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Chlorination-mediated EPS excretion shapes early-stage biofilm formation in drinking water systems

Li Liu [a], Qingyuan Hu [a], Yang Le [a], Guowei Chen [a], Zilin Tong [a], Qiao Xu [a], Gang Wang [b,*]

[a] School of Civil Engineering, Hefei University of Technology, Hefei 230009, China
[b] Department of Water and Soil Sciences, China Agricultural University, Beijing 100193, China

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ABSTRACT

Microbial surface adhesion to surfaces and subsequent biofilm establishment are ubiquitous in drinking water systems, which often contribute to deteriorated water quality. Disinfectants are common agents applied to drinking water controlling microbial propagation, yet the underlying mechanisms of how disinfectants function to regulate microbial activity and thereby biofilm development remains elusive. We experimentally studied the effects of chlorination on extracellular polymeric substance (EPS) production, and its impacts on early-stage biofilm formation in a model drinking water system. Results showed that low-level chlorine ($\leq$ 1.0 mg/L) stimulated microbial EPS (especially of proteins) excretion that favored early-stage biofilm formation. Microbes experiencing higher chlorination (>1.0 mg/L) exhibited clearly suppressed growth associated with reduced EPS release, consequently yielding less biofilm formation. Removal of cell-attached proteins and polysaccharides diminished biofilm formation, which highlighted the critical role of EPS (especially protein components) in biofilm development. A negative correlation between chlorination-mediated microbial protein production and cell surface charge suggested that chlorine disinfection may modify cell surface properties through regulation of microbial EPS excretion and thereby mediate biofilm formation. With these quantitative estimations, this study provides novel insights into how chlorination-mediated EPS excretion shapes early-stage biofilm formation, which is essential for practical functioning of drinking water systems.

1. Introduction

Despite the often heavily used chlorine dosage (0.5–4.0 mg/L) for controlling microbial propagation in drinking water, microbial colonization onto surfaces is common [1–4]. For example, the biofilms formed on pipeline surfaces in drinking water distribution systems could be accounted for over 95% of the total biomass [5], the existence of which has been reported to be associated with various problems such as water supply reduction, pathogen dispersion and degradation of water quality [6–8]. While chlorine is known to inhibit cell activity in both bulk water and biofilms, application of chlorine dosage is also one of the stress factors that lead to biofilm formation [9]. An explicit understanding of chlorination-mediated biofilm development, especially during the initial phase of the formation process, is merited for practical designing of effective biofouling control strategies in drinking water systems [10,11].

Microbial biofilms in drinking water occur as a result of cell attachment onto the surfaces, where subsequent proliferation of attached cells and strengthen of the sessile structure is often facilitated by self-produced extracellular polymeric substances (EPS) [11,12] that consists primarily of proteins (PN) and polysaccharides (PS), as well as small amounts of nucleic acids, lipids, and other substances [13,14]. Numerous functions are attributed to microbial EPS, such as protection against disinfection, nutrient reservation for biofilm-embedded cell growth, cell-cell communication stimulation, promotion of bacterial surface adhesion during early-stage biofilm formation. [15,16]. For example, studies revealed that the presence of EPS may confer disinfectant resistance for embedded microbes by preventing the transport of and interactions with disinfectants [12,17]. In addition, microbial EPS facilitate cell interactions with neighbors and with solid surfaces (e.g., pipeline surface), thereby stimulating the formation of synergistic microbial consortia [15]. As an important component of EPS, PS consist of a mixture of neutral and charged sugar residues that can hold organic or inorganic substituents that considerably influence their

* Corresponding author.
E-mail addresses: lliu@hfut.edu.cn (L. Liu), huqingyuan@mail.hfut.edu.cn (Q. Hu), 799950635@qq.com (Y. Le), gwchen@hfut.edu.cn (G. Chen), tongzl1025@126.com (Z. Tong), xiaoqiao0616@163.com (Q. Xu), gangwang@cau.edu.cn (G. Wang).

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physical and biological properties [15], and impact biofilm formation. Establishing a direct linkage between biofilm formation and microbial EPS excretion is considered essential for full understanding of the mechanisms of biofilm development origins in drinking water systems, yet this remains sketchy [18,19].

Microbes inhabiting drinking water commonly experience environmental stresses, such as low-level nutrients, chemical disinfection, hydraulic fluctuations [20]. As a consequence, microbes developed numerous strategies in response to these environmental stresses. For example, high shear stress can induce cells to secrete more EPS and thereby stabilize biofilm structure [21,22]. Carbon source availability and the balance between carbon and other limiting nutrients may also regulate collectively EPS synthesis of the biofilm-embedded cells, while the yielded EPS (typically of the most hydrophobic components) can absorb certain amounts of organic substances present in the water phase, serving as a nutrient reservoir for microbial growth [23]. Despite the numerous studies on microbial potential stress responses triggered by environmental stresses [24–26], the effects of disinfection stress on microbial activity in drinking water systems remain unclear. Our previous research investigated chlorination-mediated cell motility regulating initial surface attachment [27]; however, the effects of chlorination-mediated EPS excretion on early-stage biofilm formation in drinking water systems have not been explored.

