Contents lists available at [ScienceDirect](www.sciencedirect.com/science/journal/2589014X)

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

Evaluation of an aerobic granular sludge reactor with biological filtration (AGS-BF reactor) in municipal wastewater treatment: A new configuration

Silvio Luiz de Sousa Rollemberg, Tasso Jorge Tavares Ferreira, André Bezerra dos Santos

Department of Hydraulic and Environmental Engineering, Federal University of Ceara, ´ *Fortaleza, Ceara,* ´ *Brazil*

1. Introduction

The aerobic granular sludge (AGS) process is considered one of the most efficient and promising technologies existing in the market, presenting several advantages like simultaneous removal of carbon, nitrogen, and phosphorus, high potential of metal and several pollutants biosorption, color removal, resource recovery from the excess sludge such as polyhydroxyalkanoates (PHAs), alginate-like exopolymers (ALE), tryptophan, and polysaccharide-based biomaterial, among others ([Ferreira et al., 2021; Van Leeuwen et al., 2018](#page-7-0); [Wang et al., 2018](#page-7-0); [Lu](#page-7-0) [et al., 2016; Wang et al., 2014](#page-7-0)).

Because of these advantages, the AGS technology has been extensively studied in sewage treatment and industrial wastewater treatment, including palm oil mill effluent, livestock, leachate, dairy, textile, and other complex effluents ([Rollemberg et al., 2018\)](#page-7-0). Over ninety full-scale Nereda® AGS wastewater treatment plants are currently operating worldwide [\(Royal HaskoningDHV, 2022\)](#page-7-0). In comparison with activated sludge (AS), for example, AGS showed a significant reduction in footprint (\sim 75 %) and power consumption (30–50 %) (Bengtsson et al., [2018\)](#page-7-0).

Although aerobic granules reactors have several advantages regarding performance, footprint, energy demand, investment, and operational costs aspects, their operation on pilot- and full-scale (especially in the treatment of low-load domestic sewage and some industrial effluents) has faced some problems, such as:

- (i) Granule disintegration in long-term operation, leading to high total suspended solids (TSS) concentrations in the treated effluent, treatment performance deterioration, and, eventually, to the overall AGS reactor failure ([Rollemberg et al., 2018](#page-7-0); [Wagner et al., 2015](#page-7-0); [Wan et al., 2013\)](#page-7-0).
- (ii) Long start-up time, sometimes requiring extended periods (up to 13 months) to achieve granulation [\(Ni et al., 2009\)](#page-7-0).
- (iii) Low total nitrogen (TN) and total phosphorus (TP) removals and problems with nitrite accumulation [\(Rollemberg et al., 2020a,](#page-7-0) [2020b; Franca et al., 2018\)](#page-7-0).
- (iv) Flotation of the granules and flocs and high solids presence in the effluent due to denitrification (N_2 gas) and influent TSS and fat presence ([Van Dijk et al., 2018\)](#page-7-0).

Several modifications in the AGS reactor design and operation were suggested to overcome such problems. [Li et al. \(2014\)](#page-7-0) proposed AGS cultivation on a pilot-scale under ideal conditions with synthetic wastewater and biomass use as inoculum in full-scale wastewater treatment plants (WWTPs) after granules maturation. Although this methodology has been efficient for granule stability, it is likely

<https://doi.org/10.1016/j.biteb.2022.101172>

Available online 28 July 2022 2589-014X/© 2022 Elsevier Ltd. All rights reserved. Received 31 May 2022; Received in revised form 19 July 2022; Accepted 22 July 2022

^{*} Corresponding author at: Department of Hydraulic and Environmental Engineering, Campus do Pici, Bloco 713, Pici, CEP: 60455-900 Fortaleza, Ceara, ´ Brazil. *E-mail address:* andre23@ufc.br (A. Bezerra dos Santos).

unfeasible for full-scale application. The approach adopted by [Van Dijk](#page-7-0) [et al. \(2018\)](#page-7-0) to reduce effluent TSS was the installation of vertical baffles in front of the AGS effluent weirs. [Rollemberg et al. \(2020a, 2020b\)](#page-7-0) and [Wu et al. \(2018\)](#page-7-0) proposed sludge discharge methodologies aiming at increasing phosphorus removal (sludge age control) in addition to the selective discharge of flocculent biomass which improved the final effluent quality in terms of TSS.

All these alternatives showed important results in increasing the knowledge regarding the AGS technology. Nonetheless, a new design for AGS systems could be an approach to overcome/minimize the reported problems and increase the system reliability. This work proposes an unprecedented modification of a sequencing batch reactor (SBR) AGS system, which consists of implanting a biological filter on top of the SBR (AGS-BF) using a high surface application rate biomedia.

2. Material and methods

2.1. Experiment location and wastewater characteristics

The reactor was installed in the largest wastewater treatment plant (WWTP) of Fortaleza, Ceará, Brazil, which receives about 3.0 $\text{m}^3\text{/s}$. The sanitary wastewater used in the pilot-scale systems received coarse screening and grit removal and was stored in an equalization tank. The influent municipal wastewater composition was: chemical oxygen demand (COD) – 717 mg/L, biochemical oxygen demand $(BOD₅)$ – 313 mg/L, TSS – 167 mg/L, NH₄-N – 52.5 mg/L, and PO₄^{3–}-P (dissolved) – 7.1 mg/L (see Supplementary Material).

The influent had low load characteristics, hindering the granulation process ([Gao et al., 2011\)](#page-7-0). The methodology of sludge discharge and cycle optimization [control of the food to microorganism (F/M) ratio and control of organic loading rate through cycle optimization] presented by [Rollemberg et al. \(2020a, 2020b\)](#page-7-0) made it possible to obtain stable granulation even when applied to low-load sanitary wastewater.

2.2. Seed sludge

The aerobic flocculent biomass source was from a full-scale activated sludge reactor. The initial concentration of mixed liquor volatile suspended solids (MLVSS) was around \sim 2.0 g/L with sludge volume index at 30 min (SVI₃₀) reaching a value of around 150 mL/g, and the average size was 0.05 mm with sedimentation velocities about 3 m/h.

