



Feast/famine ratio determined continuous flow aerobic granulation

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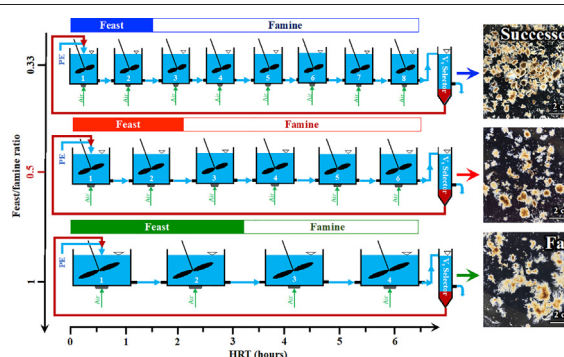
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HIGHLIGHTS

- The number of chambers used in PFR impacted aerobic granulation.
- The feast/famine ratio decreased with the chamber number increase.
- Feast/famine ratio < 0.5 is a prerequisite for continuous flow aerobic granulation.

GRAPHICAL ABSTRACT



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ABSTRACT

Plug flow reactors (PFRs) made of multiple completely stirred tank reactors (CSTRs) in series were used to cultivate aerobic granules in real domestic wastewater. Theoretically, changing the number of CSTR chambers in series will change the nature of plug flow, and thus alter the pattern of the feast/famine condition and impact the aerobic granulation progress. Therefore, PFRs were operated in 4-, 6-, and 8-chamber mode under the same gravity selection pressure (a critical settling velocity of 9.75 m h^{-1}) and hydraulic retention time (6.5 h) until steady states were reached to evaluate the effect of the feast/famine condition on continuous flow aerobic granulation. The sludge particle size, circularity, settleability, specific gravity, zone settling velocity, and extracellular polymeric substance contents were analyzed to evaluate the role that a feast/famine regime plays in aerobic granulation. It was found that aerobic granulation failed whenever the feast/famine ratio was greater than 0.5. The results support a conclusion that the feast/famine condition is likely a prerequisite for continuous flow aerobic granulation.

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1. Introduction

Aerobic granular sludge is characterized by large and spherical aggregates of microbial cells formed through cell-to-cell adhesion. It provides treatment advantages over conventional activated sludge for

their high biomass retention, easy solid-water separation, interspecies synergy in close proximity, resilience to shock loading, and smaller tankage requirements (Gao et al., 2011; Hasebe et al., 2017; Sehar and Naz, 2016). Therefore, aerobic granulation has been regarded a promising technique for domestic and industrial wastewater treatment (De Kreuk et al., 2007; Morgenroth et al., 1997; Pronk et al., 2015). This technique was first achieved in sequential batch reactors (SBRs) back in 1997 (Morgenroth et al., 1997). After that, numerous bench- and pilot-scale aerobic granulation studies were performed in SBRs (Beun et al., 2002; Isanta et al., 2012; Liu et al., 2010; Tay et al., 2001a). In

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recent years, full-scale applications of aerobic granulation in SBRs have been practiced across the world, e.g. South Africa (2006), Portugal (2008), China (2010), and Netherlands (2010 and 2013) (Giesen et al., 2013; Li et al., 2014; Niermans et al., 2014; Pronk et al., 2015). However, all these full-scale applications are limited to SBRs (Kent et al., 2018; Pronk et al., 2015), the operation of which is actually inconsistent with the continuous flow nature of most large-scale wastewater treatment plants (WWTPs) (Kent et al., 2018). Instead, the continuous flow reactors (CFRs) are more desired by utilities for their simple design and easy operation (Chen et al., 2017; Juang et al., 2010; Kent et al., 2018; Li et al., 2016). More importantly, continuous flow aerobic granulation offers the possibility to retrofit existing infrastructure in WWTPs into aerobic granulation reactors with low capital investment (Kent et al., 2018). For this reason, it is highly desired to develop a continuous flow aerobic granulation technique applicable to existing infrastructure in large-scale WWTPs (Kent et al., 2018; Sun et al., 2019). In a previous study, we demonstrated that aerobic granules can indeed form from flocculent activated sludge inoculated into a plug-flow reactor (PFR) fed with real domestic wastewater with seasonal temperature and quality variations (Sun et al., 2019).

In a previous literature review (Kent et al., 2018), it was hypothesized that the alternating feast/famine condition might be an essential factor for successful aerobic granulation in SBRs besides the gravity selection pressure on sludge settling velocity. The critical role of gravity selection in aerobic granulation has been repeatedly proven in SBR studies (Gao et al., 2011; Liu et al., 2005). Basically, gravity selection pressure washes out slow settling suspended sludge and retains only fast settling granular sludge (Qin et al., 2004a; Qin et al., 2004b). Thus, gravity selection pressure has been regarded as a driving force promoting aerobic granulation (Liu et al., 2003; McSwain et al., 2004). Yet, it was also reported by Corsino et al. (2016) that aerobic granulation cannot be achieved in completely stirred tank reactors (CSTRs) without feast/famine conditions, suggesting that some degree of substrate gradient within the reactor may be required for successful aerobic granulation (Lee et al., 2010).

It was observed that periodic feast/famine operation produces bacteria that exhibit a more hydrophobic surface property, a key factor governing the initial cell-to-cell co-aggregation (Bossier and Verstraete, 1996; Liu and Tay, 2004; López-Palau et al., 2012; Tay et al., 2001b). It was also found that increasing the relative length of the feast period by increasing nutrient availability tended to result in structural dispersal of granules or biofilms (Sauer et al., 2004; Schwarzenbeck et al., 2005). Oppositely, a longer famine period tended to cultivate granules with better structural stability (Reisner et al., 2003). In spite of these sporadic observations, the authors were unable to find a study specifically dedicated to examining the correlation between the degree of feast/famine condition provided and the success of aerobic granulation achieved (López-Palau et al., 2012). In particular, none of previous studies was able to independently vary feast/famine condition without affecting the other key operational parameters such as hydraulic retention times (HRTs) or loading rates (Liu and Tay, 2007; Liu and Tay, 2008; López-Palau et al., 2012).

