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A sustainable method for effective regulation of anaerobic granular sludge: Artificially increasing the concentration of signal molecules by cultivating a secreting strain

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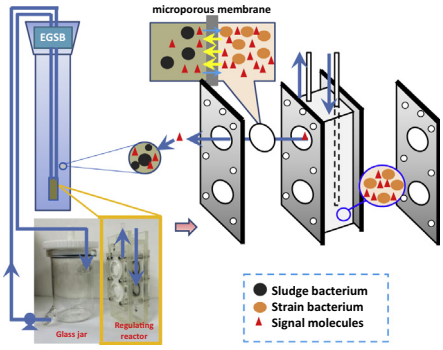
HIGHLIGHTS

- QS from signal molecule secreting strains regulates anaerobic sludge granulation.
- AI-2 QS regulation accelerates sludge aggregation.
- Aggregation yields increased extracellular polymeric substance (EPS) production.
- Granulation promotion by C4-HSL regulation was less than by AI-2.
- The negative effect of DSF-QS restrained granular diameter growth, EPS production.

GRAPHICAL ABSTRACT



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ABSTRACT

This study introduces sustainable quorum sensing (QS) granulation for anaerobic granular sludge (AnGS) and investigates the efficiency of three types of signal molecules on regulating AnGS granulation. The signal molecules of a secreting strain cultured in a QS regulating reactor increased their concentrations in an expanded granular sludge bed reactor throughout the granulating process. Increasing content of autoinducer-2 (AI-2) strengthened interspecific QS communication and gave a best performance with larger granular diameters, higher extracellular polymeric substance (EPS) production and relative hydrophobicity (RH). *N*-butyryl-homoserine lactone (C4-HSL) QS regulation was also favorable for granular growth, but its regulation was less than that of AI-2-QS. The AnGS granulated under these two types of QS regulations guided more filamentous bacteria to take part in granulation. Under diffusible signal factor (DSF)-QS regulation, the sludge had a lower granular level with a smaller granule diameter, lower EPS production (RH) when compared that of control medium.

1. Introduction

Anaerobic granular sludge (AnGS) has been used for many years to treat industrial wastewaters because of its low production of surplus sludge, high biomass concentration, and specific bioactivity (Lim and Kim, 2014). The importance of multi-species anaerobic granulation is well appreciated, but there is little understanding of the microbial process of granulation itself. In addition, the extremely long start-up period required by anaerobic reactors reduces the attractiveness and number of applications of anaerobic technology. This major drawback of AnGS also suggests that there is a strong need for further study on granulation, although numerous researchers from all over the world have studied the

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granulation process (Hulshoff Pol et al., 2004) or efficient strategies for expediting granular formation (Liu et al., 2003).

The spatial organization of bacteria in granular sludge must be regularly organized by a role or an 'invisible power' from among the numerous microbial lives. Quorum sensing (QS), a system of intercellular communication and multicellular coordination, is known as an effective way for bacteria to achieve an organized spatial structure (Liu et al., 2003). QS secrets signal molecules to regulate the behaviors and relationships between microorganisms to guide the expression of a particular gene when these signal molecules reach a threshold concentration (Shrout and Nerenberg, 2012). To date, the types of signal molecules found in water and wastewater treatment systems include *N*-acyl-homoserine lactones (AHLs) in Gram negative bacteria, autoinducer-2 (AI-2) in interspecies communication, and diffusible signal factor (DSF) in intra- and interspecies bacteria (Feng et al., 2014; Shrout and Nerenberg, 2012). There are many papers demonstrating the positive effects of AHLs or AI-2 in aerobic sludge granulation (Li and Zhu, 2014; Ren et al., 2013, 2010; Tan et al., 2014; Xiong and Liu, 2012, 2010; Zhang et al., 2011) and increasing the efficiency of the granulating process by improving granular properties. AHLs add-back studies by Tan et al. (2014) explained the promotion by AHLs in extracellular polymeric substance (EPS) synthesis and microbial aggregation. Zhang et al. (2011) speculated that the addition of boron would promote the formation of the precursor of AI-2, which resulted in accelerated aerobic granulation. After verifying the promotion of QS on aerobic granulation, Li (2014) and Wang (2014) made attempts to change the concentration of signal molecules, and both found that the granulation of aerobic granules was accelerated under an alternative feeding scheme or a comfortable pH environment with a higher concentration of AHLs.

Although previous papers have shown the application of QS regulation to aerobic sludge granulation, the drawbacks of these regulating strategies restrict their practicability to sludge granulation. For example, adding synthetic signal molecules cannot maintain a continuous positive role on sludge because they degrade considerably. High cost also limits their use to the level of laboratory research. Furthermore, variation of cultivating conditions not only adjusts the level of signal molecules, but also can change the original performance of the sludge system, which is an insoluble problem in this method. According to current knowledge, although Shapiro (1998) has investigated the multicellular cooperation in AnGS, there is still no study reported on an effective anaerobic granulating method based on a QS mechanism. An innovative method for continuous and rapidly cultivating AnGS is therefore strongly needed.

