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Molecular mechanisms governing aerobic granular sludge processes

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ABSTRACT

The aerobic granular sludge process is a promising technology for wastewater treatment. The formation and structure of aerobic granules are traditionally thought to depend highly on selection pressures, while the underlying molecular mechanisms are unclear. It is well known that bacteria coordinate their behavior using small signaling molecules, known as quorum sensing (QS). This paper is an attempt to provide updated information on QS mechanisms governing granular sludge processes. It is shown that QS-mediated cellular communication has a significant role throughout aerobic granulation, including granule development, structural stability and integrity maintenance. Such understanding is helpful for developing novel aerobic granular sludge processes.

Key words: aerobic granule, autoinducer-2, AHL, quorum sensing

INTRODUCTION

Aerobic granules, a special microbial agglomeration, have been reported from the treatment of various types of wastewater, and offer the advantages of excellent settlability, high biomass concentration, and small footprint. The consistent and reproducible development of granular biomass is a key objective in wastewater treatment, because the unique properties of granules lead to significant improvements in treatment time, as well as economic and social gains in the associated reduced footprint of the treatment plants (Yilmaz *et al.* 2008). Comprehensive understanding or manipulation of granulation process and microbial function is of great practical interest. While aerobic granule formation has been thought to depend largely on selection pressure (e.g. hydrodynamic shear force, settling time, etc), the molecular mechanisms by which selection pressure influences the formation and/or structure of granular sludge are not clear.

It is understood that some bacterial species can synchronize behavior in a coordinated manner, using a type of cell-to-cell signaling known as quorum sensing (QS). According to QS theory, the production of signal molecules depends closely on population density, i.e., when the community population density reaches a threshold, signaling molecule-mediated QS is triggered to regulate community behavior. So far, several QS signal molecules and pathways have been identified in various groups of bacterial genera: acyl-homoserine lactone (AHL), signals for Gram negative bacteria, peptide signals for Gram positive bacteria and autoinducer-2 (AI-2) molecules for interspecies signaling of both Gram negative and positive bacteria (Waters & Bassler 2005; Williams *et al.* 2007). This cellular communication plays an essential role in biofilm development. The fundamental question is whether the QS mechanism is involved and how it may affect aerobic granulation. This paper is an attempt to offer a summary of the molecular mechanisms governing aerobic granular sludge processes, which may be helpful for further engineering and optimizing the aerobic granulation process.

QUORUM SENSING IN AEROBIC GRANULATION

Microbial biofilms, as demonstrated in many single-species studies, generally develop in a step-wise manner from initial surface attachment to micro-colony, maturation and differentiation through to dispersal (Parsek & Greenberg 2005). There is evidence that biofilm formation is regulated by QS with AHLs, auto-inducing peptides and AI-2 as QS signals. In general, aerobic granules can be regarded as a special form of biofilm with highly organized, three-dimensional structures, so it is reasonable to consider that QS would also be essentially involved in aerobic granulation.

The QS ability of bacteria functions through the secretion and detection of signaling molecules, and is regulated by cell density. In order to initiate microbial aggregation, the first step is to improve biomass density through external forces and appropriate operating conditions. Various kinds of selection pressure (e.g. shear force, loading, settling time, etc.) have been identified as operation strategies for initiating granulation. So far, several studies have reported possible QS molecular mechanisms in aerobic granulation (Zhang *et al.* 2011; Xiong & Liu 2012; Ren *et al.* 2013; Tan *et al.* 2014).

Mechanisms governing settling time-induced granulation

Settling time has been considered one of the most effective selection pressures for aerobic granulation in sequencing batch reactors (SBR). In order to investigate the molecular mechanism for this type of granulation, Xiong & Liu (2012) studied aerobic granulation in three SBRs run at different settling times of 3, 30 and 60 minutes, respectively. A significant increase in EPS and AI-2 was observed at the shortest settling time, 3 minutes, suggesting that EPS and AI-2-mediated QS were probably involved in the granulation. Enhanced EPS and AI-2 production were also observed in the other reactors, after the settling times were shortened from 30 to 1 minute and from 60 to 2 minutes, respectively. This suggests that both EPS- and AI-2-mediated QS contributed significantly to aerobic granulation, but playing different roles. As can be seen in Figure 1, both EPS and QS were involved in settling time-induced aerobic granulation, but with different roles at the different development stages of aerobic granules. After subjection to a short settling time, microorganisms in the SBRs were stimulated to produce EPS, which, in turn, initiated microbial aggregation as a response against being washed out.

Figure 1 | EPS (●) and AI-2 (○) versus particle size in SBR run at a settling time of 3 minutes (Xiong 2013).

At the start, increased production of EPS induced by a short settling time can help to initiate microbial aggregation, leading to increased biomass density, whereas no AI-2-mediated QS was found to be involved in this initial aggregation (the AI-2 content was low). When the biomass density reached a critical value, AI-2-mediated QS was activated to regulate the growth of aerobic granules. It appears that environmental selection pressure (e.g. short settling time) can induce microbiological and physiological responses from microorganisms, playing essential roles at the different developmental stages of aerobic granules.

