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Enhancing aerobic granulation for biological nutrient removal from domestic wastewater

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ABSTRACT

This study focuses on the enhancement of aerobic granulation and biological nutrient removal maintenance treating domestic wastewater. Two sequencing batch reactors (SBRs) were inoculated with either only floccular sludge (100%-floc SBR) or supplemented with 10% crushed granules (90%-floc SBR). Granules developed in both reactors. The 100%-floc SBR achieved 75% of nitrogen and 93% of phosphorus removal at the end of the performance, but some floccular sludge remained in the system. The 90%-floc SBR became fully granulated and finished with 84% and 99% of nitrogen and phosphorus removal, respectively. Regarding biological phosphorus removal, nitrite was identified as an inhibitor of the process. Nitrite levels lower than 5 mg $N–NO_2^-$ L^{-1} were used for anoxic phosphate uptake while higher concentrations inhibited the process.

1. Introduction

Some of the main disadvantages in activated sludge wastewater treatment relate to the floccular nature of the sludge, as large areas for reactors and especially settlers are required (Beun et al., 1999). Aerobic granular sludge has been recently developed in laboratory-scale systems. Granules are large suspended biofilms having regular and dense structure with advantageous qualities in comparison to floccular sludge, these include: superior settling properties, high biomass retention, better able to withstand high-strength wastewater and shock loadings, and improved dewatering capabilities (Liu and Tay, 2004).

The performance of aerobic granular sludge systems has been assessed in laboratory-scale reactors while treating synthetic wastewater, or more recently to treat industrial wastewater, such as dairy or livestock (de Kreuk and van Loosdrecht, 2006; Lemaire, 2007; Yilmaz et al., 2008). Currently there are very few studies of municipal wastewater treatment by aerobic granules. Some studies have been carried out for nutrient removal treating synthetic wastewater with constituent concentrations that simulate those in domestic sewage (Coma et al., 2010; Li et al., 2007) and others have investigated nutrient removal from real domestic wastewater using acetate fed granules (Liu et al., 2007; Wang et al., 2009). However, aerobic granular sludge development with real low-strength wastewater (such as domestic) has been found unsuitable with long start-up periods when organic loads are lower than 1000 g COD m^{-3} d^{-1} (de Kreuk and van Loosdrecht, 2006). Successful COD and nitrogen removal from domestic wastewater by aerobic granules is reported when applying high loads (Liu et al., 2010) and in biofilter granular SBR systems (Di Iaconi et al., 2008; Ramadori et al., 2006). Ni et al. (2009) reported granulation in a pilot-scale reactor for removal of organic matter and ammonium at 600–1000 g COD m^{-3} d^{-1}, but neither denitrification or phosphorus removal was reported.

The majority of studies of aerobic granulation focus on organic matter removal while applying complete aerobic conditions. In order to perform both nitrogen and phosphorus removal, anaerobic and anoxic phases are also required. Nitrogen is removed in a two-stage process: oxidation of ammonium to nitrite or nitrate under aerobic conditions (nitrification) and reduction of these compounds to nitrogen gas in the presence of organic matter under anoxic conditions (denitrification). Enhanced biological phosphorus removal (EBPR) also occurs under alternating conditions; in anaerobic conditions organic matter is taken up by polyphosphate accumulating organisms (PAOs) using the glycolysis of intracellular glycogen and cleavage of polyphosphate to conserve energy and build up intracellular stores of polyhydroxyalkanoates (PHA). As a consequence phosphate is released to the media. During the following aerobic or anoxic period, PAOs use the stored PHA to replenish intracellular pools of glycogen and polyphosphate, effectively removing phosphate from the wastewater (Oehmen

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et al., 2007). Similar metabolism is performed by glycogen accumulating organisms (GAOs), utilizing the organic carbon without the phosphate transformations. Hence GAOs may compete with PAOs and are potentially detrimental to EBPR (Mino et al., 1998).

It is likely that the conditions within granules are favorable for good nutrient removal. Oxygen transfer limitation within the granules causes microbial stratification, so that simultaneous nitrification and denitrification (SND) can be well facilitated in the same granule (Lemaire et al., 2008). In addition, the fact that at least a fraction of PAOs can accumulate polyphosphate in anoxic conditions, denitrifying PAOs (DPAOs) (Meinhold et al., 1999) may be enhanced in the granule to achieve the same phosphorus removal while using less oxygen (Zeng et al., 2003a; Meyer et al., 2005; Yilmaz et al., 2008).

Biological nitrogen and phosphorus removal require the combination of different conditions and different microbial populations within a system. Consequently, one of the major challenges is to rapidly obtain granules treating domestic wastewater while simultaneously maintaining nutrient removal. Therefore, this study investigates strategies for successful granule formation while maintaining nitrogen and phosphorus removal from domestic wastewater. Additionally, an objective is to reduce the start-up granulation period by improving it with the addition of a small amount of crushed granules in the seeding sludge.

