

## Effect of the co-treatment of synthetic faecal sludge and wastewater in an aerobic granular sludge system

Barrios-Hernández, Mary Luz; Buenaño-Vargas, Claribel; García, Hector; Brdjanovic, Damir; van Loosdrecht, Mark C.M.; Hooijmans, Christine M.

**DOI**

[10.1016/j.scitotenv.2020.140480](https://doi.org/10.1016/j.scitotenv.2020.140480)

**Publication date**

2020

**Document Version**

Final published version

**Published in**

Science of the Total Environment

**Citation (APA)**

Barrios-Hernández, M. L., Buenaño-Vargas, C., García, H., Brdjanovic, D., van Loosdrecht, M. C. M., & Hooijmans, C. M. (2020). Effect of the co-treatment of synthetic faecal sludge and wastewater in an aerobic granular sludge system. *Science of the Total Environment*, 741, Article 140480. <https://doi.org/10.1016/j.scitotenv.2020.140480>

**Important note**

To cite this publication, please use the final published version (if applicable).  
Please check the document version above.

**Copyright**

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

**Takedown policy**

Please contact us and provide details if you believe this document breaches copyrights.  
We will remove access to the work immediately and investigate your claim.



# Effect of the co-treatment of synthetic faecal sludge and wastewater in an aerobic granular sludge system

Mary Luz Barrios-Hernández<sup>a,b,c,\*</sup>, Claribel Buenaño-Vargas<sup>a</sup>, Hector García<sup>a</sup>, Damir Brdjanovic<sup>a,b</sup>, Mark C.M. van Loosdrecht<sup>b</sup>, Christine M. Hooijmans<sup>a</sup>

<sup>a</sup> Department of Environmental Engineering and Water Technology, IHE-Delft Institute for Water Education, P.O. Box 3015, 2601 DA Delft, the Netherlands

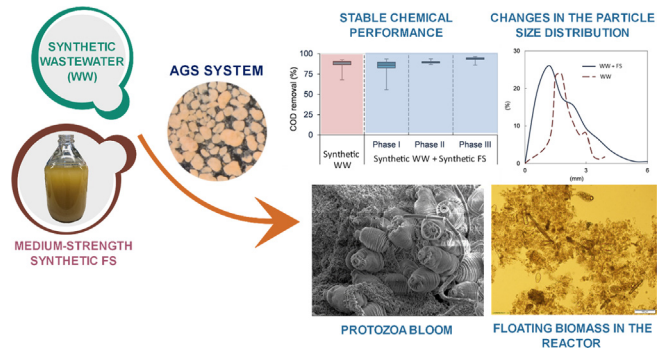
<sup>b</sup> Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, the Netherlands

<sup>c</sup> Ingeniería Ambiental, Instituto Tecnológico de Costa Rica, Cartago 159-7050, Costa Rica

## HIGHLIGHTS

- Synthetic FS well-represented digested medium-strength FS originating from septic tanks.
- The addition of FS in an AGS reactor decreased the granular sludge settleability.
- A high amount of solids accumulated in the reactor and left the system via the effluent.
- The granular sludge size was reduced, and more flocculent sludge was observed.
- A sudden bloom of ciliates protozoa occurred, contributing to solids removal.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 27 March 2020

Received in revised form 22 June 2020

Accepted 22 June 2020

Available online 23 June 2020

Editor: Yifeng Zhang

### Keywords:

Aerobic granular sludge  
Co-treatment  
Digested faecal sludge  
Suspended solids  
Protozoa  
*Vorticella* spp.

## ABSTRACT

The co-treatment of two synthetic faecal sludges (FS-1 and FS-2) with municipal synthetic wastewater (WW) was evaluated in an aerobic granular sludge (AGS) reactor. After characterisation, FS-1 showed the following concentrations, representative for medium-strength FS: 12,180 mg TSS L<sup>-1</sup>, 24,300 mg total COD L<sup>-1</sup>, 93.8 mg PO<sub>3</sub>-P L<sup>-1</sup>, and 325 mg NH<sub>4</sub>-N L<sup>-1</sup>. The NO<sub>3</sub>-N concentration was relatively high (300 mg L<sup>-1</sup>). For FS-2, the main difference with FS-1 was a lower nitrate concentration (18 mg L<sup>-1</sup>). The recipes were added consecutively, together with the WW, to an AGS reactor. In the case of FS-1, the system was fed with 7.2 kg total COD m<sup>-3</sup>d<sup>-1</sup> and 0.5 kg Nitrogen m<sup>-3</sup>d<sup>-1</sup>. Undesired denitrification occurred during feeding and settling resulting in floating sludge and wash-out. In the case of FS-2, the system was fed with 8.0 kg total COD m<sup>-3</sup>d<sup>-1</sup> and 0.3 kg Nitrogen m<sup>-3</sup>d<sup>-1</sup>. The lower NO<sub>3</sub>-N concentration in FS-2 resulted in less floating sludge, a more stabilised granular bed and better effluent concentrations. To enhance the hydrolysis of the slowly biodegradable particulates from the synthetic FS, an anaerobic stand-by period was added and the aeration period was increased. Overall, when compared to a control AGS reactor, a lower COD consumption (from 87 to 35 mg g<sup>-1</sup> VSS h<sup>-1</sup>), P-uptake rates (from 6.0 to 2.0 mg P g VSS<sup>-1</sup> h<sup>-1</sup>) and NH<sub>4</sub>-N removal (from 2.5 to 1.4 mg NH<sub>4</sub>-N g VSS<sup>-1</sup> h<sup>-1</sup>) were registered after introducing the synthetic FS. Approximately 40% of the granular bed became flocculent at the end of the study, and a reduction of the granular size accompanied by higher solids accumulation in the reactor was observed. A considerable protozoa *Vorticella* spp. bloom attached to the granules and the accumulated particles occurred; potentially contributing to the removal of the suspended solids which were part of the FS recipe.

© 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

\* Corresponding author at: Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, the Netherlands.  
E-mail address: [M.L.BarriosHernandez@tudelft.nl](mailto:M.L.BarriosHernandez@tudelft.nl) (M.L. Barrios-Hernández).

## 1. Introduction

The term faecal sludge (FS) is used to describe all materials collected from on-site sanitation solutions when the systems are deslugged. The sludge generated in such facilities is highly concentrated in suspended solids, organic matter, and other nutrients; therefore, it needs a suitable treatment option to avoid the contamination of water resources and the risk to public health. According to [Strauss and Montangero \(2002\)](#) and [Lopez-Vazquez et al. \(2014\)](#), FS can be classified based on the retention time of the sludge in the storage/collection container as either fresh or digested sludge. Fresh sludge refers to the sludge that is disposed of, for instance, after being frequently emptied from non-sewered public toilets or bucket toilets (retention times of approximately several days up to a week). Digested sludge refers to sludge that is retained in the storage/collection container for several months or even years, and has undergone a biochemical degradation process (e.g. sludge from septic tanks and/or pit latrines). This degradation process depends on factors such as temperature, retention time, presence of inhibiting substances, water content, among others ([Strauss and Montangero, 2002](#); [Tayler, 2018](#)). Indeed, it may lead to the appearance of certain chemical compounds that were not present in the original FS, for instance, different nitrogen species, i.e.  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  ([Siegrist, 2017](#)). Moreover, the sludge can also be classified considering physicochemical characteristics as low, medium, and high strength FS ([Strande et al., 2014](#); [Tayler, 2018](#)). Practices for FS treatment include landfill disposal, land treatment (agriculture practices), discharge in sludge treatment facilities (e.g. sludge drying beds, ponds, wetlands, anaerobic treatment systems, among others), and co-treatment with domestic wastewater ([Siegrist, 2017](#)).

Regarding the co-treatment with domestic wastewater, [U.S. EPA \(1994\)](#) recommends diluting FS with the wastewater 20 times (5% v/v) when FS is added before the pre-treatment of a municipal wastewater treatment plant (WWTP). Adverse effects on the performance of WWTPs can be expected especially when the facility is not designed to co-treat FS. The co-treatment of FS in conventional activated sludge (CAS) WWTPs can severely compromise the quality of the treated effluent regarding the total suspended solids (TSS), the chemical oxygen demand (COD), and the nitrogen (N) and phosphorus (P) concentrations ([Dangol, 2017](#); [Strande et al., 2014](#)). Higher oxygen demand in the biological reactor, odour issues, and the formation of scum and foam in the settling tanks can occur ([Heinss and Strauss, 1999](#)). Furthermore, based on a modelling study, [Lopez-Vazquez et al. \(2014\)](#) recommended feeding small fractions of FS, (less than 1% (v/v) of fresh or digested FS of the main influent wastewater flow, to avoid disrupting the performance of the system.

The aerobic granular sludge (AGS) process has been recently implemented as a more efficient wastewater treatment alternative compared to CAS systems ([de Kreuk et al., 2007](#); [Sepúlveda-Mardones et al., 2019](#)). Advantages of AGS include lower footprint and energy costs, and a better organic matter and nutrient removal performance ([Pronk et al., 2015b](#)). Two full-scale AGS-WWTPs are mentioned to be designed to co-treat FS from septic tanks ([Pronk et al., 2017](#)) and pit latrines ([Khan et al., 2015](#)) with wastewater. Despite the organic load variability, which is typically encountered in WWTPs co-treating FS, the authors did not report any significant adverse effects on the performance of the full-scale AGS systems, but details on the amount of FS that is co-treated are missing.

It has been demonstrated that the AGS technology can handle high organic loading rates (OLR) with a high nitrogen content when treating different kinds of substrates. [Figueroa et al. \(2011\)](#) showed organic matter and nitrogen removal efficiencies of 87% and 70%, respectively, when treating swine slurry with an OLR of  $4.4 \text{ kg COD m}^{-3}\text{d}^{-1}$  and  $0.83 \text{ kg N m}^{-3}\text{d}^{-1}$ . Moreover, [Abdullah et al. \(2013\)](#) observed the formation of compact granules when treating palm oil mill effluents ranging from  $2.5$  to  $3.5 \text{ kg COD m}^{-3}\text{d}^{-1}$ . Other researchers studied different operational conditions necessary to develop healthy granules and good

system performance when treating different proportions of highly concentrated leachate, citrus and agro-industrial digestate ([Bueno et al., 2020](#); [Carucci et al., 2020](#); [Corsino et al., 2018](#)).

However, with regards to FS, no laboratory research has been done on granular formation and system stability (cycle performance) when adding such a complex substrate to an AGS system. [Penn et al. \(2018\)](#) recommended the use of a FS simulant that can be adapted to the research requirements. The use of a reproducible and a consistent synthetic substrate can contribute clarifying the implication of co-treating FS in an AGS system with respect to overall behaviour, the granule formation/stability and the presence of protozoa community. This study aims at assessing the performance of a laboratory-scale AGS system, designed to treat only synthetic wastewater (WW), before and after replacing approximately 4% (v/v) synthetic, medium-strength FS in the total influent WW. The fraction of FS added to the system represents approximately a 20-fold dilution with a synthetic municipal wastewater, as recommended by [U.S. EPA \(1994\)](#), and is significantly higher than recommended by [Lopez-Vazquez et al. \(2014\)](#).

