), total Kjeldahl nitrogen (TKN), and ammonium nitrogen (NH$_4^+$-N) in the influent were 588.1 ± 162.5 mg L^{-1}, 304.4 ± 82.2 mg L^{-1}, 83.2 ± 18.3 mg L^{-1}, and 82.2 ± 17.8 mg L^{-1}, respectively. The resulting organic and nitrogen loading rate were, respectively, 1.07 ± 0.29 kg sCOD m^{-3} d^{-1} and 0.29 ± 0.06 kg NH$_4^+$-N m^{-3} d^{-1}. The hydraulic retention time was 6.8 hours. Activated sludge from a domestic wastewater treatment plant was used as inoculum (ETE Insular, Florianópolis, Brazil). The AGS-reactor was operated in sequencing batch mode with a cycle time of 4 hours divided into the following phases: 10–20 minutes of anaerobic static feeding from the bottom of the reactor; 183.5–218.5 minutes of aeration;

35–10 minutes of settling; and 1.5 minutes of effluent withdrawal. During the first 5 weeks of operation, the settling time was progressively reduced from 35 to 10 minutes in order to encourage granule selection. The sludge retention time (SRT) was not fixed and it was calculated taking into account the amount of biomass washout with the treated effluent. The reactor was operated at room temperature (~22 °C), with no pH or dissolved oxygen (DO) control. Air was introduced by a membrane diffusor placed at the bottom of the reactor in a superficial upflow air velocity of 1.2 cm s^{-1}.

Analytical procedures

The sCOD, NH_4^+-N, nitrite (NO_2^--N), nitrate (NO_3^--N), total suspended solids (TSS), and volatile suspended solids (VSS) were analyzed regularly according to *Standard Methods* (APHA 2005). DO concentration in the bulk liquid was measured with a multiparameter sonde (YSI 6820, Yellow Springs, OH, USA). Cycle measurements were carried out bi-weekly in order to evaluate substrate conversions. Samples for cycle measurement were collected every 15–30 minutes only during the aerobic mixing period. The sludge volume index (SVI) was determined with a mixed liquor sample taken at the end of the aerobic phase. SVI_{10} and SVI_{30} were obtained by measuring the biomass volume after a settling period of 10 and 30 minutes, respectively. Microscopy (Olympus BX40, Tokyo, Japan) was used to monitor granule formation throughout the reactor operating period. The observation of the external structure of the mature granules was performed using scanning electron microscopy (SEM) (JEOL JSM-6390LV, JEOL, Tokyo, Japan). Particle size distribution of homogeneous samples of sludge was obtained by laser diffraction (Malvern MasterSizer Series 2000, Malvern Instruments, Malvern, UK). The average granule size was determined by the software provided by the aforementioned Mastersizer, for aggregates smaller than 2,000 μm, and by image analysis (QCapture Pro software, V 7.0, QImaging, Surrey, BC, Canada), for aggregates larger than 2,000 μm.

Microbial community analysis

Biomass samples were taken periodically, fixed in 4% paraformaldehyde, placed on gelatin-coated glass slides, and fluorescent *in situ* hybridization (FISH) was performed as described by Amann *et al.* (1995). In order to obtain a homogeneous sample, before the fixation step, the granules were mechanically macerated with a glass rod and then sonicated using an ultrasonic bath (55 kHz, USC-700, Unique, Indaiatuba, SP, Brazil) at 25 °C for 5 minutes. The probes used for bacterial identification are shown in Table 1. All microbial cells were detected by staining with 1% 4,6-diamidino-2-phenylindol (DAPI) and examined with a microscope (Olympus BX41, Tokyo, Japan). In order to quantify the bacterial population, at least 10 images were recorded for each sample, and the ratio of the area of the cells labeled by the specific probe to the area of all bacteria stained by DAPI (100%) was determined by digital image.

