Science of the Total Environment 565 (2016) 40–48

Contents lists available at ScienceDirect

Science of the Total Environment

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

The effects of sodium hypochlorite against selected drinking water-isolated bacteria in planktonic and sessile states

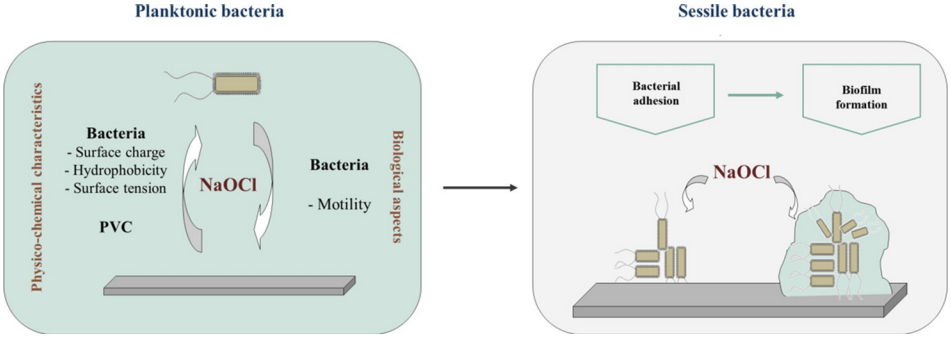
I.B. Gomes [a], M. Simões [a], L.C. Simões [b,*]

[a] LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[b] CEB — Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

HIGHLIGHTS

- *A. calcoaceticus* was more susceptible to the action of NaOCl than *S. maltophilia.*
- Biofilm removal and killing are distinct phenomena.
- High biofilm killing rates were achieved with NaOCl at residual concentrations.
- High concentrations of NaOCl cause reduced biofilm removal.
- Complementary methods to NaOCl are required for drinking water disinfection.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 16 April 2016
Accepted 17 April 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Acinetobacter calcoaceticus
Adhesion
Biofilm
Motility
Physicochemical properties
Stenotrophomonas maltophilia

ABSTRACT

Chlorine is the most commonly used agent for general disinfection, particularly for microbial growth control in drinking water distribution systems. The goals of this study were to understand the effects of chlorine, as sodium hypochlorite (NaOCl), on bacterial membrane physicochemical properties (surface charge, surface tension and hydrophobicity) and on motility of two emerging pathogens isolated from drinking water, *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia*. The effects of NaOCl on the control of single and dual-species monolayer adhered bacteria (2 h incubation) and biofilms (24 h incubation) was also assessed. NaOCl caused significant changes on the surface hydrophobicity and motility of *A. calcoaceticus*, but not of *S. maltophilia.* Planktonic and sessile *S. maltophilia* were significantly more resistant to NaOCl than *A. calcoaceticus*. Monolayer adhered co-cultures of *A. calcoaceticus-S. maltophilia* were more resilient than the single species. Oppositely, dual species biofilms were more susceptible to NaOCl than their single species counterparts. In general, biofilm removal and killing demonstrated to be distinct phenomena: total bacterial viability reduction was achieved even if NaOCl at the higher concentrations had a reduced removal efficacy, allowing biofilm reseed. In conclusion, understanding the antimicrobial susceptibility of microorganisms to NaOCl can contribute to the design of effective biofilm control strategies targeting key microorganisms, such as *S. maltophilia*, and guarantying safe and high-quality drinking water. Moreover, the results reinforce that biofilms should be regarded as chronic contaminants of drinking water distribution systems and accurate methods are needed to quantify their presence as well as strategies complementary/alternative to NaOCl are required to effectively control the microbiological quality of drinking water.

