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Advances in aerobic granular sludge stabilization in wastewater

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ABSTRACT

This study provides a comprehensive overview of the advancements made in the research concerning the stability of aerobic granular sludge, and succinctly outlines the development mechanisms of aerobic granular sludge, encompassing the "filamentous bacteria theory" and the "extracellular polymer theory", "selective pressure driven hypothesis", and "self coagulation hypothesis"; The primary elements influencing the stability of aerobic granular sludge include temperature, pH levels, the rate of organic loading, and concentrations of ammonia nitrogen, dissolved oxygen and particle size, satiety hunger period, hydraulic shear force, sludge age and toxic and harmful substances; It is discussed in detail that measures such as inhibiting filamentous bulking, promoting the secretion of extracellular polymers, enhancing the growth of slow-growing microorganisms and fortifying the central structure of granules can improve the stability of aerobic granular sludge. It is suggested that future studies should prioritize exploring the formation mechanisms and functional microbial communities associated with aerobic granular sludge. Such investigations will lay the groundwork for its broader industrial applications.

1. Introduction

Aerobic granular sludge has a relatively strong microstructure, excellent sedimentation performance, higher concentration of sludge retention, diverse microbial populations and other characteristics, so that it has a better separation of mud and water, a higher unit volume of bioreactor capacity, can withstand a high concentration of shock loads, simultaneous elimination of organic compounds and nutrients, along with elevated levels of toxic contaminants in wastewater, such as the unique ability to adapt to and deal with.

Aerobic granular sludge can be successfully cultivated in SBR, CSTR and other reactors, additionally, the integration of aerobic granular sludge with alternative reactors, such as the membrane bioreactor (referred to as AGMBR), has been documented in literature [1,2]. However, the utilization of this technology for the remediation of diverse wastewater types remains largely experimental at this juncture., and there are few reports on its actual application. During the long-term SBR test, it was found [3,4] that if the reaction conditions could not be properly controlled, the aerobic granular sludge would lose its stability frequently, which also became the biggest obstacle to its large-scale application in practical projects. The prevailing consensus among

researchers is that the destabilization and potential disintegration of particles primarily stem from particle breakdown and the overgrowth of filamentous bacteria [5,6]. In addition, through the research on the changes of extracellular polymer (EPS) of aerobic granular sludge, it is found that the disintegration of particles is also closely related to the changes of electronegativity and hydrophobicity of its surface [7]. EPS can not only stabilize the particle structure, but also protect the barrier. Consequently, the presence of EPS plays a crucial role in maintaining the stability of aerobic granular sludge [8,9].

2. Development and characterization of aerobic granular sludge

2.1. Development of aerobic granular sludge

The formation of aerobic granular sludge is affected by various factors, including the origin of the seed sludge and the composition of the substrate, organic load, water intake, reactor shape, sludge settling time, aeration, and water shear. The precise management of key influencing variables is crucial for the successful development of aerobic granular sludge.

The source of seed sludge, substrate composition and SBR control conditions (such as pH, temperature, cycle, etc.) affect the granulation of

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granular sludge. In numerous research endeavors, aerobic granular sludge formation has been observed to originate from activated sludge systems. The composition of the microbial community within activated sludge plays a crucial role in the development of aerobic granular sludge. This is attributed to the fact that hydrophilic microorganisms present in wastewater tend to attach less readily to sludge flocs compared to their hydrophobic counterparts [9]. In the seed sludge, the more hydrophobic microorganisms are, the easier aerobic granular sludge is to form and the better sedimentation is. The substrates employed for the cultivation of aerobic granular sludge encompass a range of components, including but not limited to glucose, acetate, phenol, starch, ethanol, sucrose, and various synthetic wastewater constituents. Additionally, positively charged divalent and trivalent ions, such as Ca^{2+} , Mg^{2+} , Fe^{2+} , and Fe^{3+} , have the capability to interact with negatively charged bacteria, facilitating the formation of microbial aggregates or nuclei. A study conducted by Jiang et al. also highlighted these aspects. [10] showed that the addition of positively charged ions can accelerate the formation of aerobic granular sludge. When $100\text{mgCa}^{2+}/\text{L}$ is added to the seed sludge, the granular sludge is formed in 16d; However, without adding Ca^{2+} , granular sludge will be formed in 32d. In the SBR process, aerobic granular sludge was successfully cultivated. One SBR cycle includes four parts: water inflow, aeration, sedimentation and water outflow. In the research conducted by Liu and Tay [11], it was demonstrated that an increase in the operational cycle from 1.5 h to 8 h led to a decline in the specific microbial growth rate of the granular sludge from 0.266 d^{-1} to 0.031 d^{-1} . Concurrently, the microbial yield coefficient also decreased from 0.316 gVSS/g COD to 0.063 gVSS/g COD . Zheng et al. [12] found that when the organic load was $6.0\text{ kgCOD/m}^3\text{d}$, the aerobic granular sludge structure was dense, but it would gradually lose stability due to the growth of filamentous bacteria. In the aeration stage, the larger shear force not only makes the aerobic granular sludge structure more dense. Additionally, it ensures adequate dissolved oxygen levels, which in turn minimizes the proliferation of filamentous bacteria and sustains the stability of the granular sludge.

