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Review article

# Rapid startup of aerobic granular sludge: Recent advances and future challenges

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ABSTRACT

Aerobic granular sludge (AGS) biotechnology has recently obtained considerable interest as a viable alternative to the activated sludge process (ASP) technique. This is because AGS can improve the performance of wastewater treatment and its significant capability for attaining sustainable growth. One of the main challenging issues for the aerobic granular sludge is the long startup time in wastewater treatment. This review presents a comprehensive analysis of AGS biotechnology, aiming specifically at its global adoption, startup duration, and granule stability. Aerobic granular sludge (AGS) is becoming more widespread globally. To ensure the successful implementation of AGS biotechnology, it is crucial to thoroughly identify the development of dense and stable granules, which is vital for the proper operation of wastewater treatment plants (WWTPs). Additionally, it offers a comprehensive summary of the latest advancements in the inoculum and polymer additives and their respective contributions to accelerate various processes through distinct mechanisms. In addition, this paper reviews the prevailing research patterns in the prompt initiation of rapid startup of AGS technology and outlines specific issues for future investigations.

## 1. Introduction

Compared to physicochemical techniques, biological wastewater treatment techniques are considered the most cost-effective and environmentally friendly way to eliminate organic matter and nutrients [1]. In these techniques, the standard technology for sewage treatment is the conventional activated sludge process (CAS) system. In CAS, the microbial community often develops as flocs of suspended activated sludge (AS) that must be treated. However, CAS systems have drawbacks, including separate aeration and settling tanks, low biomass concentrations, vast floor space and recycling flows [2]. The AGS technique is a potential remedy to enhance wastewater treatment efficiency, reduce resource consumption and recover valuable resources from wastewater. The typical activated sludge (AS) wastewater treatment technique has problems with nutrient removal and solid-liquid separation, which inspired this innovation [1,3]. The main shortcomings of AS technology are its wide land use, high energy consumption from the cycling of biomass and difficulty separating biomass from water, which is intended to be addressed by the AGS technology [4]. The scientific community, particularly microbiologists and wastewater engineers, has shown considerable interest in the AGS method due to its potential wastewater treatment applications [5]. AGS through SBR is a type of wastewater treatment system that uses aerobic granules instead of activated sludge to remove pollutants in batches because the sludge particles in AGS are dense and compact microbial aggregates with better settling and resistance properties than activated sludge [6]. These granules comprise different layers, as shown in Fig. 1. The outer zone of the granule is aerobics, which contains aerobic microorganisms. The middle layer has an anoxic zone, and the innermost layer consists of an anaerobic zone, which means an anaerobic microorganism due to a lake of oxygen in the depth.

In the AGS SBR system, wastewater is added to a single tank or basin and a series of treatment processes are carried out in a specific sequence,

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**Abbreviations**

| | |
|---|---|
| AGS | Aerobic granular sludge |
| SBR | Sequencing batch reactor |
| SBBR | Sequencing batch biofilm reactor |
| WWTPs | Wastewater treatment plants |
| BDL | Below detection limit |
| CAS | Conventional activated sludge |
| ASP | Activated sludge process |
| OLR | Organic loading rate |
| COD | Chemical oxygen demand |
| $BOD_5$ | 5 days Biochemical oxygen demand |
| MLSS | Mixed liquor suspended solids |
| VER | Volumetric exchange ratio |
| DO | Dissolved oxygen |
| SOUR | Specific oxygen uptake rate |
| AOB | Ammonia-oxidizing bacteria |
| NOB | Nitrite-oxidizing bacteria |
| TP | Total phosphorus |
| TN | Total nitrogen |
| TKN | Total kjeldahl nitrogen |
| SVI | Sludge volume index |
| HRT | Hydraulic retention time |
| SRT | Solids retention time |
| EPSs | Extra polymeric substances |
| PAC | Polyanionic cellulose |
| QS | Quorum sensing |
| QQ | Quorum quenching |

such as filling, aeration, settling, decanting and idle periods, as shown in Fig. 2. The AGS technology has been developed for full-scale domestic and industrial wastewater treatment plants. However, the stability of granules is still one of the significant problems for the AGS process [7]. AGS is a microbial aggregate that does not settle under low hydrodynamic shear and has benefits such as higher resistance to high organic loading, a more compact reactor with higher biomass concentration, improved nitrogen and phosphorus removal, lower sludge production, and better-settling behaviour. These factors make AGS a superior option for wastewater treatment [8].

AGS technology significantly influences the system's applied organic loading rate (OLR) because it can impact AGS Stability and physiological properties [6,9]. Long-term reactor operation does not affect the structural stability of protein-rich granules (PN). Using ammonia-nitrogen as the only nitrogen source at a COD: N ratio of 153:8 produces PN-rich granules with a PN/polysaccharides (PS) ratio greater than 20 from nitrogen lean effluent [10,11]. The granules produced can maintain structural integrity when subjected to sufficient wastewater treatment with a COD/N value of less than 500 and an OLR of 39 kg COD/$m^3$.d in an SBR mode. An enrichment of the granules was observed with Firmicutes and b-proteobacteria as the predominant isolates. In the granules, over 58% of the nitrogen introduced in nitrogen-lean effluent is changed to PN. They substituted nitrate for ammonia as the exclusive nitrogen source enriched granules with c-proteobacteria, rapidly degrading under low OLR conditions [12]. AGS can operate efficiently in high biomass retention conditions, with MLSS levels reaching 15,000 mg/L. This results in a high biomass-intensity reactor, enabling effective wastewater treatment at high volumetric loading rates [6,13]. In the AGS process, the settling velocity is three times greater than AS because of the larger particle size and density of granules [14]. Dissolved oxygen is also a crucial factor in the nitrogen conversion and removal pathways during the operation of the AGS reactor [15,16]. AGS's increased efficiency is also a result of its faster settling velocity [17]. AGS can reduce running costs and energy usage [18]. Compared to AS systems, the AGS technology cut operating costs by 20–25%, electricity use by 23–40% and space required by 50–75%. Owing to these advantages, the AGS system is thought to be more affordable and effective than traditional AS systems [19]. Due to this, AGS technology has gained more consideration today for its significant ability to reduce footprint and boost the wastewater treatment plants' efficiency. Many researchers used SBRs to support AGS technology because they can culture aerobic granules quickly, reliably and provide easy handling [20,21].

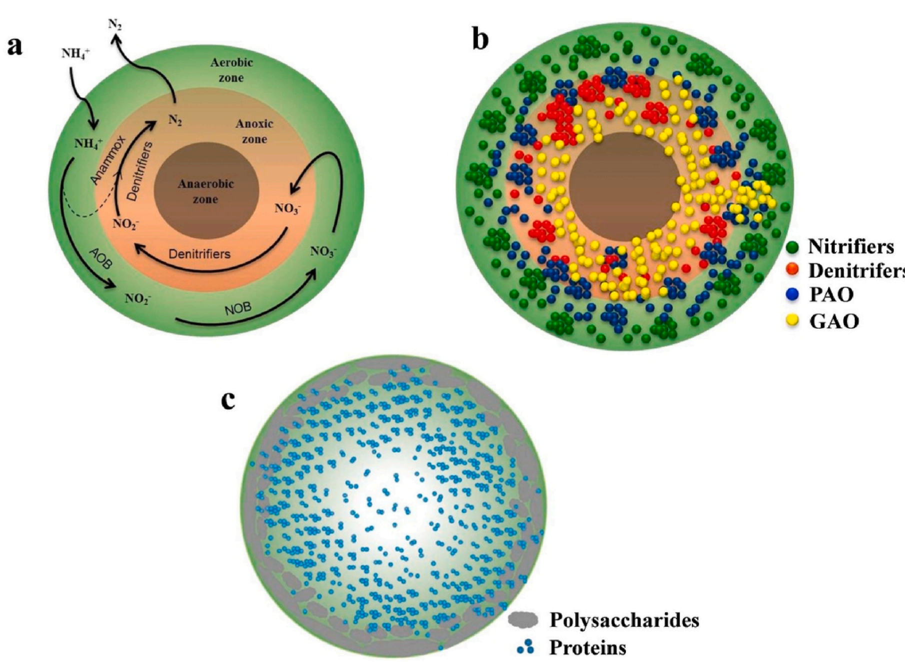


**Fig. 1.** Segregated distribution of an individual granule: a) layers and nitrogen removal pathways, b) microorganisms, and c) carbohydrates and proteins of the EPS matrix [2].

