Water Research 105 (2016) 341–350

Contents lists available at ScienceDirect

Water Research

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

Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume

Nicolas Derlon [a, c, *], Jamile Wagner [a, b], Rejane Helena Ribeiro da Costa [b], Eberhard Morgenroth [a, c]

[a] Eawag: Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland
[b] Federal University of Santa Catarina (UFSC), Department of Sanitary and Environmental Engineering, 88040-970 Florianópolis, Santa Catarina, Brazil
[c] Institute of Environmental Engineering, ETH Zürich, CH-8093 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form
5 September 2016
Accepted 6 September 2016
Available online 9 September 2016

Keywords:
Aerobic granules
Low-strength municipal wastewater
Anaerobic feeding
Wastewater upflow velocity
Simultaneous fill-draw

ABSTRACT

This study aimed at evaluating the formation of aerobic granular sludge (AGS) for the treatment of real and low-strength municipal wastewater using a column sequencing batch reactor (SBR) operated in fill-draw mode (constant volume). The focus was on understanding how the wastewater upflow velocity (V_{WW}) applied during the anaerobic feeding influenced the sludge properties and in turn the substrate conversion. Two different strategies were tested: (1) washing-out the flocs by imposing high wastewater upflow velocities (between 5.9 and 16 m h^{-1}) during the anaerobic feeding (Approach #1) and (2) selective utilization of organic carbon during the anaerobic feeding (1 m h^{-1}) combined with a selective sludge withdrawal (Approach #2). A column SBR of 190 L was operated in constant volume during 1500 days and fed with real and low-strength municipal wastewater. The formation of AGS with SVI_{30} of around 80 mL g_{TSS}^{-1} was observed either at very low (1 m h^{-1}) or at high V_{WW} (16 m h^{-1}). At 16 m h^{-1} the AGS was mainly composed of large and round granules (d > 0.63 mm) with a fluffy surface, while at 1 m h^{-1} the sludge was dominated by small granules (0.25 < d < 0.63 mm). The AGS contained a significant fraction of flocs during the whole operational period. A considerable and continuous washout of biomass occurred at V_{WW} higher than 5.9 m h^{-1} (Approach #1) due to the lower settling velocity of the AGS fed with municipal wastewater. The low sludge retention observed at V_{WW} higher than 5.9 m h^{-1} deteriorated the substrate conversion and in turn the effluent quality. High solid concentrations (and thus solid retention time) were maintained during Approach #2 (V_{WW} of 1 m h^{-1}), which resulted in an excellent effluent quality. The study demonstrated that the formation of AGS is possible during the treatment of real and low-strength municipal wastewater in a SBR operated at constant volume. Low wastewater upflow velocities should be applied during the anaerobic feeding phase in order to ensure enough biomass retention and efficient substrate removal.

1. Introduction

The formation of aerobic granules has been extensively investigated over the last 20 years (Morgenroth et al., 1997). Granular sludge is today foreseen as one of the main advanced technology for wastewater treatment (van Loosdrecht and Brdjanovic, 2014). Despite several full-scale applications of aerobic granular sludge (AGS) systems, the operating conditions required to form granules during the treatment of low-strength municipal wastewater remain unclear. Most studies on aerobic granulation were indeed performed at laboratory-scale using synthetic influents. Those synthetic influents typically contain readily biodegradable substrate such as carbohydrates or volatile fatty acids at large concentrations, ranging from few hundreds to few thousands mg_{COD} L^{-1}. Such high substrate concentrations allow growing aerobic granules within few weeks (Beun et al., 2000; Dangcong et al., 1999; Gao et al., 2011; Zheng et al., 2006). However, the aforesaid influents are not representative of real municipal wastewaters,

* Corresponding author.Eawag: Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland.
E-mail address: nicolas.derlon@eawag.ch (N. Derlon).

http://dx.doi.org/10.1016/j.watres.2016.09.007
0043-1354/

which usually contain much lower organic substrate concentrations (between 250 and 430 mg_{COD} L^{-1}) with a significant fraction in the particulate (X_S) form, e.g., 50% (Metcalf and Eddy, 2003).

Only few studies reported successful granule formation using complex influents such as municipal wastewaters (Table 1). Different observations can be drawn from these studies:

(i) Very long (more than one year) start-up periods are required to achieve full granulation. Ni et al. (2009) and Giesen et al. (2013), for example, reported periods of 10 and 13 months to achieve 85% and 90% granulation, respectively.

(ii) Aerobic granules developed with real municipal wastewater are rather small, with diameters varying between 200 and 1300 μm (Liu et al., 2010; Ni et al., 2009; Wagner and da Costa, 2013). These values are much smaller than the ones usually reported for aerobic granules cultivated with synthetic influents (diameters higher than 2000 μm).

(iii) Settling properties of aerobic granules fed with municipal wastewater are usually very good. Values for SVI_5 and SVI_{30} below 50 mL g_{TSS}^{-1} were reported (Giesen et al., 2013; Ni et al., 2009) but higher values were also observed (i.e. slightly below 100 mL g_{TSS}^{-1} for Coma et al. (2012)). A similar observation is drawn for the SVI_{30} to SVI_{10} ratio. A ratio close to 1 is representative of full granulation. However, a SVI ratio smaller than 1 are often reported (Coma et al., 2012; Wagner and da Costa, 2013).

(iv) The performances of the AGS systems in terms of substrate conversion were not clearly evaluated. Information about the denitrification or phosphorus removal are often not reported. Also, the existence of simultaneous nitrification-denitrification (as expected due to oxygen diffusion limitation inside the granules) is not clearly established based on the data reported in the literature (Giesen et al., 2013; van der Roest et al., 2011).

Even though the studies listed in Table 1 undoubtedly advanced our understanding about aerobic granule formation during the treatment of municipal wastewaters, they also display some limitations. Some studies were performed with mixed influents, e.g. mixture of municipal and industrial wastewaters (Giesen et al., 2013; Liu et al., 2010) or municipal wastewater with addition of acetate (Coma et al., 2012; Rocktäschel et al., 2015). This can result in influents with high COD concentrations that are not representative of the typical values found for municipal wastewater, e.g.1800 mg_{COD} L^{-1} (Liu et al., 2010). To what extent successful granulation can be achieved with real and low-strength municipal wastewater containing a significant fraction of organic substrate in the particulate form (X_S) still remains uncertain. Secondly, the studies listed in Table 1 did not clearly describe the system performances regarding substrate conversion, especially in terms of denitrification and phosphorus removal. In theory, a key advantage of the AGS systems is that simultaneous nitrification-denitrification can be achieved under aerobic conditions in the bulk liquid. Based on the existing literature, it remains unclear if denitrification is observed simultaneously to nitrification or if it occurs during the anaerobic feeding phase. The performances of AGS systems in terms of effluent quality must be assessed in order to evaluate the precise potential of this technology for the treatment of real and low-strength municipal wastewaters. Finally, the operating conditions to achieve granulation seem rather diverse. Sequencing batch

Table 1
Overview of the main characteristics and results of published studies about aerobic granulation using municipal wastewater.

