Rapid cultivation of aerobic granular sludge by xanthan gum in SBR reactors
Sha Liu, Hanhui Zhan, Yaqi Xie, Weijiang Shi and Siming Wang

ABSTRACT
This study focuses on the effect of xanthan gum on aerobic sludge granulation, through close monitoring of the physical and chemical changes of the aerobic granular sludge, and treatment performance. Two sequencing batch reactors (SBRs), R1 and R2, were seeded with activated sludge only (R1) and with a mixture of activated sludge and 40 mg/L of xanthan gum (R2). The results showed that granulation finished on the 20th day in R2, far faster than the granulation time of 30 days in R1. Meanwhile, there was a reliably higher sludge concentration, better settling properties and better particle mechanical strength in R2, and better removal performance of total nitrogen (TN) and chemical oxygen demand (COD). The results demonstrated that seeding xanthan gum enhanced the aerobic sludge granulation in the SBR. Maybe its anionic and hydrophilic surface characteristics facilitate interactions with cations and other polysaccharides, inducing stronger gelation, which promoted the formation of particles or increased the internal relationship between particles, thereby increasing the cohesion within the sludge, so that the granular sludge was not easily broken.

Key words | aerobic granulation, concentrated sludge, extracellular polymeric substances, gelation-facilitated biofilm formation

INTRODUCTION
Aerobic granulation is a relatively new technology for wastewater treatment, and has a number of promising features (Nancharaih & Kiran 2017). As a special form of activated sludge by microbial self-immobilization (de Kreuk et al. 2007; Franca et al. 2018), aerobic granular sludge (AGS) possesses a compact and strong microbial structure, good settling properties and high biomass retention, bringing both operational and financial advantages (Adav et al. 2008; Ni & Yu 2010; Gao et al. 2011). However, a reluctance to accept this technology largely comes from perceptions of a long start-up time and low granule stability (Lee et al. 2010; Pijuan et al. 2011). Therefore, fast granulation is crucial for making AGS technology an engineering application.

In this regard, researchers have put forward some methods to promote aerobic sludge granulation, such as artificial activated carbon particles as a carrier, so that bacteria can be more easily adsorbed on the surface to form the core, thereby reducing the granulation time to some extent (Zhou et al. 2015). Furthermore it has been reported that the addition of positive divalent and trivalent ions such as Ca\(^{2+}\), Mg\(^{2+}\) and Al\(^{3+}\) with coagulation properties shortened granulation time significantly: adding Ca\(^{2+}\) can mature AGS 16 days ahead of schedule, and the addition of Mg\(^{2+}\) can make the maturation time of AGS decrease from 32 days to 18 days. The addition of Ca\(^{2+}\) and Mg\(^{2+}\) promoted the secretion of extracellular polymeric substances (EPS) and the increase of protein and polysaccharide (Jiang et al. 2003; Li et al. 2009; Liu et al. 2014a, 2014b). These indicate that appropriate additives may be a practical way of accelerating the cultivation of aerobic granules.

EPS are made up of high-molecular-weight secretions from microorganisms, binding with cells to form a vast net-like structure for protecting cells against external stress (Dong et al. 2017). These polymers are primarily exopolysaccharides, extracellular proteins, humic acid, lipids and extracellular DNA. Most studies suggest that polysaccharides and proteins are major influencing factors in the formation of biofilms and granular sludge. Exopolysaccharides can form a three-dimensional structure by adhesion of bacteria, so that more cells and particles are attached together into a bigger and denser community (Tay et al. 2001). Sun et al. (2017) discovered that adding calcium...
alginate gel beads can promote the formation of the granular sludge, and that sodium alginate is a natural polysaccharide.

Xanthan gum is a complex microbial exopolysaccharide industrially produced from glucose via fermentation by the plant–pathogenic bacterium, *Xanthomonas campestris* pv. *campestris* (Rosalam & England 2006). In static conditions, a small amount of xanthan gum (in most foods, 0.5%) induces a large increase in the viscosity of a liquid. Moreover, unlike other gums, xanthan gum shows high stability under a wide range of temperatures and pH (Zohuriaan & Shokrolahi 2004; Sun & Gunasekaran 2009), and it is known as a thermo-stable biopolymer (Lambert & Rinaudo 1985). In addition, its anionic and hydrophilic surface characteristics facilitate interactions with cations (Bergmann et al. 2008; Nolte et al. 1992) and other polysaccharides, such as glucose, mannose (C6H12O6), potassium gluconate (C6H11KO7), acetate (CH3CO2\(-\)), and pyruvate (CH3COCOOH), inducing stronger gelation (Laneuville et al. 2006). In the field, it is applied as an additive in concrete to increase viscosity and prevent washouts. Chang et al. (2015) discovered xanthan gum has a significant strengthening effect on the treated soil. This strengthening is achieved by increasing the inter-particle relations within the soil, thereby increasing the cohesive forces within the soil.