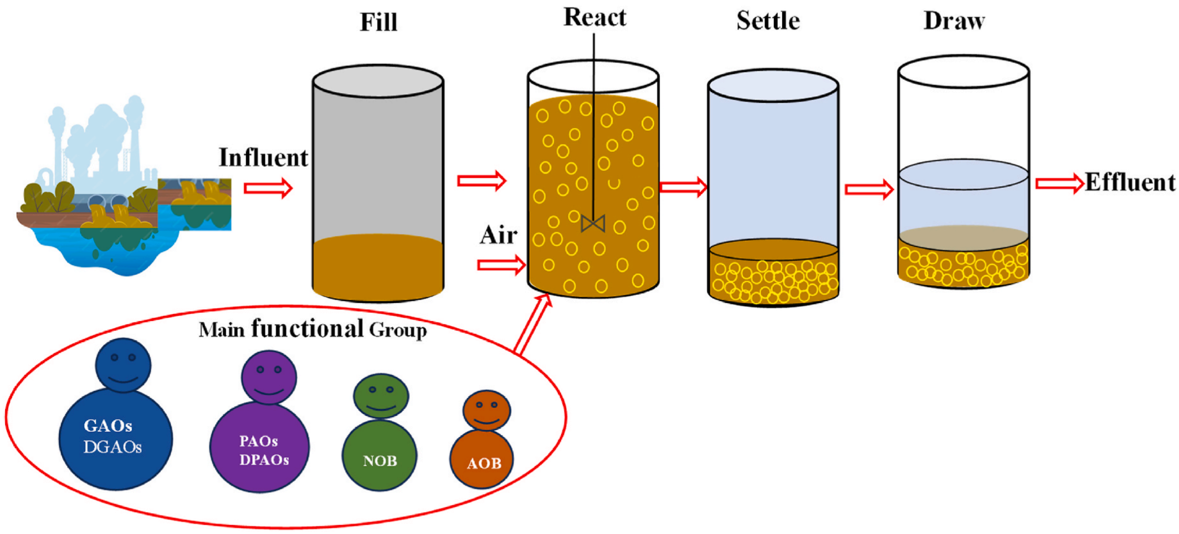


**Fig. 2.** AGS SBR operating stages.

AGS has many advantages over conventional activated sludge, such as high settling velocity, low sludge production, and high pollutant removal efficiency. However, the formation and stability of granular sludge are influenced by many factors such as slow granulation, granule disintegration, sludge washout, and sludge bulking [22]. Sludge washout is a common problem in aerobic granular sludge cultivation. Sludge washout occurs when granules are not sufficiently compact or dense to settle quickly and are carried out with the effluent. The possible causes of sludge washout are insufficient shear force, lack of feast-famine regime, and excess organic loading rate. During the operation of the AGS reactor, these parameters should be controlled to overcome these issues [23].

Researchers have also developed various rapid granulation methods to overcome these factors. Inoculum and polymer additives are the advanced mechanisms to produce stable formation of granules. These methods have been shown to reduce the time required for granulation and deliver stable, uniform granules. Quality control of operational parameters such as duration of each stage (e.g. feeding, anaerobic, aerobic, settling, and decant), cycle time in SBR, dissolved oxygen (DO) concentration and volumetric exchange ratio (VER) are essential for granulation of lumpy sludge into dense solids [24,25]. Like the C: N ratio, the granulation process rests on the characteristics of the wastewater. In addition, the shape and structure of particles are also affected by the properties of sludge seeds. The granulation development of flocculent sludge is also influenced by the consistency, hydrophobicity and microbial activity of the sludge seeds; these are the crucial factors to be considered [26]. The commencement phase of laboratory-scale aerobic granular sludge (AGS) systems typically lasted between 1 and 3 months and the maturation process of AGS systems, when seeded with floccular sludge-lasted for approximately 4–5 months during the initial startup phase. Therefore, some studies have investigated ways to start AGS processes more quickly using a different type of seed sludge instead of floccular-activated sludge. Inoculum is used in laboratory or pilot-scale systems to study the granulation process or assess the performance of various seed sludges. This technique might be time-consuming, but it gives significant information on the ideal settings for granulation and performance [8,27,28].

Over the past few years, studies have determined another aspect of the rapid startup of the aerobic granulation process: using polymers [42]. In AGS, polymers added in the process play a crucial part in stimulating the growth and stability of microbial aggregates. The extracellular polymeric substance is created by microorganisms that serve as a matrix that holds cells together and protects the granule's shape from the adverse environment [43]. Polymers retain bacterial species' progression, structure and accumulation in the AGS system [44]. Consequently, using polymers in the quick startup of AGS processes can potentially increase efficacy and shorten the beginning period for wastewater treatment. The shape and stability of granules and simplifying contaminant elimination could be easily achieved using polymers [44,45].

In addition to domestic wastewater treatment, industrial wastewater treatment has been done through AGS. These industries include petroleum, food, rubber, pig farming, pulp milling and others dealing with phenol-containing run-offs or endocrine-disrupting substances [46,47]. There are still several obstacles to be solved before AGS can completely replace activated sludge, which is its predecessor. To address these problems, much researches was done on the reactor's design, microbiological community and polymer use [47,48]. The rapid startup of AGS substantially shortens the time required to form aerobic granules from CAS. Typically, the startup period for aerobic granulation can last several months to even a year when treating low-strength municipal wastewater. However, researchers have successfully reduced the startup time with various strategies, such as optimization of operating parameters, bio-augmentation of quorum sensing strains and external supplement of metal ions. This rapid startup of aerobic granular sludge is advantageous as it allows for quicker implementation of the AGS technology in wastewater treatment, leading to improved biomass retention, settling ability and robust challenge to shock loading [49,50]. Therefore, this review identifies an overview of recent advances in the rapid startup of aerobic granulation. The applications, possible challenges, and future perspectives of AGS startups.

## 2. Applications of AGS startups

AGS biotechnology's use in treating municipal and industrial wastewater has increased because of its compact and practical structure. When applied to effluent generated by industrial activities, conventional ASP proves to be inadequate in eliminating toxic or resistant xenobiotic compounds. Retaining high concentrations of biomass and degradative strains while demonstrating increased tolerance to poisonous xenobiotics are characteristics that immobilize cell systems to enhance treatment efficacy. Anaerobic, graphene-semiconductor, palm oil mill, rubber, petrochemical, and landfill effluent are among the industrial wastewaters for which AGS has been assessed and utilized. Conversely, simulated wastewater was used in most of the investigations. Table 1 provides a comprehensive summary of various studies that have evaluated the effectiveness of AGS technology in controlling actual industrial effluent [2]. Nereda® and S: Select® are well-known names for

