Water Research 125 (2017) 309–317

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Augmentation of acyl homoserine lactones-producing and -quenching bacterium into activated sludge for its granulation

Yu-Sheng Li [a, b], Xin-Rong Pan [b], Jia-Shun Cao [a], Xiang-Ning Song [b], Fang Fang [a], Zhong-Hua Tong [b], Wen-Wei Li [b], Han-Qing Yu [b, *]

[a] Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Hohai University, Nanjing, China
[b] CAS Key Laboratory of Urban Pollutant Conversion, Department of Chemistry, University of Science & Technology of China, Hefei, China

ARTICLE INFO

Article history:
Received 27 June 2017
Received in revised form
22 August 2017
Accepted 28 August 2017
Available online 29 August 2017

Keywords:
Quorum sensing (QS)
Quorum quenching (QQ)
Acyl homoserine lactones (AHL)
Activated sludge
Granulation
Community structure

ABSTRACT

Quorum sensing (QS), especially acyl homoserine lactone (AHL)-mediated QS, in activated sludge arouses great interests because of its vital role in the formation of biofilm and aerobic granules (AG). Although QS is reported to be largely related to the properties of activated sludge, it is not economically feasible to tune QS in an activated sludge reactor through dosing pure AHL or AHL hydrolase. A more reasonable way to tune QS is to augment reactors with AHL-producing or -quenching bacteria. In this work, the impacts of continuous dose of AHL-producing or -quenching strains on the activated sludge during its granulation process were explored. Augmentation of AHL-producing or -quenching strains resulted in up- or down-regulation of the AHL concentration in the reactors. Granulation of activated sludge was also accomplished in all reactors, but the granules showed negligible or slight differences in the physicochemical properties of sludge, such as nutrients removal, biomass concentration, extracellular polymeric substances, and zeta potential. Interestingly, a smaller granule size was observed for both the reactor augmented with either an AHL-quenching strain or an AHL-producing strain, suggesting that the AHL augmentation suppressed the biofilm development. Pyrosequencing analysis reveals that the granules cultured in the reactors varied widely in bacterial community structure, indicating that the AHL augmentation had a greater impact on the bacterial community structure, rather than on the physicochemical properties of activated sludge. These results demonstrate that the role of QS in the biofilm formation in complex wastewater treatment bioreactors should be re-evaluated.

1. Introduction

Since 1970, quorum sensing (QS) has attracted considerable attention because of its relevance to bacterial virulence and biofilm. One major class of auto-inducers associated with biofilm formation is acyl homoserine lactones (AHL), which is secreted by gram-negative bacteria and can be sensed by many species (Shrout and Nerenberg, 2012). Generally, experiments with pure cultures indicate that, for AHL-producers, AHL is necessary for biofilm formation and biofilm development would be suppressed after AHL is consumed. In the past decade the role of AHL-mediated QS in activated sludge has been a research focus (Chong et al., 2012; Huang et al., 2016; Valle et al., 2004), e.g., biofouling control based on AHL degradation through quorum quenching (QQ) (Jiang et al., 2013; Oh et al., 2012).

As a specific type of biofilm, aerobic granule (AG) has several advantages over conventional activated sludge for wastewater treatment, such as excellent settling capabilities, tolerance to toxicity, and high treatment efficiency, etc. Thus, enhancing granulation process of activated sludge is highly desirable. Some previous works have revealed that there is a relationship between AG and AHL-mediated QS (Li and Zhu, 2014; Tan et al., 2014), similar to the observations for the pure culture studies. AHL is reported to be essential to the formation and stability of AG, while quenching AHL leads to the reduced granulation potential of sludge (Li et al., 2014). However, there is a lack of report about the long-term effects of AHL on the granulation of sludge.

As a potential method of tuning bacterial community, AHL manipulation in a highly complex consortium such as activated sludge should be evaluated carefully. Since activated sludge contains AHL-producing and -quenching bacteria and other groups

* Corresponding author.
E-mail address: hqyu@ustc.edu.cn (H.-Q. Yu).

http://dx.doi.org/10.1016/j.watres.2017.08.061
0043-1354/

without AHL-relevant activity (Li et al., 2016; Tan et al., 2015), the effects of AHL-manipulation might be restricted to the AHL-relevant groups only. Although the role of AHL in biofilm development has been widely reported, the results have been controversial. In some cases, AHL induces biofilm dissemble (Solano et al., 2014). In addition to the AHL-mediated QS, many other factors also have impacts on the microbial community in activated sludge (Li et al., 2008). Thus, the contribution of AHL to the affected microbial community should also be re-examined.

In this work, biological AHL-manipulation by continuous dose of AHL-producing or -quenching bacteria into activated sludge reactors was proposed and a long-term study was designed to investigate the responses of activated sludge to the AHL-manipulation in its granulation. The physicochemical properties of activated sludge, e.g., nutrient removal, biomass concentration, extracellular polymeric substances (EPS), sludge particle size and surface charge were monitored. Also, the impacts of AHL-manipulation on microbial community structure were investigated by using pyrosequencing analysis. In this way, the response of activated sludge to the augmentation with AHL-producing or -quenching bacteria in its granulation process could be elucidated.