2. Methods

2.1. Operation of sequencing batch reactors

Two lab-scale sequencing batch reactors (SBRs) were set-up. One SBR was inoculated with floccular sludge (100%-floc SBR) from a full-scale domestic wastewater treatment plant (WWTP) in Queensland (Australia) operating for BNR. The second SBR was seeded with a mixture of 10% crushed granules and 90% floccular sludge (w/w) (90%-floc SBR). The intact granules were withdrawn from a lab-scale SBR treating abattoir wastewater and were pressed through a certified sieve with a porus size of 300 μm to obtain the crushed granules. Both SBRs had a working volume of 2 L and were operated at room temperature (20–22 °C) in 6 h cycles. The SBR cycles comprised a two-step feed strategy, adding the feed under anaerobic conditions to enhance phosphorus removal. The feed volume varied from 0.5 to 1.5 L per cycle. Of the feed volume, 75% was introduced in the first feed and the remaining 25% in the second feed. After each feed a sequence of anaerobic, aerobic and anoxic stages were applied for the BNR purposes. The time period of each phase was modified according to nutrient removal performance based on analytical data evaluation. Following the two sequences the cycle ended with settling, decant and idle phases. To improve granulation the settling time was progressively decreased and the hydraulic retention time (HRT) was also decreased by increasing the feed volume. The latter increasing the reactor volume exchange ratio (VER). Table 1 summarizes the range of times applied in each phase, as well as the operational values obtained as a consequence of the settling time reduction. Dissolved oxygen set-point was maintained at 2 mg L^{-1} during the aerobic periods and N_2 was sparged during anaerobic and anoxic phases. The pH of both SBRs was not controlled and remained between 7.2 and 8.2.

The domestic wastewater comprised of 326 ± 77 mg L^{-1} total COD, 179 ± 46 mg L^{-1} soluble COD, 21 ± 11 mg C L^{-1} volatile fatty acids (VFA), 67 ± 4 mg N L^{-1} total Kjeldahl nitrogen (TKN), 51 ± 9 mg N L^{-1} ammonium, 11 ± 1 mg P L^{-1} total phosphorus (TP) and 9 ± 2 mg P L^{-1} phosphate. This wastewater was collected prior to a pre-fermentation stage, which would be included in a full-scale treatment to produce the required volatile fatty acids (VFA) for effective BNR. Consequently, acetate was added to the wastewater in order to compensate the shortfall in VFA. However, organic loading rates (OLRs) over 1000 g COD m^{-3} d^{-1} were only achieved when high VERs (62.5%) were applied in the reactors. The amount of VFA added was gradually reduced from 85.9 to 22.5 mg C L^{-1} throughout the SBR operation, which coincided inversely to the progressive increases of VER, as specified in the results Section 3. This reduction was used to avoid filamentous bacteria outgrowth and the consequence process breakdown.

2.2. Batch experiments on granular sludge

Batch tests were carried out at the end of the SBR operation with granules from the 90%-floc SBR. 0.5 L of mixed liquor of granules were withdrawn from the SBR at the end of the aerobic period, these were washed twice to ensure endogenous conditions and transferred into batch reactors. N_2 was sparged for 15 min to eliminate oxygen from the sludge before the experiment started. Four separate batch tests were conducted consisting of either anaerobic/aerobic or anaerobic/anoxic/aerobic phases. All batch tests started with an addition of 0.1 L of synthetic wastewater (500 mg L^{-1} acetate, 150 mg N–NH_4^+ L^{-1} and 30 mg P–PO_4^{3-} L^{-1}) followed by 60–90 min of anaerobic conditions. Following the anaerobic period, aerobic conditions were applied to batch A; batch B was submitted to an aerobic period with allyltiourea (ATU) to inhibit nitrification and follow phosphorus removal performance and the rest were submitted to anoxic conditions by supplying nitrite at different flows to simulate nitrification rates (7.18 mg N–NO_2^- L^{-1} h^{-1} (batch C) and 2.87 mg N–NO_2^- L^{-1} h^{-1} (batch D)). Batch C and D ended by applying aerobic conditions. N_2 was sparged during anaerobic and anoxic conditions and oxygen was supplied during aerobic periods maintaining a concentration between 2 and 2.5 mg DO L^{-1}.

In each test, mixed liquor samples were taken every 15–30 min using a syringe and immediately filtered through disposable millipore filter units (0.22 μm pore size) for the analyzes of ammonium, nitrite, nitrate and phosphate (methods given below). Sludge samples were taken before adding the synthetic feed and at the end of the anaerobic, anoxic and aerobic phases in order to analyze the PHA and glycogen content of the biomass (methods given below), which were all measured in triplicate. Anaerobic ratios were determined as phosphorus release, glycogen consumption and PHA formation per carbon uptake (P_{REL}/C_{UP}, Gly/C_{UP}, PHA/C_{UP}, respectively). Anoxic or aerobic ratios were calculated as glycogen consumption and phosphorus uptake per PHA oxidized (Gly/PHA, P_{UP}/PHA, respectively).

Table 1
Range of SBR operational times and conditions applied during the study.

