



# Granulation of susceptible sludge under carbon deficient conditions: A case of denitrifying sulfur conversion-associated EBPR process



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## ABSTRACT

Sludge granulation has been recognized as a promising biotechnology in wastewater treatment. Whereas the granulation of susceptible sludge in particular with a very low organic loading rate (OLR) ( $\leq 0.6$  kg COD/m<sup>3</sup>/day or  $\leq 120$  mg COD/g VSS/day) is a difficult task that has not been achieved in activated sludge systems yet. This study was aimed at exploring an effective strategy for sludge granulation in the recently developed Denitrifying Sulfur conversion-associated Enhanced Biological Phosphorus Removal (DS-EBPR) process using a sequencing batch pump-lift reactor. Four strategies were studied by manipulating the factors of organic loading rate (OLR), superficial upflow velocity and sludge settling time individually or collectively. Increasing both the OLR and the superficial upflow velocity effectively promoted granule formation but at the same time led to unstable and even deteriorated reactor performance. The development of granules proceeded via several stages: formation, dispersion, reformation and stabilization. Gradually increasing the superficial upflow velocity from 5.1 to 6.8 m/h and keeping the OLR at 112.4 mg COD/g VSS/day proved to be most effective strategy for accelerating granulation while simultaneously achieving stable reactor performance. Under these conditions, the granules became stable with a diameter of 375–400  $\mu$ m and displayed excellent settleability. The two major microbial groups, sulfate-reducing bacteria and sulfide-oxidizing bacteria, in the microbial community of the DS-EBPR granular sludge were enriched to 17.7% and 15.8% respectively. The newly developed DS-EBPR granular system was able to achieve an almost threefold improvement in phosphorus removal efficiency and 25% reduction in the operating cycle time compared with a flocculent DS-EBPR system.

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## 1. Introduction

Biogranulation technology has been recognized as a promising biological process for wastewater treatment. Many factors influence the granulation of flocculent sludge, including substrate composition, organic loading rate (OLR), hydrodynamic shear force, settling time, feeding strategy, and reactor configuration (de Kreuk

and van Loosdrecht, 2004; Liu and Tay, 2004; de Kreuk et al., 2007). Among these factors, the OLR is believed to be essential to sludge granulation, while a low OLR ( $< 1$  kg chemical oxygen demand (COD)/m<sup>3</sup>/day) would severely affect granule formation and could ultimately hinder the future application of this innovative technology (Ni et al., 2009; Li et al., 2008). Most of granular sludge in previous studies was cultivated under high or middle-strength organic loading rates (1.2–15 kg COD/m<sup>3</sup>/day) (Beun et al., 1999; de Kreuk and van Loosdrecht, 2004; Tay et al., 2004; Wu et al., 2010), while granulation of flocculent sludge under a low OLR has rarely been successful. In fact, this is a common challenge for cultivating granules with low-strength municipal wastewater (i.e. under a low OLR), such as in China where the COD concentration of municipal wastewater is lower than 200 mg/L (Ni et al., 2009). The

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implementation of granule based processes is therefore limited under such conditions. Meanwhile, although sludge granulation is not restricted to certain microbiological groups (Beun et al., 1999; Liu and Tay, 2004), it is particularly difficult for susceptible sludge to achieve stable granules due to their high sensitivity to environmental changes, e.g. Anammox (van der Star, 2008), nitrifying bacteria (Sun et al., 2012). The literature has not yet reported the development of granules with a low OLR (0.4–0.6 kg COD/m<sup>3</sup>/day) especially for a susceptible sludge treating saline municipal wastewater. Granulation of flocculent sludge in the recently developed Denitrifying Sulfur conversion-associated Enhanced Biological Phosphorus Removal (DS-EBPR) process presents a unique case to address this gap.

The granulation of flocculent sludge must overcome two issues. First, due to the currently low efficiency of sulfur conversion (i.e. sulfate reduction and sulfate production) involved in flocculent DS-EBPR systems, the operation cycle time is extended to  $\geq 12$  h (Wu et al., 2014; Guo et al., 2016). This long operation cycle time results in an OLR that usually does not exceed 0.6 kg COD/m<sup>3</sup>/day. This is well below the reported 1.2–15 kg COD/m<sup>3</sup>/day for successful biogranulation (Beun et al., 1999; de Kreuk and van Loosdrecht, 2004; Tay et al., 2004; Wu et al., 2010). Such a low OLR poses a great challenge to the granulation of general sludge (Ni et al., 2009). Second, the DS-EBPR system is highly sensitive to environmental changes in the reactor under an alternating anaerobic/anoxic condition because the sulfate-reducing bacteria (SRB), which serve as one of the major functional microbes in the system, are highly susceptible (Guo et al., 2016). Operating DS-EBPR stably during granulation represents another challenge not unseen in conventional EBPR sludge granulation (de Kreuk et al., 2005; Kishida et al., 2006; Wu et al., 2010; Wang et al., 2013).

With the above in mind, the present study was aimed at developing a possible strategy for granulating DS-EBPR sludge and operating the system stably. Different granulation strategies were attempted. The morphology, size and settleability of granules were characterized. The reactor performance was evaluated in terms of sulfur conversion, P release/uptake, and variation in all the major internal polymers (i.e. poly- $\beta$ -hydroxyalkanoate (PHA), glycogen, polysulfide or elemental sulfur (poly-S<sup>2-</sup>/S<sup>0</sup>), polyphosphate (poly-P)). Meanwhile, the reactor performance of the granular DS-EBPR system successfully developed in this study was carefully compared with that of a flocculent sludge DS-EBPR system developed in our previous study (Wu et al., 2014). The microbial community of granular sludge was further analyzed to explore factors that may be responsible for the successful strategy. The possible mechanism of DS-EBPR granulation was also discussed.