Influent	Substrate concentrations (mg_{COD} L^{-1})	Organic load (kg_{COD} m^3 d^{-1})	SBR operation	Time to achieve granulation	Settling properties (SVI, ratio of SVIs)	Fraction of granules	Size of granules (mm)	Effluent quality (mg L^{-1})	Reference
Municipal wastewater (China)	COD tot: 95-200 COD sol: 35-120	0.6–1	Variable volume (15–30 min settling)	10 months (300 days)	SVI_5: 40 mL g^{-1} SVI_{30}: 40 mL g^{-1} Ratio: 1	80%	0.2–0.8	Ammonia: 0.5 Nitrate: 4 Orthophosphate: 0.5 Suspended solids: 15	(Ni et al., 2009)
Municipal (40%) and industrial (60%) wastewater (China)	COD sol: 250-1800		Variable volume	400 days	SVI_{30} < 50 mL g^{-1} Ratio: 1	80-90%	0.35		(Liu et al., 2010)
Municipal wastewater (Nereda® plant, South Africa)	COD tot: 1265		Constant volume. V_{WW} unknown.		SVI_5: 30 mL g^{-1}				(Giesen et al., 2013; van der Roest et al., 2011)
Municipal + industrial wastewater (Nereda® plant in Epe, The Netherlands)			Constant volume. V_{WW} unknown	13 months	SVI_5: 60 mL g^{-1} SVI_{30}: 40 mL g^{-1} Ratio: 0.66	Around 90% under stable operating conditions		Ammonia:0.5 Nitrate: 4 Orthophosphate: 0.5 Suspended solids: 10-20	(Giesen et al., 2013; van der Roest et al., 2011)
Municipal wastewater + acetate (Australia)	[a]COD tot: 326 ± 77 [a]COD sol: 179 ± 46	Variable volume		SVI_{30} < 100 mL g^{-1}[b] Ratio: ≈ 0.9[b]				Nitrite accumulation during aerobic phase	(Coma et al., 2012)
Municipal wastewater (Brazil)[c]	COD sol: 430 ± 140	1–1.40	Variable volume (15 min settling)	140 days	SVI_{30} ≈ 53 mL g^{-1} Ratio ≈ 0.9		0.3–1.3	Ammonia: 3 Suspended solids: 25-125	(Wagner and da Costa, 2013)
Municipal wastewater + acetate (Germany)	COD tot: 287-492	0.5–2.0	Variable volume (15–30 min settling)	125 days		98%	1.1–1.8	Orthophosphate: <3 Suspended solids: 40-100	

[a] Before addition of acetate.
[b] Data of the “100%-flocs” conditions.
[c] Data of Stage I and II (without addition of acetate).

reactors (SBR) were operated either at variable volume (supernatant discharged from the mid-level of the reactor) (Ni et al., 2009; Wagner and da Costa, 2013) or at constant volume (Giesen et al., 2013; van der Roest et al., 2011). The operation at constant volume consists in feeding the reactor from its bottom with fresh wastewater while simultaneously discharging the treated water from its top. Operating AGS-SBR at constant volume is the conventional way for operating full-scale plants. However, the conditions to grow granules, in terms of wastewater upflow velocity (V_{WW}) during anaerobic feeding and excess sludge removal, are not yet clearly identified.

The objectives of this study were: (i) to evaluate to what extent aerobic granules can form during treatment of real and low-strength municipal wastewater, (ii) to better understand how the operating conditions in terms of V_{WW} influence the formation of aerobic granules, and (iii) to quantify the performance of aerobic granular sludge systems in terms of substrate conversion, especially with regard of the denitrification and biological phosphorus removal. For this purpose, a 190 L column of 4 m high was operated for a period of 1500 days as a SBR at constant volume using real and low-strength municipal wastewater. Different operating conditions in terms of wastewater upflow velocity during the anaerobic feeding were applied. The sludge properties (concentration, settling volume index, and fraction of granules) and the reactor performances (COD, N, and P removal) were monitored.

2. Materials and methods

2.1. Experimental set-up

A sequencing batch column reactor of 190 L (0.25 m of diameter and 4 m of height) was operated in fill-draw mode (constant volume). The SBR column was fed with real and low-strength municipal wastewater introduced anaerobically at the bottom of the reactor. The batch cycle lasted 4 h and it was composed of the following phases: (i) anaerobic feeding phase, (ii) anaerobic standstill phase, (iii) aerobic phase, and (iv) settling. The duration of the full anaerobic phase (feeding plus standstill) was maintained constant (90 min). The anaerobic feeding stopped after 50% of the column volume was exchanged. The duration of the feeding was thus inversely proportional to the upflow velocity of the wastewater applied in the column. The aerobic and settling phases lasted 140 min and 3–10 min, respectively. Oxygen concentration was controlled between 2 and 2.5 mg_{O2} L^{-1} during the aerobic phase using an on/off aeration control system. During the anaerobic standstill phase, air was injected every 15 min during 5 s in order to re-suspend the sludge. The air pulse did not increase the oxygen concentration in the bulk liquid (based on on-line oxygen measurements), and anaerobic conditions were thus maintained. The SBR was equipped with an oxygen sensor (Optical LDO, Endress & Hauser, Switzerland), ammonium and nitrate electrodes (ion selective electrode, Endress & Hauser, Switzerland), as wells as a pH sensor (Endress & Hauser, Switzerland). All sensors were connected to a programmable logic controller (PLC). The PLC was connected to a supervisory control and data acquisition (SCADA) system.

2.2. Experimental approach

Two different approaches were tested to study the formation of aerobic granules for the treatment of real and low-strength municipal wastewater (Table 2). The first approach consisted in washing-out the flocs by imposing a high wastewater upflow velocity during the anaerobic feeding. V_{WW} ranging from 5.9 to 16 m h^{-1} were tested for selecting granules (Approach #1, Table 2). In Approach #1 all biomass that settled slower than the V_{ww} applied during the anaerobic feeding was withdrawn. The second approach consisted in encouraging granule formation through (i) a selective utilization of organic carbon during the anaerobic feeding combined with (ii) a selective withdrawal of sludge (Approach #2, Table 2). There was no new start-up between these two experimental approaches. A very low wastewater upflow velocity (V_{WW}) of 1 m h^{-1} was applied to ensure that the organic carbon was mainly consumed by the fast-settling biomass, i.e., the granules that settled at the bottom of the reactor during the anaerobic feeding. This slow feeding procedure was combined with a selective sludge removal triggered after the settling phase (0.8 m below the liquid surface). The settling time prior the selective sludge withdrawal was gradually decreased from 10 min to 3 min (corresponding to a selection of the biomass that settle quicker than 16 m h^{-1}). Around 20 cm of the sludge bed/supernatant was removed at the end of each batch cycle during Approach #2.

2.3. Municipal wastewater and sludge inoculum

The municipal wastewater used in this study was directly pumped from the combined sewer system of the city of Dübendorf (Switzerland). The wastewater was pre-treated to remove grit and sand before primary clarification. This primary clarified wastewater was then used in all our experiments and corresponds to a low-strength influent (Table 3).

The column SBR was inoculated with activated sludge from the on-site wastewater treatment plant (WWTP) of Eawag. The activated sludge of Eawag's WWTP achieves full COD removal and nitrification but no biological-phosphorus removal. The TSS concentration and the SVI_{30} were, respectively, 2 g_{TSS} L^{-1} and 130 mL g_{TSS}^{-1} at the beginning of this study.

2.4. Sludge volume index (SVI) and sludge size distribution

SVI was measured once or twice a week using a sludge sample (1 L) taken at the end of the aerobic phase. SVI_{10} and SVI_{30} were determined by dividing the sludge bed volume (mL) measured after 10 and 30 min, respectively, by the sample volume (L) and by the TSS concentration of the sample (gTSS L^{-1}). The SVI_{30}/SVI_{10} ratio was used in order to access granule formation, as suggested by de Kreuk et al. (2007). Particle size distribution of the sludge was determined using the sieving method described by Bin et al. (2011) for the sludge grown at V_{WW} of 12.5 and 16 m h^{-1} (Approach #1), and for the sludge grown at V_{WW} of 1 m h^{-1} (Approach #2). No measurements of size-class fractions were performed for the sludge grown at V_{WW} of 5.9 and 8.5 m h^{-1}. Different sieves with decreasing mesh openings of 0.63 mm, 0.25 mm, and 0.1 mm were used. A sludge volume of 200 mL was sampled from the reactor at the end of the aerobic phase and gently mixed with additional 800 mL of tap water in order to prevent the build-up of a cake on the sieve surface. The fraction of each class of size ($d < 0.1$, $0.1 < d < 0.25$, $0.25 < d < 0.63$, and $d > 0.63$ mm) was obtained by measuring the amount of TSS retained on each sieve. The measurements used for the calculations of the mean values were performed during the last three weeks of operation at each V_{WW} (corresponding to 5 measurements for V_{WW} of 12.5 and 16 m h^{-1}, and to 2 measurements for V_{WW} of 1 m h^{-1}).

2.5. Sample collection and analytical methods

Influent and effluent samples were collected around twice a week during Approach #1 in order to monitor the reactor

Table 2
Details of the different operating conditions applied during the two experimental approaches.

