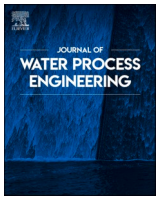
Journal of Water Process Engineering 60 (2024) 105165

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe



Achieving mainstream partial nitritation with aerobic granular sludge treating high-rate activated sludge effluent

Oriol Carbó [a,b], Jaume Teixidó [b], Joan Canals [b], Antonio Ordóñez [b], Albert Magrí [a], Mercè Baldi [b], Belén Gutiérrez [b], Jesús Colprim [a,*]

[a] LEQUIA, Institute of the Environment, University of Girona, Campus Montilivi, Carrer Maria Aurèlia Capmany 69, 17003 Girona, Catalonia, Spain
[b] GS Inima Environment, S.A., c/ Gobelas 41 1º, 28023 Madrid, Spain

ARTICLE INFO

Editor: Ludovic F. Dumée

Keywords:
AGS pilot plant
HRAS effluent
Sludge settling
Nitrite
Densified biomass

ABSTRACT

New configurations for wastewater treatment plants (WWTPs) are under development to increase their self-sufficiency and sustainability. One is the A-B process, in which the A-stage is designed to maximize the redirection of influent chemical oxygen demand (COD) to the production of biogas. A promising A-stage alternative is the high-rate activated sludge (HRAS). B-stage, on the other hand, can consist of the combined partial nitritation (PN)-anaerobic ammonium oxidation (anammox). In this study, PN with aerobic granular sludge (AGS) was attempted under mainstream conditions at pilot scale. The effluent of a HRAS system – ideally operating at 2 g/L of total suspended solids, 0.5 mg/L of dissolved oxygen and 1 h of hydraulic retention time (52 ± 13 % COD removal) – was fed to the PN-AGS reactor initially inoculated with floccular sludge. Granulation and PN were studied with daily/seasonal fluctuations and without adding external reagents. Selective wasting from the top of the settled sludge bed was found a successful strategy to trigger granular sludge formation. The maintenance of a low sludge volume index (SVI < 100 mL/g) was helpful enough for improved operation of the system (i.e., COD removal and PN). Stable PN was achieved due to the suppression of the nitrite oxidizing bacteria activity by inhibitory levels of free nitrous acid. Finally, working with fast settling biomass at long settling times reduced the influent-biomass-nitrite contact and, thus, denitritation.

1. Introduction

Wastewater treatment plants (WWTPs) play a crucial role in safeguarding public health by treating wastewater before discharge into the environment. However, conventional facilities consume substantial amounts of energy to power various treatment processes, including aeration, pumping, and sludge handling [1]. According to recent estimates, wastewater treatment accounts for about 3 % of the total electricity consumption worldwide, with significant associated greenhouse gas emissions and operational costs [2]. To address these challenges, WWTPs are adopting energy-efficient technologies and using renewable energy sources such as the biogas produced by the anaerobic digestion of the sludge [1,3]. This biogas (i.e., methane) can be used to generate electricity and heat through a combined heat and power unit [4], which offers a sustainable solution for energy management in the WWTP by reducing the dependence on fossil fuels and mitigating greenhouse gas emissions. However, in conventional WWTPs, only between 39 % and 76 % of the total energy consumed can be supplied by the biogas produced from anaerobic digestion [5]. Therefore, WWTPs are still energy-demanding facilities and new process configurations are being studied to achieve more operational self-sufficiency and sustainability. In this context, the A-B process – in which the A-stage is primarily designed to

Abbreviations: AGS, aerobic granular sludge; anammox, anaerobic ammonium oxidation; AnMBR, anaerobic membrane bioreactor; AOB, ammonia-oxidizing bacteria; CAS, conventional activated sludge; CEPT, chemical enhanced primary treatment; COD, chemical oxygen demand; DO, dissolved oxygen; EBPR, enhanced biological phosphorus removal; F/D, fill/draw; FNA, free nitrous acid; GAO, glycogen-accumulating organisms; HRAS, high-rate activated sludge; HRT, hydraulic retention time; N, nitrogen; NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; NOB, nitrite-oxidizing bacteria; OLR, organic loading rate; P, phosphorus; PAO, polyphosphate-accumulating organisms; PID, proportional-integral-derivative; PN, partial nitritation; PNA, PN-anammox; PO_4^{3-}, phosphate; SBR, sequencing batch reactor; SRT, solids retention time; SVI, sludge volume index; TN, total nitrogen; TSS, total suspended solids; VER, volume exchange ratio; WWTP, wastewater treatment plant.

* Corresponding author.
E-mail address: jesus.colprim@udg.edu (J. Colprim).

https://doi.org/10.1016/j.jwpe.2024.105165
Received 21 February 2024; Received in revised form 12 March 2024; Accepted 12 March 2024
Available online 20 March 2024

maximize the redirection of the influent chemical oxygen demand (COD) to biogas production via anaerobic digestion without producing excess sludge and the B-stage is designated for efficient nitrogen (N) removal – has been widely proposed as a promising configuration for future WWTPs [6].

Anaerobic membrane bioreactors (AnMBR), chemically enhanced primary treatment (CEPT), and high-rate activated sludge (HRAS) in the A-stage may enhance the wastewater COD capture/conversion to biogas [7]. HRAS systems are based on high-loaded activated sludge reactors that generally have a short hydraulic retention time (HRT) (<1 h), short solids retention time (SRT) (< 1 day), and low dissolved oxygen (DO) levels (< 1 mg O_2/L). These operational conditions lead to an improvement in solid-liquid separation while maximizing COD capture [8–11]. HRAS systems present several advantages over other A-stage technologies. They are not susceptible to fouling, as AnMBRs are [12], and do not require the addition of chemicals, as CEPTs do [13].

Regarding N removal in the B-stage, the application of partial nitritation (PN) coupled with anaerobic ammonium oxidation (anammox) (PN-anammox, PNA) in the mainstream could significantly reduce aeration costs. PN consists of the partial oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) instead of the conventional complete oxidation to nitrate (NO_3^-) [14], while anammox is an anaerobic oxidation of NH_4^+ using NO_2^- as an electron acceptor. This is a completely autotrophic process since COD is not necessary for denitrification. Compared with conventional biological N removal, the oxygen requirement can be lowered by 60 % and the organic carbon consumption for denitrification by up to 100 % [15]. Two configurations have been proposed with this aim: i) one-stage PNA in one reactor and ii) two-stage PNA for separated PN and anammox in two reactors running in series [16]. Under mainstream conditions, the combination of PNA in one stage [17,18] reduces infrastructure costs but may make it difficult to control the process, whereas the two-step process is more promising to achieve successful autotrophic N removal [19]. In the two-step configuration, the target of the PN reactor is to obtain a suitable NO_2^-/NH_4^+ ratio for the subsequent anammox reactor while heterotrophically removing the residual biodegradable COD arriving from the A stage [20,21]. The main challenge for PN in the first reactor is the prevention of NO_3^- formation by nitrite-oxidizing bacteria (NOB) under low N concentration and temperature and high variable flow rate due to daily and seasonal variations. Free nitrous acid (FNA, HNO_2) accumulation seems to be a good option to inhibit NOB activity [22–24]. Pilot- and full-scale experiences have been conducted to apply PNA in the mainstream [25,26], but the topics suggested above (low COD/N ratio, nitrate formation suppression, etc.) must be addressed successfully to prevent the failure due to the lack of stability and reliability over the long-term operation [15].

Aerobic granular sludge (AGS) is a technology with low energy and footprint requirements compared with conventional activated sludge (CAS) systems. The fast-settling and high-thickening properties of AGS sludge allow the settling process to be integrated inside only one treatment unit operating at an increased solids concentration, which eliminates the need for space-consuming secondary clarifiers and greatly reduces the footprint. Moreover, energy-intensive recycling flows and mixers can be avoided [27,28]. Granules are spherical biofilm structures gathering a consortium of self-aggregated microorganisms. Nitrifying bacteria grow on the oxygenated granule surface and polyphosphate accumulating organisms (PAO) grow inside the granules, where they store polyhydroxyalkanoates (PHA) and polyphosphate [29]. Thus, using AGS in the B-stage has become attractive, since the advantages of AGS can be combined with an efficient A-B configuration.

In this context, future WWTPs may combine an A-stage based in HRAS followed by a granular PNA B-stage. PNA would better be set in a two-stage configuration, with a PN AGS reactor followed by a final anammox treatment.

Experiences combining HRAS and AGS systems are scarce. Kosar et al. [30] treated a HRAS effluent in an AGS system at lab scale. Low COD-to-N ratio in the influent limited denitrification and caused high NO_3^- concentrations in the effluent. However, this can be an opportunity for the application of PN. Setting a high organic loading rate (OLR) has been considered an important parameter in aerobic granulation. However, when treating real HRAS effluent, a significant part of the raw wastewater COD has already been removed in the HRAS reactor and achieving a high OLR in the AGS reactor becomes difficult. To the best of our knowledge, there are no real full-scale applications combining AGS and PN in the mainstream of a WWTP; only lab-scale studies are available [31]. Thus, the objective of this study is to address aerobic granulation at pilot scale when treating HRAS effluent and to establish a stable PN while reducing NO_3^- formation. The novelty of this work resides in achieving aerobic granulation at a low OLR and the establishment of a stable PN avoiding NO_3^- formation with real mainstream wastewater and daily/seasonal fluctuations without the addition of external reagents.