Firstly, individuals gather to form populations; then, communities are formed on the basis of populations; then, microbial ecosystems are formed from the communities; finally, the ecosystems gradually evolve and reach relative stability through the interaction between the communities and the environment. During the formation, evolution, and maturation stages of aerobic granular sludge, the aggregation of cells and the progression from embryonic granular sludge to primary granular sludge are observed and relative stabilization of structure and function were experienced. By analyzing the literature, aerobic granular sludge formation can be divided into the following stages.

2.1.1. Phase I

Effective collisions occur between individual microorganisms or between microorganisms and inert particles. Hydrodynamics and Brownian motion are important factors that trigger effective collisions. Effective collisions form large particles, this could potentially form the central core within the sludge particles, serving as a foundation for the development of granular sludge, as illustrated in Fig. 1.

2.1.2. Phase II

Microorganisms aggregated by collision remain in stable contact, forming embryonic particulate sludge. Factors that affect the stable contact include van der Waals force, positive and negative charge

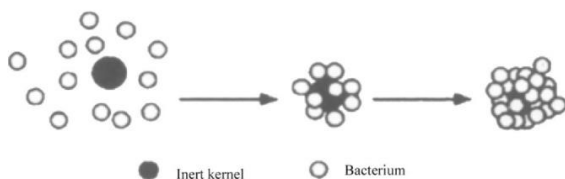


Fig. 1. The role of the inert kernel.

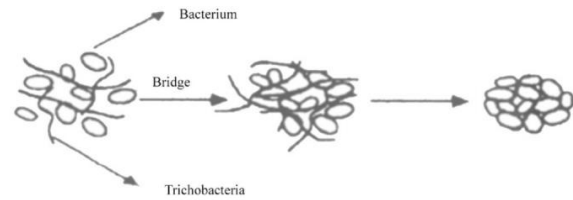


Fig. 2. Bridging action of filamentous bacteria.

attraction, surface tension, surface hydrophobicity, filamentous bacterial bridging, hydrogen bonding, during the initial stages of granular sludge formation, processes such as cell surface dehydration and cell membrane fusion are critical. Particularly, the hydrophobic nature of the bacterial surface is essential for ensuring stable interactions among microorganisms. In addition, the bridging and stabilizing effects of filamentous bacteria also contributed to the maintenance of stable contact. The filamentous bacteria intertwined with each other and formed a mesh, which played a similar role as the skeleton of steel bars in reinforced concrete in the embryonic granular sludge (Fig. 2).

2.1.3. Phase III

Microbes in embryonic granular sludge continue to grow, proliferate and aggregate, and gradually form primary granular sludge. The processes predominantly facilitating the formation of primary granular sludge by microorganisms involve the production of extracellular polymers (ECPs), cell community growth, environmental conditions and changes in biological characteristics caused by gene mutation. Extracellular polysaccharide is an extracellular polymer, which can be an important medium for cell aggregation and adhesion. Extracellular polysaccharide synthesis and metabolism are crucial for both the initiation and stability of granular sludge. Any inhibition in these processes can adversely impact the microbial aggregation within the sludge structure.

2.1.4. Phase IV

Subjected to hydraulic shear forces, the primary granular sludge developed a complex three-dimensional architecture. Hydraulic shear effect on the newborn granular sludge has shaping effect, make the granular sludge denser, the bacterial spacing is further reduced, the structure is more compact.

