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Physicochemical characteristics and microbial community evolution of biofilms during the start-up period in a moving bed biofilm reactor

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HIGHLIGHTS

- Adhesion force of biofilm during its development was characterized.
- 16S rDNA-based MiSeq sequencing for revealing microbial community structure.
- *Sphaerotilus*, *Zoogloea* and *Haliscomenobacter* were predominant in the initial stage.
- Significantly positive correlation of adhesion force and EPS content.

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ABSTRACT

This study aimed to investigate biofilm properties evolution coupled with different ages during the start-up period in a moving bed biofilm reactor system. Physicochemical characteristics including adhesion force, extracellular polymeric substances (EPS), morphology as well as volatile solid and microbial community were studied. Results showed that the formation and development of biofilms exhibited four stages, including (I) initial attachment and young biofilm formation, (II) biofilms accumulation, (III) biofilm sloughing and updating, and (IV) biofilm maturation. During the whole start-up period, adhesion force was positively and significantly correlated with the contents of EPS, especially the content of polysaccharide. In addition, increased adhesion force and EPS were beneficial for biofilm retention. Gram-negative bacteria mainly including *Sphaerotilus*, *Zoogloea* and *Haliscomenobacter* were predominant in the initial stage. *Actinobacteria* was beneficial to resist sloughing. Furthermore, filamentous bacteria were dominant in maturation biofilm.

1. Introduction

Biofilm is a structured community embedded within a self-produced matrix of EPS (Flemming and Wingender, 2010). The matrix with three-dimensional architecture provides mechanical stability of the biofilm and facilitates the microbial community more resistance to the outside world (Karunakaran et al., 2011). Biofilm-based processes have been widely applied in the field of environmental pollution control (Bassin et al., 2012; Biswas et al., 2014; Delnavaz et al., 2010), where the formation of biofilm attached on the surface of bio-carrier is vital for the bioreactor performance, especially for the start-up process during the operation of system.

Generally, a typical process of biofilm formation includes: preconditioning of the adhesion surface, attachment of planktonic cells, microbial multiplication through intercellular communication, EPS production, biofilm maturation and detachment (Ansari et al., 2012; Fernandez et al., 2008). Biofilm study in environmental system has been extensively carried out either from morphology observation by scanning electron microscope (SEM) or atomic force microscope (AFM) where AFM has been exploited to characterize microbial surface in terms of structure and function with higher resolution, various operating modes, surface feature and force measurement, as well as three–dimensional images (Abe et al., 2011; Oh et al., 2009), or from microbial community analysis by molecular-based biotechnologies, such as denaturing gradient gel electrophoresis (DGGE), fluorescence in situ hybridization (FISH) and high-throughput sequencing (Fernandez et al., 2008; Liu et al., 2014). MiSeq platform for high-through put sequencing with lower cost than other platforms, enabling microbial ecology at the greatest coverage and accurate data has been used popularly (Caporaso et al., 2012). The evolution of microbiota composition involved in biofilm formation also has been studied in aerobic or anaerobic biofilm system using DGGE and pyrosequencing

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technology (Biswas et al., 2014; Fernandez et al., 2008). However, clear descriptions of both morphology and microflora variations of biofilm coupled with the specific stages during its development, have been limited so far.

In addition, lots of researchers investigated EPS in order to understand its contribution towards biofilm adhesion and cohesion. Protein (PN) and polysaccharide (PS), as the main constituents of EPS, have been correlated with biofilm formation and adhesion (Czaczyk and Myszka, 2007; Ras et al., 2013). However, the literature about which component of those two EPS contributed to biofilm adhesion force is contradictory. PN is usually regardedKarunakaran and Biggs, 2011; Pellicer-Nacher and Smets, 2014 to have a greater contribution (). Oppositely, Ahimou et al. (2007) attributed PS to biofilm adhesion force. Recently, adhesion force determinated by AFM, a direct indicator for evaluating biofilm status, has been utilized in the area of biofouling including membrane-fouling biofilm (Ahimou et al., 2007) and drinking water pipe biofilm (Abe et al., 2011). Nevertheless, its application in attached-growth biofilm reactors has been in scarcity.

The moving bed biofilm reactor (MBBR) has been widely applied to treat both urban and industrial wastewaters due to the advantages of the biofilm process (compact, stable removal efficiency and simplicity of operation) without its drawbacks (medium channeling and clogging) (Barwal and Chaudhary, 2014; Biswas et al., 2014; Calderon et al., 2012). The objective of the MBBR systems is to achieve the growth of the biomass as a biofilm on small carriers moving around in the aeration tank under operation (Delnavaz et al., 2010). Therefore, regulation of biofilm is important for a stable and efficient operation of the system (Biswas et al., 2014). Factors such as temperature, pH, dissolved oxygen and nutrition concentration, as well as carrier type and surface characteristics involved in regulation of bioreactor performance and biofilm properties have been widely investigated (Ansari et al., 2012; Barwal and Chaudhary, 2014; Bassin et al., 2012). It is necessary to investigate biofilm variations of physicochemical characteristics and microbial community which play important roles in biofilm formation for successful MBBR start-up and operation (Biswas et al., 2014), so as to understand its formation and development, and finally the regulation of biofilm.

