



## Effect of granular activated carbon on the aerobic granulation of sludge and its mechanism



Jia Tao<sup>a</sup>, Lian Qin<sup>a</sup>, Xiaoying Liu<sup>b</sup>, Bolin Li<sup>a</sup>, Junnan Chen<sup>a</sup>, Juan You<sup>a</sup>, Yitian Shen<sup>a</sup>, Xiaoguo Chen<sup>a,c,\*</sup>

<sup>a</sup>School of Resource and Environmental Engineering, Wuhan University of Technology, Wuhan 430070, China

<sup>b</sup>School of Civil Engineering and Architecture, Wuhan University of Technology, Wuhan 430070, China

<sup>c</sup>Hubei Key Laboratory of Mineral Resources Processing and Environment, Wuhan 430070, China

### HIGHLIGHTS

- GAC accelerated the granulation of activated sludge under EBPR conditions.
- GAC had no obvious effect on the bacterial community structure of granules.
- A mechanism for GAC effect on granulation was proposed.
- Simultaneous removal of COD, nitrogen and phosphorus in aerobic granules system.

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### ABSTRACT

The granulation of activated sludge and effect of granular activated carbon (GAC) was investigated under the alternative anaerobic and aerobic conditions. The results showed that GAC accelerated the granulation, but had no obvious effect on the bacterial community structure of granules. The whole granulation process could be categorized into three phases, i.e. lag, granulation and granule maturation phase. During lag period GAC provided nuclei for sludge to attach, and thus enhanced the morphological regularization of sludge. During granulation period the granule size increased significantly due to the growth of bacteria in granules. GAC reduced the compression caused by the inter-particle collisions and thus accelerate the granulation. GAC has no negative effect on the performance of SBR, and thus efficient simultaneous removal of COD, nitrogen and phosphorus were obtained during most of the operating time.

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### 1. Introduction

The enhanced biological phosphorus removal (EBPR) is a wastewater treatment process, which operates under alternating anaerobic and aerobic conditions, with substrate supply in the anaerobic stage (Lin et al., 2003). It had been developed for efficient phosphorus (P) removal without the use of chemical precipitation and is a relatively inexpensive and sustainable method for the removal of P from wastewater (Mielczarek et al., 2013). The EBPR process has been adopted in wastewater treatment plants worldwide (Li et al., 2015). To date, most EBPR processes have been based on suspended biomass cultures, which may encounter many problems, such as sludge bulking, large treatment plant space and higher production of waste sludge (Lin et al., 2003). Recently aerobic granule system has received comprehensive concerns for

wastewater treatment. Aerobic granules are the self-immobilization of microorganisms under certain conditions in wastewater treatment processes (Liu and Tay, 2004). Compared to the conventional activated sludge, aerobic granular sludge have a regular, dense, and strong structure and good settling properties, and thus enable a high biomass retention and withstand high-strength wastewater and shock loadings (Lin et al., 2003). Therefore, the aerobic granule system has become a promising technology for wastewater treatment.

Most of the conducted research on aerobic granular sludge has focused on its ability to remove nitrogen and COD with little emphasis on the removal of phosphorus (Di Bella and Torregrossa, 2013; Liu and Tay, 2004; Zhang et al., 2016). However, aerobic granules have also been successfully developed in alternative anaerobic and aerobic SBR (Lin et al., 2003; Wu et al., 2012). This suggests that a simultaneous nitrification, denitrification, and phosphorus removal of aerobic granule is possible under EBPR conditions, which may provide a promising technology for the

\* Corresponding author at: School of Resource and Environmental Engineering, Wuhan University of Technology, Wuhan 430070, China.

E-mail address: [xiaoguo\\_chen@whut.edu.cn](mailto:xiaoguo_chen@whut.edu.cn) (X. Chen).

removal of COD, nitrogen and phosphorus simultaneously (Kagawa et al., 2015).

A long start-up period is usually required for the development of aerobic granules from flocculent sludge, especially under EBPR conditions, and the loss of biomass in this period may lead to poor nutrient removal performance (Verawaty et al., 2012). These shortcomings impede the application of this technology for real wastewater treatment. Therefore, different strategies have been proposed to enhance the formation of aerobic granules. Previous studies have showed that adding metal ions such as  $\text{Ca}^{2+}$  can accelerate the start-up of aerobic granules through forming nucleus for bacteria to attach (Liu and Tay, 2004). Recently adding granular activated carbon (GAC) was also verified to speed up aerobic granulation by providing nucleus for bacteria (Li et al., 2011; Zhou et al., 2015). In addition, the use of GAC provided aerobic granules with strong cores and thus can help maintain the long-term stability of mature granules (Li et al., 2011). Although adding GAC has been shown to be a simple and effective strategy to initiate granule formation to complete sludge granulation, its mechanism is still unclear. Moreover, whether GAC can accelerate granulation under EBPR conditions is unknown. Furthermore, a complete picture of the granulation process is still absent although several hypotheses and mathematic models for aerobic granulation have been described (Liu and Tay, 2004; Zhang et al., 2016). Therefore, further research is needed.

In this study, laboratory experiments were conducted with two sequencing batch reactors (SBR) to investigate the effects of GAC on the formation of aerobic granules under EBPR conditions. The dynamics of morphology and physical properties of the sludge in the two reactors were characterized and compared throughout the experiments. Moreover, the bacterial community structures of sludge before and after granulation were analyzed through high throughput sequencing on a HiSeq PE250 (Illumina, USA) and the effect of GAC on it was also investigated. The aims of present study were to determine the mechanism of granule formation under EBPR condition and to deduce the possible effect mechanism of GAC on the granulation process.

