ARTICLE IN PRESS

Journal of Water Process Engineering xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Effect of feeding strategy and organic loading rate on the formation and stability of aerobic granular sludge

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ARTICLE INFO

Keywords:
Aerobic granular sludge (AGS)
AGS stability
Feeding strategy
Organic loading rate
Wastewater treatment

ABSTRACT

Aerobic granular sludge (AGS) has emerged as a novel biotechnology for the effective treatment of both municipal and industrial wastewater. Two of the practical parameters that affect aerobic granule (AG) formation and stability are the feeding strategy and organic loading rate (OLR). In this paper, the impact of feeding strategy and OLR on AG formation and stability are reviewed. The feeding strategy that appears suitable for stable AG formation using all substrates is slow anaerobic feeding. Despite the long duration for AG cultivation at low OLR, compact and stable AG are formed. High OLRs result in fast AG formation but the developed granules are unstable due to the large size resulting from excessive microbial growth. The recommended strategy for stable AG formation with enhanced treatment performance is stressed (high) OLR for fast granule formation followed by reduced OLR to ensure stability. This can be combined with slow anaerobic feeding to select for slow-growing microorganisms and nutrients removers such as phosphate-accumulating organisms (PAOs). Further research is recommended to explore AG formation at high OLR using the slow anaerobic feeding strategy for long-term stability.

1. Introduction

Aerobic granular sludge (AGS) technology has emerged as a novel biotechnology for the effective treatment of both municipal and industrial wastewater. AGS technology exhibits numerous advantages over the conventional activated sludge process. These advantages include outstanding settleability, strong microbial structure, high biomass retention, high resilience to toxic chemicals, and good ability to handle high organic and shock loading rates [1–4]. Due to its strong potential to revolutionise the wastewater management industry, AGS biotechnology is considered by the International Water Association to be the most promising wastewater treatment technology of the 21 st century. AGS has been successfully used in the treatment of both municipal and a wide range of industrial wastewaters [4–6].

During the granulation process, the feeding strategy and organic loading rate (OLR) play vital roles in the type and stability of aerobic granules (AG) that form. A number of review papers have been published on AGS biotechnology in the last 20 years [1–4,6–11]. However, none of the papers focus exclusively on the influence that these key operational parameters (feeding strategy and OLR) have on aerobic granulation. While it is generally agreed that hydrodynamic shear force and settling time have major influence on AG formation and stability, the effect of feeding strategy and OLR on the formation and stability of AGS is often not highlighted. Regarding the relationship between these two parameters as applied to bioreactor operation in sequencing batch reactor (SBR) mode, OLR defines the amount of organic matter that is fed into a given reactor volume over a period of time, while the feeding strategy defines how fast organic matter is fed into the reactor and the form of carbon available at the beginning of the aeration/reaction phase of the SBR cycle. E.g. when anaerobic feeding strategy is adopted, easily biodegradable carbon in the feed is converted into slowly biodegradable storage polymers during the feeding phase. In general, OLR is a volumetric loading rate that relates to the cycle time, feed concentration, and the volumetric exchange ratio of the SBR.

The feeding strategy has been demonstrated to affect the long-term stability of AG, the microbial community, and treatment performance. It is generally accepted that the agglomeration of activated sludge into AG depends on the substrate concentration; however, AG kinetic behaviour depends on the applied substrate loading [12,13]. Although OLR has been widely viewed as not having a significant effect on AG formation [3,14], it appears that it impacts how quickly AG form, their characteristics, and stability. The focus of this review, therefore, is on

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https://doi.org/10.1016/j.jwpe.2020.101709
Received 19 May 2020; Received in revised form 18 September 2020; Accepted 19 September 2020

Please cite this article as: Oliver Terna Iorhemen, Yang Liu, *Journal of Water Process Engineering*, https://doi.org/10.1016/j.jwpe.2020.101709

the impact of these two factors on the formation, stability, and performance of AGS. The paper will be of benefit to researchers working in the field of AGS biotechnology as it highlights areas needing further research. The information contained in this review could also benefit wastewater management industry professionals in the design and successful operation of AGS bioreactors.

2. Feeding strategy

2.1. Background of the various feeding strategies

Feeding strategy, the method adopted in filling the AGS sequencing batch reactor (SBR), affects the morphology, performance and stability of granules. Five different feeding strategies have been implemented in the formation and maintenance of AG as follows:

(a) Fast static feeding strategy where the influent is fed to the reactor within a few minutes without aeration or mixing.
(b) Slow static (anaerobic) feeding strategy whereby the influent is fed to the reactor through a settled sludge bed over an extended period of time without aeration or mixing in the AGS bioreactor. Because the feeding period is long (typically about 1 h), the dissolved oxygen (DO) concentration drops, making the reactor totally anaerobic.
(c) Fast static feeding followed by anaerobic mixing which is a slight modification of the slow anaerobic feeding strategy.
(d) Aerated feeding strategy in which feeding and aeration/mixing all take place at the same time.
(e) Split anaerobic–aerobic feeding strategy which is a combination of static and aerated feeding strategies.

