Susceptibility of drinking water biofilm bacteria to sodium hypochlorite

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Biofilm formation and their resistance to disinfection have been recognized as important factors that contribute to the survival and persistence of microbial contaminations in drinking water (DW). Research on DW biofilm control will help to determine optimal disinfection parameters and lead to knowledgeable decisions regarding the management of DW distribution networks that will guarantee microbial safe and high quality DW.

The main goal of this study was to investigate the effect of sodium hypochlorite (SHC) on the control of biofilms formed by 6 DW bacteria, *Acinetobacter calcoaceticus*, *Burkholderia cepacia*, *Methylobacterium* sp., *Mycobacterium mucogenicum*, *Sphingomonas capsulata* and *Staphylococcus* sp., respectively. The bacterial genera used in this study represented above 80 % of the total genera isolated and identified from normal tap water in Braga, Portugal. Biofilms were developed in 96-wells microtiter plates for 3 days and, afterwards, were exposed to several independent SHC concentrations (0.1, 1, 10, 100 and 1000 mg/L) during 1 h. Biofilm control was assessed by means of crystal violet (CV), sodium 3,3'-[1[(phenylamino)carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate (XTT) staining and colony forming units (CFU) to assess biofilm mass, metabolic activity and culturability, respectively. The potential of biofilms to recover from disinfection was assessed 24 h after SHC exposure.

The tested biofilms had distinct susceptibilities to SHC. *B. cepacia*, *Methylobacterium* sp., *M. mucogenicum*, *Sph. capsulata* and *Staphylococcus* sp. biofilm removal increased with the increasing disinfectant concentration. *A. calcoaceticus* biofilms were significantly affected by disinfection (biofilm removal > 90 %), however, not related with the increase in SHC concentration. XTT staining and CFU experiments provided comparative results. The effects of SHC on the activity and culturability of *B. cepacia*, *Methylobacterium* sp., *M. mucogenicum* and *Staphylococcus* sp. biofilms was concentration dependent. However, total inactivation was not achieved. *Sph. capsulata* biofilms were highly sensitive to all the concentrations tested (total biofilm inactivation), while *A. calcoaceticus* biofilms had similar susceptibilities to all the concentrations tested (4 log CFU reduction). The biofilm recovery experiments demonstrated that only *B. cepacia* biofilms were not able to recover from the several SHC treatments. The other biofilms recovered their mass, activity and culturability after treatment, with the exception of those exposed to SHC at 1000 mg/L. Biofilm recovery was more significant for *A. calcoaceticus* biofilms. The recovery results propose that only very high SHC concentrations prevent biofilm recovery events.

This investigation demonstrates the distinct susceptibility of DW biofilm bacteria to SHC. Moreover, this study provides information that might help developing strategies for the efficient DW biofilm control. The identification of the species that form the biofilms with extreme resistance to inactivation and removal and the ability to recover from the disinfection process (*A. calcoaceticus* and *Methylobacterium* sp.) may provide new information for improving water quality for the consumer.