PFR offers an ideal platform for understanding the effect of feast/famine condition because it provides location snapshots of feast/famine condition along the plug flow direction independent of the sampling time at steady state. Connecting several CSTRs in series is a common way to approximate the flow pattern of PFRs. Technically, changing the number of CSTRs in series will change the nature of plug flow, and thus alter the pattern of feast/famine conditions (e.g. feast/famine ratio) without affecting HRT or loading rate. Therefore, this study aims to investigate the effect of different feast/famine condition on continuous flow aerobic granulation by alternating the number of CSTR chambers in series. It should be pointed out that the mechanism unraveled in this study may also be important to understand aerobic granulation in SBR systems.

2. Materials and methods

2.1. Reactor design

A bench-scale Plug-flow Aerobic Granulation reactor with 128 L total working volume was built and operated at the Upper Occoquan Service Authority (UOSA), a WWTP located in Centreville, VA, USA. Detailed reactor setup information can be found in a previous study by Sun et al. (2019). The bench-scale reactor consists of multiple CSTR chambers connected in series to approximate a PFR in which feast/famine conditions can be created. Gravity selection pressure was applied to drive aerobic granulation using two parallel external settling selectors placed at the end of the PFR. The selective retention of bioparticles with settling velocity greater than a critical settling velocity (V_c) was achieved by operating the settling selectors as intermittent clarifiers with a short settling time detailed elsewhere (Sun et al., 2019). As shown in Table 1, V_c was set at 9–9.75 m h⁻¹ by using a settling time of 4 min and a discharge height of 60–65 cm to selectively retain bioparticles with settling velocity greater than V_c according to a previous study (Wang et al., 2006). The influent of the bench-scale PFR was UOSA's primary effluent (PE). The characteristics of UOSA domestic wastewater during the study period are shown in Table 2 in terms of temperature, pH, total COD (tCOD), soluble COD (sCOD), 5-day biochemical oxygen demand (BOD₅), alkalinity, total suspended solid (TSS), Total Kjeldahl Nitrogen (TKN), ammonia (NH₃-N), and total phosphorus (TP). UOSA full-scale secondary treatment trains share the same influent, plug-flow nature, and HRT with the bench-scale study, while the major difference is that UOSA full-scale secondary treatment trains do not have a strong V_c as presented in the previous study by Sun et al. (2019). Basically, V_c of the full-scale treatment train is equivalent to the surface overflow rate (SOR) of its clarifier, which is as low as 1 m h⁻¹ (Table 1).

2.2. Reactor operation

The 416-day operation of the bench-scale PFR was divided into three phases, namely phases I, II, and III (Table 1). In this study, the number of CSTR chambers used in these three phases was controlled as the only variable to investigate the effects of feast/famine conditions on continuous flow aerobic granulation. Phase I lasted 118 days and used 10 CSTR chambers in series to mimic the plug-flow pattern in the bench-scale PFR. Detailed information of the phase I study can be found elsewhere (Sun et al., 2019). On day 118, because of an unexpected clogging and overflow accident, the bench-scale PFR lost more than 80% of its mix liquor suspended solid concentrations (MLSS). Right after that, the phase II operation was initiated on day 133 by reducing the chamber number from 10 to 8 with the other parameters listed in Table 1 unchanged. After a three-week steady state, phase III was started on day 253, during which the 10 CSTR chambers were divided into two PFR trains with 4 and 6 chambers in series, respectively. Both bench-scale PFRs were re-inoculated with UOSA return activated sludge (RAS) at MLSS of ~3000 mg L⁻¹ in each chamber. The detailed RAS characteristics can be found in a previous study by Sun et al. (2019). Phase III lasted for 163 days and was terminated on day 416 when no substantial change in sludge settleability can be observed for about four weeks. The operational parameters of the full-scale treatment train as shown in Table 1 remained unchanged during the entire study.

2.3. Analytical methods

As described previously (Sun et al., 2019), sludge characteristics such as 5 and 30 minute sludge volume index (SVI₅ and SVI₃₀), zone settling velocity (V_{zs}), MLSS, mix liquor volatile suspended solid (MLVSS), and specific gravity were all analyzed using standard methods (APHA, 1998). The samples for sludge characteristics analyses were taken from the last chamber of the PFR. pH was monitored using an online Liquiline M CM42 transmitter (Endress+Hauser, Greenwood, IN,

Table 1

Operational parameters of the bench-scale PFRs and the full-scale treatment train.

Phase	Duration (day)	Bench-scale			Full-scale			Study
		Number of chambers	HRT (h)	V_c (m ³ h ⁻¹)	Number of chambers	HRT (h)	V_c (m ³ h ⁻¹)	
I	0–118	10	6.5	9–9.75	4	6.5	1	Sun et al. (2019)
II	133–235	8	6.5	9.75				This study
III	253–416	4 & 6	6.5	9.75				

USA), and the temperature was monitored using an RC-4 temperature data logger (Elitech®, Milpitas, CA, USA). COD was analyzed using COD TNTplus® 820 vials and a spectrophotometer (Hach, Loveland, CO, USA), in which sCOD samples were filtered using 0.45 µm syringe filters (EZFlow®, Old Saybrook, CT, USA), and tCOD was measured in the supernatant after 30-min settling. Hence, tCOD includes particulate, colloidal and soluble COD that cannot settle with the granular sludge. BOD₅ and alkalinity were both analyzed using standard methods (APHA, 1998). TKN, TP, NH₃-N, NO₂⁻-N, and NO₃⁻-N were analyzed using a QuikChem® 8500 series 2 flow injection analysis system (Lachat, Loveland, CO, USA). Petri dish photos of mixed liquor samples were analyzed using image processing software (ImageJ 2.0.0) to determine the particle size and circularity distribution of the sludge. Extracellular polymeric substances (EPSs) extraction and quantification were performed based on the methods described by Liu and Fang (2002) and Sun et al. (2019). Briefly, 2% EDTA was used as the chemical extractant. Dialysis Kits (Spectrum™ Labs Spectra/Por™ 3500 D MWCO Standard RC Pre-Treated, Waltham, MA USA) were utilized to separate polymers with molecular weight greater than 3500 Da. A phenol-sulfuric acid method was adopted for polysaccharides (PS) analysis (Nielsen, 2010). Pierce™ modified Lowry protein assay was used for the protein (PN) analysis (Thermo Scientific™, Waltham, MA, USA).