## 2. Materials and methods

### 2.1. Research design

Two AGS laboratory-scale systems were used, one reactor fed with synthetic municipal WW and a second reactor fed a mixture of synthetic FS and WW. The FS was introduced as 4% (v/v) of the WW flow, resulting in an influent with a much higher TSS, organic matter, and nutrient concentration than the influent of a control reactor. The influent was prepared by mixing both solutions (FS and WW) before entering the system. The effects of the FS addition on the reactor performance, the granulation process and morphology, and the occurrence of protozoa in the AGS system were analysed at different operational conditions in the reactor and compared to the control.

### 2.2. Development of the synthetic FS recipe

The synthetic FS developed in this study was based on synthetic recipes obtained from the literature for faeces and urine with high organic and nutrient fractions. [Table 1](#) describes the urine and faeces composition used. The final synthetic FS recipe is based on the assumption that real FS is composed of 10% faeces, 9% urine, and 81% water.

**Table 1**

Urine composition (for 1 L of water) and measurements for the preparation of the synthetic faeces.

Classification	Components for FS recipe	
Urine FS-1	$\text{NH}_4\text{NO}_3$ (g)	19.2
	$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (g)	2.7
	KCl (g)	3.4
	$\text{KHCO}_3$ (g)	1.1
	$\text{Na}_2\text{SO}_4$ anhydrous (g)	2.3
	NaCl (g)	3.6
Urine FS-2	HCl 32% (mL)	0.4
	$\text{NH}_4\text{Cl}$ (g)	4.0
	$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (g)	3.8
	Cellulose (g)	24.1
	Psyllium husk (g)	21.1
Faeces FS-1 and FS-2	Yeast extract (10–12.5 w/w% total N) (g)	36.1
	Miso paste (g)	21.1
	Olive oil (mL)	12.1
	NaCl (g)	2.4
	KCl (g)	2.4
	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (g)	1.2
	Demineralised water (mL)	380

### 2.2.1. Faeces recipe

The synthetic faeces medium was prepared based on a recipe described by Penn et al. (2018). The recipe was adapted to simulate a medium-strength synthetic FS. Cellulose, which is insoluble in water and slowly biodegradable, mimicked the particulate fraction of faeces. Psyllium husk was used as a source of dietary fibres and carbohydrates. Yeast extract and *E. coli* represented the bacterial content. Miso paste was used as a source of proteins, fats, fibres and minerals. Olive oil introduces the fat content (oleic acids) present in faeces. Other required inorganic minerals such as NaCl, KCl, and CaCl<sub>2</sub> were added as well. All the components were mixed and dissolved in demineralised water.

### 2.2.2. Synthetic urine

Two synthetic recipes for urine were used in this research. First, the solution was prepared as proposed by Udert and Wächter (2012). Hereafter, a new urine recipe was considered better reflecting fresh (rather than stored) urine, with a much lower nitrate concentration. The variation in the urine recipes resulted in two synthetic FS recipes, FS-1 and FS-2.

### 2.3. Reactors set-up

Two double wall column reactors were installed and operated as sequencing batch AGS reactors as described in Winkler et al. (2012). Both reactors had a diameter of 60 mm and a height of 1400 mm, with 2.9 L of working volume. A bio-Controller (Applikon ADI1030, The Netherlands) and a bio-consoler (Applikon ADI1025, the Netherlands) automatically controlled the systems by continuously monitoring and adjusting the pH, dissolved oxygen (DO), and temperature; the pH was adjusted to a value of 7.1 by dosing 1 M NaOH or 1 M HCl solutions to the bulk liquid of the AGS reactors during the aerobic phase. The temperature was set to 20 °C. The air was recirculated by the aid of a recirculation pump (KNF, Germany) at an airflow rate of 6 L min<sup>-1</sup>; in such way, sufficient shear forces were provided to the reactors during the aerobic phase, while maintaining the DO concentration at the required set points of 1.8 to 4.2 mg L<sup>-1</sup>. The effluent was discharged in the middle of the column (1.5 L); therefore, the exchange ratio was approximately 49% for both reactors.

### 2.4. Experimental procedures

The two AGS reactors were inoculated with granules from a full-scale AGS-WWTP located in Vroomshoop, the Netherlands. The initial TSS and volatile suspended solids (VSS) concentrations of the inoculum were 11.7 ± 2.6 g L<sup>-1</sup> and 9.5 ± 2.1 g L<sup>-1</sup>, respectively. The sludge volume index granules after 5 (SVI<sub>5</sub>) and 30 min (SVI<sub>30</sub>) was 40.1 mL g<sup>-1</sup> and 35.0 mL g<sup>-1</sup>, respectively. Sieved granules showed 67% of the particle size bigger than 0.22 mm, 30% between 1.0 and 2.2 mm and the rest smaller the 1.0 mm. Granules were crushed after characterisation and added to the AGS reactors.

One reactor was operated as a control for 215 days fed the synthetic WW (control reactor). The second reactor was operated for 209 days (AGS-FS reactor). As described in Table 2, both systems were initially fed with the same synthetic WW (Phase I) and operated in 3-hour cycles as follows: 60 min anaerobic feeding, 110 min aeration, 5 min settling, and 5 min effluent discharge. After 68 days of operation, the synthetic FS solution was added to the AGS-FS reactor as 4% (v/v) of the total synthetic WW influent flow (Phase II). To ensure an optimal reactor performance, the 3-hour cycle was extended to 4 h as follows: (i) an additional anaerobic stand-by period of 30 min was added (to enhance the hydrolysis of the slowly biodegradable particulates from the synthetic FS); (ii) a longer settling time of 10 min was introduced; and (iii) the aeration period was extended to 135 min. During Phase III, the AGS-FS reactor operational conditions were slightly adjusted to a settling time of 5 min, and to a longer aeration time of 140 min. The

**Table 2**

Operational conditions: control and AGS-FS reactors.

Operational conditions	Control reactor	Phases in the AGS-FS reactor		
		I	II	III
Feeding (min)	60	60	60	60
Stand-by period	0	0	30	30
Synthetic WW	✓	✓	✓	✓
<i>E. coli</i> medium			✓	✓
FS			✓	✓
Aerobic phase (min)	110	110	135	140
DO (50%)	✓	✓	✓	
DO (20%)	✓			✓
Settling (min)	5	5	10–5	5
Effluent discharge (min)	5	5	5	5
Number of cycles per day	8	8	6	6
Hydraulic retention time	5.8	5.8	6.8	7.7
Cycle length (h)	3	3	4	4
OLR (kg COD m <sup>-3</sup> d <sup>-1</sup> )	2.9	2.9	7.2	8.0
NLR (kg N m <sup>-3</sup> d <sup>-1</sup> )	0.4	0.4	0.5	0.3
PLR (kg PO <sub>3</sub> -P m <sup>-3</sup> d <sup>-1</sup> )	0.06	0.06	0.06	0.06
Operational days	215	67	29	113

operational conditions on the control reactor were kept constant during the entire evaluated period.

### 2.4.1. Media composition

Both the control and the AGS-FS reactors were fed synthetic WW. The synthetic WW medium was prepared as described in de Kreuk et al. (2010) by combining a carbon source with a nutrient solution enriched with the Vishniac and Santer (1957) trace element solution. An *E. coli* solution was added to the reactor AGS-FS in Phases II and III; it was prepared by adding 25 mL of a stock solution (1 × 10<sup>9</sup> CFU 100 mL<sup>-1</sup> of *E. coli* ATCC® 25992) to a vessel of 10 L of demineralised water. During Phase II, the AGS-FS reactor fed a mixed influent flow containing synthetic WW and the prepared synthetic FS-1 solution, and synthetic FS-2 solution during Phase III. Table 2 shows the loading rates added the system per phase.

### 2.5. Analytical determinations

#### 2.5.1. Physicochemical analytical measurements

The FS recipes were characterised by measuring the total and soluble COD, nitrogen compounds, phosphate content, VSS and TSS immediately after preparation. Additionally, the nitrogen content was monitored in the FS recipes (FS-1 and FS-2) after three days of preparation to determine their variability. For the reactors, their performance was evaluated by weekly monitoring standard water quality parameters such as soluble COD, nitrogen compounds, and phosphate in samples taken from the influent, after the anaerobic feeding, after the anaerobic stand-by period and effluent of the AGS-FS and the control reactor. The following treated effluent standards were considered as reference for a good reactor performance: COD <125 mg L<sup>-1</sup>, the sum of the NH<sub>4</sub>-N, NO<sub>3</sub>-N and NO<sub>2</sub>-N < 15 mg L<sup>-1</sup> and PO<sub>4</sub>-P < 2 mg L<sup>-1</sup> (Kirhensteine et al., 2016). The COD measurement was performed according to the Closed Reflux-Colorimetric Standard Method (APHA, 2012). NH<sub>4</sub>-N, NO<sub>3</sub>-N, NO<sub>2</sub>-N and PO<sub>4</sub>-P were measured using LCK cuvette tests (manufacturer: Hach®). Samples were filtered through a 0.45 µm Millipore™ membrane filters previous the analysis. TSS and VSS were determined according to the Standard Methods for sludge and effluent samples (APHA, 2012). The solids retention time (SRT) was calculated considering TSS concentrations in both the reactor and the treated effluent. The granular bed was weekly measured using the column volume scale previously implemented in the glass columns.



### 2.5.2. Sludge settle-ability and (granular) size distribution

The sludge settle-ability was determined by the SVI<sub>5</sub> as in Pronk et al. (2015a). Samples were taken on days 69, 96, and 150 from the control reactor; while on day 57 (Phase I), 102 (Phase II) and 144 (Phase III) from the AGS-FS reactor. On day 69 and 150, the sludge was separated using a sieve with a particle size of 212 µm to determine the granular (>212 µm) and the flocculent (<212 µm) fractions. To determine the average granular size distribution, additional sludge samples were taken on day 118 from the control reactor and from the AGS-FS reactor. The size of the granules was measured by taken images with an Olympus SC50 camera adapted to an optical microscope; the images were later analysed with the ImageJ software (Rasband, 1997–2018). The information was processed using statistical distributions and presented in histograms.

### 2.5.3. Granular morphology and structural composition of the granules

The morphology and microstructural composition of the granules were observed using a scanning electron microscope (SEM). Analyses were carried out in granular samples taken on the operational days 10, 64 and 103 of the AGS-FS reactor; and on day 110 of the control reactor. For the SEM determinations, the samples were fixated with a 0.5% formaldehyde and 0.5% glutaraldehyde solution after rinsing the samples three times with a phosphate buffer solution (1 M K<sub>2</sub>HPO<sub>4</sub> and 1 M KH<sub>2</sub>PO<sub>4</sub>) at pH 7.2. After providing enough fixation time (16 h), the samples were dehydrated using first an ethanol/water solution gradually increasing the ethanol/water ratio as follows: 30, 50, 70, 80, 90, and 100%. The solution was replaced three times at each ratio every 10 min. Later, the same procedure was performed but using acetone instead of ethanol. The dried samples were finally placed on stubs using double-sided carbon adhesives double-coated with a gold sputter-coater before applying imaging in a JSM-6610 SEM at an acceleration voltage ranged from 5 to 10 kV.

