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Effects of wastewater type on stability and operating conditions control strategy in relation to the formation of aerobic granular sludge – a review

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ABSTRACT

Currently, research trends on aerobic granular sludge (AGS) have integrated the operating conditions of extracellular polymeric substances (EPS) towards the stability of AGS systems in various types of wastewater with different physical and biochemical characteristics. More attention is given to the stability of the AGS system for real site applications. Although recent studies have reported comprehensively the mechanism of AGS formation and stability in relation to other intermolecular interactions such as microbial distribution, shock loading and toxicity, standard operating condition control strategies for different types of wastewater have not yet been discussed. Thus, the dimensional multi-layer structural model of AGS is discussed comprehensively in the first part of this review paper, focusing on diameter size, thickness variability of each layer and diffusion factor. This can assist in facilitating the interrelation between disposition and stability of AGS structure to correspond to the changes in wastewater types, which is the main objective and novelty of this review.

Key words: aerobic granular sludge, multi-layer structural model, operating condition, sludge stability, wastewater treatment

HIGHLIGHTS

- There is no available standard to describe the size for dimensional multi-layer structural modal of AGS.
- Types of bacteria species that dominate the mature AGS depend on the type of wastewater being treated.
- Control strategy of operating conditions for AGS system in different type of wastewater.

1. INTRODUCTION

Intensive works on applied biological treatments over the last 20 years have found that granular sludge has the potential to be a substitute method for conventional activated sludge systems (Liu & Tay 2004; Adav *et al.* 2008). The development of aerobic granular sludge (AGS) as a wastewater treatment technology has been widely performed using the sequencing batch reactor (SBR) technique (Arrojo *et al.* 2004; Yuan & Gao 2010). SBR is commonly used to stimulate the evolution of AGS which can be easily manipulated with short cycles and reduced settling time (Wagner & Costa 2013). Due to its advantages, wastewater treatment by AGS has gained growing interest due to its outstanding settling properties which allow concentrated suspended solids in aeration tanks even at low hydraulic retention times (HRT).

Previous research has demonstrated that most AGS systems have been performed in laboratory-scale SBR using synthetic substrates including volatile fatty acids or carbohydrates rather than actual wastewater (Derlon *et al.* 2016). Positive formulation of AGS using actual wastewater has been reported for the malt industry (Corsino *et al.* 2018), slaughterhouses (Luo *et al.* 2014), rubber (Rosman *et al.* 2014), landfill leachate (He *et al.* 2017), the explosives industry (Zhang *et al.* 2011), dairy effluent (Schwarzenbeck *et al.* 2004) and sewage (Peyong *et al.* 2012; Wagner & Costa 2013; Pronk *et al.* 2015; Awang & Shaaban 2018). The kinetics of aerobic granulation vary widely between actual and synthetic wastewaters (Wagner *et al.* 2015).

Recent research trends have emphasized the emergence of AGS technology from integrated operating conditions for the formation process and chemical characteristics of extracellular polymeric substances (EPS) towards stability of AGS (Liu & Tay 2004; Adav *et al.* 2009; Abdullah *et al.* 2011; Zhang *et al.* 2016; Nancharaiah & Reddy 2018; Winkler *et al.* 2018). However, critical comparisons have been made between the effects of wastewater types on stability and crucial operating conditions which occur during the formation of AGS while referring to process performance, and physical, chemical and biological properties are rarely discussed. Therefore, in this review, the relationship between the multi-layer structural model of AGS and crucial operating conditions applied is discussed thoroughly with the aim of improving the understanding of the effect of wastewater types on stability performance. In this discussion, the wastewater is classified into two types: low-strength wastewater and high-strength wastewater. Low-strength wastewater is defined as sewage having chemical oxygen demand (COD) values of less than 2,000 mg/L (Supplementary information Table A.1), while high-strength wastewater is defined as industrial wastewater with COD values greater than 2,000 mg/L (Supplementary information Table A.2).

2. DIMENSIONAL MULTI-LAYER STRUCTURAL MODEL OF AGS

To fully understand the integration in the dimensional multi-layered structural model of AGS, an interpretation of the AGS size should be prioritized. Aerobic granule sizes indicate the biomass concentrations in the aerobic granulation. Zhou *et al.* (2014) defined aerobic granule sizes into three categories: small (0.2 to 0.6 mm), medium (0.6 to 0.9 mm) and large (0.9 to 1.5 mm). This is supported by Verawaty *et al.* (2013), for the cultivation of different size fractions of aerobic granules, namely 425 μm, 900 μm and 1,125 μm for small, medium and large size, respectively. The increase in AGS size is usually due to an increase in biomass concentration, which causes the inhabitancy of facultative anaerobic microorganisms. This condition has a preference for growth inside the granules and the minerals formed are bound in the EPS surrounding the aerobic granules that strengthen the structure (Tay *et al.* 2002; Liu *et al.* 2014).

Larger aerobic granules are actually susceptible to mixing and disintegration, and small granules tend to have lower settleability. For these reasons, significant levels of aerobic granulation are not limited to increased granule sizes (Angela *et al.* 2011). Successful granulation indicators are preferably based on particle density and settling ability. The shape of the granules is almost round and spherical (Tay *et al.* 2001a). According to Amorim *et al.* (2014), small aerobic sizes in the range of 333 up to 750 μm are relatively stable. This is based on laser scanning microscope analysis demonstrating *β*-D-glucopyranose polysaccharides, proteins and active cell present at the core of aerobic granules.

The internal composition of aerobic granules is extremely heterogeneous (Wang *et al.* 2006) and thus influences transport process and reaction in granules (Carrera *et al.* 2019). Chiu *et al.* (2007) conducted a study on the dissolved oxygen (DO) transport inside aerobic granules. The kinetic parameters showed that the surface layer thickness consisted of an active layer (cells) of 100 μm. The diffusivity of oxygen transport and the kinetics of constant oxygen intake in the cells ranged from 1.24 to 2.28×10^{-9} m^2/s for acetated granule sizes of 1.28 to 2.50 mm size, or 2.50 to $7.65 \times$ 10–10 m^2/s for details phenol-feeding sizes of 0.42 to 0.78 mm (Ramos *et al.* 2016). The diffusiveness of oxygen decreases as granulate diameter decreases due to the existence of more pores inside the granule. However, it has been reported that the interior of the aerobic granules is not uniform (Tay *et al.* 2004), in which the granules are enriched with proteins in a multi-layered structure and facilitate granulation as well as encourage stability (Awang & Shaaban 2018).

Jang *et al.* (2003) found that the multi-layered structure of granules consisted of an external layer of 80 μm of ammonium-oxidizing bacteria (AOB) and an internal anoxic layer comprising anammox. Meanwhile, aerobic granules have an external layer of AOB around 50 μm, followed by oxidizing bacteria (NOB) layer about 30 μm with the internal layer consisting of EPS (Miller *et al.* 2002). However, Winkler *et al.* (2013) found that AOB mainly presents in the upper and lower granule layers. Given a first-order nitrification reaction, the bulk of nitrification is expected to exist from the top up to 300 μm in the thickness of granules. Wang *et al.* (2006) proposed that granule cores embedded with a fully loose polysaccharide structure substance with a thickness of 200 to 300 μm (d = 3.67 mm) would result in higher stability for aerobic granules to develop in high-strength wastewater. In attempts to derive a conceptual model for simultaneous nitrification–denitrification (SND) in the aerobic granule process of low-strength wastewater, Carrera *et al.* (2019) preferred the thickness of 25 μm for all aerobic layers (d = 0.5 mm); or 25 μm for four outer layers and 150 μm for the remaining six layers of internal granules (d = 2.0 mm). While treating domestic wastewater, Guimaraes *et al.* (2017) noted that the granules consisted of bacteria and protozoa with an external layer influenced by AOB, and the thick layer of NOB nitrite and denitrifiers. The AGS method demonstrates limited nitrification in low-strength actual wastewater condition when the emission of nitrous oxide (N_2O) is lower (Derlon *et al.* 2016).

Figure 1 shows the schematic view of the multi-layered structure of aerobic granules based on the above discussion and other studies conducted by Bassin *et al.* (2012) and Winkler *et al.* (2018). As illustrated in Figure 1, the outer layer of aerobic granular sludge (AGS) is the aerobic zone. Autotrophic bacteria (AOB and NOB), heterotrophic bacteria polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs)), are present in this zone. The inner layer is the anoxic or anaerobic zone, which most likely consists of denitrifiers, anaerobic ammonium oxidizers (Anammox), denitrifying PAOs (DPAOs) and denitrifying GAOs (DGAOs). The core of aerobic granular sludge consists of dead cells and precipitates (De Kreuk & van Loosdrecht 2006; Xiong & Liu 2013; Winkler *et al.* 2013).

According to Verawaty *et al.* (2013), the mature granular sludge will subsequently disintegrate after reaching a critical size and this will cause a decrease in the size of the granules. The critical size of the granules could be defined as the diameter of the aerobic granule above the anaerobic zone or the limitation of mass diffusion (Chiu *et al.* 2007). The depth of diffusion limitation may occur in low substrate/DO conditions at depths less than 1/3 of the granule diameter, where the thickness of the anaerobic zone is twice that of the aerobic zone. To produce stable aerobic granules, Winkler *et al.* (2018) suggested to provide SND conditions by controlling DO concentrations from 0.7–1.0 mg/L and up to 2.0–7.0 mg/L. This condition proved to be effective based on a study by Liu *et al.* (2008) that used leachate as the substrate. Liu *et al.* (2008) obtained aerobic granules with a diameter of 0.3–0.6 mm when recording the DO values as low as 0.76–1.55 mg/L. By treating low-strength wastewater, a granule diameter of about 0.2 mm was typically observed with DO values exceeding 0.2 mg/L (De Kreuk & van Loosdrecht 2006).

Furthermore, DO is the main parameter for reducing organic carbon, phosphorus and nitrogen simultaneously due to critical diffusion constraints in granules. Excessive DO concentrations in turn result in a greater aerobic fraction and thus increase the nitrification rate. However, lower DO concentrations will lead to an increase in denitrification rate. Thus, by monitoring the DO cycle in SBR, excessive effluent consistency could be obtained (Pronk *et al.* 2015). This suggests that apart from the aerobic granule size, the influence of mass transfer resistance also depends on other factors, such as granule morphology, substrate concentration and diffusion (wastewater type) and the turbulence degree in the reactor (Skiadas *et al.* 2003; Liu *et al.* 2004a, 2004b). These are interrelationship processes that govern the microstructure of aerobic granules.

Based on the above discussion and Figure 1, it can be concluded that DO in the liquid phase and in the granules dictates the diversity, bacterial gene dominance and microstructure of aerobic granules. There is no conclusive standard size for a dimensional multi-layer structural model of AGS that can be set to differentiate whether the aerobic granules have been developed in low or high-strength wastewater.