Calculations

The nitrogen components were considered to be transformed via biomass assimilation, nitrification, and denitrification processes. The amount of ammonium nitrogen assimilated (g$_N$ d^{-1}) by the biomass for cell growth was estimated by the following equation (Wan *et al.* 2009):

$$N_{\text{assimilation}} = f_N(\Delta XV + VSS_{\text{out}}Q_{\text{out}}) \qquad (1)$$

Table 1 | Oligonucleotide probes used for FISH analysis

Probe	Specificity	Sequence of the probe (5′–3′)
EUBmix (I + II + III)	Most bacteria *Planctomycetales* *Verrucomicrobiales*	I – CTG CCT CCC GTA GCA II – CAG CCA CCC TAG GT GT CTG III – CCA CCC GTA GGT GT
NEU	*Nitrosomonas* sp.	CCC CTC TGC TGC ACT CTA
NSO190	Ammonia oxidizers β-Proteobacteria	CGA TCC CCT GCT TTT CTC C
NIT3	*Nitrobacter* sp.	CCT GTG CTC CAT GCT CCG
Ntspa662	*Nitrospira*	GGA ATT CCG CGC TCC TCT
THIO51	Some *Thiobacillus*	GTC ATG AAA CCC CGC GTG GT
PAE997	*Pseudomonas* spp.	TCT GGA AAG TTC TCA GCA
PAO651	*Candidatus* 'Accumulibacter'	CCC TCT GCC AAA CTC CAG

where f_N is the nitrogen fraction of the sludge; Q_{out} is the flow rate of effluent ($L\ d^{-1}$); VSS_{out} is the solids concentration present in the effluent ($g_{VSS}\ L^{-1}$); and ΔXV is the slope between two measurements of sludge concentration inside the reactor over time ($g_{VSS}\ d^{-1}$); fN was assumed to be equal to $0.1\ mg_N \cdot mg_{VSS}^{-1}$, as suggested by Lee *et al.* (2007) and Wan *et al.* (2009). Assimilation, nitrification, denitrification, and total nitrogen removal efficiencies were calculated as follows:

$$\eta_{assim}(\%) = \frac{N_{assimilation}}{(NH_4^+\text{-}N_{in})} \quad (2)$$

$$\eta_{nit}(\%) = \frac{(NH_4^+\text{-}N_{in}) - (NH_4^+\text{-}N_{out}) - N_{assimilation}}{(NH_4^+\text{-}N_{in}) - N_{assimilation}} \quad (3)$$

$$\eta_{denit}(\%) = \frac{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in}) - N_{assimilation} - (NH_4^+\text{-}N_{out}) - (NO_X\text{-}N_{out})}{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in}) - N_{assimilation}} \quad (4)$$

$$\eta_{total}(\%) = \frac{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in}) - (NH_4^+\text{-}N_{out}) - (NO_X\text{-}N_{out})}{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in})} \quad (5)$$

RESULTS AND DISCUSSION

Aerobic granulation with domestic wastewater

This study was conducted over 250 days to evaluate nitrogen removal during the formation and maturation of aerobic granules with domestic wastewater. Figure 1 shows the changing patterns of biomass concentration, settling ability, sludge volume percentage with size below 200 µm (SVP-SB200), and sludge particle size throughout the operational time. To promote aerobic granulation, during the start-up period the settling time was progressively reduced in order to selectively discharge slow-settling flocs while avoiding severe washout of the seeding sludge. This strategy resulted in a variation of biomass concentration inside the reactor and a decrease of SVI_{30} during the first 50 days of operation (Figure 1(b)). Granule formation was evident from particle size changes (Figure 1(a)) since the 10th percentile, 90th percentile, and average particle size increased, respectively, from 14.8, 163.2, and 87.7 µm on day 0 (seeding sludge) to around 80.0, 800.0, and 410.0 µm on day 56. The concept

of SVP-SB200, proposed by Liu *et al.* (2010), was used to determine the turning point at which the reactor was dominated by granular sludge. These authors considered the reactor to be granular-dominant when SVP-SB200 was below 50%. After 56 days, granules were dominant in the reactor (SVP-SB200 of 30%). However, problems in the aeration system provoked a sharp increase in the applied volumetric airflow rate (over 80 L min^{-1}) by day 74, which lasted around 3 d. The disturbance of the airflow caused an increase in shear force and therefore a partial disintegration of the formed granules. Thus, a considerable decrease of TSS and particle size, and an increase of SVP-SB200 and SVI_{30} were noticed. With the re-establishment of normal aeration conditions – airflow between 36–40 L min^{-1} – it was possible to observe the recovery of the granular biomass. After 160 days, the solids concentration,