2.4. Determination of the feast and famine conditions

In this study, the feast condition is defined as the environment with sufficient substrate to sustain microbial growth, and the other way around is the famine condition. To be specific, only when the microbial net growth is not negative, namely the theoretical growth rate ≥ theoretical decay rate, can the microbes be deemed growing under the feast condition, and vice versa. With that being said, Eq. (1) was utilized to measure whether a growth condition is feast; otherwise, it is famine,

$$-\frac{dS}{dt} \cdot Y_S \geq b \cdot X \cdot Q_{10}^{\frac{T-20}{10}} \quad (1)$$

in which S is the substrate concentration (mg COD L⁻¹), t is the time; b is the theoretical decay rate coefficient (h⁻¹) measured at 20 °C; T is the actual temperature (°C); Q_{10} is the temperature coefficient used to relate the biological kinetics change as a consequence of a 10 °C temperature change (Hegarty, 1973). A Q_{10} value of 1.63 was adopted based on

the typical value for activated sludge (Jones and Stephenson, 1996). X represents the microbial concentration as quantified by the MLVSS values, and a factor of 1.5 mg COD mg VSS⁻¹ was used for converting biomass to COD (Bullock et al., 1996). Y_S represents the theoretical growth yield of the microbes. The typical values of microbial b and Y_S in activated sludge were adapted from Activated Sludge Model No. 1 (ASM1) (Henze et al., 2000), namely 0.6 d⁻¹ and 0.67 mg COD mg COD⁻¹, respectively. The $-dS/dt$ in Eq. (1) can be estimated by assuming the first-order reaction in Eq. (2),

$$-\frac{dS}{dt} = kS \quad (2)$$

in which k is the first-order reaction coefficient. Thereby, the steady-state substrate concentration (S_i) in the i^{th} CSTR chamber of the PFR can be estimated by,

$$S_i = \frac{S_{i-1}}{1 + k \cdot \frac{\theta}{n}} \quad (3)$$

wherein n stands for the total number of chambers used in the PFR; θ stands for the total HRT of the PFR (Table 1); S_{i-1} is the influent substrate concentration from the previous, namely $i-1$ th chamber. S_0 is the PE substrate concentration in the influent to the PFR. By fitting substrate concentrations measured in each CSTR chamber together with the HRT and n used in the PFR, k values can be regressed. The degree to which the first-order reaction assumption fits the measured substrate concentration profile was evaluated by the correlation coefficient between the regressed and the measured data.

3. Results

3.1. Effect of chamber numbers on sludge settleability

It is broadly accepted that a SVI_{30} value of 60 mL g⁻¹ or lower and a $SVI_5:SVI_{30}$ ratio close to 1 are two major indicators of the successful aerobic granulation in terms of settleability (Kent et al., 2018), and thus both SVI_5 and SVI_{30} values were measured in the three phases of the study. According to Fig. 1a and b, SVI_{30} and $SVI_5:SVI_{30}$ ratios of the sludge in the 10-chamber PFR decreased from 100 mL g⁻¹ and 1.7 to around 60 mL g⁻¹ and 1.1, respectively, during the phase I study, indicating sludge with good settleability was cultivated and stabilized in the 10-chamber PFR as previously reported (Sun et al., 2019). At the beginning of phase II, SVI_{30} and $SVI_5:SVI_{30}$ of sludge in the 8-chamber PFR were around 80 mL g⁻¹ and 1.3 as a result of the accidental clogging and overflow which washed out most of the sludge (Fig. 1a and b). It took about 60 days for the PFR to regain SVI_{30} and $SVI_5:SVI_{30}$ values as low as 54 mL g⁻¹ and 1.2 (Fig. 1a and b). After SVI_{30} and $SVI_5:SVI_{30}$ values were stabilized around 50 mL g⁻¹ and 1.2 for three weeks in the 8-chamber PFR, the phase III study was initiated by dividing the 10-chamber PFR into a 4-chamber and a 6-chamber PFRs and then re-inoculating the two PFRs with the RAS from the full-scale treatment train. As can be seen from Fig. 1, the initial SVI_{30} and $SVI_5:SVI_{30}$ ratios for 4- and 6-chamber PFRs were as high as 110 mL g⁻¹ and 1.8, respectively. The decreasing trends of SVI_{30} and $SVI_5:SVI_{30}$ ratios for 4- and 6-chamber PFRs were generally identical during the first 100 days of phase III operation. However, SVI_{30} of 4-chamber PFR stopped

Table 2

Characteristics of the domestic wastewater.

Parameters	Unit	Raw influent			Primary effluent		
		Max.	Min.	Average	Max.	Min.	Average
Temperature	°C	N/A ^a	N/A	N/A	23.3	10.1	18.3
pH	N/A	7.7	6.8	7.3	7.7	6.7	7.3
tCOD	mg L ⁻¹	1320	219	554	687	150	232
sCOD	mg L ⁻¹	N/A	N/A	N/A	160	44	86
BOD ₅	mg L ⁻¹	344	63	197	148	76	116
Alkalinity	mg L ⁻¹	272	103	199	266	119	211
TSS	mg L ⁻¹	950	70	252	234	13	97
TKN	mg L ⁻¹	67.8	16.9	41.6	53.2	14.0	39.0
NH ₃ -N	mg L ⁻¹	45.8	10.2	28.2	37.3	10.0	27.7
TP	mg L ⁻¹	11.3	1.9	5.0	7.2	1.9	4.4

^a N/A: not applicable.

