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Characteristics of extracellular polymeric substances of phototrophic biofilms at different aquatic habitats

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ABSTRACT

Three different phototrophic biofilms obtained from a natural lake (Sample 1), drinking water plant (Sample 2) and wastewater treatment plant (Sample 3) were investigated. Diatoms and green algae were the dominant algae of three biofilms, and the biomass was highest in biofilm of Sample 2. The three phototrophic biofilms also had variable extracellular polymeric substances (EPS) concentrations and compositions. Total EPS concentration of 14.80 mg/g DW was highest in biofilm of Sample 2, followed by biofilms of Samples 3 and 1 (13.11 and 12.29 mg/g DW). Tightly bound EPS (TB-EPS) were the main fraction, and polysaccharides and protein were the main components of total EPS in all three biofilms. However, the compositions of loosely bound EPS (LB-EPS) and TB-EPS were different in three biofilms. Fourier-transform infrared and fluorescence spectra indicated different structure and compositions of LB-EPS and TB-EPS. These results demonstrated the characteristics of EPS produced by phototrophic biofilms varied and had compact relation to their growth environmental conditions.

1. Introduction

Phototrophic biofilms are matrix-enclosed attached microbial communities of phototrophs (cyanobacteria and microalgae) and chemotrophs (archaea, bacteria, fungi and protozoa) driven by light energy (Di Pippo et al., 2012; Guzzon, Bohn, Diociaiuti, & Albertano, 2008; Roeselers, van Loosdrecht, & Muyzer, 2008; Staal et al., 2007). Generally, oxygenic phototrophs, such as cyanobacteria, green algae and diatoms, are typically inhabited in the surface of phototrophic biofilms, which utilizing light energy and reducing carbon dioxide, providing organic substrates and oxygen (Roeselers, van Loosdrecht, & Muyzer, 2007; Roeselers et al., 2008). The produced organic substrates and oxygen would support the growth of the heterotrophs (Roeselers et al., 2007, 2008). These communities play a significant ecological role in the cycling of carbon and nutrients. For example, phototrophic biofilms can be utilized in polishing nutrient-containing effluents from wastewater treatment plant or in algal pond systems, which are generally absence in organic carbon (Roeselers et al., 2008; Wolf, Picioreanu, & van Loosdrecht, 2007). It is also a suitable candidate for bioremediation, aquaculture, etc. (Bender & Phillips, 2004; Chaillan, Gugger, Saliot, Coute, & Oudot, 2006; Di Pippo et al., 2012; Guzzon et al., 2008).

Phototrophic biofilm growth is promoted by the excretion of extracellular polymeric substances (EPS) by the cells, which serves as an adhesive agent enabling cellular attachment and form the biofilm matrix embedding the cells (Wolf et al., 2007). EPS is a complex high-molecular-weight mixture of polymers, which mainly comprise polysaccharides, proteins and humic substances. Its production and composition is influenced by nutrient, growth conditions and environmental conditions (Kavita, Mishra, & Jha, 2013; Sheng, Yu, & Li, 2010). The formed EPS was important to phototrophic biofilms. It can afford a stable environment, promoting the growth of organisms in phototrophic biofilms. Thus, it prevents the losses of organisms and retains bacterial diversity over a long period of time, allowing the development of synergistic relationships in phototrophic biofilms (Ras, Lefebvre, Derlon, Paul, & Girbal-Neuhauser, 2011). Tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS) are considered to be responsible for cell adhesion and attachment onto the carrier or cells through strong interactions (Liu et al., 2010; Nielsen & Jahn, 1999). Moreover, EPS could be used as carbon source and energy for the growth

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Table 1
The number and species of algae in three phototrophic biofilms.

Samples	Total number	Diatoms		Green algae		Cyanobacteria	
	cell/cm^2	cell/cm^2	%	cell/cm^2	%	cell/cm^2	%
Sample 1	156,561	87,146	56	50,930	33	9431	6
Sample 2	161,277	60,833	38	70,264	44	4716	3
Sample 3	257,617	170,289	66	43,664	17	35,804	14

of heterotrophs in phototrophic biofilms (Wolf et al., 2007). These characteristics of EPS had significant influence on carbon and nutrients removal by phototrophic biofilms.

Phototrophic biofilms developing highly differentiated architectures are grown on surfaces in a range of terrestrial and aquatic environments, including marine and brackish near-shore environments (Zippel, Rijstenbil, & Neu, 2007), monumental fountains (Cuzman et al., 2010), lithic surface of monuments (Rossi et al., 2012), building stone (Hallmann et al., 2013), etc. The different environmental conditions with variable light, temperature and nutrients have shown to influence microbial community and microbial metabolism of phototrophic biofilms, resulting in the variations of microbial diversity, EPS production and composition, and physicochemical and biological properties of the whole biofilm (Di Pippo et al., 2012).

