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Where are signal molecules likely to be located in anaerobic granular sludge?

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ABSTRACT

Quorum sensing is a concentration-sensing mechanism that plays a vital role in sludge granulation. In this study, the regularities of distribution of different signal molecules, including intra- and interspecific signal molecules (diffusible signal factor, DSF), interspecific signal molecules (autoinducter-2, AI-2) and intraspecific signal molecules (acyl-homoserine lactones, AHLs), from three types of anaerobic granular sludge were investigated. The results showed that 70–90% of DSF was distributed in sludge, while AI-2 in the Water phase accounted for over 80% of the total content. Interestingly, there was a positive correlation between DSF and AI-2, which played opposite roles in granulation. Moreover, more than 55% of short and medium acyl chain AHLs tended to spread in aqueous water, while the long acyl chain AHLs were closer to granular sludge than the short and medium acyl chain AHLs. With the exception of one type of sludge, the percentage of long acyl chain AHLs in the sludge phase was greater than 70%. The different distributions of signal molecules were primarily determined based on their physicochemical properties, including molecular weight and solubility in water or organic solutions. In addition, the basic properties of sludge, such as the granular level or the production of EPS, were closely related to the diversity, distribution and concentration of signal molecules. As a medium in granulation, extracellular polymeric substances production was regulated by different signal molecules from different parts of anaerobic granular sludge. This study provides a foundation for investigation of quorum sensing in the system of anaerobic granular sludge.

1. Introduction

Quorum sensing (QS), which is cell–cell communication among bacteria, has recently been intensively regulated. This process, which includes intra- and interspecies communication (Fuqua et al., 1994), is accomplished through the exchange of extracellular signalling molecules released by bacteria that are known as auto-inducers (AI). When the signalling molecules in their microenvironment reach a threshold, the signal is received and the molecules begin regulating particular gene expression. This enables bacterial populations to enhance the

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effectiveness of community cooperation (Taga and Bassler, 2003). These processes include virulence factor expression, antibiotic (Chong et al., 2012) and extracellular enzyme production (Lazar, 2011), biofilm formation (Chen et al., 2002), degradation of organic pollutants (Jiang et al., 2006; Valle et al., 2004; Yong and Zhong, 2010) and plasmid transfer (Deng et al., 2011).

QS among bacterial cells has been a microbiological research focus in environmental sciences. Previous studies demonstrated that adding boron into sequencing batch reactor (SBR) would accelerate the growth of aerobic granular sludge. This occurs because boron is an important component of AI-2, which has a close relationship with the formation of aerobic granular sludge (Zhang et al., 2011). It has been suggested that AI-2 plays an important role in maintaining the stable structure and complete form of aerobic granular sludge, but that it may not be required at the beginning of aerobic granulation (Xiong and Liu, 2010). It has also been suggested that the production and expression of QS signal chemicals from granules and granule precursors induces the gene expression of bacteria in suspensions to enable attached growth rather than suspended growth (Ren et al., 2010). Another previous study showed that QS from activated sludge flocs could regulate the enzyme activity in specific cells through the response to changes in the concentration of acyl-homoserine lactones (AHLs) such as lipase and cellulose (Chong et al., 2012). Overall, these results suggest that AHLs play an important role in the ecological environment of aerobic sludge.

However, the currently available data do not reach a consensus on how to identify signal molecules in sludge systems. Most studies conducted to date have used the supernatant to identify AHLs and AI-2 from the aerobic granular sludge (Valle et al., 2004) or sludge flocs (Wang et al., 2012). However, Chong et al. (2012) suggested that AHLs or functionally equivalent molecules were present in activated sludge flocs, but not in the bulk aqueous phase. Moreover, Xiong and Liu (2012) showed that using samples of aerobic granulation homogenized by ultrasonication to determine AI-2 provided better results than ordinary supernatant. Therefore, we wondered which position the signal molecules preferred in anaerobic granular sludge. According to the mechanism of QS, the concentration of signal molecules sensed by bacteria determined whether QS would occur or not; therefore, it is critical to determine if the concentration of molecules present and those sensed by bacteria corresponded to each other. However, no conclusions about the distribution of signal molecules in the sludge system have been reached to date. Moreover, sludge systems, especially those composed of granular sludge, have characteristics that differ from other sludge. Accordingly, it is unclear which portion of the sludge system (Water phase, Washing water phase and Sludge phase) should be used to measure signal molecules, as different types of signal molecules have their own properties, such as solubility, diffusivity, stability and other properties (Decho et al., 2011), and whether the signal molecules played a role or not is related to the concentration of the specific portions in sludge. Therefore, this study is conducted to investigate the concentration distribution of signal molecules in the sludge system.

The formation of such granular sludge is often regarded as a special case of biofilm development. Additionally, these studies have suggested that signal molecules play an important role in the formation and maintenance of aerobic granular sludge (Ren et al., 2010; Valle et al., 2004; Xiong and Liu, 2010; Zhang et al., 2011), but there have been no such studies conducted to investigate anaerobic granular sludge. Therefore, this study was conducted to confirm the types, concentrations and distribution of signal molecules in anaerobic granular sludge, and to provide a reference for further research about the mechanism of QS in anaerobic granular sludge. For example, confirming the main conditions of different types of signal molecules in sludge system, or applying QS to regulate the rapid granulation of anaerobic sludge and guiding the addition of signal molecules in practical application.

2. Materials and methods

2.1. Seeding sludge

The anaerobic granular sludge used in this experiment was obtained from three typical industrial wastewater treatment plants, a pharmaceutical factory, brewery and paper-making factory (S1, S2 and S3, respectively). The sludge was cultivated in an expanded granular sludge bed for 3–5 days using cane sugar as a carbon source. The synthetic wastewater with a COD = 2500 ± 200 mg/L was replaced every 24 h and the temperature was maintained at 30 ± 2 °C in a greenhouse. The physicochemical properties of the sludge of the three types of sludge are shown in Table 1. The average granular diameters of sludge S1, S2 and S3 were 1.76, 0.91 and 1.64 mm, respectively. Overall, the characteristics suggested that there were differences among sludge and that the indicators including the biomass and biological activities, granular diameter distribution and the composition of extracellular polymeric substances (EPS) of the sludge were correlated with each other.

2.2. Extraction of signal molecules

The signal molecules of 100 mL of the mixture of sludge and water were divided into Water phase, Washing water phase and Sludge phase, and an aqueous phase which was produced from the Water phase and the Washing water phase. The mixture of sludge and water was centrifuged at 22 091 × *g* for 5 min. For the Water phase, the supernatants were harvested and the volume was recorded, after which the pellet was resuspended to the initial volume in physiological saline (0.9% NaCl) and the supernatants were collected through centrifuging. These operations were repeated with the pellet three times, and this was noted as the Washing water phase. Subsequently, the remaining sludge was crushed in physiological saline, and this was recorded as the Sludge phase. At the same time, 1 mL of the above liquid from each process was collected, respectively, filtered through a 0.22 μm syringe filter and stored at −20 °C for the AI-2 assay. Next, 50 mL of sample were extracted with an equivalent volume of ethyl acetate, after which the mixture was shaken vigorously and the