		Anaerobic (min)	Aerobic (min)	Anoxic (min)	Settling (min)	VER (%)	HRT (h)
100% floc SBR	1st Sequence	80–100	110–140	3	40–2.5	25–62.5	24–9.6
	2nd Sequence	45–65	45–65	5–30			
90% floc SBR	1st Sequence	60–100	100–160	3	23–2	25–62.5	24–9.6
	2nd Sequence	50–70	35–50	5–40			

2.3. Analysis

Ammonium ($N–NH_4^+$), nitrate ($N–NO_3^-$), nitrite ($N–NO_2^-$) and phosphate ($P–PO_4^{3-}$) concentrations were analyzed using a Lachat Quik Chem 8000 Flow Injection Analyzer (Lachat Instrument Milwaukee. Wisconsin). VFAs were measured by Perkin–Elmer gas chromatography with column DB-FFAP 15 m_0.53 mm_ 1.0 mm (length_ID_film) at 140 °C. Total and soluble chemical oxygen demand (CODt and CODs, respectively), total Kjeldahl nitrogen (TKN), total phosphorus, mixed liquor suspended solid (MLSS) and volatile MLSS (MLVSS) were analyzed according to the standard methods (APHA, 2005). Glycogen and PHA were determined as described in Ye et al. (2010). For microbial community analysis, fluorescent in situ hybridization (FISH) was performed as described by Amann (1995) using the probes and quantitation methodology described in Zhou et al. (2008a). To determine the size distribution of the particles in each SBR, 30 mL of mixed liquor were passed through a Malvern laser light scattering instrument, Mastersizer 2000 series (Malvern Instruments, Worcestershire, UK), suitable for measurement of particle sizes in the range of 0.02–2000 μm. The granule morphology was qualitatively observed using a stereo microscope (Olympus SZH10).

The sludge volumetric index (SVI) was determined from a liter of mixed liquor from the last aerobic period of the cycle; the height of the sludge was recorded every minute during the first five minutes and every 5 min until half an hour. SVI_{10} and SVI_{30} was the sludge height at 10 and 30 min, respectively, per gram of MLSS. The settling rate was obtained as a regression line from the sludge height between 0 and 5 min.

3. Results and discussion

3.1. Aerobic granulation performance

Two SBRs were inoculated and operated for granule formation for a period of 120 days with domestic wastewater. One SBR was started-up with floccular sludge as the inoculum (100%-floc SBR), while the other SBR was seeded with a mixture of floccular sludge and 10% (w/w) crushed granules (90%-floc SBR). To enhance granulation, the settling time was decreased in both reactors according to the settling properties of the sludge.

The median particle size of both reactors reached 200 μm around day 40 of operation when the settling time was less than 10 min (Fig. 1). This is the minimum size for a particle to be considered an aerobic granule (de Kreuk et al., 2007). The 50th and 90th percentiles increased until day 80 in the 100%-floc SBR, indicating a partial granulation of the sludge. After that, these percentiles remained relatively stable (median size around 1 mm), although floccular sludge was still present in the system as shown by the low 10th percentile values (Fig. 1). The increase in particle size was slower in the 90%-floc SBR but it achieved full granulation by day 80, when the 10th percentile was higher than 200 μm. Both SBRs operated with short settling times most of the experimental period as indicated in Fig. 1.

Biomass concentration decreased at the beginning of the experiment in both reactors due to the settling time reduction (Fig. 2). The gradual loss of biomass from the 100%-floc SBR resulted in an MLSS concentration lower than 1 g L^{-1} by day 40, when the system started to granulate. Due to the low MLSS the VER was decreased (Fig. 2) to minimize biomass loss during the withdrawal of the treated wastewater and to maintain good BNR performance in the reactor. In contrast, the biomass concentration in the 90%-floc SBR rose from 3.4 to 5.8 g MLSS L^{-1} during the increase of VER up to around day 30. Following that the biomass declined to a stable concentration of around 3 g MLSS L^{-1} for the remainder of the operation and the VER was maintained at 62.5%.

Granulation, as well as biomass concentration, had different influences on the settling properties of the sludge. Settling properties of the sludge were determined during the granulation process (Fig. 3). Both reactors achieved SVI_{30} values under 100 mL g^{-1} MLSS. Nevertheless, low SVI_{30} (t = 30 min) does not necessarily imply sludge granulation and vice versa (Schwarzenbeck et al., 2004); however, the SVI_{30}/SVI_{10} ratio gives an excellent indication about the granule formation (de Kreuk et al., 2007). This ratio increased over the operational period and approached one in the 90%-floc SBR, which indicated a completely granulated system. Furthermore, the settling velocity increased in both SBRs, achieving the highest values of nearly 10 m h^{-1} with granules from the 90%-floc SBR.

