Enhanced formation of aerobic granular sludge with yellow earth as nucleating agent in a sequencing batch reactor

Q L He\textsuperscript{1}, S L Zhang\textsuperscript{1}, Z C Zou\textsuperscript{1} and H Y Wang\textsuperscript{1,2}

\textsuperscript{1}School of Civil Engineering, Wuhan University, Wuhan 430072, China

Email: hywang96@126.com

Abstract: Enhanced formation of aerobic granulation was investigated by adding yellow earth as a nucleating agent in a sequencing batch reactor with a constant setting time of 10 min. As a result, granules with an average diameter over 1 mm were obtained on the 4th day. The mature granules behaved better than the seed sludge in the water content, specific gravity, sludge volume index, settling velocity, and specific oxygen uptake rate. The yellow earth stimulated the secretion of extracellular polymeric substances, especially proteins. Both chemical oxygen demand and ammonia nitrogen had a removal rate over 90%, and more than 80% of the total inorganic nitrogen was removed even under aeration conditions due to simultaneous denitrification. The enhancement effects of the yellow earth might be based on the unique physicochemical characteristics and short settling time. A settling time of 10 min or more turned out not to be a prerequisite for a rapid granulation process.

1. Introduction

Aerobic granular sludge technology has been proposed as a novel and efficient approach in both domestic and industrial wastewater treatment. With the excellent settling velocity, separate settling tanks are not required, and an 80% area reduction is possible [1]. Aerobic granules are characterized by their compact structure, dense microbial structure, high biomass, and tolerance to high organic loading rate and toxicity. Additionally, the layered structure of aerobic granular sludge provides oxic, anoxic, and anaerobic zones from the outer layer to the deep core, thus creating shelter for co-existence of nitrifying microbes, denitrifying phosphorus removal organisms, as well as the anaerobic organisms. Therefore, simultaneous nitrogen, phosphorus, and chemical oxygen demand (COD) removal can take place from the liquid during an aeration period [2].

However, the operational conditions of aerobic granulation are strictly limited by multiple parameters, including reactor configuration, media and seed sludge, settling time, organic loading rate, volume exchange ratio, hydrodynamic shear force, feast-famine regime, cycle time, and other environmental conditions (i.e. pH and temperature) [3]. Significant efforts have been made to enhance the formation of aerobic granular sludge as well as to reveal the mechanism of aerobic granulation, which impedes the optimization and application of this technology for real use. Distinctly, aerobic granulation in both pilot-scale and full-scale reactors took much longer than in lab-scale reactors [4-7]. Thus, many studies have been conducted in the last two decades to find a way to enhance the formation of aerobic granular sludge [8].

It has been widely acknowledged that a short settling time is essential for fast granulation. To date, almost all granular sludge has been obtained at a settling time lower than 8 min, regardless of an initial low settling time of about 2-5 min or an alternation between higher and lower values [9]. Li et al. [6]
in particular realized aerobic granulation in lab-scale, pilot-scale, and full-scale tests with settling time durations as high as 10, 20 and 40 min. The seed sludge used in their study was obtained from Yancang Wastewater Treatment Plant (WWTP) in China. In this particular sludge, small particles with a low sludge volume index (SVI) of 60-79 mL·g-1 were observed. Short settling time helps discharge sludge flocs with poor settling velocity and enriches the granules. Meanwhile, the short settling time always leads to low concentrations of sludge due to sludge loss by discharge, especially those with an initial low time duration [10]. Therefore, an appropriate settling time is essential for aerobic granulation.

Multiple substrates have been added to accelerate the start-up of aerobic granular sludge by providing nucleating agents first for bacteria to attach. Metal ions (particularly calcium, magnesium, and aluminum) [11-12], metal (like zero-valent iron) [13], and mature granules were believed to be able to enhance the formation of granular sludge. Liu et al. [14-15] studied the enhancement effect of poly-aluminum chloride (PAC) on aerobic granulation and found PAC could accelerate the granulation process. Li et al. [16] reported that dried sewage sludge micropowder as a nucleating agent could enhance formation of aerobic granules. Although these strategies could shorten the start-up period to different extents, the additional cost of adding these external carrier media should be considered. Thus, applying a more economical approach to enhance the aerobic granulation process is desirable and promising for further applications.