SVP-SB200, granule average size, and SVI_{30} stabilized at around 1.8 $g_{TSS}\ L^{-1}$, 18%, 473.0 µm, and 75.6 mL g_{TSS}^{-1}, respectively. The SVI_{30}/SVI_{10} ratio for this period was around 95%, indicating, according to De Kreuk *et al.* (2007), a completely granulated system. The morphology of the mature aerobic granules grown on domestic wastewater was nearly spherical, with a dense structure and a clear outline without filamentous outgrowths (Figure 2(a)). The external structure of the granules was mainly composed by protozoa (Figure 2(b)). Lemaire *et al.* (2008) also observed the presence of these microorganisms on the surface of granules cultivated with real abattoir wastewater. According to the authors, the presence of ciliates on the surface can interfere with oxygen diffusion in the granule, creating some localized oxygen-depleted zones.

Aerobic granulation is usually a slow process in systems fed with domestic wastewater. Ni *et al.* (2009) and Liu *et al.* (2010) reported periods of 300 and 400 d to achieve 85% and 80–90% of granulation, respectively. In our previous study, around 140 d were needed to achieve a dominant granulated sludge bed (Wagner & Costa 2013). The aerobic granulation period is still a drawback for the full-scale application of this
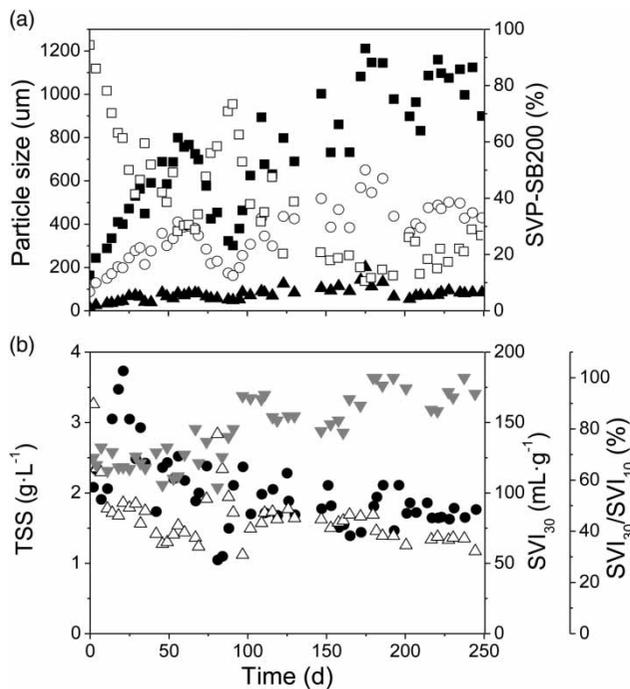
Figure 1 | Sludge particle size D_0.1 (▲), D_0.9 (■), and D_average (○), SVP-SP200 (□), TSS (●), SVI$_{30}$ (△), and SVI$_{30}$/SVI$_{10}$ ratio (▼) during the operational time. D_0.1 and D_0.9 are, respectively, the 10th and 90th percentiles of the distribution of particle size. D_average is the average size of the sludge particles.