3.2. Biological nutrient removal

An important aim of the study was to simultaneously achieve granulation and nitrogen and phosphorus removal with domestic wastewater. Organic loading rates (OLR) as COD and VFA were monitored in both SBRs. OLR above 1000 g COD m^{-3} d^{-1} was only achieved when HRT of 0.4 days (VER of 62.5%, Fig. 2) was applied in the reactors. The 100%-floc SBR was not able to cope high loadings because of the biomass washout (Fig. 2) and HRT was finally fixed at 0.67 days obtaining an OLR of 547 ± 87 g COD m^{-3} d^{-1}. In the case of 90%-floc SBR the system maintained the lowest HRT with a mean OLR of 849 ± 217 g COD m^{-3} d^{-1}. The variability of OLR values during constant HRT was due to the variable nature of the real domestic wastewater. The wastewater was supplemented with some acetate in both SBRs. However, the 100%-floc SBR had low MLSS concentrations and was not able to remove all the biodegradable organic matter, thus lower VFA were added to that SBR (23 mg C L^{-1}) to avoid filamentous bulking. For the 90%-SBR, acetate addition was progressively reduced during the operation from 147 mg C L^{-1} at day 10 to 32 mg C L^{-1} at day 100.

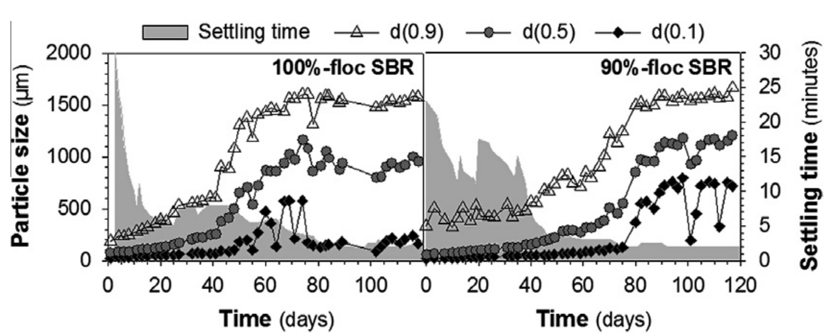

Fig. 1. Size distribution of biomass particles (10th percentile, d(0.1); 50th percentile or median size, d(0.5); 90th percentile, d(0.9)) and settling times of the 100%- and 90%-floc SBR during the experimental study.

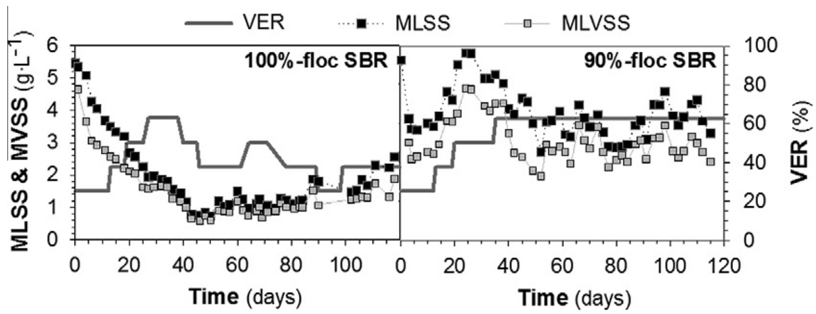

Fig. 2. Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and volume exchange ratio (VER) in both SBRs during operation.

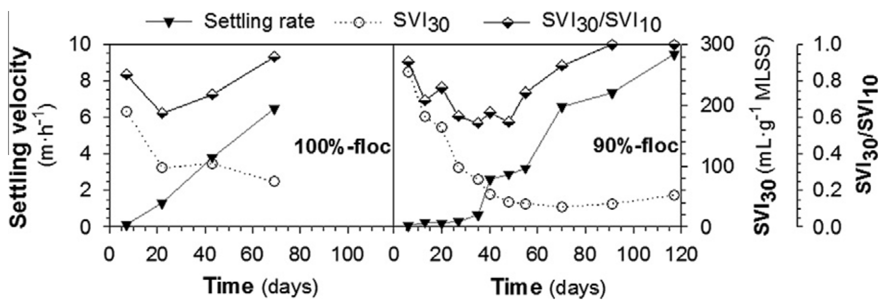

Fig. 3. The sludge settling velocity, sludge volumetric index (SVI_{30}) and SVI_{30}/SVI_{10} ratio during the experimental study.

Consequently, part of the improved performance may be attributed to the extra VFA provided to the 90%-floc SBR. Despite the differences in both reactors, organic matter was removed from all systems obtaining efficiencies of 85% and 80% for 100%-floc and 90%-floc, respectively, at the end of the performance.

To study nutrient removal performances, nitrogen and phosphorus in the influent and the effluent were analyzed (Fig. 4). In the 100%-floc SBR, nitrogen and phosphorus removal progressively decreased from the beginning of the experiment causing effluent loads to rise to 84 g N m^{-3} d^{-1} and 12 g P m^{-3} d^{-1} around day 40. At this time the reactor did not cope with the increased loading and the biomass washout (62.5% VER, 1.7 g VSS L^{-1}, Fig. 2) and poor nitrification and phosphorus removal occurred. Nevertheless, the success of granulation (around day 60) allowed a progressive increase on the biomass concentration and the recovery of nitrification and phosphorus removal. The phosphorus removal recovery could be also observed by the increase of the inorganic component of the granules, as detected by an increasing difference between MLSS and MLVSS from day 90 (Fig. 2). According to Tayà et al. (2011), when PAO are predominant, the MLVSS/MLSS ratio is lower than the average ratio of ordinary heterotrophic and nitrifying biomass due to the increased poly-P storage. At the end of the operational period, the 100%-floc SBR loadings rates were stabilized at 78 g N m^{-3} d^{-1} and 13.5 g P m^{-3} d^{-1} and nitrogen and phosphorus removal efficiencies were at 85% and 94%, respectively.