The fast static feeding strategy is done by feeding the reactor through the settled sludge within a few minutes without aeration or mixing [15–17] from the top of the reactor [18,19], or through a port on the side of the reactor. This feeding strategy has been variously employed in AG formation [15,16,20–28]. The fast static feeding strategy allows for fast AG formation. Although AG formation poses no problem, instability has been the main issue with AG formed using this feeding strategy [29,30]. Generally, the growth rate of microorganisms inside AG has been identified as a suitable parameter to enhance the stability of AGS [8]. When microorganisms within the granule grow excessively, the granule becomes big in size and its structure is weakened. The weakened granules then disintegrate, impacting treatment performance. On the other hand, slow microbial growth rates have been demonstrated to promote the formation of compact granules with high integrity and physical strength [8,31,32]. The selection for slow-growing microorganisms has been reported to suppress filamentous growth and improve AG stability [31,33,34]. This explains the choice of microbial growth rate as a suitable parameter for enhancing AGS stability. With the fast static feeding strategy, no anaerobic condition is created to convert easily biodegradable carbon into storage polymers. As such, easily biodegradable carbon is transferred to the aeration phase, promoting fast heterotrophic growth [34]. Research indicates that the growth rate of heterotrophic microorganisms decreases when the substrate is slowly biodegradable storage polymers unlike readily biodegradable carbon [35]. The excessive growth of heterotrophic microbes results in large-size granules that disintegrate over time.

The slow static (anaerobic) feeding strategy was first proposed in 2004 [36]. The rationale behind the proposal was to control the growth of filamentous organisms that ultimately result in granule instability. During feeding, the high substrate concentration in the feed penetrates the entire depth of the settled AGS bed, promoting the conversion of the available carbon to storage polymers by phosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) under anaerobic conditions. In the subsequent aeration phase, microorganisms utilise the internally stored carbon sources to grow at a low rate [8], limiting the excessive aerobic growth of fast-growing heterotrophic microorganisms on readily available carbon that causes unstable AG [34]. Additionally, this feeding strategy specifically allows for the selection of slow-growing microorganisms - PAOs and/or GAOs - which promote the formation and maintenance of compact granules. The selection for slow-growing nitrifying bacteria has also been shown to allow for stable AGS [31]. In terms of performance, the combination of PAOs with nitrifying and denitrifying bacteria enhances the removal of chemical oxygen demand (COD), total nitrogen, and phosphorus. Various other studies have utilised the slow anaerobic feeding strategy for AG formation and stability [31,34,37–39]. McSwain et al. [40] compared the slow anaerobic feeding strategy with split anaerobic–aerobic feeding at an OLR of 2.4 kg COD/m^3·d. The authors reported AG formation under both feeding strategies; however, only the reactor with the former feeding strategy formed compact and stable granules [40]. The split anaerobic-aerobic feeding strategy resulted in the selection and growth of filamentous organisms that affected the structure of the granules. Wagner et al. [41] explored the use of the slow anaerobic feeding strategy to treat wastewater containing particulate matter. They found the slow anaerobic phase to be beneficial for the formation of compact granules [41]. Slow anaerobic feeding alongside a fixed feast-famine period ratio and selective discharge of mature AG was utilised to enhance AG stability [42]. Stable reactor operation was maintained in over 240 d of the study. Numerous other studies have found the slow anaerobic feeding strategy to be beneficial for the development of compact AG with long-term stability [43–46]. Fig. 1 presents granules formed using the slow anaerobic feeding strategy.

The fast static feeding followed by a period of anaerobic mixing was explored by Rocktäschel et al. [47], who compared it to the slow anaerobic feeding strategy. They found that both feeding strategies resulted in AG formation; however, anaerobic mixing showed some merits over the anaerobic plug-flow feeding regime. Granule growth was more stable allowing for a suitable balance between the growth of heterotrophic and autotrophic microorganisms [47].

The aerated feeding strategy was found to develop a loose AG structure with a weak anaerobic core compared to the compact granules formed when the slow anaerobic feeding strategy was adopted [48]. The treatment performance in terms of total nitrogen removal was higher (mean efficiency of 91.7 ± 4.1 %) with the granules developed using the anaerobic feeding strategy. When the granules were developed using the aerated feeding strategy, the removal was poor (mean efficiency of 58.8 ± 7.4 %). It has also been shown that the aerated feeding strategy allows for the fast formation of granules but with the capability of removing only organic matter as the selected microorganisms (majorly organic matter degraders) outcompete the microorganisms responsible for nitrogen removal [45,46,49].

In the split anaerobic-aerobic feeding strategy, instead of the long slow anaerobic feeding period, a part of the feeding period is done under anaerobic conditions and the remaining period is aerated feeding

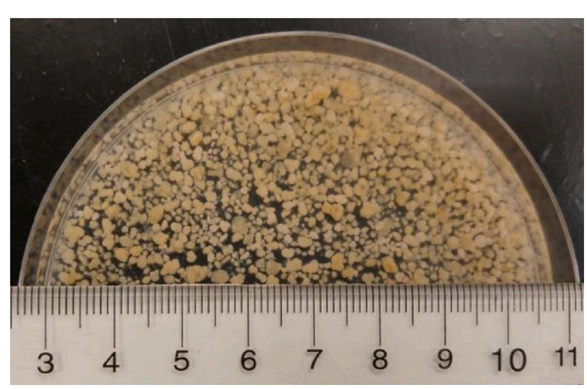

Fig. 1. Granules formed through the slow anaerobic feeding strategy.