* Corresponding author at: Centro de Engenharia Biológica, Universidade do Minho, Braga, Portugal.
E-mail addresses: up200808015@fe.up.pt (I.B. Gomes), mvs@fe.up.pt (M. Simões), luciachaves@deb.uminho.pt (L.C. Simões).

http://dx.doi.org/10.1016/j.scitotenv.2016.04.136
0048-9697/

1. Introduction

The development of a biofilm occurs in a sequential process that includes transport of microorganisms to a surface, initial (reversible and irreversible) adhesion, cell-cell communication, microcolony formation, production of extracellular polymeric substances (EPS) and biofilm maturation, with a balanced growth and dispersal (Doyle, 2000). Drinking water distribution systems (DWDS) are known to harbor biofilms, even in the continuous presence of a disinfectant. These biofilms can be a reservoir of pathogens (Li et al., 2016; Sun et al., 2014) and are a source of planktonic bacteria, which will remain present when the water is delivered through a consumer's tap (Simões and Simões, 2013). The presence of biofilms in DWDS can cause chronic drinking water (DW) contamination, reduce the esthetic quality and microbiological safety of potable water and increase the corrosion rate of pipes (Niquette et al., 2000; Percival and Walker, 1999; Simões et al., 2006; Tsai, 2005). Therefore, biofilm control is important for technical, esthetic, regulatory, and public health reasons.

Adhesion and consequent biofilm formation gives several advantages to bacteria, particularly the protection against antimicrobial agents and adverse environmental conditions. Therefore, microbial control becomes more difficult when microorganisms are embedded in biofilms (Garrett et al., 2008). Diverse strategies to control microbial adhesion and biofilm formation in DWDS were reported (Simões and Simões, 2013). The most commonly used consists on chemical disinfection, particularly with chlorine (Simões and Simões, 2013). Currently, according to the World Health Organization (WHO) the residual concentration of free chlorine leaving the treatment plant should be <1.0 mg/L and closer to 0.5 mg/L (WHO, 2011). However, the effects of chlorine on biofilm control and its mode of antimicrobial action is still poorly understood.

The use of a material that did not encourage bacterial adhesion is also of utmost importance to control the presence of biofilms in DWDS. In recent years plastic pipes have been applied in DWDS. These plastic materials have advantageous characteristics when compared to metallic and asbestos-cement pipes, particularly its low cost, the simple installation, its external and internal resistance to corrosion and its smooth surface which facilitate the removal of deposits (Rożej et al., 2015; Safe Drinking Water Committee, Board on Toxicology and Environment Health Hazards, National Research Council, 1982). Moreover, several studies demonstrated that metallic and cement-based pipes favored bacterial attachment compared to plastic pipes (Kerr et al., 1998; Niquette et al., 2000). Iron pipes can support 10–45 times more biomass than those plastic, while cement-based materials can harbor 2.6 times more biomass than polyvinyl chloride (PVC) (Niquette et al., 2000). Rożej et al. (2015) compared biofilm formation on different plastic pipes (PVC, silane cross-linked polyethylene-PEX, and high-density polyethylene-HDPE) and demonstrated that the number of bacteria was significantly lower in biofilms formed on PVC than those formed on PEX or HDPE pipes. In this study PVC was selected as substratum for bacterial adhesion due to its broad use in DWDS. For instance, it was reported in 1982 that 80% of the plastic pipes used in DWDS at USA were made of PVC (Safe Drinking Water Committee, Board on Toxicology and Environment Health Hazards, National Research Council, 1982). Two bacteria isolated from DW, *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia*, were used due to their increased relevance as opportunistic microorganisms (Gales et al., 2001). These bacteria were already associated with nosocomial infections related to the hospital water supply (Cervia et al., 2008; Talon et al., 1994; Weber et al., 1999). *A. calcoaceticus* is usually present on skin and can act as an opportunistic pathogen with highly variable degree of virulence, depending on a pre-existing break in the normal body defenses (Pal and Kale, 1981). *Acinetobacter* spp. are commonly found in water and are associated with nosocomial infections (Narciso-da-Rocha et al., 2013). In 1996, this species was confirmed as the responsible agent for the death of infants in maternity hospitals in Brazil (Penna et al., 2001). *S. maltophilia* is commonly associated with respiratory infections in humans. The treatment of *S. maltophilia* infections is problematic since it is considered an emerging multidrug resistant organism (Brooke, 2012; Denton and Kerr, 1998). Vincenti et al. (2014) isolated *S. maltophilia* from hospital tap water and 56.3–100% of the isolates were highly resistant to the 14 tested antibiotics (5 beta-lactams, 2 carbapenems, 3 aminoglycosides, ciprofloxacin, colistin, fosfomycin and trimethoprim-sulfamethoxazole). These authors considered *S. maltophilia* potentially more dangerous than other waterborne pathogens commonly described in literature.