Hence, the main purpose of this work is to investigate the feasibility of rapidly achieving aerobic granulation in a sequencing batch reactor (SBR) with xanthan gum. Physical characteristics, treatment performance and microstructure were monitored for about a month, which allowed assessment of the effectiveness of the strategy in reducing the granulation time.

**MATERIALS AND METHODS**

**Reactor operation and feed water**

Two identical Plexiglas SBRs (45 cm in height, 8 cm in internal diameter) with a working volume of 2 L were operated in parallel to cultivate aerobic granulation. Aeration was at an airflow rate of 2 L/min, equivalent to a superficial up-flow air velocity of 1.2 cm/s. A 4 hour operation cycle was implemented, including 210 min aeration, 5 min feeding, 4 min decanting and 6–18 min idling, while the settling time was decreased from 15 to 3 min according to the actual situation. Effluent was discharged at a volumetric exchange ratio of 50%. The reactor was operated at room temperature (25 ± 5°C), and the pH ranged from 6.9 to 7.3 without additional adjustment.

The components and their concentrations for the synthetic wastewater are presented in Table 1. The simulated wastewater contained sodium acetate, NH4Cl, K2HPO4 and some micronutrients. The ratio of COD:N:P was kept constant at 100:5:1. The trace element solution (Long et al. 2015) contained the following components (g/L): H3BO3, 0.5; CoCl2·6H2O, 0.5; CuCl2, 0.5; MnSO4, 0.5; AlCl3, 0.5; ZnCl2, 0.5; NiCl2, 0.5; Na2MoO4·2H2O, 0.5.

**Sludge source and seeding sludge with xanthan gum**

The seed sludge was taken from a full-scale domestic water resource recovery facility in Xuzhou, China. The treatment process of this sewage treatment plant is the traditional A-A-O process and the seed sludge was taken from its aerobic tank. The initial activated sludge was sieved through a 100 mesh sieve to remove the impurities with a diameter over 0.15 mm, then the initial mixed liquor suspended solids (MLSS) of activated sludge used as inoculum for the reactor startup was about 3,000 mg/L.

Two SBRs, seeded with different mixtures of xanthan gum and concentrated sludge, were operated to determine the effect of xanthan gum on the granulation time and reactor performance during startup. The first SBR (R1) was seeded only with concentrated activated sludge. The second reactor (R2) contained concentrated activated sludge seeded with xanthan gum, with the concentration being 40 mg/L. Considering that the mass ratio of xanthan gum to soil when xanthan gum was used to treat soil was 1–1.5% (Chang et al. 2015), and the concentration of sludge added was 3,000 mg/L in this experiment, the concentration of added xanthan gum was calculated to be 50–45 mg/L. It was discovered that the addition of 40 mg/L xanthan gum was also beneficial to mixing with sludge during the experiment. Xanthan gum was added while stirring in a six-electric stirrer containing sludge, the stirring being continued for half an hour. Then the sludge in the two reactors was pretreated to facilitate better contact between xanthan gum and sludge (Wang et al. 2016). The pretreatment process was as follows:

**Table 1 | Major composition of synthetic wastewater**

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>1–11</th>
<th>12–19</th>
<th>20–30</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>500</td>
<td>700</td>
<td>1,000</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>25</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>5</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>

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a vacuum filtration machine was used to produce dewatered activated sludge with 80% water content in both reactors. The resulting sludge cakes were gained with approximately thickness of 0.55 mm thick. Utilizing a sieve (0.55 mm aperture), the sludge cakes were cut and then put into the reactors. This pretreatment method is beneficial to the mixture of xanthan gum and sludge, and it promotes good contact between xanthan gum and the sludge.