## 2. Materials and methods

### 2.1. Reactor design and operation

Adapted from a Sequencing Batch Air-lift Reactor (SBAR) for aerobic granulation (de Kreuk and van Loosdrecht, 2004), a tight-sealed lab-scale Sequencing Batch Pump-lift Reactor (SBPR) was applied in this study as shown in Fig. 1. The SBPR has a total volume of 15 L, comprising 14 L of effective volume and 1 L of headspace. The internal diameter is 15 cm with a height of 84 cm. An internal column having a height of 60 cm and a diameter of 7.5 cm was installed 2.5 cm above the reactor bottom where a feed distributor was also installed. The internal column encouraged circulation of the internal flow and enhanced the mixing of liquor in the reactor with the help of a recirculation pump. The reactor was inoculated with 10 L of seeding sludge or 123 g of mixed liquor suspended solids (MLSS) obtained from a lab-scale flocculent sludge DS-EBPR reactor in our laboratory. The initial sludge concentration of SBPR

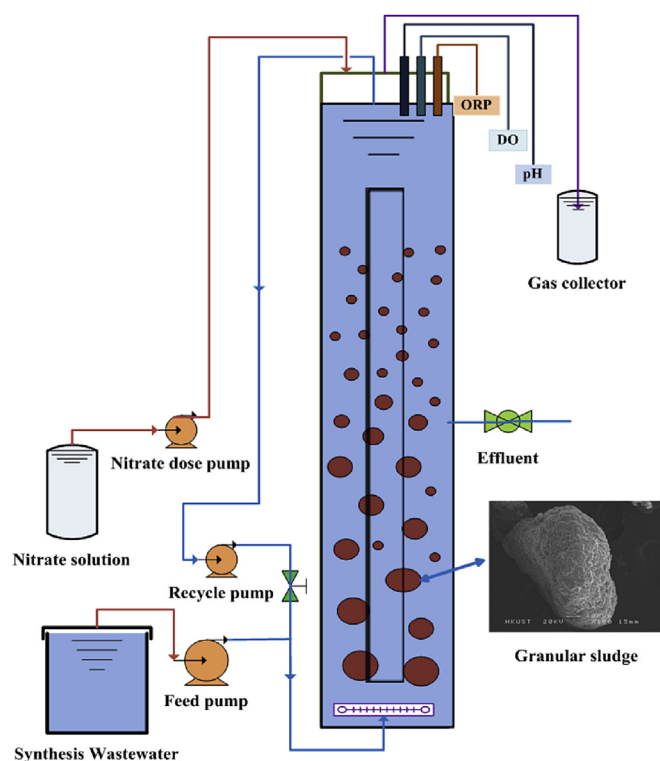


Fig. 1. Schematic diagram of the SBPR.

was set at 8.8 g MLSS/L. The SBPR was then operated under a controlled temperature of  $22 \pm 1$  °C and a controlled pH of 7.2–7.9 by alternatively adding a 0.5 N HCl solution or a 0.5 N NaOH solution whenever necessary. Synthetic saline sewage was prepared with 20% seawater and 80% freshwater ( $\sim 0.7\%$  salinity in the mixed liquor) to simulate typical saline sewage in Hong Kong (approximately 1.0 mg C/mg S). Following Guo et al. (2016), the synthetic sewage comprised 60 mg of NH<sub>4</sub><sup>+</sup>-N/L, 20 mg of PO<sub>4</sub><sup>3-</sup>-P/L, and 400 mg of COD/L, corresponding to 267 mg acetate-COD/L, 133 mg propionic-COD/L, and 150–200 mg-S/L of sulfate on average.

The SBPR was operated under an alternating anaerobic/anoxic condition following an 8–24 h operating cycle that comprised i) 10 min of 7 L feeding the synthetic sewage, ii) 3–10 h of anaerobic reaction for organic carbon consumption and P release, iii) 5 min of nitrate addition (a proper amount of 2 g N/L sodium nitrate solution pumped into the reactor, setting 40–50 mg nitrate-N/L at the beginning of the anoxic phase) for denitrifying P uptake, iv) 3–12 h of anoxic reaction, v) 3–10 min of settling, vi) 15 min of decanting 7 L of supernatant, and vii) 25–32 min of idleness. As shown in Table 1, the durations of both the anaerobic and anoxic phases varied to allow completion of phosphorus release and uptake reactions (Wu et al., 2013). The settling time was set between 3 and 10 min in order to provide suitable selecting pressure for washing small biomass particles out of the reactor (Liu and Tay, 2004; Mcswain et al., 2004).

The reactor was operated for 260 days, which can be divided into four phases according to changes in the three major influencing factors. In Phase I (days 1–25), since a higher superficial upflow velocity can improve reactor mixing and promote the formation of mature granules (Tay et al., 2001; Hulshoff Pol et al., 2004; Liu and Tay, 2004), the recirculation flow rate (R) was increased from 1.0 to 1.5 L/min to increase the superficial upflow velocity from 3.4 to 5.1 m/h, and simultaneously the settling time was decreased from 10 to 5 min to investigate the first granulation

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