(feeding, aeration and mixing). Recent research reported that the split anaerobic-aerobic feeding was suitable for the formation and maintenance of AG at low OLRs [50]. This is in contrast to an earlier study by McSwain et al. [40], in which long anaerobic feeding formed compact and stable granules compared to the split anaerobic-aerobic feeding strategy [40]. However, it must be pointed out that the substrate type and the loading rate used in the two studies were not the same. While McSwain et al. [40] used synthetic wastewater made from glucose and peptone at a loading rate of 2.4 kg COD/m^3·d, Thwaites et al. [50] utilised real municipal wastewater containing high salinity (in the range of 5,000–7,000 mg/L total dissolved solids) at loading rate of 0.76–1.15 kg COD/m^3·d. As such, the difference in findings may also be attributed to other parameters. Hence, further studies need to be conducted in this area.

The influent and effluent wastewater characteristics as applied to the different feeding strategies are presented in Table 1 below.

2.2. Perspectives on the feeding strategies

The aerated feeding strategies can lead to fast granulation, but at the expense of nutrient removal, since the favoured organisms mainly only remove organic matter and outcompete nitrifiers [51]. Both the aerated and fast static feeding strategies are applicable in situations where the removal of only organic matter is desired. Additionally, they are suitable

Table 1
Influent and effluent wastewater characteristics for feeding strategies.

Feeding Strategy	Wastewater type	Influent Characteristics	Effluent Characteristics	Reference
Fast static feeding strategy	Synthetic high-strength wastewater	2000 mg COD/L	80 mg COD/L	[21]
	Synthetic high-strength wastewater	500−3000 mg COD/L Salt conc. 2–10 g/L	16–91 mg COD/L	[22]
	Synthetic high-strength wastewater	600−2000 mg COD/L 200 mg NH_4-N/L 15 mg P/L	1.57 ± 0.39–9.15 ± 2.27 mg NH_4-N/L 3.61 ± 0.15–5.22 ± 0.14 mg P/L	[25]
	Anaerobic digester supernatant	1350 ± 145 mg COD/L 600 ± 32 mg TKN/L 493 ± 21 mg NH_4-N/L 12 ± 3.2 mg P/L	COD rem. eff. 50.8 ± 16.6–77.5 ± 7.2 % N rem. eff. 30.0 ± 5.1–73.3 ± 5.9% Effluent NH_4-N 2.5 mg/L	[15]
	Synthetic high-strength wastewater	2600 ± 450–7500 ± 600 mg COD/L 78−230 mg NH_4-N/L	Eff. COD 41.6–300 mg/L Eff. NH_4-N < 0.015 mg/L	[16]
	Synthetic oil sands process-affected water	2000 mg COD/L 80 mg N/L 8 mg P/L Naphthenic acid 16 g NA/m^3/d.	Eff. COD 78 mg/L Eff. NH_4-N 3.28 mg/L Eff. P 0.624 mg/L Mean naphthenic acid rem. 67 %	[27]
	Synthetic high-strength wastewater	2000 mg COD/L 92 mg N/L 20 mg P/L	Eff. COD 60 mg/L Eff. NH_4-N 2.76 mg/L Eff. P 11.8 mg/L	[17]
Slow static (anaerobic) feeding strategy	Domestic Sewage	330 ± 64 mg COD/L 57 ± 8 mg NH_4-N/L 70 ± 11 mg TN/L 9 ± 2 mg PO_4-P/L	170 ± 67 mg COD/L 51 ± 9 mg NH_4-N/L 60 ± 9 mg TN/L 6 ± 3.5 mg PO_4-P/L	[38]
	Municipal wastewater	Mean COD 1000 mg/L Mean NH_4-N 60 mg/L	COD rem. Eff. 80 % NH_4-N rem. 98%	[43]
	Synthetic wastewater	400 mg COD/L 60 mg N/L 20 mg P/L	P rem. eff. 36–100 %	[44]
	Synthetic wastewater	R_1 (COD only) 496 ± 42 mg tCOD/L 488 ± 43 mg sCOD/L 50 ± 5 mg NH_4-N/L 5.0 ± 0.6 mg PO_4-P/L R_2 (COD & particulate matter): 494 ± 38 mg tCOD/L 253 ± 18 mg sCOD/L 52 ± 3 mg NH_4-N/L 4.7 ± 0.5 mg PO_4-P/L	R_1 (COD only) tCOD rem. eff. 85 ± 11 % sCOD rem. eff. 96 ± 2% NH_4-N rem. 94 ± 8% PO_4-P rem. eff. 72 ± 13% R_2 (COD & particulate matter): tCOD rem. eff. 92 ± 2% sCOD rem. eff. 62 ± 18 % NH_4-N rem. 14 ± 8% PO_4-P rem. eff. 50 ± 22%	[41]
	Synthetic wastewater	509 mg COD/L 29.5 mg TN/L	7.4 mg TN/L	[34]
	Synthetic wastewater	2902 ± 130 mg COD/L 75 ± 4 mg TN/L 35 ± 4 mg PO_4-P/L	58 mg COD/L 2.98 mg TN/L 0.24 mg nitrate/L 1.75 mg PO_4-P/L	[39]
	Synthetic wastewater	300−1000 mg COD/L 15−504 mg TN/L 3 - 10 mg PO_4-P/L	Mean COD rem. eff. 98.3 ± 0.8 % NH_3-N rem. eff. 98 ± 0.5% TN rem. eff. 89 ± 6 % P rem. eff. 92 ± 13 %	[42]
Fast static feeding followed by anaerobic mixing	Synthetic wastewater	tCOD 1.92 kg/m^3·d NH_4-N 0.08 kg/m^3·d PO_4-P/L 0.012 kg/m^3·d	Eff. NH_4-N 0–2 mg/L Eff. P < 1 mg/L	[47]
Aerated feeding strategy	Synthetic wastewater	400−900 mg COD/L 25−60 mg NH_3-N/L	Eff. COD < 60 mg/L Eff. NH_3-N ~ 0 mg/L	[48]
Split anaerobic–aerobic feeding strategy	Municipal wastewater with high salinity	COD loading 0.76 kg/m^3·d Salinity 5–7 g/L total dissolved solids.	COD rem. eff. ~ 75 % NH_3-N rem. eff. ~ 97% TN rem. eff. ~ 65 % P rem. Eff. 8%	[50]

