



Understanding the granulation process of activated sludge in a biological phosphorus removal sequencing batch reactor

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ABSTRACT

The granulation of activated sludge was investigated using two parallel sequencing batch reactors (SBRs) operated in biological nitrogen and phosphorus removal conditions though the reactor configuration and operating parameters did not favor the granulation. Granules were not observed when the SBR was operated in biological nitrogen removal period for 30 d. However, aerobic granules were formed naturally without the increase of aeration intensity when enhanced biological phosphorus removal (EBPR) was achieved. It can be detected that plenty of positive charged particles were formed with the release of phosphorus during the anaerobic period of EBPR. The size of the particles was about 5–20 μm and their highest positive ζ potential was about 73 mV. These positive charged particles can stimulate the granulation. Based on the experimental results, a hypothesis was proposed to interpret the granulation process of activated sludge in the EBPR process in SBR. Dense and compact subgranules were formed stimulated by the positive charged particles. The subgranules grew gradually by collision, adhesion and attached growth of bacteria. Finally, the extrusion and shear of hydrodynamic shear force would help the maturation of granules. Aerobic granular SBR showed excellent biological phosphorus removal ability. The average phosphorus removal efficiency was over 95% and the phosphorus in the effluent was below 0.50 mg L^{-1} during the operation.

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1. Introduction

Granules are the self-immobilization of microorganisms under certain conditions in wastewater treatment processes. Granular sludge is a dense and compact microbial community with better settleability compared to floccular activated sludge (Liu and Tay, 2002). In addition, the granular sludge is known for its strong microbial structure, high biomass retention, the ability to withstand high-strength wastewater, shock loadings and tolerance to toxicity (Su and Yu, 2005; Adav et al., 2008a). Granular sludge can be formed under anaerobic and aerobic conditions. To date, anaerobic granular sludge technology has been applied widely in the treatment of municipal and industrial wastewaters (Liu and Tay, 2004), and aerobic granular sludge is also one of the promising processes in wastewater treatment (Adav et al., 2008a). The application of aerobic granular sludge for the treatment of soybean-processing, brewery and phenol wastewaters has been carried out in

bench-scale studies (Su and Yu, 2005; Jiang et al., 2006; Wang et al., 2007). Some combined processes, for example, aerobic granular sludge membrane bioreactor, were also proposed (Tay et al., 2008).

To date, previous studies have demonstrated that sequencing batch reactor (SBR) is the most suitable reactor configuration for the aerobic granulation. Formation of granules, especially for aerobic granules in SBR, has been extensively studied. Some favorable conditions for the formation of granules, in terms of seeding sludge (Wilen et al., 2008), substrates (Liu and Tay, 2004), organic loading rate (Moy et al., 2002), feeding strategy (Liu et al., 2003), reactor configuration (Liu and Tay, 2002), settling time (Liu and Tay, 2002), exchange ratio (Wang et al., 2006b), and hydrodynamic shear force (Adav et al., 2007), have been summarized in previous studies. It is generally acknowledged that (1) distinct feast-famine periods during the operation, (2) short settling time, and (3) high aeration intensity, are essential for the formation of aerobic granules in SBR. The selection pressures, such as short settling time and high aeration intensity, are the main driving force of aerobic granulation in SBR (Qin et al., 2004). The formation and maintenance of aerobic granules in SBR need relatively high cost associated with aeration, which is the main defect and limit for the scaling up of aerobic granular sludge reactors. Therefore, few

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reports concerning the engineering application of aerobic granular sludge systems can be found in literatures. Although extensive work has been done in this area, future research is also needed, for example, to decrease the energy input in aerobic granular sludge reactors.

It was reported that aerobic granules could be easily formed in the biological phosphorus removal SBR (Wu et al., 2010). An SBR with the height to diameter ratio of 6.0 has been used to investigate the characteristics of biological phosphorus removal. Strangely, the floccular activated sludge turned into granular sludge with the improvement of biological phosphorus removal ability. However, the SBR was operated under unfavorable granulation conditions with relatively long settling time of 35 min and low aeration intensity of $15.3 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$. As no filamentous bacteria were observed, Wu et al. (2010) ascribed the granulation of activated sludge to the formation of positive charged particles with the release of phosphorus during the anaerobic period, and activated sludge flocs can be aggregated around the positive particles by electronic force. It is reported that the addition of some positive ions, such as Ca^{2+} , Mg^{2+} , Fe^{2+} and Fe^{3+} , could accelerate the granulation of activated sludge because the positive ions could bind to negative charged cells to form microbial nuclei and stimulate the granulation (Jiang et al., 2003; Adav et al., 2008a). However, it is superficial to mention that cations bridge negatively charged components, such as extracellular polymeric substances (EPS) or surface of cells, considering the disproportion between the size of a cation and that of a floc or even a bacterium. The charges would be easily neutralized by attracting very few electronegative cells. It is impossible for positive charged ions to attract enough cells to form a big individual granule. The size of positive charged particles reported by Wu et al. (2010) was obviously bigger than that of the positive ions. However, the source of positive charge of these particles is still unclear, and it is still unclear how the positive charged particles work during the formation process of granules.