## 2. Materials and methods

### 2.1. Reactor setup and operation

The aerobic granules were cultivated in two identical SBR in size with 4 L working volumes. The diameter of the reactors was 15 cm with height/diameter (H/D) ratio of 2:1. Fine air bubbles for aeration were supplied through an air purge at the reactor bottom with an airflow rate of 400 ml/min. The two reactors, reactor A (RA) and reactor B (RB), were operated in parallel and inoculated with 4 g/L seeding sludge taken from a local activated sludge sewage treatment plant (Wuhan, China). As a control group, RA was run without GAC, whereas RB was inoculated with 14.5 g of GAC (diameter of 0.125–0.300 mm). The reactors were operated five cycles of alternating anaerobic-aerobic conditions every day with a volumetric exchange ratio of 50%. Each cycle lasted for 4.8 h, involving 1 min feeding of synthetic wastewater, 99 min of anaerobic stirring (110 rpm), 150–175 min of aerobic reaction, 30–5 min of precipitation, 1 min of effluent discharge, and the rest of time was idle. The stirrer speed was maintained at 110 rpm during the anaerobic and aerobic reaction periods. The temperature of the reactors were maintained at 25 °C with water bath. The reactors were operated automatically through time controllers.

### 2.2. Synthetic wastewater

The composition of the synthetic wastewater was as follows ( $\text{mg L}^{-1}$ ):  $\text{CH}_3\text{COONa}$ , 513, corresponding to a loading rate of

1.0 kg COD/( $\text{m}^3\cdot\text{d}$ );  $\text{NH}_4\text{Cl}$ , 153;  $\text{KH}_2\text{PO}_4$ , 40.6;  $\text{K}_2\text{HPO}_4$ , 46.3;  $\text{Pep-tone}$ , 26;  $\text{Na}_2\text{EDTA}$ , 38.2;  $\text{CaCl}_2$ , 100;  $\text{MgSO}_4\cdot 2\text{H}_2\text{O}$ , 138. Additionally, a trace elements solution comprised of the following components was added ( $\text{mg L}^{-1}$  in synthetic wastewater):  $\text{H}_3\text{BO}_3$ , 0.45;  $\text{FeCl}_3$ , 4.5;  $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ , 0.36;  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ , 0.09;  $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ , 0.44;  $\text{KI}$ , 0.54;  $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ , 0.18 and  $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ , 0.45.

### 2.3. Analytical methods

Parameters including TN,  $\text{NH}_4\text{-N}$ , TP,  $\text{PO}_4^{3-}\text{-P}$ , chemical oxygen demand (COD), sludge volume index ( $\text{SVI}_{30}$ ), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge specific gravity were analyzed in accordance to the standard methods (APHA, 1998). Images of sludge were taken at regular time intervals with a digital camera (Nikon Eclipse Ci) and processed for Feret diameters and roundness analysis using the ImageJ as described by Beun et al. (2002), Su and Yu (2005). Average particle size of mature granules was measured at day 150 by a laser particle size analysis system (Mastersizer 2000).

### 2.4. Microbial community composition

To investigate the dynamics of bacterial community structures during granulation and the GAC effect, the bacterial community compositions in feeding sludge and mature granules (at day 150) were investigated using next-generation sequencing (NGS). Three parallel sludge samples were taken from feeding activated sludge (AS) and mature granules in RA (GS) and RB (GSC), respectively. The bacterial DNA was extracted with a PowerSoil DNA Isolation Kit (MO-BIO, USA), according to the manufacturer's instructions. The V3–V4 region of the bacterial 16S rRNA gene was amplified using the universal primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGTATCTAAT-3') and the PCR products were mixed for each sample. After purification and quantification the amplicons were sequenced on a HiSeq 2500 (Illumina, USA) by the Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

After removing the primers from the raw sequence data, the paired-end reads from each sample were merged using FLASH. To obtain effective tags, raw tags were filtered using the QIIME software package. Then the chimera sequences were identified and removed from the effective tags by UCHIME ([http://drive5.com/usearch/manual/uchime\\_algo.html](http://drive5.com/usearch/manual/uchime_algo.html)). UPARSE pipeline were used to analyze the reads and to cluster operational taxonomic units (OTUs) at 97% sequence identity. A representative sequence was picked for each OTU by selecting the most abundant sequence in that OTU, and the RDP classifier was used to assign taxonomic data to each representative sequence with a confidence threshold of 80%. Then all samples were normalized to ensure equal number of sequences in each sample. Richness estimates and diversity indices were calculated for normalized data sets, using QIIME software package (<http://qiime.sourceforge.net/>).

## 3. Results and discussion

### 3.1. Performance of the SBR

The performance of the two reactors are shown in Fig. 1. Except for the obvious fluctuation between day 20 and day 29, the COD removal rate was higher than 80% during the experiments (Fig. 1a), whereas the  $\text{NH}_4\text{-N}$  removal rates in the two reactors were 100% in most days of the cultivation (Fig. S1). In contrast, the TN removal efficiency was lower, with average removal rate of 60% during the first 98 days, but the value reached more than 80% after that (Fig. 1b). There was no significant difference between the two reac-

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