where the wastewater has high organic matter content and fluctuations in wastewater composition are expected. In such situations, the long-term stability of the developed granules needs to be considered. Other operational parameters that control AG stability would need to be implemented when these feeding strategies are adopted.

The slow anaerobic feeding strategy has been found to form compact and stable AG where the excessive growth of heterotrophic microorganisms is kept in check, while allowing slow-growing microorganisms to thrive. Long anaerobic contact time plays three salient roles: the hydrolysis of slowly biodegradable substrates into readily biodegradable substrates, the formation of volatile fatty acids through the fermentation of slowly degradable substrates, and preferential uptake and storage of the slowly degradable substrates by slow growing PAOs and GAOs [41,45,46]. In addition to enabling AG stability, the treatment performance (particularly nitrogen and phosphorus removal) of the AG developed under this feeding strategy is enhanced. Particularly for phosphorus, the anaerobic condition during the feeding phase is essential for high removal efficiency [52,53]. Another way to look at this is to consider that the anaerobic feeding regime is the plug-flow type in which the feed passes through the settled bed of AG. As it is the plug-flow type, large-sized and dense AG are expected to settle at the bottom of the reactor, allowing higher nutrient loads to be received by these large granules [51]. This will allow substrate gradient from the reactor bottom upwards to penetrate the stratified structure of the big granules, enabling biochemical reactions to take place within the granules. Further, with fluctuating industrial effluents, this feeding strategy would be handy as it would allow adequate time for enough wastewater to accumulate and be used as feed in the next SBR cycle.

The slow anaerobic feeding period makes a simplified feeding and withdrawal system possible [36]. In order to avoid filamentous outgrowth on the granule surface, it was suggested that slowly degradable substances found in real wastewater, such as colloids and particulate matter, be run through an anaerobic hydrolysis pre-step [54,55]. The slow anaerobic feeding strategy would allow for the hydrolysis and fermentation of such slowly degradable substrates, allowing for a quick uptake in the subsequent aeration phase of the SBR cycle. This would create the desired feast-famine conditions in the reactor, which keep heterotrophic growth under check, enabling long-term AG stability and increased treatment performance. The main shortcoming of the slow anaerobic feeding strategy is the increase in the duration of the cycle which increases the hydraulic retention time of the treatment. The slow anaerobic feeding strategy is applicable in situations where the removal of both organic matter and nutrients is required, in situations of fluctuating industrial effluents, and where the wastewater contains high organic/nutrient loads as well as slowly degradable substances such as colloids and particulate matter.

As for the fast static feeding followed by the anaerobic mixing strategy, feeding under anaerobic mixing conditions at an industrial scale is suitable as it would allow complete mixing of the influent wastewater when compared to an ideal plug-flow influent stream. However, the reactor design stage would need to consider issues around the anaerobic mixing step. The issues would include the equalisation tanks required and the number of SBRs to custom build. Another issue to consider is the additional operational cost that would be required for incorporating mixing during the feeding phase of the SBR cycle. The fast static feeding followed by the anaerobic mixing strategy is applicable for industrial wastewater.

Different feeding strategies are suitable to different substrates. While fast static feeding can be suitable when using easily biodegradable substrates such as acetate, it is not desirable where slowly biodegradable substrates are involved. Substrates, such as methanol, which can be aerobically converted by relatively slow-growing bacteria, are able to form stable granules even under the fast aerobic feeding strategy [56]. Despite the positives here, it is still more advantageous to employ the slow anaerobic feeding strategy as it will allow for energy savings resulting from not using aeration during the feeding phase. And the stability made possible by the slow anaerobic feeding strategy would allow for stable reactor operation.

The split anaerobic–aerobic feeding strategy is suitable for wastewater treatment plants with low OLRs. While one study has found the split anaerobic-aerobic feeding strategy to be suitable for AGS formation and maintenance at low OLR [50], there are no similar studies reported in the literature. Further research may be needed in this area using different substrates and operating at various OLRs to establish the threshold OLR for the adoption of this feeding strategy. Another consideration is that introducing aeration during the feeding would increase the energy consumption, adding to the operational cost.