This study is conducted to introduce a revolutionary new cultivation method for AnGS. Culturing a designated microbial strain which secrets a specific type of signal molecule to increase the concentration of "beneficial" signal molecules and combine native signal molecules to accelerate the anaerobic granulation simultaneously in a traditional expanded granular sludge bed (EGSB) reactor. To accomplish this, this experiment chose AHLs and AI-2 secreting strains to compare the different achievements in two principles, and use the DSF secreting strain as a negative control to clarify the importance of correct QS regulation. The results presented here provide an in situ, sustainable, and economic QS regulation model which will benefit future applications in anaerobic granulation.

2. Materials and methods

2.1. Experimental configuration

The experiment consisted of an EGSB reactor with a total volume of 5 L and a QS regulating system. The QS regulating system comprised a regulating reactor made of a polymethyl methacrylate chamber with a volume of 70 mL, a glass jar which acted as temporary storage for the strain solution with a volume of 230 mL, a peristaltic pump, and a suitable hosepipe. The chamber (3 cm in length, 2 cm in width, and 12 cm in height) had two circular holes (1 cm in diameter) in each side. Each hole was covered by a microporous membrane (0.22 μm, Shanghai Xinya Purification Device Co., Ltd., Shanghai, China) which had a slightly larger size than that of the hole. On the outside of the membrane, a polymethyl methacrylate plate (3 cm in length, 0.5 cm in thickness, and 12 cm in height), which had roles in the same places as the reactor, was held on the side of the chamber by screws and Viton seals. With the above assembly, the EGSB reactor and regulating reactor did not have any direct microbial or macromolecular material exchange, but allowed signal molecules which were secreted by the designated strain to move from the regulating reactor to the sludge environment through the membrane. The signal molecules could easily permeate from the high to the low concentration side in a short time. Before the chamber was fixed in the bottom of EGSB reactor, two ports at the top were connected with corresponding ports in the microbial storage by two hosepipes. These components comprised a closed system containing the strain solution, which circulated under the actuation of a peristaltic pump (BQ50-1J, Longer Pump Co., Ltd., Baoding, China). The details are shown in Fig. 1.

2.2. Seed sludge, strains, and operation

The EGSB reactor containing anaerobic flocculent sludge (20 g SS/L) was incubated at 30 ± 2 °C in a greenhouse. The sludge was obtained from the wastewater treatment plant of a paper-making factory. The average granular diameter after pretreatment was about 59.45 ± 0.08 μm. The synthetic wastewater consisted of nutrients (2 g/L cane sugar, 0.38 g/L NH_4Cl, 0.09 g/L KP_2PO_4, and 2.31 g/L $NaHCO_3$) and trace elements with an organic loading rate (OLR) of 4 kg chemical oxygen demand (COD)/m^3d. The hydraulic retention time (HRT) was 12 h.

To clarify the excellent performance of QS regulation, this experiment ran four tests using the above configuration. Except for the different secreting strains in the regulating reactor, other conditions, including operating parameters and components, were constant. The regulating systems were as follows: the medium control (CK), which inoculated fresh Luria–Bertani (LB) broth to replace the strain solution; the negative control, or DSF regulation (DSF-QS), which inoculated *Xanthomonas campestris* (purchased from China General Microbiological Culture Collection Center [CGMCC], No. 1.3408) to act as the DSF secreting strain (Deng et al., 2011); the intraspecific regulation, or AHLs regulation (AHLs-QS), which inoculated *Pseudomonas aeruginosa* (purchased from CGMCC, No. 1.2814) to act as the AHLs secreting strain (Shrout and Nerenberg, 2012; Wang et al., 2012); and the interspecific regulation, or AI-2 regulation (AI-2-QS), which inoculated *Vibrio harveyi* BB170 (purchased from American Type Culture Collection [ATCC], No. ATCC #BAA-1117) to act as the AI-2 secreting strain. These secreting strains were cultured to the logarithmic growth period in LB broth at 30 °C and 130 rpm, and then inoculated into the QS regulating system. The frequency of replenishing the strain solution in the system was consistent with the HRT of the EGSB reactor. Before refreshing the strain solution in the system, three times the volume of fresh strain solution was used to wash out the outdated strain solution in the small regulating reactor. The content of signal molecules in the outdated strain solution was similar or higher than that of the fresh strain solution after one HRT. To decrease the interference of biofilm covering the membranes in the regulating reactor, the membranes were regularly replaced, typically after 4 HRT of the EGSB reactor.