**Table 1**
Experimental analyses of AGS SBR treating real industrial wastewater [2].

| Wastewater type | Wastewater Characteristics ($mgL^{-1}$) | System operational conditions | Performance of the system | Ref |
|---|---|---|---|---|
| Landfill leachate | $_t$COD:4560 ± 165; $_s$COD:1540 ± 175; $NH_4^+$ - N:945 ± 54; $NO_3$ – N:0.3 ± 0.4; TN: 845 ± 175 | Working volume:3 L; cycle time: 12 h; VER: 50%; SRT: 25–30 d | Granules size: 0.75 mm; COD: 70% removal; $NH_4^+$ - N: 59% removal; MLSS: 8 $gL^{-1}$ | [29] |
| Livestock | COD: 3600; BOD:1750; TSS: 230; TN: 650; TP: 380; pH:8.05 | Working volume: 4 L; cycle time:4 h; VER: 50% | Granules size: 3.5–4 mm; SVI: 42.1 $mLg^{-1}$; COD: 74%; TN: 73%; TP: 70%; MLSS: 10.27 $gL^{-1}$ | [30] |
| Slaughterhouse | COD:1250 ± 150; Ammonia: 120 ± 20; TP: 30 ± 5 | Working volume: 20 L; VER: 50%; cycle time: 6 h | Granules size: 1.2 mm; COD: 95.1 % removal; Ammonia: 99.3%; TP: 83.5% | [31] |
| Sugar beet processing | $_t$COD: 4280 ± 260; $_s$COD: 3055 ± 183; $NH_4^+$ - N:49 ± 5; TP: 7.8 ± 0.8 | Working volume: 2.45 L; cycle time: 6 h; VER: 50%; HRT: 12 h | Granules size: 2.59 ± 0.4 mm; $SVI_{30}$: 25 ± 1.4 $mLg^{-1}$; COD: 87% removal; TN: 57% removal | [32] |
| Swine slurry | $_s$COD: 13,689 ± 1277; $_t$COD: 15,932 ± 2627; $NH_4^+$ - N: 1823 ± 496 | Working volume:1.5 L; H/D: 5.5; cycle time: 3 h; VER: 50%; HRT: 0.25–1.88 d | Granules size: 2.1–4.9 mm; SVI: 75 $mLg^{-1}$; COD: 87% removal; TN: 70% removal; VSS: 20 $gL^{-1}$ | [33] |
| Malt industry | $_t$COD:1700; $_s$COD:470; $NH_4^+$-N:3; TN: 45 | Working volume: 12 L; cycle time: 8 h; VER: 66%; HRT: 0.5 d | SVI: 30–40 $mLg^{-1}$; COD: 80% removal; MLSS: 7$gL^{-1}$ | [34] |
| Papermaking | COD: 2100–3000; BOD: 800–1130; pH:7.8–8.5 | Working volume: 5 L; cycle time: 6 h | Granules size: 1.5 mm; SVI: 75 ± 2 $mLg^{-1}$; COD: >90% removal | [35] |
| Textile | COD: 249 ± 65; $NH_4^+$ - N: 25.6 ± 3.4; TKN: 34.2 ± 5.1 | Working volume: 9 L SBBR; HRT: 11 h | COD: 82.1 ± 3.6% removal; $NH_4^+$ - N: 95.0 ± 7.4%; TKN: 87.5 ± 5.3%; VSS: 19.3–30.7 $gL^{-1}$ | [36] |
| Soybean processing | $_s$COD: 21100 ± 2600; TN: 974 ± 112 | Working volume: 6 L; cycle time: 4 h; wastewater was diluted to get COD 2000 $mgL^{-1}$ | Granules size: 1.22 ± 0.85 mm; SVI: 30.8 ± 5.3 $mLg^{-1}$; COD: 80% removal; MLSS: 7 $gL^{-1}$ | [37] |
| Winery | COD: 2760–3350; $NH_4^+$ - N: 6.18–6.43; pH: 6.5 | Working volume: 3 L; aerobic granules were used as an inoculum; HRT: 8 h | Granules size: 3–4 mm; COD: 90% removal; MLSS: 13.2 $gL^{-1}$ | [38] |
| Dairy industry | COD: 2800; BOD:1600; TN: 40; TP: 30 | Working volume: 5 L; cycle time: 8 h; Activated sludge as an inoculum | SVI: 100 $mLg^{-1}$; COD: 90% removal; TN: 80% removal; TP: 67%; MLSS: 3.5 $gL^{-1}$ | [39] |
| Rubber industry | COD: 850; TN: 278 | Working volume: 0.6 L; cycle time: 3 h | Particle size: 1.5 mm; SVI: 22.3 $mLg^{-1}$; COD: 96.5% removal; Ammonia: 95% removal | [40] |
| Fermented soy sauce | COD: 5400; BOD: 2620; TN: 70; TP: 55 | Working volume: 3 L; aerobic granules were used as an inoculum | Granules size: 2.0–2.5 mm; SVI: 28 $mLg^{-1}$; COD: 87% removal; Ammonia: 76% removal; MLSS: 9.9 $gL^{-1}$ | [41] |

large-scale wastewater treatment plants (WWTPs) utilizing AGS. Nereda® has installed more than 90 large-scale AGS plants, and the work continues to speed up the wastewater treatment process. AGS technology has been extensively reported to treat wastewater with a daily capacity ranging from 100 to 600,000 cubic meters [18]. This approach uses aerobic microorganisms to remove organic pollutants, making it appropriate for municipal and industrial wastewater treatment. It has been established that AGS-based plants produce high-quality effluent with better nutrient removal efficiency and reduced sludge generation [51]. Observations of AGS facilities in operation from 2010 to the present reveal a substantial increase in the usage of this technology in wastewater treatment [52]. Recently, the implementation of AGS systems has significantly expanded, as shown in Fig. 3. Leading researchers' research on AGS technology and AGS startups. Several case studies demonstrate that AGS replaces earlier wastewater treatment technologies such as anoxic/oxygen CAS and oxidation ditch CAS. This adoption is prompted by the need to increase capacity and the desire to comply with progressively rigorous.

## 3. Techniques for rapid startup of AGS

### 3.1. Inoculum

The prolonged initiation phase remains a significant obstacle that hinders the full-scale implementation of AGS in real-scale treatment plants [53]. Granulation without an inoculum can enhance startup periods before achieving mature granules. The startup period without inoculum for aerobic granulation can last several months to even a year when treating real wastewater. However, researchers have successfully reduced the startup time with various strategies. The methodologies proposed for obtaining aerobic granulation have presented significant resource demands and limited clarity in their execution, prompting the need for greater insight into the underlying mechanisms governing granulation and long-term startup [54]. To overcome this limitation, it is recommended to calculate risks, like bacteria that promote microbial aggregation and accelerate production. Considering this problem and the need to find inexpensive options for improving aerobic granulation technology, AGS startups should use inoculum [55–57]. Before the reactor inoculation begins, the inoculum must undergo an adaptation procedure to ensure that the microbes assimilate the new substrate while maintaining maximal microbial diversity [6]. The adaptation procedure requires putting the inoculum in touch with the substrate at a volume ratio of 50/50, utilizing an aerated conical tank. The assimilation of the substrate is determined by monitoring the reduction of the chemical oxygen demand (COD) until it reaches a value greater than 40% [6,24,58]. Once the inoculum is ready then is used in the AGS reactor as a substrate for the development of mature granules. Four distinct stages