2. Materials and methods

2.1. Reactor and operation

Three sequencing batch reactors used in this work had a working volume of 4.0 L with a height/diameter ratio of 12. A whole operating cycle was 240 min, including 10 min feeding, 5 min discharging, and a given period of aeration (205–220 min) and settlement (5–20 min) according to the experimental design. The whole experiment duration was divided into four phases, including accommodation, AHL-manipulation, aeration intensity increase, and settling time reduction. From Phase II, at the beginning of each cycle, 50-ml *Shinella yambaruensis* AG384 solution was continuously dosed into R2 (called as AHL-enhanced reactor), 50-ml *Rhodococcus* sp. AG014 solution into R3 (called as AHL-quenched reactor), and 50 ml R2A medium (Reasoner and Geldreich, 1985) into R1. The details of the experimental setup are listed in Table 1. All reactors were equipped with thermostats and the operating temperature was set at 30 °C.

2.2. Synthetic wastewater and seed sludge

The composition of the used synthetic wastewater was as follows (mg/L): glucose 188, sodium acetate 256, sodium propionate 233, $(NH_4)_2SO_4$ 283, KH_2PO_4 13.2, $K_2HPO_4 \cdot 3H_2O$ 22.1, $CaCl_2 \cdot 2H_2O$ 25, $FeSO_4 \cdot 7H_2O$ 20, $MgSO_4 \cdot 7H_2O$ 20 and 1.0 ml trace element solution. The trace element solution contained (mg/L): H_3BO_3 50, $ZnCl_2$ 50, $CuCl_2 \cdot 2H_2O$ 30, $MnSO_4 \cdot H_2O$ 50, $Na_2MoO_4 \cdot 2H_2O$ 60, $AlCl_3$ 50, $CoCl_2 \cdot 6H_2O$ 50, and $NiCl_2 \cdot 6H_2O$ 50. Seeding sludge was collected from the Wangtang Municipal Wastewater Treatment Plant, Hefei, China. Its mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) was 3.01 g/L, 2.42 g/L and 126 ml/g, respectively.

Table 1
Reactor operating conditions.

Experiment phase	I	II	III	IV
Period (day)	0–36	37–58	59–74	75–104
Superficial flow-rate (cm/s)	0.8	0.8	1.5	1.5
Settling time (min)	30	20	20	Gradually reduced to 5
Augmentation	None	Y	Y	Y

Y: Dose of R2A medium into R1 as control, *Shinella yambaruensis* AG384 into R2 and *Rhodococcus* sp. AG014 into R3 respectively.

2.3. AHL-producing and -quenching strains and AHL determination

Shinella yambaruensis AG384 and *Rhodococcus* sp. AG014, previously isolated from a mature granular sludge reactor, were respectively used as AHL-producing and -quenching strains. The two strains were inoculated in 100 ml R2A medium and incubated at 30 °C, 180 rpm for 72 and 48 h, respectively.

AHL extraction was conducted following the methods described by Ravn et al. (2001). Briefly, after settlement of the mixed liquor, 200 ml of supernatant was obtained, acidified with formic acid (to a final formic concentration of 0.5%), extracted with 200 ml ethyl acetate for three times. The organic phase was then separated using a separating funnel, dehydrated with magnesium sulphate, and further concentrated by a rotary evaporator. When the liquid volume became less than 1 ml, it was transferred into 1.5-ml centrifuge tube and dried by nitrogen blowing instrument. The type and amount of AHL in the reactors were detected by thin layer chromatography (TLC) according to Ravn et al. (2001). TLC plate was purchased from Merck KGaA (115,683 | TLC Silica gel 60 RP-18 F_{254} s, Merck KGaA, Germany). Reporter strain *Agrobacterium tumefaciens* JZA_1 was used to detect AHL. X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside) was purchased from Sangon Biotech. Co., China.

2.4. Exopolymeric substances (EPS) extraction and determination

EPS were extracted using a cation exchange resin method as described previously (Sheng and Yu, 2006). A volume of 50 ml sludge mixed liquor was collected and centrifuged at 4 °C, 8000 rpm. The pellet was washed with 0.1 M NaCl solution and centrifuged for two times. The solid phase was then crushed and re-suspended in a beaker. A certain amount of cation exchange resin (70 g resin/g VSS) was also added into the beaker. The extraction was performed at 4 °C with magnetic stirring at 600 rpm for 6 h. After the extraction, the solution was centrifuged, filtrated through 0.45 μm acetate fiber filter, and stored at 4 °C prior to analysis. Total quantity of EPS was measured by total organic carbon analyzer (N/C 2100, Analytik Jena AG, Germany), the protein was determined using the modified Lowry method (Liu and Fang, 2002), and polysaccharide was measured by the anthrone colorimetry method (DuBois et al., 1956).

2.5. Sludge sampling, DNA extraction and high through-put sequencing

On Day 104 in the operation of reactors, sludge samples from each reactor were collected. All samples were washed with phosphate-buffered saline (PBS) for three times. DNA extraction was done by using the PowerSoil DNA isolation kit (MO BIO Laboratories Inc., USA). Raw DNA was quantified by Qubit2.0 DNA kits (Thermo Fisher Scientific, USA). The DNA samples were then amplified, targeting V3-V4 hypervariable region of 16S rRNA. Amplicons were sequenced by MiSeq platform (Illumina Co., USA).

Raw sequence reads data from MiSeq platform was merged with software FLASH (Magoč and Salzberg, 2011), the false-positive rate was controlled <0.1%. Merged sequences were spliced into each samples according to the barcodes using QIIME (Caporaso et al., 2010). Further quality control was carried out by Prinseq (Schmieder and Edwards, 2011). The threshold value was set at 40 to filter low-complexity sequences, and sequences shorter than 50 bp were also filtered. The error sequences due to pyrosequencing and chimeric sequences were removed by pre. cluster and chimera. uchime commands in Mothur (Schloss et al., 2009), respectively.