Tan *et al.* (2014) also reported the involvement of an AHLs-regulated QS system in the rapid formation of aerobic granules. The shortened settling time induced greater production of specific AHLs, and the conversion of flocculent sludge to aerobic granulation was closely associated with increased AHL concentrations. On the other hand, after the selection pressure, in terms of settling time, was withdrawn from mature aerobic granules, aerobic granule dispersal was observed, accompanied by decreased concentrations of AHLs.

Mechanisms governing shear force-induced granulation

In a study of shear force-induced aerobic granulation, Xiong (2013) provided experimental observations showing the roles of both intra-(AHLs) and inter-species (AI-2) cellular communication during the development of aerobic granules in an SBR. The concentrations of these molecules were much higher in aerobic granules than in suspended activated sludge (Figure 2). This indicates that AI-2 and AHLs may be essential for the growth of aerobic granules, but that AI-2- and AHLs-mediated cellular communication was unlikely to have been involved in the initial stage of aerobic granulation (e.g., size less than 300 μm). Tan *et al.* (2014) also found that the concentrations of specific AHLs increased up to 100-fold and were positively correlated to granulation initiation. Conversely, the concentration of AHLs decreased significantly during the granule disintegration phase. The data also show that exogenously added AHLs resulted in more EPS production, i.e., EPS production was directly regulated by an AHL-mediated QS system and QS cellular communication may play an essential role in coordinated aerobic granulation.

Figure 2 | Changes in the concentrations of AHL, AI-2 and eDNA versus the size of microbial aggregates (Xiong 2013).

Since mature aerobic granules contain much higher concentrations of signal molecules than those of suspended activated sludges, Ren *et al.* (2010) directly dosed mature granular sludge with intracellular substances to accelerate granulation in the reactor. This offers a new insight from another angle. QS signaling molecules in granular sludge facilitate the granulation process. The effect of the external addition of AI-2 on granular sludge development was also reported, e.g., the addition of the AI-2 signal precursor (R)-4,5-dihydroxy-2,3-pentanedione could significantly stimulate aerobic granulation in SBRs (Zhang *et al.* 2011).

A direct relationship between the concentration of AHLs in the community and the stages of granule development, i.e. flocs versus granules, was also established recently (Tan *et al.* 2014). Elevated levels of specific AHLs, ranging from 4 to 8 carbon-atoms, in the bulk liquid medium were strongly associated with the initiation of granule formation, while signals decreased markedly to levels below the threshold concentrations for granulation to occur in the granule disintegration phase. These highly correlated metadata strongly suggest that AHL-mediated QS plays a key role not only in initiating aerobic granulation, but also in maintaining the stability of mature granules, meanwhile highlighting the QS-mediated community assembly as one of the native roles of QS in complex communities.

Conversely, quorum quenching (QQ) induced failure of microbial granulation would support the important role of QS-mediated cellular communication. In fact, failed microbial aggregation due to QS system inhibition has been reported in pure cultures. In a study of QS and self-aggregation, Chandler *et al.* (2009) found that wild-type strain *Burkholderia thailandensis* self-aggregated and formed macroscopic clumps in a minimal medium, while the AHL synthase-deficient mutant strain could not. Furthermore, inactivation of *luxS* based on gene interruption in *Streptococcus anginosus* resulted in a mutant deficient in AI-2 activity and ultimately inhibited biofilm formation (Petersen *et al.* 2006). It is therefore possible that, in complex communities like the granular sludge system, microbial cells may adopt different strategies to modulate their interactions with their neighbors in a synergistic or antagonistic manner through their QS and/or QQ activities.

The molecular mechanisms behind the selection pressure theory for aerobic granulation are illustrated in Figure 3: (i) in the beginning, the biomass density of activated sludge is low. QS-regulated cellular communication is unlikely to be involved; (ii) biomass density can be improved through external selective forces exerted on microorganisms, which in turn can induce more EPS secretion; (iii) after biomass density has reached a threshold, QS-mediated cellular communication is activated to coordinate the community behavior of both intra-(AHLs) and inter-species (AI-2); (iv) further production of signaling molecules and eDNA would regulate granule development and structure stability.

Figure 3 | Illustration of QS-mediated aerobic granulation.

REMARKS

In this mini review, the important roles of intra- and inter-species signaling molecules (AHLs and AI-2) in aerobic granulation have been discussed. It appears that aerobic granules may be manipulated by controlling the QS system toward promoted or inhibited granulation in an engineering system. It would be interesting to look into the feasibility of coupling aerobic granule-based biotechnology

with other treatment units, such as membrane bioreactors (MBRs), to combine the advantages of both processes for efficient wastewater treatment. In future, the relationship between aerobic granulation and microbial community dynamics should also be investigated. The physiological behavior of aerobic, granule-associated bacteria, like QS-controlled gene expression and gene transfer, also need further examination.

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