### 2.6. Data analysis

Shapiro-Wilk normality test was applied to the measuring water quality parameters. Since not all the tested data were normally distributed, the non-parametric Wilcoxon rank-sum test (W) was used to compare the mean rank between the measurements taken from the influent and effluent samples both after the addition of the FS recipe (Phases II and III). A *p*-value ≤ 0.5 was used to indicate significance. Results were computed (1) to assess whether the measured parameters were significantly different from the media (substrate concentration variability); (2) to determine whether the two FS recipes were significantly different from each other, and (2) to evaluate whether the performance based on effluent concentrations of the AGS system differed when comparing the two studied phases.

Moreover, the Spearman's rank correlation ( $\alpha = 5\%$ ) was applied to the solid concentrations in the reactor vs effluent to determine the relationship between biomass grow/accumulation in the reactor and the concentration measured in the effluent. The analyses were performed using R Core Team (2020).

## 3. Results

### 3.1. Characterisation of the medium-strength synthetic FS

Table 3 shows the average characterisation achieved for the FS recipe and the composition of the combined synthetic WW + FS. Apart of the PO<sub>3</sub>-P concentrations, the Wilcoxon rank-sum test executed to the average COD and NH<sub>4</sub>-N measurements showed a significant difference (*p* < 0.05) during Phase II and Phase III (Table 4), representing two different recipes. Their addition to the AGS reactor resulted in fluctuations in the COD, nitrogen and PO<sub>3</sub>-P content of the combined influent (Fig. 1, Phase II and Phase III time 0 min). However, except for the nitrogen content, the concentrations that were weekly measured were not

**Table 3**

Chemical characterisation of municipal synthetic WW, FS synthetic recipe, and mixed influent solutions.

Parameter	Concentrations (mg L <sup>-1</sup> )			Concentrations of combined influent (mg L <sup>-1</sup> )	
	WW	FS-1	FS-2	WW + FS-1	WW + FS-2
TSS	0	12,180	9702	307	307
VSS	0	10,910	9468	250	250
COD total	403	24,300	29,740	1170	1477
COD soluble	403	11,450	9960	795	1151
NH <sub>4</sub> -N	60	325	277	86	62
NO <sub>3</sub> -N	0.0	300	18	10.8	1.3
NO <sub>2</sub> -N	0.0	0.04	0.02	12.4	0.1
PO <sub>4</sub> -P	8.5	93.8	96.7	13	12

significantly different from the median value (*p* > 0.05). The *p*-values are presented in the Supplementary material Table S1. The separated degradation test performed to the FS recipes after three days of preparation confirmed that the NO<sub>3</sub>-N concentration increased after three days from 14.9 to 22.2 mg NO<sub>3</sub>-N L<sup>-1</sup> and 16.0 to 20.5 mg NO<sub>3</sub>-N L<sup>-1</sup> in the FS-1 and FS2, respectively. This effect confirmed that the variations in the mixed influent concentrations were a result of the quick degradation of the FS recipe.

### 3.2. Evaluating the continuous performance of the AGS reactors

Fig. 1 shows the average COD, PO<sub>4</sub>-P and nitrogen concentrations for the control and AGS-FS reactor. The results are presented at different stages of the cycles of the AGS reactor as follows: (i) influent (at 0 min); (ii) after anaerobic feeding (62 min); (iii) after anaerobic standby (92 min); and (iv) treated effluent (180 min for the control, and 240 min for Phase II and III in the AGS-FS reactor). Additionally, Fig. 2 shows the cycle's profiles for the AGS-FS reactor after the addition of the FS. Weekly measurements are shown in Supplementary material Figs. S1 and S2.

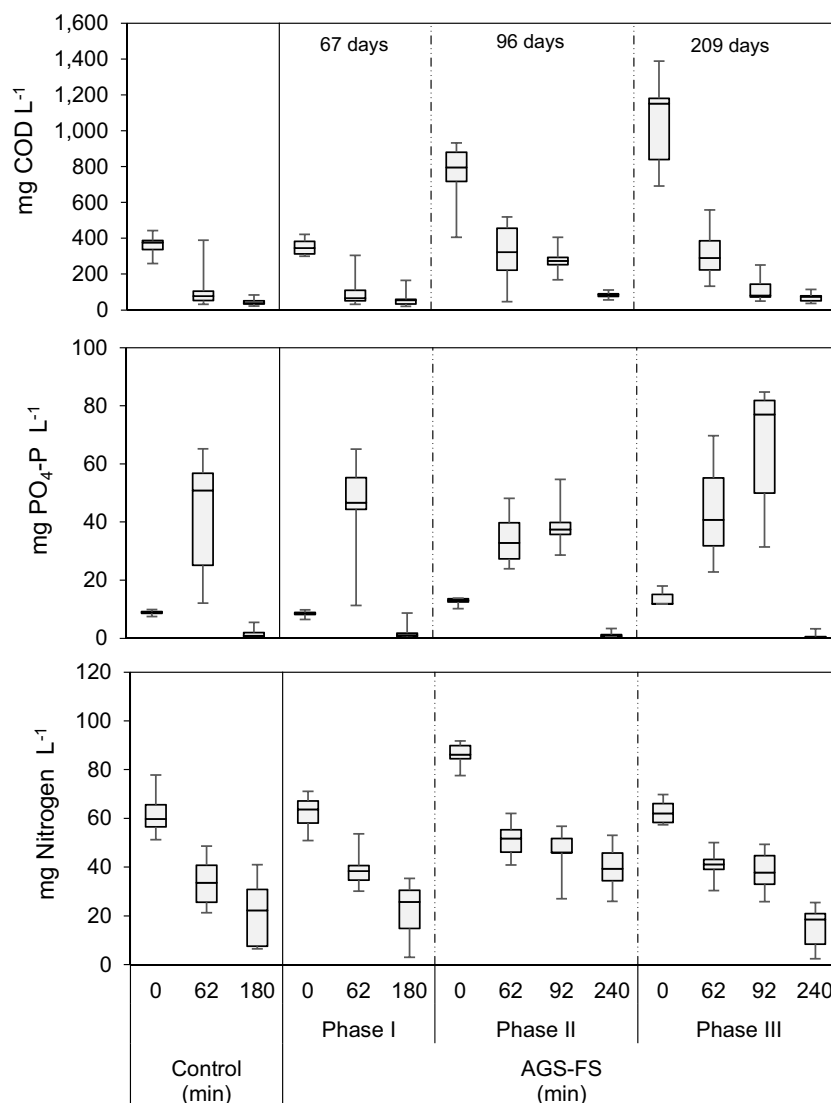
In the control reactor, on average, 80% of the COD was consumed during the anaerobic feeding (from 376 to 77 mg COD L<sup>-1</sup>). The average consumption rate during this the anaerobic feeding was 86.8 mg COD g VSS<sup>-1</sup> h<sup>-1</sup>. The rest (up to 90% of the COD) was consumed during aeration. It resulted in an effluent concentration of 38 mg COD L<sup>-1</sup>, corresponding mostly to the EDTA added in the recipe (which is non-biodegradable). Regarding the PO<sub>4</sub>-P, removal of more than 90% was observed starting on day 45 and onwards. The phosphate accumulating organisms (PAOs) community seemed to be present and active releasing PO<sub>4</sub>-P in the anaerobic phase of the cycles at concentrations ranging from 22 mg PO<sub>4</sub>-P L<sup>-1</sup> to 67 mg PO<sub>4</sub>-P L<sup>-1</sup> on operational days 27 and 67, respectively. The net PO<sub>4</sub>-P uptake during the aeration phase of the cycle was 6.0 mg P g VSS<sup>-1</sup> h<sup>-1</sup>, resulting in an average effluent concentration of 0.87 mg PO<sub>4</sub>-P L<sup>-1</sup>.

Regarding the nitrogen removal performance of the reactor, the ammonium-nitrogen concentrations in the influent (65 mg NH<sub>4</sub>-N L<sup>-1</sup>) was reduced by approximately half (32 mg NH<sub>4</sub>-N L<sup>-1</sup>) after one-hour feeding (62 min) in the anaerobic phase mostly due to

**Table 4**

*p*-Values of the comparison between the component of the FS recipe added in Phase II and Phase III in the mixed influent, and from the performance of the reactor based on effluent concentrations. The starts (\*) flag the levels of significance, "ns" = not significant. Number of samples 23.

Parameter	Mixed influent			Treated effluent		
	W	<i>p</i> -Value		W	<i>p</i> -Value	
COD	23	0.013	*	83	0.07	ns
N	126	<0.0001	****	126	<0.0001	****
PO <sub>3</sub> -P	35	0.66	ns	88	0.12	ns



**Fig. 1.** Water quality performance of the control and the AGS-FS reactor at the different sampling points: influent (0 min), after anaerobic feeding (62 min), after anaerobic stand-by (92 min); and treated effluent (180 min for the control, and 240 min for Phase II and III in the AGS-FS reactor).

dilution effects of the cycle. During the aeration cycle, an average removal rate for ammonium-nitrogen of  $2.5 \text{ mg NH}_4\text{-N g VSS}^{-1} \text{ h}^{-1}$  was reported (after operational day 47 and onwards). When starting up the control reactor, ammonium-nitrogen effluent concentrations were ranging from approximately 15 to  $41 \text{ mg NH}_4\text{-N L}^{-1}$ . Complete nitrification was observed after operating the reactor for approximately 102 days; ammonium-nitrogen concentrations lower than  $0.7 \text{ mg NH}_4\text{-N L}^{-1}$  were measured. The average nitrogen measured in the effluent was  $22 \text{ mg L}^{-1}$  for the evaluated period. The ammonium-nitrogen was almost completely nitrified and the total nitrogen observed in the effluent corresponded mostly to  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  indicating that full denitrification was not achieved. During the operation of the reactor, the DO saturation set-point was reduced from 50% to 20% (as indicated in Table 2) and improvements on the  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  effluent values were observed from 16 to  $5.4 \text{ mg NO}_2\text{-N L}^{-1}$  and from 5.7 to  $1.4 \text{ mg NO}_3\text{-N L}^{-1}$ . The AGS-FS reactor during Phase I (66 operational days) showed similar trends as the control reactor.

In Phase II of the AGS-FS reactor, starting on operational day 67, the carbon source increased to an average value of  $795 \text{ mg COD L}^{-1}$  in the anaerobic feeding phase. As can be observed in Fig. 2a, the operational changes introduced during this studied period (i.e. an extra anaerobic stand by period of 30 min) resulted in an average reduction of the organic matter concentration from  $320 \text{ mg COD L}^{-1}$  at 62 min (end of

anaerobic phase) to  $274 \text{ mg COD L}^{-1}$  at 92 min (end of stand-by period). The specific anaerobic consumption rate (including the stand-by period) of this phase was  $35.4 \text{ mg COD g VSS}^{-1} \text{ h}^{-1}$ . Contrary to Phase I, at the operational day 81 showed a potential lower PAOs activity since not all the acetate was taken up; concentrations of acetate of  $128 \text{ mg Ac L}^{-1}$ ,  $50 \text{ mg Ac L}^{-1}$ , and  $1.6 \text{ mg Ac L}^{-1}$  were reported at 62 min, 92 min, and in the effluent, respectively.

