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Drinking water biofilm cohesiveness changes under chlorination or hydrodynamic stress

L. Mathieu [a,*], I. Bertrand [b], Y. Abe [b], E. Angel [a], J.C. Block [b], S. Skali-Lami [c,1], G. Francius [b,1]

[a] Ecole Pratique des Hautes Etudes (EPHE), LCPME, UMR 7564 CNRS – Université de Lorraine, Nancy, France
[b] CNRS and Université de Lorraine, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement (LCPME), UMR 7564, Nancy, France
[c] CNRS and Université de Lorraine, Laboratoire d'Energétique et de Mécanique Théorique et Appliquée (LEMTA), UMR 7563, Nancy, France

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ABSTRACT

Attempts at removal of drinking water biofilms rely on various preventive and curative strategies such as nutrient reduction in drinking water, disinfection or water flushing, which have demonstrated limited efficiency. The main reason for these failures is the cohesiveness of the biofilm driven by the physico-chemical properties of its exopolymeric matrix (EPS). Effective cleaning procedures should break up the matrix and/or change the elastic properties of bacterial biofilms. The aim of this study was to evaluate the change in the cohesive strength of two-month-old drinking water biofilms under increasing hydrodynamic shear stress τ_w (from ~0.2 to ~10 Pa) and shock chlorination (applied concentration at T0: 10 mg Cl_2/L; 60 min contact time). Biofilm erosion (cell loss per unit surface area) and cohesiveness (changes in the detachment shear stress and cluster volumes measured by atomic force microscopy (AFM)) were studied.

When rapidly increasing the hydrodynamic constraint, biofilm removal was found to be dependent on a dual process of erosion and coalescence of the biofilm clusters. Indeed, 56% of the biofilm cells were removed with, concomitantly, a decrease in the number of the 50 –300 μm^3 clusters and an increase in the number of the smaller (i.e., <50 μm^3) and larger (i.e., >600 μm^3) ones. Moreover, AFM evidenced the strengthening of the biofilm structure along with the doubling of the number of contact points, N_C, per cluster volume unit following the hydrodynamic disturbance. This suggests that the compactness of the biofilm exopolymers increases with hydrodynamic stress.

Shock chlorination removed cells (–75%) from the biofilm while reducing the volume of biofilm clusters. Oxidation stress resulted in a decrease in the cohesive strength profile of the remaining drinking water biofilms linked to a reduction in the number of contact points within the biofilm network structure in particular for the largest biofilm cluster volumes (>200 μm^3). Changes in the cohesive strength of drinking water biofilms subsequent to cleaning/disinfection operations call into question the effectiveness of cleaning-

* *Corresponding author*. Ecole Pratique des Hautes Etudes (EPHE), LCPME, UMR 7564 CNRS – Université de Lorraine, 15 avenue du Charmois, F-54500 Vandœuvre-lès, Nancy, France. Tel.: +33 (0)3 83 682 236; fax: +33 (0)3 83 682 233.
E-mail address: laurence.mathieu@univ-lorraine.fr (L. Mathieu).

[1] Both authors contributed equally to the publication.

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in-place procedures. The combined alternating use of oxidation and shear stress sequences needs to be investigated as it could be an important adjunct to improving biofilm removal/reduction procedures.

1. Introduction

Biofilms in drinking water distribution systems are the cause of many problems such as water flow contamination by biomass detachment (Berry et al., 2006; Feazel et al., 2009), microbiologically induced corrosion (Beech and Sunner, 2004) and transitory accumulation of microorganisms of sanitary interest such as enteric viruses, *Klebsiella*, *Legionella*, *Bacillus* spores or *Cryptosporidium* oocysts (Langmark et al., 2005; Morrow et al., 2008; Helmi et al., 2008; Paris et al., 2009; Altman et al., 2009; Wingender and Flemming, 2011; Pelleieux et al., 2012).