Experimental approaches	Selective pressure	Wastewater upflow velocity (V_{WW}) during anaerobic feeding (m h^{-1})	Selective removal of sludge after settling	Duration (d)	Operation period (d)	Measured variables
Approach #1	Granulation achieved through selective removal of sludge. The selective removal is governed by the applied V_{WW}.	5.9	No	85	0–85	SVI_{10} and SVI_{30}
		8.5		55	86–141	SVI_{10} to SVI_{30} ratio
		12.5		81	142–223	Size fraction of the sludge
		16		97	223–320	TSS and VSS
Approach #2	Granulation achieved through the selective anaerobic utilization of organic carbon (slow feeding) and selective removal of sludge.	1	(Settling time prior to the selective sludge withdrawal gradually decreased from 10 min (4.8 m h^{-1}) to 3 min (16 m h^{-1}))	317	1100 −1417	Substrate and nutrient conversion (e.g. simultaneous nitrification-denitrification)

Table 3
Composition of the primary clarified municipal wastewater used in this study.

Parameters	Unit	Average concentrations
Total COD	mg_{COD} L^{-1}	304 ± 127
Soluble COD	mg_{COD} L^{-1}	127 ± 55
Total nitrogen	mg_N L^{-1}	32 ± 6
Ammonium	mg_N L^{-1}	18 ± 5
Total phosphorus	mg_P L^{-1}	5 ± 1
Orthophosphate	mg_P L^{-1}	2 ± 0,6
Total suspended solids	mg_{TSS} L^{-1}	140 ± 30
Total organic loading rate	kg_{tCOD} m^{-3} d^{-1}	0.9 ± 0.4
Soluble organic loading rate	kg_{sCOD} m^{-3} d^{-1}	0.4 ± 0.2
Nitrogen loading rate	kg_{NH4+-N} m^{-3} d^{-1}	0.05 ± 0.02
Phosphorus loading rate	kg_{PO43-P} m^{-3} d^{-1}	$6.4 \cdot 10^{-3} \pm 2 \cdot 10^{-3}$

performances. Five influent and effluent samples were collected at the end of Approach #2. These samples were collected over a time period of 24 h (composite samples) and preserved at 4 °C prior the chemical analysis. Soluble COD were measured using commercial photochemical tests (Hach Lange, Germany). Ammonium was measured using a Foss FIAstar flow injection 5000 analyzer (Denmark). The detection limit of this instrument is 0.2 $mg_{N\text{-}NH4+}$ L^{-1}. Nitrate, nitrite, and *ortho*-phosphate were measured using anion chromatography (881compact IC, Metrohm, Switzerland; detection limit for nitrite and nitrate 0.1 mg_N L^{-1} and for *ortho*-phosphate 0.1 mg_P L^{-1}). Total suspended solid concentration (TSS) in the reactor and in the effluent were obtained by using filters with a pore size of 0.45 µm (Macherey-Nagel, Önsingen, Switzerland), and determined according Standards Methods (APHA, 2005). Substrate conversion efficiencies were calculated over 18, 30, and 5 samples for V_{WW} of 12.5, 16, and 1 m h^{-1}. The morphology of the biomass was regularly observed using a stereomicroscope (Olympus SZX10, Japan). No pictures were taken for the aerobic granular sludge grown at V_{WW} of 5.9 and 8.5 m h^{-1}.

2.6. Characterization of the microbial activities during SBR cycles of Approach #2 (V_{WW} = 1 m h^{-1})

Thirteen characterisations of the microbial activities during SBR cycles were conducted during Approach #2 to evaluate substrate conversion (e.g., simultaneous nitrification and denitrification, SND, and P-uptake). Samples were thus collected every 15–30 min to determine the concentration of ammonium, nitrite, nitrate, and *ortho*-phosphate throughout the batch cycle. Sampling during the anaerobic feeding (fill-draw mode) was performed in the effluent discharge (at the top of the reactor). During the aerobic phase the reactor was mixed via an on-off aeration system. The samples were thus collected using a port located at the half of the maximum working height of the SBR. The ammonium removal and nitrite/nitrate production rates were obtained from linear regression of their corresponding concentrations. Specific rates were defined as the absolute rates relative to the VSS concentration.

2.7. Calculations

2.7.1. Solid retention time (SRT)

The mean SRT (d) was calculated according Eq. (1) (Henze et al., 2008):

$$SRT = \frac{TSS \cdot V_r}{TSS_{eff.} \cdot Q_{eff.} + TSS_W \cdot Q_W} \tag{1}$$

where:

TSS: TSS concentration in the reactor (gTSS L^{-1});
V_r: reactor volume (L);
$TSS_{eff.}$: TSS concentration in the effluent (gTSS L^{-1});
$Q_{effl.}$: effluent flow rate (L d^{-1});
TSS_W: TSS concentration of the withdrawn sludge (gTSS L^{-1});
Q_W: flow rate of sludge withdrawn (L d^{-1}).

2.7.2. Sludge production

Sludge production was approximated using Eq. (2), adapted from conventional equations used for the calculation of sludge production based on conversion yields (Metcalf and Eddy, 2003). The sludge production relative to the COD stored anaerobically is distinguished from the sludge production relative to the COD degraded aerobically:

$$P_X = P_{X_{H,STO}} + P_{X_{H,S}} \tag{2}$$

where:

$P_{X_{H,STO}}$: Sludge production resulting from growth on previously stored substrate, i.e., soluble COD stored during the anaerobic period (g_{TSS} d^{-1});
$P_{X_{H,S}}$: Sludge production resulting from direct growth of external substrate, i.e., residual soluble COD remaining after the anaerobic period and particulate organic substrate hydrolyzed during the aerobic period (g_{TSS} d^{-1}).

These two fractions of the sludge production can be calculate using the following equations:

$$P_{X_{H,STO}} = i_{S_S,BM} \times Y_{H,O_2} \times Q \times (S_{S,in} - S_{S,ana,end}) \tag{3}$$

$$P_{X_{H,Xs}} = i_{S_s,BM} \cdot Y_{H,O_2} \cdot Q \cdot (X_{S,in} - S_{S,ana,end} - S_{S,aer,end}) \tag{4}$$

where:

$i_{S_s,BM}$: Suspended solids to biomass COD ratio (0.9 g_{TSS} g_{COD}^{-1}) (Henze et al., 2000);
Y_{H,O_2}: Aerobic yield of heterotrophic biomass (0.64 g_{COD} g_{COD}^{-1}) (Henze et al., 2000);
Q: Flow rate (L d^{-1});
$S_{S,in}$: Concentration of readily biodegradable substrate in the influent (0.3 g_{COD} L^{-1} based on our measurements);
$S_{S,ana,end}$: Concentration of readily biodegradable substrate remaining at the end of the anaerobic period (0.04 g_{COD} L^{-1} based on our measurements);
$X_{S,in}$: Concentration of slowly biodegradable substrate in the influent (0.15 g_{COD} L^{-1} based on our measurements);
$S_{S,aer,end}$: Concentration of readily biodegradable substrate remaining at the end of the cycle (0.02 g_{COD} L^{-1} based on our measurements).

Default biokinetic parameters from literature were used for these calculations (Henze et al., 2000). The nitrogen requirements for cell synthesis were estimated based on the overall sludge production (g_{TSS} d^{-1}):

$$r_{N,synthesis} = i_{N,VSS} \cdot i_{VSS,TSS} \cdot P_X \tag{5}$$

where $i_{N,VSS}$ is equal to 0.08 gNgVSS^{-1} and $i_{VSS,TSS}$ is equal to 0.85 (measured).

2.7.3. Growth conditions and diffusion depth of oxygen

Comparing the specific diffusion depth of the electron donors and acceptors helps to identify what compounds that limit substrate conversion in biofilm systems. The γ coefficient is the ratio between the electron donor penetration depth ($\beta_{e,d}$) to the electron acceptor penetration depth ($\beta_{e,a}$) assuming a zero-order reaction rates (Henze et al., 2008):

$$\gamma_{e.d,e.a} = \frac{\beta_{e.d}}{\beta_{e.a}} = \sqrt{(\alpha - Y) \cdot \frac{D_{F,e.d}}{D_{F.e.a}} \cdot \frac{C_{LF,e.d}}{C_{LF,e.a}}} \tag{6}$$

where:

- α is the stoichiometric factor linking the electron acceptor and donor utilization in the catabolic reaction (4.57 g_{O2} g_N^{-1} for nitrification) (Henze et al., 2008);
- Y is the biomass yield (0.22 g_{COD} g_N^{-1} for nitrification) (Henze et al., 2008);
- $D_{F,e.d}$ and $D_{F,e.a}$ are the diffusion coefficients for the electron donor and acceptor, respectively. For the case of nitrification, the electron donor is the ammonium ($D_{F,e.d} = 149 \cdot 10^{-6}$ m^2 d^{-1}) and the electron acceptor is the oxygen ($D_{F,e.a} = 175 \cdot 10^{-6}$ m^2 d^{-1}) (Henze et al., 2008);
- $C_{LF,e.d}$ and $C_{LF,e.a}$ are the concentration of the electron donor and acceptor at the biofilm surface.