2.1.5. Phase V

The microbial ecosystem gradually evolves and stabilizes, and the aerobic granular sludge matures. Although the granular sludge formed through the above four stages has only a three-dimensional structure, its ecosystem is only a primary ecosystem, which needs to be further improved in terms of structure and function. As the hydrophobicity of the granular sludge surface intensifies and bacterial polysaccharide secretion rises, a greater number of free microorganisms can be captured and attached, enhancing the microbial diversity within the granular sludge. Various microorganisms can also adjust their spatial position through various roles to form a reasonable spatial distribution, thus optimizing their own ecological niche. Within the granular sludge, microorganisms have the capability to exchange genetic information, facilitating the cooperative evolution of metabolic pathways.

2.2. Analysis of aerobic granular sludge properties

The characteristics of aerobic granular sludge are influenced by a multitude of factors, which can be categorized into physical parameters (e.g., settling velocity, compactness, specific gravity, sludge volume index), chemical aspects (specific oxygen uptake rate, extracellular polymers), and biological factors.

The settling performance of these granules is pivotal in wastewater treatment systems as it directly affects the separation efficiency between sludge and water. Research by Qin et al. [13] indicates that the settling velocity of aerobic granular sludge typically ranges between 25 to 70 m/h, which is notably 3 to 7 times faster than conventional sludge flocs. This accelerated settling rate enhances the microbial retention within the reactor and augments the organic matter degradation capacity.

Mu and Yu [14] utilized granule diameter as an indicator to delineate the morphology of aerobic granular sludge. Nor Anuar et al. [15] explored the influence of agitation on the settling performance of aerobic granular sludge. Conversely, Mu et al. [16] discerned that the water resistance coefficient of aerobic granular sludge is inferior to that of biofilm-covered particles. Yet, Kim et al. [17] encountered challenges when attempting to characterize aerobic granular sludge properties solely based on settling performance under varying organic carbon loads.

Liu et al. [18] delved into the correlation between cell surface hydrophobicity and the characteristics of heterotrophic and nitrifying granular sludge. They discovered that the hydrophobicity of granular sludge is approximately twice that of conventional floc sludge. Intensifying hydraulic shear force can augment cell surface hydrophobicity, while organic load variations have negligible impact on this attribute. Nevertheless, the precise role of cell surface hydrophobicity in the genesis of aerobic granular sludge remains somewhat ambiguous.

Extracellular polymeric substances (EPS) are metabolites that accumulate on bacterial cell surfaces, modifying their physical and chemical properties, such as electrical charge and hydrophobicity. Adav and Lee [19] employed seven distinct extraction techniques to isolate EPS from aerobic granular sludge. Given the compact internal structure of aerobic granules, the quantity of EPS extracted varies across different methods. The polysaccharide-to-protein ratio in aerobic granular sludge can range between 3.4 to 6.2, significantly surpassing that in floc sludge (approximately 0.9).

Mcswain et al. [20] identified that an elevated protein content is pivotal for the formation of aerobic granular sludge. Modern molecular biology and microscopic techniques, including scanning electron microscopy (SEM), optical microscopy, and laser confocal microscopy coupled with fluorescence in situ hybridization (FISH) [21], offer insights into the microbial community structure of aerobic granular sludge. Research has revealed a diverse array of bacteria within aerobic granules, encompassing heterotrophic bacteria, nitrifying bacteria, denitrifying bacteria, and phosphorus-accumulating bacteria. The microbial diversity of aerobic granular sludge is intrinsically linked to its structural configuration and the composition of the culture medium. In a study on phenol wastewater treatment, Lin et al. [22] ascertained that the predominant microorganisms in aerobic granular sludge belong to the Proteobacteria phylum.

3. Factors influencing the formation of aerobic granular sludge

3.1. Incoming substrate

Aerobic granular sludge has been cultivated successfully using a diverse range of substrates, including glucose, sodium acetate, ethanol, benzene, and synthetic wastewater. These easily degradable compounds exhibit high viscosity, potentially facilitating bacterial inter-colonization. Notably, the microbial communities and structures of aerobic granular sludge developed with different carbon sources exhibit significant variations. For instance, when glucose serves as the substrate, filamentous bacteria tend to dominate the aerobic granular sludge. In contrast, cultures utilizing acetic acid as the carbon source showed an absence of filamentous bacteria [23]. The distinctions between aerobic granular sludge and activated sludge are detailed in the subsequent table. Table 1.

