Cultivation of aerobic granular sludge for the treatment of food-processing wastewater and the impact on membrane filtration properties

H. Stes, M. Caluwé, L. Dockx, R. Cornelissen, P. De Langhe, I. Smets and J. Dries

ABSTRACT

A laboratory-scale sequencing batch reactor was operated for approximately 300 days, divided into four periods based on the feeding strategy, to develop stable aerobic granular sludge (AGS) while treating chocolate processing wastewater. Application of a prolonged mixed anaerobic feeding was not sufficient to develop AGS and reach stable reactor performance. Through the application of a partially non-mixed and a partially mixed feeding strategy, the reactor performance was increased and stable AGS formation was established characterized by low diluted sludge volume index (D)SVI $DSVI_{10,30}$) values of 78 ± 27 mL·g^{-1} and 52 ± 17 mL·g^{-1}, respectively, and a capillary suction time/mixed liquor suspended solids value of 0.9 sec·(g·L^{-1})$^{-1}$. The membrane bioreactor (MBR) filtration tests showed a reduction of the fouling rate (FR) and an increase of the sustainable flux ($SF_{0.5}$) for AGS compared to flocs treating the same industrial wastewater. The $SF_{0.5}$ (FR $>$ 0.5 mbar·min^{-1}) for the flocs was 10 L·(m^2·h)$^{-1}$ while for AGS the $SF_{0.5}$ is higher than 45 L·(m^2·h)$^{-1}$ because the FR did not exceed 0.1 mbar·min^{-1}. Additionally, the AGS showed reduced irreversible fouling tendencies due to pore blocking. Our results underline the need for an increased substrate gradient during anaerobic feeding for the development and long-term maintenance of AGS under minimum wash-out conditions. The AGS–MBR filtration performance also shows strong advantages compared to a floccular MBR system due to a high increase of the $SF_{0.5}$ and reduced reversible and irreversible fouling.

Key words | aerobic granular sludge, chocolate processing wastewater, feast/famine regime, membrane bioreactor, membrane fouling

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HIGHLIGHTS

- Successful aerobic granulation while treating industrial food-processing wastewater minimum wash-out conditions.
- Combining a non-mixed/mixed anaerobic feeding strategy to obtain sufficient feast/famine conditions.
- Reduced membrane fouling tendencies for AGS compared to floccular sludge.

INTRODUCTION

The global population growth, along with industrialization and urbanization in the European region, is causing severe pressure on local water resources and the need for efficient and compact wastewater treatment technologies. In order to tackle the increasing water demand and the rising water scarcity problems, research regarding the reuse of different types of wastewater has gained more interest over the past decades (Simate *et al.* 2011; Judd 2016). Stringent discharge regulations and increased pressure towards water recycling are today's drivers in the development and application of

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alternative treatment processes. A suitable technology to achieve high effluent quality for reuse purposes involves a biological treatment combined with membranes in a membrane bioreactor (MBR) configuration. In an MBR system, microfiltration or ultrafiltration membranes are used to separate the mixed liquor from the treated water, potentially followed by nanofiltration or reversed osmosis, depending on the required effluent quality (Salgot & Folch 2018). The MBR technology is an attractive technology for the treatment of both municipal and industrial wastewater due to the high effluent quality and high biomass concentrations (Judd 2008), very controlled separation of the hydraulic retention time (HRT) from the sludge retention time (SRT) and lower sludge production compared to the conventional activated sludge systems (Judd 2008; Kraume & Drews 2010). Successful demonstrations of the MBR technology have been reported for both small and large-scale systems (Judd 2016). Even though the MBR technology shows strong advantages over conventional activated sludge systems, minimizing membrane fouling and clogging remains a primary challenge (Judd 2016). Considering the costs associated with the prevention of fouling (aeration), membrane cleaning procedures and reduced filtration efficiency, fouling is the main contributor to the overall energy demand and operating cost of MBR systems (Le-Clech *et al.* 2006). Fouling is attributed to sludge deposition and accumulation (cake layer) on the membrane surface and membrane pore clogging due to colloids and solutes (Meng *et al.* 2009). Multiple strategies have been developed over the past decades to mitigate membrane fouling and increase fouling control. One of the promising strategies is based on the integration of the aerobic granular sludge (AGS) technology in MBR systems in an innovative aerobic granular membrane bioreactor (AGS–MBR) to reduce biofouling (Truong *et al.* 2018). Similar to the MBR technology, the AGS technology has gained great interest over the past decade for the treatment of both municipal and industrial wastewater due to its efficient biological nutrient removal capacities, excellent settling properties and compact and easy-to-operate reactor design (Pronk *et al.* 2015). The AGS technology is based on the selection of slow-growing organisms through a feast/famine operational strategy combined with a hydraulic selection pressure to select for fast-settling granule structures (Pronk *et al.* 2015). One of the main challenges in AGS systems is the elevated effluent suspended solids concentrations, especially when treating industrial wastewater characterized by a high suspended solids content (Morales *et al.* 2013). The main reasons for combining both the AGS and MBR technology is an increase of the effluent quality and energy efficiency due to reduced membrane fouling while reducing the footprint. When comparing granular to floccular sludge, several authors reported improved filtration performance and thus reduced membrane fouling, as well as an increase of organic and nutrient removal efficiencies (Jing-Feng *et al.* 2012; Wang *et al.* 2013). It is believed that the increased particle size and dense structure of aerobic granules contribute to reduced and more porous bio-cake layer formation due to reduced sludge compressibility compared to floccular sludge (Lee *et al.* 2008; Jing-Feng *et al.* 2012; Wang *et al.* 2013). Therefore, the novel AGS-MBR system is believed to be an attractive technology for the treatment of municipal and industrial wastewater, including wastewater originating from the food-processing industry. Today, the long-term aerobic granule stability is still of major concern for industrial applications given the lack of a hydraulic selection pressure in an AGS-MBR system (Liébana *et al.* 2018). In this study, the feasibility of the innovative AGS technology for industrial wastewater treatment in conventional sequencing batch reactors (SBRs) was studied under minimum hydraulic pressure through the application and optimization of newly developed feeding strategies. Additionally, the impact on the overall reactor treatment performance and sludge filterability was studied while using real industrial wastewater originating from the chocolate processing industry as the feeding source. Besides the sludge dewaterability, membrane filtration performance and fouling behaviour of the stable granular sludge was compared with floccular sludge treating the same industrial wastewater. The latter comparative part of the study was performed to assess the potential of reducing membrane fouling when aerobic granules are integrated within an MBR in a novel AGS-MBR system for industrial wastewater treatment.