3. Organic loading rate

3.1. Background of the range of organic loading rate used in aerobic granulation

The OLR is one of the most salient parameters affecting biological wastewater treatment systems [14]. As applied to AGS and the sense used in this paper, reactor OLR values are divided into three categories: low (below 2 kg COD/m^3·d), moderate (2–4 kg COD/m^3·d), and high (above 4 kg COD/m^3·d). While the cultivation of AG has been possible across a wide range of OLRs: 0.6–24 kg COD/m^3·d [3,12,13,57,58], AG instability has been the major issue at high OLRs. Moy et al. [12] first studied the effect of OLR (6, 9, 12 and 15 kg COD/m^3·d) on AG formation using two substrates, glucose and acetate. While AG was formed at all the OLRs tested when glucose was the substrate, the AG formed at 6 kg COD/m^3·d were dominated by filamentous bacteria and had a loose fluffy morphology whereas AG formed at 9 kg COD/m^3·d and above were irregularly shaped and contained folds, depressions, and crevices. With acetate as the substrate, compact AG with enhanced settleability and strength were formed at 6 and 9 kg COD/m^3·d. However, the acetate-fed AG disintegrated at an OLR of 9 kg COD/m^3·d. Similarly, Tay et al. [59] found that OLR influenced AG formation as well as its characteristics and stability. At a low OLR of 1 kg COD/m^3·d, loose-structured flocs dominated the reactor without AG formation. Stable and compact AG were formed at a moderate OLR of 4 kg COD/m^3·d while at a high OLR of 8 kg COD/m^3·d, the formed AG coexisted with flocs containing pores and filament [59]. No AG formation occurred at low OLR of 1–2 kg COD/m^3·d in another study whereas operating at 4 kg COD/m^3·d resulted in stable AG [60]. At a high OLR of 8 kg COD/m^3·d, AG appeared on day 18 of operation; however, the AG exhibited instability and disintegrated within two weeks [60,61]. Using dairy wastewater as the substrate at a high OLR of 5.9 kg COD/m^3·d, filamentous growth and AG instability were reported [54].

With continuous exploration of the AG reactor operation at a high OLR, Zheng et al. [21] reported the formation of compact, round AG at an OLR of 6 kg COD/m^3·d using a sucrose substrate. However, the AG showed instability as they grew larger due to the growth of a filamentous microbial community, resulting in the reduction of hydrophobicity and specific gravity [21]. Large AG have reportedly formed at high OLR [13,32]. In the study, granule strength exhibited the opposite trend, decreasing with an increase in OLR [13]. A study found that the mean size of AG increased from 1.6 to 1.9 mm when the OLR was increased from a moderate OLR of 3 kg COD/m^3·d to high OLR of 9 kg COD/m^3·d [14]. Using glucose-fed substrate at OLRs of 1.5, 3, and 4.5 kg COD/m^3·d, Li et al. [62] reported that AG exhibited different morphologies, structural properties, and bacterial species at varying OLRs. Large and loose AG formed within a short period at a high OLR, whereas smaller and more tightly packed AG took longer to form at a low OLR. In terms of species diversity, the lowest species diversity was found at a higher OLR while the highest species diversity was found in the reactor with the lowest OLR [62]. With acetate as the carbon source, stable granules with good characteristics were obtained within the OLR range of 6–15 kg COD/m^3·d [63]. The OLR in this study was increased in a step-wise fashion from 6 to 15 kg COD/m^3·d. Adav et al. [64] cultivated

AG using acetate substrate at OLRs ranging from 9 to 21.3 kg COD/m^3·d. Whereas AG formation was successful at OLRs up to 19.5 kg COD/m^3·d, disintegration occurred at very high OLR of 21.3 kg COD/m^3·d. Another study reported the successful formation of AG at 3, 6, and 9 kg COD/m^3·d using acetate as the carbon source [65].

With more research, AG formation has been found to be feasible even at a much lower OLR than the range usually reported in the literature. Peyong et al. [57] reported the successful formation and maintenance of AG at OLR as low as 0.6 kg COD/m^3·d. It is expected that the granules formed at such low OLRs will exhibit long-term stability. The OLR has also been demonstrated to impact the microbial composition of AGS. The change in the microbial community will have an influence not only on the stability but also the performance of the AG reactor. The stepwise increase of the OLR was reported to lead to the loss of microbial diversity, with the selection of only a functional microbial consortium at high OLR [66]. With a mixture of centrate (reject from dewatering of anaerobically digested sludge) and acetate as substrate, low (0.9 kg COD/m^3·d, 1.9 kg COD/m^3·d), and moderate (3.7 kg COD/m^3·d) OLRs were tested [67]. At the different OLRs, the reactors were dominated by *Paracoccus*, *Thauera, Zoogloea*, and *Meganema* that exhibited functions such as extracellular polymeric substances (EPS) formation, polyhydroxyalkanoate (PHA) storage, and denitrification.

As a fast start-up strategy, AG formation was sped up by using a high OLR. Zhang et al. [58] applied 24 kg COD/m^3·d and reported AG formation within 7 h. However, the granules disintegrated within two days of operation. When the OLR was reduced to 12 kg COD/m^3·d and subsequently to 6 kg COD/m^3·d, AG were formed within 24 h and the granules maintained stability with steady state attained within 12 d [58]. In the same line of research, Liu and Tay [68] stressed the OLR to 12 kg COD/m^3·d during AG formation. Once the system attained steady state, the OLR was reduced to 6 kg COD/m^3·d and stability was achieved [68]. In this way, it was possible to quickly start up the AG reactor and enhance its successful operation.

