Environmental Pollution 235 (2018) 283–292

Contents lists available at ScienceDirect

Environmental Pollution

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

Extracellular polymeric substances affect the responses of multi-species biofilms in the presence of sulfamethizole☆

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ARTICLE INFO

Article history:
Received 5 October 2017
Received in revised form
15 December 2017
Accepted 16 December 2017

Keywords:
Biofilm
Extracellular polymeric substances (EPS)
Sulfamethizole
Sorption
Interaction
Bacterial community

ABSTRACT

The occurrence and transportation of antibiotics in biofilms from natural and engineered sources have attracted increasing interests. Nevertheless, the effects of extracellular polymeric substances (EPS) on the responses of biofilms to the exposure to antibiotics are not clear. In this study, the effects of EPS on the sorption and biological responses to one representative antibiotic, sulfamethizole (STZ), in model biofilms were investigated. Proteins dominated the interactions between the EPS and the STZ and the EPS from a moving bed biofilm reactor exhibited the strongest interaction with the STZ. The EPS served as important reservoirs for the STZ and the tested biofilms all showed reduced sorption capacities for the STZ after the EPS were extracted. The respiratory rates and typical enzymatic activities were reduced after the EPS were extracted. High-throughput 16S rRNA gene sequencing results confirmed that the bacterial community in the biofilm without the EPS was more vulnerable to antibiotic shock as indicated by the community diversity and richness indices. A greater increase in the abundance of susceptible species was observed in the natural biofilm. The results comprehensively suggested that the EPS played important role in biosorption of STZ and alleviated the direct damage of the antibiotic to the cells; in addition the extent of the bacterial community response was associated with the origins of the biofilms. Our study provided details on the responses of multi-species biofilms to the exposure to an antibiotic and highlighted the role of the EPS in interacting with the antibiotic, thereby providing a deeper understanding of the bioremediation of antibiotics in real-life natural and engineered biofilm systems.

1. Introduction

The sulfonamide group of antibiotics is among the most frequently used antimicrobials and is used in the treatment of bacterial infections (Dong et al., 2016; Le-Minh et al., 2010). Antibiotics including sulfonamides cannot be completely metabolized during therapeutic use and around 95% of the total amount is excreted as metabolites or parent compounds (Zhang and Li, 2011). Since the removal efficiencies by conventional processes in municipal wastewater treatment plants (WWTPs) are usually incomplete, the dissemination and feeding of antibiotics from effluents contribute to the wide occurrence of antibiotics in surface waters (Yang et al., 2012; Zhang and Li, 2011). The presence of antibiotics result in the wide spread of antibiotic resistant genes, posing serious impacts on human health and aquatic ecosystems (Martinez, 2008).

In WWTPs, the removal of antibiotics is mainly achieved using processes such as activated sludge or biofilm approaches, e.g., membrane bioreactors, biofilters and biofiltration (Goebel et al., 2007; Li and Zhang, 2010; Mazioti et al., 2015; Torresi et al., 2017). After entering surface waters, the removal and attenuation of antibiotics are mainly attributed to photolysis, sorption and biodegradation by sediments or biofilms. Biofilms are assemblages of microbial cells embedded within a complex matrix of extracellular polymeric substances (EPS), which play an important role in biogeochemical cycling as well as the transformation and fate of micro-pollutants. Biofilms are observed much less susceptible to antimicrobial agents than in their identical planktonic form. The presence of skeleton EPS in biofilm offers structural and mechanical benefits compared to free-living cells (Flemming and Wingender,

☆ This paper has been recommended for acceptance by Klaus Kummerer.

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https://doi.org/10.1016/j.envpol.2017.12.060
0269-7491/

2010; Flemming et al., 2016). Additionally, enhanced enzyme retention efficiency (Han et al., 2017) and nutrient/pollutant sorption capacities can be achieved due to the presence of EPS (Beech and Cheung, 1995; Grumbein et al., 2014; Wunder et al., 2011). Several research studies have shown that EPS protect microbes in the biofilm against toxic shocks. However, studies on the role of biofilm EPS in the sorption of antibiotics and the responses of real-life biofilms to the exposure to antibiotics are still lacking.

The origin of biofilms is another factor governing the characteristics of microorganisms in the biofilm (Writer et al., 2013). Multi-species biofilms, e.g., epiphyton, epilithon or biofilms formed on artificial substrata exhibit variations in organic contents, structural properties, and heterogeneity (Flemming et al., 2016; Wimpenny et al., 2000). Consequently, the bioactivity and sorption efficiencies of various biofilms are different (Writer et al., 2011; Wunder et al., 2011; Xu and Li, 2010). In most studies addressing the effects of antibiotics on biofilm, the influences of the antibiotics on the biological activities and microbial communities of the biofilm microorganisms are commonly discussed (Bugli et al., 2016; Grumbein et al., 2014; Proia et al., 2013). The higher degree of recalcitrance and tolerance to antibiotics by biofilms could be attributed to the combined effects of antimicrobial penetration, presence of antibiotic-modifying enzymes, reduced growth rate and oxidative stress responses in pure or mixed culture of biofilms (Hall and Mah, 2017). Proia et al. (2013) found that antibiotics markedly affected the structural and functional properties of microbial communities in a river. The microbial communities of the biofilm at the phylum, class and genus level all experienced significant shifts during the treatment of wastewater containing antibiotics in bioreactors. Highly antibiotic resistant species were also enriched in the succession owing to the increase in antibiotic loadings (Qiu et al., 2013; Zheng et al., 2016b). The higher degree of recalcitrance of the biofilm communities to the toxic substances was attributed to the antibiotic-resistant functions of the extracellular DNA (Beech and Cheung, 1995), the interactions of the antimicrobial with the biofilm components, and the actions of specific genetic determinants of antibiotic resistance (Hall and Mah, 2017). Currently, no systematic study has been conducted to investigate the sorption behaviors and microbial responses of biofilms with or without EPS to exposures to antibiotics. In light of the diffusion-reaction inhibition by EPS, the diffusion and activity of antibiotics could be retarded (Olsen, 2015). As a result, the acute damage by sulfonamides to microorganisms within the biofilm matrix might be relieved (Mulcahy et al., 2008). Accordingly, studies on the interactions between typical antibiotics and biofilms from different origins can improve our understanding of the role of EPS in protecting microbial communities; such studies are also lacking and the relevant details require clarification.