METHODS

Laboratory-scale SBR set-up and operation

The laboratory-scale SBR (see Appendix IIa) had a working volume of 11 L (height to diameter ratio = 1.1) and was operated at room temperature (18–22 °C) for approximately 300 days. The reactor was provided with a peristaltic feeding pump (Verderflex M045, UK), mechanical stirrer (IKA RW20 digital), a dissolved oxygen (DO) (Endress + Hauser, Oxymax W COS51D) and pH (Endress + Hauser, Orbisint CPS11-7AA21) sensor, and an aeration system consisting of an aeration pump (Ubbink Air 1000) and an air diffuser at the bottom of the reactor (AngelAqua DY 104-A). A Siemens

PLC (LOGO! Logic Module) was used for process control. Sensor data were recorded and visualized using an Ecograph T RSG35 graphic display recorder (Endress + Hauser). Monitoring of the DO set-points was done by the same recorder. To promote the granulation process, an anaerobic feast/aerobic famine regime was applied. In every cycle, 1.4 L of industrial wastewater was fed to the reactor, resulting in a constant volume exchange ratio (VER) of 11% and a constant HRT of 4.4 days. Every SBR cycle consisted of an aerated pre-phase (30 min), a non-aerated pre-phase (15 min), a feeding phase (127 min), a mixed feast phase and an aerated phase (data in Table 1), a long settling phase (120 min) and a final effluent withdrawal phase (60 min). The total cycle time remained constant at 720 min, resulting in two SBR cycles per day. Throughout the experiment, the feeding strategy was optimized to improve the overall granule and sludge dewaterability and filtration characteristics. First, the anaerobic feast phase was prolonged to promote anaerobic carbon uptake. Second, the mixed anaerobic feeding phase was divided into a two-step feeding phase consisting of an initial non-mixed feeding step followed by a secondary mixed feeding step. This adjustment was made to increase the in-reactor substrate gradient and the subsequent anaerobic carbon uptake. Last, the non-mixed to mixed feeding ratio was further increased. The total experimental period was divided into four periods based on the applied operational strategy. Table 1 gives an overview of the adjustments made during the experiment.

During the aerated phases, the DO concentration was maintained between 1.0 and 2.0 $mgO_2 \cdot L^{-1}$ through an on/off aeration control strategy. The average SRT was 70 days due to the automatic sludge removal of 177 mL day^{-1} during effluent withdrawal. Between day 101–118, no wastewater was available and so a period of mixing without feeding was introduced to preserve the sludge.

Industrial wastewater and SBR seed sludge

The laboratory-scale SBR was fed with chocolate processing wastewater originating from a local chocolate producing company. Wastewater batches of physico-chemically pretreated wastewater were sampled approximately every two weeks at the existing wastewater treatment plant (WWTP) and stored at 4 °C to reduce microbial activity. Nitrogen (urea, 30%) and phosphorus (phosphoric acid, 75%) were manually dosed to avoid nutrient deficiency, resulting in an average chemical oxygen demand (COD):N:P ratio of 100:2:0.5. The wastewater was consistently sieved (particle retention: 1 mm) to remove any remaining particulates and avoid clogging of the feeding tubes. Table 2 shows an overview of the chocolate processing wastewater composition throughout the experiment.

The seed sludge for AGS formation in a conventional SBR originated from a previous laboratory-scale experiment during which food-processing wastewater was treated.

Laboratory-scale MBR filtration tests

Sludge samples: origin, preparation and characterization

Two sludge samples were collected to determine the effect of the sludge granulation state on the sludge filterability: (1) AGS from the laboratory-scale SBR fed with chocolate processing wastewater and (2) activated sludge from the

Table 1 | Overview of the changes of the operational strategy

Periods Day	Period I 1–118	Period II 119–152	Period III 153–250	Period IV 251–311
Feeding strategy	Mixed feeding	Mixed feeding	Non-mixed/mixed feeding	Non-mixed/mixed feeding
Non-mixed feeding (min)	0	0	60	100
Mixed feeding (min)	127	127	67	27
Mixed feast phase (min)	3	30	15	15
Total feast phase (min)	130	157	142	142
Aerobic phase (min)	365	338	353	353
COD_{avg} ($mg\ O_2 \cdot L^{-1}$)	5,552 ± 1,454	3,003 ± 1,910	3,886 ± 1,281	3,742 ± 1,001
OLR ($kgCOD \cdot (m^3 \cdot day)^{-1}$)	1.6 ± 0.4	1.1 ± 0.7	1.3 ± 0.4	1.1 ± 0.3
F/M ($kgCOD \cdot (kgMLVSS \cdot day)^{-1}$)	0.18 ± 0.07	0.18 ± 0.10	0.13 ± 0.03	0.11 ± 0.04

F/M, feed to mass ratio; MLVSS, mixed liquor suspended solids.

Table 2 | Overview of the industrial wastewater characteristics

	SS ($mgSS \cdot L^{-1}$)	COD_t ($mgO_2 \cdot L^{-1}$)	COD_s ($mgO_2 \cdot L^{-1}$)	VFA/COD_t (%)	TN ($mgN \cdot L^{-1}$)	TP ($mgP \cdot L^{-1}$)
n	20	57	50	48	50	51
Min	45	1,784	1,546	7	21.7	6.0
Max	2,458	8,440	6,341	29	138	53.2
Average	653	4,604	3,777	16	78.4	21.6
Stdev	62	1,830	1,450	5	25.0	10.1

existing continuous WWTP treating the same wastewater. To minimize the effect of mixed liquor suspended solids (MLSS) variations on the sludge filterability, an MLSS concentration close to $10\ g \cdot L^{-1}$ was maintained for both samples through sludge dilution with effluent or sludge thickening by the use of centrifugation (Sigma 3–16 KL). Subsequently, the resulting ML(V)SS concentration and diluted sludge volume index (D)SVI were determined according to the standard methods (APHA/AWWA/WEF 1998). Additionally, microscopic analysis of the sludge, the capillary suction time (CST) and the particle size distribution analysis were performed.

