



Estimation of spatial distribution of quorum sensing signaling in sequencing batch biofilm reactor (SBBR) biofilms

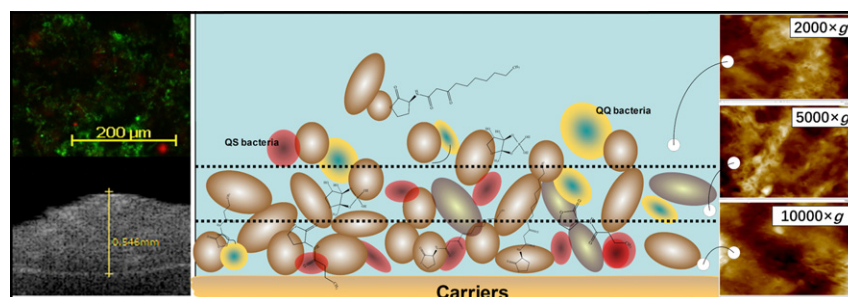
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HIGHLIGHTS

- Biofilms has been divided into three different fractions by centrifugal forces;
- SEPS and LB EPS concentration in different fractions of biofilms displayed significant positive relationship with the distribution of C12-HSL;
- Biofilm adhesion and compliance was the strongest in the tightly-bound biofilm, the weakest in the supernatant/surface biofilm;
- QS and QQ bacteria have been recognized in the wastewater treatment biofilms.

GRAPHICAL ABSTRACT



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ABSTRACT

Quorum sensing (QS) signaling, plays a significant role in regulating formation of biofilms in the nature; however, little information about the occurrence and distribution of quorum sensing molecular in the biofilm of carriers has been reported. In this study, distribution of QS signaling molecules (the acylated homoserine lactones-AHLs, and AI-2), extracellular polymeric substances (EPS) and the mechanical properties in sequencing batch biofilm reactor (SBBR) biofilms have been investigated. Using increased centrifugal force, the biofilms were detached into different fractions. The AHLs ranged from 5.2 ng/g to 98.3 ng/g in different fractions of biofilms, and N-decanoyl-DL-homoserine lactone (C10-HSL) and N-dodecanoyl-DL-homoserine lactone (C12-HSL) in the biofilms obtained at various centrifugal forces displayed significant differences ($p < 0.01$). Interspecies communication signal autoinducer-2(AI-2) in the biofilms ranged from 79.2 ng/g to 98.3 ng/g. Soluble EPS and loosely bound EPS content in the different fractions of biofilms displayed significant positive relationship with the distribution of C12-HSL ($r = 0.86$, $p < 0.05$). Furthermore, 49.62% of bacteria in the biofilms were positively related with AHLs with 22.76% was significantly positively ($p < 0.05$) related with AHLs. Biofilm adhesion and compliance was the strongest in the tightly-bound biofilm, the weakest in the supernatant/surface biofilm, which was in accordance with the distribution of C12 HSL ($r = 0.77$, $p < 0.05$) and C10-HSL ($r = 0.75$, $p < 0.05$), respectively. This study addressed on better understanding of possible methods for the improvement of wastewater bio-treatment through biofilm application.

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1. Introduction

In recent years, biofilm, in terms of dense and diverse microbial communities encased in a secreted polymer matrix, has attracted researchers' growing interests for its high efficiency of pollutants

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removal, to upgrade the capacity in Anaerobic-Anoxic-Oxic (A^2O) process in wastewater treatment systems (Bassin et al., 2012; Nadell et al., 2016; Rikmann et al., 2017). Key characteristics of biofilm used for wastewater treatment are thickness (Torresi et al., 2016), extracellular polymeric substances (EPS) (Flemming and Wingender, 2010), tolerance (Lewis, 2006), mechanical properties (Persat et al., 2015). Interestingly, chemical signals which are also used for intercellular communication, i.e., quorum sensing (QS), a mechanism controls biofilm development and the growth of bacterial populations (Williams et al., 2007), has drawn the attention of scientists and has been investigated in the special forms of biofilm, i.e., sludge (Tan et al., 2015; Tan et al., 2014). Quorum sensing communities also perform nitrogen shortcut technologies by *Pseudomonas* organism-like biofilms and autotrophic bacteria offering significant cost savings over traditional biological nitrogen removal (Zekker et al., 2015; Zekker et al., 2016). Furthermore, some function microbial, such as *Pseudomonas* (Daija et al., 2016), which uses quorum-sensing signaling systems (Chugani et al., 2001), was used as seed for many water treatment systems (Raudkivi et al., 2017; Zekker et al., 2016). However, the mechanism by which QS regulated formation of biofilm in wastewater treatment process, and the complex relation among QS, biofilm thickness and bacterial community composition needs further investigation.

