Structural Characteristics of Aerobic Granular Sludge and Factors That Influence Its Stability: A Mini Review

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Abstract: Current extensive research on aerobic granular sludge (AGS) largely focuses on improving its microbial biodiversity, settlement behavior, nitrogen and phosphorus removal efficiency, and shock load resistance. Great challenges that have to be faced are the bottleneck of slow-speed granulation and easy disintegration after granulation, which are key to the extended application of AGS technology. In the present review, the typical morphological structures of AGS are firstly summarized as well as the granulation model hypotheses, and then, we analyze the dominant microflora and their spatial distribution features. The influencing factors on particle structure stability are discussed thereafter on a macro and micro scale. Prospects and future research trends are also discussed based on the current study results for AGS technology.

Keywords: aerobic granular sludge (AGS); granular structure; zoogloea; filamentous bacteria; stability

1. Introduction

Aerobic granular sludge (AGS) can form granular biopolymers by microbial self-aggregation under suitable conditions. Compared with conventional activated sludge (CAS), due to the advantages of good settlement [1,2], better resistance to shock loads [3], low occupied area [4,5] and rich in microorganisms [6], the potential applications of AGS technology are promising [1,7]. Since Mishillla et al. [8] first discovered AGS, the technology has gone through nearly 30 years of development and practice. Currently, scholars at home and abroad have conducted much research on the physicochemical properties, decontamination characteristics, and granulation mechanism of AGS, and these results have further broadened its application range [9,10]. However, the further development of AGS technology is hindered with some serious problems that should be answered, such as how to shorten the granulation time of AGS, how to maintain its structural and functional stability in practical applications to avoid problems of particle disintegration, and problems related to sludge emersion and degradation of pollutant removal performance [11]. When the instability of AGS occurs, the sludge morphology will be degraded from dense particles to loose flocs, resulting in a significant impact on the biological activity and settling performance of sludge, which directly affects the effluent quality. Clearly, there are challenges in the instability of AGS particle structure [11–13]. Although the segregation of AGS might not be stopped, there may be an approach to deal with these problems to prolong the lifetime of the granules with stable structure in the future. Consequently, uncovering the granulation process and mechanism is of great importance requiring further study and exploration.

Up to the present, however, few specific reviews are available with respect to the maintenance strategies of the AGS structure, especially on the structural characteristics of AGS and factors that influence its stability. There is a published review that considers the granulation strategies of stable granules including changing the substrate, selective
sludge discharge, and manipulating the operational conditions [14]; however, there is no comprehensive review available on granule stability from a structural view point. A comprehensive review of the structural characteristics and stability of AGS will help us understand the connection between the morphological characteristics and influencing factors of the structural stability and find a common conclusion, which will speed up the process of the industrial large-scale application of AGS. This mini review firstly describes some typical morphological structures of AGS based on the granulation model hypotheses. Then, the dominant microflora and their spatial distribution features are analyzed to find common dominant phyla between structural stability and overall performance of different types of AGS. To further our knowledge, the main influencing factors for particle structural stability are mainly discussed on the macro and micro scale, with the aim of providing reference and suggestions for the granulation, application, and operation management related to the structural stability of AGS in the future. Last but not least, future research prospects and trends are also proposed to promote future research and its industrial application.

2. Morphology Classification of AGS

The formation of AGS is a complex process involving physics, chemistry, and biology. Scholars have explored the granulation process from different perspectives and put forward the corresponding granulation model hypothesis, but unfortunately no uncontroversial conclusion has been reached [1]. Among these, the “extracellular polymeric substances (EPS) hypothesis” [15], the “metal ion hypothesis” [16], and the “signaling molecule hypothesis” [2] all emphasize that microorganisms can secrete viscous EPS, supplemented by metal ion chelation, to form “zoogloea” type AGS, which are granulated based on floc-forming bacteria. The “Filamentous bacteria hypothesis” [2,15] proposes that filamentous bacteria, which hardly secrete EPS, can form “filamentous”-type AGS with a skeleton by wrapping each other through special filamentous shapes. The “crystal nucleus hypothesis” and “self-condensation hypothesis” [17] suggest that filamentous and floc-forming bacteria can form “integrated”-type AGS with their combined action in a suitable reaction system with appropriate environmental conditions.