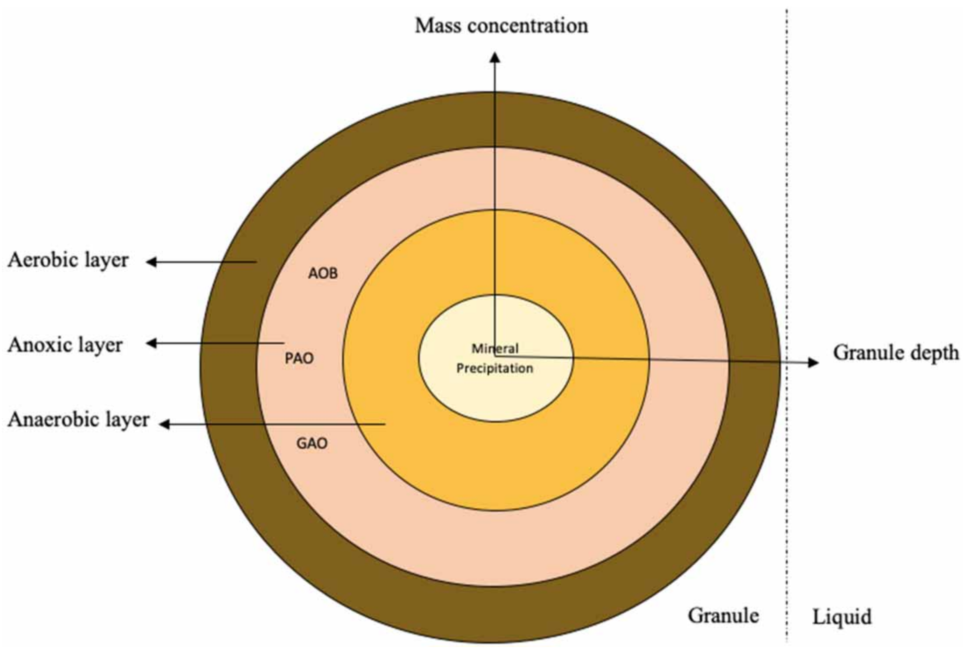

Figure 1 | Schematic view of multilayer structure of AGS.

3. OPERATING CONDITIONS CONTROL STRATEGY

In recent years, several studies have explored AGS innovations (Khan *et al.* 2013; Zhang & Li 2016; Fan *et al.* 2018). The focus of this review paper is to investigate the operating conditions that could affect the granulation procedures, EPS performance, and physical and chemical characteristics of granules. Studies on the effects of microgranulation have received little attention from fellow researchers. Improvement of aerobic granular techniques is predicted to distinguish high microbial communities (Chen *et al.* 2007). Further investigations can evaluate the impact of different types of wastewaters on AGS development with multi-layered structures. One of the factors that may influence SBR aerobic granulation is the substrate compositions. Other than that, operating conditions, which include hydrodynamic shear force, settling time and organic loading rate (OLR), have been studied (He *et al.* 2017).

An analysis of variance (ANOVA) was conducted to identify the most crucial operating conditions that have had a significant impact on the performance and formation of AGS in different types of wastewaters. The ANOVA results were collected from 30 references as summarized in Tables A.1 and A.2. The operating conditions are considered crucial if the significant value is $<$0.05–0.10 (Miller *et al.* 2002). Based on the ANOVA results (Supplementary information Table S.1 and Table B.1), superficial air velocity (SUAV), air flow rate, OLR, settling time and cycle time would be statistically significant to be discussed as the crucial operating conditions. In this section:

i. In distinguishing the relationship between the effect of wastewater type on OLR, an introductory note on substrate compositions and concentrations (can also be read as wastewater type) is discussed first in Section 3.1. The variability of substrate compositions and concentrations can be controlled and retained by applying an appropriate OLR and HRT, which is further discussed in Section 3.2.
ii. SUAV and air flow rate are merged into Section 3.3 as these two operating conditions are interrelated and play a vital role in controlling the hydrodynamic shear force inside the bioreactor.

3.1. Substrate (wastewater) composition and concentration

The composition and concentration of substrates have been reported to be the decisive factors in evaluating aerobic granules which could affect the granulation process through the collection and enrichment of specific microbial genus, and thus affect the size, kinetic behaviour and morphology of aerobic granules (Peyong *et al.* 2012).

Various substrates with organic and inorganic carbon sources have been used to cultivate aerobic granules. The carbon source positively dictates various bacterial genera with their dominance, microstructure and various biological agents including bacteria, protozoa, virus and fungi (Liu & Tay 2004; Lee *et al.* 2010). In a study conducted by Li *et al.* (2014), the sewerage system has 18 species of the genera *Georgenia, Longilinea, Desulforhabdus, Desulfuromonas, Arcobacter* and *Thauera*. Among them, pathogenic bacteria (e.g. *Enterobacter cloacae Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae* or *Proteus vulgaris*) present the most severe epidemiological risk that can cause various systemic infections, mainly to those with an immunosuppressed immune system (Morales *et al.* 2013). The dominancy of the bacteria species is due to the bacteria physiological characteristics which will subsequently affect the kinetics and stoichiometry coefficients during microbial degradation of organic substrate (Muda *et al.* 2011).

When granules are grown at low substrate concentrations, filamentous bacteria should have higher average growth rate compared with floc-forming bacteria due to their higher area to volume (A/V) ratio. In addition, higher A/V ratio of filamentous bacteria will help to facilitate the accessibility of the substrate to the filamentous cell compared with the floc-forming bacteria cells (Martins *et al.* 2004). The floc-forming bacteria use more substrate than filamentous bacteria at lower concentrations and thus control the conditions (Liu & Liu 2002).

As mentioned earlier, the influence of mass transfer resistance also depends on substrate concentration and diffusion. Under low substrate conditions, substrate diffusion can be a limiting factor in the evaluation of AGS (Liu *et al.* 2016) and therefore continue to reduce the rate of substrate removal. Peyong *et al.* (2012) highlighted that dense granules subjected to low substrate conditions would result in loose structures and the presence of filamentous bacteria. This may be due to the filamentous bacteria which fit perfectly into the loose structure and contribute to the formation of irregular shapes.

In addition, He *et al.* (2016) treated leachate as high-strength wastewater and discovered a wide number of filamentous bacteria growing on the granular surface, which looked like a hairy ‘silk-worm chrysalis’. Filamentous bacteria are an important component of large aerobic granules (Chen *et al.* 2007), but their overgrowth can make the granules fluffy and loose which will be easily washed out from the reactors (Seviour *et al.* 2012; Yuan *et al.* 2018). In addition, studies by Peyong *et al.* (2012) and Martins *et al.* (2003) deduced that filamentous bacteria are present in low-strength wastewater by the loose structure due to low substrate conditions. This may be attributed to the filamentous nature that fits in the loose structure and leads to the formation of irregular shapes.

3.2. OLR variability and HRT control

Generally, in an SBR method, the OLR rely upon substrate concentration and HRT (He *et al.* 2017). HRT represents the retention time of substrate at a certain period of cycle time over volume exchange ratio (VER). As depicted in Table A.1, typical cycle time and VER for low-strength wastewater range from 2–12 h and 40–75%, respectively, while for high-strength wastewater, the cycle time and VER range from 3–8 h and 45–60%, respectively.

In addition, the results of studies conducted by Ma *et al.* (2013) on low-strength wastewater treatment and Zhang *et al.* (2011) on treatment of various types of wastewaters were compared. Both studies used similar OLR of 0.6 kg COD/m^{3}d. Surprisingly, it can be observed that the period to achieve mature granule was not extended as the substrate (glucose) was easier to utilize than complex substrate (sewage). This is also supported by Peyong *et al.* (2012). The reason may be due to different SBR configurations and operating conditions. To accelerate the formation of AGS, Ma *et al.* (2013) and Zhang *et al.* (2011) both used pre-cultivated AGS at 1,000 mg/L acetate and glucose as seed.

In terms of OLR, Wagner & Costa (2013) and Ni *et al.* (2009) used similar OLR of 1.0 kg COD/m^{3}d with various operating conditions of SBR. The results showed that the development of granules was longer (300 d) in the study of Ni *et al.* (2009), while it took 140 d and 36 d for De Kreuk & van Loosdrecht (2006) and Wagner & Costa (2013), respectively. The variability of period to reach granules was affected by the reactor scale and the working volume.

Most of the previous studies summarized in Table A.1 investigated aerobic granules solely using low-strength wastewater sewage, while the remaining utilized pre-cultivated AGS from high concentration of synthetic wastewater as seed or inoculation to stabilize more easily in new environments compared to fresh activated sludge. However, this would not be good for the practical use of aerobic granules as a large volume of AGS seeds cultivated in high synthetic wastewater are needed to accommodate the requirements of the actual SBR. Although expensive, environmental factors such as temperature and OLR fluctuations which vary on site, can be precisely controlled in a laboratory-scale reactor.

Nevertheless, Table A.1 shows that aerobic granules cultivated from domestic wastewater could be developed at lower OLR between 0.1 to 4.0 kg COD/m^{3}d. It can be concluded that there is not much diversity in the diameter of mature granules developed in either domestic/sewage or low-strength synthetic wastewater. The typical size of mature granules is assumed to be around 0.2 mm and up to 2.00 mm depending on the selection of operating conditions.

Furthermore, based on high-strength wastewater studied by Muda *et al.* (2011), the development of granular sludge for synthetic textiles at 50% of VER increased the HRT from 6 to 24 h, resulting in the reduction of OLR. In addition, Rosman *et al.* (2014) found that the HRT of 6 h developed 2.0 $\pm$ 0.1 mm mean-sized granules with outstanding settling efficiency, high biomass density and OLR of 3.6 kg COD/m^{3}d. Studies by Su & Yu (2005), Wang *et al.* (2007), López-Palau *et al.* (2012) and Corsino *et al.* (2018) employed OLR of 6 kg COD/m^{3}d with various working volumes of 1.95 to 6 L. In contrast, Zhang *et al.* (2013) utilized 10.53 kg COD /m^{3}d OLR in their study. In addition, Kamaruddin *et al.* (2016) implied that the microorganisms in granular sludge are formed and grown rapidly with increasing OLR as well as continuous increase in particle dimensions. Furthermore, high rates of organic content will resolve mass transfer resistance to reveal greater sludge capabilities (Ferraz *et al.* 2013). Nevertheless, Table B.1 shows that the aerobic granules cultivated from high-strength wastewater could be developed at low OLR of 2.5 kg COD/m^{3}d to 27 kg COD/m^{3}d. The typical size of mature granules could be assumed to be around 0.3 to 4 mm depending on the selection of operating conditions.