The prevention of biofilm formation partly relies on drinking water nutrient reduction strategies, but the best available technology such as nanofiltration, which significantly lowers the organic matter level, is not able to radically reduce the number of cells or their cultivability within the biofilm (Sibille et al., 1997; Liu et al., 2013). Biofilm prevention is also related to disinfection practices, but again a number of studies have demonstrated their limited efficiency. Indeed, most traditional disinfectants (chlorine, chloramines) are consumed by reactions with corrosion products and deposits (Zhang and Andrews, 2012; Wang et al., 2012), pipe materials (Hallam et al., 2002; Lethola et al., 2005; Hubbard et al., 2009) and exopolymeric substances (Xue et al., 2012). Such restricted efficiency due to reaction-diffusion limited penetration has been previously reported on pure strain biofilms: *Pseudomonas aeruginosa* in alginate beads, *Staphylococcus epidermidis* in capillary flow cells, as well as binary populations of *P. aeruginosa* and *Klebsiella pneumoniae* on stainless steel coupons (De Beer et al., 1994; Chen and Stewart, 1996; Davison et al., 2011; Lee et al., 2011). As a result, even in continuously chlorinated drinking water distribution systems (e.g., 0.1–0.4 mg Cl_2/L), biofilms grow and harbour active bacteria whose species composition varies depending on the disinfectant concentration (Mathieu et al., 2009). Complementary curative strategies to remove biofilms by water flushing are hardly effective due in particular to the viscoelastic properties of the systems (Towler et al., 2003; Abe et al., 2011, 2012; Jones et al., 2011; Paul et al., 2012).

Drinking water biofilms represent a complex biophysical world embedded in an exopolymeric matrix (EPS), whose cohesion is driven by electrostatic interactions – multivalent crosslinking cations which bridge negatively charged sites (Chen and Stewart, 2002) – and hydrophobic interactions (Flemming and Wingender, 2010; Aldeek et al., 2013). As the biofilm EPS matrix plays a key role in the resistance of biofilms to disinfectants (Mah and O'Toole, 2001; Xue et al., 2012), effective cleaning procedures should break it up in order to disperse the biofilm and allow disinfectants to diffuse rapidly. Consequently, the elastic properties of bacterial biofilms should be also changed. Xavier et al. (2005) reported that chemical treatment with dilute NaOH weakened the mechanical properties of biofilm clusters, which rapidly deformed in the direction of the flow. Jones et al. (2011) and Lieleg et al. (2011) found that chlorine had a slight weakening effect on *P. aeruginosa* biofilms. Tachikawa et al. (2009) showed an apparent decrease in EPS in the biofilm matrix exposed to halogenated oxidants or ozone, and a clear relationship between the removal of EPS and the bacterial inactivation rate. Saravanan et al. (2006) demonstrated that chlorine (1 g/L) induced detachment and killing of *Pseudoalteromonas ruthenica*. Finally, Davison et al. (2011) revealed that chlorine effectiveness was due to its ability to weaken the mechanical cohesiveness of *S. epidermidis* biofilm and to erode the attached biomass.

As many industrial cleaning/disinfection procedures of distribution systems combine water flushing and/or chlorination practices, we aimed to evaluate the effect of such treatments on the removal of biofilms and on the cohesiveness of young drinking water biofilms. Indeed, the issue is of interest as hydrodynamic cleaning of drinking water biofilms has been poorly documented. Using 2-month-old multispecies drinking water biofilms grown under controlled hydrodynamic strength conditions, we tested the effect of hydrodynamic discontinuities of wall shear rate (a rapid increase from 10^3 to 10^4 s^{-1}, corresponding to shear stress values comprised between 1 and 10 Pa) or shock chlorination disinfection (applied concentration at T0: 3.7 and 10 mg Cl_2/L; 60 min contact time). We aimed to assess biofilm erosion (loss in cell density) and cohesiveness (changes in the detachment shear stress and cluster volumes) by atomic force microscopy (AFM) imaging and force measurements. The central questions addressed were whether such procedures are effective in cleaning surfaces colonized with autochthonous drinking water biofilms, and how they affect the exopolymeric matrix strength.

2. Material and methods

2.1. Drinking water biofilm formation

Drinking water biofilms were grown at 20 °C for two months on HDPE (high density polyethylene, a material representative of drinking water distribution systems) and glass (required for AFM measurements) coupons (area: 2.8 cm^2) placed in a rotating disc reactor previously described by Abe et al. (2012) and Pelleieux et al. (2012) (Fig. S1 in Supplementary data). The rotating disc was equipped with forty coupons distributed on four concentric circles corresponding to average wall shear stress values τ_w of 0.2 and 1 Pa when the rotational speed of the disc was equal to 21 or 75 rpm, respectively. The reservoir of the rotating disc reactor was continuously supplied directly with drinking tap water from the city of Nancy (pH: 8.1 ± 0.2;