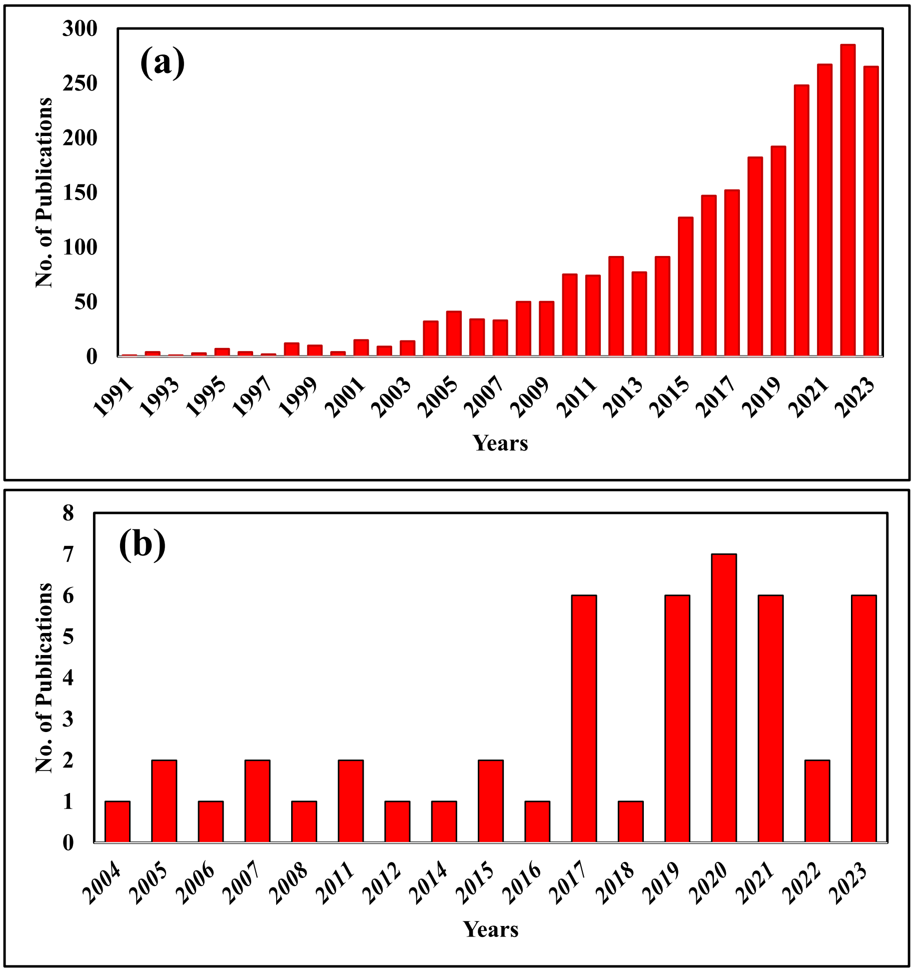


**Fig. 3.** Development of AGS technology (a) and AGS startups (b) in terms of year of publications from 1991 to 2023, Web of Science (Clarivate Analytics, USA).

for the development of AGS: i) cell-to-cell contact, ii) initial attachment of microbes to forms aggregates, iii) improved attachment by generation of EPS, and iv) shaping up of granules by hydrodynamic shear force. Since aerobic granules are produced from flocs, the first two steps, such as cell-to-cell contact and initial attachment of microorganisms, are not a prerequisite for commencing the granulation process. Granulation from identified individual bacterial cultures requires the first two processes [2,59]. The attachment and detachment processes are part of a dynamic development system; therefore, the other two processes are always occurring in AGS reactors as shown in Fig. 4. AGS with inoculum allows rapid evaluation of the capacity and potential to produce aerobic products using different inoculums [57]. Establishing a robust bacterial population in the reactor is one of the main steps that must be done for the inoculated AGS reactor. This requires adding a microbial culture to the reactor [60]. When starting aerobic granular sludge with inoculum, seed culture ensures that the appropriate bacteria are introduced into the wastewater. This facilitates speedy growth and enhances the system's stability [61,62]. Additionally, shear strength and hydraulic retention time also impact granule formation, especially on the formation and shape of the granules [63]. [64] have investigated that by storing aerobic granules as seed sludge, the time required to start the AGS system can be reduced.

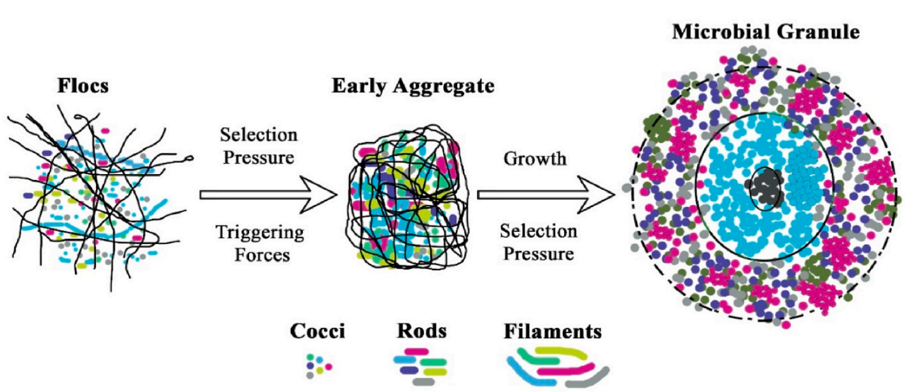


**Fig. 4.** Mechanism of formation of mature granules in AGS SBR.

In cases where stored granules are unavailable, other seed cultures can also be used for the granulation, as shown in Table 2. Table 2 provides a summary of AGS initiatives that use inoculum. The seed culture is specifically designed to grow in the reactor and improve the organic matter in the wastewater [65]. Seed cultures are available from commercial suppliers or similar wastewater treatment plants. When adding seed sludge, it is essential to maintain suitable conditions in the reactor for microbial growth. The conditions that should be kept are pH, temperature, and oxygen levels. Bacteria must be monitored regularly to ensure the reactor grows and functions appropriately. The effectiveness of these products in purifying wastewater lies in their superior ability to decompose organic compounds, surpassing the capabilities of

