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Biofilm formation inside drinking water distribution systems (DWDS) constitutes one of the major microbial problems in the distribution of safe water. Biofilms in DWDS can act as a reservoir of pathogenic microorganisms and consequently constitute a threat to public health. Therefore, the control of biofilm development inside the pipes of DWDS is a concern for drinking water companies being the use of chlorine one of the most commonly used disinfecting strategies to avoid microbial growth. The aim of this work was to understand the effects of sodium hypochlorite (NaOCl) at residual and high doses against biofilms formed by two bacteria isolated from a DWDS (Acinectobacter calcoaceticus and Stenotrophomonas maltophilia, two emergent pathogens) on polyvinyl chloride. The NaOCl effects were evaluated in bacterial membrane properties and in biofilm cohesion. NaOCl demonstrated action on the bacterial membrane, particularly on the surface hydrophobicity of A. calcoaceticus and on the surface charge of S. maltophilia. NaOCl also caused motility inhibition of A. calcoaceticus. The use of residual concentrations to control bacterial adhesion was inefficient. High concentrations were able to reduce significantly the number of adhered bacteria. However, mature biofilms formed by A. calcoaceticus and S. maltophilia were highly resistant to the combination of chemical and mechanical stresses. In conclusion, the overall results demonstrated a significant action of NaOCl on A. calcoaceticus and S. maltophilia planktonic cells and monolayer adhered cells. However, their mature biofilms were not controlled even when high biocide doses and mechanical stress were applied alone and in combination.