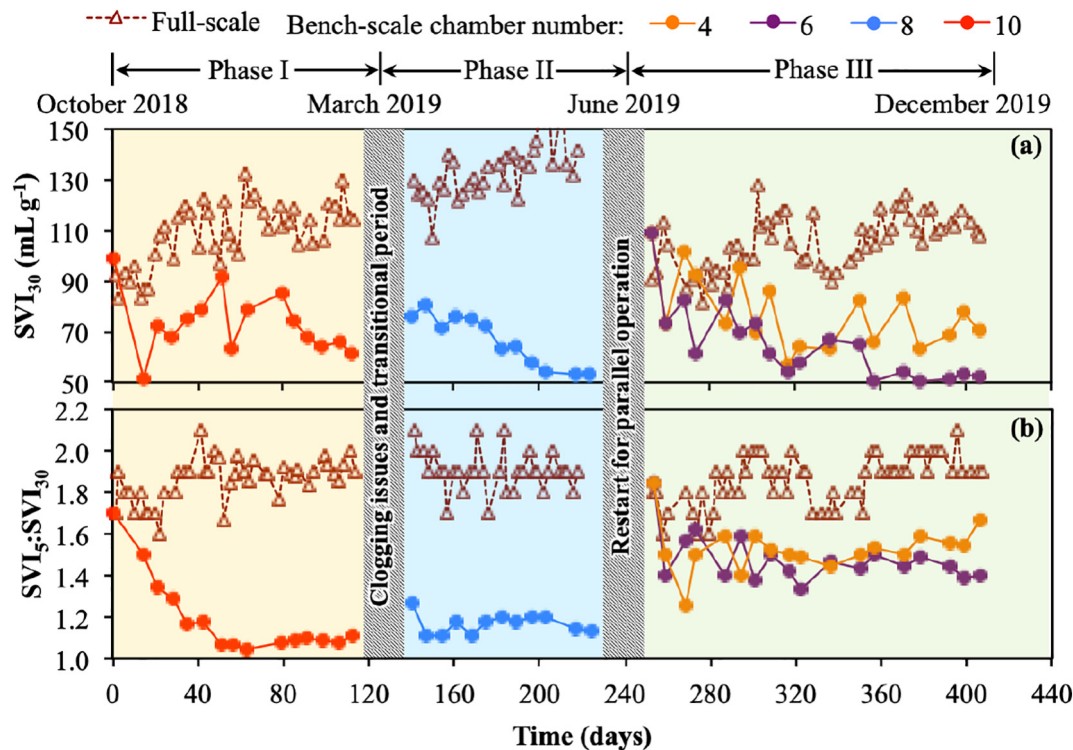


Fig. 1. Profiles of (a) SVI_{30} and (b) $SVI_5:SVI_{30}$ measured from the last chamber of the bench-scale PFRs and the full-scale treatment train over phase I, phase II, and phase III studies. Phase I data has been published in Sun et al. (2019). Operational parameters of the bench-scale PFRs and the full-scale treatment train during the three phases are shown in Table 1.

decreasing around the 110th day after the phase III startup and was finally stabilized around 70 mL g^{-1} . On the contrary, SVI_{30} of 6-chamber PFR continued to decrease until reaching around 50 mL g^{-1} at the 360th day and then was stabilized. Likewise, $SVI_5:SVI_{30}$ ratios for 4- and 6-chamber PFRs were stabilized around 1.6 and 1.4, respectively (Fig. 1). In contrast, the values of SVI_{30} and $SVI_5:SVI_{30}$ in the full-scale treatment train with the same influent water and HRT but a much lower V_c (1 m h^{-1} , Table 1) remained above 110 mL g^{-1} and 1.7 throughout the phase I, II, and III (Fig. 1). Such values are not uncommon for conventional activated sludge (Świątczak and Cydzik-Kwiatkowska, 2018; Wan and Sperandio, 2009), and in particular for this full-scale treatment plant. The steady-state values of SVI_{30} and $SVI_5:SVI_{30}$ ratios for the 4-, 6-, and 8-chamber PFRs are also summarized in Table 3. These results suggest that sludge settleability and degree of granulation improve as the number of CSTR chambers increases at the same HRT and V_c .

The V_{zs} is another indicator of sludge settleability (Sun et al., 2019), and the results in Table 3 merit additional discussion. V_{zs} of the sludge in the 4-chamber PFR was only 7.9 m h^{-1} . It becomes higher, e.g. 11.8 m h^{-1} , in the 6-chamber PFR, and even higher, e.g. 15.0 m h^{-1} , in the 8-chamber PFR. It is known that V_{zs} can be impacted by the MLSS concentration in that higher MLSS leads to smaller V_{zs} (Vanderhasselt and Vanrolleghem, 2000). According to Table 3, the reactor with the highest MLSS (e.g., 8-chamber) actually exhibited the highest V_{zs} , indicating that the increase of V_{zs} for that experimental run was not due to lower MLSS. The values of V_{zs} in 6- and 8-chamber PFRs were both greater than the V_c of the selector (9.75 m h^{-1}), indicating that the sludge adapted to the reactor's gravity selection pressure. On the contrary, the V_{zs} in the 4-chamber reactor was lower than the V_c of the selector, indicating that the sludge was not adapted to the gravity selection pressure in the 4-chamber PFR.

It is generally accepted that the sludge with better settleability may possess a higher specific gravity relative to water (Liu et al., 2009). According to literature, the specific gravities of traditional activated sludge are within the range of 1.001–1.01 (Cassidy and Belia, 2005; Li et al.,

2007; Su and Yu, 2005), while the granular sludge with good settleability can possess specific gravities as high as 1.03–1.2 (Cassidy and Belia, 2005; Liu et al., 2004; Tao et al., 2017; Zheng et al., 2005). As shown in Table 3, the specific gravities of the sludge in 4-, 6-, and 8-chamber PFRs were all within this high range and increased with the increase of chamber number used.