2. Materials and methods

2.1. Pilot plant: HRAS + AGS

A pilot plant formed by an HRAS unit plus an AGS reactor was installed in La Garriga WWTP (Catalonia, Spain) to treat real raw wastewater in situ under mainstream conditions. The HRAS unit had already been described elsewhere [8,9]. The main operational conditions applied in this case were: total suspended solids (TSS) in the reactor controlled at 2 g/L, DO adjusted to 0.5 mg/L, and HRT of 1 h. The HRAS pilot plant effluent was directly pumped as influent to the AGS reactor.

The AGS reactor was a column reactor 4.5 m high and 0.5 m in diameter (0.9 m^3). The feeding and recirculation streams were both pumped to the bottom of the reactor by centrifugal pumps controlled with electromagnetic flowmeters (PID control). The effluent of the reactor overflowed from the top and passed through a 50-L tank before discharge, where a TSS probe (Hach) was installed. Aeration, which may be supplied using coarse or fine bubbles, was controlled by mass flow controllers (Bronkhorst High-tech B.V., Netherlands) and a DO probe (Hach Lange GmbH, Germany). The pH, redox potential, and temperature in the reactor were monitored on-line using specific probes (Hach Lange GmbH), which were all placed at 2/3 of the reactor's height. The wasting of solids from the reactor was performed gravimetrically through a pneumatic valve controlled with an electromagnetic flow totalizer (Fig. S1).

The AGS reactor was operated as a sequencing batch reactor (SBR) at constant volume for 780 days to develop granular biomass for COD removal and PN. The reactor was inoculated at day 0 with floccular sludge from a CAS system, which had a sludge volume index (SVI) of 150 mL/g. Throughout the experimental time, there was no control of temperature, which ranged from 12 °C to 27 °C. Each operational cycle in the SBR comprised: i) simultaneous fill/draw (F/D), ii) anaerobic reaction, iii) aerobic reaction, and iv) settling (Fig. S2). The total cycle length ranged from 160 to 360 min and the DO concentration in the aerobic phases was maintained above 2.0 mg/L. The volume exchange ratio (VER) was adjusted to 50 %.

2.2. AGS influent

In this study, the AGS reactor was fed with effluent from the HRAS unit, which treated raw wastewater arriving at La Garriga WWTP (sidestream wastewater returning to the headworks was also included). The raw wastewater entering the HRAS system contained 797 ± 348 mg/L of total COD and 145 ± 38 mg/L of filtered COD at 0.2 μm. The average composition of the influent to the AGS reactor was total COD: 391 ± 84 mg/L, filtered COD: 129 ± 30 mg/L, TSS: 130 ± 43 mg/L, NH_4^+-N: 40 ± 13 mg/L, PO_4^{3-}-P: 4.5 ± 1.6 mg/L, and alkalinity: 359 ± 34 mg $CaCO_3$/L.

2.3. Analytical methods and sampling

Three automatic samplers (mod. AS950, Hach) were used to take integrated 24-h samples of the HRAS influent, the AGS influent, and the AGS effluent once or twice a week. Moreover, periodic samples from the AGS and HRAS reactors and waste were collected for analysis several times a week. They were analyzed according to the *Standard Methods for the Examination of Water and Wastewater* [32]. Total suspended solids (TSS) were determined gravimetrically after sample filtration with a glass microfiber filter and drying to constant weight at 105 °C. Alkalinity was determined by acid titration to a pH-endpoint of 4.5 and expressed as $CaCO_3$. COD was determined through the closed reflux and colorimetric method (test LCK 714, spectrophotometer mod. DR 3900, Hach Lange GmbH). The concentration of NH_4^+, NO_2^-, NO_3^-, and phosphate (PO_4^{3-}) were determined using ion chromatography (mod. ICS-5000, Dionex, USA), after filtering samples with 0.2 μm nylon filters. The SVI was measured in a 1-L graduated cylinder. The height of the sludge was recorded at different times within with an interval of 5 to 30 min. Thus, the SVI_5 and SVI_{30} (SVI) were the sludge blankets at 5 and 30 min, respectively, divided by TSS concentration. Average sludge particle size and distribution in a range from 0 to 2500 μm were measured by a laser diffraction analysis system (Mod. Malvern Mastersizer Series 2600, Malvern Instruments Ltd., UK). Moreover, the sludge size distribution was also determined by the sieving method [33]. The morphology of the sludge was qualitatively observed with a stereomicroscope (mod. Stereo Discovery V12, Zeiss, Germany).

2.4. Calculations

The concentration of FNA (HNO_2) was calculated according to the measured total NO_2^- concentration (C_{NO2}), temperature (T), and pH [34] (Eq. (1)):

$$FNA = \frac{C_{NO_2}}{1 + e^{\left(\frac{-2300}{273+T}\right)} \cdot 10^{pH}} \quad (1)$$

The SRT was calculated with the TSS content in the reactor (TSS_r), waste (TSS_w) and reactor effluent (TSS_{eff}), the reactor volume (V_r), and the waste (Q_w) and effluent (Q_{eff}) volumetric flow rates, as follows (Eq. (2)):

$$SRT = \frac{TSS_r \cdot V_r}{TSS_w \cdot Q_w + TSS_{eff} \cdot Q_{eff}} \quad (2)$$

TSS_r and TSS_p were measured in the laboratory through TSS analysis whereas TSS_{eff} was measured in situ using the solids probe placed in the 50-L effluent tank (Fig. S1). Q_p and Q_{eff} were measured by flowmeters.

2.5. AGS cycle analysis

Several times throughout this study (days 70, 556, 673, and 778), the performance of a representative SBR cycle was analyzed by the collection of samples at 1/3 of the reactor's height. Samples were immediately filtered and stored at 4 °C for the subsequent analysis of COD, NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}. Moreover, the DO, temperature, and pH were recorded by the installed probes (2/3 of the reactor's height). During the F/D and anaerobic phases, the content was not homogeneous in the reactor, which operated as a plug flow system. Otherwise, in aerobic phases, the reactor content was homogeneous due to the mixing induced by the aeration.

3. Results

3.1. Operational strategies to move from floccular to aerobic granular biomass

A combined system based on HRAS and AGS was employed to treat real raw wastewater from La Garriga WWTP (Catalonia, Spain) for 781 days. During this time, various sequential strategies were applied to the AGS SBR reactor to granulate conventional activated sludge in order to enhance the system performance. Three different experimental periods were established based on the most significant changes (Table 1). In Period I the granulation strategy was focused on the washout of the floccular sludge in F/D phases through the effluent withdrawal by increasing the influent wastewater upflow velocity (F/D v_{up}) – from 2.3 to 4.5 m/h – while reducing the settling time (t_{set}) – from 30 to 5 min (Fig. S3). In Period II, an intense lateral wasting at the end of the settling phase was implemented to increase the washout of floccular sludge (Fig. S4). Finally, in Period III, the cycle length was extended to enable nitrification, and a selective wasting from the top of the settled sludge bed was implemented in each operational cycle.

3.1.1. COD removal in the HRAS and AGS reactors

COD removal efficiencies in the HRAS and AGS reactors were rather stable throughout the operation. Despite the large fluctuations in the composition of the raw wastewater, the HRAS unit acted as a buffering system, homogenizing the AGS influent COD over time (Fig. 1). The HRAS reactor achieved an average COD removal of 52 ± 13 %, while COD removal in the AGS reactor was 81 ± 8 %. The OLR applied in the AGS reactor averaged 1.70 ± 0.4 kg COD/(m^3·day) during periods I and II, while it decreased to 1.05 ± 0.3 kg COD/(m^3·day) in period III. Such a reduction in the loading rate was caused by the extension of the cycle length, applied since day 600 to enhance nitrification (Fig. 1).

3.1.2. Aerobic granulation

Period I started with the inoculation of the reactor with floccular activated sludge (SVI of 150 mL/g). The applied granulation strategy was based on the washout of flocs together with the discharge of the effluent in the simultaneous F/D phases, fixing a wastewater upflow velocity of up to 4.5 m/h (Table 1). From day 0 to 150, the TSS content in the reactor remained rather stable at 2.9 ± 0.6 g/L with the SVI as 116 ± 23 mL/g (Fig. 2A). Around day 150, the first granules appeared and the TSS content sharply increased up to 8.4 g/L with an SVI of 29 mL/g. From day 220 onwards a gradual reduction of the TSS to 2.0 g/L and an increase of the SVI to 86 mL/g were observed. Such evidence coincided in time with a water temperature drop from 18 °C to 13 °C (due to the arrival of seasonal winter conditions). During the first days (0 to 86 d), the average sludge particle size reached values of up to 297 μm (Fig. 2B). Afterwards, particle size was not measured since big aggregates (> 2 mm) started to form and were not measurable with the particle-size analyzer. Starting then, the sieving method was used to characterize the biomass-size distribution. Big aggregates and granules coexisted with flocs (< 0.5 mm) in the reactor (Fig. 3AI). It was only at the end of Period I (days 220 to 300), when temperatures dropped to 13 °C, that big aggregates disappeared, and particle size could representatively be measured again with the analyzer. By then, a mean diameter as small as 65 μm was detected, showing a clear particle-size distribution of flocs in comparison with the beginning of the period (Figs. 2B and 3BI). The morphology of the biomass was observed with a stereoscopic microscope (Fig. 4). After inoculation, flocs rapidly transitioned to spherical but not dense aggregates (day 32). Such aggregates disappeared after day 40 and a mixture of conventional flocs and small granules predominated (day 78). It was on day 200 when big granules (> 2 mm) with finger-type outgrowth on the surface were abundant in the reactor. Such finger-like outgrowths were mostly attributable to protozoan colonies. On day 276, the big granules broke into little pieces, forming little granules of about 100 μm, which coexisted with flocs.