Based on the studies, it can be inferred that when the AGS system is subjected to low-strength wastewater or run over a long period, reducing the cycle time, or rising the VER will contribute to high OLR, and maintain a balance food to microorganism (F/M) ratio. A balance between cycle time and VER will ensure enough time for the system to suppress the suspended growth as well as optimize the substrate accumulation, and enough food for microbial growth (Wang *et al.* 2014). Meanwhile, high-strength wastewater can be treated with aerobic granules as mentioned by Sheng *et al.* (2010); when the organic content is higher, the growth rate of microorganisms can lead to greater particle size and substrate mass transfer, and thus produce lower particle density and mechanical strength. Thus, the research on high-strength wastewater has concluded that treatment with aerobic granules is reasonably important (Zhao *et al.* 2013).

3.3. Intensity of hydrodynamic shear force

Similar to the evaluation of biofilm and anaerobic granules, the key element in the development of structure and stability of aerobic granules is the hydrodynamic shear force resulting from hydraulics and particle collision (Tay *et al.* 2001a; Liu *et al.* 2008). In a column-type upflow reactor such as SBR, the effect of hydrodynamic shear force is usually determined by the strength of the SUAV. As previously reported, shear forces are among the factors that will influence the formation of AGS and its characteristics. This shear force is usually quantified in the AGS reactor by dividing the aeration rate in the cross-sectional area of the SBR into SUAV.

According to Tay *et al.* (2001b), for the production of regular, rounder, solid and dense granules in low-strength wastewater, greater shear force was preferable. Bioflocs and regular granules were observed to be present in a reactor with SUAV of 0.3 cm/s or 1.2 cm/s, respectively. At SUAV of 1.2 cm/s, it was considered a threshold value for the AGS production. In the thermodynamics rules as stated by Liu *et al.* (2004a), a larger height to diameter (H/D) ratio of column-type upflow reactor can reflect longer circular flow paths, which can result in efficient microbial hydraulic rotation. Furthermore, the circular hydraulic attraction produced can support the microbial aggregates to granules in high cell surface hydrophobicity or low surface Gibbs free energy.

As shown in Table A.1, most researchers tended to manipulate either the aeration flow rate or H/D ratio over 10 to achieve a higher shear force, which in turn achieved SUAV of above 1.2 cm/s as reported in studies by Ma *et al.* (2013) and Zhu *et al.* (2013) for low-strength wastewater treatment. This method would restrict the practical use of aerobic granules, as most recent SBRs have low H/D ratios and increase the operational cost for aeration energy requirement.

The structure and shape of aerobic granules are affected by shear forces. Most laboratory-scale reactors of AGS are built as bubble columns, where the aeration rate, expressed as SUAV, generates shear forces usually within the range of 1 to 1.2 cm/s. On another note, granules did not develop SUAV greater than 1.2 cm/s (Ma *et al.* 2013). High aeration rates provide a conducive environment which promotes better granule development, with phenol acting as a carbon source that produces small granules which differ from intermediate aeration levels, and are composed of a huge number of EPS or polysaccharide ratio (Tay *et al.* 2001b; Adav *et al.* 2007). In a study by Liu & Tay (2012), they achieved stable granules during the famine process with the aeration rate decreased by 0.55 cm/s when the growing rate and oxygen consumption were small. Furthermore, Luo *et al.* (2014) reported that 0.8 cm/s SUAV formed granules with large porous and fragile structures.

As a result, granules may be developed at low speed of 0.42 cm/s during low-strength wastewater treatment. However, this may not be possible for intermediate or high-strength wastewater treatment (Devlin *et al.* 2017). The granules displayed poorer settling properties with decreased polysaccharide ratio at low SUAV but higher rates of nitrogen removal. Dong *et al.* (2017) claimed that the evaluation of granules depends on several parameters of rapid-growing aerobic microorganisms at higher OLRs and requires more shear or shear off the surface of the microorganisms, while less shear is needed at lower OLRs.

3.4. Short settling time for microbial selection

The settling time is considered as one of the significant inventions of AGS. Short settling time aids in selecting the fast-settling aggregates while slowly washing out impurities (Nancharaiah & Reddy 2018). In the SBR system, biosolids–liquid separation takes place prior to the effluent being withdrawn at the end of each cycle. Biomass or sludge with lower settleability will be washed out within the given settling time, leaving the fast-settling bacteria more space for their growth. This is supported by the findings from Qin *et al.* (2004a, 2004b) where short settling period for the growth of fast-settling bioparticles was preferred. In several studies, it has been identified that settling time is one of the important parameters in the selection of granules.

Greater exchange ratio for granule selection in the reactor with a short decanting step contributes to faster granulation (Sheng *et al.* 2006). Consequently, Gao *et al.* (2011) employed various strategies for enhancing AGS development and ensured that rapid granulation was obtained by reducing the settling time from 15 to 5 minutes in 11 days. The results indicated that the granules had a better settle rate (32–95 m/h), storage stability (99%) and higher EPS (100.8 mg/g SS) content than those developed under other conditions. On another note, Adav *et al.* (2009) emphasized that the option of settling period needs to be critically opted only at the initial cycle since most of the cycle duration is at the aeration phase. The implementation of short settling period at the initial stages of the cultivation procedure would make sure a washout of non-flocculating sludge seed strains which correlate to the shift in the granular sludge of microbials. Therefore, the initial stage settling time must be longer than the final cycle settling time (Nancharaiah & Reddy 2018).

Table A.1 demonstrates that when treating low-strength wastewater, SBRs are typically run with short settling time ranging from 2 to 10 minutes. A study by Wang *et al.* (2009) found that higher VER in the reactor resulted in rapid AGS development between 20% to 80%. In the settling period, the volumetric exchange ratio and the discharge time are among the most important parameters for rapid selection of solution aggregates to produce AGS as the basic form of biomass. Ni *et al.* (2009) and Sguanci *et al.* (2019) emphasized that effective granulation occurred in pilot reactors fed by low-strength wastewater within the settling time of 15 to 30 minutes, while the exchange rate was in the range of 50 to 75%. Therefore, it is noted that the settling time on full-scale AGS plants could be longer, approximately around 30 minutes due to the mixture of grains and floculose sludge provided (Pronk *et al.* 2015). Meanwhile, Hailei *et al.* (2006) adopted 30 minutes settling time for a full-scale AGS plant for treating high-strength wastewater using aerobic granules seed previously cultivated in pilot-scale reactor with settling time of not more than 15 minutes.

As shown in Table A.2, Corsino *et al.* (2018), Kocaturk & Erguder (2015), Wu *et al.* (2017) and Wang *et al.* (2006) succeed in obtaining mature aerobic granules from high-strength wastewater (malt industry, sugar beet processing, landfill leachate and brewery) within 21 to 35 days at 5 minutes settling time and 50% exchange ratio. For synthetic wastewater treatment, the settling time was 10 minutes and the exchange ratio was 40%, signifying a lower exchange ratio with longer settling time, which should be considered as important aspects for the cultivation of granules in the reactor (Nancharaiah & Reddy 2018).

Hence, a settling period in the range 1 to 30 minutes has been employed by different researchers as summarized in Tables A.1 and A.2 to enhance granulation cycle. SBRs are usually run with short settling times when forming AGS from 2 to 10 minutes for low-strength wastewater as reported by Carrera *et al.* (2019), Fan *et al.* (2018), Wang *et al.* (2017), Ma *et al.* (2013), Zhu *et al.* (2013), Peyong *et al.* (2012), Sun *et al.* (2017); Zhang *et al.* (2011), Wang *et al.* (2009) and De Kreuk & van Loosdrecht (2006). For high-strength wastewater, short settling times were recorded in the range of 2 to10 minutes as reported by Hamza *et al.* (2018), Corsino *et al.* (2018), Kocaturk & Erguder (2015), Liu *et al.* (2014), Harun *et al.* (2014), Zhang *et al.* (2013), Wu *et al.* (2017), López-Palau *et al.* (2012), Wang *et al.* (2006), Su & Yu (2005), Arrojo *et al.* (2004) and Schwarzenbeck *et al.* (2004). Therefore, better sludge settling in SBR is important for aerobic granulation.

3.5. Cycle time reduction

The AGS sequencing batch reactor (SBR) has demonstrated its versatility in the treatment of low-strength wastewater such as sewage, and thigh-strength wastewater which includes rubber, landfill leachate, dairy, brewery, and textile. Meanwhile, the AGS formation requires a long time for low-strength wastewater treatment. Strategies of increased volumetric flow by means of short cycles and mixing of sewage with industrial wastewaters can promote AGS formation while treating low-strength sewage. As demonstrated by Li *et al.* (2014), sewage and industrial wastewater are not claimed as low-strength wastewater. The reason is because the divalent and monovalent ions contained in industrial wastewater contribute to increased granulation.

In treating low-strength wastewater, two factors that contribute to efficient granulation are low sludge loading rate and high initial seed sludge to rapidly promote the development of granules. The study by Seviour *et al.* (2011) noted that a 12-hour cycle produced a granule diameter of 0.75 mm, while Pronk *et al.* (2015) recorded 6.5-hour cycle to obtain a granule diameter of 0.2 mm. Generally, when treating low-strength wastewater, a long cycle is needed to achieve an efficient granulation, while, a short cycle can contribute to a mature granulation in treating high-strength wastewater. This is because the monovalent and divalent ions present in the industrial wastewater contribute to improved granulation. Thus, granule maturation is efficient in high-strength wastewater treatment rather than low-strength wastewater due to the existing high-strength wastewater ions which result in successful granulation (Chen *et al.* 2019).

4. STABILITY OF AGS SYSTEM IN DIFFERENT TYPES OF WASTEWATERS

Based on the discussion above, it is noteworthy that aerobic granule could be produced as through several working conditions and types of substrates. As previously defined, aerobic granules formed in the reactor may consist of microbial communities that remove nitrogen and phosphorus, accumulate glycogen, degrade the carbon source and remove autotrophic nitrogen by anammox (Seviour *et al.* 2012). Here, aerobic granules stability is also characterized as the capability of microbial granules to avoid hydrodynamic shear forces and also mechanical aspects (Sheng *et al.* 2010). Aerobic granular stability is usually determined as bioactivity, microbial community composition and granule characteristics properties (Chen *et al.* 2007). Furthermore, Sheng *et al.* (2006) reported a multi-layer structure of AGS with two specific regions which are identical with the proposed AGS multi-layer structural model as shown in Figure 1. Moreover, it is noted that the middle part of aerobic granules provides its stability, which is the residual sludge cells fused with EPS which is of non-readily extractable EPS. The outer region is vice versa.

In addition, the effects of EPS on biofilm with anaerobic granules were established in 2001 by Tay *et al.* (2001a) which examined the function of EPS in aerobic granule formation and stability. The following study by McSwain *et al.* (2005) showed that EPS contributes significantly to the stability of the microbial granules. Comprehensive work has been developed to characterize the function of EPS in aerobic granules development and stability. Other researchers, Adav *et al.* (2008); Lee *et al.* (2010) and Liu *et al.* (2016) reviewed in detail the aerobic granule characteristics, EPS extraction process, spatial distribution and role in regulating evaluation and stability. Nevertheless, none of the authors has clearly elucidated the mechanism of AGS development and stabilization among certain intermolecular interactions. To date, only Liu & Tay (2004) briefly described the interactive forces involved in aerobic sludge formation without further elaboration on the interactive mechanism or patterns.