3.2. Effect of chamber numbers on sludge morphology

The sludge morphology in 4-, 6-, and 8-chamber PFRs at the steady state is shown in Fig. 2. According to the first petri dish photo in Fig. 2a, it can be seen that the majority of the sludge in the 4-chamber PFR was in the form of fluffy, large, and irregular bioflocs, and no granular sludge can be seen. This is consistent with the particle size and circularity distributions shown in Fig. 2b and c. Basically, the size of 60% of the sludge were greater than 8 mm, and more than 80% of the sludge biomass possesses the circularity less than 0.2 (Fig. 2b and c). In the 6-chamber PFR, although most sludge was still fluffy and loose, their

Table 3
Comparison of steady-state sludge characteristics in the bench-scale PFRs.

Parameters	Units	Chamber number		
		4	6	8
SVI_{30}	mL g^{-1}	68 ± 9	53 ± 7	52 ± 3
$SVI_5:SVI_{30}$	N/A ^a	1.56 ± 0.06	1.42 ± 0.04	1.18 ± 0.03
V_{zs}	m h^{-1}	7.9 ± 1.2	11.8 ± 1.5	15.0 ± 0.7
d_{50}	mm	12.4	4.1	2.1
Circularity (median value)	N/A	0.09	0.34	0.55
Specific gravity	N/A	1.03 ± 0.09	1.12 ± 0.07	1.19 ± 0.07
PS	mg g^{-1}	9.5 ± 0.5	9.9 ± 0.7	12.0 ± 0.8
EPS		20.4 ± 1.0	22.6 ± 1.5	38.2 ± 2.0
MLSS	mg L^{-1}	942 ± 122	2169 ± 145	2299 ± 177
MLVSS	mg L^{-1}	798 ± 102	1857 ± 129	2012 ± 172
SRT	days	2.0	5.0	6.4

^a N/A: not applicable.

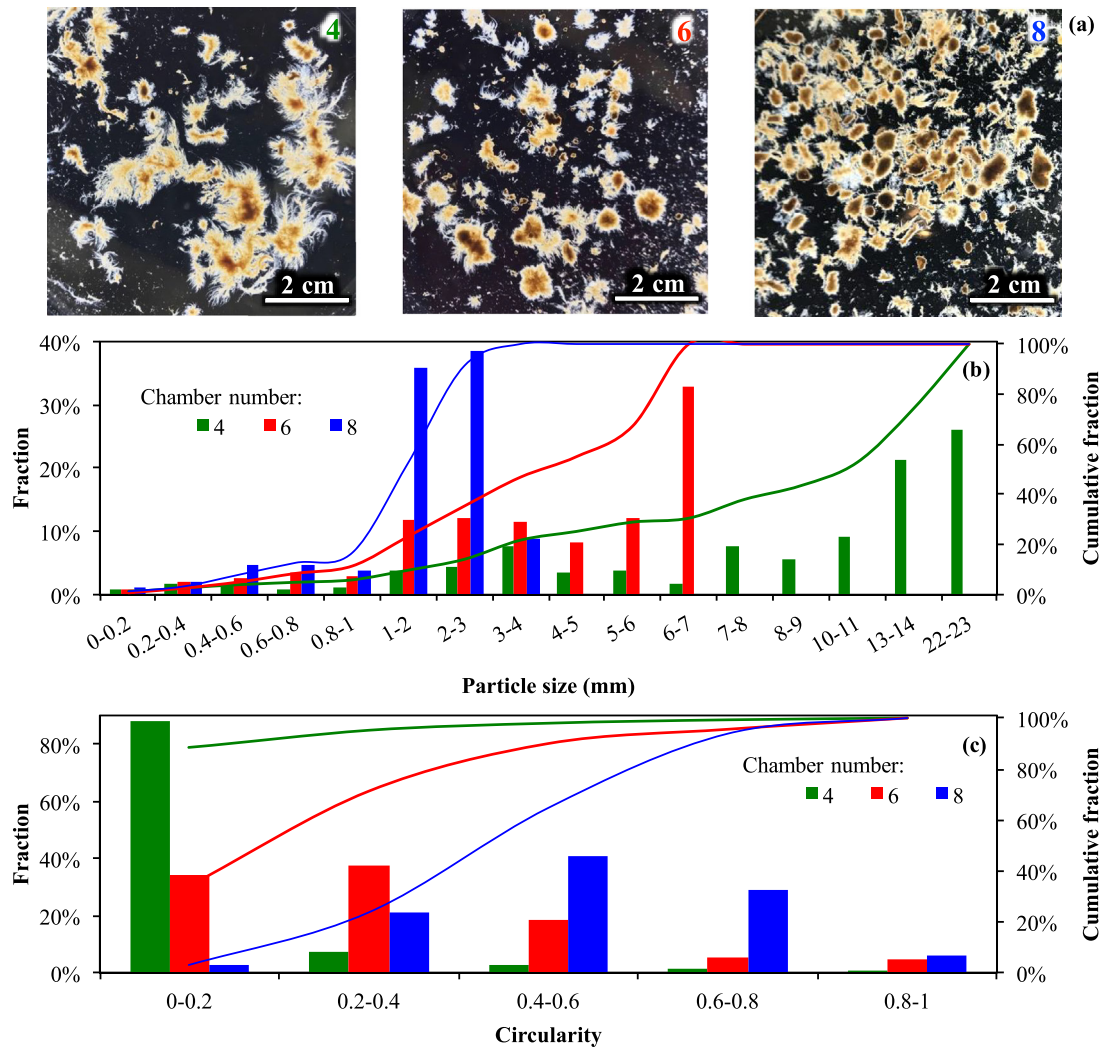


Fig. 2. Steady-state sludge morphology observed in the last chamber of 4-, 6-, and 8-chamber bench-scale PFRs: (a) petri dish photos, (b) particle size, and (c) circularity distributions.

shape became more regular, and those large bioflocs that appeared in the 4-chamber PFR did not show up (Fig. 2a). According to the particle size distribution shown in Fig. 2b, more than 50% of the particles were within the size range of 4–7 mm. As shown in Fig. 2c, sludge circularities in the 6-chamber PFR also shifted to greater values. In the 8-chamber PFR, it is noteworthy that dense and spherical granules already became dominant, and about 75% of granules were within the range of 1–3 mm, indicating sizes of granule were more uniform and smaller than those fluffy bioflocs in the 4- and 6-chamber PFRs (Fig. 2a). Besides, Fig. 2c showed that the sludge circularities shifted to even higher values in the 8-chamber PFR, indicating the granules in the 8-chamber PFR were indeed more spherical as compared to the fluffy bioflocs in the 4- and 6-chamber PFRs. As summarized in Table 3, the steady-state sludge circularities were positively correlated to the chamber number, and vice versa for the median size (d_{50}).