Table 1
SBR operational parameters applied in the experimental periods.

Period	Day (d)	SBR phase lengths (min)				Cycle time (min)	F/D v_{up} (m/h)	Solids wasting
		F/D	Anaerobic	Aerobic	Settling			
I	0–300	30	30	68–105	30–5	160–180	2.3–4.5	(a)
II	301–458	30	30	100–105	10	180	4.5	(b)
III	459–781	30–60	0	100–269	30	180–360	4.5–2.3	(c)

Solids wasting strategy: (a) With the withdrawal of the treated effluent; (b) intense lateral wasting introduced at the end of the settling time; or (c) from the top of the settled sludge bed.

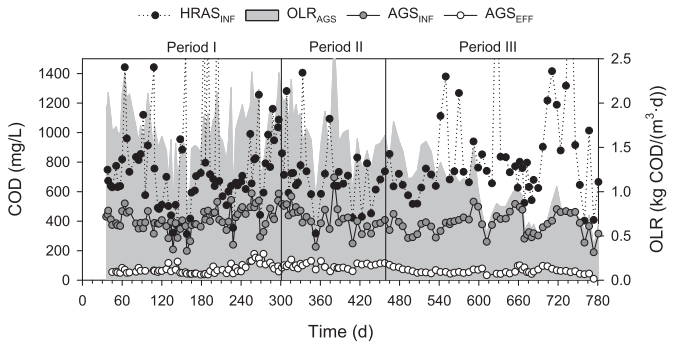


Fig. 1. Evolution of the total COD content (HRAS influent, AGS influent and AGS effluent) and the OLR applied in the AGS reactor throughout the experimental period.

In Period II, to enhance floccular biomass washout and increase the granular biomass concentration, an intense wasting (i.e., up to 44 % of the reactor's volume) was introduced at the end of the settling phase, by considering different wasting heights and number of wastings per day (Fig. S4). The intense wasting entailed that the TSS content in the reactor fell to values as low as 1.0 ± 0.6 g/L, with a minimum value of 0.41 g/L. The SVI changed notably in this period, with values ranging from 20 to 160 mL/g. An average sludge particle size of 166 ± 57 μm was measured throughout this period (Fig. 2B). In this case, the particle-size analyzer was used, despite the presence of big, unmeasurable aggregates, to check the particle-size distribution of flocs and possible protogranules. The sludge was constituted by a mixture of big granules/aggregates (> 2 mm) and small flocs (< 0.2 mm) (Fig. 3AII and BII). Microscope observations on day 344 (Fig. 4) showed that the big aggregates had a similar morphology to those formed in Period I, with finger-type protozoan outgrowth. The floccular sludge fraction turned into a kind of filamentous structure.

Finally, Period III was characterized by the implementation of automatic wasting from the top of the settled sludge bed at the end of every settling phase. This was a selective wasting, since only those flocs with poorer settling capacity were removed from the system. At this point, the granulation process started and the TSS content in the bioreactor reached high values (e.g., 14.9 g/L on day 532) due to granule formation and accumulation. Such an increase in the TSS content was followed by a gradual diminution to and final stabilization at 6.2 g/L on day 585. From days 586 to 620, the TSS content in the reactor stabilized at 6.7 ± 1.5 g/L with a SVI_5/SVI_{30} ratio near 1.0 and an SVI of 20 ± 4 mL/g (Fig. 2A and B). Next, since only granules were present in the reactor, the wasting from the top of the settled sludge bed started to include granules. Previously, only flocs were removed during wasting and granules remained mostly in the system. From day 621 to the end of this study (day 781), such phenomena led to a gradual accumulation of flocs in the system, which stably coexisted with granules. This was evidenced by an increase in the SVI to about 100 mL/g and an increase in the SVI_5/SVI_{30} ratio to approximately 2.5 (Fig. 2A and B). An initial accumulation of big aggregates (> 2.0 mm) was observed during the first days of Period III, but from then onwards, the big aggregates evolved towards perfect granules of about 1 mm (Fig. 3AIII). An average sludge particle size as big as 807 μm was reported on day 610 (Fig. 2B). During this period, and for the first time, size fractions from 0.2 to 2 mm accounted for a significant biomass-size fraction (Fig. 3AIII), shaping a classic granular particle-size distribution (Fig. 3BIII). High variability in the particle-size distribution was observed in this period. Period III started with a high accumulation of very big (about 5 mm) protozoan-dominated aggregates on day 512 (Fig. 4). At the beginning, the

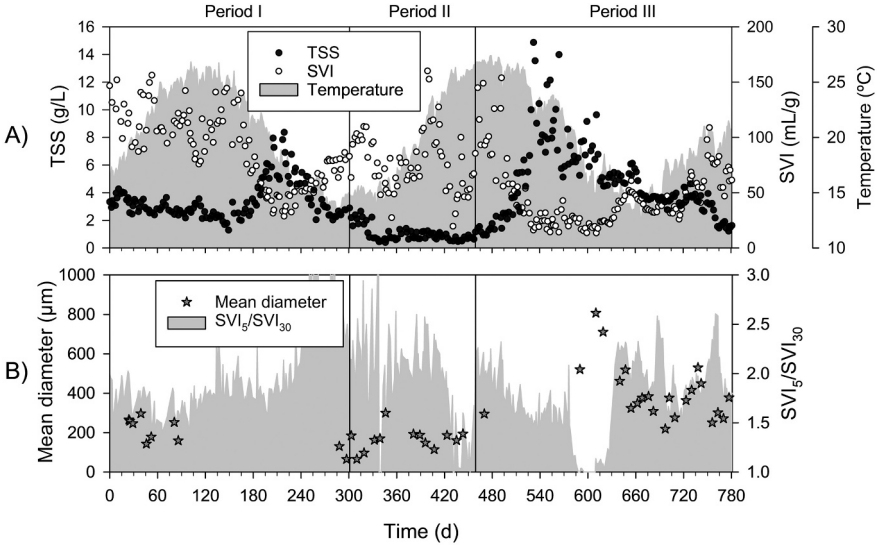


Fig. 2. Evolution of the (A) TSS content, SVI, and water temperature in the AGS reactor, and (B) SVI_5/SVI_{30} ratio and average sludge particle size (mean diameter) throughout the experimental period.

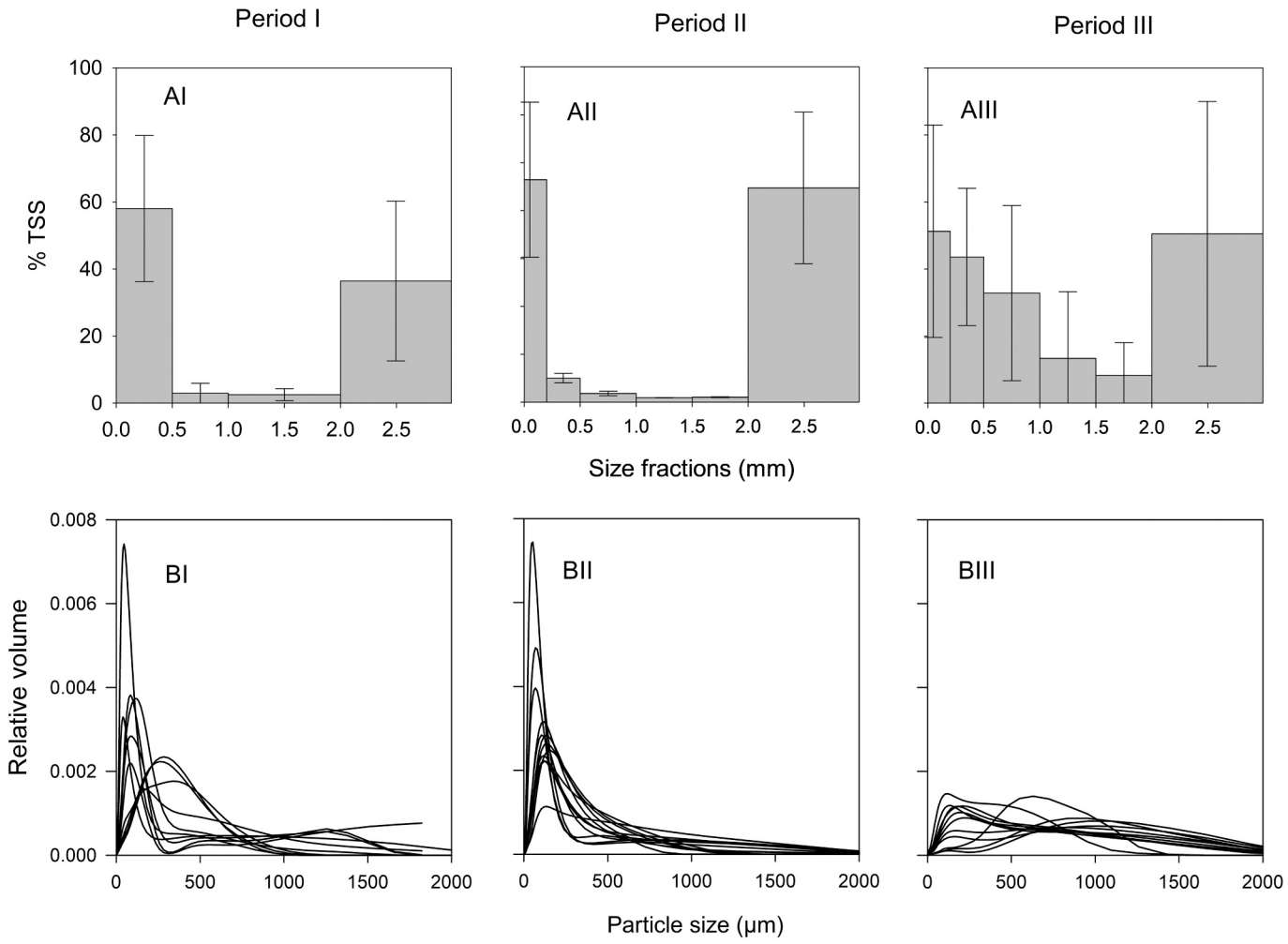


Fig. 3. Mean TSS size fractions in the reactor determined by sieving in periods (AI) I, (AII) II, and (AIII) III. Volumetric sludge particle size distributions determined in the particle-size analyzer in periods (BI) I, (BII) II, and (BIII) III.