Alternating between varying levels of OLR values has been explored as a way to enhance AG stability. Yang et al. [69] applied alternating OLR feeding to speed up AG formation. Alternating between 4.4 and 17.4 kg COD/m^3·d, they found that the stress the microbial community was subjected to allowed for the secretion of the alginate-like exopolysaccharides, which accumulated and eventually enhanced the AG structure [69]. Compared to the operation at a constant 8 kg COD/m^3·d OLR, granulation was faster with the alternating OLR approach with the increase in the amount of EPS and cell adhesiveness. For granule stability, Long et al. [70] found that AGs exhibited structural stability at OLRs $<$ 15 kg COD/m^3·d; however, OLRs $>$ 18 kg COD/m^3·d resulted in instability. This is because an increase in the size of AG results in an increase in the growth of anaerobic cores inside the granules formed by massive dead cells [70].

In general, while operation at low and moderate OLRs takes longer to form granules, it allows for stable granules. High OLRs allow for fast granulation but at the expense of AGS stability. At high OLRs ($>$ 4 kg COD/m^3·d), the rate of granulation, granule structure and morphology also depend on the substrate type. For example, energy-rich substrates such as glucose may allow for the proliferation of filamentous bacteria which result in loose morphology and fluffy surface [8]. Additionally, whereas simple carbon sources e.g. acetate select for simple and uniform microstructures, relatively complex carbon sources such as carbohydrates produce granules with layered and complex microstructures and a greater diversity of microorganisms. This implies real wastewater which typically contain a complex composition of different substrates are likely to exhibit different substrate degradation rates which would impact AG stability and the microbial community within AG [54,71].

3.2. Perspectives on the organic loading rate

The formation of AG has been demonstrated to be possible at both a very low OLR (0.6 kg COD/m^3·d) and a very high OLR (24 kg COD/m^3·d). The variable result is the time it takes for AG to form and the type of the resulting granules. AG formation at a low OLR takes several weeks while only a few hours or days are required at a high OLR. Where there is enough time for granule formation, AG formation at a low OLR would be advantageous since stable small-size granules are formed. In situations in which time is of the essence, the application of a high OLR to form granules is desired. However, the major concern is the maintenance of AG stability at a high OLR since increasing the OLR leads to an increase in granule size, which ultimately results in anaerobic conditions within the granule core due to oxygen diffusion limitations. This weakens the structural integrity of the granule, ultimately leading to granule disintegration. With compact and small granules, oxygen transfer limitation into the granule does not pose any problem. It keeps the development of anaerobic conditions within the granule core in check.

Current research efforts are geared towards enhancing AG integrity by exploring ways to control the granule size. Previous studies have explored alternating between high and low OLR to maintain granule integrity. It is suggested here to conduct research on the combination of high OLR and slow anaerobic feeding strategy to enhance granule stability. The slow anaerobic feeding strategy has been demonstrated to select for slow growing microorganisms [31,36] that control the excessive growth of heterotrophic organisms in the subsequent aeration phase within the SBR cycle. The combination of high OLR and slow anaerobic feeding strategy will allow for the hydrolysis of slowly biodegradable substrates in the feed into readily biodegradable substrates during the feeding phase. This will create the desired feast-famine conditions within the SBR cycle necessary to maintain granule integrity. A filling period of at least 1 h is suggested to allow adequate time for the feed to slowly penetrate through the settled granule bed. Even at high OLRs, such a long filling time would prove to be beneficial in allowing a small amount of the substrate into the bioreactor at a time.

In addition, the newly developed strategy of stressing the OLR (using a high OLR) to form granules and lowering the OLR once a steady state is reached [58,68] can be further exploited for AG stability. The stressed OLR will serve the purpose of obtaining granules within the shortest possible time. This will be useful for faster start-ups of AG plants. Once the purpose of granule formation has been achieved, it may be worth exploring the possibility of enhancing the treatment performance of the AG reactors since granule formation at high OLR results in the loss of diversity of the microbial species. It is suggested here to adopt the slow anaerobic feeding strategy at the reduced OLR after attaining granulation. This would allow for the development of a microbial community necessary to enhance the removal of both organic matter and nutrients. Such a feeding arrangement would permit the growth of slow growing microorganisms that are also nutrient removers [31,36]. A similar strategy has been recommended for the enhancement of treatment performance in situations where an increase in OLR is required, such as a switch from lab-scale to pilot- or full-scale or where a change in the type of wastewater to be treated is desired. A gradual increase of the OLR was earlier suggested for high treatment performance in terms of nitrogen and phosphorus removal [46]. This will maintain the microbial community within the system. In practice, while municipal wastewater treatment plants operate at low OLRs with no issues of AG instability, it is desirable to operate AG reactors at high OLRs for high-strength organic wastewaters to allow for compact reactors with a small footprint. A stepwise increase of OLR would have to be adopted during such a switch. A stepwise increase of OLR was previously demonstrated to select for a functional microbial consortium at a high OLR [66].