technology for domestic wastewater treatment. Furthermore, the obtained biomass is usually a mixture of granules and flocs, which can affect the quality of the treated effluent. Since the sedimentation time is usually short in AGS reactors, the presence of flocs can lead to a high concentration of solids in the effluent. However, even though the floccular biomass corresponded to around 18% of the total biomass in our study, the concentration of solids in the treated effluent was relatively low (around 45 mg$_{TSS}$ L^{-1} after granule formation). Despite the long period required
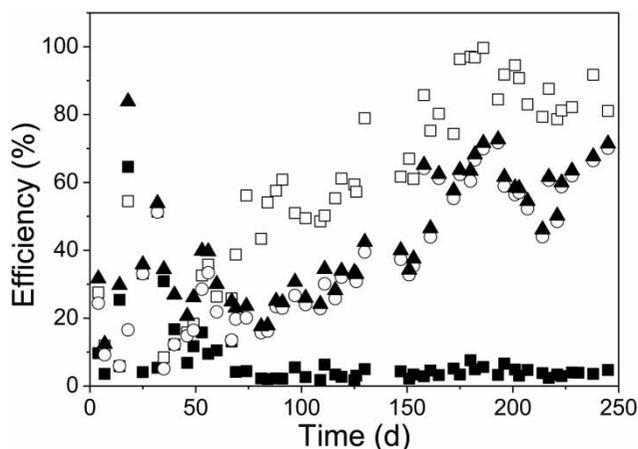
for aerobic granulation with domestic wastewater, the obtained granules remained stable for over 100 days after formation. Moreover, they were able to quickly recover after the partial disintegration induced by the problems in the aeration system. Zhu et al. (2013) mentioned that after the disintegration of granules, the sludge debris with good settling performance can act as a nucleus to which the microorganisms can attach, leading to rapid granule formation. Following the same principle, Coma et al. (2012) demonstrated that adding a low fraction of crushed granules to the seed sludge enhances aerobic granulation when using domestic wastewater.

Nitrogen removal

During the formation and maturation of the AGS, the nitrogen compounds were considered to be transformed via assimilation, nitrification, and denitrification (Figure 3). The ammonium assimilation for cell growth was more evident during the first 67 days of operation (between 3.5 and 64.6%). After this period, assimilation accounted for less than 5% and nitrogen was mainly removed by simultaneous nitrification and denitrification (SND). Nitrification efficiency was not stable during the first days of operation as a result of the constant biomass washout, but tended to increase with the formation of granules. However, with biomass loss due to the disintegration of granules, the SRT decreased to around 4 d; thus, nitrification efficiency also decreased. The recovery of the biomass led to an increase in the SRT to around 14 d, which allowed the enrichment of nitrifying organisms. An average efficiency of 86.6% for nitrification, 59.5% for denitrification, and 60.5% for total nitrogen, was reached after 160 days of operation, which corresponds to the moment when the granulation process
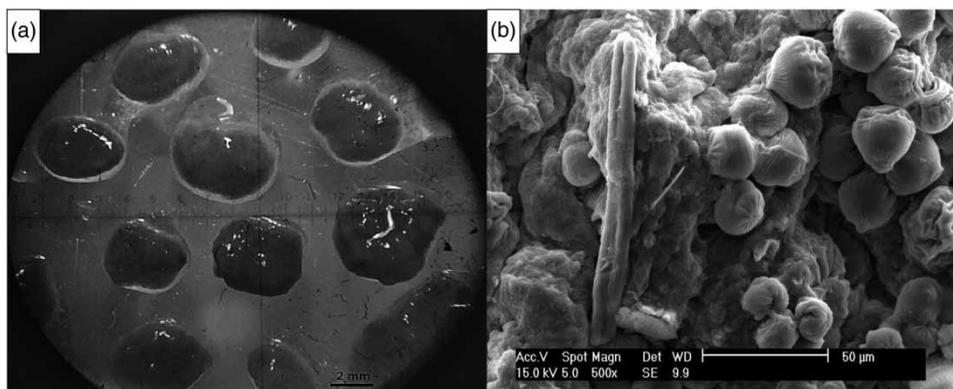

Figure 2 | Images of the mature aerobic granules grown on domestic wastewater: (a) optical microscopy (bar 2 mm); (b) SEM (bar 0.05 mm).

Figure 3 │ Nitrogen removal by the aerobic granules grown on domestic wastewater during the operational time: assimilation (■), nitrification (□), denitrification (○), and total nitrogen (▲).