Table 1
Comparative analysis of aerobic granular sludge and activated sludge characteristics.

	Aerobic granular sludge	Activated sludge
Average diameter (mm)	2.4 ± 0.71	0.15
Sludge volume index (mL/g)	51-85	150-250
Settlement speed (m/h)	35 ± 8.5	< 10
Sludge concentration in reactor (g/L)	8.0-15.0	3.0-5.0
Density (g/L)	41.1 ± 6.9	~
Granular sludge strength (%)	98 ± 0.9	Weaker
Aspect ratio	0.79 ± 0.06	Irregular shape
Hydrophobicity (%)	68.0 ± 3.9	~
Oxygen consumption rate (SOUR, mgo ₂ /g/hr)	69.4 ± 8.8	100
COD removal rate (%)	96.6 ± 1.6	90 %

3.2. Apparent gas velocity and dissolved oxygen levels

The observed gas velocity influences the hydraulic shear force exerted and concurrently impacts the dissolved oxygen concentration. Changing the proportion of anaerobic and aerobic bacteria in the sludge particles has a great impact on the sludge granulation process. Lu [24] examined the impact of varying dissolved oxygen (DO) levels and hydraulic shear forces on aerobic granular sludge within an SBR reactor setting. When the aeration rate is 0.3 L/min, the appropriate hydraulic shear force and DO mass concentration will enable microorganisms to gather together to form new regular granular sludge; When the aeration rate continues to increase to 0.4 mL/min, the excessive hydraulic shear force will cause the particle structure of sludge to be washed and destroyed, accelerating the disintegration of granular sludge. Ji et al. [25] explored the degradation capabilities of aerobic granular sludge when treating saline wastewater under conditions of low surface gas velocity. Maintaining a surface gas velocity of 0.0056 m/s led to the rapid formation of both light yellow and black granular sludge within the reactor. Within the black granules, a diverse range of inorganic salt crystals and filamentous bacteria were found to coexist. This coexistence enhanced the robustness of the granular sludge and decreased the substrate's mass transfer resistance.

On the other hand, Mosquera-Corral et al. [26] investigated the growth patterns of granular sludge at 40 % and 100 % dissolved oxygen saturation levels. Their findings indicated that particles cultivated under higher dissolved oxygen concentrations exhibited larger particle sizes and superior sedimentation capabilities. Thus, elevated levels of dissolved oxygen and hydraulic shear force appear to be more favorable for the development of well-formed sludge particles.

3.3. Metal ions

Metal ions, including calcium (Ca²⁺) and magnesium (Mg²⁺), serve as essential trace elements for microbial proliferation. These ions can accelerate microbial growth rates, enhance the microbial diversity index, and facilitate the granulation process of aerobic sludge. Hao et al. [27] reported the impact of different types and concentrations of metal ions on the microbial adsorption capacity and group receptivity of flocculent activated sludge and mature granular sludge. It was found that adding 2 mg/L Cu²⁺ and Fe²⁺ to the floc sludge could significantly promote the sludge granulation. Liu et al. [28] studied and compared the effects of adding Ca²⁺ and Mg²⁺ on the formation of aerobic granular sludge. The results showed that when the contents of Ca²⁺ and Mg²⁺ were both 40 mg/L, the granular sludge containing Ca²⁺ formed faster and had excellent physical properties; However, the granular sludge containing Mg²⁺ has a higher content of extracellular polymers, more abundant biological phase and better sewage