Biofilm formation and microbial attachment on the bio-carriers are multiple-step processes. Physicochemical forces (hydrodynamic force, gravity force, etc.) and biological forces (production of extracellular polymer, growth of bacteria clusters, etc.) play significant roles in these processes (Boelee et al., 2011). A good understanding of the mechanical forces of biofilms which provide mechanical stability of microorganisms requires additional structural and compositional analysis of the samples (Persat et al., 2015). Mechanical properties of different types of wastewater treatment biofilms have been studied under various modes of loading as given in a comprehensive review by Safari et al. (2015). The significant variations in the reported data were not surprising as different bacterial species produced various types of EPS under different environmental conditions (Williams and Bloebaum, 2010). EPS immobilize microbes in biofilms and keep them in close proximity, thus providing a continuous on-off mechanism for modifying the concentration of these molecules in the biofilm matrix. The matrix are consisting of polysaccharides, proteins, lipids and extracellular DNA (eDNA), released by microbes and adhered onto the cell surfaces of activated sludge, granular sludge, and biofilms in wastewater treatment (Flemming and Wingender, 2010; Yu et al., 2008). Pellicer-Nàcher et al. (2013) assayed EPS in sludge flocs, and their findings indicated that EPS were composed of soluble EPS (SEPS) and bound EPS, which can be further classified into loosely bound extracellular polymeric substances (LB-EPS) and tightly bound EPS (TB-EPS). The adhesion of SEPS to cells is weak; as a result, SEPS are often dissolved in solution. Furthermore, TB-EPS have a certain morphology while LB-EPS have no distinct boundary (Liu and Fang, 2002).

Despite the complexity of biofilm matrix and communities, cell-cell interactions and communication have played significant roles in biofilm spatial structuring (Battin et al., 2016). Biofilm formation enables effective intercellular communication, either using chemical or electrical signals, even in habitats where signaling molecules are not contained by the biofilm would be readily diffused (Flemming et al., 2016). It has been suggested that the production of QS signal chemicals from biofilms induces the gene expression of bacteria in suspensions to enable attached growth rather than suspended growth (Ren et al., 2010). Numerous QS signal molecules and circuits involved in acylated homoserine lactones (AHLs) have been identified and genes regulated by QS have been defined in a diverse group of bacteria genera, which are closely related with EPS production in natural and manmade systems (Tan et al., 2014). Furthermore, quorum quenching (QQ) bacteria, which is possible to degrade the QS signal by secretion of certain enzymes, has drawn close attention. QQ bacterial were found to be distributed in the sludge environment (Tan et al., 2015). Indeed, the complex

relationship between QS and QQ needs further investigation. However, the majority of previous research studies have mainly focuses on the effect of QS on biofilm attachment on pure cultures or mechanism of bacteria granulation at bench scale, rather than in the real packing (Hu et al., 2016; Tan et al., 2014). Moreover, whether wastewater treatment or bioremediation can be enhanced and optimized by promoting specific QS signaling pathways for beneficial biofilms, is still unknown and requires further research, especially for the relationship among QS signal distribution, biofilm structure and energy efficiency.

Up to now, the studies of mature biofilms have been focused on the whole character, but the heterogeneity of biofilms makes it complex and hard to evaluate different properties of different parts of the biofilm. A systemic investigation was necessary to present the inside world of biofilm and to bring a better understanding of wastewater treatment biofilms. In this study, the SBBR biofilm have employed and detached into different fractions. We aimed to investigate the distribution of signals (acylated homoserine lactones-AHLs, and autoinducer-2-AI-2) in the fractions of biofilm. And the role of QS signaling in the production of EPS, communities assemble and further highly complex biofilm mechanical properties has been investigated.

2. Material and methods

2.1. The sequencing batch biofilm reactor (SBBR) operation

The bio-carriers had a 0.95 g/cm^3 density, a $10 \times 25 \text{ mm}$ dimension and a specific area for biofilm growth of $460 \text{ m}^2/\text{m}^3$ (Jiangsu Yulong Environment Protection Co., Ltd., China) (Zhu et al., 2015). The bio-carriers were used for the SBBR experiments.

Cylindrical SBBR had an effective volume of 9 L (45 cm height, 16 cm diameter). The cycle time of the reactor was 12 h, and the wastewater cycle was comprised of the following phases: 15 min feeding, 11 h 30 min aeration, and 15 min drainage. No settling phase was needed since the biomass was attached to plastic carriers (Zhu et al., 2015). The amount of carriers corresponded to a volume fraction of 35% ($V_{\text{support}}/V_{\text{reactor}}$). Moreover, an aerator was placed at one side of the bottom of the tank. The SBBR was initially fed with a synthetic medium and was inoculated with aerobic activated sludge collected from the secondary sedimentation tank of cyclic activated sludge technology (CAST) in Xianlin municipal WWTP in Nanjing, China. After 24 h, the inoculated sludge was discharged from the reactor when the microorganism of sludge adhered to the surface of carriers adequately. The reactor operated with synthetic wastewater and a detailed list of components is in Table 1. For the biofilm formation process, the concentration of 500 mg/L COD were selected as the higher concentration of municipal wastewater (Zhu et al., 2015), and shock resistance was conducted by a higher concentration of 1000 mg/L COD. For the SBBRs, The dissolved oxygen (DO) concentration was varied along the operational cycle, around 4.0–5.0 mg/L. The temperature was kept at $25 \pm 2^\circ\text{C}$, pH was maintained between 7.2 and 7.5 (Zhu et al., 2015).

2.2. Removal of the biofilm from the substratum at three different centrifugal speeds

After 100-day reactor operation, the bio-carriers were harvested and used for further study. In order to examine the inside of biofilms,

Table 1
The specific components of synthetic wastewater.

Components	Concentration (mg/L)	Components	Concentration (mg/L)
Glucose	500.0	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	5.08
NH_4Cl	200.0	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.28
KH_2PO_4	19.0	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.44
Na_2CO_3	53.6	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.39
$\text{FeCl}_3 \cdot 3\text{H}_2\text{O}$	2.42	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.42
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.37	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	1.26

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