morphology of these aggregates was similar to that previously observed in periods I and II. However, on day 539, a sort of compaction was observed in the structure of the finger-type outgrowths and the core of the aggregates. It was on day 562 when the first perfectly shaped 1 mm granules were observed in the reactor. A mixture of the compacted protozoan-dominated aggregates and perfect granules sizing 1 mm dominated the reactor at this point. From day 596 onwards, aggregates disappeared and only 1 mm granules constituted the sludge bed in the reactor. Later, on days 617 and 674, a mixture of small flocs and 1 mm granules was stably observed. That morphology was maintained until the end of the study.

3.2. NH_4^+ oxidation and N and P removal

To understand the nutrient removal performance of the reactor, it should be taken into account that, in periods I and II, the objective was mainly focused on the granulation process, while in Period III the aim was to achieve both granulation and nitrification. The SRT varied significantly throughout the operation of the pilot plant because of the granulation strategies considered (Fig. 5A), thus affecting the microbial communities involved in nitrification. In Period I, the SRT averaged 3.1 ± 1.2 days until day 174. From day 175 to 327 – when big granules appeared – it reached values of 8.4 ± 4 days. By the end of Period I, the SRT decreased to 2.2 ± 1.0 days (Fig. 5A) due to the worsening of the SVI. Since in Period I the solids wasting was performed through the effluent, the worsening of the SVI implied an increased release of solids and, therefore, the TSS content and the SRT in the reactor decreased. In Period II, as a result of the intense lateral wasting, the resulting SRT was as low as 1.1 ± 0.3 days. Finally, in Period III, the SRT increased to 27 ± 15 days from day 519 to day 700, owing to the high amount of TSS accumulated in the reactor. From day 700 to the end of the study (day 781), the SRT was maintained at 10 days (targeted value) by adjusting the wasted sludge volume in each cycle. In summary, the SRT in the AGS reactor in Periods I and III was long enough to allow nitrifying bacteria to grow [35]. In contrast, that was not the case in Period II due to the intensive solids wasting and the resulting short SRT.

When nitrification occurred (periods I and III), NH_4^+ oxidation was partial, and NO_2^-, but not NO_3^-, was detected in the effluent (Fig. 5B). The formation of NO_2^- instead of NO_3^- was analyzed in representative SBR cycle profiles of periods I and III (Fig. 6A–D). Even though a complete N removal was not the main objective of the AGS reactor (i.e., only partial nitritation was targeted to feed a subsequent anammox reactor), denitrification occurred during the operation. During the first part of Period I (days 0 to 200), a significant N removal efficiency of 57 ± 15 % was achieved. Cycle profiles (Fig. 6A) show that denitrification started from NO_2^- rather than from NO_3^-. Indeed, NO_3^- was practically not observed during the SBR cycles throughout the whole operation (i.e., its concentration was always below 1.0 mg NO_3^--N/L). By the end of Period I, the entire Period II and the beginning of Period III (from days 200 to 528), N removal efficiency decreased to 10 ± 12 %. Such a reduction was not caused by nitrification and denitrification, which did not occur during those days, but by N assimilation due to the formation of new

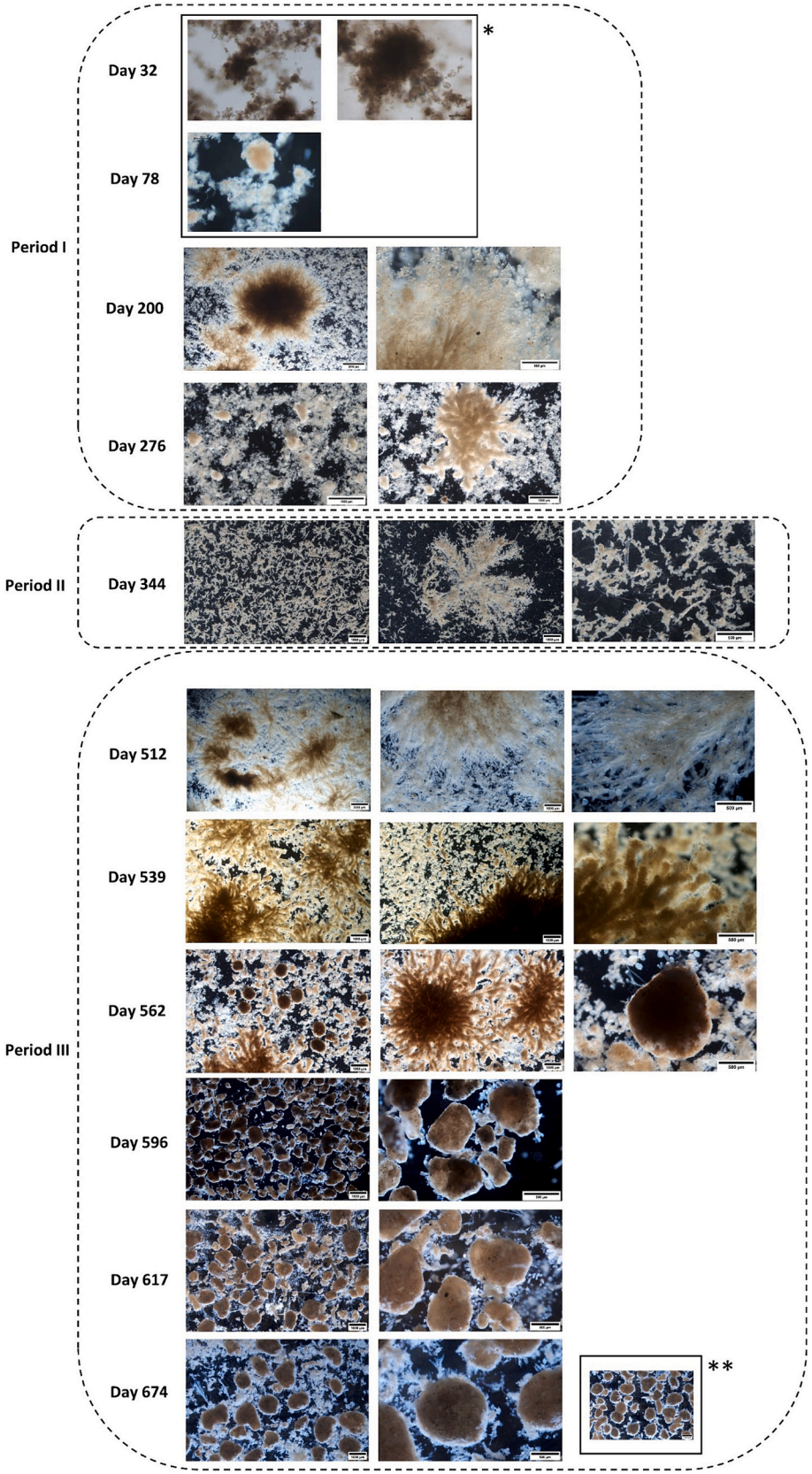


Fig. 4. Microscopic views of the sludge throughout the operation. (*): pictures taken by an optical microscope; the rest were taken by a stereoscopic microscope. (**): selected and washed granules.