It is well known that EPS is essential to aerobic granule long-term stability (Adav *et al.* 2008). Owing to its dense structure, it is more difficult to extract EPS from granules than activated sludge. In addition, various types of extraction and analytical procedures are used to provide different outcomes of EPS function in granulation (McSwain *et al.* 2005). Conversely, Adav *et al.* (2009) reported the individual functions of EPS elements on structural stability by phenol-fed granules and found that extracellular proteins, α-polysaccharides and lipids have little impact, while granulate integration is formed when β-polysaccharides are hydrolysed. Exopolysaccharides present in aerobic granules are determined as gellin agents (Seviour *et al.* 2012). In treating high-strength wastewater, granular stability can be achieved by the concentration of suspended solids on the effluent. For example, the capacity of the granules to purify the effluent through sedimentation of active sludge increases the concentration of suspended solids. Stability of granules is significant for effluent to control lower concentrations of suspended solids (Wu *et al.* 2017).

Furthermore, the mechanisms that can cause good diffusion can be clogged due to the presence of particles and colloids in the wastewater as well as the production of EPS (Harun *et al.* 2014). Starvation and anaerobic reaction/process surrounding the granules result in endogenous cell respiration and cell lysis which at best lead to porous cavities and disintegration of granules (Corsino *et al.* 2018). The variety of OLRs offers different biomass growth rates in the reactor and thus can affect the concentration of the effluent suspended solids. In addition, Liu *et al.* (2014) found that the concentrations of effluent suspended solids were higher in the excess OLR at the beginning of the AGS reactors by different OLRs. For matured granules, the difference in the concentration of effluent suspended solids has decreased and, over the years, the F/M ratio has played a significant role in promoting the stability of the system.

4.1. Microbial distribution

A moderate number of filamentous bacteria is beneficial as they can serve as binders. However, the proliferation of filamentous bacteria, which float freely or spread from the flocs will cause deterioration of sludge settleability and result in a bulking sludge effect (Gao *et al.* 2011). However, the dense structure of granules is able to prevent the growth of filamentous bacteria (Liu & Liu 2006; Fang *et al.* 2009). Filamentous bacteria such as *Chloroflexi, Sphaerotilus* and *Thiothrix* will grow to excess. If bacteria have low hydrogen potential (pH), low DO concentrations and/or high OLR, it can lead to AGS instability (Figueroa *et al.* 2015). This is evidenced in a study conducted by Aqeel *et al.* (2016) that filamentous bacteria of *Janthinobacterium* and *Auxenochlorella* can form bulking of sludge, while the chitinophithic activity of *Chitinophaga* can inhibit the growth of *Auxenochlorella* and increase AGS development. The genus *Chitinophaga* is in the Bacteroidetes phylum that exists in wastewater systems and can be found in oceans around the world. *Chitinophaga* can be defined as chitinolytic, gliding, filament and heterotrophic bacteria.

In AGS bioreactors, various genera have been found when treating various types of wastewaters, for example, *Acinetobacter, Acidovorax, Flavobacterium, Paracoccus* and *Zoogloea* which are often detected are bacteria. Generally, one of the types of filamentous bacteria present in high-strength wastewater is Flavobacterium, which is a common genus in AGS fed by synthetic wastewater (Hamza *et al.* 2018), whereas, filamentous bacteria such as *Thiothrix* exists in low-strength wastewater due to excessive amounts of organic acids (Sguanci *et al.* 2019). Their numbers are lower in contrast to other genera, such as *Paracoccus*, *Zoogloe* and *Thauera* (Wan *et al.* 2015). Meanwhile, *Flavobacterium* dominates pharmaceutical wastewater (Amorim *et al.* 2014) which may reflect wastewater toxicity. In addition, *Paracoccus* is only dominant bacterium at high OLR (Szabó *et al.* 2017), while *Thauera* and *Zoogloea* are observed at low and high OLR (Lv *et al.* 2013; Zhao *et al.* 2013).

Studies by previous researchers (Lemaire *et al.* 2008; Kagawa *et al.* 2015) have concluded that aerobic granules have a layered structure and the distribution of microbial species from one layer to another may differ due to the restriction of oxygen and nutrients. However, several studies have been conducted to comprehensively investigate microbial communities inside and outside of the aerobic detail (Zhang *et al.* 2011). This can be ascertained in the study of Sun *et al.* (2017) where they separated aerobic granules into shell and core. The study further stated that the inner core is dominated by the family *Moraxellaceae* or *Rhodocyclacea*e, and the outer spherical shell is dominated by the families *Xanthomonadaceae*, *Rhodobacteraceae*, *Microbacteriaceae* and *Flavobacteriaceae*. Therefore, a community of core bacteria probably exists in aerobic granules and are important for granulation and other AGS behaviour. Moreover, previous studies have found that *Accumulibacter* sp. is generally separated in the outermost areas of the details, while *Competibacter* sp. is grouped in the core details (Xu *et al.* 2010).

Other researchers, (Lemaire *et al.* 2008; Weissbrodt *et al.* 2013) generally studied the microbial communities inside and outside aerobic granules. Furthermore, Sun *et al.* (2017) discovered that the inner core is influenced by *Moraxellaceae*, while the outer spherical shell is influenced by *Xanthomonadaceae, Flavobacteriaceae, Microbacteriaceae, Rhodobacteraceae* and *Rhodocyclaceae* families. In addition, research on the ability of *Zoogloea* to produce EPS has been well established. A study by Weissbrodt *et al.* (2013) proved that *Zoogloea* abundance can induce an increment of 2 to 38% during the granulation process, which is persistent among EPS content fluctuations. Moreover, other genera such as *Devosia, Meganema, Rhodocyclus, Stenotrophomonas* and *Thauera* contribute to the production of EPS (Zhang *et al.* 2011; Szabó *et al.* 2017), while *Sphingobacteriales* and *Cytophag*e are established to consume EPS (Matsuyama *et al.* 2008).

The stability of AGS is influenced by aerobic granules and it can be observed that it is a significant ecosystem or ecological balance in the bioreactor. Stability can be achieved due to the high retention capacity of slow-growing bacteria, and the number of nitrifying bacterial communities in granules should be slightly higher than in flocular sludge (Zhu *et al.* 2012). In addition, the populations of *Nitrosomonas* or *Nitrosospira* are dominant over AOBs in conventional wastewater treatment (Kowalchuk & Stephen 2001). There are also several studies reporting that the amounts of *Nitrosomonas* or *Nitrosospira* typically differ with the particles formed (Winkler *et al.* 2013), and only *Nitrosomonas* bacteria are present in aerobic granules (Sun *et al.* 2017).

In addition, denitrification also play an important role in AGS. Denitrifiers have an impact in AGS and its cumulative relative amount can contribute up to 45–60% in aerobic granules (Szabó *et al.* 2017). *Accumulibacter* is formed in AGS with slaughter wastewater (Lemaire *et al.* 2008), with both *Competibacter* and *Accumulibacter* present in the AGS fed with propionate and glucose (Weissbrodt *et al.* 2013). Moreover, a study by Henriet *et al.* (2016) found that the genus *Accumulibacter* tends to be enriched with compact granules, and prolonged settling times can control a huge distribution of granular size and also density. This results in better phosphorus removal. In fact, *Rhodocyclus* and *Accumlibacter* are used to synthesize polyhydroxyalkanoate (PHA) anaerobically and accumulate polyphosphate aerobically as observed in AGS (Zhang *et al.* 2011). Additionally, Li *et al.* (2014) noted that bacterial of *Thiothrix* filaments in AGS help in promoting the accumulation of deposited phosphates.

On the other hand, *phylum Proteobacteria* has an active role in damaging the organic compounds of phenolic or anillin. As can be seen in the phylum, *Thauera* can reduce the *p-chloroaniline* (Dong *et al.* 2017), while *Comamonas* and *Pseudomonas* can reduce the anilin (Jang *et al.* 2003) and *Rhodococcus* can reduce 2-fluorophenol (Rollemberg *et al.* 2019). Jemaat *et al.* (2014) emphasized that the genus *Acinetobacter* can be responsible for the biodegradation of o-cresol in AGS. Fang *et al.* (2009) proved that *Defluviicoccus* was involved in the reduction of azo dyes in AGS. In addition, members in other phyla may also be important for the removal of organic pollutants. Kong *et al.* (2015) claimed that *Chloroflexi* and *Bacteroidetes* are rich in aerobic granules exposed to cefalexin. In contrast to conventional active sludge, it can be concluded that the AGS technology demonstrates good achievement in treating wastewater including flammable organics, such as phenolic, dye, amine and pharmaceutical wastewaters (Chen *et al.* 2007; Amorim *et al.* 2014; Dong *et al.* 2017).

Therefore, this review paper highlights the physicochemical properties of aerobic granules and the dynamics of the microbial community as temporary instability of this structure due to filaments growth on a laboratory-scale SBR. As mentioned earlier, the formation of aerobic granule sludge at low OLR is often subjected to the domination of filamentous bacteria. To control the growth of filamentous bacteria, manipulation of operating conditions depending on wastewater type is crucial. Tables A.1 and A.2 present an overview of published data on various SBR configurations with different operating conditions for treatment of low and high-strength wastewater, while Table B.2 exhibits the control strategies to serve the stability concerns from AGS formation.

4.2. Selective EPS secretion

In the treatment of low-strength wastewater, Luong *et al.* (2018) inoculated a pilot plant fed by actual wastewater and discovered that it required 400 days to achieve a stable period, with few floccular sludge still existing inside the pilot plant. The microbial community composition was estimated by denaturing gradient gel electrophoresis (DGGE), which was close to the granules and floccular sludge. The study suggested that, for certain microorganism classes, there was no clear microbial selection to produce granules. For example, Pronk *et al.* (2015) analysed the microbial community and noted no substantial differences in microbial community among sludge such as seeding, floccular and granules, with a large number of bacteria belonging to Flavobacteria, phyla β-Proteobacteria and Sphingobacteria.

In addition, to treat low-strength wastewater, Adav *et al.* (2008) determined the part of the individual EPS element of structural stability by phenol-fed granules. The study found that selective enzymatic hydrolysis of extracellular proteins, lipids and α-polysaccharides gave a smaller effect, while granule integration was formed when β-polysaccharides were hydrolysed. Exopolysaccharides in the activated sludge were observed as a gelling medium in aerobic granule, which was clearly more attached than EPS (Seviour *et al.* 2011). In addition, Tay *et al.* (2004) noticed that the extracted exopolysaccharides were similar to alginate from aerobic granules developed in the pilot plants.

