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Short Communication

Quorum quenching is responsible for the underestimated quorum sensing effects in biological wastewater treatment reactors

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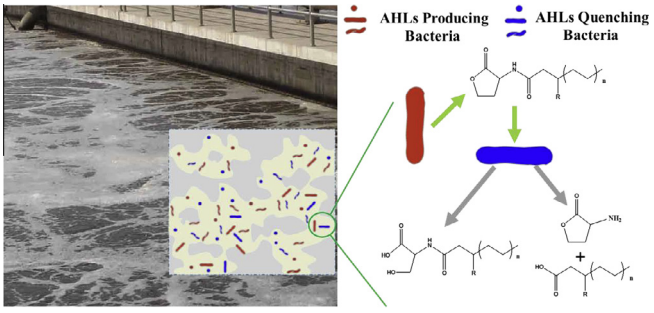
HIGHLIGHTS

- Quorum quenching (QQ) activity was evaluated *in situ* in an activated sludge reactor.
- The coexistence of quorum sensing (QS) and QQ in activated sludge was confirmed.
- The developed method could be used to monitor QQ in other biosystems.

GRAPHICAL ABSTRACT



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ABSTRACT

Quorum sensing (QS) and quorum quenching (QQ) are two antagonistic processes coexisting in various bacterial communities in bioreactors, e.g., activated sludge for biological wastewater treatment. Although QS signal molecules are detected in activated sludge reactors and known to affect sludge properties and reactor performance, there has been no direct evidence to prove the endogenous existence of QQ effects in activated sludge. In this study, for the first time, acyl homoserine lactones-degrading enzymatic activity, a typical QQ effect, was discovered in activated sludge and found to considerably affect the QS detection results. The coexistence of QS and QQ bacteria in activated sludge was further confirmed by bacterial screening and denaturing gradient gel electrophoresis analysis. The method developed in this study could also be used to evaluate QQ activities in bioreactors, and a possible way is provided to tune bioreactor performance through balancing the QS and QQ processes.

1. Introduction

Bacterial communities can coordinate their behaviors as a group through a process called quorum sensing (QS), in which bacteria secrete small-molecular signal chemicals, i.e., autoinducers, sense them, and consequently modulate cellular physiology as a feedback to population density (Miller and Bassler, 2001). Several types of autoinducers have been identified, among which acyl homoserine lactones (AHLs) are a major class of autoinducers commonly secreted and sensed by many bacterial species (Miller and Bassler, 2001). In biological wastewater treatment systems there are complex microbial communities, thus AHLs are considered to play an important role in adjusting these bacterial communities. So far, AHLs have been detected in various wastewater treatment

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bioreactors, which contain large quantities of dense microbial consortia in the form of flocs, granules or biofilm, and are found to affect activated sludge properties and reactor performance (Shrout and Nerenberg, 2012). Specifically, the microbial communities and pollutant degradation ability of activated sludge (Valle et al., 2004), the granulation of sludge (Tan et al., 2014), and the biofilm formation processes (Ren et al., 2013) are all affected by dosing AHLs.

An antagonistic process of QS is quorum quenching (QQ), in which the autoinducers such as AHLs are degraded or the receptors of AHLs are inhibited (Dong and Zhang, 2005). Several AHL-hydrolyzing enzymes including acylase have been identified (Dong and Zhang, 2005). The acylase, or bacteria capable of secreting these hydrolyzing enzymes, have been used in wastewater treatment bioreactors to mitigate biofouling through hydrolyzing AHLs (Oh et al., 2012). These studies indicate that the performance of wastewater treatment bioreactor can be tuned through selectively manipulating either QS or QQ, i.e., raising or lowering AHL activity. Thus, a deep understanding about these processes is essential.

Until now, most studies on QQ effect in bioreactors were conducted by exogenously dosing AHL-degrading enzymes or bacteira into reactors (Oh et al., 2012; Yeon et al., 2009). Several AHLs-producing bacteria, including *Acinetobacter* spp., *Aeromonas* spp. and *Pseudomonas* spp., have been identified in wastewater treatment bioreactors (Kämpfer et al., 1996). Meanwhile, some bacteria with QQ activity have been isolated from bioreactors (Cheong et al., 2013). By producing enzymes, which could cleavage of amide or lactone bonds (Uroz et al., 2009), these QQ bacteria, together with QS bacteria, control the concentration of AHLs and exert consequent biological effects. Although external dose of these bacteria was found to affect the QS and bioreactor performance, there is no direct evidence to support the existence of endogenous QQ in wastewater treatment bioreactors yet. QQ and QS, as the response of microbial community to environmental conditions, are thus very sensitive to the fluctuation of these conditions. Operational conditions, wastewater compositions, microbial diversity, etc., can affect the occurrence and balance of QQ and QS caused by diverse bacteria in bioreactors. In previous studies regarding QQ in bioreactors, QQ bacteria are usually isolated from the mixed cultures in bioreactors and then explored in terms of their QQ roles. Such a manner inevitably changes the status of the QQ bacteria, and thus it is impossible to judge whether these bacteria really play their QQ roles in bioreactors or not. So far, there is no report to directly reveal the native and endogenous QQ activity *in situ*. In other words, there is no clear information about the QQ in bioreactors, as well as whether QS and endogenous QQ would synergistically regulate the behaviors of microbial consortia in wastewater treatment bioreactors. Therefore, the main objective of this study was to examine whether there was an coexistence of endogenous QQ and QS activity in activated sludge process, with a membrane bioreactor (MBR) as an example, and explore the feasibility to improve reactor performance through regulating endogenous QQ activity.