Nutrient removal performance was more stable in the 90%-floc SBR, even though higher loads than in the 100%-floc SBR were treated. This was due to the higher and more stable levels of biomass in the reactor (Fig. 2), which performed to remove nitrogen and phosphorus to low concentrations in the effluent while the VER was increased from 25% to 62.5%. At the end of the operating period the system achieved 85% and 94% nitrogen and phosphorus removal, respectively, when loads of 122 g N m^{-3} d^{-1} and 20.8 g P m^{-3} d^{-1} were applied in the 90%-floc SBR.

To obtain details of the BNR performance reactor cycle analyzes were carried out on the 100%-floc and 90%-floc SBRs. SND efficiency (denitrification/ nitrification during aerobic phases, Zeng et al. (2003a)), nitrates and nitrites obtained in the effluent and

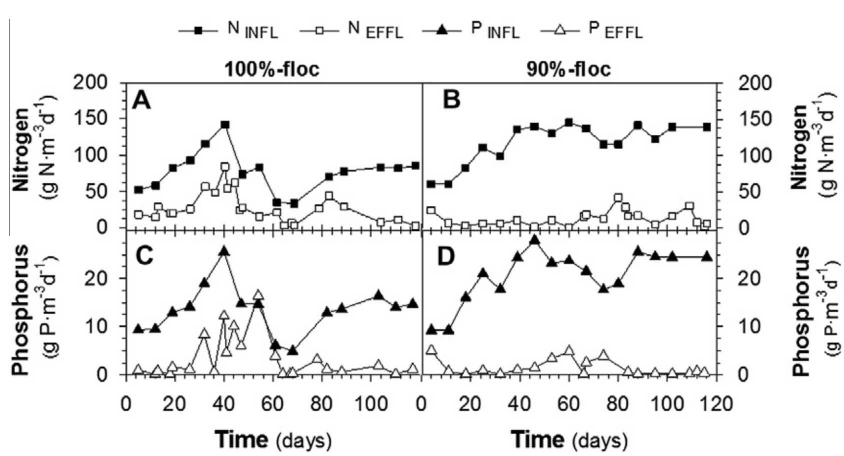

Fig. 4. Influent and effluent loads for nitrogen and phosphorus from 100%-floc (A and C) and 90%-floc (B and D) SBRs.

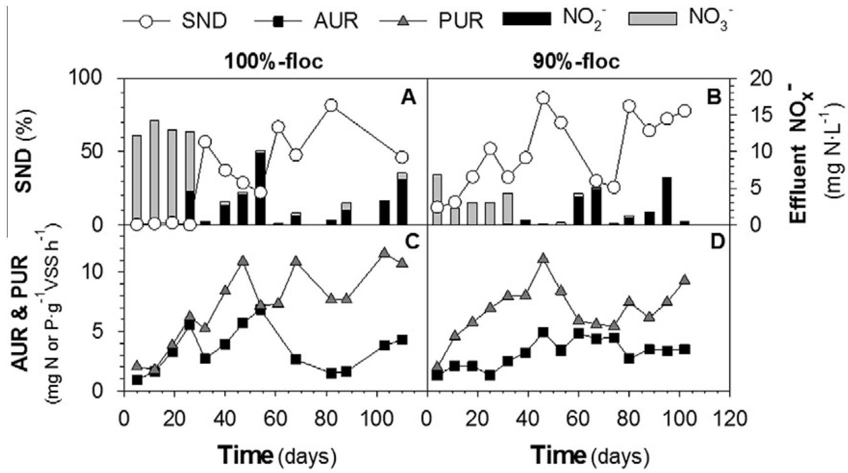

Fig. 5. SBR performance comparison of SND efficiencies, effluent nitrate and nitrite levels, ammonium uptake rates (AUR) and phosphorus uptake rates (PUR) from the 100%-floc (A and C) and the 90%-floc (B and D) SBRs.

ammonium and phosphate uptake rates evaluated from cycle studies are presented in Fig. 5.

Nitrite was the main product of nitrification after day 40 (Fig. 5A) when granules started to develop (Fig. 1). Nitrite concentration in 90%-floc SBR was lower than that in 100%-floc SBR with similar ammonium concentrations in the influent. The fact of a higher total nitrogen removal in a granular reactor compared with a floccular reactor was also stated by Gao et al. (2011). A major presence of granules in the 90%-SBR compared to the 100%-floc SBR (Fig. 1) may have enhanced denitrifying activity due to larger anoxic zones existing inside the granules than the floccular sludge. SND in both reactors increased over 50% when nitrite instead of nitrate was present at low concentrations in the effluent. However, when high values of effluent nitrite were obtained (>5 mg $N–NO_2^-$ L^{-1}), SND was reduced to 20% in 100%-floc SBR at day 55 and in 90%-floc SBR at day 65. Moreover, phosphate uptake rates (PUR) were rising when nitrate was the main product of nitrification (from day 0 to day 50 in both reactors). However, these rates decreased when nitrite was at high levels. The fact that PUR as well as SND activities were lower when nitrite was present could be due to both processes occurring together by denitrifying PAOs (DPAOs). This would explain both lower SND activity and lower PUR measured during aerobic periods from granular sludge due to nitrite presence. Thus, as it is stated in the literature, nitrite or free nitrous acid is an inhibitory compound for phosphorus removal and DPAO activity as well (Saito et al., 2004; Zhou et al., 2008b; Pijuan et al., 2010).