Yellow earth, as a common and inexpensive material [17], contains mainly silicon dioxide and aluminum oxide. Contrastingly with the agents mentioned above, yellow earth provides a novel and cost-effective alternative in the enhancement of aerobic granular sludge. For the first time, an initial settling time of a constant 10 min was selected for the granulation process. Ultimately, then, this paper proposes a new approach involving yellow earth in the acceleration of granulation. The present study’s main objectives were to (1) confirm whether a short settling time was a prerequisite for granulation, and (2) whether the granulation process could be accelerated with yellow earth as a nucleating agent.

2. Materials and methods

2.1. Reactor and operation

A column reactor made of Plexiglas was used in the present study (shown in figure 1). In brief, the reactor was 120 mm in diameter and 800 mm in total height, giving an effective volume of 7.0 L with a working height of 700 mm. Thus, the reactor’s effective ratio of height to diameter (H/D) was about 5.2. 3.5 L of synthetic wastewater was fed into the reactor at the beginning of each cycle at a volume exchange ratio of 50%. Air was introduced by a fine-bubble pump at the bottom of the reactor at a constant rate of 2.5 L min⁻¹. A mechanical stirrer was configured for the reactor at a constant speed of 250 rpm. The experiment was conducted at a water temperature of 18 ± 0.5 °C. The reactor was operated sequentially in 3 h per cycle, consisting of 2 min of feeding, 166 min of aeration (stirring meanwhile), 10 min of settling, and 2 min of effluent discharge.
2.2. Seed sludge, agent and media
The seed sludge obtained from Shahu Wastewater Treatment Plant (China) operated with an A/A/O process configuration. The seed sludge was inoculated into the reactor with an initial mixed liquor suspended solid (MLSS) of 7440 mg L\(^{-1}\) after appropriate pretreatment. After a 2-cycle operation (6 h equally), 1 g L\(^{-1}\) of the yellow earth passed through a 50-mesh (corresponding to a diameter of 0.27 mm) sieve was added to the reactor.

Synthetic wastewater used in the present study was made up of the following compositions (per liter): 500 mg COD, 25 mg ammonia nitrogen (NH\(_4^+\)-N), 5 mg total phosphorus (TP), and 1 mL trace solution per 15 L influent as our previous compositions \[18\]. Influent pH was adjusted around 7.5 without control during the operation.

2.3. Analytical methods
COD, NH\(_4^+\)-N, nitrite (NO\(_2^-\)-N), nitrate (NO\(_3^-\)-N), TP, settling velocity (SV), SVI, MLSS, mixed liquor volatile suspended solids (MLVSS), water content and specific gravity were measured according to standard methods \[19\]. A pHS-25 meter and YSI5000 meter were used to measure the pH and DO. Extant respirometry was used to measure the specific oxygen uptake rate (SOUR), following the procedures by Abdullah et al. \[20\]. Total inorganic nitrogen (TIN) was the sum of NH\(_4^+\)-N, NO\(_2^-\)-N and NO\(_3^-\)-N. The morphology was observed, photos were taken, and granule size analysis was conducted via an image analysis system (Image-Pro Plus, V6.0, Media Cybernetics) with an Olympus Microscope (SZX9, Japan). EPS was extracted following a modified heat extraction method by Yang et al. \[21\]. Protein (PN) content was determined by a modified Lowry method, and the content of
polysaccharides (PS) was analyzed using a phenol-sulphuric acid method [9]. EPS was regarded as the sum of PN and PS.

3. Results

3.1. Formation and characterization of granules
Yellow earth was added to the reactor to investigate its effects on the enhanced formation of aerobic granular sludge. The yellow earth distributed itself well in the water solution, and particles of different sizes could be seen clearly by the Telegraph (shown in figure 2a). Small granules (figure 2b and figure c) were observed on the 2nd and 3rd day. On the 4th day, no obvious sludge flocs could be seen in the reactor. Regular and bright yellow granules with an average of 1.06 mm were visible from the outside of the reactor, indicating the completion of the start-up of the granulation process. During the whole operation period, biofilm adhesion phenomenon was observed on the surface of the reactor, implying a moderate biological selector [9]. The granule size analysis was conducted on the aerobic granular sludge on the 4th day and the results are shown in figure 3. As seen in figure 3, the average diameter of the granules was 1.06 mm, and a majority of particles (83%) were in the range from 0.7 to 1.3 mm.