2.3. Experimental setup

Two identical sequencing batch reactors (SBR) were used, with a diameter of 100 mm, height of 1000 mm, working volume of 7.2 L, and a ratio of height to diameter (H/D) of 10. Reactor R1 was a conventional AGS system that worked as a control, while R2 was a modified AGS system by applying a biological filter (BF) compartment in the upper zone. For this reactor, a false bottom was inserted to support the high surface application rate biomedias. The BF compartment volume was approximately 10 % of the useful SBR volume. The biomedia was made of polyurethane foam, commercially called Monera Bio Power®, being considered, for this research, a surface area of $1000 \text{ m}^2/\text{m}^3$. Different sludge discharge points were included along the AGS heights and BF compartment. [Fig. 1](#page-2-0) shows the schematic of the research reactors.

Air was pumped to the bottom of the reactors with an aeration rate of 10.0 L/min, keeping the dissolved oxygen (DO) concentration in the mixed liquor between 2 and 3 mg/L. The exchange ratio was 50 %, and the ambient temperature was about 28 \pm 2 °C. The initial cycle of the SBR reactors was 6 h, consisting of anaerobic feeding (60 min), oxic phase (280–295 min), and settling (20–5 min). The hydraulic retention time (HRT) was around 12 h. The systems were operated in four periods, varying the sedimentation time: 20 min (Period I), 10 min (Period II), 5 min (Period III), and the cycle time reduction from 6 h to 4 h after granule maturation (Period IV). Such reduction in the cycle time,

keeping the same exchange ratio of 50 %, resulted in the HRT decrease to 8 h.

The SBR operation was as simultaneous fill-and-draw mode or constant volume, in which influent was pumped to the reactor bottom while the treated wastewater leaves it from the top. The sludge discharge protocol was carried out according to [Rollemberg et al. \(2022\).](#page-7-0) The selective sludge discharge through SRT control (the SRT was maintained around 20 days) was applied after the sedimentation period. Bottom sludge and flocculent sludge discharges were implemented, constituting 30 % and 70 % of the total volume of sludge discarded, respectively. Bottom and flocculent sludge discharges were done through specific registers located along the reactor height. The idea was to withdraw both old P-rich/saturated granule present at the reactor bottom and the filamentous/flocculent sludge existing at the reactor top. All excess sludge retained on the BF compartment was recirculated twice a week to the bottom of the AGS reactor.

2.4. Sludge production and theoretical yield coefficient (Y)

Mass balance to define the volume of discharge sludge was carried out from the yield coefficient (Y_{obs}) calculation, considering the sludge withdraw, the sludge production and effluent solids, and dividing by the accumulated TCOD removed, according to the literature [\(Metcalf, 2003\)](#page-7-0) Eq. (1):

$$
Y_{obs} = \frac{[(X_2 - X_1)V_r + X_eV + X_sV_s]}{(COD_{in} - COD_{out})V} [gVSS/gCOD_{rem}]
$$
 (1)

where X_2 and X_1 are the biomass concentrations (gVSS/L) at the day (n) and (n-1), V_r is the reactor working volume, X_e (gVSS/L) is the effluent biomass concentrations, V is the daily volume of wastewater treated, X_s is the waste biomass concentration (gVSS/L), V_s is the daily volume of waste sludge, and COD_{in} and COD_{ef} are the influent and effluent COD concentration (g/L), respectively.

2.5. Analysis

COD, pH, ammonium, nitrite (NO₂), nitrate (NO₃), total phosphorus (TP), total and volatile suspended solids (TSS and VSS), and sludge volume index at 10 and 30 min (SVI_{10} and SVI_{30}) were determined according to [APHA \(2012\).](#page-7-0) Dissolved oxygen (DO) was measured by a YSI 5000 m (YSI, USA). The aeration system was carried out through an air compressor (Yuting SUN, China). The extracellular polymeric substances (EPS), i.e., proteins (PN) and polysaccharides (PS) contents, were determined according to [Rollemberg et al. \(2019\).](#page-7-0) The reactors were subject to mechanical stirring (Magnetic stirrer, WEA, 30 rpm) through the anaerobic, oxic and anoxic phases to prevent settling. More details about types, brand, and origin of all instruments are described in Rollemberg et al. (2021).

For the determination of NO₂, NO₃, and PO₄⁻ ions, ion chromatography was performed on a Dionex™ ICS-1100 ion chromatograph according to [Do Nascimento et al. \(2021\).](#page-7-0)

The method described by [Lin et al. \(2013\)](#page-7-0) was used to extract the ALE from the aerobic granular sludge. A dried biomass (0.5 g) was lyophilized for 5 min (Freeze Dryer L 101, Liotop, Brazil), and the ALE were extracted by using 80 mL of a 0.2 M Na₂CO₃ solution at 80 °C for 1 h. After centrifugation at 15,000 rpm for 20 min, the pellet was discarded. The mass value was expressed following the recommendations of [Felz et al. \(2016\).](#page-7-0) The method used for P determination in the biomass was described elsewhere (Rollemberg et al., 2021).

The sludge settling velocity was determined similarly to Wang et al. [\(2018\)](#page-7-0) using an acrylic column with a working height of 0.4 m and a diameter of 75 mm. The granules collected from the SBRs were introduced on the top of the column, and, then, the time for their complete settling was measured in triplicate.

Fig. 1. Schematic diagram of the reactors: Conventional AGS, control (R1) (a) and AGS with a biological filter (R2) (b).

2.6. Statistical methods

The non-parametric Mann-Whitney test was used to compare the reactors' performance at a confidence level of 95 %, in which the data groups were statistically different when $p \leq 0.05$.

3. Results and discussion

The reactors R1 (conventional, control) and R2 (modified AGS with BF compartment) were evaluated in terms of (i) performance in the removal of C, N, and P; (ii) pollutant removal mechanism; (iii) sludge production and characterization of excess sludge; (iv) energy consumption and engineering aspects.