In terms of the wastewater type (substrate) to be treated, the impact that OLR has on AG formation and stability is substrate-specific. With glucose as the substrate, fluffy AG with filamentous growth formed while acetate-grown AG exhibited a more round and compact structure. Compact AG show high hydrophobicity, a characteristic that allows for granule stability. On the other hand, large filamentous AG formed when glucose was the substrate exhibited low hydrophobicity and specific gravity. As the OLR increased, the compact acetate-fed granules grew to a stage where problems associated with diffusion limitation probably became insurmountable. With acetate, OLR above 21 kg COD/m^3.d resulted in AG disintegration [64]. As such, before initiating granulation at pilot- or full-scale, it may be worth conducting lab-scale testing on the substrate to ascertain the threshold OLR that can allow for both AG formation and long-term stability.

Where it is practically possible, alternating between low and high OLR can also enhance AG stability. It was previously reported that alternating between a feed OLR of 4.4 and 17.4 kg COD/m^3·d resulted in the accumulation of secreted alginate-like exopolysaccharides that strengthened the AG structure [69]. This is an interesting area for further research as it may open the door to co-mingling different industrial wastewater types for effective treatment in addition to enhancing AG stability. Quite often, situations arise where effluents from different industrial processes are to be treated. The alternation between low and high OLR would allow for treatment in the same bioreactor. Additionally, where industrial effluent discharge fluctuates, dilution would allow for maintaining the same flow rate whilst allowing for the alternation between low and high OLR that enhance granule stability.

AGS stability is adversely affected at high OLRs due to the large size of the cultivated granules as a result of the increase in the growth of the anaerobic core inside the granules, formed by massive dead cells [70]. A topic for further research in this regard is the combination of a high OLR with a slow anaerobic feeding strategy. At high OLR, there is abundant food available for microorganisms; and, this allows for excessive growth of the microbes. With the unlimited food and excessive microbial growth, the granule size becomes large leading to the weakening of the granule structure. The slow anaerobic feeding, on the other hand, selects for slow-growing microbes that allow for compact granules as well as enhance treatment performance. As such, the adoption of slow anaerobic feeding at high OLR would enable the proliferation of slow-growing microbial species that would keep the granule size in check despite the abundance of food. This operating strategy would contribute to granule stability as well as increase the microbial diversity necessary for enhanced treatment performance of the bioreactor.

4. Discussion

Feeding strategy and OLR, the main operational parameters discussed in this paper, are not the only operational parameters that enhance granule stability. The other salient parameters necessary for both AG formation and stability include hydrodynamic shear force, settling time, cycle time, volumetric exchange ratio (VER) (the ratio of the volume of the effluent withdrawn to total working volume of the reactor) and solids retention time (SRT). The hydrodynamic shear force and settling time are particularly important for granule formation. These other salient parameters are briefly discussed here.

The hydrodynamic shear force, in the form of up-flow superficial air velocity, is a prime parameter for both AG formation and stability. Generally, higher shear force enhances AG formation and stability. A threshold value of 1.2 cm/s is reported in the literature as the minimum up-flow superficial air velocity required for granule formation [60,72]. High up-flow superficial air velocities allow for dense and stable AG by detaching filamentous outgrowth, and by increasing the production of exopolysaccharides, AG-specific gravity, and hydrophobicity [6]. From a cost-savings perspective, it is desirable to operate at lower up-flow superficial air velocities that allow for reduced aeration intensity, resulting in reduced energy demand. Combined with other parameters, studies have reported granule formation at 0.8 cm/s [73], 0.6 cm/s [74], 0.42 cm/s [75], and 0.41 cm/s for low-strength wastewater [76]. However, the AG formed at the lower up-flow superficial air velocities had a loose microbial structure, exhibiting instability [73,74]. Devlin et al. [76] demonstrated that at 0.41 cm/s, stable AG could only be formed using low-strength (COD of 340 mg/L) but not medium-strength (630 mg/L) or high-strength (1300 mg/L) wastewater. This demonstrates that the threshold up-flow superficial air velocity for stable AG depends on the OLR. Operating at low OLRs would require low values of up-flow superficial air velocities, implying reduced energy demand as a result of the reduced aeration intensity. Using high-strength wastewater (2000 mg COD/L), Chen et al. [73] reported successful granulation at up-flow superficial air velocities in the range of 0.8–3.2 cm/s. However, AG formed at the low values of 0.8 and 1.6 cm/s were large and had filamentous bacteria, a loose structure, and an irregular shape that resulted in instability. While formation is possible at low up-flow superficial air velocities, long-term stability is an issue at such low values. The combination of the slow anaerobic feeding strategy and low superficial upflow air velocity could prove vital in this regard. A better understanding of the self-immobilisation of microbes under these operating conditions is required to elucidate our understanding of AG formation and stability.

The settling time is an important hydraulic selection pressure on the microbial community for AG formation. It is a significant operational parameter for AG formation. Settling time determines the quantity and type of sludge to be accumulated in the reactor, which will eventually agglomerate to form granules. Long settling times will result in the accumulation of both fast settling and slow settling sludge, resulting in the formation of flocculated biomass. Short settling times select fast-settling bacteria while allowing the poorly settling flocs to wash out, thus enhancing granulation [3]. However, extremely short settling times allow the washout of even fast-settling sludge, leading to the accumulation of insufficient biomass for granulation [20]. Generally, a 5-min settling time has been indicated to be essential for granulation [77,78]. This allows for the formation of granules with outstanding settleability, which is essential for the effective functioning of biological wastewater treatment systems. For the start-up of AGS bioreactors, settling times are usually long (about 30 min) at the beginning and progressively reduced to 2–5 min as granulation proceeds.