Such calculation can be performed for one electron donor only. Thus, if oxygen is limiting for one electron donor (e.g. ammonium), it will be more limiting for two electron donors (ammonium and organic substrate). Ammonium was thus considered as electron donor for this calculation. Ammonium concentrations was varied between 20 and 0 mg_N L^{-1} for the calculations (i.e., full range of ammonium concentration during the aerobic phase assuming full nitrification). Oxygen concentration of 2.5 mg_{O2} L^{-1} was considered for the electron acceptor.

The calculation of the γ coefficient helps to predict if this is the electron donor or the electron acceptor that limits the substrate conversion within the biofilm. Two cases can then be differentiated:

- If $\gamma_{NH_4^+,O_2} > 1$ then oxygen is potentially limiting inside the granule and ammonium will fully penetrate the granule.
- If $\gamma_{NH_4^+,O_2} < 1$ then ammonium is potentially limiting inside the granule and oxygen will fully penetrate the granule.

The penetration depth of the electron donor or acceptor can be calculated according Eq. (7) (Henze et al., 2008):

$$Z_{penetration} = \beta \cdot L_F = \sqrt{\frac{2 \cdot D_F \cdot C_{LF}}{k_{0,F} \cdot X_F}} \tag{7}$$

The total oxygen consumption is the sum of the oxygen consumption by the heterotrophic bacteria ($X_{H,F}$) and autotrophic bacteria ($X_{AUT,F}$) that grow within the biofilm (Henze et al., 2008). D_F is the diffusion coefficient for oxygen ($175 \cdot 10^{-6}$ m^2 d^{-1}) (Henze et al., 2008) and C_{LF} is the oxygen concentration at the surface of the granules (2 mg_{O2} L^{-1} in our study). X_F is the concentration of biomass in the granules (10 000 gCOD m^{-3}) (Henze et al., 2008).

$$k_{0,F} \cdot X_F = k_{0,O2,H} \cdot X_{H,F} + k_{0,O2,AUT} \cdot X_{AUT,F} \tag{8}$$

Where $k_{0,O2,H}$ is the zero-order constant of the heterotrophic bacteria for oxygen (7.2 g_{O2} g_{COD}^{-1} d^{-1}) and $k_{0,O2,AUT}$ is the zero-order constant of the autotrophic biomass for oxygen (18.8 g_{O2} g_{COD}^{-1} d^{-1}), $X_{H,F}$ and $X_{AUT,F}$ are the heterotrophic and autotrophic biomass concentrations, respectively. It was assumed that autotrophs represent 2% of the total biomass ($0.02 \times 10{,}000 = 200$ g_{COD} m^{-3}) while heterotrophs represent the rest (Henze et al., 2008).

3. Results

3.1. Sludge volume index and solids concentrations

The different sludge properties were monitored for each operating condition in terms of V_{WW} (Fig. 1). During Approach #1, increasing the V_{WW} from 5.9 to 16 m h^{-1} resulted in improved settling properties, i.e., decreased SVIs. The SVI_{30} indeed decreased from 200 to less than 50 mL g_{TSS}^{-1} when increasing the V_{WW} from 5.9 to 16 m h^{-1} (Fig. 1a). A similar observation was done for the SVI_{10}. Ultimately, the SVI_{30} to SVI_{10} ratio increased from 0.6 to around 0.8 (Fig. 1b and c). When operating the reactor at 1 m h^{-1} (Approach #2), the SVI_{30} and SVI_{10} decreased from 150 to 65 mL g_{TSS}^{-1} and from 190 to 75 mL g_{TSS}^{-1}, respectively (Fig. 1a and b). An average SVI_{30} of 84 ± 12 mL g_{TSS}^{-1} was calculated after day 1200 (22 measurements). Also, the SVI_{30} to SVI_{10} ratio increased from 0.7 to 0.95 under the operating conditions of the Approach #2 (Fig. 1c).

Increasing V_{WW} from 5.9 to 16 m h^{-1} during the anaerobic feeding also led to a gradual decrease of the solids concentration, in terms of TSS, inside the reactor (Approach #1) (Fig. 1d). The TSS concentration indeed decreased from 4 g_{TSS} L^{-1} to 1.2 ± 0.3 g_{TSS} L^{-1} when V_{WW} increased from 5.9 to 16 m h^{-1}. The decreasing biomass concentration resulted from a massive loss of biomass with the treated effluent: 172 ± 100, 143 ± 40, 100 ± 20, and 117 ± 45 mg_{TSS} L^{-1} at 5.9, 8.5, 12.5, and 16 m h^{-1}, respectively. This biomass loss exceeded the approximated biomass production (91 g_{TSS} d^{-1}) and ultimately decreased the mean SRT from

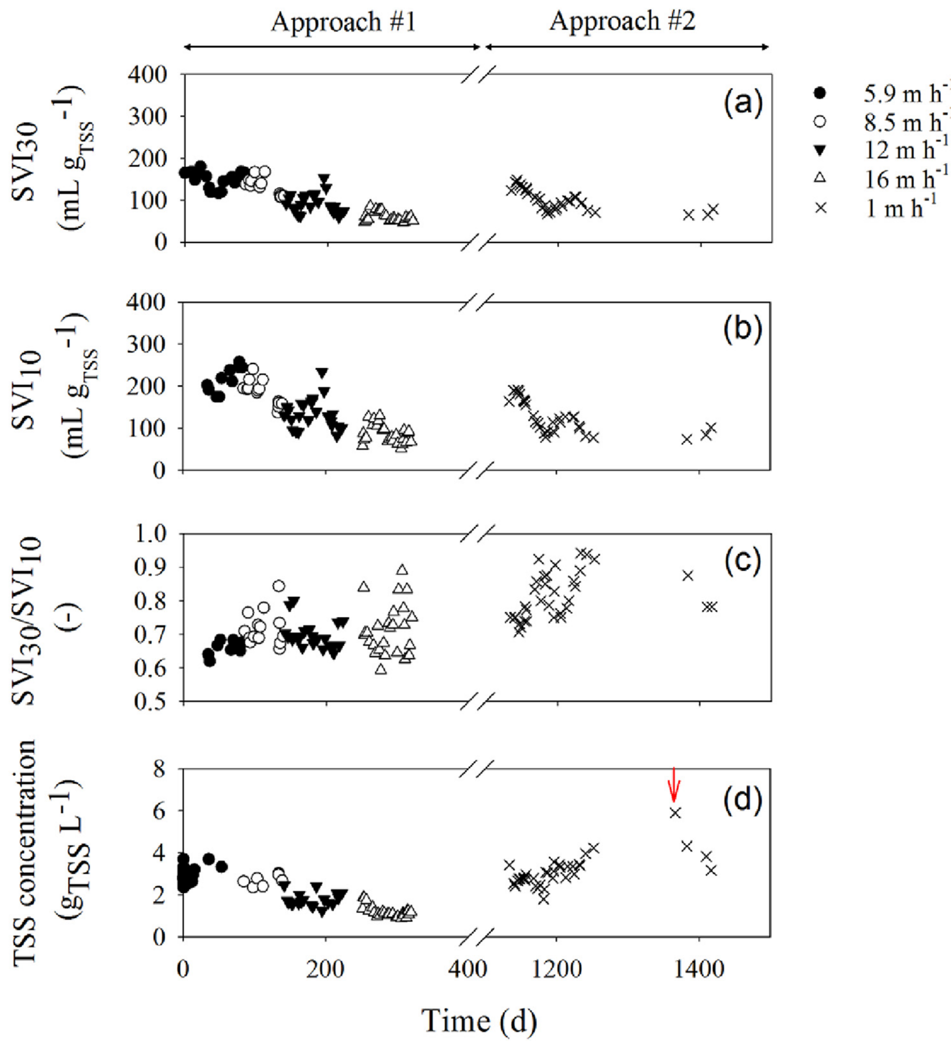

Fig. 1. Changes in the (a) SVI_{30}, (b) SVI_{10}, (c) SVI_{30} to SVI_{10} ratio, and (d) TSS concentration in the reactor for the different operating conditions in terms of wastewater upflow velocity during the anaerobic feeding phase. The red arrow shown on Fig. 1d indicates involuntary sludge removal (independent of this study) that lead to a significant decrease of the sludge concentration inside the SBR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5.1 ± 0.5 d to 3.6 ± 0.6 d (Approach #1). A much lower TSS concentration (9 ± 5 mg_{TSS} L^{-1}) was however measured in the effluent of the system operated at a V_{WW} of 1 m h^{-1} (Approach #2). A significant amount of sludge was thus maintained in the SBR (>6 gTSS L^{-1} at day 1417and mean SRT of 17 ± 7 d).