3.1. Reactor start-up

As mentioned, the 1st part of the investigation consisted of reducing the sedimentation time from 20 min to 10 min and then to 5 min, a common procedure in AGS research, aiming at selecting granules by washing the filaments and obtaining mature granules. Although the reactors started with similar conditions (MLVSS of 2000 mg/L; SVI_{30} of 180 mL/g), considering that the same inoculum (activated sludge) and the same concentration were used, the behavior of the systems during the experimental period was considerably distinct (Fig. 2).

Initially, both reactors showed a significant reduction in SVI_{30} , which is expected since the aerobic granulation process promotes the improvement of biomass sedimentability by transforming activated sludge flocs into aerobic granules induced by several selection pressures ([Rollemberg et al., 2018](#page-7-0)). The ones adopted in the current research were: (i) sedimentation time reduction to keep only the biomass with a sedimentation velocity above 0.2 m/min; (ii) selection of slow-growing bacteria that use intracellularly stored PHA through an anaerobic period followed by aerobic periods with extended famine (substrate absence, which was consumed around 70–80 % in the anaerobic period); (iii) selective sludge discharge, where the filaments present in the upper layer of the sludge blanket and floating biomass were selectively removed ([De Kreuk et al., 2007](#page-7-0)7; [Rollemberg et al., 2018](#page-7-0)).

As mentioned, the reactors showed different behaviors. There was considerable instability (partial disintegration) in R1, especially between days 25–30 and 60–65, with an SVI_{30} increase in addition to an MLVSS concentration decrease (Fig. 3a). Several works ([Franca et al.,](#page-7-0) [2018; Wagner et al., 2015\)](#page-7-0) had reported this instability, with a partial

Fig. 2. SVI₃₀ variation of the control reactor (R1, square) and AGS with a biological filter (R2, circle) throughout the operational periods.

Fig. 3. MLVSS concentration (a) and effluent VSS concentration (b) of the control reactor (R1) and AGS with a biological filter (R2) throughout the operational periods.

disintegration of the granules when AGS was applied to treat sanitary wastewater. On the other hand, in R2, no record of instability was observed, either in terms of SVI₃₀ (Fig. 2) or MLVSS (Fig. 3a). This difference in behavior is related to R2 modification. The biological filter in the upper part of the reactor played a decisive role in retaining biomass originating from the granules that went through a disintegration/ breakdown process.

A significant difference was found concerning MLVSS concentration (Fig. 3a). While R1 had an average of 2 g/L, a typical value of AGS reactors in sanitary wastewater treatment on a lab- and pilot-scale ([Roll](#page-7-0)[emberg et al., 2020a, 2020b; Wagner et al., 2015](#page-7-0)), R2 had an average value of 3.5 g/L. This difference can also be attributed to the modification performed in R2. Fig. 3 evaluated the MLVSS and effluent VSS concentrations, showed that the biological filtration helped to retain the biomass, which was recirculated to the lower compartment (aerobic granular biomass compartment).

Regarding effluent VSS (Fig. 3b), higher values were observed in R1, especially during the instability period. On the other hand, in R2, no instability was observed, and low VSS values in the effluent ($\approx 10 \text{ mg/L}$) were found. The effluent VSS concentrations were statistically different $(p = 0.04)$ (data not shown), with mean values of 39.7 ± 3 mg/L in R1

and 15.4 ± 4 mg/L in R2. In terms of effluent TSS, the average values are 44.1 and 17.1 mg/L for R1 and R2, respectively. Therefore, it is clearly observed that in R2, a significant reduction of effluent solids was found due to the BF compartment inclusion. Solids' loss in AGS reactors effluent has been reported in many works, as a result of granule disintegration, biomass washout by denitrification or due to the presence of fats ([Rollemberg et al., 2020a, 2020b](#page-7-0); [Franca et al., 2018;](#page-7-0) Rocktäschel [et al., 2015;](#page-7-0) [Li et al., 2008](#page-7-0)). Generally, well-operated AGS systems in full-scale applications such as WWTP Garmerwolde [\(Pronk et al., 2015\)](#page-7-0) and WWTP Dinxperlo [\(van der Roest et al., 2011\)](#page-7-0), have presented TSS values close to 20 mg/L.

Some works have proposed methodologies to reduce TSS concentration in the effluent. [Rollemberg et al. \(2020a, 2020b\)](#page-7-0) proposed tertiary filtration, obtaining high-quality effluent (TSS below 15 mg/L and turbidity below 3.5 NTU). TSS near 8 mg/L and turbidity close to 5.0 NTU were obtained during vertical baffles installation in front of the AGS effluent weirs ([Van Dijk et al., 2018\)](#page-7-0). This research adopted a simple solution, which provided an effluent with a low TSS concentration without the need of an additional tertiary treatment unit (filtration). Other aspects related to reactor operation are described in Table 1.

3.2. Granule characteristics

The reactor configuration also influenced biomass characteristics. Granules' average size (\approx 1.2 mm) and sedimentation velocities (\approx 20 m/h) were close in both systems. However, there was a significant difference in the EPS content ($p \approx 0.03$), where notably, there was a greater accumulation of PS and mainly PN in the R2 granules.

One of the most important parameters in granulation is the EPS, substances secreted by bacteria under specific conditions and mainly composed of proteins, polysaccharides, humic acids, and lipids ([Adav](#page-7-0) [and Lee, 2008\)](#page-7-0). The PN/PS ratio generally increases in EPS after granulation ([Rollemberg et al., 2020a, 2020b](#page-7-0); [Zhang et al., 2017\)](#page-7-0). There is a consensus in the literature that PN is responsible for the structure and stability of granules, while PS is important for acting as a biological glue in the aggregation of granular biomass. In general, PN is the most abundant substance in the EPS of stable aerobic granules. Therefore, it is important to evaluate the PN/PS ratio ([McSwain et al., 2005](#page-7-0)).