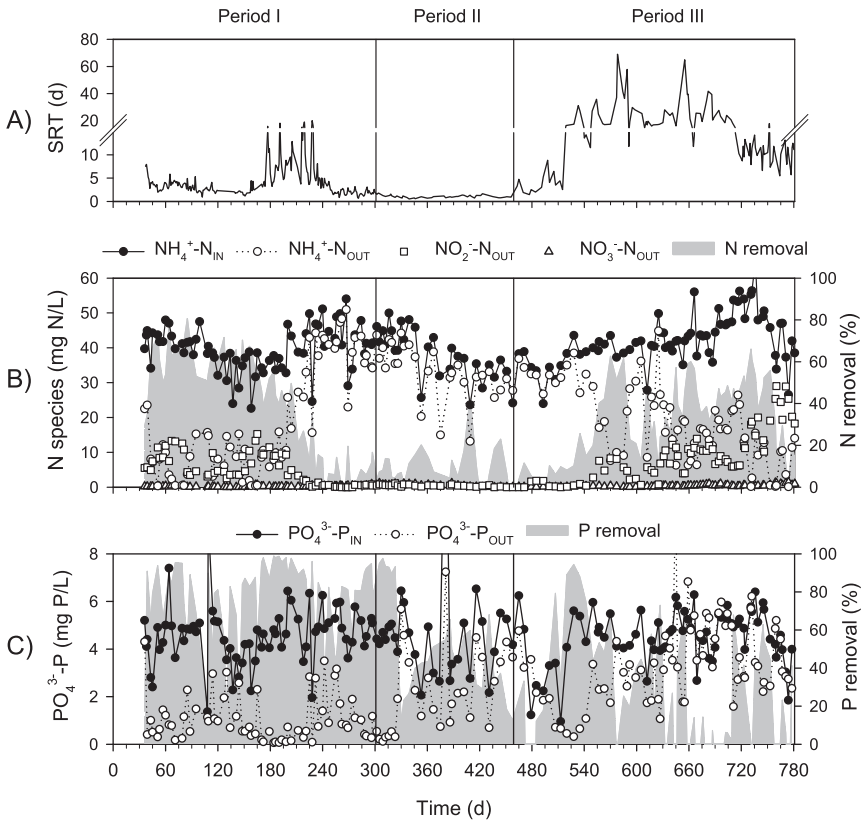


Fig. 5. Evolution of the (A) SRT, (B) influent and effluent N species and N removal efficiency, and (C) influent and effluent PO_4^{3-}-P and P removal efficiency in the AGS reactor throughout the experimental period.

cells and subsequent waste. According to the mass balance, the N loss matched with the biomass produced, considering a 5 % of N per gram of VSS. During Period III, once granular biomass was formed (days 528 to 781), the N removal efficiency increased to 37 ± 16 %, also occurring via NO_2^- because of the suppression of NOB activity (Fig. 6B–D).

According to the data presented in Fig. 6, the difference between total nitrogen (TN) concentration (NH_4^+-N + NO_2^--N + NO_3^--N) at the beginning and at the end of aerobic phases is 3 ± 1 mg N/L (Table S1), which represents 9 ± 3 % of the TN concentration at the beginning of aerobic phases. Such low N removal during the aerobic phases can be attributed to biomass assimilation but not due to a significant simultaneous nitrification-denitrification. The main part of the N removal occurs via denitritation during F/D phases, when the influent COD comes into contact with the biomass and the NO_2^- present in the reactor (Fig. 6A–D).

The highest N removal efficiencies (57 ± 15 %) were reached in Period I (days 0 to 200), when flocs predominated in the reactor (Figs. 2 and 3). Otherwise, in Period III (days 528 to 781), lower N removal efficiencies were achieved (37 ± 16 %), when granules and flocs coexisted in the reactor.

N removal of the PN effluent through the anammox process was preliminary assayed under pilot-scale conditions in a perfectly mixed reactor (0.33 m^3) for a short period of 40 days reaching an average rate of 0.0039 ± 0.0021 kg N/(g VSS d) and a N-removal efficiency of 73 ± 16 % (Fig. S5). TN < 10 mg N/L was targeted in the effluent. The anammox sludge was inoculated from the sidestream of a WWTP (one-stage granular reactor, Paques®).

In Period I and part of Period III, the SBR cycles evidenced PAO activity due to the existence of a significant P release under anaerobic conditions and P uptake under aerobic conditions (Fig. 6A and B). Concentrations up to 20 mg P/L were reached in the reactor at the end of anaerobic phases on day 70, with P release and uptake rates of 0.74 and 0.49 mg P/(L·min) (Table S1), respectively. Such an observation matches with the P removal efficiency of 76 ± 23 % achieved in Period I (Fig. 5C). In period II, the P removal efficiency decreased – 40 ± 17 % from days 330 to 458 – owing to the short SRT applied. Finally, in the first part of Period III (day 556), concentrations up to 40 mg P/L were measured at the end of F/D phases (Fig. 6B), with P release and uptake rates of 0.54 and 0.52 mg P/(L·min), respectively. P removal efficiencies up to 94 % were achieved. However, at the end of this period, when the SRT reached values around 27 days, the PAO activity stopped and neither P uptake nor release was observed (Fig. 6C), and high PO_4^{3-} concentrations were detected in the effluent (Fig. 5C). From day 700 onwards, the SRT was controlled at 10 days and the PAO activity was partially recovered, as P release and uptake were restored (Fig. 6D). Nevertheless, the concentration of P in the effluent was still high, with an average value of 3.6 ± 1.3 mg P/L.

4. Discussion

4.1. Aerobic granulation while treating an HRAS effluent

Many operational strategies promoting the formation and stability of aerobic granules have been identified in the literature. The following are some of the most frequently referred to: high OLR applied to the reactor [36], selective carbon utilization by the microorganisms promoted by a slow anaerobic bottom-feeding [37], high shear force induced by aeration [38], and selective biomass wasting promoted by a low settling time before discharge or by a high influent wastewater upflow velocity applied during simultaneous F/D [39]. However, there is no general consensus and system dependency still governs the application of the AGS technology. Recently, selective carbon utilization and biomass wasting have been proposed as the most promising options to effectively promote granulation when treating real wastewater [40,41].

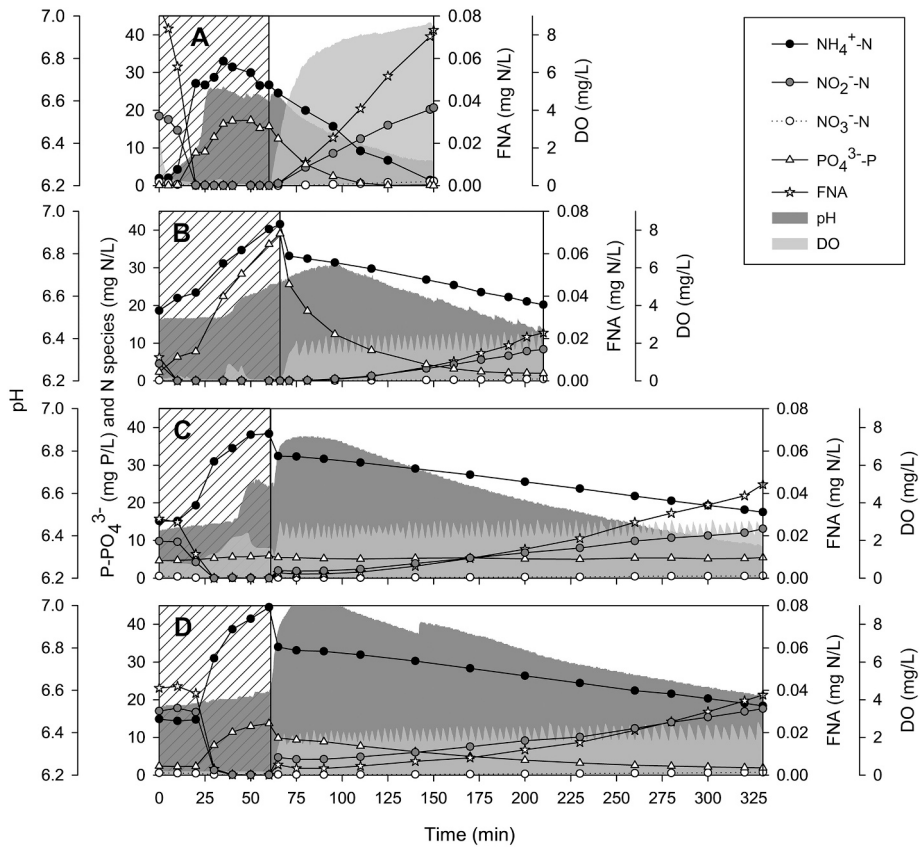


Fig. 6. Evolution of the concentration of the N species, FNA, PO_4^{3-}-P, pH, and DO in representative operational SBR cycles: (A) day 70 (Period I, 150 min), (B) day 556 (Period III, 210 min), (C) day 673 (Period III, 330 min), and (D) day 778 (Period III, 330 min). The concentration of the N species and PO_4^{3-}-P correspond to the concentration at 1/3 of the reactor's height, while pH and DO correspond to the values at 2/3 of such height. (▨): fill/draw and anaerobic phase, no homogeneity in the reactor, plug flow regime. (▭): aerobic phase, homogeneous reactor content because of aeration.

As a result, the characteristics of the wastewater fed to the AGS reactor are a critical aspect to be considered to trigger the formation of stable granules. Working with high-strength wastewater will facilitate the application of a high OLR to the bioreactor, while shorter HRTs are necessary when dealing with low-strength wastewater to maintain a high loading rate. Besides, the nature of the available COD (i.e., soluble or particulate) directly affects the carbon utilization pathway. In this regard, the abundance of soluble biodegradable COD (i.e., volatile fatty acids) facilitates selective anaerobic carbon storage, which benefits the formation of granules [42]. Conversely, a higher presence of particulate COD challenges the anaerobic storage since hydrolysis needs to take place first. In this study, a real HRAS effluent was used as influent for the AGS reactor. Such an influent is prone to contain less total, particulate, and soluble COD compared with raw or primarily settled wastewater, since the wastewater will have already been pre-treated in the HRAS bioreactor and settler. A literature review presented in an Excel file as supplementary material summarizes the most relevant studies performing aerobic granulation by treating real, low-medium strength urban wastewater in SBR reactors. (Our study is also included for comparison purposes.) According to this review, this is the first study performing aerobic granulation (and PN) in a SBR by treating a real HRAS effluent.