3.2. Sludge size fractions and morphology

The different size fractions of the sludge were only quantified when the system was operated at V_{WW} of 12.5, 16, and 1 m h^{-1} (Fig. 2). Flocs and granules co-existed under all applied conditions in terms of feeding velocities. Increasing the wastewater upflow velocity during the anaerobic feeding (Approach #1) helps to select for large granules (d > 0.63 mm) (Fig. 2a and b). The total fraction of granules, here referred to aggregates with diameter higher than 0.25 mm (d > 0.25 mm), was slightly higher than 60% and 70% at V_{WW} of 12.5 and 16 m h^{-1}, respectively. However, filamentous structures could be observed at the surface of these large granules (Fig. 3a and b). During Approach #2 (V_{WW} of 1 m h^{-1} combined with selective excess sludge removal), the sludge was mainly composed of small granules (0.25 < d < 0.63 mm: 60 ± 1%) (Figs. 2c and 3c). The total fraction of granules (d > 0.25 mm) reached around 70% at the end of this experimental period. The change in the sludge fraction during the granulation process was monitored during Approach #2 (Figure SI-1). The sludge was mainly composed of flocs, here referred to aggregates with diameter smaller than 0.25 mm, at the beginning of the Approach #2 (d < 0.25 mm: around 70% between days 1100 and 1150) (Figure SI-1). The fraction of flocs subsequently decreased to around 30% at day 1250, i.e., within 3 months, and then remained at this level until day 1417. Meanwhile the fraction of small granules (0.25 < d < 0.63 mm) increased from around 20% (day 1100) to 60% (day 1417).

3.3. System performances

3.3.1. Carbon and nutrient removal efficiencies

High soluble COD removal efficiencies (>80%) were measured for all the applied conditions in terms of V_{WW} (Fig. 4). Average soluble COD concentrations of 34 ± 7 and 33 ± 6 mg_{COD} L^{-1} were measured in the effluent at V_{WW} of 12.5 and 16 m h^{-1} (Approach #1), respectively. Lower average concentrations (22 ± 4 mg_{COD} L^{-1}) were measured in the effluent when operating the SBR under the conditions of Approach #2 (V_{WW} of 1 m h^{-1}). The extensive floc washout observed at V_{WW} of 12.5, and 16 m h^{-1}(Approach #1) resulted in a low mean SRT, and in turn in partial ammonium and phosphorus removal. Ammonium removal efficiencies of 58 ± 24%

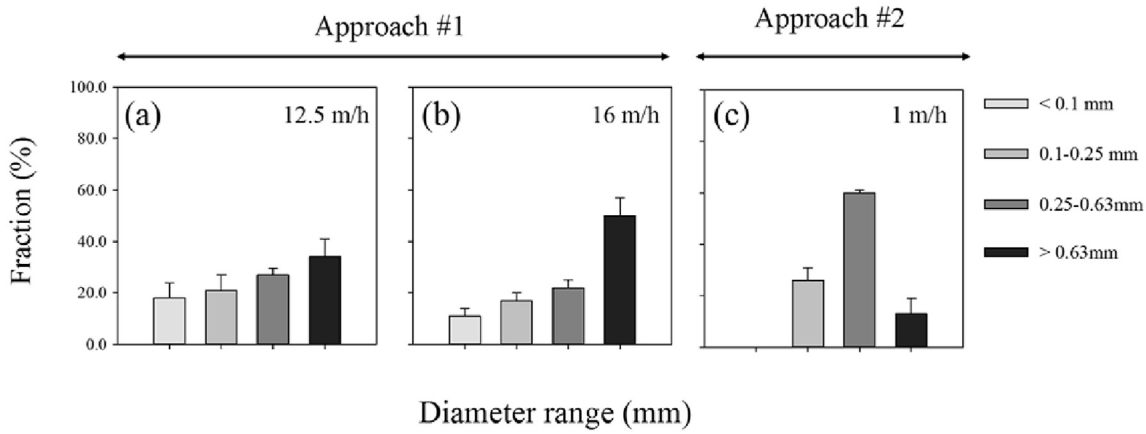

Fig. 2. Particle size distribution of the sludge measured for the different conditions in terms of wastewater upflow velocity during the anaerobic feeding. The sludge fractions are presented as averages values of five measurements performed over the three last weeks of operation at (a) V_{WW} of 12.5 and (b) 16 m h^{-1} (5 measurements each) (Approach #1), and at (c) V_{WW} of 1 m h^{-1} (2 measurements) (Approach #2). No measurements of size-class fractions were performed for the sludge grown at 5.9 and 8.5 m h^{-1}.

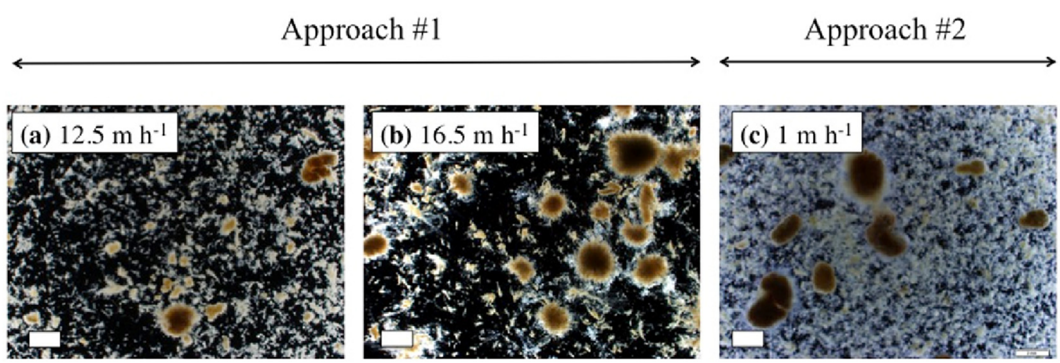

Fig. 3. Stereomicroscopic images of the aerobic granular sludge grown in the reactor at the different conditions in terms of wastewater upflow velocity during the anaerobic feeding phase. The presented images were obtained during the last week of each operation period at (a) V_{WW} of 12.5 and (b) 16 m h^{-1} (Approach #1), and at (c) V_{WW} of 1 m h^{-1} (Approach #2). No pictures were taken of the aerobic granular sludge grown at V_{WW} of 5.9 and 8.5 m h^{-1}. White bars indicate a size of 1 mm.

and of 28 ± 18% were measured at V_{WW} of 12.5 and 16 m h^{-1}, respectively. Ammonium and phosphorus concentrations of 8 ± 4 mg_N L^{-1} and 0.9 ± 0.25 mg_P L^{-1} were measured in the effluent at 12.5 m h^{-1}. At 16 m h^{-1}, higher effluent ammonium and phosphorus concentrations were observed: 13 ± 5 mg_N L^{-1} and 1.7 ± 0.6 mg_P L^{-1}, respectively. The nutrient removal capacity was more pronounced when operating the AGS-SBR at V_{WW} of 1 m h^{-1} (Approach #2). Average removal efficiencies of 96 ± 4% for ammonium and 89 ± 7% for phosphorus were measured, resulting in an excellent effluent quality. The ammonium, nitrite, and *ortho*-phosphate concentrations were indeed below the limit of quantification during Approach #2. An average nitrate concentration of 3.9 ± 2 $mg_{N\text{-}NO3\text{-}}$ L^{-1} was measured in the effluent during this period.