The P-uptake ratio was also reduced compared to the performance of the reactor during Phase I, from  $6.0$  to  $2.0 \text{ mg P g VSS}^{-1} \text{ h}^{-1}$ . P-release values during the anaerobic feeding (62 min) from 24 to  $48 \text{ mg PO}_4\text{-P L}^{-1}$  (average of  $41 \text{ mg PO}_4\text{-P L}^{-1}$ ) were measured; lower than the P-release values in Phase I (from 22 to  $67 \text{ mg PO}_4\text{-P L}^{-1}$ ). No additional activity was observed by the PAOs during the extra (added) anaerobic period. The observed concentration in the treated effluent was on average  $0.7 \text{ mg PO}_4\text{-P L}^{-1}$ .

On the subject of the nitrogen removal, the average ammonium-nitrogen removal rate dropped from 2.5 (Phase I) to  $1.2 \text{ mg NH}_4\text{-N g VSS}^{-1} \text{ h}^{-1}$ ; consequently, the average ammonium-nitrogen concentration in the treated effluent increased up to  $19 \text{ mg NH}_4\text{-N L}^{-1}$ . In addition, the average nitrite concentrations increased from 7.6 to  $15 \text{ mg NO}_2\text{-N L}^{-1}$ , while nitrate went from 4.1 to  $5.2 \text{ mg NO}_3\text{-N L}^{-1}$ . However, it is important to highlight that the AGS-FS (Phase II) influent contains both  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  as described in Table 3 originated from the

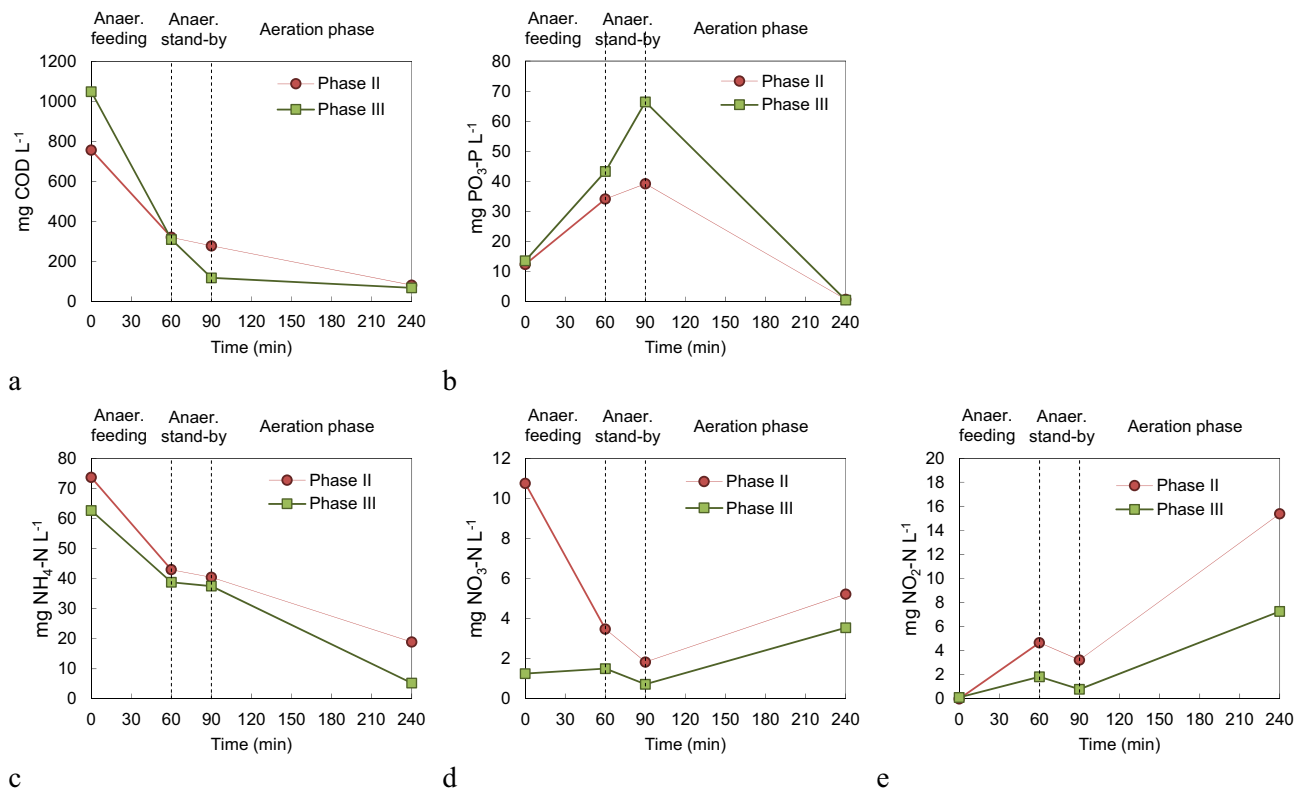


Fig. 2. Average water quality parameters profiles per cycle after addition of the FS (Phase II and III) in the AGS-FS reactor.

initial FS components (miso paste) and its potential decomposition. 39 mg Nitrogen  $\text{L}^{-1}$  was measured in the treated effluent; although more than 50% was removed, nitrification and denitrification did not occur at full extent.

During Phase III of the operation of the AGS-FS reactor, the composition of the synthetic FS recipe was modified (as described in Table 3). The new FS recipe contained a higher organic content, resulting in a higher COD concentration (average 1152 mg COD  $\text{L}^{-1}$ ) in the AGS-FS during this phase (Fig. 2a). Moreover, nitrite (Fig. 2e) was not added to the new synthetic FS, resulting in a better P uptake performance during the anaerobic feeding phase. The average COD uptake during the anaerobic period in Phase III increased, the COD concentration went from

290 mg COD  $\text{L}^{-1}$  at 62 min to 80 mg COD  $\text{L}^{-1}$  after the anaerobic stand-by period (92 min). Moreover, an average COD concentration in the effluent of 73 mg  $\text{L}^{-1}$  was reported, corresponding to a removal efficiency of above 96%. When comparing with the previous Phase II performance, COD effluent values were not significantly different (Table 4).

An increase of the P-release (Fig. 2b) after the anaerobic feeding (62 min) was observed, the phosphate concentration went up to 41 mg  $\text{PO}_4\text{-P L}^{-1}$  (compared to 31 mg  $\text{PO}_4\text{-P L}^{-1}$  in Phase II). After the stand-by period (92 min) the average P-release was 77 mg  $\text{PO}_4\text{-P L}^{-1}$ . After the operational day 160, the P-release reached a steady-state value of above approximately 80 mg  $\text{PO}_4\text{-P L}^{-1}$  almost twice the value that was measured at 62 min. The P-uptake rate remained similar as in

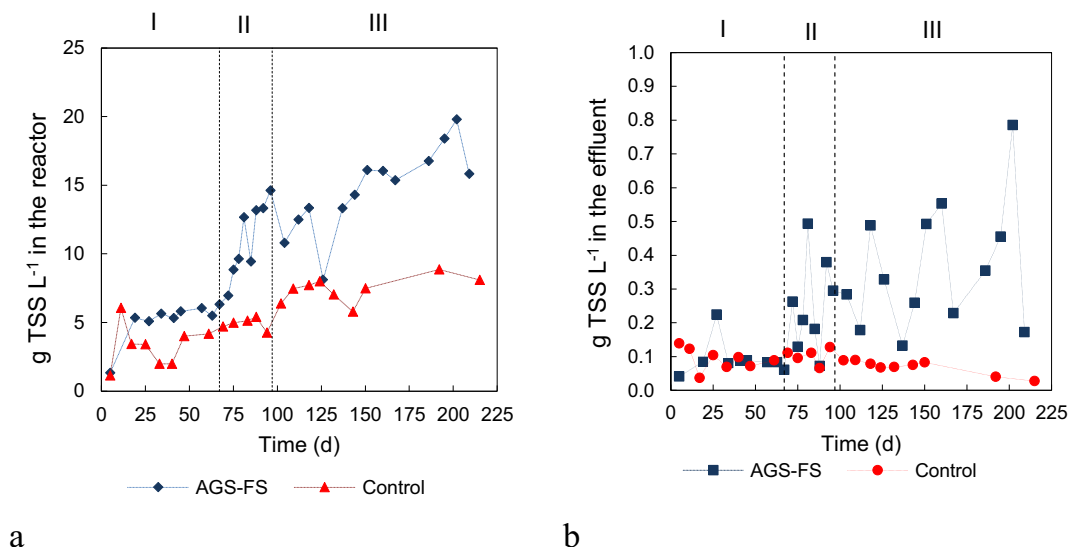


Fig. 3. TSS concentration in the control and the AGS-FS reactors (a) and treated effluents (b).

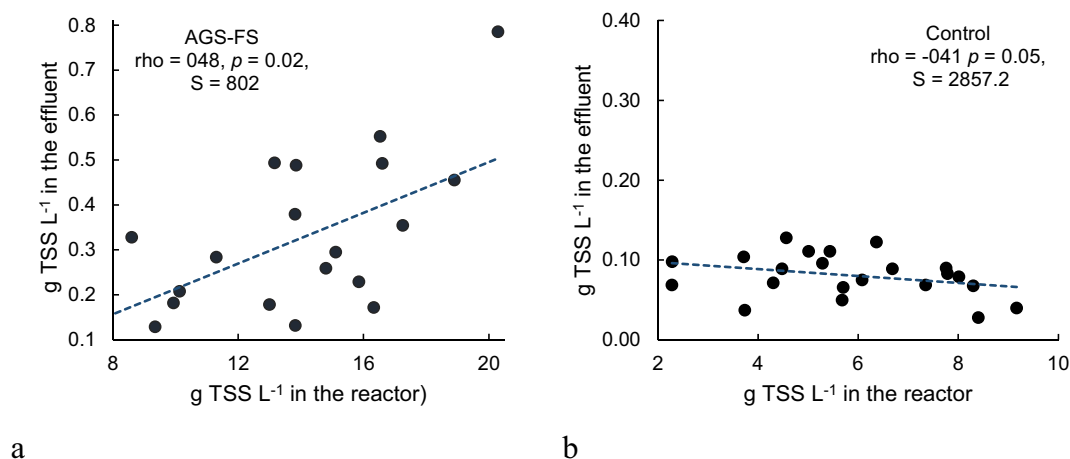


Fig. 4. Relationship between the solids concentration in the effluent and the solids in the AGS-FS (a) and control reactor (b).

Phase II (2.0 mg P g VSS<sup>-1</sup> h<sup>-1</sup>); however, the P concentration in the effluent was reduced to 0.3 mg PO<sub>4</sub>-P L<sup>-1</sup>.

Regarding the performance on nitrogen removal during Phase III (Fig. 2c–e), the ammonium-nitrogen conversions slightly increased to 1.4 NH<sub>4</sub>-N g VSS<sup>-1</sup> h<sup>-1</sup>, and much lower nitrate and nitrite concentrations were measured compared to Phase II. Nitrate and nitrite concentrations of 1.3 mg NO<sub>3</sub>-N L<sup>-1</sup> and 0.11 mg NO<sub>2</sub>-N L<sup>-1</sup> were measured for the treated effluent, respectively. The system achieved concentrations lower than 15 mg Nitrogen L<sup>-1</sup> in the effluent after 186 days of operation. Thus, the lower nitrate concentration of the FS recipe seemed to have had a positive effect both on the nitrification and denitrification processes observed in the AGS-FS reactor.