was complete in the reactor. In this period, partial nitrification up to nitrite was observed in the reactor (Figure 4b); thus, denitrification occurred via nitrite. The volume of the anoxic zone inside the aerobic granules was probably
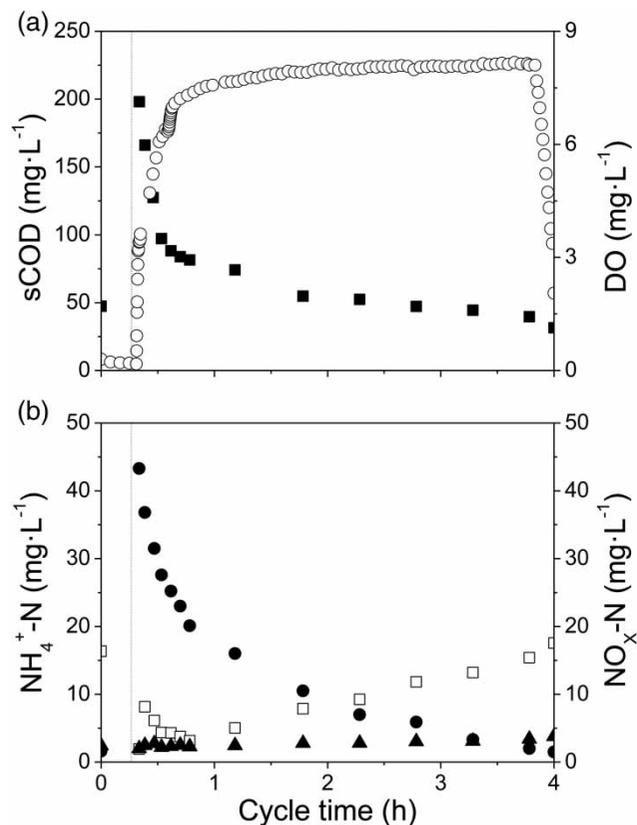
Figure 4 │ Profiles of sCOD (■), DO (○), NH_4^+-N (●), NO_2^--N (□), and NO_3^--N (▲) during an operational cycle of the reactor.

small and not enough to promote complete denitrification of nitrite in nitrogen gas, and nitrite accumulated in the reactor (data not shown). Although nitrogen removal via the nitrite pathway has some advantages, such as faster denitrification rates and reduced demand for organic substrate, high concentrations of nitrite in the treated effluent can have toxic effects on the receiving waters. In this case, nitrogen removal could be enhanced by controlling the applied oxygen concentration during the aerobic phase using an on/off aeration control system or including a non-aerated phase during the SBR cycle.

From the results obtained in the present study it can be seen that the assimilation by the biomass for cell growth was an important process for nitrogen removal only during the formation of the granules. The mature granules grown on sewage had a low ability to assimilate nitrogen and SND was the main process occurring in the system. Opposite results were reported by Mosquera-Corral *et al.* (2011) while studying the aerobic granulation process during the treatment of synthetic wastewater at low organic loads. The authors evaluated the effects of different carbon to nitrogen ratios (C/N) in the feeding and estimated that 90% of nitrogen removal was due to the assimilation for biomass production, and that the nitrification process was almost absent. Biomass concentration varied between 3 and 7 g_{TSS} L^{-1}. However, the authors attributed the absence of nitrification to a low SRT.

The compounds necessary for bacterial development, such as nitrogen, are adsorbed onto the surface of the granule. After adhering, they are transported through the granules via diffusion mechanisms, where they are metabolized by the microorganisms for growth and reproduction (Von Sperling 2007). Bassin *et al.* (2011), working with AGS reactors fed with synthetic wastewater, noticed that ammonium concentrations after anaerobic feeding were lower than expected (based on influent concentration and dilution in the reactor). The authors estimated that the synthetic granules adsorbed a considerable percentage of the influent ammonium (18–37%) and exhibited much higher adsorption capacity compared to flocs. Biomass concentration in the reactors was kept roughly constant and equal to 12 g_{VSS} L^{-1}. Yu *et al.* (2014) stated that the adsorption capacity of the aerobic granules tends to increase with the increase of biomass concentration in the system. Thus, at typical sludge concentration in AGS systems fed with synthetic influent (usually around 10 g_{TSS} L^{-1}), a significant fraction of ammonium will be adsorbed to the granular biomass (Lin *et al.* 2012). Furthermore, in long-term operation of AGS reactors, adsorption is not such an important

consideration for the nitrogen balance calculation, since ammonium will be desorbed from the granules and therefore available for nitrification (Lin *et al.* 2012). These results suggest that the presence of flocs (smaller adsorption capacity than granules), the low biomass concentration in the reactor, and the maturation of the granules (steady-state conditions) can lead to a decrease in the amount of ammonium nitrogen that is adsorbed and thus assimilated. This may explain why the assimilation ability of the mature granules grown on domestic wastewater was lower than the commonly reported results obtained for synthetic granules.