2.1. Zoogloea Type AGS

The zoogloea-type AGS can adsorb free microbes, organic matter, and metal ions in water to form zoogloeal using the EPS secreted by microorganisms as “adhesive substance”. Then, the floc-forming bacteria continuously integrate together and develop under the action of external forces, and a surface regular and dense AGS (Figure 1) can be finally formed [18]. A zoogloeleal, which is formed from the adsorption of microbial cells by EPS, can aggregate, oxidize, and decompose macromolecular organic compounds in water, and then it can provide nutrients and living environment for the growth of bacteria, protozoa, and other microorganisms [15], thus forming a high-density microbial symbiotic system. In this process, the coagulation of microbes in the symbiotic system is regulated by the EPS content. Wang et al. [19] found that there was a directly proportional relationship between the self-aggregation of bacterial strain (Enterobacter sp. strain FL) extracted from activated sludge and its secreted EPS content. Meanwhile, Liu et al. [20] found that when adopting some measures such as changing the hydraulic conditions and adding poly aluminum chloride (PAC), microbial cells can be stimulated to secrete more EPS, thereby enhancing the coagulation of microorganisms and accelerating the formation of AGS.
2.2. Filamentous Type AGS

The AGS formed from filamentous bacteria does not require the participation of EPS and floc-forming bacteria at the initial stage of granulation. It mainly relies on the steric hindrance and mechanical action of filamentous bacteria to form a porous network structure with large specific surface area, which can capture free microorganisms and organic matter in water to form a dense AGS (Figure 2) [15,22]. It is well known that activated sludge bulking is mainly caused by the excessive reproduction of filamentous bacteria. However, a study has shown that far from sludge bulking with the presence of filamentous bacteria, the biological compactness and sludge settling performance may be improved, and even filamentous-type AGS can be cultivated in turn [23]. Du et al. [24] found that promoting the growth of filamentous bacteria can significantly shorten the granulation time of filamentous bacteria AGS. Li et al. [22] found that the structure morphology (divergence or density) and stability of filamentous-type AGS were significantly affected by the hyphae species and winding pattern of filamentous bacteria, and a single strain with irregular growth was more likely to form AGS with dense particles, a stable structure, and good physicochemical properties. Therefore, the cultivation of filamentous-type AGS can provide new ideas for exploring rapid granulation conditions and solving the problem of sludge bulking.

![Figure 1. SEM images of the outer surface and interior of zoogloea type AGS. Adapted from reference [21] (copyright reserved Elsevier, 2018).](image)

2.3. Integrated Type AGS

There are two dramatically different academic views on the granulation process of integrated-type AGS. Some scholars believe that the integrated-type AGS is initially formed with EPS as the skeleton and supplemented by filamentous bacteria (Figure 3a). Chen et al. [25] found that mature AGS was formed through the penetration of filamentous bacteria with protein (PN) as the core and polysaccharide (PS) as the skeleton. As a hydrophobic group in EPS, PN plays a determinantal effect on the granulation feature and structural morphology of AGS [26]. While the PS in EPS, especially the β-polysaccharide with certain mechanical strength and high adhesion properties, can be used as the skeleton of AGS to form a network structure that directly adheres to free filamentous bacteria and other microorganisms [27,28], which also determines the structural stability of the particles. On the other hand, some scholars state that the integrated-type AGS is initially formed

![Figure 2. SEM images of the outer surface and interior of filamentous-type AGS. Adapted from reference [22] (copyright reserved Elsevier, 2010).](image)
with filamentous bacteria as the skeleton (Figure 3b). Shao et al. [29] effectively promoted the formation of AGS by prolonging the influent time and reducing the concentration of acetic acid, and it was found that a large number of filamentous bacteria became granular skeletons in the early stage of AGS formation, and within which EPS was intertwined as an adhesive substance. Chen et al. [30] also found that filamentous bacteria can become the core of granular sludge in the initial formation stage, followed by the zoogloea attaching to the filamentous skeleton, and then, mature AGS could be formed under fluctuations of salinity.