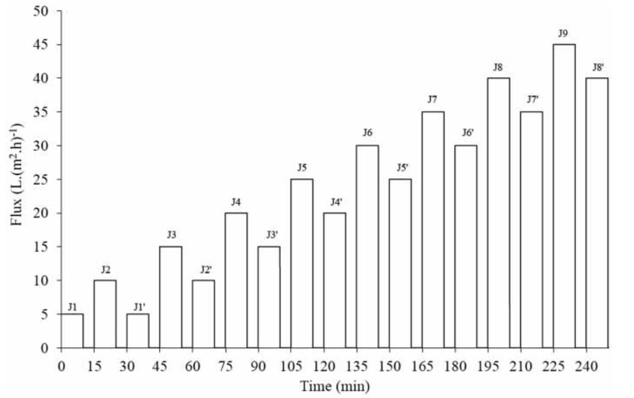

Figure 1 | Applied flux-step profile for the sludge filtration experiments.

MBR set-up and operation

To confirm the effect of the sludge granulation state on the sludge filterability within conditions that are more closely related to the MBR crossflow conditions, a small-scale filtration test was designed. A Plexiglas laboratory-scale submerged MBR (sMBR) (see Appendix IIb) was used which was provided with a submerged A4 Kubota membrane (average pore size: 0.2 μm) and a bubble aerator at the bottom of the reactor (aeration rate: $125\ L \cdot h^{-1}$ or $1.1\ Nm^3 \cdot (m^2 \cdot h)^{-1}$). The membrane was directly connected to a pressure transducer and subsequently a peristaltic pump (Masterflex L/S Series) which was controlled by LABVIEW^{TM} software (National Instruments). The applied flux and corresponding pressure data were also recorded by LABVIEW. The applied fluxes increased step-wise as described by Van De Staey *et al.* (2015) with fluxes ranging from $5\ L \cdot (m^2 \cdot h)^{-1}$ up to $45\ L \cdot (m^2 \cdot h)^{-1}$, with intervals of 5 $L \cdot (m^2 \cdot h)^{-1}$. Between every 10 min of filtration, the membrane was physically cleaned through air scouring during 5 min of membrane relaxation. A graphical overview of the applied flux profile is given in Figure 1.

From the filtration experiment, the transmembrane pressure (TMP) profiles are generated and used to determine the average TMP and fouling rate (FR) for every applied flux step. From Figure 1 it is clear that every flux is applied twice (J_1, J_1', J_2, J_2', J_n, J_n',…, J_9' with J_n=J_n') and so every flux generates two TMP_{avg} values, one for each applied flux step, i.e. $TMP_{avg,J1}$ and $TMP_{avg,J1'}$. The sustainable flux ($SF_{0.5}$) was calculated and defined as the flux for which the FR is above the threshold value of $0.5\ mbar \cdot min^{-1}$. When the TMP exceeded 150 mbar the filtration protocol was automatically stopped to prevent damaging the pressure transducer.

Clean water filtration test

To gain more insight into the degree of irreversible fouling due to internal membrane pore blocking, a clean water filtration (CWF) test was designed and performed before and after each sludge filtration test. At the start, the CWF protocol was performed using a clean membrane in the MBR unit, filled with demineralized water followed by the actual sludge filtration test. Subsequently, the laboratory-scale MBR unit was cleaned using demineralized water while the membrane cake layer was manually removed by flushing the membrane surface using demineralized water. Thereafter, it was repositioned into the laboratory-scale MBR unit and a second CWF protocol was performed. The CWF filtration protocol consisted of five cycles of alternating filtration (flux = 30 L.(m^2·h)$^{-1}$) and relaxation steps of 10 and 5 min, respectively, while filtering demineralized water. The loss of membrane permeability due to pore blocking during the sludge filtration test will result in a net increase of the total membrane resistance R. The membrane resistance analysis was made using Darcy's Law as described by Wang *et al.* (2013). After each experiment, the membrane was intensively chemically cleaned as described by Van De Staey *et al.* (2015) to restore the original membrane permeability.

Analytical measurements and sludge characterization

Influent and effluent compositions were determined using Hach test kits (Mechelen, Belgium) measuring the following parameters: total COD (COD_t) and soluble COD (COD_s) (LCK014, LCK514), total nitrogen (TN-N) (LCK 338, LCK138), total phosphorus (TP-P), phosphate phosphorus (PO_4^{3-}-P) (LCK350 and LCK348) and volatile fatty acids (VFA) (TNT872). Before measuring the soluble COD and PO_4^{3-}-P concentrations, samples were filtered using glass microfibre filters (particle retention: 0.6 μm, Macherey-Nagel MN GF-3). During reactor operation, the MLSS concentration was measured by filtering 5 mL of a homogeneous sludge mixture over a glass microfibre filter, which was subsequently washed with demineralized water and dried for 24 h at 105 °C. The influent suspended solids (SS) concentration was determined in the same way, except that instead of 5 mL, a volume of 20 mL was filtered. The (D)SVI and MLSS before the filtration experiments were determined as described by APHA/AWWA/WEF (1998). The particle size distribution by volume (DV) of sludge samples were measured using a Malvern Mastersizer 3000 (Malvern, UK) as described by Stes *et al.* (2018). Weekly analysis of the sludge was performed to investigate the evolution of the sludge morphology using a CX43 Olympus microscope (phase contrast). Additionally, the sludge filterability/dewaterability was regularly determined through a chromatography-based test using CST measurements (Triton Electronics). The concentration and characteristics of structural gel-forming extracellular polymeric substances or alginate-like exopolysaccharide (ALE) were determined according to the high temperature–sodium based extraction protocol as described by Felz *et al.* (2016), including some practical modifications, i.e. centrifugation at room temperature instead of at 4 °C. To compare the sludge rheology characteristics, the sludge viscosity was determined using a HAAKE RheoStress 1 rheometer (Thermo Electron Corporation). Sludge viscosity was determined using a PP60 plate, while the *g* rotation speed gradually decreased from 150 s^{-1} to 120 s^{-1} at a constant temperature (20 °C), which is in line with the operating conditions of both the SBR and the MBR.