3.3. Effect of chamber numbers on feast/famine ratios

The Eq. (3) simulated substrate concentration profiles were plotted along with the actual data measured from the experiment with all regressed k and R^2 values presented in Fig. 3. As can be seen, Eq. (3) provided excellent fitness to the substrate profiles in all PFRs with R^2 values greater than 0.9, indicating that the first-order reaction assumption is acceptable. Most importantly, k values in Eq. (3) was found to increase

with the PFR chamber numbers at the same HRT, e.g. $k = 1.4, 2.0$, and 2.2 h^{-1} when $n = 4, 6$, and 8 (Fig. 3). This is not difficult to understand in that higher MLVSS were retained in PFRs with more chamber numbers by virtue of the better aerobic granulation (Table 3).

The substrate utilization rate ($-dS/dt$) in each chamber of the in 4-, 6-, and 8-chamber PFRs was calculated using Eq. (2) and then plotted in Fig. 4 as solid horizontal lines. The minimum $-dS/dt$ that can sustain microbial growth in each PFR was also calculated using Eq. (1) and plotted as dashed horizontal lines in Fig. 4. The feast phase of the feed regime is differentiated from the famine phase based on the last chamber with the growth rate $\geq$ the decay rate according to Eq. (1). This technique allows for a visualization of the feast and famine phase lengths in 4-, 6-, and 8-chamber PFRs, which are shown in the three colored boxes on the top of Fig. 4. According to Fig. 4, the number of feast chambers to famine chambers ratios, namely feast/famine ratios, for 4-, 6-, and 8-chamber PFRs were determined to be 1, 0.5, and 0.33, respectively.

4. Discussion

4.1. Feast/famine ratio dependent sludge characteristics

Fig. 2 shows that the success of aerobic granulation was compromised when the chamber number in PFR was reduced from 8 to 6 and then totally failed when further reduced to 4. Fig. 4 revealed that the

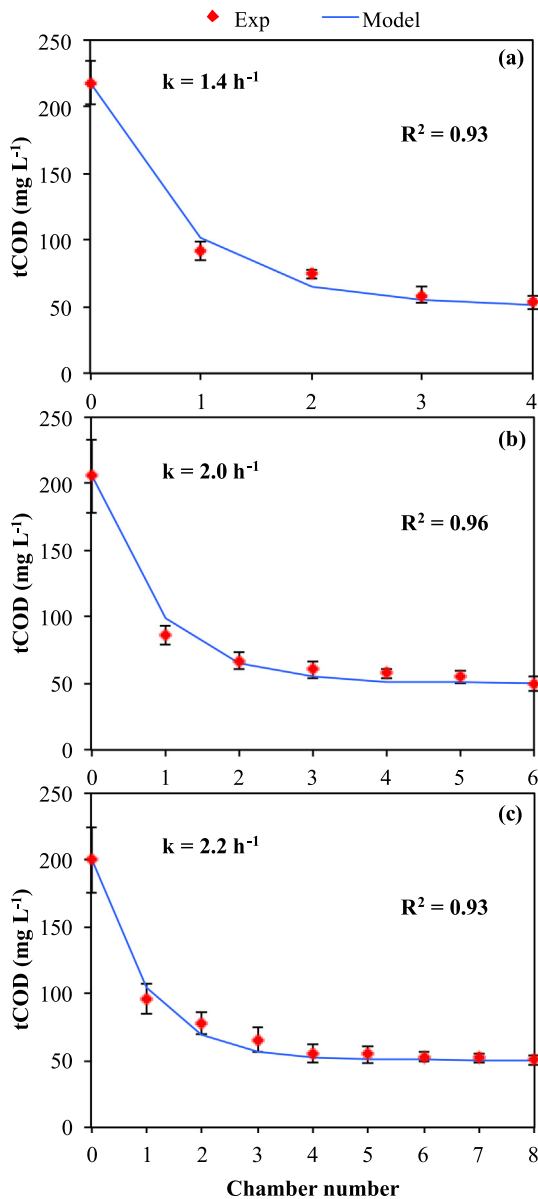


Fig. 3. Experimental and Eq. (3) simulated tCOD profiles at steady state in (a) 4- (b) 6-, and (c) 8-chamber bench-scale PFRs. Chamber number 0 represents the influent concentration.

number of chambers used in PFRs was actually highly associated with the feast/famine ratio resulted in PFRs. Therefore, it is a reasonable consideration that the feast/famine ratio might have played an essential role in determining the sludge morphology and settleability. This can be seen from the monotonic relationship ($R^2 > 0.85$) between the feast/famine ratio and almost all the sludge characteristics such as circularity, d_{50} , specific gravity, V_{zs} , SVI_{30} , SVI_5/SVI_{30} , MLVSS, MLSS, and k as shown in Fig. 5. It should be noted that all feast/famine ratios determined in Fig. 4 are steady-state values which can be approximated as constants. In contrast, the dynamic feast/famine ratios during reactor startup are variables and thus not considered in this study. It is clear from Fig. 5 that a feast/famine ratio as low as 0.33 can cultivate spherical, dense, and fast settling granular sludge in PFRs. Additionally, as the feast/famine ratio increased from 0.33 to 0.5, the sludge morphology and density became worse (Fig. 5a and b), while the settleability (SVI_{30}) did not change much (Fig. 5c). This phenomenon implies that the sludge cultivated under the feast/famine ratio of 0.5 did not look like granular sludge but has similar settleability to granular sludge.