In contrast, three main granulation strategies have been used in this work: i) concomitant application of high influent wastewater upflow velocity and short settling time, ii) intensive biomass lateral wasting after a short settling time, and iii) selective wasting from the top of the settled sludge bed after a long settling time (Table 1). The aim in Period I was to achieve granulation through the application of a wastewater upflow velocity of 4.5 m/h and a settling time of 5 min. This strategy was like the one used by Derlon et al. [43], although they used primary settled wastewater instead of HRAS effluent. Following this strategy, a mixture of small flocs (< 0.2 mm) and big granules (> 2 mm) with finger-type outgrowth on the surface was formed in the reactor (Figs. 3 and 4), but no mature granules with smooth, rounded shapes appeared. Weber et al. [44] stated that the appearance of big granules/aggregates with finger-type outgrowth (mostly formed by ciliated protozoa) is the first step in the aerobic granulation mechanism. This is in line with the observations made in this study, since big granules formed in Period I presented abundant aggregations of protozoa. Thus, in Period I, aerobic granulation seemed to have begun but not to have finished. At the end of this first period, low temperatures (i.e., winter conditions) could have hindered sludge settleability (Fig. 2A), leading to the washout of solids and the disappearance of big aggregates. In Period II, the implementation of a massive lateral wasting at the end of the settling phase (which lasted 10 min per cycle) did not result in the formation of granules or big aggregates. However, a large washout of floccular solids from the reactor did occur, leaving inside only biomass with good settling properties (Fig. 2A). Aerobic granules could not grow under these conditions since the washout of solids during the wasting(s) was too severe and unselective. Period III started with the increase of the settling time to 30 min and the application of a selective wasting from the top of the settled sludge bed. Again, a mixture of flocs and big aggregates appeared in the reactor (Fig. 4), but later in this period granules were formed (Fig. 4) and stably coexisted with flocs until the end of the study (Figs. 2 and 3). Thus, the strategy followed in Period III can promote a rather short start-up time when inoculating a plant with floccular sludge in comparison to the total experimental period (I-III). This is of paramount importance, since long start-up times will condition the consolidation of the AGS technology in real implementations.

Settling time, wastewater upflow velocity, and intensive wasting

seemed not to be the most critical parameters to effectively trigger the formation of granules in this study, whereas a selective solids wasting from the top of the settled sludge bed to the sludge line was found to be a successful strategy. Settling time was an important parameter that could help to selectively waste the solids. A longer settling time could promote the removal of flocs from the top of the settled sludge bed. Wastewater upflow velocity and intensive wastings were therefore assumed to be factors with a weak influence in the granulation process.

Under the studied conditions, achieving full granulation of the biomass (i.e., SVI_{10}/SVI_{30} ratio close to 1 [44]) was not seen as a crucial aspect compromising the proper performance of the reactor treating real HRAS effluent. Thus, the maintenance of a low SVI (< 100 mL/g) was helpful enough for improved operation. In this regard, the recently defined 'densified biomass' [45], which consists of a mixture of dense flocs and granules with a whole diluted SVI (at 2 g/L TSS) between 30 and 80 mL/g, perfectly matches the characteristics of the biomass obtained in Period III. Effluent COD was always below 100 mg/L, demonstrating that the presence of granular sludge in the reactor was not strictly necessary to reach such low COD levels (Fig. 2). The low availability of COD in the effluent is of capital importance in view of the subsequent application of an anammox process since influent COD could compromise its performance [46].

4.2. N removal and partial nitritation

Regarding N removal, the objective of the AGS reactor studied was to achieve stable PN in the mainstream in view of a subsequent anammox reactor. To understand the performance of N removal in the study, the dynamics of a cycle need to be explained in detail. During the F/D phase, a non-homogeneous plug-flow regime is achieved and is governed by the influent wastewater upflow velocity. This phase performs a double function. On the one hand, it allows the anaerobic storage of the influent COD by PAO and GAO, on the other, it causes the denitrification of a part of the NO_x formed during the previous aerobic phase. Both phenomena (i.e., storage and denitrification) depend on the simultaneous contact between the influent COD, the biomass, and the NO_x available in the reactor. In addition, such contact depends on the settled sludge bed expansion and fluidization during the F/D phase. Therefore, the more expanded and fluidized the settled sludge, the greater the denitrification reached (i.e., greater contact between influent COD and reactor's biomass and NO_x). The F/D phase can then be followed by an anaerobic phase in which the recirculation pump continues the plug flow regime by pumping from the top to the bottom of the reactor. During this phase, storage and denitrification may continue to occur. Afterwards, a homogeneous aerobic phase is introduced. Air is fed from the bottom of the reactor and the content is completely mixed. Finally, a settling time is applied before a new F/D phase.

In this study, which was conducted over the long term by treating a real HRAS effluent (NH_4^+-N of 40 ± 13 mg/L and alkalinity of 359 ± 34 mg $CaCO_3$/L), seasonal and daily temperature and load fluctuations occurred according to the performance of a real WWTP. A constant VER of 50 % was applied to the SBR and no NO_3^- accumulation was observed in the effluent throughout the whole operational period (Fig. 5B). Under the operational conditions referred to above, NOB activity was suppressed. This can be explained by the high concentrations of FNA reached in the reactor during the SBR cycles (Fig. 6). According to Zhou et al. [47], the range of FNA that starts to negatively affect the NOB activity has been reported as 0.011–0.08 mg HNO_2-N/L, whereas complete inhibition has been observed at 0.026–0.22 mg HNO_2-N/L. On the other hand, ammonia-oxidizing bacteria (AOB) have been described as significantly less sensitive to FNA, with the corresponding 50 % inhibitory level as high as 0.42–1.72 mg HNO_2-N/L [47]. In this study, inhibitory concentrations of FNA for NOB were reached in the SBR cycles during Period I (e.g., 0.075 mg HNO_2-N/L as shown in Fig. 6A) and III (e.g., 0.021–0.044 mg HNO_2-N/L as shown in Fig. 6B–D), while AOB could grow in the reactor, leading to a stable PN. In this regard, values for FA will not be inhibitory under mainstream conditions even in summer, when temperature is higher (e.g., 25 °C), owing to the values of pH and NH_4^+-N concentration in water (pH 6.5 and 25 mg NH_4^+-N/L are the mean values of Fig. 6). In these circumstances (0.046 mg FA-N/L), FA concentration is below the value of 0.082 mg N/L stated by [22] as the initial threshold for NOB inhibition (without negative effect for AOB).

Significant N removals of 57 ± 15 % (Period I, days 0 to 200) and 37 ± 16 % (Period III, days 528 to 781) were reached (Fig. 5B). Such observations match the occurrence of PN, which enabled denitritation also to occur. The N removals mostly took place due to denitritation (i.e., via NO_2^-) during the F/D phases (Fig. 6) – rather than N assimilation (Table S1) – once the influent wastewater, biomass, and NO_2^- came into contact. Therefore, the higher N removal achieved in Period I would have occurred because of the greater wastewater-biomass-NO_2^- contact during F/D phases. Such increased contact could be attributed to the higher presence of flocs in the reactor during Period I (Figs. 2 and 3) and the low settling time applied (e.g., 10 min, Fig. S3). Since flocs have worse settleability than granules (i.e., higher SVI), during the F/D phases of Period I, the settled sludge bed could have expanded more easily. Moreover, with the short settling time applied (10 min), at the beginning of F/D, most of the biomass would still be settling through the reactor at a considerable height, instead of being compacted at the bottom (i.e., the settled sludge bed would already be expanded). On the other hand, in Period III, since a mixture of granules and flocs coexisted (i.e., better overall settleability with lower SVI) and the settling time applied was higher (e.g., 30 min, Table 1), during the F/D phases the settled sludge bed would not have expanded enough and the biomass would have mostly been compacted at the bottom of the reactor, reducing the wastewater-biomass-NO_2^- contact and, therefore, the N removal efficiency.

The simultaneous presence of NH_4^+ and NO_2^- in the effluent of the AGS reactor indicates an eventual suitability of this stream for the application of the anammox process, whose ratio needs to be adjusted first to a value close to 1.32 g NO_2^--N/g NH_4^+-N [20,48]. If this ratio is achieved, the percentage of N removed via denitritation will directly affect the amount of NH_4^+ to be oxidized to NO_2^-. Thus, the higher the N removal (i.e., denitritation) achieved, the higher the amount of NH_4^+ to be oxidized to meet the 1.32 theoretical ratio. If an ideal 0 % N removal is assumed, then 57 % NH_4^+ needs to be nitritated, but if the amount of N removed is higher, more NH_4^+ will need to be oxidized, which implies greater energy consumption for aeration. Nevertheless, if N removal via denitritation occurs, lower NO_2^- values will be present in the effluent of the AGS reactor, reducing the inhibition risk in the potential anammox reactor [49].

4.3. P removal

There are many parameters and circumstances that can affect the stability of microbial communities present in wastewater treatment bioreactors (e.g., SRT, inhibition, etc.). Nitrifiers and PAO have different sensibilities to FNA inhibition. Zhou et al. [47] stated that AOB are the least sensitive to FNA, with values as high as 0.42–1.72 mg HNO_2-N/L responsible for 50 % reduction of their activity, as previously mentioned. On the other hand, PAO are the most sensitive to FNA, with an inhibitory threshold of 0.0005 mg HNO_2-N/L. The sensitivity of NOB to FNA is, thus, between that of AOB and PAO. The SRT is another critical parameter that can affect PAO and nitrifiers. There is a specific minimum SRT for each microbial community to ensure their growth and there is also a maximum beyond which growth is compromised. In the case of PAO, 20 days SRT has been regarded as the maximum value enabling their growth, with values below 10 days being optimal to achieve effective enhanced biological P removal (EBPR) in the system [50].