**Table 2**
AGS startups with inoculum for treatment of wastewater.

| Wastewater type | Wastewater characteristics (in mg $L^{-1}$) | Scale | Inoculum type | duration | System operational conditions | Performance of the system | Ref |
|---|---|---|---|---|---|---|---|
| Synthetic | COD: 400 | Pilot | Stored granules | 40 days | Working volume: 34L; internal dia:19 cm; height: 160 cm; cycle time: 4h; airflow rate: 20 $Lmin^{-1}$; velocity:1.2 $cms^{-1}$; temp: 25 °C | Granules size: 1.28 ± 0.03 mm; SVI: 28.4$mLg^{-1}$; COD: 96% removal | [64] |
| Synthetic | COD: 4500–6000; $_S$COD: 3000–3600; TKN: 400–450; $NH_4^+$-N: 250-290 | Lab | Flocculent sludge | 105 days | Working volume: 5L; dia: 10 cm; height: 80 cm; effective height: 64 cm; headspace: 16 cm; central draft tube depth: 60.5 cm; airflow: 3.5 $Lmin^{-1}$; HRT: 20 h; SRT: 20d; DO: 1–2 $mgL^{-1}$; VER: 50%; cycle time: 10 h; Temp: 29–31.5 °C; pH: 7.25-7.30 | Granular size: 1.8–3.2 mm; SVI: 16–20 $mLg^{-1}$ TS. COD: 97.7 ± 1.5% removal; $NH_4^+$-N: 87.1 ± 11.8% removal; TN: 74.43 ± 11.7% removal | [81] |
| Municipal | MLSS: 5000; COD: 1000; $NH_4^+$-N: 30; TP: 5 | Pilot | Activated sludge & mature AGS (70 + 30%) | 20 days | Working volume: 105.5L; dia: 27.7 cm; height: 175 cm; H/D: 6.3; HRT: 4 h; VER: 60%; cycle time: 6 h | Granules size: 1.62 mm COD: >91% removal; TP: >85% removal; TN: 90% removal | [93] |
| Synthetic | COD: 300 TN: 70 TP: 10 | Lab | Mature granular sludge | 160 days | Working volume: 22L; height: 100 cm; dia: 18 cm; cycle time: 6 h; HRT: 9 h; SRT: 22–33 days; Temp: 20-2 °C | Granules size: 800–850 μm; COD: >90% removal; TP: 90% removal; TN: 60% removal | [83] |
| Synthetic | COD: 220–250; $NH_4^+$-N: 20–24; TP: 3–4; $CaCl_2$: 10; $MgSO_4.7H_2O$: 10 | Lab | Activated sludge | 110 days | Reactor volume: 2.2L; internal dia: 100 mm; height: 300 mm; H/D: 3; cycle time: 6 h; VER: 50%; HRT: 12 h; DO: 3–4 $mgL^{-1}$; Temp: 19-2 °C | COD: 24.37 $mgL^{-1}$; $NH_4^+$-N: 0.25 $mgL^{-1}$; TN: 7.89 $mgL^{-1}$; TP: 0.12 $mgL^{-1}$ | [82] |
| Municipal | COD: 400; TN: 22; P: 3.6; Ca: 80; Mg: 20 | Lab | Sludge from Municipal WWTP | 160 days | Reactor volume: 31.4L; dia: 20 cm; height: 100 cm; cycle time: (8 h, 6 h, 4 h); VER: (35%, 45%, 50%); HRT: (22.8 h, 13.3 h, 8 h) | Granule fractions were dominant, with an average diameter of 2.35 mm and a maximum diameter of 7 mm at HRT 4 h, VER 50%. Pollutant removal efficiency: COD: 87%; TSS 0.88 $gL^{-1}$ | [94] |
| Domestic | COD: 317; $BOD_5$: 123; $NH_4^+$-N: 30.1; P: 4.3; TSS: 21 | Lab | Stored granules | 60 days | Working volume: 2.35L; dia: 6 cm; height: 100 cm; VER: 36%; DO 1.5–2.0 $mgL^{-1}$; cycle time: 3 h; pH: 6.85. | Granules size: 2–3 mm; $SVI_5$: 62 $mLg^{-1}$; settling velocity: 62.18 $mh^{-1}$; MLSS: 3450 $mgL^{-1}$ | [95] |
| Synthetic | COD: 300; $NH_4^+$-N: 60 | Lab | Floc sludge | 100 days | Working volume: 8L; internal dia: 8 cm; height: 170 cm; H/D: 21.2; VER: 50%; Aeration rate: 2-3$Lmin^{-1}$; DO: 6 $mgL^{-1}$; Temp: 15 °C; cycle time: 12 h | Granules size: R1: 1.0–2.0 mm; R2: 2.0–3.0 mm; R3: >3 mm; $SV1_{30}$: 33$mLg^{-1}$; COD: 47.2 $mgL^{-1}$; $NH4^+$-N: R1: 36.2%; R2: 77.2%; R3: 94.9% | [52] |
| Synthetic | COD: 192.6–645; $NH_4Cl$: 43.4–79 $KH_2PO_4$: 3.8–8.6 | Lab | Returned activated sludge | 142 days | Working volume: 8L; H/D: 20; Temp: 26 ± 2 °C; cycle time: (6 h Phase 1–4, 8 h Phase 5); HRT: (12 h (Phase 1–4, 16 h Phase 5); DO: (7–8 $mgL^{-1}$ Phase 1–4; 3 ± 0.5 $mgL^{-1}$ Phase 5); pH: 7–8.5 | Granules size: 667.7 μm; $SVI_{30}$: 30 $mLg^{-1}$; $NH_4^+$-N: 100% removal; TN: 84.3% removal; TP: 91.8% removal; SNED: 61.6% | [8] |
| Synthetic | COD: 200–300; $NH_4^+$-N: 50–60; TP: 2~3.5 | Lab | Aerobic granules | 60 days | GSBR with volume: 1.5L; cycle time: 12 h; VER: 50%; DO concentration: Stage I (1–10 d); 2.0 $mgL^{-1}$; Stage II (11–20 d); 1.2 $mgL^{-1}$; Stage III (21–60 d); 0.8 $mgL^{-1}$; Temp: 28~30 °C | COD; Stage I: 90.13% removal; Stage II: 88.55% removal; Stage III: 84.15% removal; $NH_4^+$-N; Stage I: 90% removal; Stage II: 82.70% removal; Stage III: 90.88% removal; TP: Increased from Stage I – stage III 39.90% 85.78% removal | [80] |
| Synthetic | COD: 2600 ± 450 | Lab | Activated sludge | 100 days | Working volume: 16L; internal dia: 150 mm; air flow rate: 28$Lmin^{-1}$; air velocity: 2.8 $cms^{-1}$; cycle time: 4 h | Granules diameter: 952–1330 μm; SVI <50 $mLg^{-1}$; COD: 96 ± 2.7% removal; $NH_4^+$-N: 92% removal; $PO_4$–P: 96% removal | [74] |
| Domestic | COD: 200–400; TN: 30–45; $NH_4^+$-N: 10–20; TP: 1-4 | Pilot | Dewatered sludge | 80 days | Reactor volume: 140L; Length: 1.68 m; width: 0.22 m; dia: 0.4 m; HRT: 16 h; Temp: 25 ± 5 °C | $SVI_5$: 40 $mLg^{-1}$; COD: 83.23–93.03% removal; $NH_4^+$-N: 85.13–100% removal; TN: 22.0–2.9% removal | [96] |
| Synthetic | COD: 8000; TN: 450; TP: 90 | Pilot | Activated sludge & AGS (75 + 25%) | 24 days | Working volume: 105.46L; dia: 27.7 cm; height: 175 cm; H/D ratio: 6.3; VER: 60%; cycle time: 6 h; superficial gas velocity: 1.2-2 $cms^{-1}$; Temp: 15–20 °C | Average particle size: 1.58 mm; SVI: 67.64 $mLg^{-1}$; SVI30/$SVI_5$: 0.91 $mLg^{-1}$; EPS: 268.90 mg $EPSg^{-1}$ MLSS; Water content: 98.16% | [97] |
| Municipal | COD: 800; TN: 40; TP: 8 | Lab | Dewatered sludge | 120 | Working volume: 3.2L; dia: 9 cm; height: 52.1 cm; Temp: 25–30 °C; HRT: 5.6, 6.4; 7.2, 8 h | 70.25% of granules size range 0.5–2 mm; COD: >90% removal; TN: 80% removal; TP: 90% removal | [98] |
| Synthetic | sCOD: 1500; $NH_4^+$-N: 43 ± 5; $NO_2^-$N: 43 ± 5; $NO_3^-$N: 43 ± 5; $PO_4^{3+-}$P: 43 ± 5 | Lab | Dried granules | 60 | Working volume: 4.4L; internal dia: 8.9 cm; HRT: 11.4 h; VER: 35%; cycle time: 4 h | Average granules size: 2.7–2.9 mm; COD: 97% removal; $NH_4^+$-N & $PO_4^{3+-}$P: BDL | [99] |

conventional wastewater treatment methods. Once the bacteria are cultivated and functioning efficiently, the aerobic granular sludge reactor can initiate the standard wastewater treatment process [57,66,67]. It is also vital to maintain optimum conditions and regularly observe the reactor to guarantee the proper functioning of the microorganisms [20].

AGS formation has been significantly simplified using a quick settling time. The rapid formation of granules depends on a brief settling period [68,69]. However, directly utilizing a very short settling period may lead to insufficient granule retention, which would shorten the formation duration or an ineffective startup of AGS. Therefore, the suitable duration of settling time plays a vital role in achieving the rapid development of AGS [70,71]. Liu and Tay [72] addressed the development of aerobic granules is also significantly inclined by organic loading when exposed to a high OLR of 8–12 kg COD/$m^3$·d. The granulation of aerobic granular sludge (AGS) occurred rapidly, taking around 120–180 h, resulting in an average particle size of approximately 800 μm. Han et al. [73] have summarized that an increase in OLR within a particular range (2.5–15 kg COD/$m^3$.d) resulted in a higher rate of granulation, larger particle diameter, and reduced particle density. Using seed sludge with a high degree of hydrophobicity characteristic can speed up granulation in AGS systems. Hydrophobicity is the tendency of certain substances to repel water molecules, and various microorganisms exhibit this trait. Seed sludge with high hydrophobicity characteristics can promote microorganisms' initial adhesion to the granules' surface, which can further aggregate and form a cohesive matrix, creating stable, compact granules [74]. The scientific literature has demonstrated that improved granulation can be achieved by introducing metal ions, altering the seeding sludge composition, and bio-augmentation using specialized strains [73]. Extracellular polymeric substances (EPS) have a substantial effect on the aggregation, granulation, and stability of activated sludge by microorganisms. Diverse variations of EPS possess distinct physical and chemical characteristics that produce various impacts on the properties of sludge [74,75].