RESULTS AND DISCUSSION

AGS-SBR performance

The conventional SBR was operated to promote aerobic granule formation through a sole metabolic selection pressure, i.e. by applying a slow anaerobic feast feeding strategy followed by an aerobic famine phase. The SBR was operated under realistic feeding rates for full-scale application and minimum wash-out conditions via a prolonged settling phase (120 min) to create operating conditions that were comparable to an MBR system treating real industrial wastewater. Due to the prolonged settling phase, no additional hydraulic selection pressure was applied to separate well-settling particles from slow-settling particles, which is more comparable to conditions in MBR systems. A constant VER of 11% was applied while the influent composition showed strong variations (Table 2), resulting in a fluctuating organic loading rate (OLR) throughout the operational periods. The increase of the OLR from 1.1 up to 2.5 kgCOD·(m^3·day)$^{-1}$ during Period I had a negative impact on the effluent quality. The effluent COD_s values reached up to a maximum value of 124 mgO_2·L^{-1} (day 86) while COD_t increased up to 179 mgO_2·L^{-1}, which is above the Flemish discharge limit of 125 mgO_2·L^{-1} (see Figure 2). The increased effluent COD_s concentration suggests that the biomass was not able to fully metabolize the temporarily increased carbon loading. Additionally, despite the long settling phase, elevated effluent SS concentrations were observed by the increased difference between the effluent COD_t and COD_s values. From Period II, the OLR and reactor performance were more stable. Thereafter, during Periods III and IV, strong variations of the OLR between 0.6 and 2.0 kgCOD·(m^3·day)$^{-1}$ were once more observed. However, these varying OLR had a less negative impact on the overall reactor performance because COD_t values were consistently below 80 mgO_2·L^{-1} from the beginning of Period III until the end of the experiment. An overview of the effluent COD_t and COD_s concentrations and COD removal efficiencies throughout the experimental periods are given in Figure 2.

As can be seen from Figure 2, during Periods I and II both the effluent COD_t and COD_s concentrations were temporarily higher and overall less stable under varying OLRs

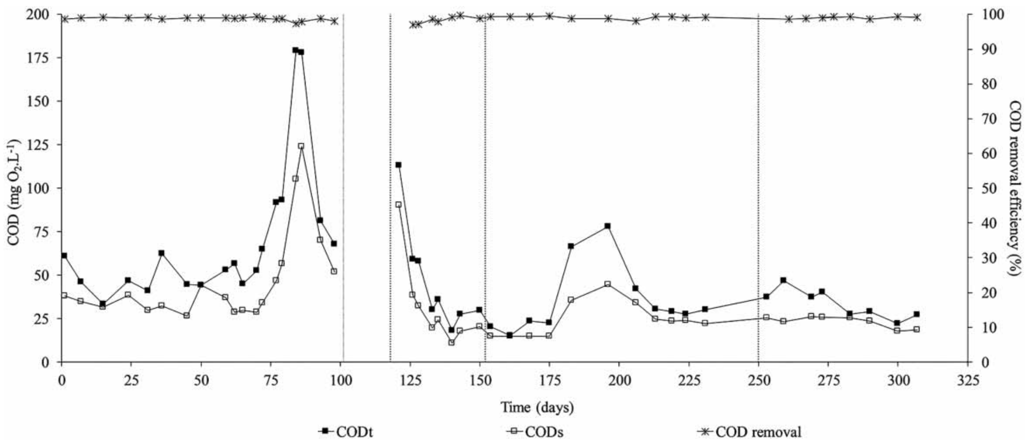

Figure 2 | Effluent COD concentrations and COD removal efficiencies for the treatment of chocolate processing wastewater. (Dotted lines: change of operational period; day 101–119: period of mixing without feeding).

compared to Periods III and IV. During Periods I and II, the complete anaerobic mixed feeding strategy prevented the enrichment of granule forming organisms (see next paragraph). Additionally, increased OLRs led to worsening of the sludge settling properties and subsequently to increased effluent SS concentrations. It is proposed that, during Periods III and IV, the increased substrate concentration during the anaerobic feast phase as a result of the partially non-mixed feeding phase had a positive impact on the enrichment of granule forming organisms, leading to more stable settling characteristics (see Figure 4) and reactor performances. These findings concerning the resilience of AGS towards variating OLRs are correspond with the general knowledge that, compared to conventional activated sludge, AGS has a greater ability to withstand shock loadings (Liu & Tay 2004). The improved stability of the effluent quality during Periods III and IV indicate that the optimization of the operational strategy, merely the feeding strategy, had a positive impact on the sludge settling characteristics and stability of the overall removal performance. It should be noted that the suspended solids content in the chocolate processing industrial wastewater was sometimes very high during the SBR operation (Table 2). In general, the presence of particulates in the influent is known to cause elevated SS in the effluent in AGS systems, especially when treating industrial influents (Morales *et al.* 2013). However, the reactor performances during Periods III and IV were found to be more resistant towards variations in OLR and influent particulates compared to Periods I and II.

Granule formation and sludge filterability

To evaluate the evolution of the sludge granulation characteristics, the sludge settling behaviour, morphology and particle size distribution were regularly determined. The evolution of the sludge morphology is shown in Figure 3.

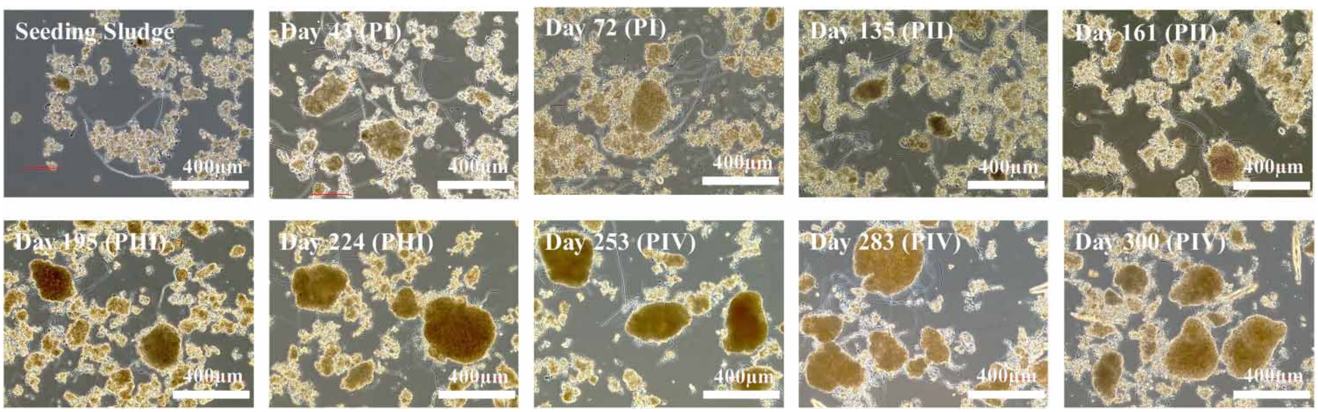

Figure 3 | Evolution of the sludge morphology (calibration curve: 400 µm, ×400 magnification).