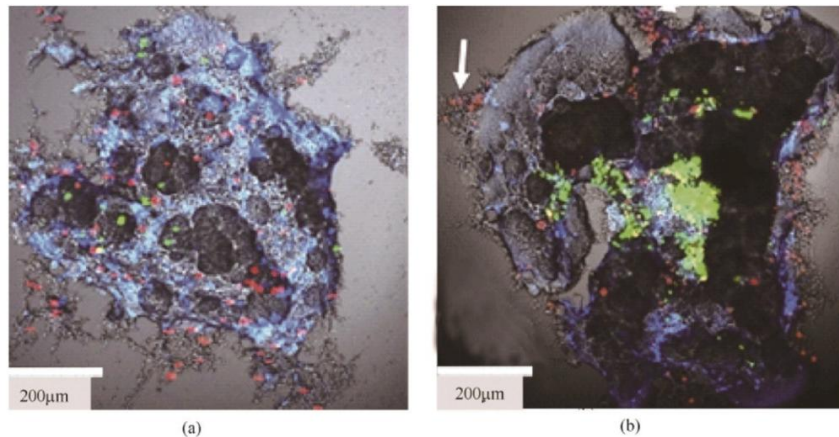


Fig. 3. Confocal laser scanning microscope image of flocculated sludge attached to the particle surface on day 26 of reactor operation. Among them, the pictures (a) and (b) are fluorescent confocal laser scanning microscope pictures with bright vision, showing that the marked floculent sludge (red) is attached to the surface of broken particles (green).

treatment effect. Wang et al. [29] investigated the impact of introducing Cu^{2+} and Ni^{2+} into the SBR system, which already contained pre-cultured particles. Their findings indicated that the presence of Cu^{2+} led to a notable decrease in biomass concentration, microbial diversity, and microbial activity within the aerobic particles. Conversely, the addition of Ni^{2+} showed minimal influence on the performance of the particles. Wang et al. [30] studied the cultivation of granular sludge by adding polyaluminum chloride at different times. It was found that adding polyaluminum chloride on the 8th to 14th days advanced the formation time of granules by 6 days, and the formed granules had regular shape, uniform size, good pollutant removal performance, and had more extracellular polymers and protein content. These studies demonstrate that multivalent cations influence the development of granular sludge, the physicochemical characteristics of aerobic granular sludge, and its efficacy in wastewater treatment.

3.4. Settling time

In the sequencing batch reactor (SBR) system, the adjustment of settling duration can effectively remove smaller dispersed and poorly settling flocculent sludge, which significantly influences the development of aerobic granular sludge. Aerobic granular sludge predominates when the settling time in the SBR is set to 5 min. However, at settling times of 20, 15, and 10 min, a mixture of aerobic granular sludge and suspended sludge is observed. A shorter settling duration tends to promote the secretion of extracellular polymeric substances (EPS) and enhances cell surface hydrophobicity. Thus, selecting an appropriate settling duration is crucial for the successful aerobic granulation process. Elevated chemical oxygen demand (COD) levels promote granular sludge growth. Prolonged settling times can result in the accumulation of flocculent sludge, while shorter settling times may wash out a significant amount of activated sludge, leading to reduced mixed liquor suspended solids (MLSS) and COD removal efficiency in the reactor. Therefore, under high COD loads, it is essential to adjust the settling time to eliminate slow-settling suspended and flocculent sludge, ultimately favoring the development of well-settling granular sludge.

3.5. Metabolic patterns

Different metabolic pathways can influence the granulation process of activated sludge. Commonly observed granulation methods include aerobic granulation and anaerobic/aerobic sequential granulation. The structural impact of prolonged anaerobic conditions and alternating anaerobic/aerobic phases on the configuration of activated aerobic granular sludge during the starvation period is also noteworthy, under both conditions, the morphology of the final particles in the starvation

period has changed. Under the condition of intermittent anaerobic/aerobic starvation, the loss of particle density is faster and more significant; Under this condition, the release of Ca^{2+} indicates the degradation of EPS; During anaerobic and intermittent anaerobic/aerobic starvation periods, the activity of nitrifying bacteria decreased by 20 % and 36 % respectively. Wang et al., the obtained granular sludge has a lower volume index ($\text{SVI}_{30} = 45 \text{ mL/g}$) and a higher concentration of mixed liquid suspended solids ($\text{MLSS} = 9\text{--}10 \text{ g/L}$) [30].