Figure 2. Morphology of yellow earth solution and sludge: (a) yellow earth dispersed in the water phase; (b) sludge flocs on day 2; (c) granules on day 3; and (d) mature granular sludge on day 4.
Compared with the seed sludge, the granular sludge showed improved physiochemical and biological characteristics (Table 1). As an important index for settling performance of activated sludge, the sharp decrease of SVI from 75 to 23 mL g\(^{-1}\) indicated the tight structure of granules and successful granulation, as does the rising of values of SV\(_{30}\)/SV\(_{5}\) from 0.65 to 0.96 [22]. Water content revealed the profile of hydrophilic and hydrophobic cells within granules. In the present study, the water content fell from 99.67% to 98.26%, indicating the enrichment of hydrophobic cells [23]. The increase of specific gravity also confirmed the results [22]. SOUR suggested microbial activity in granular sludge and the significant enhancement of SOUR from 13.58 to 42.8 mg O\(_2\) g MLVSS\(^{-1}\) h\(^{-1}\), indicating that granules were stronger in the organism’s activity than in inoculated activated sludge [20].

Table 1. Attributes of activated sludge in present study (granules on day 4).

| Sludge | SVI (mL/g) | SV\(_{30}\)/SV\(_{5}\) | Water content (%) | Specific gravity | SOUR (mg O\(_2\) g MLVSS\(^{-1}\) h\(^{-1}\)) |
|--------|-----------|----------------|-----------------|-----------------|----------------|
| Seed   | 75        | 0.65          | 99.67           | 1.008           | 13.58          |
| R\(_e\) | 23        | 0.96          | 98.26           | 1.022           | 42.8           |

The profile of MLSS and MLVSS/MLVSS is shown in figure 4. By the visible insight from outside the reactors, the reactors suffered a loss of sludge during the first 7 days. Then it rose slightly afterward. However, the ratio of MLVSS to MLSS increased gradually from 0.47 for the seed sludge to 0.733 for granular sludge on the 11th day, which indicated the higher bioactivity and lower inorganic composition of the granular sludge than the flocs [24].
3.2. EPS and PN/PS
EPS is a vital substance that has long been believed to aid the granulation process [25]. The variations of EPS and PN/PS over operation were shown in figure 5. Compared with the EPS content of seed sludge (92.16 mg g MLSS$^{-1}$), it rose sharply during the formation of granular sludge, which reached a peak (345.92 mg g MLSS$^{-1}$) on the 7th day. In general, adding earth on the first day significantly enhanced the amount of EPS and maintained this trend throughout the operation. The ratio of PN to PS (PN/PS) greatly increased during the granulation process from 1.21 to about 2.45.
3.3. Reactor performance

COD and nitrogen removal performance are presented in Figure 6. As shown in Figure 6(a), over 90% of influent COD was removed throughout the operation with the stable effluent concentration of about 20 mg L\(^{-1}\). As for nitrogen removal (Figure 6(b)), 95% of the influent NH\(^4\)+-N was reduced while about 80% of TIN was eliminated as the effluent NO\(^3\)-N, and NO\(^2\)-N were about 1.6~5.3 and 0~0.4 mg L\(^{-1}\) respectively. At constant aeration conditions, though, obvious nitrogen loss was observed and it could be concluded that denitrification took place during this oxic phase.

Interestingly, the TP removal rate varied from 17 to 46% (data not shown), demonstrating the simultaneous nitrogen and phosphorus removal (SNPR) capacity of both reactors [2].

![Figure 6](image1)

Figure 6. Reactor performance: (a) COD removal and, (b) nitrogen removal.

4. Discussion

A short settling time under 8 min has long been assumed as a prerequisite for aerobic granulation in SBR. The present study proposes a different view. Successful aerobic granulation was realized with a constant settling time of 10 min. Settling time, as a hydraulic selection pressure, was once believed to drive the hydraulic retaining of the granular type biomass [26] by discharging the sludge flocs with poor settling ability. The settling time in combination with the height of the reactor allowed only particles with a settling velocity larger than 2.1 m·h\(^{-1}\) to be maintained in the reactor. However, mature aerobic granules cultivated in lab-scale (51 m·h\(^{-1}\)) or pilot-scale (18~40 m·h\(^{-1}\)) reactors had a much higher settling velocity. The results of the present work suggest that a short settling time is not a prerequisite for an aerobic granulation process under such conditions.