Figure 3. Schematic diagram of granulation process of integrated-type AGS with skeleton of (a) EPS and (b) filamentous bacteria.

3. Microbial Characteristics of AGS

3.1. Dominant Bacterial Group

Although during the granulation process of flocculent activated sludge, the selective tress can cause a significant reduction in the microbial biomass of sludge granulation, which results in a decrease in microbial diversity and richness of AGS [31,32], some microorganisms that are beneficial to the structural stability and pollutant removal of AGS are instead retained and enriched [31]. Therefore, a mature AGS not only has considerable biological activity and decontamination performance comparable with inoculated sludge, but also maintains the stability of its own structure [33]. Studies have confirmed that the niche of AGS is significantly affected by substrate composition, thus resulting in bacterial communities with large individual differences [34], additionally resulting in the distinct structural morphology of AGS. It can be safely concluded that AGS cultured with glucose as a carbon source is more conducive to the enrichment of glycogen-accumulating organisms (GAOs); on the contrary, the survival of phosphorus-accumulating organisms (PAOs) can be hindered, thus reducing the phosphorus removal ability of AGS. He et al. [35] found that PAOs could become the dominant strain of AGS when sodium succinate and sodium acetate were used as mixed carbon sources, which effectively inhibited the enrichment of GAOs and then improved the structural stability and decontamination performance of AGS.

The microbial population of different type AGS has certain specificity; however, AGS formed in different growth environments may still have a similar dominant bacterial group and play a decisive role in sludge granulation and its pollutant removal performance [6,36]. The dominant phyla of AGS include phylum Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Acidobacteria, Verrucomicrobia, Chloroflexi, and Saccharibacteria [6,33,37]. Xu et al. [38] found that Proteobacteria and Bacteroidetes were the dominant phyla of AGS formed in a continuous-flow reactor by the addition of dewatered sludge; among them, the phylum Proteobacteria was reported with high adhesion force by secreting much EPS and played a predominant role in the pollutant removal process, while the phylum Actinobacteria was related to sludge filamentation bulking. Xia et al. [33] also discovered that the dominant phyla of AGS cultured in a pilot-scale SBR reactor were phylum Bacteroidetes and
Proteobacteria, and the abundance of Proteobacteria was the highest. Whereas at the class level, the abundance of β-Proteobacteria with nitrogen and phosphorus removal functions accounted for the highest percentage, which allowed AGS to show good nitrogen and phosphorus removal performance. Generally, it can be found that similar dominant bacterial groups across various types of AGS are controlled by substrate composition, where Proteobacteria is the predominant phylum in the AGS, followed by Bacteroidetes in zoogloea-type AGS, Actinobacteria in filamentous-type AGS, and Bacteroidetes and Actinobacteria in integrated-type AGS. Meanwhile, these dominant bacterial groups play a decisive role in the granular structure and pollutant removal performance.