During the initial phase of the aerobic granular sludge system, the incorporation of ground granular sludge can influence the commencement period of the aerobic granular sludge system. Research conducted by Pijuan et al. [31] demonstrated that the introduction of crushed granular sludge into the reactor effectively decreased the start-up duration of the aerobic granular sludge system. The findings indicated that an increased amount of crushed granular sludge led to a more expedited initiation of the aerobic granular sludge system. Especially when the added crushed granular sludge accounts for 50 %, the shortest granulation time can be achieved for 18 days. The particles formed have good nitrogen removal function, but the phosphorus accumulating bacteria may be inhibited due to the temporary accumulation of nitrite, and the phosphorus removal effect will be affected to a certain extent. Long et al. [32] also achieved 18 days of granular sludge formation time by inoculating 75 % activated sludge and 25 % granular sludge. The research of Su et al. [33] further proved this conclusion. By using the aerobic sludge self-coagulation immobilization culture technology and adding a certain amount of crushed granular sludge, ordinary activated sludge can be domesticated into aerobic sludge granules with dense structure, uniform particle size, clear edges and regular shape within one month, greatly reducing the start-up time of the entire process. To investigate the mechanism behind the enhanced granulation of fragmented particles, Verawaty et al. [34] carried out the subsequent experiments: Fluorescent microsphere particles measuring m in diameter were employed to label both the flocculent sludge and the crushed particles' surfaces. These labeled sludges were introduced into a laboratory-scale wastewater treatment facility. The particle aggregation process was observed using a confocal laser scanning microscope (CLSM) (refer to Fig. 3). The findings indicated that the flocculent sludge attached to the surface of the seeded particles, resulting in the formation of larger aggregates. This phenomenon minimized sludge washout and expedited the granulation process. Such experimental outcomes suggest that particles can serve as nucleation sites, facilitating the adherence of flocculent particles and thereby hastening particle formation.

3.6. other influencing factors

Since sludge particles are essentially the growth of microbial flora aggregation, its influence factors are very many, in recent years,

numerous researchers have undertaken extensive studies on aerobic granular sludge particles, reactor structure, ambient temperature, cellular hydrophobicity and many other factors have a greater impact on the process of granulation.

4. Factors affecting the stability of aerobic granular sludge

The current inability to maintain the stability of aerobic granular sludge over prolonged periods hinders its industrial scalability. This instability primarily manifests as filamentous overgrowth, degradation of the granular sludge's intrinsic metabolism, decreased microbial activity, and other factors, ultimately resulting in the destabilization and breakdown of aerobic granular sludge structures. The primary factors contributing to the destabilization of aerobic granular sludge are delineated as follows.

4.1. Temperature

Temperature significantly influences the growth and viability of microorganisms. Currently, the majority of research on aerobic granular sludge has been conducted at ambient temperatures, typically ranging from 20 to 25 °C. Song et al. [35] investigated the cultivation of aerobic granular sludge at varying temperatures of 25 °C, 30 °C, and 35 °C, revealing that sludge matured at 30 °C exhibited a more compact structure, enhanced sedimentation performance, and superior biological activity. Cui et al. [36], after initiating cultivation at 20 °C, increased the temperature to 26 °C. Subsequent observations over 47 days indicated a decrease in the protein-to-polysaccharide ratio within the extracellular polymer of the aerobic granular sludge, culminating in sludge disintegration. Additionally, Winkler et al. [37] observed a decline in the settling performance of granular sludge as temperature decreased. In research by Kreuk et al. [38], aerobic granular sludge cultivated at 8 °C exhibited irregular and unstable morphologies, accompanied by a proliferation of filamentous bacteria, which eventually resulted in significant sludge loss. However, when the initiation temperature was set at 20 °C and subsequently reduced to 15 °C and 8 °C, the stability of the granular sludge remained unaffected.

4.2. pH value

Different strains have pH values suitable for their growth, and low pH value is the main reason for filamentous bulking of aerobic granular sludge. Ji et al. [39] used the identification method of filamentous microorganisms to determine that the microorganism causing filamentous bulking of aerobic granular sludge is a filamentous fungus, and this kind of microorganism is very easy to grow and reproduce under low pH environmental conditions. Yang et al. [40] found that under the pH value of 4.0, the particle size of aerobic granular sludge can reach 7.0 mm, and fungi are dominant strains with loose structure; However, when the pH value is 8.0, the particle size of aerobic granular sludge is only 4.8 mm, and bacteria are dominant strains with compact structure. Seviour et al. [41] studied the EPS gel properties of aerobic granular sludge under different pH values, and found that when pH < 9, the extracellular polymer of granular sludge showed strong gel properties.