We detected that PUR and SND increased from around day 80 until the end of the operation in the 90%-floc SBR (Fig. 5B). This could be explained by the increase in granule size up to 1200 μm during this period (Fig. 1). According to the literature, oxygen and substrate mass transfer through the granule would enhance SND because of stratified bacterial distribution in the particle (Carvalho et al., 2006). The enhanced SND would lead to reduced nitrite accumulation and avoid free nitrous acid (FNA) inhibition of the nutrient removal processes.

The microbial communities were monitored by FISH to determine the percentages of nitrifying bacteria, PAOs and GAOs during the operational periods in both reactors (Table 2). Both SBRs had low abundance of PAOs initially, but this increased (as did the GAOs) as the sludges became more granular.

3.3. EBPR in domestic granules

Denitrification in SNDPR systems is ideally carried out by denitrifying PAOs (DPAOs), but denitrifying GAOs (DGAOs) may also play an important role in nitrogen removal (Zeng et al., 2003a). Cycle study analyzes of the SBRs indicated that denitrification was occurring in aerobic conditions and that nitrite was the main oxidized nitrogen species present in the system. Consequently, batch studies were conducted to evaluate the denitrification activity and its effect on phosphorus removal in the granular sludge. In all batch studies phosphorus was released while VFA (in form of acetate) was consumed under anaerobic conditions (Fig. 6). During the aerobic phase ammonium was gradually oxidized to nitrite while phosphate was taken up (Fig. 6A). However, the PUR decreased as nitrite increased to around 5 mg $N–NO_2^-$ g^{-1} VSS. In contrast, in batch B when ATU was added to stop nitrification, nitrite was not detected and phosphate uptake continued throughout that phase to achieve near complete removal (Fig. 6B). The slight decrease of ammonium (2 mg $N–NH_4^+$ L^{-1}; 1.1 mg $N–NH_4^+$ g^{-1} VSS) in batch B is then likely due to biomass growth.

In batch C, 7.2 mg $N–NO_2^-$ L^{-1} h^{-1} was added during an anoxic phase (Fig. 6C). During the early stage of the anoxic period some phosphate uptake occurred. Denitrification was evident as the detected nitrite level was less than the amount added. However, 12.3 mg $N–NO_2^-$ L^{-1} (accounting for 5.8 mg $N–NO_2^-$ g^{-1} VSS) had accumulated at the end of the anoxic phase. The anoxic PUR for batch C was lower than in the no nitrite addition, batch B. In the aerobic phase of batch C phosphate uptake was not detected, however 2.6 mg $N–NH_4^+$ g^{-1} VSS was nitrified and 2.3 mg $N–NO_2^-$ g^{-1} VSS was produced and accumulated. In contrast, in batch D, nitrite was provided at a lower rate (2.87 mg $N–NO_2^-$ L^{-1} h^{-1}). In this batch, 6.43 mg $N–NO_2^-$ g^{-1} VSS was added at the end of the anoxic phase and nitrite did not accumulate. A steady PUR occurred during the entire anoxic phase (DPAO activity) and this increased strongly when aerobic conditions were applied (Fig. 6D).

Table 2
Polyphosphate accumulating organisms (PAO), glycogen accumulating organisms (GAO) and ammonium oxidizing bacteria (AOB) populations in 100%- and 90%-floc SBRs.

	100%-floc SBR			90%-floc SBR		
	PAO (%)	GAO (%)	AOB (%)	PAO (%)	GAO (%)	AOB (%)
Day 0	6.5 ± 2.3	2.8 ± 1.9	5.2 ± 1.7	5.6 ± 2.2	2.2 ± 1.5	2.4 ± 0.6
Day 35	3.7 ± 1.9	2.7 ± 0.8	2.5 ± 1.0	3.5 ± 1.0	7.9 ± 1.7	3.0 ± 0.6
Day 60	9.1 ± 3.5	6.0 ± 3.5	9.6 ± 3.2	3.4 ± 2.0	6.5 ± 2.0	9.1 ± 2.0
Day 100	NA	NA	NA	13.0 ± 2.6	16.9 ± 2.0	5.4 ± 2.2

NA: not analyzed.

