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Microbial communities, extracellular proteomics and polysaccharides: A comparative investigation on biofilm and suspended sludge

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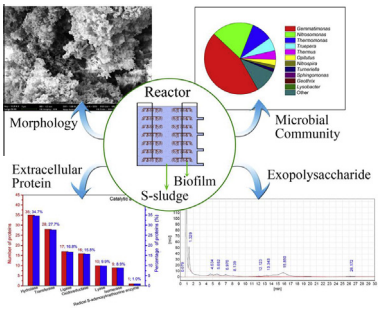
HIGHLIGHTS

- The S-sludge had a more flexible and loose spatial structure than the biofilm.
- Denitrification was mainly performed in the S-sludge.
- 103 and 126 proteins were classified in biofilm and S-sludge, respectively.
- The results could elucidate the why of the two aggregate architectures diversity.

GRAPHICAL ABSTRACT



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ABSTRACT

Biofilm and suspended sludge (S-sludge) floc exhibit distinct physicochemical properties and process performances in an integrated fixed-film and suspended growth sequencing batch reactor. However, the mechanisms of governing these differences between the two aggregates were unknown. Current work evaluated the diversity of morphologies, microbial communities, extracellular proteins and polysaccharides between the biofilm and S-sludge. Contrast to biofilm, the denitrification was much more extensive performed in S-sludge. Furthermore, many microbial cells in the biofilm acted as the backbone of aggregates and maintained the structure stability. An extracellular protein observed only in the biofilm can promote the cell adhesion. In contrast, more extracellular proteins related to catalytic activity in the S-sludge could decrease the compactness of floc. In addition, the monosaccharide compositions from the two aggregates were various. These results could elucidate how the diversities of architecture and biochemical process between the two aggregates occurred.

1. Introduction

Integrated fixed-film and suspended growth sequencing batch reactor (SBR) has captured many researchers' attentions in the investigations on emerging contaminants degradation, nitrogen and phosphorus removal by alternating anoxic and aerobic conditions (Jin et al., 2012; Cai et al., 2013). Both biofilm and suspended sludge (S-sludge) floc are the mainly types of microbial aggregate in an integrated fixed-film and suspended growth SBR system. The microorganisms exist in solution as the floc form for S-sludge, whereas they adhere on carrier as the form of tightly aggregation for biofilm. The S-sludge and the microorganisms dispersed from biofilm will drift with the water flowing at the aeration stage,

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and they will settle on the bottom of reactor or adhere onto biofilm at anaerobic stage.

Different types of microbial aggregate, such as granular sludge, S-sludge and biofilms, play various roles in overall performance based on different microbial communities, surface physicochemical properties and physiological functions (Falas et al., 2012, 2013). Winkler et al. (2013) revealed that the dominant ammonium oxidation bacteria were *Nitrosomonas* in S-sludge, whereas in granular sludge, *Nitrosomonas* and *Nitrosospira* were equivalent, even though they fed with the same wastewater. Biofilms have a lower negative surface charge and are more hydrophilic than S-sludge flocs, and the microbial community compositions of the biofilm are distinct from the S-sludge flocs in integrated fixed-film activated sludge systems (Mahendran et al., 2012). On the contrary, Chao et al. (2014) stated that more hydrophobic bacteria were present in biofilms rather than S-sludge. These inconsistencies could be associated with the microbial species and extracellular polymeric substances (EPS) in the surface of aggregate. In these microbial aggregates, EPS act as a protagonist is closely related to the microbial growth and surface physicochemical properties, and exerts a crucial function in pollutant removal as well (Flemming and Wingender, 2010; Su and Yu, 2005).

EPS is a complex high-molecular-weight mixture of polymers which most main compositions are extracellular proteins and polysaccharides. Extracellular protein enzymes broke down biopolymers to small molecular products, which are taken up and utilized as carbon and energy sources by microorganisms. Extracellular proteins can promote the aggregation of S-sludge flocs, the formation of biofilm and granules, and maintain the stability of the microbial aggregates (Flemming and Wingender, 2010; Lv et al., 2014). Furthermore, some exopolysaccharides could also play an important role in granulation (Seviour et al., 2009). β-polysaccharides act as the backbone of microbial aggregates and associate with structural integrity of granules (Adav et al., 2008). In addition, exopolysaccharides, polypeptides and proteins make the primary contribution to the interaction with environmental pollutants (Guibaud et al., 2003; Moreau et al., 2007). Since the EPS composition was complicated, the separation and identification of major compositions from them are indispensable to further investigation.