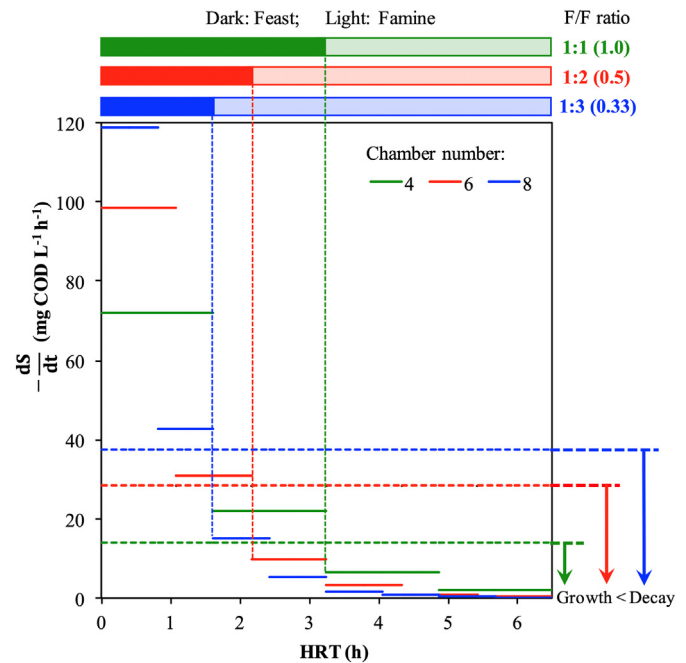


Fig. 4. Steady-state tCOD utilization rate ($-dS/dt$, solid horizontal lines) and the minimum $-dS/dt$ that can sustain microbial growth (dashed horizontal lines) in each chamber of PFRs calculated according to Eq. (1), from which the feast/famine (F/F) ratios were determined (colored boxes on the top) in 4- (red), 6- (green), and 8-chamber (blue) bench-scale PFRs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Thus, the sludge cultivated under this condition is more like a transitional form between granular sludge and floc-like sludge. Such a transitional form can also be seen from the second petri dish photo in Fig. 2a. Hence, 0.5 looks like a critical feast/famine ratio required for aerobic granulation, i.e., a feast/famine ratio > 0.5 is very likely to result in aerobic granulation failure, and vice versa. This conclusion can also be supported by many previous findings of aerobic granulation in SBRs. For example, successful aerobic granulation has been reported in SBRs when feast/famine ratios were as low as 0.16, 0.25, 0.33, and 0.42 (Liu and Tay, 2006, 2008; López-Palau et al., 2012; López-Palau et al., 2009), which were all below the critical feast/famine ratio of 0.5 identified in this study. Besides, López-Palau et al. (2012) and Liu and Tay (2008) both reported that aerobic granulation began to fail in SBRs when feast/famine ratios increased from values smaller than 0.5 (e.g., 0.25 and 0.42) to 0.66. Furthermore, Corsino et al. (2016) reported that aerobic granules cannot form in CSTRs without feast/famine conditions. All of this information points toward the concept that a feast/famine ratio less than around 0.5, as identified in this study, is a critical prerequisite for successful aerobic granulation. Furthermore, it is important to note that the aforementioned studies (including this one) were conducted in different kinds of reactors (e.g., SBRs and CFRs), with different operational parameters (e.g., loading rate, cycle time, HRT), variable influent sources (Liu and Tay, 2008; López-Palau et al., 2012), and by different research institutes, implying that the necessity of an acceptable feast/famine ratio is a universal microbial condition for all aerobic granulation applications.

4.2. Feast/famine condition is a prerequisite for continuous flow aerobic granulation

EPSs have been regarded as the cementing materials excreted by bacteria to bind themselves to each other into the form of aerobic granular sludge or biofilms (Adav et al., 2008; Lee et al., 2010). PS and PN are the two major components of EPSs. PS content was believed to be responsible for binding small bioflocs into larger aggregates (Gao et al.,

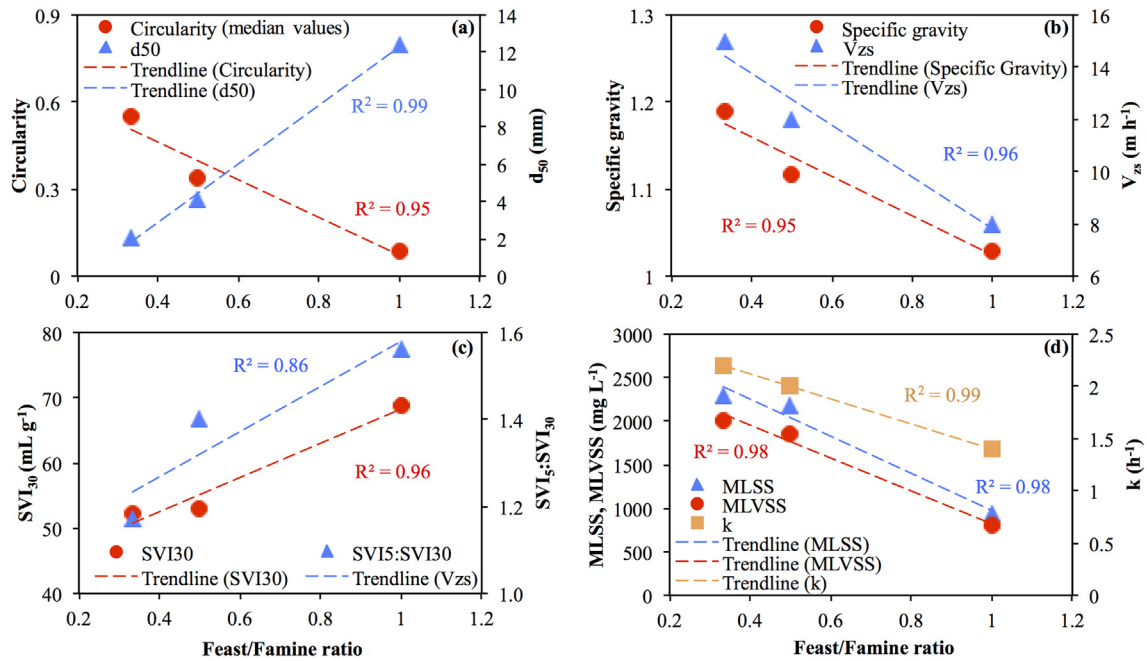


Fig. 5. Dependence of sludge characteristics such as (a) circularity and d_{50} , (b) specific gravity and V_{zs} , (c) SVI_{30} and $SVI_5:SVI_{30}$ ratios, (d) MLSS, MLVSS, and k on feast/famine ratios at the steady state. Standard deviations for all experimental values are shown in Table 3.