### 3.1.1. Inoculum selection and preparation

The startup process of the AGS reactor involves crucial steps such as inoculum selection and preparation. To ensure the successful commencement of the process, it is essential to select an appropriate inoculum to provide the necessary microbial diversity and activity required for stable granule production [57,76]. The type of wastewater, efficacy of existing treatment plants, composition, and role of microbial organisms are some factors to consider when choosing an inoculum [77,78]. Wastewater treatment plants that have used AGS in the past would be good inoculum sources. This approach facilitates obtaining thriving and heterogeneous populations based on specific wastewater components [79]. However, the inoculum is found in wastewater treatment plants and natural environments such as rivers, lakes, and wetlands. This means that microbes can cause AGS to occur [80]. The inoculum must be prepared and processed before starting the reactor. This must be adjusted on the inoculum to ensure the reactor's operating parameters and desired effluent quality. Batch experiments have been performed to ensure the effectiveness of microbial cultures. In these studies, the inoculum was exposed to varying temperatures, pH, and organic loading levels in wastewater. These studies evaluated microbial activity and setting capability based on two critical aspects of AGS reactor startup [8,81]. It is also important to condition the inoculum with nutrients and follow trace minerals to encourage organism development and digestion. Giving carbon and nitrogen sources, such as glucose and ammonium, can successfully stimulate microbial development and contribute to the formation of granules. Expanding the following components, like iron and phosphorus, can improve microbial digestion and lighten any nutrient lacking [82]. Appropriate planning of the inoculum can lead to viable and feasible wastewater treatment, upgrading the productivity and solidness of AGS reactor startup [83–85].

### 3.1.2. Factors affecting AGS reactors with inoculum

The inoculum is the initial biomass that is used to start the reactor and influence the granulation process [86]. Many operational factors affect how an inoculated aerobic granular sludge reactor starts. These factors can affect the performance of the microbial community within the reactor, which can affect growth and stability [87]. Some of the factors that affect the performance of AGS reactors with different types of inoculums are:

3.1.2.1 Inoculum source: The source of the inoculum can be activated sludge, mature granules, or other types of biomasses. The inoculum source determines the microbial diversity, the granulation time, and the pollutant removal efficiency of the AGS reactor. For example, using mature granules as inoculum can shorten the start-up period and improve the stability of the granules, compared to using activated sludge [86].

3.1.2.2 Inoculum concentration: The inoculum concentration is the amount of biomass that is added to the reactor at the beginning. The inoculum concentration affects the initial organic loading rate, the settling velocity, and the granule size of the AGS reactor. For example, using a high inoculum concentration can increase the organic loading rate and the settling velocity, but decrease the granule size, compared to using a low inoculum concentration [88].

3.1.2.3 Aeration flowrate: the aeration flowrate is the amount of air that is supplied to the reactor to provide oxygen and mixing. The aeration flowrate affects the dissolved oxygen level, thehydrodynamic shear, and the aerobic fraction of the AGS reactor. For example, increasing the aeration flowrate can increase the dissolved oxygen level and the aerobic fraction, but decrease the granule size and the denitrification capacity, compared to decreasing the aeration flowrate [86]

Operating parameters such as temperature, pH, and dissolved oxygen (DO) concentration can significantly impact microbial activity and metabolism in AGS reactors [85]. AGS reactors generally operate between 20 and 30 °C because these temperatures encourage bacterial growth and granule formation [62]. The availability and solubility of nutrients and the composition of microbial communities are generally affected by pH level. AGS reactors generally need to start in the 6.5–8.0 range. Air velocity also impacts oxygen transfer and aeration rate, two processes essential for microbial metabolism and particle production [89]. Initially, the recommended DO range for AGS reactor is generally 2–4 mg/L because this supports both anaerobic and aerobic microbial activity [90]. Microbial species, activity, and solubility are examples of inoculum variables that can affect the startup of the AGS reactor [91]. Inoculum conditioning can improve these properties, increasing the AGS reactors' stability and performance. However, the availability and composition of the substrate can also affect the AGS reactor startups, which in turn affects microbial growth and metabolism. Selecting substrate components should ensure carbon and nutrient balance while eliminating interfering substances that may interfere with microbial activity and particle formation. The microbial culture and the amount of organic loading rate are affected by substrate availability, which in turn affects the formation of the granules [20,65,92].

## 3.2. Polymer additives

AGS reactors rely on forming dense, compact granules of microorganisms that settle quickly and efficiently, allowing for high removal rates of organic matter. However, the slow startup of AGS reactors has been identified as a significant challenge for their widespread implementation. Developing stable and mature granules can take several months, resulting in high operational costs and delayed implementation. Using polymers in the startup offers a promising solution to this obstacle [100,101]. Polymers are essential in AGS treatment because they can enhance the formation of the granular sludge and improve the efficacy of the biological treatment process. Typically, polymers intended for wastewater treatment are available in dry granular or liquid form. Chemical agents facilitate solid-liquid separation by inducing the formation of flocs from colloids.

The colloid's charge, chemical composition, and particle size influence the coagulation processes. Sludge particles usually have a negative charge, but some may also have a positive charge [100,102]. Polymers used in the AGS reactors have two methods. One which is described by Zou et al. [103], prepared the polymer base sludge aggregates through batch study and then used that polymer-based sludge in the AGS reactor. The second one which is described by Jalali et al. [104], optimized the polymer dosages and then used that dosage into the AGS reactor. The dewatering efficiency can be enhanced by using cationic and anionic polymer combinations. Chemical conditioning alters the sludge structure and creates more gasps between the particles. This facilitates water removal during dewatering. The coagulation/flocculation process aggregates small colloidal particles into large flocs, enhancing dewatering efficiency. The efficiency and advantages of dry and liquid polymers used in operation depend on their respective concentrations. For instance, dry polymers typically exhibit more than 90% chemistry, while emulsion polymers contain approximately 30% [44]. According to some research reports, aerobic granulation can be promoted by metal cations and polyaluminium chloride, which aid in bacterial accumulation by neutralizing negative charges on the microbial surface and stimulating the creation of extracellular polymeric substances (EPSs) [105]. However, Polymer additives are better than other metal ion additives for the rapid startup of aerobic granular sludge because they can stimulate the production of extracellular polymeric substances (EPS), which are essential for the formation and stability of granules. EPS acts as a glue that binds the microbial cells together and protects them from environmental stress. Polymer additives, such as chitosan, alginate, and polyacrylamide, can enhance the EPS content, hydrophobicity, and mechanical strength of granules, and reduce the granulation time and sludge volume index [105,106]. Metal ion additives, such as calcium, iron, and aluminum, can also improve the granulation process by neutralizing the negative charges on the cell surface and forming bridges between EPS and cells. However, metal ion additives may have some drawbacks, such as increasing the metal content in the effluent, affecting the microbial community structure, and inhibiting biological nutrient removal [107,108]. Therefore, polymer additives are more environmentally friendly and effective than metal ion additives for the rapid startup of aerobic granular sludge.