Our previous work demonstrated that the biomass activities, e.g. nitrification, of biofilm were significantly higher compared with those of the S-sludge in a completely autotrophic nitrogen removal over nitrite process (Chen et al., 2013a). Carriers can potentially affect the production and characteristics of biofilm EPS (Chen et al., 2013b). Furthermore, the aggregation potential of biofilm cells was higher than that of S-sludge cells, and the EPS in S-sludge exhibited important roles in flocculation and aggregation (Zhang et al., 2014). Therefore, it is indispensable to investigate the microbial communities, extracellular proteins and polysaccharides in different types of microbial aggregate to further analysis of their respective functions. In this work, the microbial morphologies and communities from biofilm and S-sludge in an integrated fixed-film and suspended growth SBR were analyzed by microscope and Illumina MiSeq sequencing, respectively. The proteins separated from EPS were identified by shotgun proteomics, and their origins and functions were investigated. In addition, the exopolysaccharides were also analyzed by determining the Fourier transform infrared (FT-IR) spectra and monosaccharide compositions.

2. Methods

2.1. Biofilm and S-sludge

Both biofilm and S-sludge samples were collected from an integrated fixed-film and suspended growth SBR with more than 2 years of steady operation. The reactor was fed with synthetic inorganic wastewater, which the details on the composition of the synthetic wastewater as follows: 1975 mg/L NH_4HCO_3, 328 mg/L $NaHCO_3$, 70 mg/L KH_2PO_4, 2 mL of trace element solution [5.0 g/L EDTA, 1.6 g/L $CoCl_2{\cdot}6H_2O$, 2.2 g/L $ZnSO_4{\cdot}7H_2O$, 5.1 g/L $MnCl_2{\cdot}4H_2O$, 1.6 g/L $CuSO_4{\cdot}5H_2O$, 1.1 g/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 5.5 g/L $CaCl_2{\cdot}2H_2O$, 5.0 g/L $FeSO_4{\cdot}7H_2O$]. The temperature of the reactor was controlled at 30 to 34 °C. Dissolved oxygen was kept using intermittent aeration, and which was 1.8 mg/L at aeration stage. pH (8.0 ± 0.2) and hydraulic retention time (2 d) of the reactor were maintained. The process performance of reactor is shown in Table 1, which indicated that the system exhibited satisfactory nitrogen removal efficiency.

2.2. Environmental scanning electron microscope (ESEM) and SEM

ESEM was used to observe and analyze sample in natural state without sample pretreatment, which can feature more realistic apparent and complete observation to sample. The biofilm and S-sludge samples were imaged directly using an ESEM (Quanta 450, FEI, USA). The images with higher resolution were obtained using an SEM. The biofilm and S-sludge samples were fixed by glutaraldehyde, and then rinsed three times using phosphate buffer (0.1 mM, pH 7.0). After dehydration with ethanol, the samples were lyophilized and observed by a focused ion beam-SEM (Auriga, Zeiss, Germany).

2.3. Illumina MiSeq sequencing

DNA of the biofilm and S-sludge cells were extracted using 3S column centrifugal environmental sample DNA extraction kit (Shennengbocai Biotech, Shanghai, China). 16S rRNA gene PCR amplification, Illumina MiSeq sequencing, and data analysis were performed by Personalbiology Biotechnology (Shanghai, China) Co., Ltd. Primers 520F (5-AYTGGGYDTAAAGNG-3) and 802R (5-TACNVGGGTATCTAATCC-3) which target V4 regions of bacterial 16S rRNA genes were selected.

2.4. Extracellular proteome assays

2.4.1. Extraction and purification of protein

The extracellular proteins were analyzed by shotgun proteomics. The EPS in biofilm and S-sludge were extracted using cationic exchange resin (CER) method. In general, 20 mL of microorganism suspension and CER (70 g/g VSS) were transferred to a conical flask. The extraction was conducted in a shaking incubator at 250 rpm for 2 h. Then the suspension was centrifuged at 10,000*g* for 20 min. The acquired supernatant was filtered through 0.45 μm filter and stored for protein purification. The protein in EPS was separated by the modified method of trichloroacetic acid (TCA) precipitation (Silva et al., 2012). In general, the TCA (100%, w/v) was added to the EPS sample solution to a final concentration of 13%, and the mixture was incubated overnight after a thoroughly mixed. Then the mixture was centrifuged at 13,000*g* for 20 min, and the supernatant was removed. The residual TCA in the precipitates was washed using acetone and centrifuged at

Table 1
The process performance of the integrated fixed-film and suspended growth SBR at the steady state.

Inffluent (mg/L)	Effluent (mg/L)				Removal rates (%)	
NH_4^+–N	NH_4^+–N	NO_2^-–N	NO_3^-–N	TN	NH_4^+–N	TN
~350	⩽5	⩽3	60–75	65–75	⩾98	~80