3.2. Spatial Distribution Characteristics of Microflora

A mature and stable AGS has clear delamination interfaces in space and appreciable microflora stratification. Specifically, rod-shaped bacteria in integrated-type AGS are tightly packed on the surface of particles, while filamentous bacteria play a skeleton role throughout the particles [6]. However, there is a proportion of cases (such as adding special substrates, changing hydraulic shear conditions, etc.) in which filamentous bacteria will also be entangled on the surface of the particles, and the bacterial cells, zoogloea, and organic matter will be connected and aggregated by adsorption bridging [15]. The mass transfer and diffusion of dissolved oxygen (DO) and substrate in AGS will be limited with the increase in particle size and the blocking of matrix inside the particle, and thus the aerobic zone, anoxic zone, and anaerobic zone will be formed spatially in particles from outside to inside. Meanwhile, the heterogeneity of the spatial mass transfer of AGS further led to the stratification of microflora. Sun et al. [36] divided the spatial structure of AGS into the outer sphere dominated by aerobic and facultative microbes and the inner core dominated by anaerobic microbes (Figure 4). Among them, the outer sphere and the inner core had a similar Shannon index (p > 0.05), while the Simpson index was significantly different (p < 0.05). This result showed that microbial communities could be distributed in the outer sphere and inner core of AGS at the same time, but the heterogeneity of the spatial mass transfer caused differences in microflora richness. In parallel, the oxygen environment partition structure formed in AGS causes it to selectively enrich the microflora conducive to particle structural stability and pollutant removal, thereby improving the pollutant removal performance of AGS. However, in the reaction system with alternating DO concentrations, PAOs and GAOs are enriched simultaneously in mature AGS, making it a dominant flora and accounting for 74% of the total bacterial population [39]. Under anaerobic conditions, the removal of exogenous COD by PAOs and GAOs can effectively prevent the excessive proliferation of filamentous bacteria and maintain the stability of the granular structure. However, the competition of GAOs for carbon sources will affect the phosphorus removal capacity of PAOs. Accordingly, how to inhibit the biological activity of GAOs and improve the phosphorus removal performance of AGS has been a currently focus of research.

![Figure 4. Division of DO concentration of AGS.](image-url)
4. Macroscale Influencing Factors on AGS Structural Stability

4.1. Reactor

4.1.1. Operating Mode and Aspect Ratio (H/D)

From the existing literature, an SBR reactor is usually used for activated sludge granulation, primarily due to its fill-and-draw running and aerobic starvation stage. Firstly, as a filling-and-draw reactor, the settling time of SBR is short, which is conducive to the granulation of AGS [40]. On the other hand, there are two independent stages of sufficient substrate activity and substrate starvation in SBR, in which aerobic starvation can account for more than 75% of the total cycle [11]. Microorganisms consume massive amounts of EPS during aerobic starvation, reducing the surface-negative charge of the sludge and enhancing its hydrophobicity, which can induce sludge to form dense and stable AGS [41]. Santo et al. [42] cultured aerobic granular sludge in different cycles and found that longer aerobic starvation was more conducive to the stability of particles, further indicating the importance of an aerobic starvation period on the granulation and structural stability of AGS. It is conventionally assumed that a high aspect ratio (the H/D ratio of 15–30) is conducive to promoting the ripening of AGS with a dense surface [34]. However, Hamiruddin et al. [43] found that AGS with a dense structure can be formed in an SBR reactor even under the condition of low H/D. These studies have highlighted that the H/D ratio of an SBR reactor is not a necessary condition for microbial aggregation and particle structure maintenance when the operational conditions are appropriate.

4.1.2. Hydraulic Shear Stress

In order to cope with the shear force caused by water flow, AGS can regulate the biological process inside the particles by changing the composition and concentration of EPS to maintain relative stability with the external environment [44]. Hydraulic shear stress is generally characterized by apparent gas velocity, and studies have confirmed that too low hydraulic shear stress will lead to excessive reproduction of filamentous bacteria, and only when the apparent gas velocity is greater than 1.2 cm/s can AGS be formed [45]. At the same time, higher hydraulic shear stress can also stimulate cells to secrete more EPS and promote the formation of sludge particles with dense structure and intact appearance. Under the action of hydraulic shear stress, the hydrophobicity of granular surface gradually increases, which further improves the structural stability of AGS. However, too high hydraulic shear stress not only carries high operation costs, but also increases particle abrasiveness, resulting in the destruction of particle mechanical strength [46]. Additionally, the effect of hydraulic shear stress on sludge granulation and particle structural stability also depends on the substrate concentration, such as in the study by Devlin et al. [47] where they found that wastewater with low concentrations of COD (<300 mg/L) formed stable granular sludge even at a low superficial gas velocity of 0.41 m/s, but when the COD concentration increased to 600 mg/L and above, the same hydraulic shear stress was unable to form AGS.