4.3. Hydrolysis of aerobic particulate cores

During the development of aerobic granules, the limited transfer of oxygen often results in the formation of an anaerobic core. The stability of this anaerobic core plays a pivotal role in determining the overall stability of the aerobic granules. Zheng et al. [12] suggested that the mass transfer limitations in larger granules can induce the formation of an anaerobic core, thereby stimulating anaerobic microbial activity and potentially causing granule disintegration. Adav, Lemaire, and their colleagues [42] employed a combination of optical microscopy, scanning and transmission electron microscopy, copolymerization laser

scanning, fluorescence in situ hybridization, as well as dissolved oxygen and pH electrodes to investigate the granule structure. Their findings indicated that the disintegration of mature granules was attributed to pore blockages, which impeded nutrient uptake by the microorganisms. Prolonged periods of starvation were found to render the granular structure more susceptible to breakage. Research has also indicated that the stability and activity loss of granules during extended idle periods can be influenced by their storage temperature.

Kreuk et al. [38] explored the recovery of high salinity aerobic granules following low-temperature storage by gradually increasing the organic load in the influent. Their observations revealed that the external characteristics of the high salinity aerobic granules remained largely unchanged after six weeks of low-temperature storage, whereas noticeable alterations in color and internal structure were evident. This transformation can be attributed to the conditions of low-temperature storage devoid of dissolved oxygen and nutrients. Under such conditions, heterotrophic microorganisms within the granules are predisposed to endogenous respiration and intracellular hydrolysis, resulting in diminished biological activity of the granules.

4.4. Dissolved oxygen and particle size

Dissolved oxygen plays a vital role in the formation and stability of aerobic granular sludge. Low dissolved oxygen will lead to insufficient internal oxygen supply of granular sludge, which is prone to anaerobic metabolism in the granular core. Mosquera-Corral et al. [26] began to disintegrate the granular sludge after the dissolved oxygen was reduced by 40 %. Liu et al. [18] mentioned that the mature granular sludge under low DO condition will lose stability due to filamentous bulking. The particle size will affect the mass transfer resistance and oxygen transfer efficiency of the matrix. Wang et al. [29] inoculated aerobic granular sludge in the MBR reactor and found that granular sludge with a particle size greater than 0.9 mm collapsed after 24 days of operation, while granular sludge with a particle size between 0.18 mm and 0.9 mm showed a growing trend. Li et al. [43] analyzed the characteristics of aerobic granular sludge selected from ordinary push flow aeration tank of urban sewage plant, and found that the diameter of granular sludge is mainly 0.2–0.8 mm. Therefore, controlling the particle size of aerobic granular sludge is one of the factors to maintain the stability of aerobic granular sludge.

4.5. Overgrowth of filamentous microorganisms

The density and robustness of aerobic particles exhibit an inverse relationship with microbial growth rates. Rapid microbial proliferation results in an accelerated particle size increment. Moreover, an overabundance of filamentous bacteria can compromise the compactness of particle structures, diminish sedimentation efficiency, and render the particles susceptible to washout. Liu et al. [10] demonstrated that under low dissolved oxygen (DO) conditions, an overgrowth of filamentous microorganisms can precipitate operational disruptions in the reactor. This phenomenon arises due to the gradual dominance of filamentous bacterial populations within the particles, thereby limiting mass transfer. Consequently, the nutrient availability for anaerobic microorganisms residing within the particles becomes insufficient, leading to particle disintegration. Li et al. [43] observed that elevated COD concentrations can stimulate filamentous bacterial proliferation, subsequently suppressing the extracellular polymer secretion and compromising the structural integrity of granular sludge. Additionally, the propensity of filamentous microorganisms to obstruct pipelines can further exacerbate reactor system operational challenges. Adav and Lee [19] compared three identical reactors treating phenol wastewater under different aeration intensities (1–3 L air/min) to observe the granulation process. No particles are formed under low aeration intensity (1 L/min), but mature and stable particles with a compact core with a diameter of 1–1.5 mm will be formed under high aeration

intensity (3 L/min). They found that strong shear force will inhibit the growth of filamentous bacteria in long-term operation.

4.6. Hydraulic shear

The strength of hydraulic shear force has a great influence on the stability of sludge granulation. The higher hydraulic shear force can not only make the granular sludge more compact and stable, but also have a certain inhibitory effect on filamentous bacteria. Liu and Tay [11] found that higher hydraulic shear force can cultivate granular sludge with smooth surface, dense structure, high mechanical strength and high microbial content, and also found that higher hydraulic shear force can stimulate aerobic granular sludge to secrete more extracellular polymers (EPS). Adav and Lee [19] also investigated the effect of hydraulic shear on the formation of aerobic granular sludge with phenol as the substrate and the content of EPS. High hydraulic shear force can not only promote sludge granulation, but also provide sufficient oxygen to inhibit the proliferation of filamentous bacteria, which is conducive to the long-term stable operation of aerobic granular sludge.