Polymers can help to improve the aggregation and adhesion of microorganisms, increase the mechanical strength and compactness of the granules, and reduce the sludge volume and excess sludge production. However, the type and dosage of polymers may have different effects on the granulation process and the performance of AGS because a consistent and excessive introduction of polymers into the AGS results in the precipitation of these polymers, leading to an elevated ash content in the sludge and a reduction in bioactivity [104]. The selection of polymer dosages and their types is also a very important factor because of the long-term impacts that could be on increasing the EPS content and sedimentation in the AGS reactor. The timing and frequency of the polymer addition should be aligned with the operational cycle and the granulation process to achieve optimal effects and minimize the negative impacts of the polymer addition [109]. Settling time and reactor exchange ratio are the most effective factors in forming aerobic granular sludge. SVI is a usual standard parameter that simultaneously considers these two factors. For obtaining optimum cationic polymer concentration, the changes in SVI value versus polymer concentration should be plotted [104]. Although polymer additives may improve the granule formation without reducing treatment efficiencies, there are still environmental concerns due to the fate and toxicity of discharged excess sludge. Biopolymers from natural sources, often used in food preparation, are attractive candidates for AGS additives. Polysaccharides derived from microbial or other natural sources, including alginate, chitosan, agar, xanthan gum, cellulose, and polyhydroxyalkanoates, are biodegradable, non-toxic, and structurally like EPS-AGS. Therefore, biodegradable polymers are the best choice to overcome these types of issues during the operation of the AGS reactor [103]. Biodegradable polymers used as a coagulant can neutralize the electrostatic charge of sludge particles and promote their coalescence, including that of suspended solids. This destabilization leads to particle aggregation and eventual sedimentation. The choice of coagulant largely influences the effectiveness of the dewatering process, as each type possesses unique structural properties [102,104].

Furthermore, the intermixing conditions dictating the amalgamation of sludge and chemical additives represent a crucial aspect that should not be disregarded [110]. Empirical evidence has revealed that the polymer and subsequent mixing significantly influence the distribution of floc sizes as a result. Synthetic organic polymers are commonly employed as conditioning agents for waste sludge, primarily due to their cationic nature that minimizes the electrostatic repulsion between polymer molecules and biogenic sludge particles, consequently facilitating the formation of larger and more robust flocs. However, the harmful effect of these polymers on aquatic systems has been brought to light [111].

The maximum size of granules in AGS reactors using polymer solutions varies depending on the type and concentration of polymers, the hydraulic shear force, the feeding mode, and the microbial composition [104]. The average granule size using polymers can range from 0.5 mm to 4 mm [101]. The surface area of the granules offers a source of easily accessible carbon for the bacteria to utilize. The organic matter in the wastewater may degrade more quickly and entirely because of the bacteria's ability to multiply and increase more swiftly [112]. The process generally involves establishing a microbial community in the reactor and providing the necessary nutrients and circumstances for the bacteria to produce and form granules [111,113]. Different studies on the evaluation of AGS technology using polymers for treating wastewater are summarized in Table .3. Polymers can be injected into the reactor during the preliminary stages of the AGS reactor process to promote the growth and development of granules. They can enhance granule stability, reduce startup time, and improve the overall performance of AGS reactors [102]. Therefore, polymers play a significant role in sludge dewatering for rapid granulation and to reduce startup time. Two types of polymers have been extensively investigated for their effectiveness in the fast startup procedure of AGS reactors: natural and synthetic polymers [114].

### 3.2.1. Natural polymers

Natural polymers are utilized in the AGS process to enhance the granules' mechanical strength and settling properties [101]. They can also serve as substrates for the development of microbes, which may aid in the granules' formation [75]. Natural polymers can increase the aerobic granular sludge process's cost-effectiveness, energy, and treatment efficiency. Biological polymers can be obtained from plants, animals, and microorganisms and are biodegradable. In aerobic granules, natural polymers include proteins like gelatin and collagen and polysaccharides like chitosan, alginate, and cellulose [114,115]. Numerous studies have indicated how natural polymers can help promote mature granules during the startup of an AGS reactor. For instance, it has been demonstrated that using chitosan, a biopolymer made from chitin, increases the creation of compact and solid granules and raises the reactor's nutrient removal efficiency [101,102]. The capability of the polysaccharide alginate, made from brown seaweed, to aid in granule formation and stability has also been researched. The natural polymers alginate and cellulose can support granules' structure and steady development [113]. Natural polymers facilitate sludge dewatering by inducing flocculation, a process wherein a network of particles is formed and binds together to produce larger, heavier aggregates. This can separate sludge from water efficiently. The efficiency of sludge dewatering through polymers can depend on the type and concentration of polymers and the percentage of solids in the sludge. Every polymer has different properties when used for sludge dewatering [100,111].

**Table 3**
AGS startups using polymers as an additive for wastewater treatment.

| Wastewater type | Wastewater characteristics (in mg $L^{-1}$) | Scale | Duration (days) | Polymer type | dosage strategy | System operational conditions | Performance of the system | Ref |
|---|---|---|---|---|---|---|---|---|
| Synthetic | COD: 350; $NH_4^+$-N: 28; $PO_4^{3-}$ -P: 7 | Lab | 60 | Natural | Chitosan-based sludge aggregate with 0.5%, 1%, 2%, 5%, 10%, 20% dosage | Working volume: 3L; height: 32 cm; dia: 11 cm; VER: 50%; Temp: 20 ± 2 °C; air flow rate: 2.5 $Lmin^{-1}$; cycle time: 6 h; HRT: 12 h | $SVI_{30}$: 90.1 $mLg^{-1}$ Granules size: 1300 μm; COD: <40 $mgl^{-1}$; $NH_4^+$-N:71.6% removal; $PO_4^{3-}$ -P: <0.5 $mgl^{-1}$ | [101] |
| Synthetic | COD: 750; $NH_4^+$-N: 50 | Lab | 50 | Synthetic | Polyaluminum chloride (PAC) with 500 $mgL^{-1}$ dosage | Working volume: 2L; height: 1.5 m; dia: 0.05 m; air flow rate: 2.0 $Lmin^{-1}$; Temp: 22–25 °C; pH: 7.2–7.8; cycle time: 6 h | $SVI_{30}$: 38 $mLg^{-1}$; Particles size: 3.2 mm | [112] |
| Synthetic | COD: 1500 | Lab | 50 | Synthetic | Polyacrylamide cationic polymer 0–150 ppm (optimum dose obtained 30 ppm) | Working volume: 32L; dia: 15 m; height: 2 m; cycle period: 12 h; superficial up-flow velocity: 3.6 $cms^{-1}$ | $SVI_{15}$ < 30 $mLg^{-1}$; COD: >90% removal | [104] |
| Synthetic | COD: 300–500; $NH_4^+$-N: 30; $PO_4^{3-}$ -P: 10 | Lab | 60 | Synthetic | Magnesium & PAC augmentation with 20 $mgL^{-1}$ & 1 $gL^{-1}$ | Working volume: 13L; dia: 4 cm; VER: 50%; cycle time: ~ 8 h | In the R2 reactor: Sludge granulation was reduced from 28 days to 14 days. The mean diameter of granules in R2: 2.5 mm, 1.5 mm in R1 | [102] |
| Real | TS: 5780; COD: 6910 | Lab | – | Synthetic | Cationic polyacrylamide (C-PAM): 3 mg $g^{-1}$ TS | Batch study | Granules size: 219.1 μm; Sludge SRF: 87%; water content: 95.04% | [44] |
| Synthetic | COD: 500 | Lab | 56 | Natural | Bone glue: 40 $mgL^{-1}$ | Working volume: 5L; VER: 50%; cycle time: 6 h; Temp: 25 ± 1 °C; pH: 7.0–7.2; SRT: 30d | Granules size: 0.5–3 mm; COD: 86.7% removal; $NH_4^+$-N: 90.6% removal; $PO_4^{3-}$-P: 93.8% removal | [119] |
| Municipal | COD: 720–880; BOD: 300–375; TSS: 335–400; VSS: 300–362; TKN: 35–48; TP: 23–35; VFA: 10-20 | Lab | 100 | Natural | Locust bean gum: 20 $mgg^{-1}$ TSS | Working volume: 6L; dia: 0.1–0.25 m; height: 1 m | COD: 83% removal; SVI: 26 $mLg^{-1}$ | [120] |
| Textile | COD: 2250; $BOD_5$: 144; SS: 172.58; DO: 3.09; TDS: 4961.79 | Lab | – | Synthetic | ZOPAT: 737 $mgL^{-1}$ | Batch study | Average particle size: 96.7 μm; COD: 80% removal; Turbidity: 100% removal; SS: 100% removal; Color: 93% removal | [121] |