Therefore, the characteristics of EPS produced by phototrophic biofilms from a natural lake, drinking water plant and wastewater treatment plant were investigated in this work. In addition, the information about the structure and functional properties of EPS was obtained using Fourier-transform infrared (FTIR) spectrophotometer and three-dimensional excitation-emission-matrix (EEM) fluorescence spectrometry. Hopefully, the results of this work would provide a better understanding of the characteristics of phototrophic biofilm and their significant metabolic substrates of EPS under different environmental conditions. With the study of EPS of phototrophic biofilms, we hope to provide useful information on tertiary water treatment with phototrophic biofilms.

2. Materials and methods

2.1. Resources of the phototrophic biofilms

Three different phototrophic biofilms were sampled in spring of 2011 when the biofilms grown vigorously. Sample 1 was collected from a natural lake in Nanjing city, Jiangsu province of China (N 32.03637°, E 118.76111°), which was coated in rock along the shore under water about 10–20 cm. Sample 2 was obtained from the tank wall of clarifier under water 10–20 cm in a drinking water plant of Nanjing city (N 32.11796°, E 118.77004°). Sample 3 was collected from the wall of secondary settler under water 10–20 cm in a wastewater treatment plant (N 32.01975°, E118.69340°). Biofilms were transported to the laboratory and stored at 4 °C for analysis and EPS extraction.

2.2. EPS extraction

The EPS extraction process was performed according to the protocol described by Liang, Li, Yang, and Du (2010). Biofilm LB-EPS was extracted and harvested by using the regular centrifugation method described by Zhang and Bishop (2003) with modification. About 1.0 g biofilm (dried weight) was put into a centrifugation tube along with 10 mL MilliQ water and 0.06 mL formamide (37%) was added into suspension. The formamide was used to enhance efficiency of LB-EPS extraction and decrease contamination by intracellular substances (Liang et al., 2010). The tube was shaken gently in shaking incubator at 150 rpm and 4 °C for 1 h and then centrifuged at 5000 × *g* and 4 °C for 15 min. The supernatant was filtered through 0.45 μm acetate cellulose membranes to represent LB-EPS.

The biofilm pellet was re-suspended again with 10 mL buffer (2 mM $Na_2PO_4{\cdot}12H_2O$, 4 mM $NaH_2PO_4{\cdot}H_2O$, 9 mM NaCl, 1 mM KCl, pH = 7), followed by the cation exchange resin (CER) addition with a dosage of 70 g/g volatile suspended solids (VSS). These suspensions were stirred for 1 h at 600 rpm and 4 °C. After removing CER by settlement, the solutions were centrifuged at 10,000 × *g* and 4 °C for 15 min to remove remaining biofilm components. The supernatants were then filtered through 0.45 μm cellulose acetate membranes to represent TB-EPS.

2.3. Analytic methods

The biofilm biomass was determined with biofilm lipid phosphorus method (Wu et al., 2010), which was expressed as the number of cells. The algae number was measured with microscope count method (Jiang & Tu, 1990). The concentrations of chlorophyll a, chlorophyll b and chlorophyll c were measured using the standard methods (APHA, 1998). The content of carbohydrates in EPS was measured with the anthrone method (Raunkjaer, Hvitved-Jacobsen, & Nielsen, 1994), and the contents of proteins and humic substances were determined using the modified Lowry methods (Frolund, Palmgren, Keiding, & Nielsen, 1996).

2.4. EEM and FTIR spectrometry

Three-dimensional excitation-emission-matrix (EEM) fluorescence spectrometry was used to characterize the EPS of biofilm. All EEM spectra were measured using a luminescence spectrometry (F7000, Hitachi, Japan). The EEM spectra were collected with subsequent scanning emission spectra from 200 to 450 nm at 0.5 nm increments by varying the excitation wavelength from 200 to 600 nm at 10 nm increments. Excitation and emission slits were both maintained at 10 nm, and the scanning speed was set at 1200 nm/min for all measurements. The spectrum of the deionized water was recorded as the blank (Sheng & Yu, 2006). The software MatLab 7.0 (MathWorks Inc., USA) was employed for handling EEM data.

The EPS from all extracted samples was dried at 60 °C. The compositions of the EPS were compared using a Fourier-transform infrared (FTIR) spectrophotometer. The powders were mixed with IR grade KBr powders at a mass ratio of 1:100 (Liang et al., 2010). The prepared pellets were placed in a sample cell that was fitted to the sample compartment of the FTIR spectrometer (Nexus870, Nicolet, USA).

3. Results and discussion

3.1. Characteristics of phototrophic biofilms

A large number of rod-shaped, spherical, rhombic, navicular, slug and filamentous bacteria, algae and organic, inorganic debris were observed in three biofilms through optical microscope. The dominant algae species in different biofilms are listed in Table 1 by microscopic counting. Diatoms, green algae, cyanobacteria,