The aim of this study was to evaluate the effects of chlorine, as sodium hypochlorite (NaOCl), on the control of bacterial adhesion and biofilms on PVC and to understand how this biocide can act on bacterial surface physicochemical properties, charge and motility.

2. Material and methods

2.1. Bacteria and culture conditions

A. calcoaceticus and *S. maltophilia* were isolated from a DWDS in Braga (Portugal) and identified by 16S rRNA gene sequencing as described previously by Simões et al. (2007a).

Bacterial cells were grown overnight using a synthetic nutrient medium (glucose 5 g/L, peptone 2.5 g/L, yeast extract 1.25 g/L and 0.2 M phosphate buffer at pH 7) (Simões et al., 2009) at room temperature (23 ± 3 °C) and under agitation (120 rpm) in an orbital incubator (New Brunswick Scientific, I26, USA). All growth medium compounds were purchased from Merck (VWR, Portugal). Cells were harvested by centrifugation (Eppendorf centrifuge 5810R) at 3777 *g*, 12 min, washed twice with 0.2 M phosphate buffered saline (PBS) at pH 7 and resuspended in the same buffer or appropriated medium in order to achieve the bacterial concentration required for further experiments.

2.2. Minimum inhibitory concentration of NaOCl

The minimum inhibitory concentration (MIC) of sodium hypochlorite - NaOCl (Sigma, Sintra, Portugal) for *A. calcoaceticus* and *S. maltophilia* was determined by the broth microdilution method according to McBain et al. (2004). A pre-culture grown as described previously was used as inoculum (≈10^8 CFU/mL). MIC was determined in polystyrene 96-well microtiter plates (Orange Scientific, Belgium). NaOCl was prepared at diverse concentrations (0.1–5000 mg/L) from a 10% (v/v) stock solution. A volume of 20 μL of each NaOCl solution was added to each well containing 180 μL of cell culture in synthetic nutrient medium. The optical density was measured in a microtiter plate reader at 610 nm (SpectraMax M2E, Molecular Devices) before and after 24 h incubation at room temperature (23 ± 3 °C) and 120 rpm. The MIC corresponds to the lowest concentration of NaOCl at which no growth was found. Each condition was tested in triplicate with three independent experiments.

2.3. Substratum for bacterial adhesion and biofilm formation

PVC coupons (1 × 1 cm) were used as adhesion substratum. PVC was selected as a representative pipe material from DW networks (Simões et al., 2007a). In order to prepare PVC for further analysis, this material was immersed in a solution of commercial detergent (Sonasol Pril, Henkel Ibérica S. A.) in ultrapure water for 30 min. The coupons were rinsed in ultrapure water and subsequently immersed in ethanol at 96% (v/v) for 1 min in order to remove any remaining detergent (Simões et al., 2007a). Afterwards they were rinsed three times with ultrapure water and dried at 65 °C for 3 h, before being used for contact angle measurements, zeta potential assessment, adhesion and biofilm assays.

2.4. The effect of NaOCl on bacterial surface charge - zeta potential measurement

The zeta potential of PVC and bacteria, before and after contact with NaOCl (0.5 mg/L and MIC for *S. maltophilia* and *A. calcoaceticus*)