In this study, the characteristics of activated sludge were investigated in two parallel SBRs. One was operated in biological nitrogen removal process and the other was operated changing from biological nitrogen to phosphorus removal process. The granulation was observed in the P removal SBR and then the mechanism of granulation was proposed. The results presented would be helpful to understand the granulation characteristics driven by biological phosphorus removal. More importantly, it provides the potential application of aerobic granules for biological phosphorus removal in wastewater treatment.

2. Materials and methods

2.1. Reactor and operation

Two parallel SBR reactors (termed as R1 and R2) with the working volume of 4 L were used in this study. The id and height of the reactor are 15 cm and 40 cm, respectively. R1 was operated in biological nitrogen removal mode. R2 was operated in biological nitrogen removal process for 30 d and then changed to biological phosphorus removal period. The operation of biological nitrogen removal mode was the same for R1 and R2. The SBRs were operated with a cycle time of 8 h during the operation. The biological nitrogen removal cycle consisted of 5 min feeding, 240 min aeration, 135 min denitrification with dose of methanol, 40 min settling, 15 min decanting and 45 min idling while the biological phosphorus removal cycle included 5 min feeding, 115 min anaerobic period, 300 min aeration, 40 min settling, 15 min decanting and 5 min idle. The solids retention time of biological nitrogen and phosphorus removal period were 20 and 10 d controlled by biomass wasting. 3 L synthetic wastewater was pumped into the

reactor during each feeding. Nitrogen gas was used to mix the liquid and keep anoxic or anaerobic condition during the operation. Compressed air was supplied to the SBR at a flow rate of 120 L h^{-1} during the aerobic period. pH was not controlled during the operation. The typical variations of pH and dissolved oxygen (DO) during the biological nitrogen and phosphorus removal are shown in Supplementary material (SM), Figs. SM-1 and SM-2. The seeding sludge was taken from a local anoxic/oxic wastewater treatment plant in Harbin, China. The seeding concentration of the activated sludge was about 2500 mg L^{-1} .

2.2. Wastewater composition

In this study, propionate was used as the sole carbon source in the raw wastewater. NH_4Cl and KH_2PO_4 were used to provide the N and P sources in the synthetic wastewater. The COD, $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ concentrations were 250, 50, 2.5 and 400, 20, 12 mg L^{-1} during nitrogen and phosphorus removal periods, respectively. Other compositions of the synthetic wastewater were the same, including (per liter tap water): 0.030 g MgSO_4 , 0.450 g NaHCO_3 , 0.020 g CaCl_2 and 1 mL trace elements solution. The composition of the trace elements solution was the same as the description of Adav et al. (2007).

2.3. Chemical analyses

Orthophosphate-phosphorus, ammonia, nitrite and nitrate were analyzed according to the Standard Methods (APHA, 1998). All the samples were filtered with the $0.45 \mu\text{m}$ cellulose acetate membrane before analyzing. The measurements of polyhydroxyalkanoate (PHA) and glycogen in the cells were the same as the description of Wu et al. (2010).

2.4. Analysis of particles size and zeta potential

1000 mL supernatant was taken at the end of aeration during the biological phosphorus removal period in R2. Biomass-free medium was obtained by filtering the supernatant with $0.45 \mu\text{m}$ cellulose acetate membrane to intercept the bacteria cells and other components that can affect the measurement of particle size and zeta potential. Then proper amount of KH_2PO_4 was added into the biomass-free medium to simulate the phosphorus release during the anaerobic period at 0, 60 and 120 min according to the measured P concentrations in the SBR. pH, propionate and NH_4^+ were also adjusted according to the values in the SBR during the anaerobic period. The composition of other compounds, such as MgSO_4 , CaCl_2 and NaHCO_3 in the prepared simulation medium, was the same as that in the synthetic wastewater. Particles size and distribution in the simulation medium were analyzed by a particle analyzer for liquids (Royco 970, HIAC, USA). Zeta potential of the simulation solution was determined by a zeta potential analyzer (Nano-Z, Malvern, UK).

2.5. Image analysis

The pretreatment of granules for scanning electronic microscopy (SEM) observation was the same as the description of Wu et al. (2010). A total of 0.5 mL simulation medium was filtered through a $0.45 \mu\text{m}$ cellulose acetate membrane and then the membrane was air dried for the particles SEM observation. SEM and SEM-energy dispersive X-ray (EDX) analysis were taken with a Hitachi S-4700 field emission SEM equipped with an EDX detector to determine the selected element composition of the particles formed in the simulation medium. Elements of C, N, O, Na, Mg, P, and Ca were monitored. The preparation of the granules for transmission electronic microscopy (TEM) observation was performed

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