### 3.2.1. Suspended solids in the long term reactor performance

Both reactors showed similar granular formation and steady bed volumes of approximately 400 mL. Fig. 3a and b show the TSS concentration in the reactors, as well as in the treated effluent. The TSS concentration in the control reactor increased from 4 g TSS L<sup>-1</sup> to almost 9 g TSS L<sup>-1</sup> with an average VSS/TSS ratio of 0.81. The TSS concentration in the AGS-FS reactor also increased during the first 19 days of operation (from 1.2 to 5.3 g TSS L<sup>-1</sup>). During Phase I, the TSS concentration in the reactor reached a value of approximately 5.6 g TSS L<sup>-1</sup> with a VSS/TSS ratio of 0.81. The sludge bed in the AGS-FS reactor was comprised mostly of granules (>95%) and the rest of flocs at this stage. An average TSS concentration in the treated effluent of 0.1 g TSS L<sup>-1</sup> was observed for both the control and the AGS-FS reactor (Phase I).

After the addition of the synthetic FS to the AGS-FS reactor (Phase II), the sludge bed volume increased mostly due to the higher concentration of suspended solids in the influent wastewater compared to the municipal synthetic WW. As described in Table 3, the concentration of solids in the influent was on average 307 ± 96 mg TSS L<sup>-1</sup>; i.e., 460 mg of solids added per cycle of the AGS reactor. The TSS concentration in the reactor increased steadily during Phase II as observed in Fig. 3a. As can be seen in Fig. 3, the solids concentration in the reactor and the effluent showed some peaks and valleys, caused by extra sludge wastage via the effluent on top of the regular sludge waste (via taken weekly samples).

During the final stage (AGS-FS, Phase III), the TSS concentration in the influent remained approximately the same as during Phase II. The TSS concentrations in the reactor increased from 10.1 g TSS L<sup>-1</sup> on day 104 to 19.9 g TSS L<sup>-1</sup> on day 202. Low values were observed during day 125 and 200 that are attained to analytical measurements. The TSS concentration in the effluent increased from 0.1 g TSS L<sup>-1</sup> at the beginning of the Phase II to 0.3 g TSS L<sup>-1</sup> at the end of Phase III. There was a significant Spearman's rank ( $p = 0.02$ ) correlation between the solids concentration in the reactor and the solids leaving the system via the effluent (Fig. 4a). Moreover, it was observed that the sludge bed in the

AGS-FS reactor comprised of approximately 60% of granules and 40% of flocs at the end of Phase III. The dynamics of the granular and floating bed can be observed in the Supplementary materials (Fig. S3).

From the reactors' start-up to the operational day 83, the control reactor had an SRT value of 16 days; after day 83, the SRT value of the system was 20 days. The AGS-FS reactor (Phase I) operated on an average SRT value of 18 days. After the faecal sludge addition (Phase II), the SRT was reduced to 10 days. Due to the reactor operational changes, the SRT value reached 14 days at the end of Phase III.

### 3.3. Effects of FS on the granular settle-ability and size distribution

Both granular beds (control and AGS-FS reactors) exhibited a good settle-ability with SVI<sub>5</sub> values of 63 (at operational day 69) and 85 mL g<sup>-1</sup> (operational day 57), respectively. They showed the same SVI<sub>5</sub> value of 36 mL g<sup>-1</sup> on operational days 96 and 102, respectively. Hereafter, the SVI<sub>5</sub> in the control reactor decreased to 30 mL g<sup>-1</sup> (on operational day 150), and in the AGS-FS reactor (Phase III) increased up to 52 mL g<sup>-1</sup> on the operational day 144 (Phase III).

Regarding the particle size distributions, Fig. 5 shows the granular size obtained from both the control and Phase III of the AGS-FS reactor on days 112 and 118, respectively. For the control reactor, an average granule diameter of 2.1 ± 0.5 mm was measured, and 95% of the granules were in a range between 1.2 and 3.0 mm. For the AGS-FS reactor, the

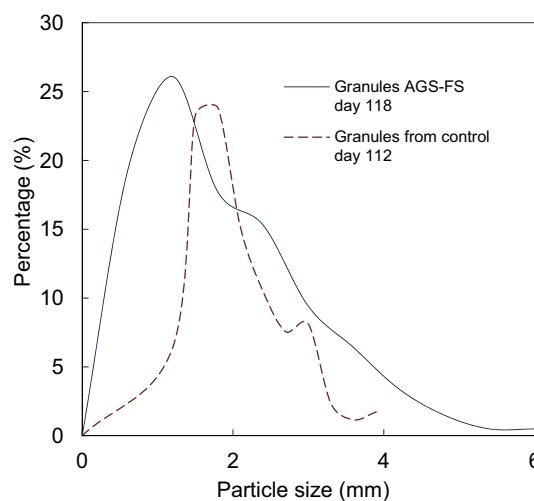


Fig. 5. Particle size distribution of the granules in the control reactor and after the addition of the synthetic FS in the AGS-FS.

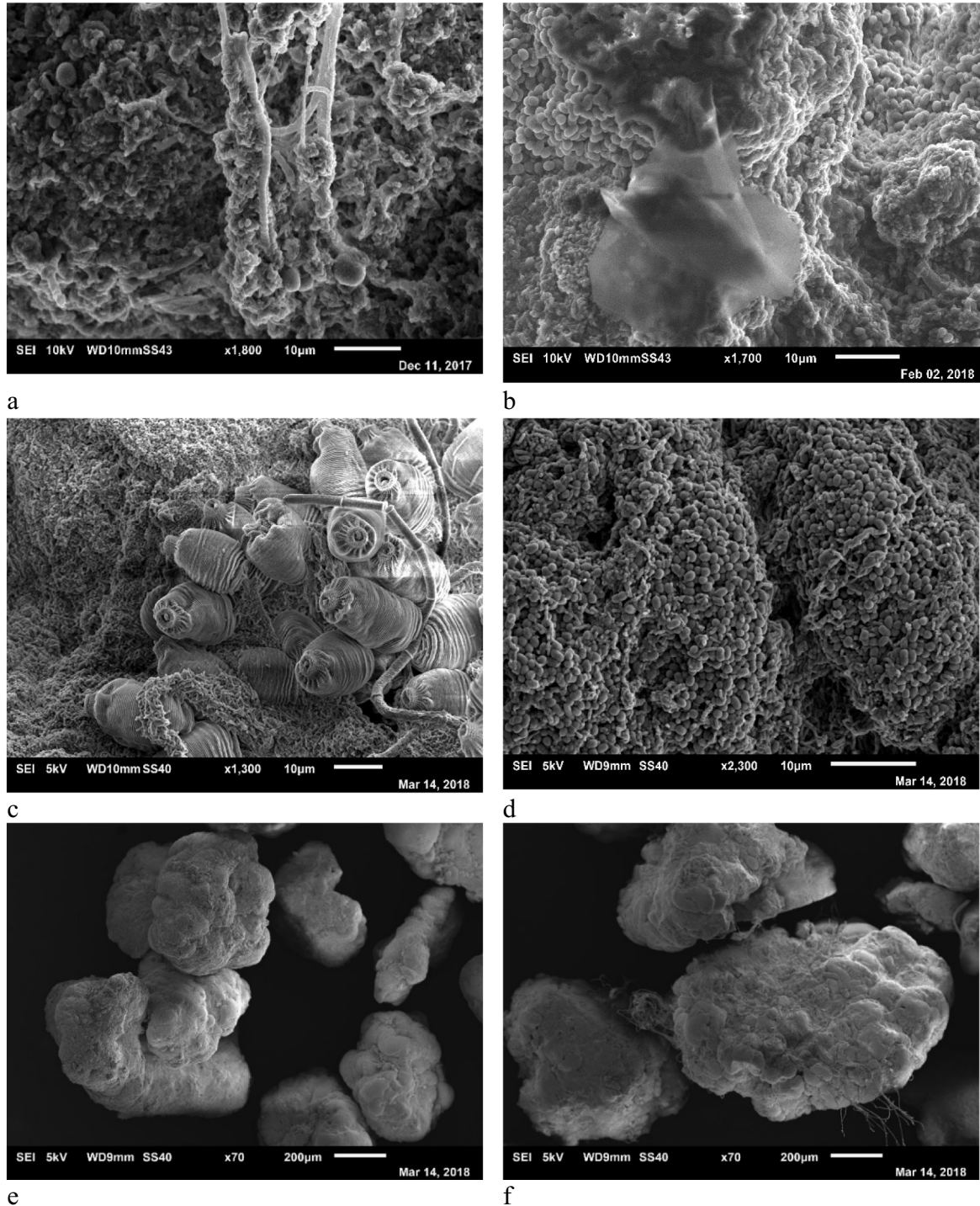


size of the granules showed more variation. An average granule diameter of  $2.1 \pm 0.8$  mm was measured and reported; however, only 69% of the granules were in a range between 1.2 and 3.0 mm. For 20% of the granules, the average diameter was reduced to  $0.6 \pm 0.1$  mm, and the remaining 11% had an average diameter between 3.6 and 6.0 mm.

#### 3.4. Effects of the synthetic FS on the occurrence of protozoa

The protozoa community presence was evaluated in the control reactor and at the three different stages (Phases I, II, and III) of the AGS-

FS reactor by SEM and optical observations. Both reactors (control and AGS-FS) were inoculated with 400 mL of crushed granular sludge and initially fed only municipal synthetic WW based on acetate as the carbon source. Fig. 6a shows the SEM of a sludge sample taken from the AGS-FS reactor on operational day 10. The granules are under formation; therefore, on the surface of such granules, the SEM observations indicated the presence of agglomerations of cocci-shaped bacteria attained to stalks. A substantial reduction in the protozoa community population was observed; protozoa previously found in the inoculum (Fig. 7a and b) including rotifers and the typical genus of *Peritrich*



**Fig. 6.** Morphology of the granules: (a) After operational day 10 (Phase I of AGS-FS); (b) After operational day 64 (AGS-FS - Phase I); (c) After operational day 103 (AGS-FS reactors - Phase III); (d) After operational day 110 (control reactor); (e) and (f) Appearance of the formed granules from the AGS-FS and control reactors after operational day 103, respectively.

ciliates subclass; i.e., *Vorticella* spp., *Carchesium* spp., and *Epistylis* spp., among others, were no longer present on the granular surface or in the liquid bulk. After operational day 64 of the AGS-FS reactor (Fig. 6b) and 110 days of the control reactor (Fig. 6d), systems were in a steady-state condition, and well-shaped granules were observed in the systems.

