



Short Communication

Effect of N-acy-L-homoserine lactones-like molecules from aerobic granules on biofilm formation by *Escherichia coli* K12

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HIGHLIGHTS

- Demonstration of the quorum sensing effect of aerobic granules on *Escherichia coli* biofilm formation.
- Granular sludge promoted *E. coli* biofilm formation more than activated sludge.
- Detection of N-acy-L-homoserine lactonesin (AHL) by thin-layer chromatography (TLC).
- AHL-like signal molecules produced by granules for attached growth of *E. coli* K12.

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ABSTRACT

A laboratory study was conducted to investigate the production of quorum sensing (QS) molecules by aerobic granules in membrane-partitioned bioreactor. Flow-chamber (FC) tests with *Escherichia coli* K12 demonstrated that granules induced more attached growth of *E. coli* cells than activated sludge flocs, leading to more cell adhesion and biofilm formation on the FC cover slide. Using the thin-layer chromatography, N-acy-L-homoserine lactones (AHLs) with acyl chains shorter than 10 carbons were detected in the liquid phase of granular sludge. Organic substances extracted with acidified ethyl acetate from the supernatant of granular sludge promoted the adhesion and growth of *E. coli* cells on the glass surface. AHL-like signal molecules were apparently produced by granules and might be involved in the formation of granules and the maintenance of granular structures during wastewater treatment.

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1. Introduction

Aerobic sludge granulation is a process that has the potential to become one of next-generation biological wastewater treatment technologies (Tay et al., 2005; Ni et al., 2009). Compared to conventional activated sludge (AS), aerobic granular sludge (GS) has a compact structure and excellent settleability that allow for rapid sludge-effluent separation, a high level of biomass concentration and a greater organic loading capacity than the AS process (Tay et al., 2005; Abdullah et al., 2011). Aerobic granules are a special form of biofilms formed in sludge suspension in sequencing batch reactors (SBRs) (Li and Li, 2009; Guo et al., 2011); however, the controlling factors for granule formation and the underlying mechanism of aerobic granulation are not completely understood.

A bacterial community may regulate its action by exchanging chemical signals to coordinate the response of bacteria to environmental challenges and opportunities, a phenomenon denoted as quorum sensing (QS) (Raina et al., 2010). Microorganisms secrete a wide variety of small molecules that can be self-recognized in a concentration-dependent manner and subsequently induce or repress the expression of QS-controlled genes. It is also possible that signaling between bacterial cells may have functions for purposes other than the census taking (Camilli and Bassler, 2006; Christiaen et al., 2011). Previous studies on biofilms have shown that signaling chemical molecules released by the bacteria in biofilm affect the growth mode of bacteria inducing attached growth rather than suspended growth (Cha et al., 2008; Dunn and Stabb, 2007), which is essential to the growth and maintenance of biofilms in biological wastewater treatment. QS signal chemicals are often referred to as auto-inducers and can be classified based upon their molecular structures (Camilli and Bassler, 2006; Shroff and Nerenberg, 2012). N-acy-L-homoserine

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