2011). PN content was reported to be positively correlated with cell hydrophobicity (Dignac et al., 1998; Sponza, 2003) probably due to the hydrophobic side groups in the amino acids (Chen et al., 2009). These trends are in line with EPS data in Table 3, i.e., both PS and PN contents decreased as the feast/famine ratio increases, especially when the feast/famine ratio is greater than 0.5 when chamber number < 6. Jimenez et al. (2015) revealed that bacteria tended to synthesize and accumulate EPSs during the feast phase, and the EPS production increased with the organic carbon removal rate. It was also recognized that a portion of the EPS is actually biodegradable and thus can be used by bacteria to sustain their lives during the starvation (Wang et al., 2007). Therefore, a decent length of famine phase for bacteria will trigger excessive EPSs production during the feast phase (Rahman et al., 2017), providing the basic materials for granular sludge formation. Other than EPS production, the starvation phase was also reported to increase bacterial surface hydrophobicity which initiates the reversible cell-to-cell co-aggregation in preparation for irreversible granular sludge formation via EPS binding (Liu et al., 2004; Tay et al., 2001b). That being said, the feast/famine condition produces a favorable environment inducing bacteria to produce EPS which provides one of the fundamental construction materials needed for granular sludge formation.

However, even with construction materials present, e.g. EPSs, aerobic granules still may not form unless additional selection pressure is present to provide a growth advantage to bacteria that choose to co-aggregate. It is known that feast/famine alternation may improve sludge settleability to a certain degree but not necessarily lead to granular sludge formation (Rahman et al., 2017). This can be seen from the SVI_{30} and $SVI_5:SVI_{30}$ values of the full-scale treatment train shown in Fig. 1, i.e., although the full-scale treatment train was also PFRs with feast/famine alternation as described in a previous publication (Sun et al., 2019), granular sludge was not formed because the V_c was too low (Table 1). Technically, dispersed cells grow faster than granulated cells due to the increased mass diffusion limitation in denser aggregates. This can be seen from the solids retention time (SRT) values in Table 3. Therefore, granulated cells were not able to compete with dispersed cells for the limited substrate availability unless a settling selective pressure, e.g. V_c , is introduced to wash out dispersed cells so as to favor the growth of granulated cells that are retained. This explains why aerobic granule formation is not generally reported in feast/famine reactor

regimes configured in conventional SBRs and PFRs. This concept emphasizes the important role of gravity selection pressures provided by either a V_c selection arrangement or hydrocyclones for SBRs or PFRs (Kent et al., 2018; Liu et al., 2005). A similar mechanism was actually also discovered in a research of pure culture responding to feast/famine conditions (Merritt and Kuehn, 2018). The investigators discovered that an alternating feast/famine condition triggered the phenotype change of *E. coli* cells which formed aggregates growing at a much lower rate than that of the dispersed *E. coli* cells. A washout mechanism had to be put in place to get rid of the fast-growing, dispersed *E. coli* cells so as to favor the growth of the slower growing *E. coli* aggregates. It is probable that repeated exposure of microbial sludge to a feast/famine ratio smaller than 0.5 is an environmental factor that triggered phenotype change of microbial sludge to aggregated growth. This aggregated phenotype can dominate only if the strength of the gravity selection pressure is high enough to favors its growth. This mechanism appears to be independent of the microbial species because the same phenomenon was observed in both mixed and pure culture studies with a variety of different types of substrates (Liu and Tay, 2006, 2008; López-Palau et al., 2012; López-Palau et al., 2009; Merritt and Kuehn, 2018).

It appears from this study that a feast/famine ratio smaller than 0.5 is required besides the driving force role of V_c around 10 m h⁻¹ toward successful continuous flow aerobic granulation as reported in previous studies (Sun et al., 2019; Wang et al., 2006). Therefore, a proper plug flow condition that can provide a feast/famine ratio smaller than 0.5 should be considered for future full-scale continuous flow aerobic granulation design. In contrast, CSTRs without feast/famine condition should be avoided. This is especially important in view of numerous reports of full-scale continuous flow applications without granular sludge formation even though hydrocyclones were installed (Ford et al., 2016; Van Winkel et al., 2016; Willoughby et al., 2016). This reinforces the concept that besides the driving force role of gravity selection pressure, the prerequisite role of a proper feast/famine condition that provides a prominent substrate gradient is also required for the success of continuous flow aerobic granulation.

5. Conclusions

Three major conclusions can be drawn as a result of this study:

- 1) The formation of aerobic granular sludge was achieved in 8- and 10-chamber PFRs but was compromised when chamber numbers were reduced to 6, and then completely failed when the chamber number was further reduced to 4.
- 2) The feast/famine ratios for 4-, 6-, and 8-chamber PFRs were defined by a metric related to whether the microbial growth rate is above (feast) or below (famine) the microbial decay rate. The characteristics of the sludges observed were dependent on the feast/famine ratios established in the PFRs.
- 3) In addition to adequate external gravity selection pressure, a feast/famine ratio smaller than 0.5 is likely a prerequisite for continuous flow aerobic granulation in a PFR and should be considered in full-scale design of this nature.

CRedit authorship contribution statement

Yewei Sun: Investigation, Formal analysis, Writing - original draft.
Bob Angelotti: Methodology, Supervision, Writing - review & editing.
Matt Brooks: Methodology, Supervision, Writing - review & editing.
Zhi-Wu Wang: Formal analysis, Methodology, 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.

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