Cycle time determines the feast-famine regimes in the reactor. Short cycle times result in short hydraulic retention times that favour rapid granulation. Such short cycle times stimulate the microbial activity and production of cell polysaccharides, and improve cell hydrophobicity. A wide range of cycle times has been reported in the literature: 1.5–24 h. While short cycle times favour AG formation, granules formed at such short cycle times exhibit instability. For long-term AG stability, a suitable feast-famine regime should be created in the reactor. Periodic starvation strongly impacts cell hydrophobicity. A long starvation period was shown to favour AG stability [16,79]. The cycle starvation period for AG stability reported in the literature is in the range of 60–80 % [80,81]. This requirement for long-term stability will impact the cycle time as slowly degradable substrates such as industrial wastewater require long cycle times to achieve the feast-famine regime required for AG stability. As such, the cycle time to be adopted depends on the type of wastewater to be treated. For instance, saline wastewater with a phenol concentration of 1000 mg/L required a 17 h cycle time for 99 % treatment efficiency [82]. Treatment of dairy wastewater for 90 % COD removal required an 8 h cycle time [54], and a 6 h cycle time was required for an AGS reactor treating brewery wastewater [83]. A 3–6 h cycle time was required for rubber wastewater [84,85]. Corsino et al. [86] reported successful AG formation using brewery wastewater at a 6 h cycle time but the granules were unstable. Stability was restored by extending the cycle time to 12 h. This extended the famine conditions that favour slow-growing bacteria [86]. Hence, for each type of wastewater to be treated, cycle test analyses would need to be conducted to determine when the substrate is completely degraded within the cycle. From there, the cycle time could be adopted.

The VER at the end of each SBR cycle acts as the selection pressure to get rid of non-granular sludge from the reactor. The granulation process can be enhanced at a higher VER. With a VER from 20 to 80 %, it was reported that faster AG formation occurred at a higher VER [87]. This is expected because a high VER would allow for more substrate to be loaded into the reactor in each cycle, allowing for the increased growth of microorganisms. Pilot-scale and full-scale SBRs have been operated at a VER of 50–75%, allowing for compact and dense AG [88–91]. The choice of the VER depends on other practical factors as well. For low-strength wastewater, it may be desirable to operate at a higher VER in order to increase the quantity of substrate in the reactor for faster granulation. Granulation using medium- or high-strength wastewater has been possible at 50 % or less VER [36,84,92,93]. Hence, the choice of the VER to be adopted depends on the wastewater type. The majority of studies (and full-scale applications) have utilised a 50 % VER. To retrofit existing reactors into AGS bioreactors, for practical reasons the VER to be adopted depends on the reactor geometry.

The SRT in AGS bioreactors is an important parameter for AG stability and treatment performance. AG formation has been possible across a wide range of SRT: 2–40 d [52,94,95], implying that it has no effect on granulation. It has been shown that long SRTs are detrimental to AG stability [96]. Granule disintegration impacts treatment performance since some microbial species are located at AG outer layers. Nitrifiers were found to be specifically located at the outer AG layers (with a mean SRT of 11 ± 3 d) with PAOs and GAOs located both on the outer and inner layers (with a mean SRT of 13 ± 4 d). The mean SRT of the nitrifiers was shorter than the mean reactor SRT (14 ± 4 days) [97], implying granule disintegration will impact the AGS bioreactor's capacity to remove nutrients. For long-term AG stability and AGS bioreactor treatment performance, the trend now is to fix the SRT through the selective withdrawal of mature granules from the bottom of the reactor [42,96,98]. This strategy allows for a good mixture of both old and newly formed AG with suitable micro-environments for the removal of organic matter and nutrients. Typical SRTs used for AG stability and enhanced AGS treatment performance lie in the range of 9–15 d.

5. Conclusion

The impact of feeding strategy and OLR on AG formation and stability has been reviewed. The feeding strategy that appears suitable for stable AG formation using various substrates is the slow anaerobic feeding strategy. Compact and stable granules are formed at low OLRs although the formation time is long. Operating at high OLRs results in fast granule formation but these granules are unstable. For the formation of stable granules with enhanced performance, it is recommended to use a strategy of stressing (high) OLR for fast granule formation followed by reducing OLR to ensure stability. This can be combined with anaerobic slow feeding to select for slow growing microorganisms and nutrients removers such as PAOs. Further research can explore granule formation at high OLR with a slow anaerobic feeding strategy to assess the long-term stability of such aerobic granules.

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.

Acknowledgement

The authors acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC) , CanadaIndustrial Research Chair (IRC) Program in Sustainable Urban Water Development (Liu, Y.) through the support of EPCOR Water Services, Canada, EPCOR Drainage Services, Canada, and Alberta Innovates, the Canada Research Chairs, Canada (CRC) in Future Water Services (Liu, Y.) and the NSERC Postdoctoral fellowship (Iorhemen, O.T.).

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