2. Experimental

2.1. Chemicals and bacterial strains

N-(3-oxooxtanoyl)-L-homoserine lactone (3-oxo-C8-HSL) was purchased from Sigma–Aldrich Co., USA. 5-Bromo-4-chloro-3-indolyl-beta-D-galactopy-ranoside (X-gal), phenylmethanesulfonyl fluoride (PMSF) and o-nitrophenyl-β-D-galactopyranoside (ONPG) were purchased from Sangon Co., China. AHLs were detected by *Agrobacterium tumefaciens* KYC55, which shows a sensitive response to a wide spectrum of AHLs (Joelsson and Zhu, 2006). Another reporter strain *Aeromonas* sp. (pBB–LuxR) expressing green fluorescent protein (GFP) under induction of AHLs was used to *in situ* detect AHLs in the activated sludge (Chong et al., 2012).

2.2. Sludge extracts and autoclaved sludge extracts

MBR operation and sludge characteristics are described in Supplementary Materials. Aliquots of 4 ml sludge samples from an MBR were centrifuged at 5000×*g* for 10 min. The pelleted sludge was washed three times using equal volume of phosphate buffered saline (PBS) solution containing 1.0 mM PMSF to inhibit proteases, and then resuspended in 1 ml PBS-PMSF. The sludge incubated in an ice-water bath was subjected to ultrasonic disruption (80 w power output, 300 times, each time for 3 s followed by 3-s interval) using an ultrasonic cell disruptor (Ningbo Scientz Biotechnology Co., China) to lyse cells, followed by centrifugation at 12,000×*g* for 20 min. The extracted supernatants were filtered through a 0.22 μm filter. The filtrates were used to analyze AHL-degrading activity and autoclaved at 105 °C for 20 min to inactivate the AHL-hydrolyzing or AHL-modifying enzymes when necessary.

2.3. AHL degradation tests

The sludge extracts and autoclaved ones were mixed with equal volume of PBS solution containing 2 μM AHL. Here, 3-oxo-C8-HSL, which is widely utilized by a variety of bacteria (Barnard and Salmond, 2007; Horng et al., 2002), was chosen as a model AHL. The corresponding mixed solutions without AHL were used as two negative controls, and 1-μM AHL PBS solution was used as positive control. These five mixtures were incubated at 30 °C. Specified volumes of liquid samples were taken out every 2 h, and then incubated in a water bath at 90 °C for 10 min to inactivate enzymes. The as-obtained liquid samples were stored at −20 °C before analyzing the residual 3-oxo-C8-HSL. The protein contents of the five mixtures were determined by the bicinchoninic acid assay using protein assay kit (Sangon Co., China).

The relative content of 3-oxo-C8-HSL in the samples was determined following the method described by Joelsson and Zhu (2006). The assay of β-galactosidase activity was conducted according to Miller and the specific activity was expressed in Miller Units (Miller, 1972).

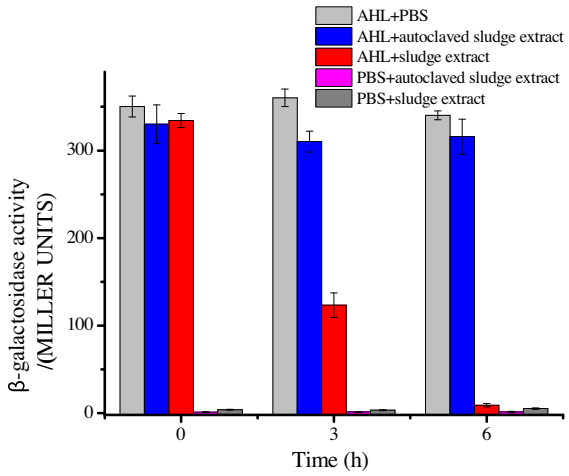

Fig. 1. Residual AHLs detected by *A. tumefaciens* KYC55 after 6-h incubation. β-Galactosidase activity of AHL + PBS, AHL + autoclaved sludge extract, AHL + sludge extract, PBS + autoclaved sludge extract, and PBS + sludge extract.