In this study, we aimed to quantify the effects of chlorination stress on microbial EPS production, and its impacts on early-stage biofilm formation. In addition, we compared biofilm formation dynamics of treated cells (by removing the cell surface-attached EPS) with those of untreated cells to estimate the influence of cell surface associated EPS on early-stage biofilm formation in drinking water.

2. Materials and methods

2.1. Water sample collection and solution preparation

Water samples were collected from a drinking water tap in Hefei, China. Prior to the experiments, the collected water was stored at room temperature (20 ± 2 °C) for 24 h to allow residual chlorine decay until the detected chlorine was less than the detection limit of 0.01 mg/L [28]. Chlorine stock solutions were prepared with 10.0% sodium hypochlorite (100.0 g/L, Guangfu, Shanghai) in sterile deionized water. The chlorine concentration in the water samples was estimated using a chlorine colorimeter (SYL-1, Xinrui, Shanghai, China) according to the N, N-diethyl-pphenylenediamine (DPD) method.

2.2. Experimental set-up and EPS removal tests

2.2.1. Experimental systems

The experimental system consisted of five parallel cylindrical batch reactors made of polyvinyl chloride with an effective volume of 1.5 L (Ø10 × 26 cm). All reactors were autoclaved (LX-B50, Huatai, Hefei) at 121 °C for 15 min and then were UV sterilized (20 w) for 30 min prior to use. Twelve polyethylene (PE) coupons (length × width × thickness = 3.0 cm × 1.0 cm × 0.2 cm) were placed on the bottom of the experimental system as an attachment substratum. Prior to use, the PE coupons were sterilized with a 75% solution of ethanol and then were autoclaved at 121 °C for 15 min.

2.2.2. Experimental procedure

The batch reactor was initially filled with a 1.0 L water sample and was then operated at room temperature for 6 h in stagnant conditions. To understand the effect of chlorine stress on EPS production and the impact on early-stage biofilm formation, initial chlorine concentrations of 0, 0.5, 1.0, 2.0 and 3.0 mg/L were applied to five sets of parallel reactors representing common scenarios observed in drinking water systems, with the other operational conditions applied identically for all scenarios. The PE coupons and bulk water samples were collected at 1, 2, 4 and 6 h after exposure. All experiments were conducted with three replications in this study.

2.2.3. EPS removal tests

The drinking water microbes used for EPS removal tests as well as the control were collected from 400 mL of tap water, which was pre-stored to remove the residual chlorine and was then cultured at 37 °C for 24 h with 1 mL sodium acetate (5 mg/L) for microbial growth. Bacterial solution was centrifuged at 1000 g for 10 min and was then re-suspended in sterile normal saline to eliminate redundant sodium acetate.

To quantify the roles of microbial extracellular PS and PN on early-stage biofilm formation, 50.0 mmol/L sodium metaperiodate (SCR, Shanghai, AR) and 1.0% trypsin (Gibco, Canada) solutions were used to remove the cell surface-attached PS and PN, respectively, prior to inoculation [29]. The cells suspended in the sodium metaperiodate and trypsin solutions were incubated at 22 °C and 37 °C, respectively, for 24 h [29] and were then centrifuged at 1000 g for 10 min to harvest the cells. The harvested cells were then re-suspended respectively in sterile normal saline solution. Three sets of batch experiments were conducted in the experimental systems under 1.0 mg/L of initial chlorine, with one inoculated with cells (of 50.0 mL cell suspension, $OD_{600} = 0.15 \pm 0.02$) without the PS and PN removal treatments that served as the controls, and the other two inoculated with the sodium metaperiodate and trypsin pre-treated cells (identical 50.0 mL cell suspensions, $OD_{600} = 0.15 \pm 0.02$), respectively. Three other scenarios with identical inoculums, in the absence of initial chlorine, were conducted in identical systems.

2.3. Harvest of biofilm cells and cell resuspension

Biofilm cells were removed from the PE coupons and were then re-suspended according to Gagnon and Slawson [30]. Specifically, the PE coupons were gently dip-rinsed in sterile distilled water to remove any loosely attached cells [31]. A sterile cotton wool swab was used to remove the remaining biofilm cells on the coupon. The swab was then aseptically placed into a sterilized tube (size of 20 mL) containing 10.0 mL of phosphate buffer saline (PBS) to release the biofilm cells (into the PBS solution) by applying 4 min of sonication (Shumei, KQ3200E) at 40 kHz [30,32].

2.4. Live/dead bacterial cell staining procedure

The Live/Dead BacLight bacterial viability kit (L-7012, Invitrogen), consisting of Syto 9 and propidium iodide, was used for distinguishing the live and dead bacteria cells from the biofilm samples. The Syto 9 and propidium iodide components were mixed together (2.0 μL + 2.0 μL) in a 5 mL centrifuge tube [33]. The cell suspension (2.0 mL) of biofilm samples was loaded into the centrifuge tube and was then placed in the dark for 20 min. The stained sample was filtered through a 0.2 μm pore-size Nuclepore black polycarbonate membrane filter (Whatman, Nuclepore, Clifton, NJ) [34], and after filtering, the filtered sample was ready for fluorescence microscope (IX73, Olympus, Japan) observation. Cell numbers were estimated manually from 10 randomly selected microscopic fields at a magnification of 200 (10 × 20) and expressed as cells/cm^2.

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