In the presence of calcium chloride, the exopolysaccharides displayed gel-forming characteristics and crucially contributed to the elastic structure and hydrophobicity. To identify the granulation mechanisms, the process to characterize these exopolysaccharides should be further developed to point out the individual polymers and their aggregate interactions (Seviour *et al.* 2012).

Furthermore, quorum sensing (QS) is a significant mechanism involved in granulation to treat high-strength wastewater (Zhang & Li 2016). Among bacteria, QS control has different purposes (Wang *et al.* 2007). The importance of QS for granulation and granular stability has been demonstrated in several studies (Liu *et al.* 2005; Wan *et al.* 2013; Li & Zhu 2014). For example, the concentrations of acyl homoserine lactones (AHL), a common inducer in Gram-negative bacteria developed by granulation (Jang *et al.* 2003) and granular sludge, have been seen to have a greater AHL content compared to floccular sludge (Lv *et al.* 2013, 2014a, 2014b).

Higher EPS formation is due to higher hydrophobic effects, resulting in increased aggregation and granule stability that are closely related to higher QS activity throughout granulation (Li & Zhu 2014; Li *et al.* 2014). In addition, SBR inoculated with flocculated sludge and fed by synthetic wastewater demonstrated changes in community composition and AHL concentration, as well as a positive correlation in the growth of EPS. However, there was a correlation between granular disintegration and then reduced AHL content (Wang *et al.* 2009; Wu *et al.* 2017).

Higher QS activity is associated with higher potential for microbial growth (Li & Zhu 2014; Li *et al.* 2014). Suspended bacterial intake is higher when mature granular supernatant is used in contrast to flocks, possibly due to higher auto-inducer concentrations in containers (Tay *et al.* 2002; Liu *et al.* 2016). *Nitrosomonas*-dominated nitrification sludge can be enhanced with the addition of exogenous AHL, thereby enhancing extracellular protein and autotrophic organisms (He *et al.* 2016).

He *et al.* (2016) also ran the AGS reactor fed by high-strength synthetic wastewater with simultaneous removal of COD at low OLR. During granulation by *Bacteroidetes, Proteobacteria, Chloroflexi, Actinobacteria* and *Firmicutes* as abundant bacteria at the phylum level, the microbial community exhibited significant changes in microbial diversity and richness. A study by Aqeel *et al.* (2016) examined the dynamics of microbial composition in laboratory-scale reactors fed with synthetic wastewater for COD removal throughout granulation. It was found that, during granulation, microbial diversity and richness decreased with the *Rhodanobacter* genus dominating at the granulation maturation stage, which coincided with the increase in EPS protein content.

Subsequently, Fan *et al.* (2018) compared granulation for domestic and synthetic wastewaters in similar reactors at the same CODs. The outcomes depicted a strong decrease in bacterial diversity by distinct bacterial population structures in different granules to seed sludge during granulation.

It is well known that EPS is essential for the long-term stability of aerobic granules (Adav *et al.* 2008). However, a contradictory hypothesis may occur when taking into account the diffusion limitation factor for mature and dense aerobic granules as discussed in Section 2.0. It is more complex to extract EPS from aerobic granules compared to activated sludge, as various extraction and analytical procedures are required. McSwain *et al.* (2005) provided different theories on the role of EPS in granulation process. The study concluded that microbial selection is caused by working parameters such as F/M ratio, OLR, solid retention time, settling time and type of substrates. AGS is known to have higher microbial diversity compared to flocular sludge as it indicates more ecological niches due to the slope of the substrate produced between the aggregates. Moreover, it is the same for microorganisms' functional groups that exist in granular and flocular sludge but with division differences between phylogenetic groups at the phylum or class level (Isanta *et al.* 2012; Winkler *et al.* 2013; Winkler *et al.* 2015).

4.3. Cell surface hydrophobicity

Hydrophobicity and surface charge are important factors in estimating the characteristics of the aerobic granule (Liu *et al.* 2014), where throughout granulation, biomass contributes to increasing hydrophobic granules (Isanta *et al.* 2012). Simultaneous increase in cell hydrophobics can lead to a decrease in Gibbs surface-free excess energy affecting thermodynamics, which in turn encourages cell self-aggregation from the liquid phase into new solid phases (Liu & Tay 2004). Changes in cells facilitate bacteria to aggregate (Gao *et al.* 2011). The increase in hydrophobicity could be due to the higher protein/polysaccharide ratio caused by changes in EPS and bacterial community composition (He *et al.* 2017). Moreover, an increase in the polysaccharide ratio should reduce the negative surface charge of the granules, as well as reduce the electrostatic repulsion among bacterial cells and thus increase the granulation process.

A study by Wan *et al.* (2015) suggested that negatively charged bacteria next to the calcium inorganic core and phosphate precipitates produce exopolysaccharides, as well as promoting microbial aggregation. The cell aggregation in granules follows similar colloidal interactions and mechanisms in activated sludge, and is affected by the same parameters, yet not limited to Derjaguin–Landau–Verwey–Overbeek (DLVO)-type/colloid chemistry theory interactions, EPS connectivity by cations hydrophobic interactions, cell surface charge and water phase surface tension (Lettinga *et al.* 1980; Mishima & Nakamur 1991; Morgenroth *et al.* 1997; Tebbutt 1998). In addition, Muda *et al.* (2011) noted that carbohydrate substrates are related to granules by layered structures, while substrates such as formate and acetate are formed in granules with moderately uniform micro-structures. The degradation of relatively complex organic substrates such as carbohydrates is a step-by-step process consisting of a few groups of degradation, and this can identify the complex micro-granule structures by greater diversity of microorganisms. Simple substrates can use simple micro-granule structures influenced by a handful of bacteria. Granulation with higher EPS production in turn will subsequently contribute to the increase in aggregation and granules stability (Zhou *et al.* 2014). Wang *et al.* (2006) visualized aerobic granules by section using an image analyser (Olympus imaging analysing system SZX9, Japan) and discovered that the granule outer shell has a much higher hydrophobicity associated with β-linked EPS.

Cellular interactions have been demonstrated to play a major role in the formation of granules. Figure 1 depicts that there are three layers that have sufficient capacity for wastewater treatment. The inner part of the aerobic zone is known as an anoxic zone with low oxygen concentration. The anoxic zone is known to be an important micro environment for denitrification and biological phosphorus removal. Finally, the core granules are completely free of microbial cells as an anaerobic layer. Due to the granule structural composition and the stability of the microenvironment, there exists a huge gradient distribution of electron donors or electron (Nancharaiah & Reddy 2018). The aerobic region offers a conducive environment for microbial communities that perform oxidation of organic matter and nitrification, both of which rely on oxygen as the terminal electron receiver. Furthermore, the outer layer is capable of oxidizing organic matter in the form of COD (Xiong & Liu 2013; He *et al.* 2017).

4.4. Instability of AGS system

Nevertheless, several previous studies have accentuated the instability of AGS system mainly in a long-term operating period, which will hinder its practical application. Martins *et al.* (2003) proposed four mechanisms to account for the loss of aerobic granule stability, namely the outgrowth of filamentous organism, anaerobic core hydrolysis, functional strain loss and EPS role. Filamentous organisms can be controlled by various methods such as oxidizing chemical and chlorine doses. However, with high chlorine doses, this can cause floc break-up due to inhibition of nitrification and organic matter removal. Therefore, the oxidizing chemical is currently the only effective strategy applicable for all wastewater treatment plants (WWTP). In addition, Lee *et al.* (2010) and Luong *et al.* (2018) have narrowed this down to two possible causes, namely a significant reduction in net tyrosine production of EPS and microbial shifts leading to the collapse of the granule structure. Here, it can be hypothesized that the sturdiness of the aerobic granule structure plays an important role in ensuring its stability.

Under normal or static conditions, the size of aerobic granules will govern microbial activity inside the granule. An increase in aerobic size proportional to time is an unstoppable process and will subsequently lead to the hydrolysis of the anaerobic core, whereby the hydrolysis process is attributed to the gases and organic acids produced (Tay *et al.* 2001a) and mass transfer resistance that hinders nutrient intake for microbial activity (Tay *et al.* 2001b). Later, microbial activity shifts to fit its own physiological requirement, in which filamentous organisms will dominate the aerobic granules. Thus, this will alter the EPS matrix and lead to the loose structure of aerobic granules. At this point, an external force will disintegrate the aerobic granules into small flocs that will continue to regrow with the stability of time.

Although the AGS instability mechanism is still poorly accepted, investigation on short settling time, hydraulic shear strength and feast famine activity are needed to maintain granular stability (Fan *et al.* 2018). High pressure can affect microorganisms with good ability to remain in the reactor and present as control functional species when selected under pressure. However, it is difficult to note the selection of adequate pressure for these practical bacteria in a dry storage environment. During low-strength wastewater treatment with increasing AGS size, anaerobic cores are produced regularly in granules due to resistance in oxygen transfer. Moreover, anaerobic activity in the anaerobic cores is known to be the leading cause of granular instability in working conditions (Luong *et al.* 2018). This effect is also formed in the stored granules, and the restriction of oxygen transfer is more severe on the agar block. About half of the mature AGS of the archaeal genera disappears during storage, of which methanogenic Archaea remained the dominant genera after recovery.

The structure of aerobic granules begins to deform and demonstrates a reduction in mass simultaneously with increased metabolic activity and concentration of anaerobic microbes, and consumption of nutrients from the dead cells by microbes to proliferate (Zhang *et al.* 2016). This is due to the transformation of a large number of cellular materials into odor, carbon dioxide and methane (He *et al.* 2016). Anaerobic bacteria and metatrophic organisms (*Clostridium III, Macellibacteroides* and *Paludibacter*) degrade dead aerobic bacteria (*Nitrosomonas, Aquimonas, Arenimonas, Brevifollis, Ferruginibacter, Roseibacillus, Reyranella* and *Zoogloea*) as nutrients. Meanwhile, methanogenic Archaea further uses its fermentation materials, such as alcohols, amines and organic acids as carbon sources (Gao *et al.* 2011). Therefore, metabolites are transformed into methane and other gases that are discharged into the environment, which ultimately contribute to the degradation in granular structure (Chen *et al.* 2007; He *et al.* 2017).

However, microorganisms with rapid settling speed and good cohesive potential should be maintained at the reactor and act as a control of functional species in high-strength wastewater. Due to its particular stratified structure, Xu *et al.* (2010) revealed that stable AGS is inhabited by huge amounts of functional bacteria (*Thauera, Zoogloea, Flavobacterium* and *Chryseobacterium*) that are depleted due to insufficient nutrients and oxygen. The destruction of functional bacteria indicates decreased EPS secretion and poor inter-cell cohesion (Yuan & Gao 2010). Studies have proven that EPS secreted by *Zoogloea* and *Thauera* has adhesive characteristics that help to facilitate the development of granules (Xiong & Liu 2013). Therefore, the loss of *Zoogloea* and *Thauera* due to decreased EPS secretion will prolong the period to achieve mature granules.