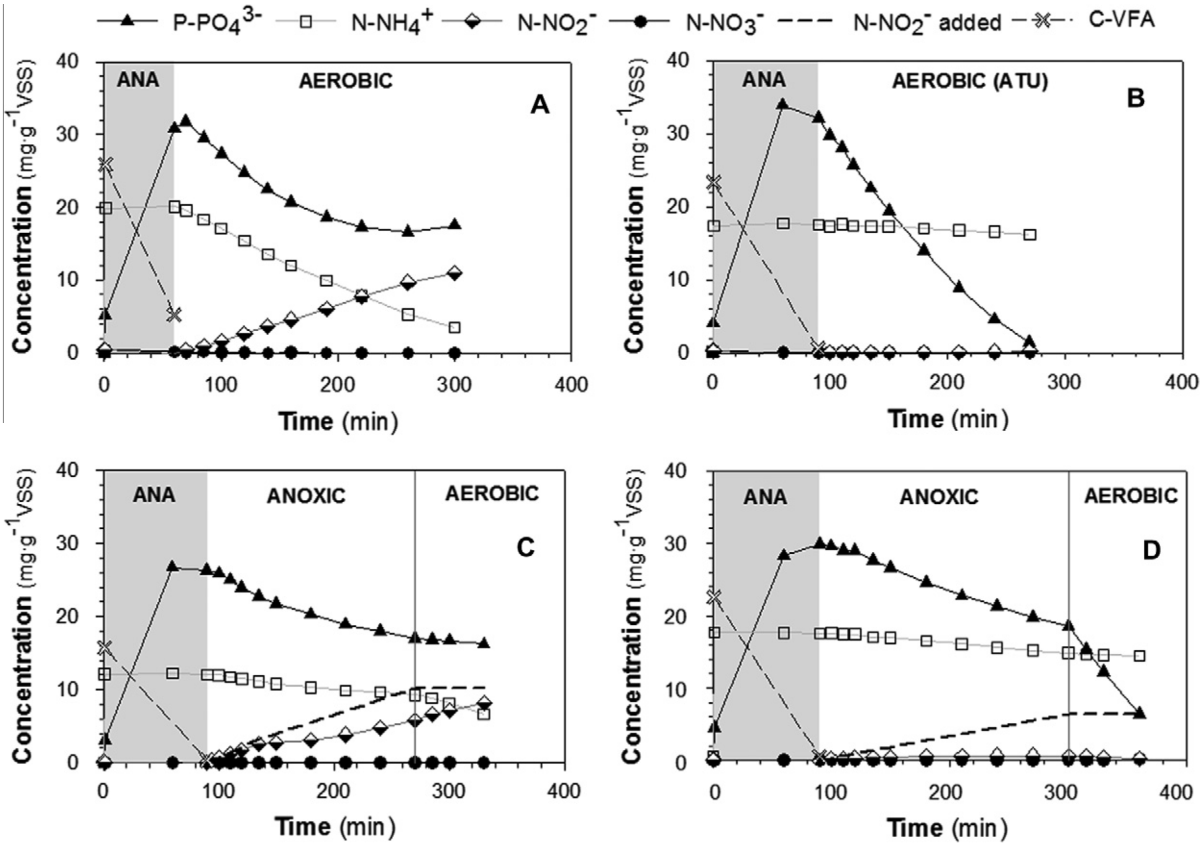

Fig. 6. Concentrations of nitrogen, phosphorus and volatile fatty acids obtained from batch studies of 90%-floc SBR granular sludge. (A) Anaerobic–aerobic batch test; (B) anaerobic–aerobic batch test with allyltiourea (ATU) to inhibit nitrification; (C) anaerobic–anoxic–aerobic batch test with 7.2 mg $N–NO_2^-·L^{-1}$ h^{-1} during anoxic phase; (D) anaerobic–anoxic–aerobic batch test with 2.9 mg $N–NO_2^-$ L^{-1} h^{-1} during anoxic phase.

These microbially mediated transformations, pertaining to the nutrient removal, detected in the batch tests (Table 3) were compared with models for PAO and GAO activities (see supplementary information). The range of values for P_{REL}/C_{UP} obtained in all batch tests were closer to the PAO model presented by Smolders et al. (1995) (0.5 mol P/molC) rather than GAO model (0 mol P/mol C, Zeng et al., 2003b). Also for glycogen degradation, Gly/C_{UP}, the batch tests rates were closer to the PAO model (0.5 mol C/mol C) than to the GAO model (1.12 mol C/mol C). For PHA production, a range of 0.20–0.26 mol C/mol C for the PHV/C_{UP} ratio is detected in the batch tests. The PAO model, and studies on phosphorus removal, did not contemplate a PHV production (Smolders et al., 1995; Zhou et al., 2008a). In contrast, according to the model GAOs have PHV/C_{UP} ratio of 0.46 mol C/mol C on (Zeng et al., 2003b; Zhou et al., 2008a). These results suggest that some GAO activity is detected on batch test, as values obtained were between PAO and GAO theoretical performance. This would be in agreement with microbial community structure determined by FISH (14.7% of PAO and 8.6% of GAO during batch test experiments).