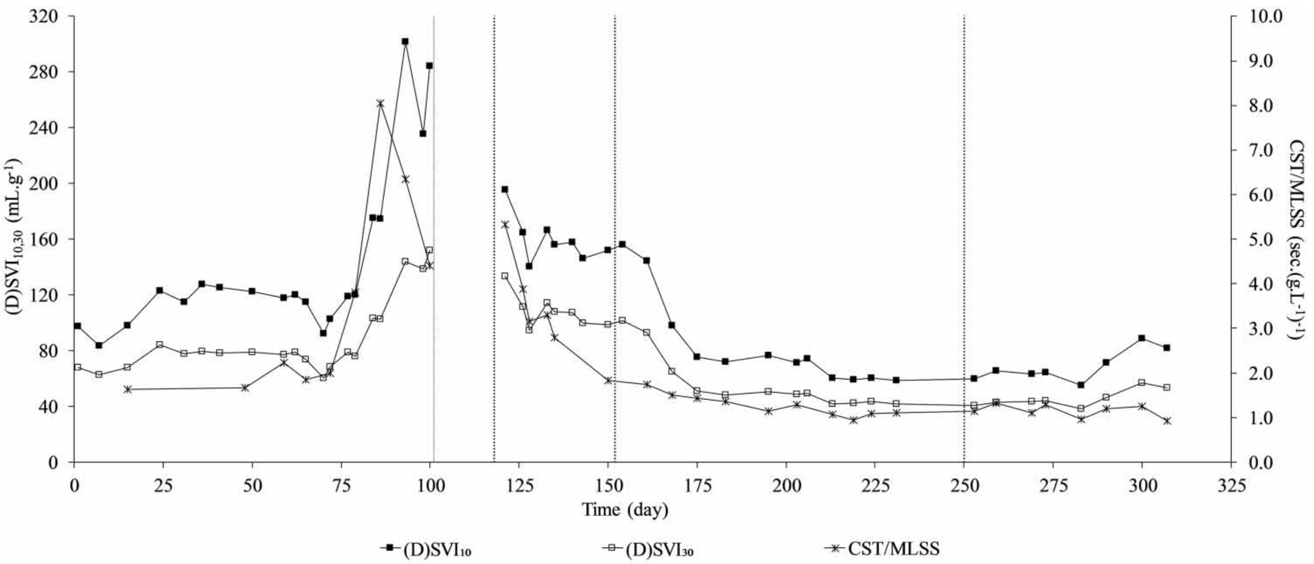

Figure 4 | Evolution of the sludge settling and filtration characteristics. (Dotted lines: change of operational period; day 101–119: period of mixing without feeding).

It is clear that implementation of the initial operational strategy during Periods I and II (day 1–152) had no major impact on the sludge morphology and thus on the development of aerobic granules in the system. The mixed liquor was dominated by small, loose flocs and only minor dens structures were present in the system. The median particle size by volume (DV_{50}) remained below 100 μm and DV_{90} below 250 μm during Periods I and II, which confirms the microscopic observations. To assess the effect of the granule characteristics on the sludge dewaterability/filterability, the CST was frequently determined (see Figure 4). The CST measurement was found to be a highly informative method for evaluating the sludge filterability characteristics (Scholes *et al.* 2016). However, the biomass concentration had a strong impact on the CST, and therefore the CST was normalized against the MLSS concentration (Scholes *et al.* 2016). The sludge settling and filterability behaviour varied strongly during Periods I and II, resulting in very high maximum $(D)SVI_{10,30}$ values of 302 $mL{\cdot}g^{-1}$ and 144 $mL{\cdot}g^{-1}$, respectively, and elevated CST/MLSS ratios reaching up to 8.1 $sec{\cdot}(g{\cdot}L^{-1})^{-1}$.

From day 101–119, a period of mixing without feeding was applied due to the temporary lack of wastewater production at the chocolate processing plant. During Periods III and IV, the OLR again showed strong variations; however, Figures 3 and 4 show that the introduction of the combined non-mixed/mixed feeding strategy (Periods III and IV) had a major impact on the sludge morphology and stability of the sludge settling and dewaterability. From the beginning of Period III (day 153) until the end of the experiment, instead of loose floc structures, large, dense granular structures developed together with small, dense granules (Figure 3). These findings are confirmed by an increase in the particle size distribution, with $DV_{50,90}$ values up to 128 ± 0.8 μm and 288 ± 16 μm, respectively, during Period III and up to 145 ± 2.0 μm and 395 ± 26 μm during Period IV, respectively. Accordingly, it was only during Periods III and IV that the sludge settling behaviour improved by a steep decrease of the $(D)SVI_{10,30}$. Both $(D)SVI_{10}$ and $(D)SVI_{30}$ values remained consistently below 100 $mL{\cdot}g^{-1}$ since day 163 and reached average values of 78 ± 27 $mL{\cdot}g^{-1}$ and 52 ± 17 $mL{\cdot}g^{-1}$, respectively. These results indicate the presence and stability of fast-settling granular biomass in the system during Periods III and IV. The results do not show an additional improvement of the settling or filtration characteristics after increasing the non-mixed feeding from 60 to 100 min; however, the $DV_{50,90}$ values increased. This may be explained by the increasing concentration gradient allowing the development of larger granules due to increased diffusion potential.