In addition, other microbes use EPS as a carbon source (Rollemberg *et al.* 2019) which also causes a decrease in EPS. Fractures of a large number of granules after re-aeration at the beginning of the recovery stage also indicate defects in the granule structure over storage (Chen *et al.* 2007). Moreover, the *Zoogloea* genus is composed mainly of aerobic bacteria, and a decrease in its abundance also causes a decrease in specific rate of oxygen uptake (SOUR). Thus, microbial community formation is a source of granular stability loss in storage, and structural destruction and property degradation are beyond the changes of microbial metabolism and communities. Consequently, new species of EPS secretion agents, such as *Streptococcus* and *Lactococcus*, play an important role in rebuilding damaged granular structures to control aerobic granule sludge instability, especially in the long-term operation phase, which will be the main barrier in the practical application of filamentous species, anaerobic core hydrolysis, loss of functional strains and position of EPS.

5. RECOMMENDATION FOR CONTROL STRATEGY

Factors affecting the formation of AGS in SBR such as operating conditions include settling time, OLR and hydrodynamic shear force (He *et al.* 2017). Throughout long-term operation, applying appropriate operating conditions such as selective sludge discharge, settling time, aerobic–anaerobic phase, shear force and cycle time are necessary for granular sludge stability (Isanta *et al.* 2012; Liu & Tay 2012; Zhu *et al.* 2013). Results from selective sludge discharge controls proved that aerobic granular size has played an important role in its stability. To overcome the problems with filamentous bacteria, the growth rate of aerobic granules was controlled by a deliberate increase in the N/COD ratio (Hamza *et al.* 2018). In addition, positive recovery of aerobic granules after exposure to external carbon sources, toxic shock loading, breakage, drying and storage has ascertained its stability (Rollemberg *et al.* 2019; Barros *et al.* 2020). Storage can be performed at low temperatures with the aim of minimizing bacterial growth rates and further enhancing the stability of granules (Chen *et al.* 2019).

For low-strength wastewater, aerobic granules can be produced at low SUAVs as low as 0.42 cm/s, but this is not the case for high-strength wastewater (Devlin *et al.* 2017). SUAV of 1.2 cm/s has become an essential value for AGS development (Liu & Tay 2004) lower than the value stated above, which would not form granules (Tay *et al.* 2001b). This is because antecedent researches revealed that granules formed at SUAV of 0.8 cm/s were more likely to exhibit highly porous characteristics leading to unstable structures (Jemaat *et al.* 2014). Therefore, it is suggested that SUAV should not be lower than 1.2 cm/s for treating any type of wastewater to obtain compact and dense aerobic granules.

This review indicates that AGS can be developed at OLRs as low as 0.1 kg COD/m^{3}d (low-strength wastewater) and up to 27 kg COD/m^{3}d (high-strength wastewater). Although aerobic granules can be possibly developed close to OLR range, it is crucial for HRT control, cycle time, settling time and volumetric exchange rate to ensure sludge retention time (SRT) and F/M ratio in bioreactor remains at a working standard threshold value. It can be concluded that there is not much variation in the diameter of mature granules developed in either domestic/sewage or low-strength synthetic wastewater. The typical size of mature granules could be considered around 0.75 mm and up to 2.00 mm based on the selection of operating conditions. Meanwhile, the typical size of mature granules developed in high-strength wastewater is assumed to be around 0.3 to 4 mm depending on the selection of operating conditions.

Although a short settling time is recommended for selection of floc/aerobic granules with high rate settling ability and biomass washout (floc/aerobic granules) due to excessive filamentous microbial growth when OLR and F/M ratio are not within working standard threshold value. Thus, the most important aspect of obtaining compact granules in a short period of time is to regulate the settling time with continuous monitoring of the sludge volume index and oxygen uptake rate.

Details of operating conditions are summarized in Tables A.1 and A.2 with the proposed development of AGS in SBR as follows:

- Fast settling time of 2 to 10 minutes with exchange ratio of 20 to 80% for all types of wastewater.
- A settling time of 15 to 30 minutes in exchange ratio between 50 to 75% is needed to achieve effective cultivation by low-strength wastewater from pilot-scale reactor.
- The settling time at the full-scale AGS plants is longer, about 30 minutes, providing a mixture of granules and floccules sludge.
- For high-strength wastewater, a settling time of 30 minutes is recommended for full-scale AGS plants, whereas a pilot-scale study can be successfully cultivated with an operating SBR settling time of not more than 15 minutes for cultivation of granules in the reactor.
- Cycle hours are reduced during the period of AGS formation to balance the F/M ratio from 6 to 3 or 2 hours for low-strength wastewater and 24 to 12 or 8 to 6 hours for high-strength wastewater.
- AGS can be developed across all OLR ranges as long as the ratio for F/M is well maintained above 0.2 g COD/g MLVSS d.
- Although AGS can be developed in SBR with threshold value of SUAV as low as 0.13 cm/s, a longer period of time to obtain mature AGS is expected to be in line with the increase in working volume and H/D ratio.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

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

REFERENCES

Abdullah, N., Ujang, Z. & Yahya, A. 2011 Aerobic granular sludge formation for high strength agro-based wastewater treatment. *Bioresour. Technol.* **102** (12), 6778–6781.

Adav, S. S., Lee, D.-J. & Lai, J. Y. 2007 Effects of aeration intensity on formation of phenol-fed aerobic granules and extracellular polymeric substances. *Appl. Microbiol. Biotechnol.* **77** (1), 175–182.

Adav, S. S., Lee, D.-J. & Tay, J.-H. 2008 Extracellular polymeric substances and structural stability of aerobic granule. *Water Res.* **42** (6–7), 1644–1650.

Adav, S. S., Lee, D.-J. & Lai, J.-Y. 2009 Potential cause of aerobic granular sludge breakdown at high organic loading rates. *Appl. Microbiol. Biotechnol.* **85** (5), 1601–1610.

Amorim, C. L., Maia, A. S., Mesquita, R. B., Rangel, A. O., van Loosdrecht, M. C. M. & Castro, P. M. 2014 Performance of aerobic granular sludge in a sequencing batch bioreactor exposed to ofloxacin, norfloxacin and ciprofloxacin. *Water Res.* **50**, 101–113.

Angela, M., Béatrice, B. & Mathieu, S. 2011 Biologically induced phosphorus precipitation in aerobic granular sludge process. *Water Res.* **45** (12), 3776–3786.

Aqeel, H., Basuvaraj, M., Hall, M., Neufeld, J. & Liss, S. 2016 Microbial dynamics and properties of aerobic granules developed in a laboratory-scale sequencing batch reactor with an intermediate filamentous bulking stage. *Appl. Microbiol. Biotechnol.* **100** (1), 447–460.

Arrojo, B., Mosquera-Corral, A. G. & Méndez, R. 2004 Aerobic granulation with industrial wastewater in sequencing batch reactors. *Water Res.* **38** (14–15), 3389–3399.

Awang, N. A. & Shaaban, M. G. 2018 Effect of variable and low organic loading rate on formation aerobic granular sludge in sewage treatment. *J. Adv. Res. Mater. Sci.* **48** (1), 1–10.

Barros, A. R., Rollemberg, S. L., Amorim de Carvalho, C. d., Moura, I. H., Firmino, P. I. & Santos, A. B. 2020 Effect of calcium addition on the formation and maintenance of aerobic granular sludge (AGS) in simultaneous fill/draw mode sequencing batch reactors (SBRs). *J. Environ. Manag.* **255**, 109850.

Bassin, J., Kleerebezem, R., Dezotti, M. & van Loosdrecht, M. C. M. 2012 Simultaneous nitrogen and phosphate removal in aerobic granular sludge reactors operated at different temperatures. *Water Res.* **46** (12), 3805–3816.

Carrera, P., Campo, R., Méndez, R., Di Bella, G., Campos, J. L., Mosquera-Corral, A. & Val Del Rio, A. 2019 Does the feeding strategy enhance the aerobic granular sludge stability treating saline effluents? *Chemosphere* **226**, 865–873.

Chen, Y., Jiang, W., Liang, D. T. & Tay, J. H. 2007 Structure and stability of aerobic granules cultivated under different shear force in sequencing batch reactors. *Appl. Microbiol. Biotechnol.* **76** (5), 1199–1208.

Chen, T., Zhu, Z., Yu, W. & Zhang, C. 2019 Granulation process and mechanism of aerobic granular sludge under salt stress in a sequencing batch reactor. *Tecnología Y Ciencias del Agua* **10** (4), 156–181.

Chiu, Z. C., Chen, M. Y., Lee, D. J., Wang, C. H. & Lai, J. Y. 2007 Oxygen diffusion and consumption in active aerobic granules of heterogeneous structure. *Appl. Microbiol. Biotechnol.* **75** (3), 685–691.

Corsino, S. F., Trapani, D. 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. Manage.* **214**, 23–35.

De Kreuk, M. K. & van Loosdrecht, M. C. M. 2006 Formation of aerobic granules with domestic sewage. *J. Environ. Eng.* **132** (6), 694–697.

Derlon, N., Wagner, J., da Costa, R. H. & Morgenroth, E. 2016 Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume. *Water Res.* **105**, 341–350.

Devlin, T. R., di Biase, A., Kowalski, M. & Oleszkiewicz, J. A. 2017 Granulation of activated sludge under low hydrodynamic shear and different wastewater characteristics. *Bioresour. Technol.* **224**, 229–235.

Dong, J., Zhanga, Z., Yua, Z., Daia, X., Xua, X., Alvarez, P. J. & Zhua, L. 2017 Evolution and functional analysis of extracellular polymeric substances during the granulation of aerobic sludge used to treat p-chloroaniline wastewater. *Chem. Eng. J.* **330**, 596–604.

Fan, X.-Y., Gao, J.-F., Pan, K.-L., Li, D.-C., Li-Fang, Z. & Wang, S.-J. 2018 Shifts in bacterial community composition and abundance of nitrifiers during aerobic granulation in two nitrifying sequencing batch reactors. *Bioresour. Techno.* **251**, 99–107.

Fang, F., Liu, X.-W., Xu, J., Yu, H.-Q. & Li, Y.-M. 2009 Formation of aerobic granules and their PHB production at various substrate and ammonium concentrations. *Bioresour. Technol.* **100** (1), 59–63.

Ferraz, F. M., Povinelli, J. & Vieira, E. M. 2013 Ammonia removal from landfill leachate by air stripping and absorption. *Environ. Technol.* **34**, 13–16.

Figueroa, M., Val del Río, A., Campos, J., Méndez, R. & Mosquera-Corral, A. 2015 Filamentous bacteria existence in aerobic granular reactors. *Bioprocess. Biosyst. Eng.* **38** (5), 841–851.

