Comparison of methods to assess biofilm disinfection and recovery by drinking water-isolated bacteria

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Drinking water (DW) distribution systems are known to harbour biofilms even in the presence of disinfectants. DW biofilms are constituted by microbial communities adapted to low nutrient concentrations and high chlorine levels. Biofilm formation and resistance to disinfection have been recognized as important factors that contribute to the survival and persistence of microbial contamination in DW.

The purpose of this work was the comparison of diverse methods to assess the disinfection of biofilms formed by six DW-isolated opportunistic bacteria (*Acinetobacter calcoaceticus*, *Burkholderia cepacia, Methylobacterium* sp., *Mycobacterium mucogenicum, Sphingomonas capsulata* and *Staphylococcus* sp.) by sodium hypochlorite (SHC). Single and multi-species biofilms (composed of combinations of 6 and 5 bacteria) were developed in 96-wells microtiter plates for 3 days, afterwards, were exposed to several independent SHC concentrations (0.1, 0.5, 1 and 10 mg/L) during 1 h. The potential of biofilms to recover was assessed 24 h after disinfection. The disinfection efficacy and recovery were assessed in terms of variation in: biofilm mass (crystal violet staining); metabolic activity (XTT staining); cultivability (CFUs) and viability (Live/Dead staining).

The results indicated that biomass removal increased with increasing SHC concentration, but total biofilm mass removal was not achieved. The effects of SHC on the biofilm activity, cultivability and viability were also concentration dependent. Total biofilm inactivation was achieved only for *A. calcoaceticus* biofilms and for multi-species biofilms without *A. calcoaceticus*, when exposed to high SHC concentrations. Almost all multi-species biofilms were more resistant to removal and inactivation than the single biofilms. *Methylobacterium* sp. and *A. calcoaceticus* formed the most resistant and the most susceptible biofilms, respectively. On the other hand, biofilm combination with the six DW bacteria was the most resistant to SHC and combination without *A. calcoaceticus* was the least resistant, for all concentration tested. The several methods used to assess of biofilm activity (metabolic activity, cultivability and viability) provided comparable results. However the viability results provide the worst case scenario in terms of biofilm control analysis (higher number of viable cells for all the SHC concentrations tested). The recovery results demonstrated that only biofilms without *A.calcoaceticus* were not able to recover their biomass from the SHC treatments. Also, those biofilms had a decreased ability to recover their metabolic activity, cultivability and viability. Conversely, multi-species biofilms without *Staphylococcus* sp. had the highest ability to recover from disinfection. Biofilm mass and activity recovery were not correlated for all the biofilms tested. However, the data of biofilm recovery in terms of metabolic activity, cultivability and viability also provided comparable results.

Keywords: biofilm disinfection; drinking water bacteria; methods, recovery; resistance; sodium hypochlorite

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