In this study, good P removal efficiencies with low P concentrations in the effluent were reached in Period I (76 ± 23 % P removal), whereas

higher P levels were measured in the effluent in periods II and III (Fig. 5C). In Period I, a rather short SRT of 4.1 ± 3.4 days (< 10 days) was maintained and allowed a good performance, while in Periods II and III very short (2.2 ± 1.0 days) and long (> 20 days) SRTs were reached. It was only at the end of Period III (day 711 to the end of the study) that the SRT was controlled and stabilized at 10.6 ± 2.4 days, allowing for partial recovery of the PAO activity (Fig. 6D). Biological P removal is system-dependent and, under the applied conditions (high FNA, SBR mode, 50 % VER, etc.), resulted to be strongly SRT-dependent, being necessary to maintain the SRT below 10.6 ± 2.4 and above 2.2 ± 1.0 days, respectively, to effectively achieve EBPR. Operating at an SRT lower than 10 days can negatively affect nitrification, especially in winter, when water temperatures are below 20 °C [35], therefore, special care should be taken in those periods to maintain both nitritation and EBPR. Further research should be focused on the duration (or the homogenization reached) of the F/D phases, because PAO activity can be enhanced by lengthening the anaerobic period while ensuring wastewater-biomass contact to promote organic storage. By enhancing its activity, PAO might be able to perform correct EBPR at higher SRTs, without compromising nitritation and, consequently, adding robustness to the system.

5. Conclusions

- In this study, a selective wasting from the top of the settled sludge bed was found to be a successful strategy to effectively trigger the formation of AGS, whereas the settling time, influent wastewater upflow velocity, and intensive wasting seemed not to be the most critical parameters.
- The achievement of full granulation was not seen as crucial to the proper performance of the reactor treating real HRAS effluent. Conversely, the maintenance of a low SVI (< 100 mL/g) was helpful enough for an improved operation of the system (i.e., COD removal, PN and EBPR).
- Stable PN was achieved under mainstream conditions by treating a real HRAS effluent with daily and seasonal temperature and load fluctuations, in line with the performance of a real WWTP. NOB activity was repressed by reaching inhibitory FNA concentrations in the reactor.
- Working with good settling biomass and long settling times reduced the influent-biomass-NO_2^- contact and, therefore, denitritation. By reducing denitritation, less NH_4^+ oxidation is required to achieve a 1.32 g NO_2^--N/g NH_4^+-N ratio and less oxygen is consumed.

CRediT authorship contribution statement

Oriol Carbó: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jaume Teixidó:** Investigation. **Joan Canals:** Formal analysis, Conceptualization. **Antonio Ordóñez:** Supervision, Resources, Project administration, Funding acquisition. **Albert Magrí:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Mercè Baldi:** Writing – review & editing, Visualization, Resources, Project administration, Formal analysis. **Belén Gutiérrez:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Jesús Colprim:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This project has received funding from the Spanish Centre for Industrial Technological Development (CDTI) [Ref. IDI-20200648]. The first author acknowledges the industrial predoctoral grant received from the Ministry of Science, Innovation and Universities [Ref. DIN2019-010700/AEI/10.13039/501100011033]. The authors would like to thank the support received from the Consorci Besòs Tordera [EXP 2019/138]. The research group LEQUIA has been recognized as consolidated research group (Ref. 2021-SGR-1352) by the Catalan Ministry of Research and Universities. LEQUIA is recognized as a TECNIO group by the Government of Catalonia's Agency for Business Competitiveness (ACC1Ó). The authors thank Research Technical Services of the University of Girona for the experimental support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2024.105165.

References

[1] O. Nowak, P. Enderle, P. Varbanov, Ways to optimize the energy balance of municipal wastewater systems: lessons learned from Austrian applications, J. Clean. Prod. 88 (2015) 125–131, https://doi.org/10.1016/j.jclepro.2014.08.068.
[2] W.-W. Li, H.-Q. Yu, B.E. Rittmann, Reuse water pollutants, Nature 528 (2015) 29–31, https://doi.org/10.1038/528029a.
[3] P.L. McCarty, J. Bae, J. Kim, Domestic wastewater treatment as a net energy producer-can this be achieved? Environ. Sci. Technol. 45 (2011) 7100–7106, https://doi.org/10.1021/es2014264.
[4] L. Appels, J. Baeyens, J. Degrève, R. Dewil, Principles and potential of the anaerobic digestion of waste-activated sludge, Prog. Energy Combust. Sci. 34 (2008) 755–781, https://doi.org/10.1016/j.pecs.2008.06.002.
[5] G. Silvestre, B. Fernández, A. Bonmatí, Significance of anaerobic digestion as a source of clean energy in wastewater treatment plants, Energy Convers. Manag. 101 (2015) 255–262, https://doi.org/10.1016/j.enconman.2015.05.033.
[6] Y. Liu, J. Gu, M. Zhang, A.-B. Processes, Towards Energy Self-Sufficient Municipal Wastewater Treatment, IWA Publishing, London, UK, 2019.
[7] J. Wan, J. Gu, Q. Zhao, Y. Liu, COD capture: a feasible option towards energy self-sufficient domestic wastewater treatment, Sci. Rep. 6 (2016) 25054, https://doi.org/10.1038/srep25054.
[8] J. Canals, A. Cabrera-Codony, O. Carbó, J. Torán, M. Martín, M. Baldi, B. Gutierrez, M. Poch, A. Ordóñez, H. Monclús, High-rate activated sludge at very short SRT: key factors for process stability and performance of COD fractions removal, Water Res. 231 (2023) 119610, https://doi.org/10.1016/j.watres.2023.119610.
[9] J. Canals, A. Cabrera-Codony, O. Carbó, M. Baldi, B. Gutiérrez, A. Ordoñez, M. J. Martín, M. Poch, H. Monclús, Nutrients removal by high-rate activated sludge and its effects on the mainstream wastewater treatment, Chem. Eng. J. 479 (2024) 147871, https://doi.org/10.1016/j.cej.2023.147871.
[10] J. Carrera, O. Carbó, S. Doñate, M.E. Suárez-Ojeda, J. Pérez, Increasing the energy production in an urban wastewater treatment plant using a high-rate activated sludge: pilot plant demonstration and energy balance, J. Clean. Prod. 354 (2022) 131734, https://doi.org/10.1016/j.jclepro.2022.131734.
[11] J. Jimenez, M. Miller, C. Bott, S. Murthy, H. De Clippeleir, B. Wett, High-rate activated sludge system for carbon management - evaluation of crucial process mechanisms and design parameters, Water Res. 87 (2015) 476–482, https://doi.org/10.1016/j.watres.2015.07.032.
[12] J. Kim, K. Kim, H. Ye, E. Lee, C. Shin, P.L. McCarty, J. Bae, Anaerobic fluidized bed membrane bioreactor for wastewater treatment, Environ. Sci. Technol. 45 (2011) 576–581, https://doi.org/10.1021/es1027103.
[13] H. Wang, F. Li, A. Keller, R. Xu, Chemically enhanced primary treatment (CEPT) for removal of carbon and nutrients from municipal wastewater treatment plants: a case study of Shanghai, Water Sci. Technol. 60 (2009) 1803–1809, https://doi.org/10.2166/wst.2009.547.
[14] S. Wang, Q. Li, P. Zhang, J. Li, J. Zheng, F. Meng, Y. Li, J. Yu, Start-up a novel coupling process with partial nitritation, partial denitrification and anaerobic ammonia oxidation in a single sequencing batch reactor, J. Water Process Eng. 45 (2022) 102472, https://doi.org/10.1016/j.jwpe.2021.102472.
[15] X. Li, S. Klaus, C. Bott, Z. He, Status, challenges, and perspectives of mainstream nitritation–anammox for wastewater treatment, Water Environ. Res. 90 (2018) 634–649, https://doi.org/10.2175/106143017X15131012153112.