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

Guimaraes, L. B., Mezzari, M. P., Daudt, G. C. & da Costa, R. H. 2017 Microbial pathways of nitrogen removal in aerobic granular sludge treating domestic wastewater. *J. Chem. Technol. Biotechnol.* **92** (7), 1756–1765.

Hailei, W., Guangli, Y., Guosheng, L. & Feng, P. 2006 A new way to cultivate aerobic granules in the process of papermaking wastewater treatment. *Biochem. Eng. J.* **28**, 99–103.

Hamza, R. A., Sheng, Z., Terna Iorhemen, O., Zaghloul, M. S. & Tay, J. H. 2018 Impact of food-to-microorganisms ratio on the stability of aerobic granular sludge treating high-strength organic wastewater. *Water Res.* **147**, 287–298.

Harun, H., Anuar, A. N., Ujang, Z., Rosman, N. H. & Othman, I. 2014 Performance of aerobic granular sludge at variable circulation rate in anaerobic-aerobic conditions. *Water. Sci. Technol.* **69** (11), 2252–2257.

He, Q., Zhou, J., Wang, H., Zhang, J. & Wei, L. 2016 Microbial population dynamics during sludge granulation in an A/O/A sequencing batch reactor. *Bioresour. Technol.* **214**, 1–8.

He, Q., Zhang, W., Zhang, S. & Wang, H. 2017 Enhanced nitrogen removal in an aerobic granular sequencing batch reactor performing simultaneous nitrification, endogenous denitrification and phosphorus removal with low superficial gas velocity. *Chem. Eng. J.* **326**, 1223–1231.

Henriet, O., Meunier, C., Henry, P. & Mahillon, J. 2016 Improving phosphorus removal in aerobic granular sludge processes through selective microbial management. *Bioresour. Technol.* **211**, 298–306.

Isanta, E., Suárez-Ojeda, M. E., Val del Río, Á., Morales, N., Pérez, J. & Carrera, J. 2012 Long term operation of a granular sequencing batch reactor at pilot scale treating a low-strength wastewater. *Chem. Eng. J.* **198–199**, 163–170.

Jang, A., Yoon, Y.-H., Kim, I. S., Kim, K.-S. & Bishop, P. L. 2003 Characterization and evaluation of aerobic granules in sequencing batch reactor. *J. Biotechnol.* **105** (1–2), 71–82.

Jemaat, Z., Suárez-Ojeda, M. E., Pérez, J. & Carrera, J. 2014 Partial nitritation and o-cresol removal with aerobic granular biomass in a continuous airlift reactor. *Water Res.* **48**, 354–362.

Kagawa, Y., Tahata, J., Kishida, N., Matsumoto, S., Picioreanu, C., van Loosdrecht, M. C. & Tsuneda, S. 2015 Modeling the nutrient removal process in aerobic granular sludge system by coupling the reactor- and granule-scale models. *Biotechnol. Bioeng.* **112** (1), 53–64.

Kamaruddin, M. A., Yusoff, M. S., Aziz, H. A. & Alrozi, R. 2016 Current status of Pulau Burung Sanitary Landfill leachate treatment, Penang Malaysia. *AIP Conf. Proc.* **1774**, 030–014.

Khan, M. Z., Mondal, P. K. & Sabir, S. 2013 Aerobic granulation for wastewater bioremediation: a review. *Can. J. Chem. Eng.* **91** (6), 1045–1058.

Kocaturk, I. K. & Erguder, T. H. 2015 Investigation of the use of aerobic granules for the treatment of sugar beet processing wastewater. *Environ. Technol.* **36** (20), 2577–2587.

Kong, Q., Wang, Z., Shu, L. & Miao, M. 2015 Characterization of the extracellular polymeric substances and microbial community of aerobic granulation sludge exposed to cefalexin. *Int. Biodeterior. Biodegradation.* **102**, 375–382.

Kowalchuk, G. & Stephen, J. 2001 Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Ann. Rev. Microbiol.* **55**, 485–529.

Lee, D.-J., Chen, Y.-Y., Show, K.-Y., Whiteley, C. G. & Tay, J.-H. 2010 Advances in aerobic granule formation and granule stability in the course of storage and reactor operation. *Biotechnol. Adv.* **28** (6), 919–934.

Lemaire, R., Webb, R. I. & Yuan, Z. 2008 Micro-scale observations of the structure of aerobic microbial granules used for the treatment of nutrient-rich industrial wastewater. *ISME. J.* **2** (5), 528–541.

Lettinga, G., van Velsen, A., Hobma, S., de Zeeuw, W. & Klapwijk, A. 1980 Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnol. Bioeng.* **22** (4), 699–734.

Li, Y.-C. & Zhu, J.-R. 2014 Role of N-acyl homoserine lactone (AHL)-based quorum sensing (QS) in aerobic sludge granulation. *Appl. Microbiol. Biotechnol.* **98** (17), 7623–7632.

Li, J., Ding, L.-B., Cai, A., Huang, G.-X. & Horn, H. 2014 Aerobic sludge granulation in a full-scale sequencing batch reactor. *Biomed. Res. Int.* **155**, 268–789.

Liu, Y. & Liu, J.-H. 2002 The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge. *Water Res.* **36** (7), 1653–1665.

Liu, Y. & Liu, Q.-S. 2006 Causes and control of filamentous growth in aerobic granular sludge sequencing batch reactors. *Biotechnol. Adv.* **24** (1), 115–127.

Liu, Y. & Tay, J.-H. 2004 State of the art of biogranulation technology for wastewater treatment. *Biotechnol. Adv.* **22** (7), 533–563.

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** (22–24), 2619–2626.

Liu, Y., Yang, S.-F. & Tay, J.-H. 2004a Improved stability of aerobic granules by selecting slow-growing nitrifying bacteria. *J. Biotechnol.* **108** (2), 161–169.

Liu, Y.-Q., Liu, Y. & Tay, J.-H. 2004b The effects of extracellular polymeric substances on the formation and stability of biogranules. *Appl. Microbiol. Biotechnol.* **65** (2), 143–148.

Liu, Y., Wang, Z.-W., Qin, L., Liu, Y.-Q. & Tay, J.-H. 2005 Selection pressure-driven aerobic granulation in a sequencing batch reactor. *Appl. Microbiol. Biotechnol.* **67** (1), 26–32.

Liu, L., Li, Y. & Tollefsbol, T. O. 2008 Gene-environment interactions and epigenetic basis of human diseases. *Curr. Issues Mol. Biol.* **10** (1–2), 25–36.

Liu, Y., Liu, Z., Wang, F., Chen, Y., Kuschk, P. & Wang, X. 2014 Regulation of aerobic granular sludge reformulation after granular sludge broken: effect of poly aluminum chloride (PAC). *Bioresour. Technol.* **158**, 201–208.

Liu, X., Sun, S., Ma, B., Zhang, C., Wan, C. & Lee, D.-J. 2016 Understanding of aerobic granulation enhanced by starvation in the perspective of quorum sensing. *Appl. Microbiol. Biotechnol.* **100** (8), 3747–3755.

López-Palau, S., Pinto, A., Basset, N., Dosta, J. & Mata-Álvarez, J. 2012 ORP slope and feast-famine strategy as the basic of the control of a granular sequencing batch reactor treating winery wastewater. *BioChem. Eng. J.* **68**, 190–198.

Luo, J., Hao, T., Wei, L., Mackey, H., Lin, Z. & Chen, G.-H. 2014 Impact of influent COD/N ratio on disintegration of aerobic granular sludge. *Water Res.* **62**, 127–135.

Luong, V. T., Kurz, E. E., Hellriegel, U., Luu, T. L., Hoinkis, J. & Bundschuhc, J. 2018 Iron-based subsurface arsenic removal technologies by aeration: a review of the current state and future prospects. *Water Res.* **133**, 110–122.

Lv, Y., Wan, C., Liu, X., Zhang, Y., Lee, D.-J. & Tay, J.-H. 2013 Drying and re-cultivation of aerobic granules. *Bioresour. Technol.* **129**, 700–703.

Lv, J., Wang, Y., Zhong, C., Li, Y., Hao, W. & Zhu, J. 2014a The effect of quorum sensing and extracellular proteins on the microbial attachment of aerobic granular activated sludge. *Bioresour. Technol.* **152**, 53–58.

Lv, J., Wang, Y., Zhong, C., Li, Y., Hao, W. & Zhu, J. 2014b The microbial attachment potential and quorum sensing measurement of aerobic granular activated sludge and flocculent activated sludge. *Bioresour. Technol.* **151**, 291–296.

Ma, J., Quan, X. & Li, H. 2013 Application of high OLR-fed aerobic granules for the treatment of low-strength wastewater: performance, granule morphology and microbial community. *J. Environ. Sci.* **25** (8), 1549–1556.

Martins, A. M., Heijnen, J. J. & van Loosdrecht, M. C. M. 2003 Effect of feeding pattern and storage on the sludge settleability under aerobic conditions. *Water Res.* **37** (11), 2555–2570.

Martins, A. M., Pagilla, K., Heijnen, J. J. & van Loosdrecht, M. C. M. 2004 Filamentous bulking sludge–a critical review. *Water Res.* **38** (4), 793–817.

Matsuyama, H., Katoh, H., Ohkushi, T., Satoh, A., Kawahara, K. & Yumoto, I. 2008 *Sphingobacterium kitahiroshimense* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.* **58** (7), 1576–1579.

McSwain, B., Irvine, R., Hausner, M. & Wilderer, P. 2005 Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. *Appl. Environ. Microbiol.* **71** (2), 1051–1057.

Miller, R. L., Acton, C., Fullerton, D. A., Maltby, J. & Campling, J. 2002 Analysis of variance (ANOVA). In: *SPSS for Social Scientists* (Campling, J., ed.). Palgrave Macmillan, UK, pp. 145–154.

Mishima, K. & Nakamur, M. 1991 Self-immobilization of aerobic activated sludge–a pilot study of the aerobic upflow sludge blanket process in municipal sewage treatment. *Water Sci. Technol.* **23** (4–6), 981–990.

Morales, N., Figueroa, M., Fra-Vázquez, A., Val del Río, A., Campos, J., Mosquera-Corral, A. & Méndez, R. 2013 Operation of an aerobic granular pilot scale SBR plant to treat swine slurry. *Process. Biochem.* **48** (8), 1216–1221.

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

Muda, K., Aris, A., Salim, M. R., Ibrahim, Z., van Loosdrecht, M. C. M., Ahmad, A. & Nawahwi, M. Z. 2011 The effect of hydraulic retention time on granular sludge biomass in treating textile wastewater. *Water Res.* **45** (16), 4711–4721.

Nancharaiah, Y. V. & Reddy, G. K. 2018 Aerobic granular sludge technology: mechanisms of granulation and biotechnological applications. *Bioresour. Technol.* **247**, 1128–1143.

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

Peyong, Y. N., Zhou, Y., Abdullah, A. Z. & Vadivelu, V. 2012 The effect of organic loading rates and nitrogenous compounds on the aerobic granules developed using low strength wastewater. *BioBiochem. Eng. J.* **67**, 52–59.