In this study, we aim to elucidate how EPS affect the responses of multi-species biofilms in the presence of a typical sulfonamide, i.e., sulfamethizole (STZ). Three representative biofilms were selected, including a natural biofilm from a hypereutrophic lake, a cultivated biofilm originated from lake sediments, and a biofilm from a moving bed biofilm reactor (MBBR). Batch tests were performed to determine the effects of the EPS on the sorption of STZ into biofilms. The respiratory rate and typical enzymatic activities of the biofilm microorganisms were measured. The composition and distribution of the bacterial microbial communities of the biofilms with and without EPS extraction were determined and compared using high-throughput sequencing analysis. The findings of the study will provide new insights into the role of biofilm EPS against antibiotic shock and provide a better understanding of the bioremediation processes in natural and artificial biofilm systems.

2. Materials and methods

2.1. Collection and cultivation of biofilms

The natural biofilm was collected from Xuanwu Lake (32°4′8″N, 118°48′17″E), a representative hypereutrophic lake in Nanjing in the Jiangsu Province of China. The biofilm sample was obtained 3–10 cm below the water surface with a sterile spatula near the lake shore. The biofilms were then washed with sterile deionized water and filtered through a 0.85-mm pore size screen to remove large particles. Afterwards, the biofilm sample was placed in a sterile plastic tube and stored at 4 °C for use. Sediment from Lake Taihu (31°16′996″N, 120°00′297″E) was sampled to cultivate the biofilm in the lab. The sediment samples were homogenized and wet-sieved to less than 2 mm particle size and were maintained at 4 °C prior to the experiment. The detailed process is provided in the supplementary materials (SM). The biofilm from an aeration tank in an MBBR was collected from the Shengke Water Treatment Company. The activated sludge and the packings from the aeration tank were collected. Subsequently, only the packings were mixed with distilled water and sonicated for 30 min to harvest the biofilm samples. The concentrations of suspended solids (SS) and volatile suspended solids (VSS) in the three biofilm bulk solutions were measured in duplicate following the standard methods (APHA, 2005). The VSS contents (%) of the natural biofilm, cultured biofilm, and MBBR biofilm were 15 ± 5%, 24 ± 4%, and 55 ± 6%, respectively.

2.2. EPS extraction and sorption tests

To obtain EPS-extracted biofilms for subsequent experiments, the extraction time, EPS yield, destruction and lysis of cells should all be taken into account (Torelli et al., 2017; Zhang et al., 2013). A modified cation exchange resin (CER) method was used to extract the EPS from the biofilms following the method described by Zhang et al. (2013); this method is suitable for EPS extraction and results in high extraction efficiency and low cell lysis. The extraction approach is provided in SM. The extracted EPS solutions were concentrated and washed with distilled water using an ultrafiltration device with a 5000 Da polyethersulfone ultrafiltration membrane (MSC80005, Mosu Corporation, China) to remove small residues and salts, followed by lyophilization. The biofilm after the EPS extraction is referred to as biofilm without EPS in the following sections. In order to reveal the amount of EPS extracted, the concentrations of polysaccharide, proteins and humic substances were measured (detailed in SM). The total amount of released EPS were 21.1 ± 1.7, 12.2 ± 0.2 and 27.6 ± 0.8 mg/g SS from the natural biofilm, the cultivated biofilm and the MBBR biofilm, respectively, suggesting the extraction process did not have a significant effect on the total biomass (Fig. S1).

STZ (≥98% purity) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and its physicochemical properties are shown in Table S1. All other chemicals were purchased from Chemical Reagent Co. (Shanghai, China) and were of analytical grade. The sorption of the STZ into the biofilms with and without EPS was studied over a dose range of 0–1000 μg/L. After the STZ was added to 25 mL of the biofilm samples, the pH was adjusted to 7.0 ± 0.2 with 0.1 M HCl or NaOH. The flasks were placed on a rotary shaker operating at 160 rpm for 12 h at 25 °C. Thereafter, 5 mL of the supernatant was centrifuged at 8000 rpm for 1 min and then passed through a 0.22-μm cellulose filter prior to the analysis. The STZ contents were determined using a high-performance liquid chromatography (1260 Infinity, Agilent Co., USA) instrument equipped with a 5 μm × 4 mm × 150 mm Eclipse XDB-C_{18} column and a UV detector at a wavenumber of 280 nm. The mobile phase was a