[16] S. Lackner, E.M. Gilbert, S.E. Vlaeminck, A. Joss, H. Horn, M.C.M. van Loosdrecht, Full-scale partial nitritation/anammox experiences - an application survey, Water Res. 55 (2014) 292–303, https://doi.org/10.1016/j.watres.2014.02.032.
[17] T.R.V. Akaboci, F. Gich, M. Ruscalleda, M.D. Balaguer, J. Colprim, Effects of extremely low bulk liquid DO on autotrophic nitrogen removal performance and NOB suppression in side- and mainstream one-stage PNA, J. Chem. Technol. Biotechnol. 93 (2018) 2931–2941, https://doi.org/10.1002/jctb.5649.
[18] M. Hoekstra, S.P. Geilvoet, T.L.G. Hendrickx, C.S. van Erp Taalman Kip, R. Kleerebezem, M.C.M. van Loosdrecht, Towards mainstream anammox: lessons learned from pilot-scale research at WWTP Dokhaven, Environ. Technol. 40 (2019) 1721–1733, https://doi.org/10.1080/09593330.2018.1470204.
[19] A. Pedrouso, N. Morales, B. Rodelas, D. Correa-Galeote, A. Val del Rio, J. L. Campos, J. Vázquez-Padín, A. Mosquera-Corral, Rapid start-up and stable maintenance of the mainstream nitritation process based on the accumulation of free nitrous acid in a pilot-scale two-stage system, Sep. Purif. Technol. 317 (2023) 123851, https://doi.org/10.1016/j.seppur.2023.123851.
[20] A. Magrí, M. Ruscalleda, A. Vilà, T.R.V. Akaboci, M.D. Balaguer, J.M. Llenas, J. Colprim, Scaling-up and long-term operation of a full-scale two-stage partial nitritation-anammox system treating landfill leachate, Processes 9 (2021) 800, https://doi.org/10.3390/pr9050800.
[21] S.W.H. Van Hulle, H.J.P. Vandeweyer, B.D. Meesschaert, P.A. Vanrolleghem, P. Dejans, A. Dumoulin, Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams, Chem. Eng. J. 162 (2010) 1–20, https://doi.org/10.1016/j.cej.2010.05.037.
[22] A.C. Anthonisen, R.C. Loehr, T.B.S. Prakasam, E.G. Srinath, Inhibition of nitrification by ammonia and nitrous acid, J. Water Pollut. Control Fed. 48 (1976) 835–852. http://www.jstor.com/stable/25038971.
[23] A. Pedrouso, A. Val del Río, N. Morales, J.R. Vázquez-Padín, J.L. Campos, R. Mendez, A. Mosquera-Corral, Nitrite oxidizing bacteria suppression based on in-situ free nitrous acid production at mainstream conditions, Sep. Purif. Technol. 186 (2017) 55–62, https://doi.org/10.1016/j.seppur.2017.05.043.
[24] V.M. Vadivelu, Z. Yuan, C. Fux, J. Keller, The inhibitory effects of free nitrous acid on the energy generation and growth processes of an enriched *Nitrobacter* culture, Environ. Sci. Technol. 40 (2006) 4442–4448, https://doi.org/10.1021/es051694k.
[25] M.S. Kowalski, T.R. Devlin, A. di Biase, J.A. Oleszkiewicz, Effective nitrogen removal in a two-stage partial nitritation-anammox reactor treating municipal wastewater – piloting PN-MBBR/AMX-IFAS configuration, Bioresour. Technol. 289 (2019) 121742, https://doi.org/10.1016/j.biortech.2019.121742.
[26] S.M. Podmirseg, M. Gómez-Brandón, M. Muik, B. Stres, M. Hell, T. Pümpel, S. Murthy, K. Chandran, H. Park, H. Insam, B. Wett, Microbial response on the first full-scale DEMON® biomass transfer for mainstream deammonification, Water Res. 218 (2022) 118517, https://doi.org/10.1016/j.watres.2022.118517.
[27] M. Pronk, M.K. de Kreuk, B. de Bruin, P. Kamminga, R. Kleerebezem, M.C.M. van Loosdrecht, Full scale performance of the aerobic granular sludge process for sewage treatment, Water Res. 84 (2015) 207–217, https://doi.org/10.1016/j.watres.2015.07.011.
[28] S. Berzio, J.M. Araújo, T. Gehring, L.C. Phan, B. Teichgräber, M. Lübken, M. Wichern, Compact pilot-scale aerobic granular sludge system treating real wastewater continuously for over 500 days, J. Water Process Eng. 53 (2023), https://doi.org/10.1016/j.jwpe.2023.103847.
[29] M. Sepúlveda-Mardones, J.L. Campos, A. Magrí, G. Vidal, Moving forward in the use of aerobic granular sludge for municipal wastewater treatment: an overview, Rev. Environ. Sci. Biotechnol. 18 (2019) 741–769, https://doi.org/10.1007/s11157-019-09518-9.
[30] S. Kosar, O. Isik, B. Cicekalan, H. Gulhan, S. Cingoz, M. Yoruk, H. Ozgun, I. Koyuncu, M.C.M. van Loosdrecht, M.E. Ersahin, Coupling high-rate activated sludge process with aerobic granular sludge process for sustainable municipal wastewater treatment, J. Environ. Manag. 325 (2023) 116549, https://doi.org/10.1016/j.jenvman.2022.116549.
[31] K. Zhao, T. Zhang, Y. Tian, H. Li, J. Wan, Y. Wang, Efficient partial nitrification with hybrid nitrifying granular sludge based on a simultaneous fill/draw SBR mode, Chemosphere 313 (2023) 137579, https://doi.org/10.1016/j.chemosphere.2022.137579.
[32] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 23rd ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA, 2017.
[33] M.C.M. van Loosdrecht, P.H. Nielsen, C.M. Lopez-Vazquez, D. Brdjanovic, Experimental Methods in Wastewater Treatment, IWA Publishing, London, UK, 2016.
[34] R. Ganigué, H. López, M.D. Balaguer, J. Colprim, Partial ammonium oxidation to nitrite of high ammonium content urban landfill leachates, Water Res. 41 (2007) 3317–3326, https://doi.org/10.1016/j.watres.2007.04.027.
[35] C. Hellinga, A.A.J.C. Schellen, J.W. Mulder, M.C.M. van Loosdrecht, J.J. Heijnen, The SHARON process: an innovative method for nitrogen removal from ammonium-rich waste water, Water Sci. Technol. 37 (9) (1998) 135–142, https://doi.org/10.1016/S0273-1223(98)00281-9.
[36] M. Coma, Biological Nutrient Removal in SBR Technology: From Floccular to Granular Sludge, University of Girona, 2011. http://hdl.handle.net/10803/32025 (PhD Thesis).
[37] M.K. De Kreuk, J.J. Heijnen, M.C.M. van Loosdrecht, Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge, Biotechnol. Bioeng. 90 (2005) 761–769, https://doi.org/10.1002/bit.20470.
[38] J.H. Tay, Q.S. Liu, Y. Liu, The effect of upflow air velocity on the structure of aerobic granules cultivated in a sequencing batch reactor, Water Sci. Technol. 49 (11–12) (2004) 35–40, https://doi.org/10.2166/wst.2004.0798.
[39] Y.V. Nancharaiah, G.K.K. Reddy, Aerobic granular sludge technology: mechanisms of granulation and biotechnological applications, Bioresour. Technol. 247 (2018) 1128–1143, https://doi.org/10.1016/j.biortech.2017.09.131.
[40] N. Derlon, M.G. Villodres, R. Kovács, A. Brison, M. Layer, I. Takács, E. Morgenroth, Modelling of aerobic granular sludge reactors: the importance of hydrodynamic regimes, selective sludge removal and gradients, Water Sci. Technol. 86 (2022) 410–431, https://doi.org/10.2166/wst.2022.220.
[41] M. Pronk, E.J.H. van Dijk, M.C.M. van Loosdrecht, Aerobic granular sludge, in: G. Chen, G.A. Ekama, M.C.M. van Loosdrecht, D. Brdjanovic (Eds.), Biological Wastewater Treatment: Principles, Modeling and Design, IWA Publishing, London, 2020, pp. 497–522, https://doi.org/10.2166/9781789060362_0497.
[42] J. Wagner, D.G. Weissbrodt, V. Manguin, R.H.R. da Costa, E. Morgenroth, N. Derlon, Effect of particulate organic substrate on aerobic granulation and operating conditions of sequencing batch reactors, Water Res. 85 (2015) 158–166, https://doi.org/10.1016/j.watres.2015.08.030.
[43] N. Derlon, J. Wagner, R.H. Ribeiro da Costa, E. Morgenroth, Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume, Water Res. 105 (2016) 341–350, https://doi.org/10.1016/j.watres.2016.09.007.
[44] S.D. Weber, W. Ludwig, K.H. Schleifer, J. Fried, Microbial composition and structure of aerobic granular sewage biofilms, Appl. Environ. Microbiol. 73 (2007) 6233–6240, https://doi.org/10.1128/AEM.01002-07.
[45] C. Roche, S. Donnaz, S. Murthy, B. Wett, Biological process architecture in continuous-flow activated sludge by gravimetry: controlling densified biomass form and function in a hybrid granule–floc process at Dijon WRRF, France, Water Environ. Res. 94 (2022) 1–18, https://doi.org/10.1002/wer.1664.
[46] M. Jia, K. Solon, D. Vandeplassche, H. Venugopal, E.I.P. Volcke, Model-based evaluation of an integrated high-rate activated sludge and mainstream anammox system, Chem. Eng. J. 382 (2020) 122878, https://doi.org/10.1016/j.cej.2019.122878.
[47] Y. Zhou, A. Oehmen, M. Lim, V. Vadivelu, W.J. Ng, The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants, Water Res. 45 (2011) 4672–4682, https://doi.org/10.1016/j.watres.2011.06.025.
[48] T. Lotti, R. Kleerebezem, C. Lubello, M.C.M. van Loosdrecht, Physiological and kinetic characterization of a suspended cell anammox culture, Water Res. 60 (2014) 1–14, https://doi.org/10.1016/j.watres.2014.04.017.
[49] T. Lotti, W.R.L. van der Star, R. Kleerebezem, C. Lubello, M.C.M. van Loosdrecht, The effect of nitrite inhibition on the anammox process, Water Res. 46 (2012) 2559–2569, https://doi.org/10.1016/j.watres.2012.02.011.
[50] A. Onnis-Hayden, N. Majed, Y. Li, S.M. Rahman, D. Drury, L.A. Risso, A.Z. Gu, Impact of solid retention time (SRT) on functionally relevant microbial populations and performance in full-scale enhanced biological phosphorus removal (EBPR) systems, Water Environ. Res. 92 (2020) 389–402, https://doi.org/10.1002/wer.1185.