Pronk, M., de Kreuk, M. K., Bruin, B. d., Kamming, P., Kleerebezem, R. & van Loosdrecht, M. C. M. 2015 Full scale performance of the aerobic granular sludge process for sewage treatment. *Water Res.* **84**, 207–217.

Qin, L., Tay, J.-H. & Liu, Y. 2004a Effect of settling time on aerobic granulation in sequencing batch reactor. *Biochem. Eng. J.* **21**, 47–52.

Qin, L., Tay, J. H. & Liu, Y. 2004b Selection pressure is a driving force of aerobic sequencing batch reactors. *Process Biochem.* **39** (5), 579–584.

Ramos, C., Suárez-Ojeda, M. E. & Carrera, J. 2016 Biodegradation of a high-strength wastewater containing a mixture of ammonium, aromatic compounds and salts with simultaneous nitritation in an aerobic granular reactor. *Process Biochem.* **51** (3), 399–407.

Rollemberg, S. L., de Oliveira, L. Q., Barros, A. R., Melo, V. M., Firmino, P. I. & Santos, A. B. 2019 Effects of carbon source on the formation, stability, bioactivity and biodiversity of the aerobic granule sludge. *Bioresour. Technol.* **278**, 195–204.

Rosman, N. H., Anuar, A. N., Chelliapan, S., Md Din, M. F. & Ujang, Z. 2014 Characteristics and performance of aerobic granular sludge treating rubber wastewater at different hydraulic retention time. *Bioresour. Technol.* **161**, 155–161.

Schwarzenbeck, N., Erly, R., Mc Swain, B. S. & Wilderer, P. A. 2004 Treatment of malting wastewater in a granular sludge sequencing batch reactor (SBR). *Acta Hydrochimical et Hydrobiologica.* **32** (1), 16–24.

Seviour, T. W., Lambert, L. K., Pijuan, M. & Yuan, Z. 2011 Selectively inducing the synthesis of a key structural exopolysaccharide in aerobic granules by enriching for *Candidatus* 'Competibacter phosphatis'. *Appl. Microbiol. Biotechnol.* **92** (6), 1297–1305.

Seviour, T., Yuan, Z., van Loosdrecht, M. C. & Lin, Y. 2012 Aerobic sludge granulation: a tale of two polysaccharides? *Water Res.* **46** (15), 4803–4813.

Sguanci, S., Lubello, C., Caffaz, S. & Lotti, T. 2019 Long term stability of aerobic granular for the treatment of very low strength real domestic water. *J. Cleaner Prod.* **222**, 882–890.

Sheng, G.-P., Yu, H.-Q. & Li, X.-Y. 2006 Stability of sludge flocs under shear conditions: roles of extracellular polymeric substances (EPS). *Biotechnol. Bioeng.* **93** (6), 1095–1102.

Sheng, G.-P., Yu, H.-Q. & Li, X.-Y. 2010 Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. *Biotechnol. Adv.* **28** (6), 882–894.

Skiadas, V. I., Gavala, H. N., Schmidt, J. E. & Ahring, B. K. 2003 Anaerobic granular sludge and biofilm reactors. *Adv. Biochem. Eng. Biotechnol.* **82**, 35–67.

Su, K.-Z. & Yu, H.-Q. 2005 Formation and characterization of aerobic granules in squencing batch reactor treating soybean processing wastewater. *Environ. Sci. Technol.* **39** (8), 2818–2827.

Sun, H., Yu, P. L., Ren, H., Liu, B., Ye, B. & Zhang, X.-X. 2017 Transformation of anaerobic granules into aerobic granules and the succession of bacterial community. *Appl. Microbiol. Biotechnol.* **101** (20), 7703–7713.

Szabó, E., Liébana, R., Hermansson, M., Modin, O., Persson, F. & Wilén, B.-M. 2017 Microbial population dynamics and ecosystem functions of anoxic/aerobic granular sludge in sequencing batch reactors operated at different organic loading rates. *Front. Microbiol.* **8**, 770.

Tay, J. H., Liu, Q. S. & Liu, Y. 2001a The role of cellular polysaccharides in the formation and stability of aerobic granules. *Lett. Appl. Microbiol.* **33** (3), 222–226.

Tay, J. H., Liu, Q. S. & Liu, Y. 2001b The effects of shear force on the formation, structure and metabolism of aerobic granules. *Appl. Microbiol. Biotechnol.* **57** (1–2), 227–233.

Tay, J. H., Ivanov, V., Pan, S. & Tay, S. T.-L. 2002 Specific layers in aerobically grown microbial granules. *Lett. Appl. Microbiol.* **34** (4), 254–257.

Tay, J.-H., Pan, S., He, Y. & Tay, S. T. 2004 Effect of organic loading rate on aerobic granulation. I: reactor performance. *J. Environ. Eng.* **130** (10), 1094–1101.

Verawaty, M., Tait, S., Pijua, M., Yuan, Z. & Bon, P. L. 2013 Breakage and growth towards a stable aerobic granule size during the treatment of wastewater. *Water Res.* **47** (14), 5338–5349.

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

Wagner, J., Weissbrodt, D. G., Manguin, V., da Costa, R. H., 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.

Wan, C., Zhang, P., Lee, D.-J., Yang, Z., Liu, X., Sun, S. & Pan, X. 2013 Disintegration of aerobic granules: role of second messenger cyclic di-GMP. *Bioresour. Technol.* **146**, 330–335.

Wan, C., Chen, S., Wen, L., Lee, D.-J. & Liu, X. 2015 Formation of bacterial aerobic granules: role of propionate. *Bioresour. Technol.* **197**, 489–494.

Wang, Z., Liu, L., Yao, J. & Cai, W. 2006 Effects of extracellular polymeric substances on aerobic granulation in sequencing batch reactors. *Chemosphere* **63** (10), 1728–1735.

Wang, X., Zhang, H., Yang, F., Xia, L. & Gao, M. 2007 Improved stability and performance of aerobic granules under stepwise increased selection pressure. *Enzyme Microb. Technol.* **41** (3), 205–211.

Wang, F., Lu, S., Wei, Y. & Ji, M. 2009 Characteristics of aerobic granule and nitrogen and phosphorus removal in a SBR. *J. Hazard. Mater.* **164**, 1223–1227.

Wang, B.-B., Peng, D.-C., Hou, Y.-P., Li, H.-J., Pei, L.-Y. & Yu, L.-F. 2014 The important implications of particulate substrate in determining the physicochemical characteristics of extracellular polymeric substances (EPS) in activated sludge. *Water Res.* **58**, 1–8.

Wang, S., Shi, W., Tang, T., Wang, Y., Zhi, L., Lv, J. & Li, J. 2017 Function of quorum sensing and cell signaling in the formation of aerobic granular sludge. *Environ. Sci. Bio/Technol.* **16**, 1–13.

Weissbrodt, D. G., Neu, T. R., Kuhlicke, U., Rappaz, Y. & Holliger, C. 2013 Assessment of bacterial and structural dynamics in aerobic granular biofilms. *Front. Microbiol.* **4**, 175.

Winkler, M.-K. H., Kleerebezem, R., Strous, M., Chandran, K. & van Loosdrecht, M. C. M. 2013 Factors influencing the density of aerobic granular sludge. *Appl. Microbiol. Biotechnol.* **97** (16), 7459–7468.

Winkler, M.-K. H., Le, Q. H. & Volcke, E. I. 2015 Influence of partial denitrification and mixotrophic growth of NOB on microbial distribution in aerobic granular sludge. *Environ. Sci. Technol.* **49** (18), 11003–11010.

Winkler, M.-K. H., Meunier, C., Henriet, O. M., Suárez-Ojedad, M. E., De Sanctise, G. D., Di Iaconie, C. & Weissbrodtf, D. G. 2018 An integrative review of granular sludge for the biological removal of nutrients and recalcitrant organic matter from wastewater. *Chem. Eng. J.* **336**, 489–502.

Wu, L.-j., Li, A.-j., Hou, B.-l. & Li, M.-X. 2017 Exogenous addition of cellular extract N-acyl-homoserine-lactones accelerated the granulation of autotrophic nitrifying sludge. *Int. Biodeterior. Biodegradation.* **118**, 119–125.

Xiong, Y. & Liu, Y. 2013 Importance of extracellular proteins in maintaining structural integrity of aerobic granules. *Colloids Surf. B. Biointerfaces* **112**, 435–440.

Xu, H.-C., He, P.-J., Wang, G.-Z., Yu, G.-H. & Shao, L.-M. 2010 Enhanced storage stability of aerobic granules seeded with pellets. *Bioresour. Technol.* **101** (21), 8031–8037.

Yuan, X. & Gao, D. 2010 Effect of dissolved oxygen on nitrogen removal and process control in aerobic granular sludge reactor. *J. Hazard. Mater.* **178**, 1–3.

Yuan, S., Gao, M., Afzal, M. H., Z, M., Wang, Y.-K., Wang, M. & Wang, X.-H. 2018 Qualitatively and quantitatively assessing the aggregation ability of sludge during aerobic granulation process combined XDLVO theory with physicochemical properties. *J. Environ. Sci.* **67**, 154–160.

Zhang, W. & Li, C. 2016 Exploiting quorum sensing interfering strategies in Gram-negative bacteria for the enhancement of environmental applications. *Front. Microbiol.* **6**, 1535.

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.

Zhang, Q., Hu, J. & Lee, D.-J. 2016 Aerobic granular processes: current research trends. *Bioresour. Technol.* **210**, 74–80.

Zhang, Y., Zhao, M., Sawalha, A. H., Richardson, B. & Lu, Q. 2013 Impaired DNA methylation and its mechanisms in $CD4^{+}$ T cells of systemic lupus erythematosus. *J. Autoimmun* **41**, 92–99.

Zhao, Y., Huang, J. & Zha, H. Y. 2013 Microbial community and N removal of aerobic granular sludge at high COD and N loading rates. *Bioresour. Technol.* **143**, 439–446.

Zhou, D., Niu, S., Xiong, Y., Yang, Y. & Dong, S. 2014 Microbial selection pressure is not a prerequisite for granulation: dynamic granulation and microbial community study in a complete mixing bioreactor. *Bioresour. Technol.* **161**, 102–108.

Zhu, L., Qi, H. Y., Lv, M. L. K., Yu, Y. W. & Xu, X. Y. 2012 Component analysis of extracellular polymeric substances (EPS) during aerobic sludge granulation using FTIR and 3D-EEM technologies. *Bioresour. Technol.* **124**, 455–459.

Zhu, L., Lv, M., Dai, X. L., Zhou, J.-L. & Xu, X. Y. 2013 The stability of aerobic granular sludge under 4-chloroaniline shock in a sequential air-lift bioreactor (SABR). *Bioresour. Technol.* **140**, 126–130.

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