While the PN/PS ratio in R1 was close to 1.1, values close to 1.5 were observed for R2, indicating greater granules' stability. This result agrees with what was discussed in Section 3.2, where a greater oscillation in the MLVSS content in R1 was observed, caused by granules' disintegration at two different times. Therefore, the reactor modification, with the BF compartment inclusion and sludge recirculation to the aerobic granular sludge zone, promoted a higher EPS concentration, mainly PN, acting on the stability of the granules.

There was a tendency towards a yellowish color in R1 and a brown color in R2 (probably due to the higher P content in $R2$ – see Section

Table 1

Granule's characteristics of the control reactor (R1) and AGS with a biological filter (R2) throughout the operational periods.

Sludge characteristics		Period I	Period II	Period III	Period IV
MLVSS (g/L)	R1	1.3 ± 0.5	1.7 ± 0.8	$1.7 + 0.5$	2.0 ± 0.2
	R ₂	$2.6 + 0.5$	$3.2 + 0.7$	$3.7 + 0.6$	$3.8 + 0.4$
SVI_{30}/SVI_5	R1	0.89	0.9	0.93	0.92
	R ₂	0.91	0.92	0.95	0.96
Mean diameter (mm)	R1	0.9	1.3	$1.2\,$	1.2
	R ₂	0.8	1.2	1.1	1.3
Settling velocity (m/h)	R1	18	14	19	21
	R ₂	17	16	22	24
PS (mg/mgVSS)	R1	144	149	145	144
	R ₂	129	155	143	153
PN (mg/mgVSS)	R1	117	151	157	155
	R ₂	180	204	215	215
PN/PS ratio	R1	0.8	1.1	1.1	1.1
	R ₂	1.3	1.3	1.5	1.4

[3.5\)](#page-6-0). The aerobic granules obtained in R1, and the biomass obtained in R2 (granules from the lower zone – AGS zone; adhered and flocculent sludge obtained from the upper zone – biological filtration zone) are shown in the Supplementary Material.

3.3. Systems' performance

Systems' performance in terms of C, N, and P removals was evaluated and the results are shown in Table 2.

As mentioned, the experiment was divided into four periods, varying the sedimentation time: 20 min (Period I), 10 min (Period II), 5 min (Period III), and the cycle time reduction from 6 h to 4 h after granule maturation (Period IV). BOD removal was high in both systems, with values above 90 % observed in all periods, with no significant differences ($p = 0.06$) between the reactors. The values found are similar to those of several other studies that observed high BOD removals (*>* 90 %) in AGS systems treating sanitary/municipal wastewaters [\(Rollemberg](#page-7-0) [et al., 2020a, 2020b;](#page-7-0) [Pronk et al., 2015\)](#page-7-0).

Similar to BOD removal, COD removal was also high in both systems. However, in some operation periods, lower removals were achieved in R1, especially in Periods I and II, where system instability was observed. Overall, COD removals of close to 90 % were found.

Removal of NH $_4^+$ -N was also evaluated in the two systems, in which a significant difference between Periods I and II was found $(p = 0.04)$. In this regard, the lower efficiency found in R1 can be explained by the lower MLVSS concentration. Moreover, the instability and biomass loss may be related to the retention of ammonia-oxidizing bacteria (AOB). After the stability and maturation of the granules, ammonia removals above 90 % were observed, with no significant effect upon cycle time reduction (Period IV).

A tendency of nitrite accumulation in R1 was observed, which in turn

Table 2

Performance of the control reactor (R1) and AGS with a biological filter (R2) throughout the operational periods.

Parameter	Period I		Period II			Period III		Period IV	
	R1	R ₂	R1	R ₂	R1	R ₂	R1	R ₂	
BOD _{inf}	341 ± 53	327 ± 45	349 ± 34	319 ± 40	338 ±76	385 \pm 81	396 ± 53	427 ±49	
(mg/L) BOD _{eff}	19	14	16	11	17	$9+$	13	$9 +$	
(mg/L)	±13	± 8	±7	$±$ 4	± 8	3	± 9	3	
$\mathrm{BOD}_{\mathrm{rem}}$	92	91	90	95	91	96	93	95	
(%)	± 6	± 5	±4	$±$ 4	$±$ 4	\pm 3	\pm 5	± 3	
	$\%$	$\frac{0}{0}$	$\frac{0}{0}$	$\%$	$\%$	$\frac{0}{0}$	$\%$	$\%$	
COD_{inf}	809	867	773	805	721	705	719	682	
(mg/L)	±45	± 39	\pm	± 91	± 63	± 27	± 61	±49	
			112						
COD _{eff}	48	26	41	22	33	17	24	19	
(mg/L)	\pm 21	±7	± 9	± 5	±10	± 6	±10	± 8	
$\mathrm{COD_{rem}}$	89	96	90	94	90	95	91	95	
(%)	±7	±7	± 2	±2	±7	± 4	± 5	± 4	
	$\%$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\%$	$\frac{0}{0}$	$\%$	$\%$	
TP_{inf} (mg/	$9 +$	$8\pm$	$9 \pm$	$9 +$	$9 +$	$9 \pm$	$9+$	$9 +$	
L)	3	3	$\overline{2}$	$\overline{2}$	$\overline{2}$	1	1	$\mathbf{1}$	
TP_{eff} (mg/	3±	3±	3±	$2\pm$	$2\pm$	$2\pm$	$2\pm$	$2\pm$	
L)	$\overline{2}$	$\mathbf{1}$	$\overline{2}$	$\mathbf{1}$	$\mathbf{1}$	$\mathbf{1}$	1	1	
TP_{rem} (%)	64	63	69	74	75	72	73	71	
	±7	± 3	± 2	± 3	± 3	± 3	± 2	\pm 3	
NH_4^+ - N_{inf}	63	59	48	46	52	53	61	65	
(mg/L)	± 9	±15	±7	± 5	\pm 9	±7	± 6	± 3	
NH_4^+ -N _{efl}	11	5±	7 _±	$6\pm$	$4\pm$	5±	$6\pm$	7 _±	
(mg/L)	±7	$\overline{4}$	3	3	$\overline{2}$	$\overline{2}$	$\overline{2}$	3	
$NO2 - Neff$	$8\pm$	$2\pm$	$8\pm$	3±	$9+$	$4\pm$	10	3±	
(mg/L)	8	3	5	$\mathbf{1}$	$\overline{4}$	3	± 3	$\mathbf{1}$	
NO_3^- - N_{eff}	$1 \pm$	$1 \pm$	$2\pm$	3±	5±	3±	$2\pm$	$1 \pm$	
(mg/L)	$\overline{2}$	$\overline{2}$	$\overline{2}$	$\overline{2}$	$\overline{2}$	$\mathbf{1}$	$\overline{2}$	$\mathbf{1}$	
NH_4^+ - N_{rem}	82	91	83	88	93	91	91	90	
(%)	±4	± 3	± 3	± 3	±2	± 2	± 3	± 1	
TN_{rem} (%)	75	86	77	85	78	85	76	86	
	± 5	± 2	± 4	± 5	± 3	± 5	± 2	± 3	