**Analytic methods**

**Physical and chemical index**

Chemical oxygen demand (COD), total nitrogen concentration (TN), ammonia nitrogen concentration (NH₄-N), nitrate nitrogen concentration (NO₃-N), nitrite nitrogen concentration (NO₂-N), MLSS, sludge volume index (SVI) were measured according to Standard Methods (APHA 1998). Quick determination of COD by the potassium dichromate method, and TN, NH₄-N, NO₂-N and NO₃-N were determined by the alkaline potassium persulphate digestion-UV spectrophotometric method. Nessler’s reagent spectrophotometry, N-(1-naphthyl)-ethylenediamine photometric method, and ultraviolet spectrophotometry, respectively. The SVI value was measured after the mixture had precipitated for 30 minutes. MLSS were determined by gravimetric method. The pH in the reactors was tested by pH1 meter. Size distribution of the particles was measured by the wet sieving separation method. The size distribution was obtained by recording the proportions of the granular sludge with certain size of the total sludge’s MLSS. 100 mL samples of SBR mixed liquor taken at the end of the aeration period were filtered through a series of sieves with apertures of 1 mm, 0.55 mm, 0.38 mm, and 0.21 mm. The granulation time was defined as the time when the 10th percentile bacterial aggregate size was larger than 200 μm (i.e. 90% of the particles were larger than 200 μm) (De Kreuk et al. 2007; Pijuan et al. 2011). The mechanical strength is expressed in terms of integrity coefficient (%), which is defined as the ratio of residual granules to the total weight of the granular sludge after 5 min of shaking at 200 rpm on a platform shaker (Liu et al. 2014a, 2014b).

**Microscopy and morphology of granules**

The appearance of aerobic granules was observed with a digital camera. The microstructure and morphology of the aerobic granules were observed with a scanning electron microscope (SEM) (FEI Quanta TM 250, USA). Aerobic granule samples were first fixed with 2.5% glutaraldehyde and then dehydrated by successive passages of 50, 70, 80, 90, and 100% ethanol solution. The dehydrated granules were replaced by isoamyl acetate and then dried for 8 hours in a silica gel desiccator. The dried granules were sprayed with a thin conductive coating and then observed by SEM.

**Extracting extracellular polymeric substances**

Seviour et al. (2009) have proposed that the stability of AGS is closely related to the EPS of sludge. Therefore, the variation of EPS content and components in the sludge from both reactors was measured. The extraction of EPS from AGS and the polysaccharide and protein contents of the EPS are described by Hong et al. (2017). The heat extraction method was used to extract the EPS from the activated sludge and granules. Polysaccharide content was determined using the anthrone–sulfuric acid method with glucose as the standard. Protein content was analyzed using the modified Lowry method with bovine serum albumin as the standard. The EPS composition was measured by a three-dimensional fluorescence spectrometer (Aqualog-UV-NIR-800-C). The three-dimensional fluorescence spectrum was scanned from an excitation wavelength of 200 nm–500 nm in 2 nm increments, and scanned from an emission wavelength of 250 nm–500 nm in increments of 2 nm. The scanning speed was 0.1 nm/ms and the response time was 0.1 seconds. The spectral data were analyzed with Origin 8.0.

**RESULTS AND DISCUSSION**

By contrast to flocculent sludge, aerobic granule sludge has excellent physical characteristics usually used as symbols of granulation, such as large size, high biomass, simultaneous nitrification and denitrification. Thus, particle size, SVI, MLSS and substrate degradation rate (COD and TN) were determined to know whether efficient granulation had been achieved.

**Morphological change**

The appearance of mature AGS is usually orange yellow or light yellow and regular, smooth and nearly round in shape (Long et al. 2015; Nguyen et al. 2016). After pretreatment, the initial sludge had a compact structure, a cubic shape, dark brown color and about 0.55 mm size. With the experiment running, part of the sludge crushed and the granular shape in two reactors became circular or oval under the action of the hydraulic shear force, while the color of the sludge
gradually changed from dark brown to yellowish-brown. On the seventh day, the granular color had faded a little in the two reactors, but granular color in R2 (Figure 1(c)) was slightly darker and bigger than R1 (Figure 1(a)) owing to R1’s higher sludge damage rate. On the 26th day, when the settling time was decreasing, the aerobic granules became compact and denser with a spherical and smooth outer morphology, and the size increased to 0.5–1 mm. Moreover, AGS in R2 completely changed to yellow, while some sludge remained dark brown in R1. Hence, rapid granulation is more feasible with xanthan gum than without xanthan gum in the same conditions.

**Formation of AGS**

Granule size is a direct parameter to show the growth and aging process in sludge granulation. Granules broke into small particles and flocculent sludge under aeration shear stress after being added to the reactors. 78% of granules turned into flocculent sludge (particle diameter smaller than 0.21 mm) without xanthan gum (R1). However, since broken granules in R2 with xanthan gum only made up 37% after the fifth day (Figure 2), it can be seen that the granules with added xanthan gum were not easily broken in the reactor, so xanthan gum has a significant strengthening effect, which is achieved by increasing the inter-particle relations within the sludge, thereby increasing the cohesive forces within the sludge (Chang et al. 2015). In the subsequent culture, we discovered the granular quantity in two reactors was enhanced from the fifth day to the 20th day, yet R2 always had more microbial biomass than R1 during the following trials.