Even though variations of the OLR occurred throughout all four experimental periods, reactor performance and sludge behaviour were more stable in the presence of AGS in the system. These findings correspond to the results described by Morales *et al.* (2013) who reported that the AGS performance was not affected by varying OLRs while treating industrial swine slurry wastewater. The development and stability of the AGS had a strong influence on the stability and decrease of the CST/MLSS ratio, with average values of 1.3 ± 0.3 sec·$(g{\cdot}L^{-1})^{-1}$ and 1.1 ± 0.1 sec·$(g{\cdot}L^{-1})^{-1}$ for Periods III and IV, respectively. The simultaneously improved sludge settling and dewaterability characteristics during the development of mature granules are in line with the results reported by Basuvaraj *et al.* (2015) who found a strong correlation between the floc size, SVI and CST values for AGS, stating that large granular structures showed better settling and dewaterability characteristics. They reported the DO and F/M ratio to be important parameters for obtaining granules with good dewaterability performances. In this study, granular sludge was successfully developed during Periods III and IV without the use of a hydraulic selection pressure while treating industrial wastewater. The granules were found to be able to withstand variations of the OLRs in terms of effluent quality, sludge settling and dewaterability. Therefore, our results imply that the feeding strategy was decisive and had the most impact on the overall reactor performance and sludge characteristics when operating in non-hydraulic selection conditions. This corresponds with the findings by Stes *et al.* (2018) where successful AGS formation was achieved in treating industrial brewery wastewater by applying sufficient feast/famine conditions through a non-mixed pulse (10 min) feeding strategy, followed by a prolonged mixed anaerobic feast phase while operating under minimum wash-out conditions. However, short feeding phases are not feasible in full-scale applications due to the high flows required, and so longer feeding times are typically applied. As a result, an increase of the substrate gradient in a prolonged feeding phase is necessary to obtain sufficient feast/famine conditions. Stable AGS requires the presence of slow-growing organisms which are known to enrich under feast/famine conditions. During the anaerobic feast conditions, i.e. high substrate concentrations in the absence of an electron acceptor, these organisms take up the substrate and store it intracellularly as polymers, after which they use these stored polymers for microbial growth during aeration (de Kreuk & van Loosdrecht 2004). When in-reactor substrate concentrations remain low due to slow-mixed feeding, diffusion limitations may limit anaerobic carbon uptake and therefore the enrichment of slow-growing organisms. Additionally, diffusion limitations of the substrate towards the inner core of flocs or small granules may inhibit the development of larger structures. A sufficiently high substrate concentration during the feast was therefore achieved through the combination of a non-mixed and mixed feeding phase, which seems to be important for developing and maintaining stable granular sludge structures. This may be of concern in the development of AGS-MBRs, considering MBRs are typically operated in a continuous or moderate batch mode without applying stringent feast/famine conditions (Lin *et al.* 2012). In multiple studies concerning the filtration performance of AGS, the granules are cultivated under unrealistic operations for full-scale applications, i.e. extremely fast feeding and extreme wash-out conditions (Wang *et al.* 2013; Sajjad *et al.* 2016). Corsino *et al.* (2016) also addressed the importance of feast/famine conditions for maintaining stable granules in a continuous AGS-MBR treating synthetic wastewater. To our knowledge, it is the first time that the feeding strategy of an SBR was linked to the sludge dewaterability while treating real industrial wastewater.

Granular vs. floccular filtration tests – general sludge characterization

From these results, it is clear that the formation of AGS showed enhanced and stable dewaterability performance during Periods III and IV. Sludge dewaterability and filterability are found to be closely related (Sawalha & Scholz 2010); however, Scholz (2005) addressed the importance of different floc sizes and structures. To determine the effect of the AGS morphology on the actual sludge filtration behaviour, sludge from two different reactors treating the same industrial influent were seeded to the laboratory-scale MBR filtration unit and subjected to a flux-step protocol: (1) floccular sludge originating from the existing chocolate processing wastewater treatment plant and (2) the cultivated AGS from the laboratory-scale SBR. Several authors have reported inconsistent results concerning the impact of the biomass concentration on the sludge filtration characteristics (Le-Clech *et al.* 2006). To avoid possible effects of varying MLSS concentrations, both samples were brought to an MLSS concentration of about $10\,g{\cdot}L^{-1}$, which was found to be a suitable biomass concentration for an sMBR configuration (Judd 2016). In Table 3, an overview of the resulting sludge characteristics is given, including sludge concentration, settling and dewatering behaviour, the particle size distribution, viscosity and ALE content.

The results in Table 3 clearly show the differences in sludge characteristics between the two samples. Microscopic analysis (see Appendix I) shows that the floccular sludge sample, originating from the existing full-scale plant treating chocolate processing wastewater, is characterized by the presence of mainly (very) small loose flocs, minor filaments and some medium–large flocs. The median particle size by volume (DV_{50}) of the floccular sample is half of

Table 3 | Characterisation of floccular and AGS

	MLSS ($g \cdot L^{-1}$)	(D)SVI_{10} ($mL \cdot g^{-1}$)	(D)SVI_{30} ($mL \cdot g^{-1}$)	DV_{50} (μm)	DV_{90} (μm)	CST/MLSS ($sec \cdot (g \cdot L^{-1})^{-1}$)	Viscosity (mPa·s)	ALE (%)
Flocs	10.1 ± 0.3	297	112	76.8 ± 0.4	170 ± 8	2.7	2.43 ± 0.04	16
AGS	9.5 ± 0.1	69	46	147 ± 2	355 ± 11	1.8	2.92 ± 0.02	16

that of the AGS, indicating the presence of larger granule structures in the AGS. Sludge settleability as well as sludge dewaterability show significant differences indicated by elevated (D)SVI and CST/MLSS values for the floccular sludge and lower values for the AGS. The difference in sludge viscosity between the two sludge samples was found to be rather small. With both values below 3.0 mPa·s, these values are low compared to the mean viscosity value of 11.6 ± 0.6 mPa·s for industrial sludges reported by Buzatu *et al.* (2018). The rheology of the industrial samples in this study seem to be more similar to the municipal sludge samples reported by Buzatu *et al.* (2018) with a mean value of 2.1 ± 0.4 mPa.s. Additionally, the ALE extraction resulted in a similar yield of 16% for both sludge type, while for both samples the extracted ALE showed positive gel-forming behaviour.

Granular vs. floccular filtration tests – sludge filterability

In this experimental set-up, the MBR unit is operated by a step-wise increase of the flux where membrane fouling and clogging lead to an increase of the TMP. The flux steps were divided by 5 min relaxation periods, during which reversible membrane fouling, i.e. cake layer, is partially removed due to air scouring. For each flux step, the average TMP (TMP_{avg}) was calculated. For practical reasons, the data set used to determine the TMP_{avg} values consisted of all TMP measurements taken arbitrary 60 s after the start of the flux step until the end of filtration. In this study, the membrane fouling was noted in terms of an increase of the TMP over time (t). From the resulting TMP profiles, the FR was determined for every flux step, which is defined as the rate at which the TMP increases at a certain flux and is calculated using the following formula:

$$FR\,(\mathrm{mbar} \cdot \mathrm{min}^{-1}) = \frac{\sum (TMP_n - TMP_{avg}).(t_n - t_{avg})}{\sum (t_n - t_{avg})^2}$$

The resulting values for both sludge types at each flux-step are given in Figure 5. As previously mentioned, the filtration experiment is stopped when TMP exceeds 150 mbar. For the AGS, the maximum TMP remained very low for all flux steps, reaching a maximum value of 53 mbar at the end of filtration at a flux of 45 $L \cdot (m^2 \cdot h^{-1})$. However, for the floccular sludge sample, the maximum TMP of 150 mbar was reached after 6.6 min of filtration at a flux of 40 $L \cdot (m^2 \cdot h)^{-1}$, after which the filtration experiment was automatically stopped.