impacted the TN removal. Other AGS reactors have faced problems with nitrite accumulation (partial nitrification) [\(Coma et al., 2012](#page-7-0)), when the COD available for denitrification is low ([Derlon et al., 2016](#page-7-0)). The better denitrification in R2 was explained by two mechanisms: (i) In the aerobic period, the DO concentration in the AGS zone was 2.0 mg/L, but below 0.5 mg/L in the BF (upper zone), therefore, likely promoting the simultaneous nitrification and denitrification - SND mechanism, as it occurs in the aerobic granular sludge, improving TN removal; (ii) during an anaerobic period a high sludge denitrified activity was found in the two stages (aerobic granular sludge and BF zones); (iii) higher sludge concentration in R2, due to the greater biomass retention and sludge recirculation, enhancing denitrification, mainly endogenous, i.e., the denitrification that occurs when there is an absence of substrate and the sludge uses the material stored internally as an electron donor.

Unlike TN removals, TP removals were not impacted by the reactor configuration. After systems stabilization, removal values close to 70 % were observed. Several factors impact TP removal, the main ones being: (i) sludge age control, which is carried out by controlling sludge

Fig. 4. Concentration of organic matter (a), phosphorous (b) and nitrogen species (c-e) throughout the cycle: R1 (square), R2 – AGS zone (triangle) and R2 biofilter zone (circle).

discharge; (ii) type of substrate, being known that acetate and propionate favor the development of polyphosphate-accumulating organisms - PAOs; and (iii) cycle configuration, i.e., the existence of an anaerobic period followed by an aerobic period [\(Rollemberg et al., 2018](#page-7-0); [Nan](#page-7-0)[charaiah and Reddy, 2018](#page-7-0)). Considering that all these factors were the same in the two systems, the configuration change in R2 did not significantly impact the TP removal.

3.4. Removal mechanisms and sludge production

In order to understand the pollutants removal processes, influent and effluent samplings were carried out during an SBR cycle. For R2 [\(Fig. 4](#page-5-0)), another sampling was included immediately before the BF compartment. The simultaneous nitrification, denitrification, and phosphorus removal (SNDPR) process was initially evaluated. In the R1 system, it was verified that the main nitrogen removal mechanism occurred through the SND in the granule during the aerobic period, with a slight nitrite accumulation. Also important is the process of phosphorus release in the anaerobic period, followed by sequestration in the aerobic period. At the end of the experiment (reactors' stabilization), TN removal was favored in R2 [\(Table 2\)](#page-4-0) due to the biomedia presence that helped in the additional denitrification. Therefore, SND occurred in both systems due to the presence of the granules ([Fig. 4c](#page-5-0) and d), but only R2 presents an additional denitrification due to the attached-growth zone.

Endogenous denitrification was also observed in the sludge adhered to the biomedia. TP removal occurred mainly in the aerobic granular sludge ([Fig. 4](#page-5-0)e). This justifies the similar efficiencies found in the two reactors. The lower PAOs activity in the biomedia may be related to the high density of denitrifying bacteria (dispute for the substrate, i.e., VFA, in the anoxic period). This can be explained due to direct competition between PAO bacteria and denitrifying bacteria [\(Rollemberg et al.,](#page-7-0) [2018; Nancharaiah and Reddy, 2018](#page-7-0)). The upward reactor flow favors the initial substrate contact with the aerobic granular biomass during the anaerobic feeding. Therefore, the low organic load in the upper zone (biological filter) may explain the low favorability of PAOs bacteria in the biomedias.

Finally, the high capture of filamentous solids detached from the aerobic granular zone, which are adhered by the biomedias in the upper layer (biological filter), also stands out. This mechanism provided an effluent with lower turbidity and TSS. In addition, it is important to mention that the BF compartment insertion increases system reliability, especially during periods of instability where the granules' fragments (due to breakage) increase the effluent TSS, turbidity, and COD.

In addition to these parameters, the sludge production in both systems was also evaluated through respirometry, as presented by [Corsino](#page-7-0) [et al. \(2016\)](#page-7-0) and [Rollemberg et al. \(2019\)](#page-7-0). According to [Marais and](#page-7-0) [Ekama \(1976\),](#page-7-0) in aerobic degradation, 1/3 of the energy in organic matter is used to catabolism and 2/3 to anabolism. It is emphasized that in an aerobic system is usually adopted a yield coefficient (Y) of 0.4 to 0.5 gVSS/gCODrem ([van Haandel and Lubbe, 2011](#page-7-0)) and 0.05 to 0.15 gVSS/gCOD_{rem} for anaerobic biomass ([van Haandel and Lettinga, 1994](#page-7-0)).

Sludge production has been evaluated in some AGS studies. The theoretical yield coefficients (Y) of aerobic granules were estimated at 0.2 [\(Liu et al., 2005\)](#page-7-0) and 0.3 gVSS/gCODrem ([Rollemberg et al., 2019\)](#page-7-0) for municipal wastewater. Compared with the conventional AS system, in which a sludge growth yield of around 0.45 gVSS/gCOD_{rem} is found ([Metcalf, 2003\)](#page-7-0), there is a sludge production decrease by around 30 % in AGS systems.