### *3.2.2. Chemical/synthetic polymers*

Chemical polymers, frequently employed in wastewater treatment procedures to enhance the settling and dewatering of sludge, can be advantageous for sludge dewatering. Chemical polymers function by aggregating solid particles into flocs, which settle more quickly and are easier to dewater than single particles. These polymers are artificial and not biologically produced. These polymers contain properties like coagulating and flocculating suspended particles or adsorbing contaminants [102,112]. Polyacrylamides are polymers that can flocculate suspended particles and be easily separated from water. It is possible to use polyethyleneimine to coagulate and flocculate suspended wastewater particles, polyvinyl alcohol to make gels that help remove heavy metals and other pollutants, and polystyrene sulfonate to absorb contaminants from wastewater [116]. By producing the flocculation of fine particles, synthetic polymers like polyacrylamide-based flocculants can accelerate dewatering and improve fine retention. Flocculation combines smaller particles into larger ones (flocs) by adding a material (polymer) that makes them adhere to one another. This can be achieved by gentle mixing that increases interparticle collisions. The optimum dosage of different polymers varies depending on the characteristics of the sludge and the polymer [100,101].

### *3.2.3. Factors affecting AGS startups using polymer additives*

The stability of granules is generally related to microbial actions and particle size, as well as the absence of granules breakdown and washout from the reactor. The relationship between granule stability, reactor operating parameters, microbial population, feeding regime, and OLR rate has been studied [109,117]. The feeding regime strongly influences the stability and growth of the granules. A technique has been adopted to address this issue, such as a single aerobic reaction phase, which might be used as the feeding regime [118]. In most studies, the researchers used synthetic wastewater, as shown in Table 3, because synthetic wastewater mainly comprises readily degradable carbon sources. This specified nature of synthetic wastewater shows precise interactions between polymers and AGS [47]. Furthermore, the presence of dominating microorganisms may be affected by the polymer interaction and the usage of real wastewater [89]. When treating sanitary wastewater, longer granulation times and smaller particle sizes are observed. So, further studies should be done to test polymer-based granulation in real-scale wastewater treatment plants.

## 4. Prospects and challenges in AGS startup

Since its inception, extensive research and development have been conducted to study the structure of AGS, factors affecting microbial aggregation, and various operational strategies that can be used to achieve different treatment goals [122–124]. Granular size increased in response to an increase in organic load rate. To guarantee the diameter of particles of the AGS granules, it is necessary to investigate operational strategies. This allows for the reduction in reactor size while maintaining the same level of removal efficacy [125]. To control the size of granules in the reactor, several factors need to be considered and managed effectively such as operational parameters, nutrients balance, pH control, biomass retention, etc. [126]. An increasing number of new WWTPs around the globe are utilizing AGS to recover valued resources from wastewater, including energy, phosphorus, and polymers, which indicates well the future of AGS applications in optimizing treatment capacity [100]. Although there have been attempts to improve the implementation of the AGS process on a global scale, there is still insufficient understanding of certain aspects of this technology [55,85]. Practical implementation and realizing this technology's benefits are inhibited by the absence of comprehension of the fundamental mechanisms regulating biofilm formation, an extended granulation period, and AGS breakdown during continuing operation [97].

Furthermore, the impacts of different modes of operation and effluent composition on microbial ecology and EPS remain unknown [57]. Recent research has investigated how interspecies interactions are crucial in regulating community QS signaling within the microbial community. EPS synthesis and control are closely connected to these interactions. This investigation has utilized biological paradigms known as quorum sensing (QS) and quorum quenching (QQ) [124,127,128]. N-acyl homoserine lactones (AHLs), among the diverse QS signal molecules (autoinducers), facilitate the maturation of granules through the regulation of EPS synthesis [87,129]. Research is needed to examine the utilization of QS and QQ mechanisms in the context of granule formation, the sustained stability of AGS over time, and the obtaining of valued resources from the granules. Environmental stress conditions significantly impact EPS production as well. Toxic compounds, including emerging contaminants and heavy metals, salinity, tensile forces, and the presence of such substances, all induce EPS production as a defensive mechanism.

While these results may indicate that the extracellular proteins generated would facilitate granulation by promoting nucleation, an overabundance of EPS may harm the performance of the granular system [17,130]. As the most appropriate form of the reactor is SBR, this system is utilized for most of the aerobic granulation in the process. However, since most WWTPs use continuous–flow reactors, using AGS startup in a complete–scale treatment plant is challenging [100,101]. To overcome this limitation, various strategies have been implemented. These strategies include incorporating mature granules grown in continuous flow reactor SBRs, introducing novelties in the shape of these reactors, and amalgamating built-in baffles to create aeration and sedimentation in the reactors [102,131]. Keeping the selective pressures of different settling speeds between flocculent and granular sludge and hydraulic shear forces encourages microbial aggregation, EPS secretion, and mature granules' development. This is why the productive growth of AGS is essential in continuous flow reactors [115,124]. When it comes to updating existing facilities with space limits for growth, it has been brought to everyone's attention that for AGS to obtain a modest benefit over competing technologies, aerobic granulation must be shown in a continuous plug flow regime [101,124,132]. It is essential that nutrients, particularly nitrogen and phosphorus, be eradicated effectively. Enhanced nutrient removal efficacy might necessitate implementing optimization strategies [104]. Aeration is a crucial parameter for developing mature granules into the system, but it decreases the removal efficiency of biological nutrients. Thus, different processes should be considered to create and treat wastewater from these mature granules. Furthermore, VFA may be utilized as a carbon and electron donor source for better removal of nutrients [133].

In summary, considering existing research issues and limitations, the following should be the focus of future research on the full-scale applications of rapid startup of AGS.

- To avoid biomass washout and sludge bulking to ensure long-term stability.
- To implement AGS technology from lab or pilot scales to full-scale WWTPs without compromising the system's performance.

## 5. Conclusion

This review provides a complete summary of the primary tactics employed to achieve a rapid startup of AGS, which encompasses the utilization of AGS with inoculum and adding polymers. AGS startups with inoculum are highly effective methods for rapidly developing granules. Nevertheless, operational variables can substantially influence microbial activity and metabolism. Polymers have proven to be a highly efficient approach for rapidly initiating AGS in the past decade. With the addition of polymer, the average duration for granulation is reduced because polymers act as binding agents to agglomerate particles, forming larger and denser clusters. Polymers can help to improve the aggregation and adhesion of microorganisms, increase the mechanical strength and compactness of the granules, and reduce the sludge volume and excess sludge production. However, the type and dosage of polymers may have different effects on the granulation process and the performance of AGS because a consistent and excessive introduction of polymers into the AGS results in the precipitation of these polymers, leading to an elevated ash content in the sludge and a reduction in bioactivity. Biopolymers from natural sources, often used in food preparation, are attractive candidates for AGS additives. Polysaccharides derived from microbial or other natural sources, including alginate, chitosan, agar, xanthan gum, cellulose, and polyhydroxyalkanoates, are biodegradable, non-toxic, and structurally like EPS-AGS. In summary, the rapid initiation of AGS utilizing polymers is a noteworthy alternative for AGS cultivation, and it is expected to get more attention in the future.

## CRediT authorship contribution statement

**Sajid Hussain:** Writing – original draft, Investigation, Conceptualization. **Roberta Ferrentino:** Validation, Data curation. **Khakemin Khan:** Writing – review & editing, Data curation. **Zulfiqar Ali:** Validation. **Muhammad Yousuf:** Validation, Data curation. **Gianni Andreottola:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

## 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.

## Data availability

Data will be made available on request.

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