4.1.3. Organic Loading Rate (OLR)

In general, AGS can withstand an OLR of 2.5–15 kgCOD/(m$^3$·d) [48]. An OLR that is too low or fluctuates greatly in short time can lead to the proliferation of filamentous bacteria in the granules, which makes the granular structure loose and porous, and thus results in poor stability of the AGS structure and affects the performance of the system. Although AGS can be formed under high OLR, the granular structure is not stable, and particle disintegration is prone to occur after long-term operation. For example, Liu et al. [49] found that when AGS was cultured under an OLR of 12 kgCOD/(m$^3$·d), AGS could be formed after 3 days, but the sludge particles gradually expanded and disintegrated after 2 weeks. When the OLR was reduced to 6 kgCOD/(m$^3$·d), the rapid formation of AGS could maintain particle structural stability for a long time. Meanwhile, studies have shown that only when OLR is lower than 9 kgCOD/(m$^3$·d) can the structural stability of AGS be sustained in the long term [50]. This is because under low load conditions, functional
bacteria that can secrete EPS are easily enriched in sludge, especially the accumulation of tight bound extracellular polymers (TB-EPS), which can significantly regulate the structural stability of AGS [51]. Furthermore, dynamic changes in OLR are also helpful for the granulation of sludge and the maintenance of the particles’ structural stability. This finding is reinforced by other studies, such as that of Zhang et al. [50], who found that when a progressive decline of OLR from 5.5 to 3.5 kgCOD/(m$^3$·d) occurred, the granulation ratio and particle structural stability were significantly improved.

4.1.4. Other Factors

Sludge retention time (SRT) is not a necessary factor affecting the aggregation and granulation of microorganisms, but it is a key parameter in the maintenance of the stability of the granular structure [52]. Liang et al. [53] found that too long an SRT will lead to aging sludge that cannot be discharged from the reactor, thus resulting in the disintegration of AGS. Therefore, the selection of appropriate SRT to control sludge volume is conducive to the structural stability of AGS. The concentration of DO in the reactor not only affects microbial metabolism, but also affects granular structure and stability [54]. Too low a DO concentration will result in gas generated through anaerobic metabolism inside the particles, coupled with the proliferation of filamentous bacteria under low DO conditions, resulting in easy internal disintegration of AGS, whereas long-term aeration causes the DO to remain at a high level accompanied by excessive hydraulic shear force, and it has been confirmed that granules are also easily broken up under this condition [4]. Research gas demonstrated that only when the DO concentration is controlled at 50% of its saturated concentration can the stability of AGS structure be maintained [52].

4.2. Reaction System

4.2.1. Substrate Conditions

Different substrate conditions (such as carbon source, metal ions, etc.) have great influence on the structural stability of AGS. It was found that when glucose was used as the sole carbon source, heterotrophic microorganisms would become the dominant bacteria, the rapid proliferation rate of which could significantly increase the sludge biomass, but this would also lead to excessive growth of filamentous bacteria causing structural instability of AGS; whereas, when acetate and succinate were used as mixed carbon sources, the cultured AGS was dominated by rod-shaped bacteria with higher structural stability and denitrification ability [13,35]. Metal cations and surface-negative charges of sludge can form a stable three-dimensional structure by binding or bridging, accelerating the granulation process of sludge and maintaining its stable structure [55]. Sajjad et al. [56] found that Ca$^{2+}$ was easily combined with OH$^-$ in PS to form sludge particles with large viscosity, while Mg$^{2+}$ had a strong complex with amide groups in PN, which facilitated the formation of hydrophobic AGS. Yang et al. [57] found that divalent metal ions such as Ca$^{2+}$ and Fe$^{2+}$ are conducive to sludge granulation. In addition, some metal ions can also stimulate microbes to secrete more EPS, which in turn improve the adhesiveness within the particles, and thus effectively avoid the loss of biomass and the disintegration of particle structure [58].