The Y coefficient found in this research was 0.33 and 0.37 gVSS/ gCOD_{rem} in R1 and R2, respectively, and the Y coefficient of R2 was 11 % higher than that of R1. Therefore, the sludge growth and sludge filtration on BF carrier are the main reasons for the high Y coefficient of R2. As noted, the biomass production rate in R2 was slightly higher than that found in R1. However, such an impact of the BF compartment was not as significant because most of the influent COD was removed in the AGS zone, as previously explained.

3.5. Resource recovery from excess sludge and engineering aspects

This work evaluated the presence of phosphorus and ALE in the AGS granules (R1 and R2) and in the BF compartment sludge (R2). The ALE found in the granules have chemical and mechanical properties (gelforming capability) that allow industry applications. The high potential of ALE recovery from AGS provided a new Nereda® project through Royal HaskoningDHV, called Kaumera Nereda® Gum, aiming at the production of bio-based resources to a variety of oil-based materials. The first large-scale Kaumera production unit is currently in operation in Zutphen, The Netherlands ([Royal HaskoningDHV, 2022;](#page-7-0) Rollemberg et al., 2021). The processes involving the production of ALE in AGS and the accumulation of P in the granules were described in some review papers [\(Carvalho et al., 2021](#page-7-0); [Ferreira et al., 2021\)](#page-7-0).

In R1, values of 0.012 gP/gTSS and 0.15 gALE/gVSS were observed. On the other hand, R2 granules presented values close to 0.014 gP/gTSS and 0.17 gALE/gVSS. Values close to 0.002 gP/gTSS and 0.07 gALE/ gVSS were observed in the BF compartment sludge (most adhered to the biomedia).

The results showed that granular biomass has a significantly greater capacity to accumulate phosphorus and ALE than cultivated sludge adhered to biomedia. This result confirms preliminary studies carried out elsewhere showing that AGS has a greater capacity to accumulate P and produce ALE due to the greater abundance of slow-growing microorganisms compared to attached-growth systems [\(Nancharaiah and](#page-7-0) [Reddy, 2018](#page-7-0)).

Regarding sludge discharge, the internal recirculation protocol from the biological filtration zone to the aerobic granular zone provided a sludge with high digestibility content. VSS/TSS ratio in the discharged sludge was below 0.7, which could also be related to the high phosphorus content in the granular biomass, as shown in Section 3.5.

Close energy demand was found for the two systems evaluated. In other words, the biological filter insertion in the SBR upper zone did not require greater aeration, in which a consumption close to 0.3 kWh/m^3 was observed. As mentioned, the BF compartment remained anoxic most of the time, justifying the high N removal in this system. The advantages found in R2 over R1 are summarized in the Supplementary Material.

4. Conclusions

The BF compartment in the AGS reactor (R2) provided a high-quality effluent in terms of TSS and turbidity, and excellent TN removals were found. Sludge production was evaluated, and the yield coefficient (Y, $gVSS/gCOD_{rem}$) and sludge concentration were different in the two reactors. As expected, no increase in energy demand in R2 was found. The new AGS system can be an interesting alternative to improve some aspects of conventional AGS reactors, such as denitrification and nitrite accumulation, effluent TSS concentration, and system reliability increase during periods of instability and granules breakage.

CRediT authorship contribution statement

Silvio Luiz de Sousa Rollemberg: Writing – original draft, Writing – review & editing. **Tasso Jorge Tavares Ferreira:** Writing – original draft. **André Bezerra dos Santos:** Writing – review & editing, Funding acquisition.

Uncited references

- [26](#page-7-0)
- [27](#page-7-0)
- [31](#page-7-0)
- [22](#page-7-0)
- [23](#page-7-0)
- [37](#page-7-0)
- [42](#page-7-0)

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

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at [https://doi.](https://doi.org/10.1016/j.biteb.2022.101172) [org/10.1016/j.biteb.2022.101172.](https://doi.org/10.1016/j.biteb.2022.101172)