Complete granulation was defined as the time when more than 90% of microbial aggregates were larger than 0.20 mm in

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**Figure 1** | Morphologic change of sludge in two reactors on the seventh and 30th days, respectively: R1 (a) and (b); R2 (c) and (d).

**Figure 2** | Variation of sludge particle size distribution.
this experiment. A granulation time of 20 days was obtained with xanthan gum, whilst more than 30 days were needed for aerobic granules to develop without xanthan gum, which indicated that granulation time could clearly be decreased through seeding concentrated activated sludge with xanthan gum. Hence, the period of sludge granulation was shortened owing to adding xanthan gum reducing the initial broken granules.

Physical characteristics of AGS

Sludge concentration and settling properties during operation

A concentration of 40 mg/L xanthan gum was added with the influent, the reactor was inoculated with 2 L of activated sludge and the initial MLSS was 3,000 mg/L. The MLSS in R2 was always more than R1 after running the experiment (Figure 3(a)) owing to the unbreakable granular sludge of R2 which was not easily washed out and therefore a large amount of biomass was preserved in the reactor. After the 26th day, the MLSS still increased to nearly 9,000 mg/L in R2 while the MLSS in R1 was always below 6,653 mg/L, which indicated that the aerobic granules in R2 had higher biomass retention at ambient temperature.

SVI is an index to measure the settling performance of activated sludge, which can reflect the loose structure and the coagulation performance of sludge. The profile of SVI showed a declining trend during the operation (Figure 3(b)), which indicated that the sludge's setting property was getting better along with the increase of selection pressure (the sludge settlement time was reduced, more floc sludge was discharged from the reactor and granular sludge was selected). The pretreated sludge was cubic, which has better settling performance, and the SVI in R1 was 80–85 mL/g in the first 14 days, while in R2 it was stable below 68 mL/g. After the 14th day, it reduced rapidly in both R1 and R2 with the final value of 58.7 mL/g and 42 mL/g on the 26th day, respectively. This was mainly caused by the short settling time, after which plenty of the non-fast settling biomass was washed out from the reactor. The SVI change in the two reactors indicated that sludge seeded with xanthan gum had better settling properties.

Mechanical strength of granules

In order to withstand extensive shear stress during operation, sufficient structural integrity in sludge is essential. Cohesion of sludge was measured to reveal how the sludge can resist shear stress as well as control surface detachment and breakage. The mechanical strength measured after filtering through a sieve with an aperture of 0.55 mm on the eighth, 16th and 24th days is shown in Table 2. The granular mechanical strength in R1 was below 90% and inclined to reduce during operation. However, the aerobic granules seeded with xanthan gum had greater mechanical strength and the strength of mature granular sludge was above 95%. Thus xanthan gum improves the mechanical strength and maintains stability under hydraulic shear force.

Reactor performances

COD removal

The COD and nitrogen removal performance of the two SBRs during the granulation period are shown in Figure 4.
R2 slightly had worse removal capability during the first 4 days because of the xanthan gum; xanthan gum is a complex exopolysaccharide therefore it enters the water and raises the COD in the water when a small amount of granular sludge is broken. Subsequently, the removal effect of the two reactors was gradually enhanced and R2 had a slightly better removal capability. After the 20th day, the influent COD concentration was increased from 700 mg/L to 1,000 mg/L, consequently the removal rate in R1 and R2 had decreased to 89.8% and 92.7%, respectively, on the 21th day. This might relate to adaptation to the new environment of the sludge. Finally, the removal rate in R1 and R2 was maintained around 95.7% and 96.1%, respectively.

Hence, the addition of xanthan gum in the formation process of AGS was more suitable for a bioreactor in long-term steady operation which also had the better biodegradation ability.

**TN removal**

Stratification of granules provided aerobic and anaerobic/anoxic layers, which allowed the simultaneous removal of carbon, nitrogen, and phosphorus. The overall profile of effluent TN and NH₄⁺-N showed a downward trend, while their removal rate gradually increased. Effluent NO₃⁻-N was always at a low level during the operation in each reactor.
reactor, almost under the detection limit. In the first 11 days, effluent NO₃-N in each reactor remained under 0.55 mg/L. Accumulation of NO₃-N during the domestication period was because of the high nitrogen loading initially; with the increase in influent concentration of NH₄-N, NO₃-N concentration in R1 was at 2–3 mg/L while it in R2 was stable below 2 mg/L.