At fluxes up to 15 $L(m^2 \cdot h)^{-1}$, the TMP_{avg} was approximately 2–4 times lower for the AGS compared to the floccular sludge treating the same industrial wastewater. At fluxes above 15 $L \cdot (m^2 \cdot h)^{-1}$, the average TMP was up to eight times lower for AGS compared to the flocs, indicating a substantial improvement of the sludge filterability due to the changes in the operational strategy of the bioreactor. The sustainable flux ($SF_{0.5}$) in this study is defined as the flux at which the FR remains below a threshold value of 0.5 $mbar \cdot min^{-1}$, as suggested by Van De Staey *et al.* (2015). The concept of a sustainable flux noted as an FR has shown to be an appropriate way to relate to the operational and economic sustainability of the filtration process (Bacchin *et al.* 2006). As can be seen from Figure 5, the FR for the AGS did not exceed 0.1 $mbar \cdot min^{-1}$ even at fluxes up to 45 $L \cdot (m^2 \cdot h)^{-1}$ (FR = 0.08 $mbar \cdot min^{-1}$), meaning that the $SF_{0.5}$ was above 45 $L(m^2 \cdot h)^{-1}$ for the AGS. Thus, the sustainable flux for the AGS was not yet reached at a flux of 45 $L(m^2 \cdot h)^{-1}$. However, due to practical restrictions, higher fluxes could not be applied. On the contrary, the floccular sludge showed FR of 0.25 $mbar \cdot min^{-1}$ and 0.51 $mbar \cdot min^{-1}$ at fluxes of 10 $L(m^2 \cdot h)^{-1}$ and 15 $L(m^2 \cdot h)^{-1}$, respectively, indicating that $SF_{0.5}$ lies between 10–15 $L(m^2 \cdot h)^{-1}$ for the floccular sludge. Findings reported by Wang *et al.* (2013) are in line with our results, considering they both found granules to show improved filtration characteristics compared to flocs. It is believed that the granular sludge structures tend to remain in suspension, while flocs tend to settle easily on the membrane surface and subsequently form a sludge cake layer, leading to reduced membrane permeability (Truong *et al.* 2018). Corsino *et al.* (2016) and Truong *et al.* (2018) FR underlined the importance of feast/famine conditions for maintaining stable granules and subsequently reduced membrane fouling tendencies.

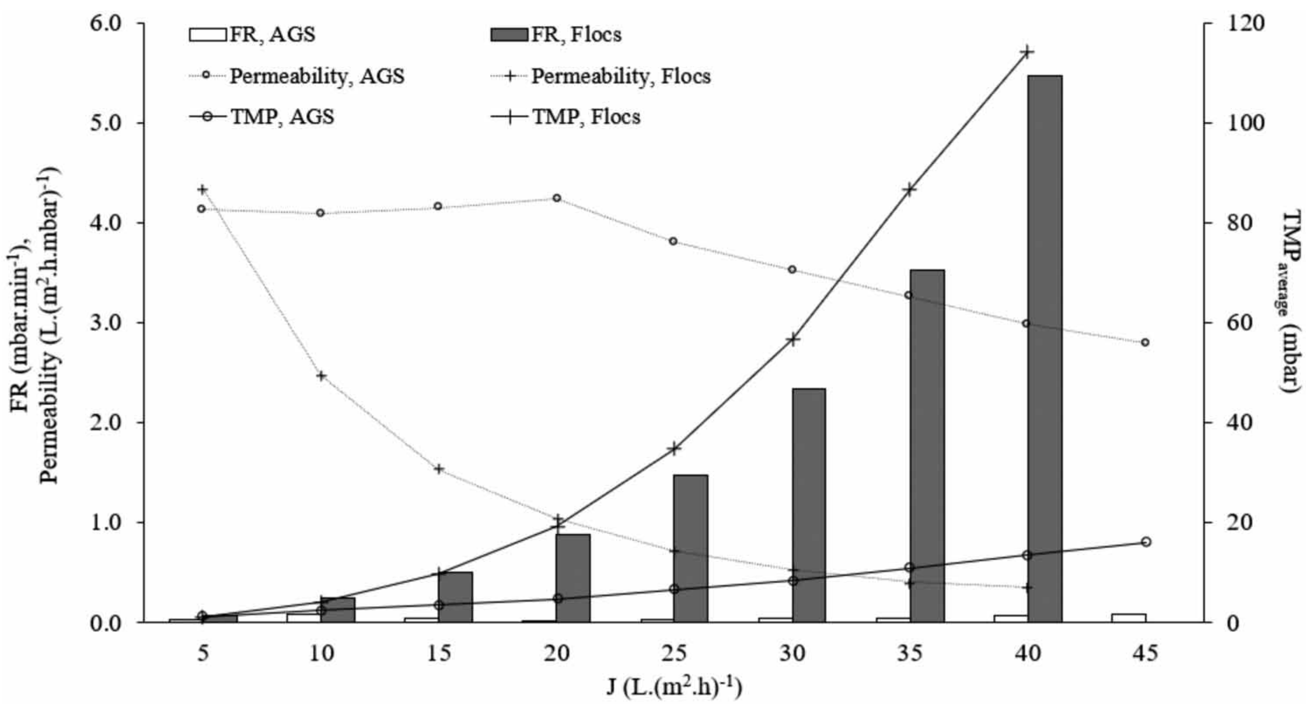

Figure 5 | Calculated average TMP, FR and permeability for each increasing flux step (J_n) for both flocs and AGS sludge.