4.2.2. C/N Ratio

The carbon source is the electron donor of energy required for microbial metabolism, and the nitrogen source is the main component of proteins in microorganisms, which means that the C/N ratio is an important factor affecting microbial growth. Some studies have confirmed that C/N has a significant impact on the structural morphology of sludge granulation. For example, Zhao et al. [59] cultivated AGS under the conditions of influent C/N of 100:5 to 100:50. It was found that with a C/N of 100:10, AGS had dense structure, uniform particle size, good sedimentation performance, and good simultaneous nitrification and denitrification performance; AGS with small C/N ratio had a large particle size, loose structure, and poor settlement performance; while AGS formed under a higher C/N ratio had dense structure, and the small particle size accounted for a large proportion and
was not conducive to sludge settlement. Similarly, Zhang et al. [60] found that when the C/N ratios were 5 and 15, the C/N ratio of AGS with the best structural stability was 5. Although too low or too high a C/N ratio is unfavorable for the formation of structurally stable AGS, mature AGS can still show good structural stability and denitrification ability in the treatment of high-concentration ammonia nitrogen wastewater with a low C/N ratio (less than 1) [61]. This is because of the unique layered structure of AGS, which can ensure the simultaneous nitrification and denitrification process within the granular sludge under the external high DO environment, resulting in an effective decrease in the demand for carbon sources. This feature of AGS characterized by a layered structure has broad application prospects in treating wastewater with low C/N ratios.

4.2.3. Inoculated Sludge

The inoculated sludge concentration had little effect on the cultivation of AGS, and the appropriate MLSS concentration was 1–20 g/L, and the SVI value was 7–220 mL/g [43]. However, the characteristics of inoculated sludge have a significant impact on the process of microbial coagulation during sludge granulation. Song et al. [62] found that the sludge from beer wastewater treatment plant is more suitable as seeding sludge than municipal sewage sludge. This is because the sludge contains some bacteria with strong EPS secretion ability and high cohesion, which is more likely to form stable AGS. For example, the self-aggregation indexes of *Klebsiella pneumoniae* strain B and *Pseudomonas veronii* strain F are 65% and 51%, respectively. A large amount of AGS with a complete structure and dense surface can be formed after eight days of inoculation, and the structural stability of AGS is higher than that of other inoculated sludges [63]. In addition, inoculation with granules or debris particles can effectively shorten the granulation time of flocculent sludge. Lei et al. [64] cultivated mature saline AGS within 52 days by inoculating anaerobic granular sludge, and successfully transformed the inoculated anaerobic granular sludge into aerobic granules while maintaining the integrity of the granular structure. Wang et al. [65] found that adding stored granular sludge can significantly shorten the granulation time of sludge in comparison to directly inoculating flocculent sludge, and AGS with good settlement performance and stable structure can be formed after 22 days. By adding complete granular sludge, Zou et al. [66] not only effectively inhibited sludge bulking, but also obtained stable AGS.

4.2.4. pH

The pH is an important indicator of the microbial growth process, which not only affects the physicochemical properties of sludge, but also regulates the metabolism of bacteria and changes the composition and content of EPS, resulting in fluctuations in the structural stability and pollutant removal performance of sludge particles [67,68]. Jiang et al. [68] found that AGS can maintain good structural stability and sedimentation performance in weak acid (pH = 5.5) and neutral environments, while alkaline environments can cause an irreversible negative impact in a short time. Similarly, too low a pH (pH = 3.0) can cause EPS hydrolysis, resulting in instability and disintegration of the AGS [11]. Therefore, alkaline and extremely acidic environments should be avoided, and neutral environments (pH = 7–7.5) should be adopted to maintain the structural stability of AGS [4].