R2 showed a stronger denitrifying capability owing to having more particles and a greater proportion of large particles, offering more anoxic zones, after the TN of influent increased to 35 mg/L. The removal rate of TN in R1 decreased to 63.1%, while in R2 it was above 77.5%. When the influent TN increased from 35 to 50 mg/L, the removal rate in R1 dropped to 62.2%, while TN removal had a rate of above 70% in R2. The removal rate in R2 decreased more than in R1 due to the increase of influent nitrogen concentration, indicating that the activated sludge seeded with xanthan gum had a superior ability to resist the impact load.

Variation of EPS content and component

The changes in EPS content were analyzed (Figure 5), revealing that proteins were mainly higher than polysaccharides (Campo et al. 2018). The results in the start-up stage showed that the protein content in R2 increased from 56.67 mg/g MLSS of seed sludge to 79.9 mg/g MLSS of initial granular sludge (12th day). However, there was no obvious variation in polysaccharide content: the concentration varied over the range 18.9–23.8 mg/g MLSS in R2 during the whole process. The addition of xanthan gum had a significant effect on the polysaccharide content of the EPS, xanthan gum being an extracellular polysaccharide secreted by Xanthomonas campestris, which results in a higher polysaccharide content in the sludge after the reactor operation. Furthermore, with the increasing degree of granulation, the protein content continued to increase and reached 89.9 mg/g MLSS on the 26th day. This result illustrated that the high total protein content could be the mechanism by which AGS formed. The relatively high protein content was essential for the cultivation of AGS, consistent with the results of Adav et al. (2008). The ratio of protein to polysaccharide in the two reactors showed an upward trend during the whole operation, from 2.8 to 3.7 when the granulation was complete.

Further to the quantitative analysis of EPS, its main components were analyzed by three-dimensional fluorescence excitation-emission matrix (3D-EEM). Figure 6 shows the 3D-EEM results of EPS in R1 and R2. Each 3D-EEM spectrum provides information about six major substances of EPS (Chen et al. 2003). Figure 6 shows that the 3D-EEM characterization of AGS and its EPS demonstrated two major peaks in the spectrum with excitation-emission (Ex/Em) wavelengths at 200–250/280–380 nm (peak A) and 250–310/320–380 nm (peak B). Peaks A and B were in regions II (aromatic proteins) and IV (tryptophan and protein-like), respectively, proving that protein was the key component for aerobic granulation, consistent with the results of Wang et al. (2013). Note that the protein content in AGS (Figure 6(b)) is higher than that in R1 (Figure 6(a)), which indicates that the xanthan gum affected the amount of EPS but did not affect the type of protein.
Microstructure of aerobic granules observed by SEM

The microstructure of AGS in R1 and R2 is shown in Figure 7; it was observed by SEM on the 32nd day. It was observed that a large number of bacteria were distributed on the surface of the particles (Figure 7(c)). It could be seen that the granules were rich in biological species inside, including bacillus, coccus and filamentous fungus. The cultivated AGS was spherical or ellipsoidal and pale yellow in two reactors. Rough and irregular surfaces could
further provide a larger and rougher surface for floccular biomass attachment under the existing shear forces. There are obvious depressions and voids on the surface of the granular sludge in R1 (Figure 7(a) and 7(d)), while the surface of the granular sludge in R2 is smooth without larger gaps, confirming that granules seeded with xanthan gum would have better mechanical strength, and the depressions and voids of granules in R1 may be caused by hydraulic shear force. Moreover, more spherical microorganisms were attached to the surfaces of the granular sludge in R2 (Figure 7(b)). The granular sludge in R2 has more abundant pores (Figure 7(e) and 7(f)), which are the transport channels for nutrients and gases, and are also the channel for the release of metabolites from microbial discharges within granular sludge, providing a survival and metabolic environment for a variety of metabolic species of microorganisms.

CONCLUSION

The influence of xanthan gum on aerobic sludge granulation was assessed in this study by adding xanthan gum to an SBR. Taking 20 days, granulation efficiently completed in R2 with an extremely high MLSS of 8,961 mg/L, and the AGS had better COD and TN removal efficiencies. The addition of xanthan gum in the formation process of AGS is more suitable for bioreactors in long-term steady operation, so the granules can maintain good morphology and smooth appearance. Its anionic and hydrophilic surface characteristics facilitate interactions with cations and other polysaccharides, inducing stronger gelation. Cultivating granules with xanthan gum produced granular sludge that was not easily broken, effectively avoiding biomass loss that typically occurs during granulation and maintaining the pollutant removal capability of the sludge during granulation. From an economic point of view, xanthan gum has very extensive applications in many fields, it is cheap and the amount used in this experiment was low. However, the optimal dosage of xanthan gum remains to be elucidated. Further experimental studies are required to fully reveal the interactions between xanthan gum and activated sludge during granulation.

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