In the present study, specific stages during the start-up period of biofilm development in MBBR were characterized. AFM and high-throughput 16S rRNA gene amplicon sequencing using the MiSeq platform were applied to reveal the morphology, adhesion force and microbial community evolution of biofilm, respectively. To our best knowledge, it is the first attempt to combine the two technologies to characterize biofilms coupled with the specific stages in the MBBR during the start-up period. EPS including PN and PS, and the biomass expressed by volatile solid (VS), were also investigated. The relationship between EPS and adhesion force was finally presented. This study may provide clear information on key characteristics elaborating biofilm development and the basis for optimizing MBBR start-up.

2. Methods

2.1. MBBRs configuration and operation

The experiment was conducted in a cylindrical MBBR with an effective volume of 9 L (45 cm height, 16 cm diameter). The reactor operated in a sequencing-batch mode as illustrated in Fig. 1 and filled with suspended bio-carriers (0.95 g/cm^3 density and 10 × 25 mm dimension). The cycle time of the reactor was 12 h, and the cycle comprised the following phases: 15 min feeding, 11 h 30 min aeration, and 15 min withdraw. No settling phase was needed since the biomass was attached to the plastic carriers. The specific area for biofilm growth is 460 m^2/m^3. The amount of carriers corresponded to a volume fraction of 35% ($V_{support}/V_{reactor}$). Moreover, an aerator was placed at one side of the bottom of the tank. Besides providing good oxygen transfer into the synthetic wastewater, air generated by this device also allowed the carriers to circulate around the whole chamber.

The MBBR was initially fed with synthetic medium and was inoculated with 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. And the reactor operated with synthetic wastewater. Synthetic wastewater with influent COD of 390 ± 10 mg/L and NH_4^+-N of 40 ± 5 mg/L was selected in the feed. The synthetic wastewater contained glucose 400 mg/L, NH_4Cl 240 mg/L and KH_2PO_4 19 mg/L as primary nutrients, as well as $CaCl_2$ 30 mg/L, $FeSO_4$ 2.49 mg/L, $MnCl_2{\cdot}4H_2O$ 0.28 mg/L, $CuSO_4{\cdot}5H_2O$ 0.39 mg/L, $ZnSO_4{\cdot}7H_2O$ 0.44 mg/L and $CoSO_4{\cdot}7H_2O$ 0.5 mg/L as trace nutrients. Na_2CO_3 120 mg/L was added to adjust pH.

For the MBBRs, The dissolved oxygen (DO) concentration was varied along the operational cycle, around 4.0–7.5 mg/L. The temperature was kept at 25 ± 2 °C, pH was maintained between 7.5 and 8.0.

2.2. Sample preparation

Influent and effluent of the reactor were sampled for water quality analysis. Representative bio-carriers contained biofilms were removed from the reactor at different times. After collected, a piece of the carrier was to be cut carefully with a dimension about 4 × 10 mm by a scalpel in order to keep the original biofilm structure and analyzed immediately for AFM experiment in situ. Samples for biomass, PN and PS were stored at −80 °C until analysis. Biofilms for microbial community determination were detached from bio-carriers and filtrated through 0.22 μm filter paper, and the filter was immersed in 50% ethyl alcohol and stored at −20 °C before DNA extraction. Biofilms for analyzing VS, PN, PS and microbial community were manually scraped from the carriers using tweezers and mixed with sterile saline solution firstly. After that, the treated carriers were placed in sterile containers, added with sterile saline solution and vortexed for 1 min (Biswas et al., 2014; Calderon et al., 2012). The suspended biofilm material was collected by centrifugation at 3000*g* for 10 min.

2.3. Analytical methods

Volatile solids (VS) of the attached biomass, and chemical oxygen demand (COD), ammonium (NH_4^+-N), nitrate nitrogen (NO_3^-) and nitrite nitrogen (NO_2^-) of the wastewater samples were all directly measured according to Standard Methods (APHA, 2005). Dissolved oxygen, pH and temperature values were recorded using oxygen (SG6, METTLER TOLEDO Inc., USA) and pH meters (FE20, METTLER TOLEDO Inc., USA). Meanwhile, the extraction of PN and PS from biofilms has been described in the previous study (Ahimou et al., 2007). PS concentration was measured using the phenol sulfuric acid method with glucose as standard (Nielsen and Jahn, 1999). PN concentration was determined by the bradford assay with bovine serum albumin (BSA) as standard (Bradford, 1976).

2.4. Morphology and adhesion force determination

Morphology and adhesion force of biofilms was performed by using AFM. Biofilm morphology in air was carried out after drying