3.3.2. Microbial activities during SBR cycles

Batch experiments were performed in order to get further insights into the substrate conversions occurring during the whole SBR cycle. A representative example of the substrate profile observed during Approach #2 (V_{WW} of 1 m h^{-1}) is given on Fig. 5. At a V_{WW} of 1 m h^{-1}, the anaerobic period consisted of 90 min of feeding (no anaerobic standstill phase). During the anaerobic feeding the remaining nitrate from the previous cycle was denitrified. The nitrate concentration thus decreased from 6 to 0.2 mg L^{-1} (specific denitrification rate of 0.045 $g\cdot g_{VSS}^{-1}\cdot d^{-1}$) (Fig. 5a). At the same period the ammonium, nitrite, and *ortho*-phosphate concentrations remained constant in the discharged treated water. During the following aerobic phase the ammonium concentration measured in the mixed-liquor decreased from 12 to 0.5 $mg_{N\text{-}NH4+}$ L^{-1} (Fig. 5b). Meanwhile the nitrate concentration increased from 0.2 to 8 $mg_{N\text{-}NO3\text{-}}$ L^{-1} and no nitrite accumulation occurred in the reactor. Nitrogen requirement for the synthesis of cells was estimated to around 5 mg N/L from Eq. (5). This value was in the same order of magnitude than the difference between the oxidized ammonium and the nitrate produced by nitrification. No significant simultaneous nitrification-denitrification was thus observed in our study. The amount of phosphate in the beginning of the aerobic phase was much higher than the average values usually measured in the wastewater influent. This indicates that phosphate release occurred within the sludge bed during the anaerobic feeding phase. Aerobic phosphate uptake then occurred during the aerobic phase, as indicated by the rapid decrease in the phosphate concentration: from 7.3 to 0.2 $mg_{P\text{-}PO43\text{-}}$ L^{-1} in 40 min.

The calculation of the γ coefficients for the aerobic phase presented in Fig. 5 indicated that the substrate conversion is limited by the diffusion of oxygen. The γ coefficient decreased from 4.1 (t = 100 min, ammonium concentration of 11.6 mg L) to 1.3 (t = 220 min, ammonium concentration of 1.1 mgN L) (Eq. (6)). The penetration depth of oxygen was approximated to 107 μm using Eq. (7).

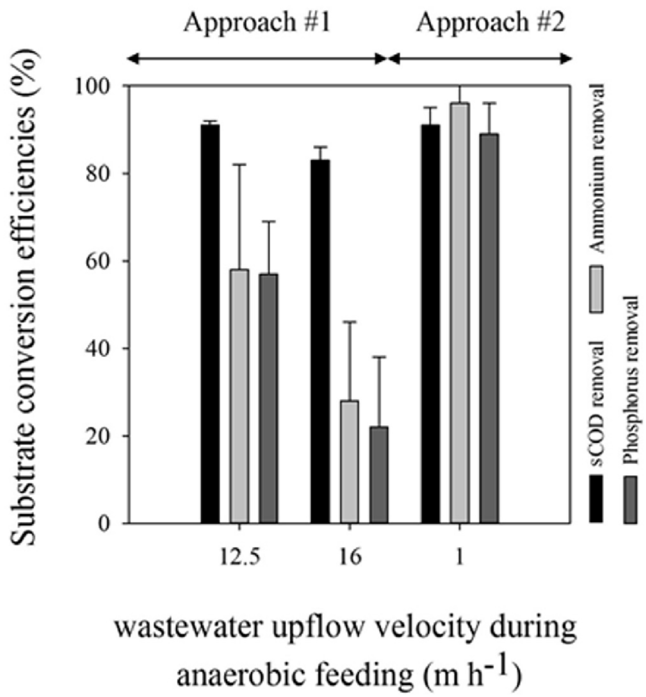

Fig. 4. Efficiencies of substrate conversion measured for the different conditions in terms of wastewater upflow velocity during the anaerobic feeding phase. Conversion efficiencies are presented as averages values calculated based on 18, 30, and 5 samples for experiments conducted at 12.5, 16, and 1 m h^{-1}, respectively.

4. Discussion

4.1. Successful granulation can be achieved using real and low strength municipal wastewater

Several full-scale AGS installations are currently in operation worldwide but few information are available regarding the operating conditions required to start-up these systems. Our study aimed at evaluating two different approaches to cultivate aerobic granules during the treatment of real and low-strength municipal wastewater. The first approach consisted in washing-out the flocs by imposing a high wastewater upflow velocity during the anaerobic feeding (Approach #1, Table 2). The second approach consisted in encouraging granule formation through a selective utilization of organic carbon during the anaerobic feeding combined with a selective withdrawal of sludge (Approach #2, Table 2). Both strategies were successful in cultivating AGS with a dominant granule fraction (>70%) and very good settling properties (low SVIs and high SVI ratio).

In SBRs operated at constant volume without selective sludge withdrawal, floc removal takes place during the discharge of the treated effluent. The removal of flocs with the effluent occurs if the V_{WW} applied during the anaerobic feeding phase exceeds the floc settling velocity. Settling velocities lower than 7 m h^{-1} are usually reported for floccular biomass (Ni et al., 2009) while settling velocities larger than 30 m h^{-1} are reported for aerobic granules fed with synthetic influents (Beun et al., 1999; Morgenroth et al., 1997). Lower settling velocities are however reported for aerobic granules fed with real municipal wastewater: between 18 and 40 m h^{-1} (Ni et al., 2009). The measurements of settling velocities reported by Ni et al. 2009, suggest that a feeding velocity between 7 and 18 m h^{-1} should be applied to washout the flocs and select the granules. This is confirmed by our results, which indicated that increasing the V_{WW} from 5.9 to 16 m h^{-1} efficiently selected granules due to the continuous floc washout (Figs. 1 and 2). The slow settling velocity of AGS fed with influents containing particulate organic substrates can explain the continuous solid washout observed at V_{WW} ranging from 5.9 to 16 m h^{-1} (de Kreuk and de Bruin, 2004; Wagner et al., 2015). Reducing the selective pressure for slow settling biomass was required to grow aerobic granules for the treatment of synthetic influent that contained particulate organic matter (Wagner et al., 2015). A adaptation of the operating conditions was also required during treatment of pre-treated sewage (de Kreuk and de Bruin, 2004). In the aforementioned study, a selection of the biomass that settled between 2 and 5 m h^{-1} was performed (de Kreuk and de Bruin, 2004). In our study, the biomass loss observed in the effluent at high V_{WW} (5.9–16 m h^{-1} - Approach #1) was in the same order of magnitude than the estimated sludge production. Limited granule

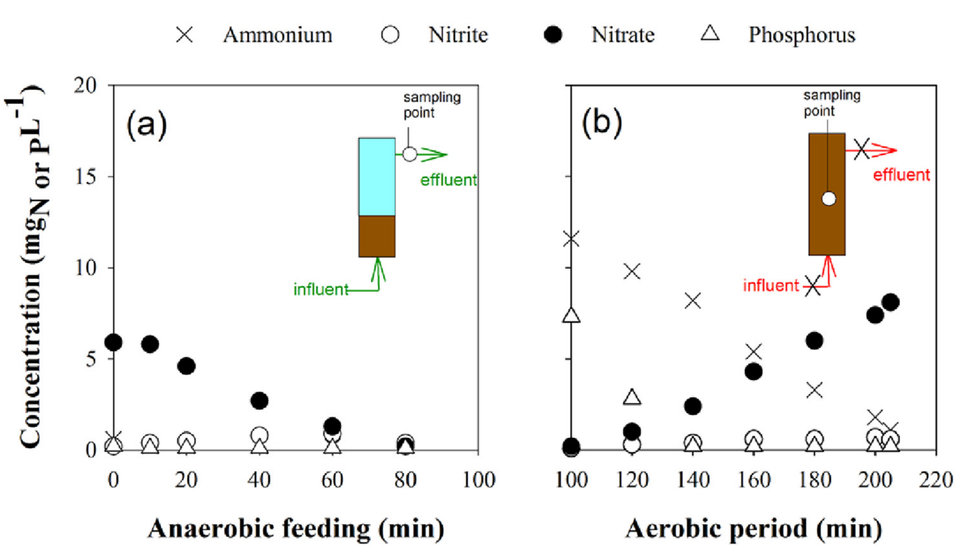

Fig. 5. Representative example of the substrate conversion throughout a batch cycle monitored during Approach #2 (V_{WW} of 1 m h^{-1}): (a) anaerobic feeding (samples from the effluent) and (b) aerobic period. During the feeding the reactor is not mixed and the samples were thus collected in the upper part of the reactor. The presented values correspond to the effluent concentrations of ammonium, nitrite, nitrate, and phosphorus. During the aerobic phase, the reactor is mixed and the samples were thus collected using a port located at the half of the maximum working height of the reactor.