4.2.5. Temperature

Temperature is one of the basic regulatory parameters affecting microbial activity, and the suitable temperature for most microorganisms to survive is 20–25 °C. Too low a temperature (T < 10 °C) will lead to the excessive growth of filamentous bacteria in AGS, resulting in particle disintegration and biomass loss, while high temperatures (T > 30 °C) can cause protein denaturation and even enzyme inactivation, resulting in the instability of the whole system [69]. In order to further broaden the possibility of engineering application of AGS in extremely cold or tropical regions, some scholars have successfully domesticated AGS adapted to cold conditions (T < 7 °C) with good decontamination performance and
structural stability [70]. Ab Halim et al. [71] obtained AGS with good sedimentation performance under high temperature conditions (30–50 °C) by gradually increasing the reaction temperature; unfortunately, the decontamination ability of AGS was inhibited due to the influence of high temperature.

5. Microscale Influencing Factors on AGS Structural Stability

5.1. Microorganisms

5.1.1. Microbial Community Structure

Microbial community structure and biodiversity have significant effects on the decontamination performance and structural stability of AGS. As an influencing factor that is difficult to regulate, microbial community structure is regulated by substrate composition, temperature, pH, AGS granulation stage, and spatial location, which makes AGS show significant differences [14]. Appropriate amounts of filamentous bacteria can be used as a particle skeleton to trap free substances and promote the formation and stability of AGS, but excessive proliferation of filamentous bacteria will destroy its structural stability, resulting in loose sludge structure and flocculent [72]. If PAOs become the dominant bacteria in sludge granules, they can cause phosphorus precipitation in AGS. In such a case, $\text{PO}_4^{3-}-\text{P}$ is precipitated in the form of calcium phosphate or magnesium phosphate inside the granules, which hinders the bridging effect of metal cations and EPS and destroys the structural stability of granules [5]. In addition, some proteases secreted by microorganisms can also decompose PN in EPS and reduce particle hydrophobicity. The polypeptides, amino acids, organic acids, and other substances produced by PN decomposition also hinder the formation of rigid bridge structure between EPS and polyvalent cations [5], which makes sludge particles prone to disintegration and even structural instability.

5.1.2. Microbial Quorum Sensing Effect

In recent years, the microbial quorum sensing (QS) caused by signal molecules has become a research hotspot with respect to the effects of AGS granulation and structural stability. In an AGS system, QS can promote bacteria to secrete EPS by secreting, accumulating, and sensing signal molecules, improve the surface adsorption performance and overall adhesion ability of particles in turn, and thus accelerate the granulation speed of AGS and enhance its structural stability [73–75]. Acyl-homoserine lactones (AHLs) signal molecules are secreted and synthesized by Gram-negative bacteria, which can spread extracellularly, and when the concentration reaches a certain threshold, AHLs can enter the intracellular area again for reaction and information transmission [41,74]. Zhang et al. [74] found that the QS effect was significantly enhanced in the granulation process of AGS, and the two AHLs signaling molecules (C8-HSL and 3OHC8-HSL) showed statistically significant positive correlations with the contents of tryptophan and protein-like substances in EPS. The results showed that AHL signaling molecules could promote EPS secretion and maintain the adhesion and stability of particles (Figure 5) [75,76]. The signal directly received by cell surface receptors is called the first messenger, and the signal transmitted to the intracellular after conversion is the second messenger. The second messenger (HSL-like substances) can activate the activity of enzymes and non-enzyme proteins and plays an important role in QS [74]. Cyclic diguanylic acid (c-di-GMP) is a second messenger ubiquitous in bacteria, which can promote the secretion of EPS, especially conducive to the synthesis of PN and PS and microbial aggregation [77]. It can also transform single, free microorganisms into multi-cell adhesive microorganisms, which plays a positive role in promoting AGS granulation and maintaining its structural stability.
5.2. Physicochemical Properties of Sludge