SEM images showed a well-arranged granular surface fully formed of rod-shape bacteria lacking protozoa organisms in the granular surface of both samples. Interestingly, the inoculum used in the study was from a WWTP treating besides domestic wastewater, industrial WW discharged by a plastic recycling company and a particulate piece of plastic was observed on the granule surface (Fig. 6b). Fig. 6c shows how the granules formed of rod-shaped bacteria were fully colonised by stalked ciliates from the Peritricia subclass, mainly *Vorticella* spp. after the operational day 103 of the AGS-FS reactor (Phase III) (Li et al., 2013). At this operational stage, granules in the reactor were healthy but slightly smaller than the control (Fig. 6e and f). Additional optical microscopy (4× magnified) observations showed a condensed population of such organisms; they colonised both the granular surface (Fig. 7c), as well as most of the particle surfaces present in the FS recipe (Fig. 7d).

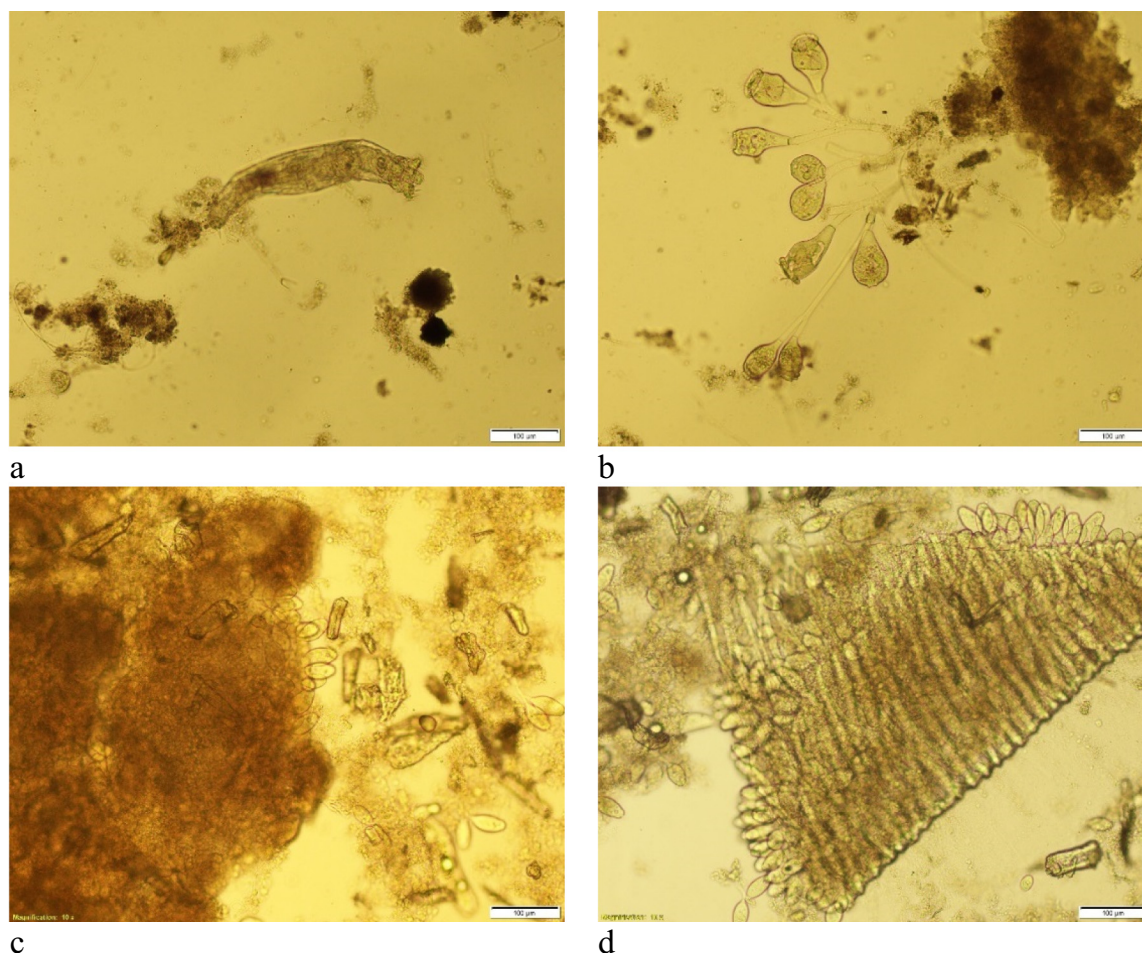
## 4. Discussion

### 4.1. Consideration for the development of a medium-strength synthetic FS

This study assessed the effect of co-treating two different synthetic FS recipes with synthetic municipal WW in a long-term operational

AGS reactor. The use of the medium-strength FS recipe aimed at assisting countries with extensive on-site sanitation coverage (septic tanks) to better understand the dynamics of co-treatment of FS with wastewater in AGS systems (Bower, 2014; Ingallinella et al., 2002). The synthetic FS was used to simulate the variety of biochemical components present in real FS such as bacteria debris, fibres, fats, minerals, proteins and carbohydrates. In regards to the urine simulant, initially, the solution used for FS-1 was developed based on the suggestions giving by Penn et al. (2018), and assuming that the urea was hydrolysed to ammonia, that 25% of the ammonia was already volatilised and that 50% of the remaining ammonia was oxidised to nitrate (Udert and Wächter, 2012). However, the final nitrate concentration was unrealistically high compared to real FS. Therefore, a second urine solution was developed and used in this study (FS-2) containing less nitrate.

The final FS simulant (FS-2) developed met the terms of the highly variable characteristics presented in Strande et al. (2014) for septic tank sludge. Nevertheless, when using the FS recipe during the experiments, it was observed that the mixed influent exhibited high variability of COD and N components although it was kept at 4 °C and replaced every three days. Changes might have been caused by the use of miso paste in the faeces simulant. This paste is made from soybeans, rice, salts, water and the filamentous fungus *Aspergillus oryzae*, which is rich in hydrolytic enzymes for the fermentation process (Marui et al., 2013), although *Lactobacillus* and *Bacillus* species may additionally be used (Methven, 2012). Nout (2015) stated that of all the nitrogen present in this type of products, half is present as amino-nitrogen. Because of the presence of easily available carbon and nitrogen, protein



**Fig. 7.** Optical microscopic observations. (a) and (b) Higher organisms found in the inoculum; (c) Ciliates attached to the granular surface. (d) Ciliates grazing/attached on the psyllium husk particle.



degradation and growth might have taken place in the mixed influent medium resulting in the high variability of COD and N components.

#### 4.2. Effects of the FS on the continuous performance of the AGS reactor

For the AGS control reactor, a stable performance was observed during the entire operational time, and stable and mature granules were reported. The AGS-FS reactor also showed stable conditions and mature/stable granules during the operational conditions set for Phase I. The biological phosphorus removal observed in both reactors (control and AGS-FS Phase I) agreed with previous AGS studies reported in literature using a similar influent P/Ac ratio (Bassin et al., 2012b; Schuler and Jenkins, 2003). After the operational day 57 (for the control reactor) and 75 (for the AGS-FS reactor Phase I), acetate was fully consumed in both reactors as can be expected for a healthy acetate-fed system (Lopez-Vazquez et al., 2009). The resulting P-uptake of 6.6 and 6.0 mg P/gVSS<sup>-1</sup> h<sup>-1</sup> for the control and the AGS-FS reactor was in agreement with the findings reported by Bassin et al. (2012a).

Regarding the N removal, the initial reduction in the NH<sub>4</sub>-N concentration at the end of the anaerobic feeding phase (after 62 min) in both reactors (control and AGS-FS Phase I) was due to a dilution effect and to a potential absorption of ammonium-nitrogen onto the surface of the granules (Bassin et al., 2011). The high concentrations of nitrite and nitrate observed after the aeration phase are due to an inefficient denitrification process observed both at the control and the AGS-FS reactors. The initial relatively high DO concentration set in the reactors (4.5 mg L<sup>-1</sup>) may have negatively impacted the denitrification process. A well-adjusted growth rate is necessary for anoxic conversions in the inner layers of the granule (Bassin et al., 2012a; Winkler et al., 2012). Longer operational times could have been required to reach an optimal nitrification/denitrification rate in the systems. Therefore, the DO concentration set-point was reduced in order to reach a DO concentration of 1.8 mg L<sup>-1</sup> in the reactor. Hereafter, lower nitrite and nitrate concentrations were measured in the effluent (Fig. 2d–e); however, complete denitrification was not observed during the entire operation of the reactors and high total nitrogen concentrations were always measured in the effluent. A better adjustment of the DO set-point in the reactors may be needed to achieve complete denitrification.

After the synthetic FS addition, multiple effects on the system performance were observed. The extension of the anaerobic phase by an added stand-by period of 30 min seemed to facilitate the hydrolysis of the organic matter to more simple components (Fig. 2a) (Corsino et al., 2017; de Kreuk et al., 2010; Morgenroth et al., 2002). However, it was observed that the increased anaerobic period was not long enough to ensure the complete organic matter uptake as occurred in the control reactor fed with synthetic WW. It seems that the PAOs were not able to store all the COD at the end of the anaerobic phase as can be seen by the high COD measured and the lower acetate consumption. One of the reasons was the high NO<sub>3</sub>-N concentration present in the FS causing the anaerobic phase to be anoxic affecting the PAOs which could not sufficiently accumulate acetate or the hydrolysed substrate disrupting the P-uptake during the aeration phase (Bassin et al., 2012b; de Kreuk et al., 2010; Saito et al., 2004). Moreover, the increased denitrification affected the granular settle-ability and resulted in a higher solids wash-out via the effluent.

The SRT of the AGS-FS reactor decreased from 18 days in Phase I to 10 days in Phase II, which is unfavourable for slow-growing organisms such as ammonium or nitrite oxidisers (Szabó et al., 2016); consequently, less ammonium was oxidised during the aeration phase impacting on the expected simultaneous denitrification process. With the lower NO<sub>3</sub>-N concentrations present in the FS synthetic recipe for Phase III, nitrite/nitrate no longer accumulated in the anaerobic feeding; hence, the PAOs activity improved by increasing the COD uptake/P release after the anaerobic phase as can be seen during 62 min in Fig. 1 of Phase III compared to Phase II. Besides, due to operational changes and modification of the FS recipe, less biomass was wasted via the

effluent, and the reduction of DO to 20% saturation enhanced the simultaneous nitrification/denitrification capacity of the system (Mosquera-Corral et al., 2005).

Moreover, as previously reported by Rocktäschel et al. (2015), the presence of the high concentration of TSS in the influent and its accumulation in the reactor led to high solids concentrations in the treated effluent. The TSS went from values lower than 0.01 g L<sup>-1</sup> for both the control and Phase I of the AGS-FS reactor to 0.8 g TSS L<sup>-1</sup> in the operational day 200 (phase III) for the AGS-FS reactor (Fig. 3). The solid accumulation showed a significant relationship with the solids concentration in the effluent as well (Fig. 4a and b). Even though such values are unusual in AGS full-scale WWTPs (Khan et al., 2015; Pronk et al., 2015b), the results from our study showed implications for effluent quality that may require further attention. However, as mentioned by van Dijk et al. (2018), the solids concentrations can be better studied in full-scale WWTPs where the sludge withdrawal take place in a separate process to enhance the effluent quality.