Fouling is typically divided into two components: (1) reversible fouling, which can easily be removed by the implementation of physical cleaning, e.g. relaxation, and (2) irreversible fouling, which includes the deposition of material that can only be removed through chemical cleaning. In general, reversible fouling is attributed to the formation of the cake layer, while the irreversible fouling is caused by pore blocking or clogging. To determine the reversibility of the impact of a certain flux increase, the flux was increased by 10 $L(m^2{\cdot}h)^{-1}$ and subsequently decreased by 5 $L{\cdot}(m^2{\cdot}h)^{-1}$ while the TMP was measured (see Figure 1). To compare the TMP values at a certain flux, the calculated TMP_{avg} was used. During the sludge filtration experiment, the flux was increased to a flux J_{n+1} and subsequently again decreased to a lower flux (J_n'). If at a flux $J_{n'}$ (=J_n) the $TMP_{avg,n'}$ is equal to $TMP_{avg,n}$ no irreversible fouling has been built up. However, if the resulting $TMP_{avg,n'}$ was increased compared to $TMP_{avg,n}$ (arbitrary, >5%), irreversible fouling has occurred during the previous flux step (J_{n+1}), indicating the exceedance of the critical flux for irreversibility. The critical flux for irreversibility is therefore defined as the flux at which membrane fouling is not removed through intermediate physical cleaning (van der Marel *et al.* 2009), e.g. relaxation in our study. For the AGS sample, the critical flux for irreversibility was found to be 25 $L(m^2{\cdot}h)^{-1}$. Below this critical flux, the difference in TMP_{avg} for the first and second filtration cycles per flux was negligible (<5%), indicating the absence of irreversible fouling. However, when the flux was increased to 25 $L(m^2{\cdot}h)^{-1}$ and subsequently reduced to 20 $L(m^2{\cdot}h)^{-1}$, the second filtration cycle was characterized by an increase of the TMP_{avg} value, with 0.6 mbar from 4.7 mbar (first filtration cycle) to 5.3 mbar (second filtration cycle). Due to the relatively large flux steps of 5 $L(m^2{\cdot}h)^{-1}$, it can be mentioned that the actual critical flux for irreversibility lies between 20 and 25 $L(m^2{\cdot}h)^{-1}$ for the AGS. For the floccular sludge sample, the critical flux for irreversibility was already reached at a flux of 10 $L(m^2{\cdot}h)^{-1}$. The first and second filtration cycles at a flux of 5 $L(m^2{\cdot}h)^{-1}$ were characterized by a TMP_{avg} value of 1.2 mbar and 2.1 mbar, respectively, indicating that the intermediate flux of 10 $L(m^2{\cdot}h)^{-1}$ caused irreversible fouling that could not be removed through relaxation. These results indicate that irreversible fouling was more severe for the floccular sludge sample at relatively low fluxes compared to the granules. From an operational point of view, this is of great importance, considering that for the floccular sludge sample, after a peak flux because of, for example, a temporary increase of the hydraulic loading, the initial filtration performance could not be recovered solely by reducing the applied flux. Instead, a chemical cleaning procedure will be needed to remove the build-up of irreversible fouling.

This experiment was designed to gain insight into the difference in sludge filtration behaviour when submitted to operational conditions closely related to full-scale operations, such as high MLSS concentrations (~10 g·L^{-1}) and moderate aeration flow rates (125 L·h^{-1}). It is clear that the introduction of a feast/famine regime in the SBR resulted in great improvements, not only in the stability of reactor performance and sludge settling and dewaterability, but also in actual sludge filterability performances. Even though the resulting granules in our study are relatively small (see Table 3) compared to other studies (>0.5 mm) (Wang *et al.* 2013; Corsino *et al.* 2016), the results clearly indicate that the membrane fouling occurs to a lesser extent with AGS compared to flocs under realistic conditions when cultivated under non-wash-out feast/famine conditions. To the best of our knowledge, our study proposes, for the first time, the application of only a metabolic selection pressure using a prolonged anaerobic feeding strategy as a way to successfully maintain AGS in a SBR. This operational strategy is believed to be feasible for full-scale applications in industrial wastewater treatment processes.

Granular vs. floccular filtration tests – membrane pore blocking

To assess the difference in irreversible membrane pore-blocking tendency more in-depth, an additional membrane resistance analysis was performed based on the results of the CWF tests. The clean membrane resistance (R_m) and membrane resistance due to pore blocking (R_p) are determined respectively before (Equation (1)) and after (Equation (2)) the sludge filtration experiment using demineralized water. A flux of 30 L(m^2·h)$^{-1}$ was applied five times for a duration of 10 min, and divided by 5 min of relaxation. From the resulting TMP values, R_m and R_p are calculated using the following formula:

$$R_m = \frac{TMP_{avg}}{\mu \cdot J} \tag{1}$$

$$R_{irreversible} = R_m + R_p = \frac{TMP_{avg}}{\mu \cdot J} \tag{2}$$

with $R_{irreversible}$ as the total irreversible membrane resistance during the CWF experiment, μ the viscosity for demineralized water (=1 mPa·s) and J the applied flux (=30 L(m^2·h)$^{-1}$). For the floccular sludge sample, the membrane resistance due to pore blocking was up to 21 times higher compared to the AGS. The average membrane resistances due to pore blocking (R_p) were 0.5×10^{11} m^{-1} and 1.1×10^{12} m^{-1} for the AGS and the flocs, respectively. It is reasonable to conclude that long-term MBR operation with the floccular sludge would result in a substantially higher operational cost due to the need for more frequent chemical cleaning procedures to remove the irreversible fouling. Several authors have proposed that membrane fouling is reduced using AGS due to the large particle size resulting in a more porous, loose-cake structure, and thus an increased permeability (Liébana *et al.* 2018; Truong *et al.* 2018). In literature, both pore blocking and cake deposition were found to be the main contributors to the overall membrane resistance for AGS, while for floccular sludge, the cake resistance was found to be the main contributor (Wang *et al.* 2013; Corsino *et al.* (2016), Truong *et al.* 2018). From the results presented in this study, it is unclear whether the enhanced sludge filtration performance with AGS was solely due to the reduced pore-blocking tendency or whether it can also be attributed to a reduced cake layer resistance. However, our results clearly show a strong reduction of the overall membrane fouling and additional pore-blocking tendency with AGS compared to floccular sludge.

CONCLUSIONS

From an operational point of view this research gives a clear message concerning the impact of the feeding strategy on the stability of the reactor performance and sludge filterability when treating industrial wastewater. Application of a slow non-mixed/mixed feeding strategy was found to be suitable to obtain sufficient feast/famine conditions and to successfully maintain AGS stability under minimum wash-out conditions. Even though the cultivated granules were relatively small, membrane fouling tendencies were up to eight times lower for the AGS compared to floccular sludge treating the same chocolate processing wastewater. Additional membrane resistance analysis showed up to 21 times lower irreversible fouling due to pore blocking for AGS compared to the floccular sludge. The results indicate that the use of AGS would reduce both the capital and operational cost of a AGS-MBR compared to a conventional MBR.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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