5.2.1. PN/PS

At present, there are some divergences regarding the effects of PN and PS on the granulation and structural stability of AGS. Some scholars believe that PS can fix microorganisms and organic matter by improving the internal adhesion of particles to prevent the loss of biomass, thereby improving the structural rigidity of AGS [34]. Therefore, a lower PN/PS value (PN/PS ≈ 1) is more conducive to the stable structure of AGS [54]. He et al. [78] found that the content of PS increased significantly with the increase in salt content in the substrate, indicating that PS could regulate the structural stability of AGS to adapt to salinity stress. At the same time, the decrease in PN/PS also significantly improves the cell surface hydrophobicity and particle stability [79]. Other scholars believe that PN can improve the hydrophobicity of sludge and improve the structural stability of particles by changing the surface charge of cells [19]. Wang et al. [80] found that during the granulation process of AGS, a large amount of PN was secreted to regulate its structural stability to adapt to the large fluctuation of influent water quality, so the PN/PS value increased from 4.27 to 7.54. Meanwhile, the PN/PS value also showed an upward trend during the granulation and ripening of AGS because PN was more conducive to the stable structure of AGS than PS, and the larger the PN/PS value was, the more conducive to the granulation of AGS [81].

5.2.2. Particle Size

An excessive particle size of AGS hinders DO and nutrient mass transfer inside the particles, resulting in excessive growth of filamentous bacteria due to insufficient DO, reducing sludge settling performance, and even damaging the structural stability of the particles. At the same time, the larger anaerobic region will also lead to the decrease in AGS biological activity resulting in particle instability [21]. Smaller particles usually have poor sedimentation performance and smaller biomass [49]. Therefore, it is necessary to control the AGS particle size within a suitable range. Long et al. [82] found that the optimum particle size range of AGS was 2–3 mm, and the particle size increased slowly in this range, which was beneficial to the maintenance of particle structure. Even after ultrasonic crushing, the broken particles could be re-granulated in a short time.

6. Conclusions and Future Perspectives

In this mini review, some research progress in improving the structural stability of AGS during/after granulation in recent years has been summarized. The morphological structures of AGS can be mainly grouped into three categories, namely, zoogloea-, filamentous-, and integrated-type AGS, corresponding to the different formation meth-
ods. The morphology classification of AGS can be controlled by substrate composition; however, the same layer-by-layer structure and similar dominant bacterial groups across various types of AGS, where Proteobacteria, Bacteroidetes, and Actinobacteria are the dominant phyla, play a decisive role in granular structure and pollutants removal performance. Finally, it has been confirmed that some manipulation strategies for external environmental conditions can be used to enhance the structural stability of AGS, including suitable hydraulic shear stress, appropriate OLR and SRT, changing the substrate composition and contents, selective inoculated sludge, and controlling the growth environment for microbes, which, in turn, can also affect microbial community structure and regulate the QS effect at the micro scale, resulting in changing physicochemical properties of sludge. However, it should be pointed out that there is no consensus on the recommendations for rapid granulation of AGS with a stable structure nor on the maintenance of the long-term stability of its structure in practical application. As most previous works have focused on granular stability through some enhancement method(s), the exact mechanisms involved and the scaling up of rapid AGS formation and long-term structural stability maintenance strategies still require further investigation in order to avoid practical application problems, such as particle disintegration, sludge floating, and degradation of overall performance. Comparing and classifying improvement strategies for the three types AGS regarding granulation and granule stability can help obtain AGS with improved structural stability and promote the practical large-scale application of AGS technology. Finally, regulating the QS behavior of flora can provide new regulatory strategies for the structural stability of AGS.

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