References

- [Adav, S.S., Lee, D.J., 2008. Extraction of extracellular polymeric substances from aerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643336658) [granule with compact interior structure. J. Hazard. Mater. 154 \(1](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643336658)–3), 1120–1126.
- [APHA, 2012. Standard Methods for the Examination of Water and Wastewater, 22. ed.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270644015398) [American Public Health Association, American Water Works Association, Water](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270644015398) [Environment Federation, Washington, DC](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270644015398).
- [Barros, A.N.de, Lira, V.N.S.A., Firmino, P.I.M., Dos Santos, A.B., Rollemberg, S.L.de S.,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646098806) [2019. Comparison of the dynamics, biokinetics and microbial diversity between](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646098806) [activated sludge flocs and aerobic granular sludge. Bioresour. Technol. 294, 122106.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646098806)
- [Barros, A.R.M., de Lima, J.P.M., Santos, A.F., Firmino, P.I.M., dos Santos, A.B.,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645415507) [Rollemberg, S.L.de S., 2019. Influence of sequencing batch reactor configuration on](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645415507) [aerobic granules growth: Engineering and microbiological aspects. J. Clean. Prod.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645415507) [238, 117906.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645415507)
- [Bengtsson, S., de Blois, M., Wilen, B.M., Gustavsson, D., 2018. Treatment of municipal](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643336048) [wastewater with aerobic granular sludge. Crit. Rev. Environ. Sci. Technol. 48 \(2\),](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643336048) 119–[166](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643336048).
- [Carvalho, C.A., Santos, A.F., Ferreira, T.J.T., Lira, V.N.S.A., Barros, A.R.M., Dos](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646346456) [Santos, A.B., 2021. Resource recovery in aerobic granular sludge systems: is it](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646346456) [feasible or still a long way to go? Chemosphere 274, 129881.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646346456)
- [Coma, M., Verawaty, M., Pijuan, M., Yuan, Z., Bond, P.L., 2012. Enhancing aerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643333498) [granulation for biological nutrient removal from domestic wastewater. Bioresour.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643333498) [Technol. 103 \(1\), 101](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643333498)–108.
- [Corsino, S.F., Capodici, M., Torregrossa, M., Viviani, G., 2016. Fate of aerobic granular](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643331158) [sludge in the long-term: the role of EPSs on the clogging of granular sludge porosity.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643331158) [J. Environ. Manag. 183, 541](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643331158)–550.
- [De Kreuk, M.K., Kishida, N., Van Loosdrecht, M.C.M., 2007. Aerobic granular sludge](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643322638) [state of the art. Water Sci. Technol. 55 \(8](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643322638)–9), 75–81.
- [Derlon, N., Wagner, J., da Costa, R.H.R., Morgenroth, E., 2016. Formation of aerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643324828) [granules for the treatment of real and low- strength municipal wastewater using a](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643324828) [sequencing batch reactor operated at constant volume. Water Res. 105, 341](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643324828)–350.
- [Do Nascimento, J.G. da S., de Araújo, M.H.P., dos Santos, A.B., da Silva, M.E.R.,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1015) [Firmino, P.I.M., 2021. Redox mediator, microaeration, and nitrate addition as](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1015) [engineering approaches to enhance the biotransformation of antibiotics in anaerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1015) [reactors. J. Hazard. Mater. 403, 123932.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1015)
- [dos Santos, A.F., Ferreira, T.J.T., Firmino, P.I.M., dos Santos, A.B., Rollemberg, S.L.de S.,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645090277) [2021. Evaluation of the production of alginate-like exopolysaccharides \(ALE\) and](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645090277) [tryptophan in aerobic granular sludge systems. Bioprocess Biosyst. Eng. 44,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645090277) 259–[270](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270645090277).
- [Felz, S., Al-Zuhairy, S., Aarstad, O.A., van Loosdrecht, M.C., Lin, Y.M., 2016. Extraction](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1020) [of structural extracellular polymeric substances from aerobic granular sludge. J. Vis.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1020) [Exp. 26 \(115\), 54534.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1020)
- [Ferreira, T.J.T., Rollemberg, S.L.S., Barros, A.N., Lima, J.P.M., Dos Santos, A.B., 2021.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646330636) [Integrated review of resource recovery on aerobic granular sludge systems:](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646330636) [Possibilities and challenges for the application of the biorefinery concept. J. Environ.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646330636) [Manag. 291, 112718.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646330636)
- [Franca, R.D.G., Pinheiro, H.M., van Loosdrecht, M.C.M., Lourenço, N.D., 2018. Stability](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643321268) [of aerobic granules during long-term bioreactor operation. Biotechnol. Adv. 36 \(1\),](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643321268) 228–[246](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643321268).
- [Gao, D., Liu, L., Liang, H., Wu, W.M., 2011. Aerobic granular sludge: characterization,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643317268) [mechanism of granulation and application to wastewater treatment. Crit. Rev.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643317268) [Biotechnol. 31, 137](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643317268)–152.
- [Li, A.J., Yang, S.F., Li, X.Y., Gu, J.D., 2008. Microbial population dynamics during](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643310818) [aerobic sludge granulation at different organic loading rates. Water Res. 42,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643310818) [3552](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643310818)–3560.
- [Li, J., Ding, L.B., Cai, A., Huang, G.X., Horn, H., 2014. Aerobic sludge granulation in a](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646319876) [full-scale sequencing batch reactor. BioMed Res. Int. 268789.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646319876)
- [Lin, Y.M., Sharma, P.K., van Loosdrecht, M.C.M., 2013. The chemical and mechanical](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643309528) [differences between alginate-like exopolysaccharides isolated from aerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643309528) [flocculent sludge and aerobic granular sludge. Water Res. 47, 57](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643309528)–65.
- [Liu, Y., Wang, Z.W., Tay, J.H., 2005. A unified theory for upscaling aerobic granular](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643305158) [sludge sequencing batch reactors. Biotechnol. Adv. 23, 335](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643305158)–344.
- [Lu, H., Wan, J., Li, J., Shao, H., Wu, Y., 2016. Periphytic biofilm: a buffer for phosphorus](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643395018) [precipitation and release between sediments and water. Chemosphere 144,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643395018) [2058](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643395018)–2064.
- [Marais, G.V.R., Ekama, G.A., 1976. The activated sludge process. Part I steady state](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643285484) [behaviour. Water SA 2 \(4\), 163](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643285484)–200.
- [McSwain, B.S., Irvine, R.L., Hausner, M., Wilderer, P.A., 2005. Composition and dis](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643379248)[tribution of extracellular polymeric substances in aerobic flocs and granular sludge.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643379248) [Appl. Environ. Microbiol. 71, 1051](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643379248)–1057.
- [Metcalf, L., 2003. Wastewater Engineering: Treatment and Reuse. Metcalf](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647592015) & Eddy Inc. [Nancharaiah, Y.V., Reddy, G.K.K., 2018. Aerobic granular sludge technology:](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647466325)