4.7. Sludge age

The maximum specific growth rate of filamentous bacteria was lower than that of bacterial colloid, and longer sludge residence time would be favorable for the growth of filamentous bacteria. Wang et al. [44] identified that the filamentous microorganisms appearing in aerobic granular sludge generally appeared in the system with long sludge age. However, too short sludge age is difficult to carry out nitrification and denitrification, which is not conducive to the growth of aerobic granular sludge.

4.8. Toxic and hazardous substances and other factors

Among the diverse wastewater treatment technologies addressing both municipal and industrial effluents containing toxic and recalcitrant contaminants, aerobic granular sludge technology stands out due to its significant potential. Nevertheless, the genesis and structural integrity of aerobic granular sludge are notably susceptible to the influence of toxic compounds. Zhu et al. [45] investigated the repercussions of the prevalent toxic pollutant, p-chloroaniline, on granule morphology and performance. Their findings revealed that an influent concentration of 200 mg/L p-chloroaniline precipitated the disintegration of aerobic granular sludge, compromising its pollutant removal efficacy. Examination of the extracellular polymeric substances (EPS) from both intact and disintegrated granules indicated a reduction in EPS protein content. Notably, a decline in the content of amide I3 trans helix, β -folding, and a decrease in aspartic acid composition were identified as detrimental to the stability of aerobic granular sludge. Microbial community profiling further highlighted the disappearance of dominant species such as *Kineosphaeralimos* and the emergence of *Acinetobacter*, potentially exacerbating EPS reduction and granule disintegration.

Wei et al. [46] explored the implications of low concentrations of 4-chlorophenol (4-CP) on the stability of aerobic granular sludge. Their investigations indicated that granules cultured with acetate as a substrate could effectively degrade 4-CP and withstand elevated influent concentrations of the compound. This suggests that acclimated granules can achieve a commendable 4-CP removal rate.

Kreuk et al. [47] delved into the effects of quinolone antibiotics, namely ofloxacin, norfloxacin, and ciprofloxacin, on aerobic granules. Their research discerned that the overall COD removal remained unaffected by the presence of these quinolones throughout the treatment process.

5. Conclusion

Aerobic granular sludge, as a burgeoning wastewater treatment modality, boasts attributes such as a compact architecture, efficient settling capabilities, diverse microbial consortia, and elevated biological retention efficiency. These characteristics offer potential solutions to the limitations inherent in conventional activated sludge processes. Nevertheless, the intricate mechanisms underpinning the formation of granular sludge remain insufficiently elucidated. Granular sludge represents a distinct microbial aggregation structure, naturally evolving in response to various environmental parameters like nutrient levels, hydraulic shear forces, and dissolved oxygen concentrations. The complex nutritional interdependencies among diverse microbial species within these granules, coupled with their synergistic roles in organic pollutant degradation, remain areas of limited understanding. This gap in knowledge consequently hampers a comprehensive grasp of the cultivation mechanisms of aerobic granular sludge. Consequently, there is an imperative need for deeper investigations into the formation dynamics of aerobic granular sludge, the determinants of its stability, and the mechanisms governing its multi-species synergistic interactions. Such endeavors will be pivotal in augmenting our comprehension of aerobic granular sludge, thereby expediting its industrial adoption and widespread application in wastewater treatment.

The future of aerobic granular sludge (AGS) technology research lies in a multidisciplinary approach that fuses molecular biology, computational modeling, and process optimization. Key areas for exploration include the intricate microbial ecosystems within AGS and their response to various stressors, aiming to uncover the mechanisms underlying granule formation and stability. Advanced molecular techniques, such as next-generation sequencing, offer promising avenues to identify and enhance the roles of crucial microbial species. Additionally, computational fluid dynamics could revolutionize reactor design, optimizing conditions for AGS development while mitigating challenges like filamentous overgrowth. Addressing these aspects is essential for extending AGS applications to treat a wider range of wastewaters, including those with high variability and pollutant loads. Ultimately, this research direction holds the potential to significantly improve the efficiency and sustainability of wastewater treatment systems worldwide, marking a significant step forward in environmental protection and resource recovery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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