accumulation was thus observed under the conditions of Approach #1, as confirmed by the low solid concentrations maintained in the system (Fig. 1d). The limited accumulation of biomass at high V_{WW} might also result from a partial anaerobic utilization of the organic substrates. At high V_{WW}, the permeation rate of the organic substrates through the settled bed of granules might exceed the substrate utilization rate (i.e., storage rate). If the organic substrate storage is incomplete during the anaerobic feeding period, the substrates are then degraded aerobically. Aerobic growth on organic substrates ultimately favours the formation of fluffy granules (de Kreuk et al., 2010) and the loss of biomass with the treated effluent. Our microscopic observations of fluffy granules developed at high V_{WW} supports the hypothesis that significant utilization of organic substrate under aerobic conditions occurred in our system (Fig. 3a and b).

A higher biomass retention was observed when a slow feeding strategy was coupled with a selective excess sludge removal (Approach #2). The values of SVI_{30} measured in our study during this period (SVI_{30} of around 80 mL g_{TSS}^{-1}) are in the same order of magnitude than those reported for aerobic granules fed with real municipal wastewaters (Giesen et al., 2013; Liu et al., 2010; Ni et al., 2009; van der Roest et al., 2011). We expect that lower SVI values would have been measured after a longer operation of the SBR as more than one year is usually requested for the start-up of AGS reactors fed with real and low-strength wastewaters. However, a low SVI_{30} does not automatically imply full granulation and the SVI_{30}/SVI_{10} ratio should also be considered when evaluating the extent of granulation. This ratio indeed increases with an increasing granule fraction. In our study an average SVI_{30}/SVI_{10} ratio of 0.85 ± 0.06 was measured (Fig. 1c). This value is slightly lower than those reported in previous studies (0.9–1) (Coma et al., 2012; Giesen et al., 2013; Liu et al., 2010; Ni et al., 2009; van der Roest et al., 2011) and indicate that some flocs remained in the reactor. The presence of flocs in the AGS was confirmed by our measurements of the sludge size fractions (30% of flocs with d < 250 μm after 3 months of cultivation – Approach #2) (Fig. 2c). In previous studies on aerobic granulation for the treatment of municipal wastewaters, almost full granulation (granules fraction > 90%) was achieved. However, extended periods (more than 1 year) were necessary to reach granule fractions higher than 90% (Giesen et al., 2013; Liu et al., 2010; Ni et al., 2009; van der Roest et al., 2011). The fraction of granules, for example, increased from 40% to 90% in around 1.5 years in the study of van der Roest et al. (2011). In our study the fraction of granules (d > 0.25 mm) increased from 44 to 75% in 317 days (Approach #2). The fraction of flocs is also directly linked to the selective excess sludge removal. It is expected that a lower fraction of flocs could be maintained with a more selective sludge removal (e.g. shorter settling phase prior removal or larger volume removal). This might in turn result in a selection for larger granules.

Overall our results demonstrate that successful granulation in terms of settling properties (SVI_{30} < 80 mL g_{TSS}^{-1} and SVI_{30}/SVI_{10} ratio of 0.85 ± 0.06) can be achieved within few months during the treatment of real and low strength wastewater through (i) a selective utilization of organic carbon during the anaerobic feeding combined with (ii) a selective withdrawal of sludge (Approach #2).

4.2. Excellent effluent quality in terms of COD, ammonium, phosphorus, and TSS is achieved

Our study demonstrates that excellent effluent quality can be achieved when feeding the reactor from the bottom at low wastewater upflow velocity ($V_{WW} = 1$ m h^{-1} – Approach #2) while simultaneously discarding the effluent from the top (fill-draw operation). High COD removal, nitrification, and biological phosphorus removal were achieved under these operating conditions. The high mean SRT (17 ± 7 d) maintained under these conditions allowed the growth of heterotrophs (ordinary and storing) and nitrifiers. The ammonium, nitrite, and phosphorus concentration measured in the effluent were below the limits of quantification of our instruments (0.2 mg N L^{-1} for ammonium, 0.1 mg N or P L^{-1} for nitrite and *ortho*-phosphate). Carbon and *ortho*-phosphate can thus be removed by almost 100% with aerobic granular sludge treating real and low-strength municipal wastewater. Batch cycle analysis displayed that phosphorus is released during the anaerobic feeding and then incorporated to the biomass in the subsequent aerobic phase. The release of phosphorus under anaerobic conditions and low phosphorus concentrations in the effluent are not only helpful to meet discharge limits, but also to achieve a stable growth of aerobic granules (Rocktäschel et al., 2015). Such excellent performances of aerobic granular sludge system were already reported for full-scale plants (Giesen et al., 2013; van der Roest et al., 2011).

When operating the SBR under the condition of Approach #2, very low TSS concentrations (9 ± 5 mg_{TSS} L^{-1}) were measured in the effluent. This value is in the same range than those reported by Ni et al. (2009) (15 mg_{TSS} L^{-1}) or by Giesen et al. (2013) for the Nereda® process of Epe (10–20 mg_{TSS} L^{-1}). In our study low TSS effluent concentrations were achieved even when a significant fraction of flocs was present in the sludge (around 30%). Instead, the fraction of flocs was very low (around < 10%) in the study of Ni et al. (2009) and of Giesen et al. (2013). The higher fraction of flocs measured in our study thus did not deteriorate the effluent quality in terms of TSS concentration. Also, a too high fraction of the granules often increases the TSS effluent concentrations (Rocktäschel et al., 2015). Rocktäschel et al. (2015) therefore recommends granule fractions between 70 and 80% to achieve TSS effluent concentrations below 50 mg_{TSS} L^{-1}.

4.3. But only partial denitrification is observed

Partial denitrification was measured in our study when operating the system at $V_{WW} = 1$ m h^{-1} (Approach #2), resulting in effluents with an average nitrate concentration of around 4 $mg_{N\text{-}NO3\text{-}}$ L^{-1}. Despite this, it is relevant to discuss why denitrification was incomplete during the treatment of municipal wastewater with aerobic granules. In AGS systems, mass-transfer is limited by diffusion. Thus, denitrification can in theory occur simultaneously to nitrification during the aerobic phase (simultaneous nitrification-denitrification, SND) due to the formation of an anoxic zone in the granule centre. In our study, denitrification occurred mainly during the anaerobic feeding of the reactor. No or very little SND occurred during the aerobic phase. Two mechanisms can explain the quasi absence of SND: (i) the diffusion of electron donors and acceptors that determine the anoxic volume within the granules and/or (ii) the availability of organic substrates. The extent of SND is directly linked to the fraction of the granules that is exposed to anoxic conditions and thus to the granule size (Rocktäschel, 2013). The ratio of denitrified nitrate to the produced nitrate increases with the mean granule diameter, i.e., with an increasing anoxic volume (Rocktäschel, 2013). Chen et al. (2011) reported an increase of the total nitrogen removal efficiency of 67.9–71.5% with the increase of the size of the granules of 0.7–1.5 mm. Our calculations of γ coefficients indicated that oxygen was the limiting compound and that anoxic conditions thus existed within the granules. The penetration depth of oxygen was roughly estimated at around 107 μm based on zero-order equations. The estimated oxygen penetration was thus slightly thinner than the radius of the granules (minimum 125 μm, i.e., diameter of 250 μm). This calculation supports the fact that an anoxic layer existed within the granules but did not result in high SND. This anoxic layer was rather thin (around 18 μm), according to our estimation. The availability of organic substrates in the anoxic zone of the granules might also explain the limited SND. During the aerobic phase the organic substrate is available (i) in the form of stored COD by phosphorus- and glycogen-accumulating organisms, (ii) in the form of soluble COD that was not fermented during the anaerobic phase, and (iii) in the form of particulate COD that was not hydrolyzed during the anaerobic period. However, those different organic substrates are used for denitrification only if they are available in the anoxic zones of the granules. Organic substrates in the particulate form cannot diffuse through granules and were likely not available for denitrification in the anoxic core of the granule. Mainly the stored COD and residual soluble COD could thus support denitrification. However, if the stored COD is available in the aerobic zone of the granules, it will not be used for denitrification. The same applies for the soluble COD that remains in the bulk liquid at the end of the anaerobic feeding. Thus, no or very little simultaneous nitrification and denitrification will occur.