- [mechanisms of granulation and biotechnological applications. Bioresour. Technol.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647466325) [247, 1128](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647466325)–1143.
- [Ni, B.J., Xie, W.M., Liu, S.G., Yu, H.Q., Wang, Y.Z., Wang, G., Dai, X.L., 2009.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643372948) [Granulation of activated sludge in a pilot-scale sequencing batch reactor for the](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643372948) [treatment of low-strength municipal wastewater. Water Res. 43, 751](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643372948)–761.
- [Peyong, Y.N., Zhou, Y., Abdullah, A.Z., Vadivelu, V., 2012. The effect of organic loading](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647455925) [rates and nitrogenous compounds on the aerobic granules developed using low](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647455925) [strength wastewater. Biochem. Eng. J. 67 \(15\), 52](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647455925)–59.
- [Pijuan, M., Werner, U., Yuan, Z., 2011. Reducing the startup time of aerobic granular](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643351988) [sludge reactors through seeding floccular sludge with crushed aerobic granules.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643351988) [Water Res. 45 \(16\), 5075](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643351988)–5083.
- [Pronk, M., et al., 2015. Full scale performance of the aerobic granular sludge process for](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643350308) [sewage treatment. Water Res. 84, 207](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643350308)–217.
- 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](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1025) [effluent of a pilot scale sequencing batch reactor operated with aerobic granular](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1025) [sludge. Sep. Purif. Technol. 142, 234](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1025)–241.
- [Rollemberg, S.L.S., Barros, A.R.M., Firmino, P.I.M., dos Santos, A.B., 2018. Aerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647449255) [granular sludge: cultivation parameters and removal mechanisms. Bioresour.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647449255) [Technol. 270, 678](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647449255)–688.
- [Rollemberg, S.L.S., de Oliveira, L.Q., Barros, A.R.M., Melo, V.M.M., Firmino, P.I.M.,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647440835) [Santos, A.B., 2019. Effects of carbon source on the formation, stability, bioactivity](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647440835) [and biodiversity of the aerobic granule sludge. Bioresour. Technol. 278, 195](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647440835)–204.
- [Rollemberg, S.L.S., de Oliveira, L.Q., Firmino, P.I.M., Dos Santos, A.B., 2020. Aerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647431525) [granular sludge technology in domestic wastewater treatment: opportunities and](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647431525) challenges. Eng. Sanitária Ambient. 25, 439-449.
- [Rollemberg, S.L.S., de Oliveira, L.Q., de Barros, A.N., Firmino, P.I.M., dos Santos, A.B.,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647356245) [2020. Pilot-scale aerobic granular sludge in the treatment of municipal wastewater:](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647356245) [optimizations in the start-up, methodology of sludge discharge, and evaluation of](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647356245) [resource recovery. Bioresour. Technol. 311, 123467](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647356245).
- [Rollemberg, S.L.S., Barros, A.N., Firmino, P.I.M., dos Santos, A.B., 2022. Evaluation of](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1010) [sludge discharge methodologies in aerobic granular sludge reactors. Bioresour.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1010) [Technol. Rep. 18, 101018](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1010).
- Royal HaskoningDHV, 2022. Available in the website: [https://global.royalhaskoningdhv.](https://global.royalhaskoningdhv.com/nereda) [com/nereda.](https://global.royalhaskoningdhv.com/nereda) Accessed in 22/06/22.
- [van der Roest, H.F., de Bruin, L.M.M., Gademan, G., Coelho, F., 2011. Towards](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647343525) [sustainable wastewater treatment with Dutch Nereda](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647343525)® technology. Water Pract. [Technol. 6, 3](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647343525)–6.
- [Van Dijk, E., Pronk, M., van Loosdrecht, M., 2018. Controlling effluent suspended solids](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647323245) [in the aerobic granular sludge process. Water Res. 147, 50](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647323245)–59.
- [van Haandel, A.C., Lettinga, G., 1994. Anaerobic Sewage Treatment, , 1. ed.1. John](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647311095) [Wiley and Sons, Chichester. 226p](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647311095).
- [van Haandel, A.C., Lubbe, J.V.D., 2011. Handbook Biological waste water treatment](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647043796) [second edition. In: Design and Optimization of Activated Sludge Systems, , 01. ed.01.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647043796) [IWA Publishing of Alliance House, London. 816p](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270647043796).
- [Van Leeuwen, K., De Vries, E., Koop, S., Roest, K., 2018. The energy](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1005) & raw materials [factory: role and potential contribution to the circular economy of the Netherlands.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1005) [Environ. Manag. 61, 786](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf1005)–795.
- [Verawaty, M., Pijuan, M., Yuan, Z., Bond, P.L., 2012. Determining the mechanisms for](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643349068) [aerobic granulation from mixed seed of floccular and crushed granules in activated](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643349068) [sludge wastewater treatment. Water Res. 46, 761](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643349068)–771.
- Wagner, J., Guimarães, L.B., Akaboci, T.R.V., Costa, R.H.R., 2015. Aerobic granular [sludge technology and nitrogen removal for domestic wastewater treatment. Water](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643346338) [Sci. Technol. 71 \(7\), 1040](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643346338)–1046.
- [Wan, C., Zhang, P., Lee, D.J., Yang, X., Liu, X., Sun, S., Pan, X., 2013. Disintegration of](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643345008) [aerobic granules: role of second messenger cyclic di-GMP. Bioresour. Technol. 146,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643345008) 330–[335](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643345008).
- [Wang, S.G., Liu, X.W., Gong, W.X., Gao, B.Y., Zhang, D.H., Yu, H.Q., 2007. Aerobic](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643340028) [granulation with brewery wastewater in a sequencing batch reactor. Bioresour.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643340028) [Technol. 98, 2142](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643340028)–2147.
- [Wang, J., Li, W.W., Yue, Z.B., Yu, H.Q., 2014. Cultivation of aerobic granules for](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643343338) [polyhydroxybutyrate production from wastewater. Bioresour. Technol. 159,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643343338) 442–[445](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643343338).
- [Wang, L., Wu, L., Li, A., Hou, B., Jiang, X., 2018. Synergy of N-\(3-oxohexanoyl\)-l](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643342008)[homoserine lactone and tryptophan-like outer extracellular substances in granular](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643342008) [sludge dominated by aerobic ammonia-oxidizing bacteria. Appl. Microbiol.](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643342008) [Biotechnol. 102 \(24\), 10779](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643342008)–10789.
- [Wu, D., et al., 2018. Optimization of F/M ratio for stability of aerobic granular process](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646356186) [via quantitative sludge discharge. Bioresour. Technol. 252 \(866\), 150](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270646356186)–156.
- [Zhang, D., Li, W., Hou, C., Shen, J., Jiang, X., Sun, X., Li, J., Han, W., Wang, L., Liu, X.,](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643338758) [2017. Aerobic granulation accelerated by biochar for the treatment of refractory](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643338758) [wastewater. Chem. Eng. J. 314, 88](http://refhub.elsevier.com/S2589-014X(22)00229-8/rf202207270643338758)–97.