An aerobic phosphate uptake rate of 11.7 mg P g^{-1} VSS h^{-1} was detected in batch B where ATU was added to inhibit nitrification (Fig. 6B). This rate was reduced to 8.0 mg P g^{-1} VSS h^{-1} when nitrification occurred in batch A and nitrite accumulated in the reactor (Fig. 6A). Both anoxic rates of phosphate uptake obtained in batch C and D were lower than aerobic rates but similar at 4.0 and 3.5 mg P g^{-1} VSS h^{-1}, respectively. In contrast, the subsequent aerobic phosphate uptake rate for batch D increased to be similar to batch B, at 12.1 mg P g^{-1} VSS h^{-1}, whereas the aerobic rate was severely diminished for C (0.7 mg P g^{-1} VSS h^{-1}) when nitrite was present. Both aerobic and anoxic rates obtained were lower than the ones obtained by Chen et al. (2011). Chen and co-authors stabilized P removal due to carrying out nitrification in a separate reactor. This fact allowed strict anaerobic environment weakening the negative effect of nitrate on phosphate release and a shorter SRT which favored the growth of PAOs. In the present study, a mixture of nitrifying and phosphorus removal bacteria was obtained within granules, so biomass selection by SRT was not suitable. Furthermore, ammonium was oxidized to nitrite instead of nitrate

Table 3
Experimentally observed ratios under anaerobic, anoxic and aerobic conditions from batch tests.

	Anaerobic			Anoxic		Aerobic	
	P_{REL}/C_{UP} (mol P/molC)	Gly/C_{UP} (mol C/molC)	PHA/C_{UP} (mol C/molC)	Gly/PHA (mol C/molC)	P_{UP}/PHA (mol P/molC)	Gly/PHA (mol C/molC)	P_{UP}/PHA (mol P/molC)
Test a	0.48	0.44	NA	–	–	NA	NA
Test b	0.48	0.35	1.20	–	–	0.39	0.44
Test c	0.59	0.25	1.03	0.43	0.30	0.45	0.27
Test d	0.45	0.33	1.54	0.10	0.44	0.46	0.32

NA: Not Analyzed.
See Section 2 for ratios definition.

during aerobic conditions and the reduction of phosphate uptake during the presence of nitrite could be explained as FNA inhibition on PAO transformations (Zhou et al., 2008b; Pijuan et al., 2010).

All batch experiments showed similar values for glycogen production to PHA oxidation ratios in the anoxic and aerobic conditions except batch D when nitrite was not detected in the media, in which the *GLY/PHA* ratio dropped from around 0.40 to 0.10 mol C/mol C. Also, for batch D under anoxic conditions obtained the same value of P_{UP}/PHA ratio as batch B (aerobic conditions) where total phosphorus removal activity was found (0.44 mol P/mol C). The results suggest that DPAOs are regulating their activity so that energy is preferentially used for anoxic phosphate uptake rather than for glycogen synthesis.

These batch tests assessed the BNR performance of the aerobic granules by examining the biochemical transformations during simulated SBR cycles. Granules performed good phosphate uptake in aerobic conditions only when nitrite levels were low. However, during high nitrification rates nitrite would accumulate in the system, leading to a lower denitrification and phosphorus removal, probably due to free nitrous acid inhibition (Zhou et al., 2008b). That fact was also observed during 100% and 90%-floc performance as SND was reduced when higher nitrite concentrations were detected in the effluent (Fig. 5). Contrarily, lower nitrification in granular sludge would avoid nitrite accumulation and allow better DPAO and SNDPR activities.

3.4. Advantages of adding crushed granules in the seeding sludge

A granulation strategy was compared by providing different innoculum to the start up of two laboratory scale SBRs. The settling time was sequentially reduced as a strategy to select for the granules. When only a floccular sludge was used to inoculate the granular system (100%-floc SBR), granules appeared after only 40 days of operation. However, by applying this strategy, biomass loss occurred causing disturbed BNR performance. Additionally, even after the MLSS and nutrient removal had recovered, floccular sludge was not totally washed out from the system while applying a low settling time (2.5 min).

A similar start-up strategy was used for 90%-floc SBR except this was seeded with floccular sludge containing 10% crushed granules. It has been previously demonstrated that adding crushed granules to the seeding sludge significantly reduced the start-up time of aerobic granular sludge reactors when dealing with a nutrient rich abattoir wastewater (Pijuan et al., 2011). In this study, a shorter start-up time was not so evident. However, the nutrient removal performance, the more stable biomass and superior granule formation were all features in the sludge amended with crushed granules. The biomass loss was negligible even after progressively reducing the settling time and floccular sludge was completely removed from the system. In addition, the BNR did not experience any destabilization due to granular formation, even when applying a high VER of 62.5%, which was not achieved during the granulation of the fully floccular system. Therefore, the use of crushed granules as seed sludge, even at low percentages, improves the start-up performance of a domestic granular system.

4. Conclusions

Aerobic granular sludge can be successfully formed from floccular sludge while removing nitrogen and phosphorus from sewage. The following main conclusions can be drawn from this study:

- Granulation from floccular sludge is possible using low-strength domestic wastewater, even though a substantial loss of biomass during the start-up is observed.
- A low fraction of crushed granules in the seed sludge (10%) enhances the start-up by avoiding nutrient removal disturbances and biomass loss.
- A fully granular system enhances the simultaneous nitrification, denitrification and phosphorus removal in VFA limited influents such as domestic wastewater.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.10.014.

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