#### 4.3. Effects of FS on granular formation and stability

Granules from the control and AGS-FS reactor Phase I showed a good settle-ability with an SVI<sub>5</sub> of 38 mL g<sup>-1</sup>. This is slightly higher than the value reported by de Kreuk et al. (2005) for granules full-grown with acetate (SVI<sub>5</sub> 24 mL g<sup>-1</sup>) but considerable lower than for flocs in CAS systems (100–150 mL g<sup>-1</sup>). The measured granular diameter (from 1.2 and 3.0 mm) was in line with the classification previously described by de Kreuk et al. (2007) and Corsino et al. (2018). Moreover, the biomass yield measured in this study was in agreement with previous laboratory-scale studies confirming healthy granular systems using acetate as a substrate (Rocktäschel et al., 2015; Wagner et al., 2015).

In line with Cetin et al. (2018) and Corsino et al. (2018), the high amount of solids and the higher OLR as a result of the FS synthetic recipe in the influent had an impact on the particle size distribution of the granules. The size of 20% of the granules of the AGS-FS reactor was reduced to a range of 0.6 to 1.2 mm. However, the size remained within the reported standards for AGS systems (de Kreuk et al., 2007). Based on the water quality parameters, there were no noticeable adverse effects in case of the AGS-FS reactor. The average granular size was between the optimal size (0.7–1.9 mm) suggested by Zhou et al. (2016) to enhance nitrogen removal efficiencies which was also shown in this study.

Moreover, a higher fraction of flocculent sludge (40%) was observed (Wagner et al., 2015). Similar effects were also reported by Liu and Tay (2012), they encountered a detrimental effect on the granular fraction and faster growth of the flocs. They also found that the composition of the microbial community of the flocculent fraction hardly differed from the granular fraction when feeding a reactor with industrial wastewater (with concentrations ranging from 250 to 1800 mg COD L<sup>-1</sup> and 39 to 93 mg NH<sub>4</sub>-N L<sup>-1</sup>). Selection pressure by reducing the settling time was as a measurement taken to enhance the granule fraction, which was also applied in this study (after initially increasing the settling time to 10 min, was reduced again to 5 min) to maintain an optimal particle granular size.

#### 4.4. Effects of the synthetic FS on the occurrence of protozoa

The inoculum used for seeding the AGS-FS reactor contained a variety of organisms (i.e., bacteria and eukaryotes), EPS, and other kinds of components such as (small) amounts of micro-plastics – originating from discharge to the sewer by a connected plastic recycling company. Rotifers and protozoa, which have been previously observed during the start-up of two AGS laboratory-scale reactors fed with particulate materials such as starch and maltose (de Kreuk et al., 2010), were also observed. According to Zhang et al. (2011), it is assumed that the filaments and stalks of those higher organisms generate a support structure for the growth of the bacteria and the granule formation. Weber et al.

(2007) explained the development of the granules in sequencing batch reactors systems (i.e., malthouse, brewery, and synthetic WW) in three phases. Firstly, protozoa get attached to both flocs and free-swimming particles developing a tree-like colony of ciliate stalks. Subsequently, bacteria colonise the stalks leading to the development of the granules. Later, some of the protozoa die or leave the biofilm through the treated effluent as free-swimming ciliates. In this study, during the start-up and maturation of the system, granules larger than 2.0 mm were observed in both reactors (control and AGS-FS). Moreover, an agglomeration of bacteria was observed which was present in the inoculum as shown in Fig. 6a and b; however, no protozoa were found on the surface of the granules or in the bulk liquid. They may have been washed-out during the effluent withdrawal. The remaining protozoa could have been embedded in the biofilm entirely covered by the same bacteria forming smooth and compact granules; therefore, protozoa were not seen under the light microscope.

From Phase II onwards, in line with Weber et al. (2007), de Kreuk et al. (2010) and Corsino et al. (2018) findings, an outgrowth of filamentous bacteria together with finger-type structures were expected to occur; this because of the addition of particulate material on the FS recipe. However, it was not the case in this study, a sudden bloom of protozoa occurred right after the addition of the synthetic, medium-strength FS. This present study validates Cetin et al. (2018) findings, the addition of high solids concentrations in the systems will not always result in a filamentous organisms out-growth. Eventually, compounds such as microcrystalline cellulose, yeast extract, and psyllium husk could have induced the growth of the protozoa (which were most likely embedded in the granules). Unfortunately, no protozoa enumeration was done in this study for determining the growth and decay rate of these microorganisms in the reactors.

#### 4.5. The relevance of the findings for future applications

The addition of synthetic FS to a healthy granular system helped to better understand the co-treatment dynamics in an AGS reactor. Our study showed that the AGS-FS system was able to treat synthetic FS being 4% of municipal synthetic WW total influent flowrate during 186 days of operation. Results confirmed that a functional AGS system can handle the co-treatment of FS with wastewater (Pronk et al., 2017). The good treatment performance was possible due to an extended duration of the anaerobic phase, a reduction on the DO set-point, and the changes in the settling time. Probably, those parameters are part of the operational conditions applied in full-scale AGS systems co-treating FS but are not yet reported. However, the operation of full-scale AGS systems will require attention when dealing with the high organic and nitrogen loads resulting from co-treating FS. Feeding conditions (i.e., FS dilution range and required pre-treatment) will need to be considered concerning the WWTP design capacity; as it was observed in this study that the additional particulate organic matter and the high nitrogen content coming from the FS impacted the AGS system performance. Furthermore, real FS has a high variation in composition that will require a proper characterisation before its addition into an AGS system. The behaviour of these different types of substrates (i.e., fresh or digested FS coming from pit latrines or septic tanks, among others) needs to be further evaluated.

Granules in full-scale systems tend to be larger than the ones in laboratory-scale systems and with a more diverse microbial population since the microstructure and diversity of the aerobic granules are related to the type of substrate used (Pronk et al., 2015a; Tay et al., 2001). It would be interesting to determine the differences in the composition of the bacterial and protozoa community present in the granules and compare it with our findings using synthetic FS. Furthermore, considering the role of protozoa in the pathogen removal in AGS systems (Barrios-Hernández et al., 2020), it would be relevant to evaluate the contribution of the FS addition on the occurrence and removal of the pathogenic organism. Further studies are needed to assess the effect

of excessive protozoa grazing of (pathogenic) bacteria and particles on the granulation process, and to determine whether the effects perceived in this study (i.e., treatment performance, granular formation/stability, and presence of protozoa) will be observed.

## 5. Conclusions

The co-treatment of synthetic faecal sludge with synthetic wastewater was evaluated in AGS laboratory-scale reactors. The two adapted synthetic medium-strength FS (low and high NO<sub>3</sub>-N) recipes well-represent digested medium-strength FS originating from septic tanks. Special attention needs to be given regarding degradability when using proteinous components such as miso paste which can contribute to nitrogen augmentation in the FS recipe.

The co-treatment of FS with synthetic wastewater required operational adjustments to prevent the deterioration of the effluent quality. The high NO<sub>3</sub>-N concentrations present in the first FS recipe led to a disruption of the anaerobic conditions required for an optimal aerobic granular sludge system performance causing a floating sludge bed and solids wash-out. The second FS recipe with lower NO<sub>3</sub>-N levels in the influent produced a better organic matter and nutrients removal. However, the addition of FS decreased the sludge settle-ability, and an accumulation of solids in the reactor occurred for both FS recipes. Moreover, due to the addition of particulate biodegradable organics, the average granular size was reduced, a higher fraction of flocculent sludge was perceived, and a sudden bloom of ciliates protozoa occurred. Further studies are necessary to determine the effect of real FS and its variabilities on full-scale AGS systems.

## CRediT authorship contribution statement

**Mary Luz Barrios-Hernández:** Conceptualization, Formal analysis, Investigation, Writing - original draft, Visualization. **Claribel Buenaño-Vargas:** Conceptualization, Investigation, Writing - review & editing. **Hector García:** Conceptualization, Writing - review & editing. **Damir Brdjanovic:** Supervision. **Mark C.M. van Loosdrecht:** Supervision. **Christine M. Hooijmans:** Conceptualization, Project administration, Writing - review & editing, Supervision.

## 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.

## Acknowledgements

M.L. acknowledges the Technological Institute of Costa Rica for providing the fellowship (Grant Number 007-2014-M) to pursue her PhD programme (2016–2020) at IHE-Delft, the Netherlands. The authors thank the laboratory staff at IHE-Delft institution for their technical support.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.140480>.

## References

- Abdullah, N., Yuzir, A., Curtis, T.P., Yahya, A., Ujang, Z., 2013. Characterization of aerobic granular sludge treating high strength agro-based wastewater at different volumetric loadings. *Bioreour. Technol.* 127, 181–187. <https://doi.org/10.1016/j.biortech.2012.09.047>.