Once a stable granulation system had been achieved, cycle studies were carried out in order to evaluate the reactor's performance throughout a cycle (Figure 4). The influent had a concentration of 375.0 mg L^{-1} of sCOD and 74.7 mg L^{-1} of NH$_4^+$-N. The starting values depicted at time 0 refer to the substrate concentrations at the end of the previous operational cycle. The subsequent sample was collected 2 minutes after the aeration phase had started, i.e., after complete homogenization. The separation between feeding and aerobic phases is depicted by a dotted vertical line. During the feeding, the DO concentration in the reactor was below 0.2 mg L^{-1}. Part of the nitrite remaining from the previous cycle was diluted with the subsequent feed and also denitrified during the 20 minutes of anaerobic feeding. The sCOD and NH$_4^+$-N concentrations sharply decreased at the beginning of the aeration phase. Assimilation accounted for less than 3% and ammonium nitrogen was mainly oxidized to nitrite, although some small amounts of nitrate were also measured during the cycle. The DO limitation in the bulk solution, due to depletion of sCOD and NH$_4^+$–N in the first 30 minutes of the aeration phase, resulted in an anaerobic core inside the aerobic granules, which favored the denitrification of the nitrite produced during nitrification. After that, DO concentration increased until reaching a saturated value of 8.1 mg L^{-1}, leading to an increase of oxygen diffusion inside the granules. Furthermore, at this point, the COD/N ratio was around 3 and thus not enough for complete denitrification. As a result, the denitrification process stopped and nitrite accumulated in the reactor, reaching a concentration of 17.3 mg L^{-1} at the end of the cycle. The sCOD and total nitrogen removal efficiency were 91.6% and 70.1%, respectively.

Microbial populations

A considerable increase in the abundance of ammonia oxidizing bacteria (AOB) (probes NEU and NSO190) was noticed throughout the operational time, reaching around 35% after granule formation. These results are in line with the considerable high nitrification efficiency observed during this period. For the nitrite oxidizing bacteria (NOB), no positive results were obtained for *Nitrobacter* (probe NIT3), confirming that low nitrite oxidation activity was detected and that denitrification occurred mainly via nitrite. One possible reason for the occurrence of partial nitrification up to nitrite is the inhibition of NOB by the presence of free ammonia (FA). The FA concentrations in the reactor were calculated by the equation proposed by Anthonisen *et al.* (1976), and varied between 0.2 and 7.2 mg L^{-1}, with an average value of 1.5 mg L^{-1}. The estimated average FA concentration exceeds the inhibition threshold of 0.1–1.0 mg L^{-1} for *Nitrobacter* reported by Anthonisen *et al.* (1976). On the other hand, *Nitrospira* (probe Ntspa662) were detected in the mature granules with an abundance of 6%. *Nitrospira* are usually the dominant NOB in the majority of full-scale domestic wastewater treatment plants (Daims *et al.* 2009). As for the denitrificants, *Thiobacillus* were not detected during the whole operational time but *Pseudomonas* spp. were present in the mature granules, with a percentage of around 15%. The polyphosphate-accumulating organisms (probe PAO651), *Candidatus Accumulibacter*, were also found in the granules, in an abundance of approximately 15%. Some clades of these organisms are able to use nitrite or nitrate as electron acceptors instead of oxygen, resulting in simultaneous N and P removal (Kuba *et al.* 1993; Flowers *et al.* 2009). However, it is important to mention that the denitrifiers and PAO probes used in the present study were not targeting all bacteria capable of performing denitrification and/or phosphorus removal.

CONCLUSIONS

- The formation of aerobic granules was achieved in an SBR by using real domestic wastewater as substrate. The obtained granules were able to quickly recover after the stress induced by the problems in the aeration system, and they remained stable for over 100 days after formation.
- Nitrogen from domestic wastewater was mainly removed via partial nitrification up to nitrite followed by denitrification of nitrite. The assimilation ability of the mature granules grown on domestic wastewater was lower than that of the granules cultivated with synthetic wastewater.

ACKNOWLEDGEMENTS

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