The profile of MLSS was not synchronous with the granulation process. Complete granulation was obtained on the 4th day, while the MLSS kept dropping until the 7th day. This phenomenon is aligned with the findings of Liu et al. [10], who also detected a time lag between granulation and biomass growth. According to Li et al. [27] and Liu et al. [14], granules with external carrier media like PAC or dried sludge contained less organic compounds, explaining the relatively low MLVSS/MLSS value for mature granules in the present study.

This study’s granulation speed is comparable to previous studies [9, 12, 27-29]. First, the readily available and inexpensive sources of the yellow earth used as a nucleating agent in the present study provide an economically-viable alternative for wider application. The unique characteristics of the yellow earth used were key for rapid aerobic granulation. According to the formation process of this study, a yellow-earth-based process was proposed for its enhancement effects, as shown in figure 7. Yellow earth’s chemical components (mainly made up of silicon dioxide and aluminum oxide) and physical size (above 0.27 mm) make it ideal as a nucleating agent to induce bacteria attachment and aggregation of granular-type microbes, as well as the shaping of particles in combination with the constant aeration and stirring conditions. Namely, the small flocs act as the nuclei for granular-type biomass to attach, and stimulates the secretion of EPS (especially the PN). Ones with media sizes are embedded, and the larger ones help shape the small and irregular granules into regular ones [6, 15].
addition, the yellow earth particles may have a bridging effect among particles, microbes and EPS [27]. EPS also plays a vital role in aggregation and cohesion of different types of biomass as well as yellow earth particles [30].

**Figure 7.** Formation of aerobic granular sludge enhanced by yellow earth.

With a settling time of 10 min, aerobic granulation was realized within 4 days in a sequencing batch reactor with yellow earth as the nucleating agent. The granular sludge showed distinct attributes including well-settling performance, low water content and high specific gravity, high microbial activity, and abundant EPS content (especially PN content). Excellent COD and nitrogen removal were achieved during the start-up period, and the denitrification process was detected due to the layered structure of the sludge. This study proves that settling is not a prerequisite for aerobic granulation in the aforementioned conditions. Rapid granulation was enhanced by the physicochemical characteristics of the yellow earth. The research presented in this paper offers an improved and cost-effective alternative for the enhanced formation of aerobic granular sludge.

**References**

[1] Bruin L M, Kreuk M K, Roest H F, Uijterlinde C and Loosdrecht M C 2004 Aerobic granular sludge technology: an alternative to activated sludge? Water Sci. Technol. 49: 1-7

[2] Kreuk M K, Heijnen J J and Loosdrecht M C 2005 Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge Biotechnol Bioeng 90: 761-69

[3] Jang A, Yoon Y H, Kim I S, Kim K S and Bishop P L 2003 Characterization and evaluation of aerobic granules in sequencing batch reactor J. Biotechnol 105: 71-82

[4] Ni B J, Xie W M, Liu S G, Yu H Q, Wang Y Z, Wang G and Dai X L 2009 Granulation of activated sludge in a pilot-scale sequencing batch reactor for the treatment of low-strength municipal wastewater Water Res. 43: 751-61

[5] Isanta E, Suárez-Ojeda M E, Val Del Río Á, Morales N, Pérez J and Carrera J 2012 Long term operation of a granular sequencing batch reactor at pilot scale treating a low-strength wastewater Chem. Eng. J. 198-199 163-70

[6] Li J, Ding L B, Cai A, Huang G X and Horn H 2014 Aerobic sludge granulation in a full-scale sequencing batch reactor Biomed Res. Int. 2014 :2687-89

[7] Pronk M, Kreuk M K, Bruin B, Kamminga P, Kleerebezem R and Loosdrecht M.C 2015 Full scale performance of the aerobic granular sludge process for sewage treatment Water Res. 84: 207-17

[8] Liu Z, Liu Y, Kuschk P, Wang J, Chen Y and Wang X 2016 Poly aluminum chloride (PAC)
enhanced formation of aerobic granules: Coupling process between physicochemical–biochemical effects Chem. Eng. J. 284:1127-35

[9] Long B, Yang C Z, Pu W H, Yang J K, Jiang G S, Dan J F, Li C Y and Liu F B 2014 Rapid cultivation of aerobic granular sludge in a pilot scale sequencing batch reactor Bioresource Technol. 166: 57-63