- APHA, 2012. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
- Barrios-Hernández, M.L., Pronk, M., Garcia, H., Boersma, A., Brdjanovic, D., van Loosdrecht, M.C.M., Hooijmans, C.M., 2020. Removal of bacterial and viral indicator organisms in full-scale aerobic granular sludge and conventional activated sludge systems. *Water Research* X 6, 100040. <https://doi.org/10.1016/j.wroa.2019.100040>.
- Bassin, J.P., Pronk, M., Kraan, R., Kleerebezem, R., van Loosdrecht, M.C.M., 2011. Ammonium adsorption in aerobic granular sludge, activated sludge and anammox granules. *Water Res.* 45 (16), 5257–5265. <https://doi.org/10.1016/j.watres.2011.07.034>.
- Bassin, J.P., Kleerebezem, R., Dezotti, M., van Loosdrecht, M.C.M., 2012a. Measuring biomass specific ammonium, nitrite and phosphate uptake rates in aerobic granular sludge. *Chemosphere* 89 (10), 1161–1168. <https://doi.org/10.1016/j.chemosphere.2012.07.050>.
- Bassin, J.P., Winkler, M.K.H., Kleerebezem, R., Dezotti, M., van Loosdrecht, M.C.M., 2012b. Improved phosphate removal by selective sludge discharge in aerobic granular sludge reactors. *Biotechnol. Bioeng.* 109 (8), 1919–1928. <https://doi.org/10.1002/bit.24457>.
- Bower, K.M., 2014. Water supply and sanitation of Costa Rica. *Environ. Earth Sci.* 71 (1), 107–123. <https://doi.org/10.1007/s12665-013-2416-x>.
- Bueno, R.d.F., Faria, J.K., Uliana, D.P., Liduino, V.S., 2020. Simultaneous removal of organic matter and nitrogen compounds from landfill leachate by aerobic granular sludge. *Environ. Technol.*, 1–15 <https://doi.org/10.1080/09593330.2020.1740798>.
- Carucci, A., Cappai, G., Erby, G., Milia, S., 2020. Aerobic granular sludge formation in a sequencing batch reactor treating agro-industrial digestate. *Environ. Technol.*, 1–22 <https://doi.org/10.1080/09593330.2020.1769742>.
- Cetin, E., Karakas, E., Dulekgurgen, E., Ovez, S., Kolukirik, M., Yilmaz, G., 2018. Effects of high-concentration influent suspended solids on aerobic granulation in pilot-scale sequencing batch reactors treating real domestic wastewater. *Water Res.* 131, 74–89. <https://doi.org/10.1016/j.watres.2017.12.014>.
- Corsino, S.F., di Biase, A., Devlin, T.R., Munz, G., Torregrossa, M., Oleszkiewicz, J.A., 2017. Effect of extended famine conditions on aerobic granular sludge stability in the treatment of brewery wastewater. *Bioresour. Technol.* 226, 150–157. <https://doi.org/10.1016/j.biortech.2016.12.026>.
- Corsino, S.F., Di Trapani, D., Torregrossa, M., Viviani, G., 2018. Aerobic granular sludge treating high strength citrus wastewater: analysis of pH and organic loading rate effect on kinetics, performance and stability. *J. Environ. Manag.* 214, 23–35. <https://doi.org/10.1016/j.jenvman.2018.02.087>.
- Dangol, B., 2017. Co-treatment of faecal sludge with wastewater treatment systems: a systematic review. *Journal of Environment and Public Health* 1 (1), 51–57.
- de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C.M., 2005. Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. *Biotechnol. Bioeng.* 90 (6), 761–769. <https://doi.org/10.1002/bit.20470>.
- 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. <https://doi.org/10.2166/wst.2007.244>.
- de Kreuk, M., Kishida, N., Tsuneda, S., Van Loosdrecht, M., 2010. Behavior of polymeric substrates in an aerobic granular sludge system. *Water Res.* 44 (20), 5929–5938. <https://doi.org/10.1016/j.watres.2010.07.033>.
- Figuerola, M., Val del Rio, A., Campos, J., Mosquera-Corral, A., Mendez, R., 2011. Treatment of high loaded swine slurry in an aerobic granular reactor. *Water Sci. Technol.* 63 (9), 1808–1814. <https://doi.org/10.2166/wst.2011.381>.
- Heinss, U., Strauss, M., 1999. Co-Treatment of Faecal Sludge and Wastewater in Tropical Climates. SOS-management of Sludges From On-site Sanitation. EAWAG/SANDEC.
- Ingallinella, A., Sanguinetti, G., Koottatep, T., Montangero, A., Strauss, M., 2002. The challenge of faecal sludge management in urban areas-strategies, regulations and treatment options. *Water Sci. Technol.* 46 (10), 285–294. <https://doi.org/10.2166/wst.2002.0355>.
- Khan, A.A., Ahmad, M., Giesen, A., 2015. NEREDA®: an emerging technology for sewage treatment. *Water Practice and Technology* 10 (4), 799–805. <https://doi.org/10.2166/wpt.2015.098>.
- Kirhensteine, I., Cherrier, V., Jarrit, N., Farmer, A., de Paoli, G., Delacamara, G., Psomas, A., 2016. EU-level Instruments on Water Reuse. Final Report to Support the Commission's Impact Assessment. European Union, Luxembourg.
- Li, J., Ma, L., Wei, S., Horn, H., 2013. Aerobic granules dwelling vorticella and rotifers in an SBR fed with domestic wastewater. *Sep. Purif. Technol.* 110, 127–131. <https://doi.org/10.1016/j.seppur.2013.03.022>.
- Liu, Y.-Q., Tay, J.-H., 2012. The competition between flocculent sludge and aerobic granules during the long-term operation period of granular sludge sequencing batch reactor. *Environ. Technol.* 33 (23), 2619–2626. <https://doi.org/10.1080/09593330.2012.673011>.
- Lopez-Vazquez, C.M., Oehmen, A., Hooijmans, C.M., Brdjanovic, D., Gijzen, H.J., Yuan, Z., van Loosdrecht, M.C., 2009. Modeling the PAO–GAO competition: effects of carbon source, pH and temperature. *Water Res.* 43 (2), 450–462. <https://doi.org/10.1016/j.watres.2008.10.032>.
- Lopez-Vazquez, C.M., Dangol, B., Hooijmans, C.M., Brdjanovic, D., 2014. Co-treatment of faecal sludge in municipal wastewater treatment plants. In: Strande, L., Ronteltap, M., Brdjanovic, D. (Eds.), *Faecal Sludge Management—Systems Approach Implementation and Operation*. IWA Publishing, London, UK, pp. 177–198.
- Marui, J., Tada, S., Fukuoka, M., Wagu, Y., Shiraishi, Y., Kitamoto, N., Sugimoto, T., Hattori, R., Suzuki, S., Kusumoto, K.-I., 2013. Reduction of the degradation activity of umami-enhancing purinic ribonucleotide supplement in miso by the targeted suppression of acid phosphatases in the *Aspergillus oryzae* starter culture. *Int. J. Food Microbiol.* 166 (2), 238–243. <https://doi.org/10.1016/j.jfoodmicro.2013.07.006>.
- Methven, L., 2012. Natural food and beverage flavour enhancer. In: Baines, D., Seal, R. (Eds.), *Natural Food Additives, Ingredients and Flavours*. Woodhead Publishing, Cambridge, pp. 76–99.
- Morgenroth, E., Kommedal, R., Harremoës, P., 2002. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment—a review. *Water Sci. Technol.* 45 (6), 25–40. <https://doi.org/10.2166/wst.2002.0091>.
- Mosquera-Corral, A., de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C., 2005. Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor. *Water Res.* 39 (12), 2676–2686. <https://doi.org/10.1016/j.watres.2005.04.065>.
- Nout, R., 2015. 18 - quality, safety, biofunctionality and fermentation control in soya. In: Holzapfel, W. (Ed.), *Advances in Fermented Foods and Beverages*. Woodhead Publishing, pp. 409–434.
- Penn, R., Ward, B.J., Strande, L., Maurer, M., 2018. Review of synthetic human faeces and faecal sludge for sanitation and wastewater research. *Water Res.* 132, 222–240. <https://doi.org/10.1016/j.watres.2017.12.063>.
- Pronk, M., Abbas, B., Al-zuhairi, S.H.K., Kraan, R., Kleerebezem, R., van Loosdrecht, M.C.M., 2015a. Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. *Appl. Microbiol. Biotechnol.* 99 (12), 5257–5268. <https://doi.org/10.1007/s00253-014-6358-3>.
- Pronk, M., de Kreuk, M.K., de Bruin, B., Kamminga, P., Kleerebezem, R., van Loosdrecht, M.C.M., 2015b. Full scale performance of the aerobic granular sludge process for sewage treatment. *Water Res.* 84, 207–217. <https://doi.org/10.1016/j.watres.2015.07.011>.
- Pronk, M., Giesen, A., Thompson, A., Robertson, S., van Loosdrecht, M.C.M., 2017. Aerobic granular biomass technology: advancements in design, applications and further developments. *Water Practice and Technology* 12 (4), 987–996. <https://doi.org/10.2166/wpt.2017.101>.
- R\_Core\_Team, 2020. R: a language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria <https://www.R-project.org>.
- Rasband, W.S., 1997–2018. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA <https://imagej.nih.gov/ij/o>.
- 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, 234–241. <https://doi.org/10.1016/j.seppur.2015.01.013>.
- Saito, T., Brdjanovic, D., Van Loosdrecht, M., 2004. Effect of nitrite on phosphate uptake by phosphate accumulating organisms. *Water Res.* 38 (17), 3760–3768. <https://doi.org/10.1016/j.watres.2004.05.023>.
- Schuler, A.J., Jenkins, D., 2003. Enhanced biological phosphorus removal from wastewater by biomass with different phosphorus contents, part I: experimental results and comparison with metabolic models. *Water Environment Research* 75 (6), 485–498. <https://doi.org/10.2175/106143003X141286>.
- Sepúlveda-Mardones, M., Campos, J.L., Magrí, A., Vidal, G., 2019. Moving forward in the use of aerobic granular sludge for municipal wastewater treatment: an overview. *Rev. Environ. Sci. Biotechnol.* 18 (4), 741–769. <https://doi.org/10.1007/s11157-019-09518-9>.
- Siegrist, R.L., 2017. *Treatment Using Septic Tanks. Decentralized Water Reclamation Engineering: A Curriculum Workbook*. Springer International Publishing, pp. 237–288.
- Strande, L., Ronteltap, M., Brdjanovic, D., 2014. *Faecal Sludge Management: Systems Approach for Implementation and Operation*. IWA Publishing.
- Strauss, M., Montangero, A., 2002. *FS Management—Review of Practices, Problems and Initiatives*. EAWAG/SANDEC publications, Dübendorf.
- Szabó, E., Hermansson, M., Modin, O., Persson, F., Wilén, B.M., 2016. Effects of wash-out dynamics on nitrifying bacteria in aerobic granular sludge during start-up at gradually decreased settling time. *Water* 8 (5), 172. <https://doi.org/10.3390/w8050172>.
- Tay, J.H., Liu, Q.S., Liu, Y., 2001. Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor. *J. Appl. Microbiol.* 91 (1), 168–175. <https://doi.org/10.1046/j.1365-2672.2001.01374.x>.
- Taylor, K., 2018. *Faecal Sludge and Septage Treatment: A Guide for Low and Middle Income Countries*. Practical Action Publishing.
- U.S.\_EPA, 1994. *Guide to Septage Treatment and Disposal*. Report No. 625/6/R-94-002. U.S. Environmental Protection Agency.
- Udert, K.M., Wächter, M., 2012. Complete nutrient recovery from source-separated urine by nitrification and distillation. *Water Res.* 46 (2), 453–464. <https://doi.org/10.1016/j.watres.2011.11.020>.
- van Dijk, E.J., Pronk, M., van Loosdrecht, M.C.M., 2018. Controlling effluent suspended solids in the aerobic granular sludge process. *Water Res.* 147, 50–59. <https://doi.org/10.1016/j.watres.2018.09.052>.
- Vishniac, W., Santer, M., 1957. *The thiobacilli*. *Bacteriol. Rev.* 21 (3), 195.
- 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. <https://doi.org/10.1016/j.watres.2015.08.030>.
- Weber, S., Ludwig, W., Schleifer, K.-H., Fried, J., 2007. Microbial composition and structure of aerobic granular sewage biofilms. *Appl. Environ. Microbiol.* 73 (19), 6233–6240. <https://doi.org/10.1128/AEM.01002-07>.
- Winkler, M.K.H., Bassin, J.P., Kleerebezem, R., Sorokin, D.Y., van Loosdrecht, M.C.M., 2012. Unravelling the reasons for disproportion in the ratio of AOB and NOB in aerobic granular sludge. *Appl. Microbiol. Biotechnol.* 94 (6), 1657–1666. <https://doi.org/10.1007/s00253-012-4126-9>.
- Zhang, B., Ji, M., Qiu, Z., Liu, H., Wang, J., Li, J., 2011. Microbial population dynamics during sludge granulation in an anaerobic-aerobic biological phosphorus removal system. *Bioresour. Technol.* 102 (3), 2474–2480. <https://doi.org/10.1016/j.biortech.2010.11.017>.
- Zhou, J.-h., Zhang, Z.-m., Zhao, H., Yu, H.-t., Alvarez, P.J.J., Xu, X.-y., Zhu, L., 2016. Optimizing size distribution for aerobic granular sludge stability: effect of a novel funnel-shaped internals on hydraulic shear stress. *Bioresour. Technol.* 216, 562–570. <https://doi.org/10.1016/j.biortech.2016.05.079>.