



## The potential of OXONE as an alternative disinfectant to control drinking water biofilms

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### Introduction

Chlorination is the most widely used method for the disinfection of drinking water (DW) (Ellis, 1991). However, the production of toxic disinfection by-products and the occurrence of biofilms in chlorinated drinking water distribution systems (DWDS) have been frequently reported (Andersson et al., 2019; Liu et al., 2016). Biofilms are responsible for several undesirable effects on water quality and constitute a reservoir of pathogenic microorganisms (Simões and Simões, 2013).

Given the health concerns of the side reactions of chlorine compounds and the increase of microbial tolerance to conventional chlorination, new alternatives for DW disinfection are required. Therefore, the main purpose of this study was to evaluate the antibiofilm efficacy of pentapotassium bis(peroxymonosulphate) bis(sulphate) (OXONE) compared to the common free chlorine disinfection.

### Materials and methods

The effects of OXONE and free chlorine from calcium hypochlorite were evaluated against 48 h-old *Stenotrophomonas maltophilia* biofilms. *S. maltophilia* was previously isolated from a DWDS in Braga (Portugal) and was selected as model microorganism given its importance as emerging pathogen. Polyvinyl chloride (PVC) and stainless steel (SS) coupons (1 × 1 cm) were used as substrata for biofilm formation and were selected as representative pipe material from DW networks. The antibiofilm activity was analyzed in terms of biofilm culturability (log (CFU/cm<sup>2</sup>)) immediately after a 30 min exposure to the disinfectants at 10 × minimum bactericidal concentration (MBC) and biofilm regrowth for 24 h after chemical exposure. The MBC of OXONE and free chlorine against *S. maltophilia* (344 and 0.8 mg/L, respectively) were already determined in a previous work (Oliveira et al., 2022).

Additionally, the changes in the viscoelastic properties of biofilms resulting from the 30 min exposure to the disinfectants were evaluated by rheometry. Colony biofilms of *S. maltophilia* were subjected to oscillatory tests performed using a parallel plate rheometer.

### Results and discussion

The 30 min exposure to OXONE allowed a reduction in the biofilm culturability up to 7 log, resulting in CFU levels significantly lower than the biofilms unexposed to disinfectants ( $P < 0.05$ ). The biofilms exposed to OXONE showed CFU numbers of  $0.0 \pm 0.0$  and  $3.6 \pm 0.7$  log (CFU/cm<sup>2</sup>) on SS and PVC, respectively, in contrast to unexposed biofilms with  $7.2 \pm 0.3$  and  $7.2 \pm 0.1$  log (CFU/cm<sup>2</sup>) on SS and PVC, respectively.

The reposition of nutrients after disinfectant exposure allowed biofilm regrowth, even in biofilms that had been exposed to OXONE or free chlorine. However, the biofilms previously exposed to OXONE showed CFU levels significantly lower than the biofilms unexposed to disinfectants ( $P < 0.05$ ). After 24 h of biofilm regrowth, the biofilms previously exposed to OXONE presented CFU numbers of  $2.6 \pm 2.3$  and  $6.1 \pm 0.5$  log (CFU/cm<sup>2</sup>) on SS and PVC, respectively. In contrast, the unexposed biofilms showed CFU numbers of  $7.8 \pm 0.3$  and  $7.9 \pm 0.3$  log (CFU/cm<sup>2</sup>) on SS and PVC, respectively.



In both tests, the antibiofilm activity of OXONE was significantly better than the activity of free chlorine against biofilms formed on SS ( $P < 0.05$ ). The CFU numbers on SS coupons immediately after free chlorine exposure and after 24 h of biofilm regrowth were  $4.3 \pm 0.9$  and  $6.7 \pm 0.5$  log (CFU/cm<sup>2</sup>), respectively.

The rheometry analysis demonstrated that independently of the disinfectant treatment the *S. maltophilia* biofilms behaved as viscoelastic solid material, with a higher elastic shear modulus ( $G'$ ) than viscous shear modulus ( $G''$ ). Nonetheless, biofilms exposed to OXONE had complex shear modulus ( $G^*$ ) significantly lower than the values of unexposed biofilms and biofilms treated with free chlorine ( $P < 0.05$ ) (Table 1). Therefore, the 30 min exposure to OXONE reduced the stiffness of the biofilm, weakening its cohesiveness.

**Table 1.** Complex shear modulus ( $G^*$ ) of colony *S. maltophilia* biofilms after 30 min exposure to OXONE and free chlorine at  $10 \times$  MBC.

Treatment	Complex shear modulus ( $G^*$ , kPa)
Control	$140 \pm 29$
Free chlorine	$104 \pm 70$
OXONE	$69 \pm 21$

### Conclusions

This study demonstrated that OXONE presented significant action against *S. maltophilia* biofilms. In biofilms formed on SS, OXONE had better efficiency in reducing biofilm culturability and limiting biofilm regrowth than free chlorine. The antibiofilm activity of OXONE was related to changes in the viscoelastic properties of the biofilms, reducing their cohesiveness and promoting the disinfection of bacteria. This study reinforces OXONE as promising alternative to free chlorine for DW biofilms disinfection and highlights the importance of understanding biofilm rheology to improve biofilm control strategies.

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