Overall, a better understanding of the factors that limit SND during the treatment of real municipal wastewaters with AGS is thus required to accurately evaluate the relevance of such technology. With more stringent requirements on total nitrogen removal, a partial SND might limit the application of AGS technology.

5. Conclusions

The main conclusions of this study are:

(i) Successful granulation in terms of settling properties was achieved during the treatment of real and low-strength municipal wastewater in a sequencing batch reactor operated at constant volume. Better results were obtained when applying (a) a selective utilization of organic carbon by fast-settling biomass during the anaerobic feeding combined with (b) a selective withdrawal of slow-settling sludge (Approach #2, V_{WW} of 1 m h^{-1}). The AGS contained a significant fraction of granules (>70%) after 3 months of operation under these conditions but the granule size was rather small (0.25 < d < 0.63 mm) despite very good settling properties (SVI_{30} of 65 mL g_{TSS}^{-1} and SVI_{30}/SVI_{10} ratio of around 0.85).

(ii) No simultaneous nitrification and denitrification was observed in the AGS reactor dominated by small granules cultivated under the operating conditions of Approach #2. The absence of simultaneous nitrification and denitrification can be explained either by the limited anoxic volume maintained within the granules during the aerobic phase, or by the limited availability of organic substrate in the anoxic zones of the granules.

(iii) Excellent effluent quality was achieved during Approach #2: high COD, ammonium, and phosphorus removal were observed. TSS in the effluent remained lower than 10 mg_{TSS} L^{-1}. Effluent nitrate concentrations were low (<5 mg_N L^{-1}) and denitrification occurred mainly during the slow anaerobic feeding period. Overall, it can be concluded that low wastewater upflow velocities should be applied when operating the AGS reactors at constant volume, in order to ensure enough biomass retention and efficient substrate removal.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.09.007.

References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, twentyfirst ed. (Washington, DC).

Beun, J.J., Hendriks, A., Van Loosdrecht, M.C.M., Morgenroth, E., Wilderer, P.A., Heijnen, J.J., 1999. Aerobic granulation in a sequencing batch reactor. Water Res. 33 (10), 2283–2290.

Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J., 2000. Aerobic granulation. Water Sci. Technol. 41 (4–5), 41–48.

Bin, Z., Zhe, C., Zhigang, Q., Min, J., Zhiqiang, C., Zhaoli, C., Junwen, L., Xuan, W., Jingfeng, W., 2011. Dynamic and distribution of ammonia-oxidizing bacteria communities during sludge granulation in an anaerobic–aerobic sequencing batch reactor. Water Res. 45, 6207–6216.

Chen, F.Y., Liu, Y.Q., Tay, J.H., Ning, P., 2011. Operational strategies for nitrogen removal in granular sequencing batch reactor. J. Hazard. Mater. 189, 342–348.

Coma, M., Verawaty, M., Pijuan, M., Yuan, Z., Bond, P.L., 2012. Enhancing aerobic granulation for biological nutrient removal from domestic wastewater. Bioresour. Technol. 103 (1), 101–108.

Dangcong, P., Bernet, N., Delgenes, J.-P., Moletta, R., 1999. Aerobic granular sludge—a case report. Water Res. 33 (3), 890–893.

de Kreuk, M., de Bruin, L.M.M., 2004. Aerobic Granule Reactor Technology. STOWA Report. IWA Publishing. ISBN: 1 84339 067 1.

de Kreuk, M.K., Kishida, N., Van Loosdrecht, M.C.M., 2007. Aerobic granular sludge – state of the art. Water Sci. Technol. 55 (8–9), 75–81.

de Kreuk, M.K., Kishida, N., Tsuneda, S., van Loosdrecht, M.C.M., 2010. Behavior of polymeric substrates in an aerobic granular sludge system. Water Res. 44 (20), 5929–5938.

Gao, D., Liu, L., liang, H., Wu, W.-M., 2011. Comparison of four enhancement strategies for aerobic granulation in sequencing batch reactors. J. Hazard. Mater. 186 (1), 320–327.

Giesen, A., de Bruin, L.M.M., Niermans, R.P., van der Roest, H.F., 2013. Advancements in the application of aerobic granular biomass technology for sustainable treatment of wastewater. Water Pract. Technol. 8 (1), 47–54.

Henze, M., Gujer, W., Mino, T., van Loosdrecht, M., 2000. Activated sludge models ASM1, ASM2, ASM2d and ASM3. In: IWA Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment. IWA Scientific and Technical Report. ISBN: 1 900222 24 8. ISSN: 1025–0913.

Henze, M., van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D., 2008. Biological Wastewater Treatment. Principles, Modelling and Design. IWA Publishing.

Liu, Y.Q., Moy, B., Kong, Y.H., Tay, J.H., 2010. Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment. Enzyme Microb. Technol. 46 (6), 520–525.

Metcalf & Eddy, 2003. In: Tchobanoglous, G., Burton, F.L., Stensel, H.D. (Eds.), Wastewater Engineering: Treatment and Reuse, fourth ed. McGraw-Hill, Boston; Montreal.

Morgenroth, E., Sherden, T., van Loosdrecht, M.C.M., Heijnen, J.J., Wilderer, P.A., 1997. Aerobic granular sludge in a sequencing batch reactor. Water Res. 31 (12), 3191–3194.

Ni, B.J., Xie, W.M., Liu, S.G., Yu, H.Q., Wang, Y.Z., Wang, G., Dai, X.L., 2009. Granulation of activated sludge in a pilot-scale sequencing batch reactor for the treatment of low-strength municipal wastewater. Water Res. 43 (3), 751–761.

Rocktäschel, T., 2013. Treatment of Municipal Wastewater with Aerobic Granules - Influence of Feeding Strategies. PhD Thesis. Technische Universität München, Chair of Urban Water Systems Engineering, Germany.

Rocktäschel, T., Klarmann, C., Ochoa, J., Boisson, P., Sørensen, K., Horn, H., 2015. Influence of the granulation grade on the concentration of suspended solids in the effluent of a pilot scale sequencing batch reactor operated with aerobic granular sludge. Sep. Purif. Technol. 142 (0), 234–241.

van der Roest, H.F., de Bruin, B., van Loosdrecht, M.C.M., Pronk, W., 2011. Dutch sustainable Nereda technology takes an impressive step towards maturity. Budapest, Hungary. In: 11th IWA Specialised Conference on Design, Operation and Economics of Large Wastewater Treatment Plants. 4-8 September, 2011, pp. 177–182.

van Loosdrecht, M.C.M., Brdjanovic, D., 2014. Anticipating the next century of wastewater treatment. Science 344 (6191), 1452–1453.

Wagner, J., da Costa, R., 2013. Aerobic granulation in a sequencing batch reactor using real domestic wastewater. J. Environ. Eng. 139 (11), 1391–1396.

Wagner, J., Weissbrodt, D.G., Manguin, V., da Costa, R.H.R., Morgenroth, E., Derlon, N., 2015. Effect of particulate organic substrate on aerobic granulation and operating conditions of sequencing batch reactors. Water Res. 85, 158–166.

Zheng, Y.-M., Yu, H.-Q., Liu, S.-J., Liu, X.-Z., 2006. Formation and instability of aerobic granules under high organic loading conditions. Chemosphere 63 (10), 1791–1800.