[10] Liu Y Q and Tay J H 2015 Fast formation of aerobic granules by combining strong hydraulic selection pressure with overstressed organic loading rate Water Res. 80: 256-66

[11] Wan C, Lee D J, Yang X, Wang Y, Wang X and Liu X 2015 Calcium precipitate induced aerobic granulation Bioresource Technol. 176: 32-37

[12] Wang S, Shi W, Yu S, Yi X and Yang X 2012 Formation of aerobic granules by Mg$^{2+}$ and Al$^{3+}$ augmentation in sequencing batch airlift reactor at low temperature Bioproc Biosyst Eng. 35: 1049-55

[13] Kong Q, Ngo H H, Shu L, Fu R S, Jiang C H and Miao M S 2014 Enhancement of aerobic granulation by zero-valent iron in sequencing batch airlift reactor J. Hazard Mater 279: 511-17

[14] Liu Z, Liu Y, Kuschk P, Wang J, Chen Y and Wang X 2016 Poly aluminum chloride (PAC) enhanced formation of aerobic granules: Coupling process between physicochemical biochemical effects Chem. Eng. J. 284: 1127-35

[15] Liu Z, Liu Y, Zhang A, Zhang C and Wang X 2014 Study on the process of aerobic granule sludge rapid formation by using the poly-aluminum chloride (PAC) Chem. Eng. J. 250: 319-25

[16] Li Y, Zhang Y, Zhao Z, Sun S, Quan X and Zhao H 2016 Enhancement of sludge granulation in hydrolytic angiogenesis by denitrification Appl. Microbiol Biotechnol 100:3313-20

[17] Bindhu B and Madhu G 2015 Influence of three selection pressures on aerobic granulation in sequencing batch reactor India J. Chem.. Techn.. 22: 241-47

[18] He Q, Wang H, Yang X, Zhou J, Ye Y, Chen D and Yang K 2015 Culture of denitrifying phosphorus removal granules with different influent wastewater Desalin Water Treat 1-8.

[19] APHA 2005 Standard methods for the examination of water and wastewater American Public Health Association (APHA): Washington, D.C, USA

[20] Abdullah N, Yuzir A, Curtis T P, Yahya A and Ujang Z 2013 Characterization of aerobic granular sludge treating high strength agro-based wastewater at different volumetric loadings Bioresource Technol. 127: 181-87

[21] Yang Y C, Liu X, Wan C, Sun S and Lee D J 2014 Accelerated aerobic granulation using alternating feed loadings: alginate-like exopolysaccharides Bioresource Technol. 171: 360-66

[22] Kreuk M K, Pronk M and Loosdrecht M C 2005 Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures Water Res. 39: 4476-84

[23] Lochmatter S, Gonzalez G G and Holliger C 2013 Optimized aeration strategies for nitrogen and phosphorus removal with aerobic granular sludge Water Res. 47: 6187-97

[24] Yu S, Sun P, Zheng W, Chen L, Zheng X, Han J and Yan T 2014 The effect of COD loading on the granule-based enhanced biological phosphorus removal system and the recoverability Bioresource Technol. 171: 80-87

[25] Verawaty M, Pijuan M, Yuan Z and Bond P L 2012 Determining the mechanisms for aerobic granulation from mixed seed of floccular and crushed granules in activated sludge wastewater treatment Water Res. 46: 761-71

[26] Zhou D, Niu S, Xiong Y, Yang Y and Dong S 2014 Microbial selection pressure is not a prerequisite for granulation: dynamic granulation and microbial community study in a complete mixing bioreactor Bioresource Technol. 161: 102-08

[27] Li J, Liu J, Wang D, Chen T, Ma T, Wang Z and Zhuo W 2015 Accelerating Aerobic Sludge Granulation by Adding Dry Sewage Sludge Micro powder in Sequencing Batch Reactors Int.
[28] Moy B Y, Tay J H, Toh S K, Liu Y and Tay S T 2002 High organic loading influences the physical characteristics of aerobic sludge granules Lett. Appl. Microbiol. 34: 407-12

[29] McSwain B S, Irvine R L and Wilderer P A 2004 The influence of-settling time on the formation of aerobic granules Water Sci. Technol. 50: 195-202

[30] Gao D, Liu L, Liang H and Wu W M 2011 Aerobic granular sludge: characterization, mechanism of granulation and application to wastewater treatment Crit. Rev. Biotechnol 31: 137-52