

# Behavior of single and mixed biofilms subjected to benzalkonium chloride: the impact of using chemical-adapted bacteria

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## Abstract

The majority of lab-scale studies involves a single species to form biofilms and to later evaluate the effect of the antimicrobials against these biofilms. However, in real systems, biofilms can be found as a complex consortium of species where the biofilm behaviour is the sum of the synergistic and antagonistic interactions between the different species. It is not surprising that disinfection protocols developed with a single species biofilm can fail when applied to a real system. The central query of this study was to understand the behaviour of binary biofilms when exposed to high doses of benzalkonium chloride (BZK), a general disinfectant in health care facilities.

*P. aeruginosa* (ATCC 10145), *P. aeruginosa* adapted to 4.0 mM of BZK (PA4) and *Escherichia coli* k12 were the strains used to form single and binary biofilms. The mixed-species biofilms were obtained by a combination of 50% of suspended inocula of each specie: *E. coli* with *P. aeruginosa* and *E. coli* with PA4. The role of surface preconditioning with BZK on biofilm properties was also investigated. Biofilms were formed for 24 h in 96 plates (non- and conditioned with 0.7 mM of BZK for 30 min), being then treated with 1.0 mM of BZK. Crystal violet (CV) together with XTT, were used to assess, respectively, total attached biomass and respiratory activity. The number of CFU/cm<sup>2</sup> was also determined.

The data emphasised that the characteristics of the binary biofilms depend on the strains that formed them. In fact, when the adapted strain is involved, the resulting biofilms have less mass, activity and cells. It is also observed that, for both single- and dual-species biofilms, surface preconditioning significantly favoured biofilm formation. Although the BZK preconditioning of the surfaces increased biomass, it simultaneously appeared to weak bacterial adhesion, since BZK attack reduced significantly the amount of biofilm comparing

to the clear surfaces, for both single- and mixed-species. Concerning the data related with BZK attack, for both non- and conditioned surfaces, there was a notorious reduction of the bacterial activity and number of cells, in both single and mixed biofilms. But, the PA4 and *E. coli* binary biofilms seemed to reveal recalcitrant behaviour since the CFU still unaltered after BZK attack. These data suggest that when biofilms are formed by the PA4 and *E. coli*, it seems to exist a competitive behaviour between the bacteria that give rise to a mixed biofilm with fewer mass and cells. However, these biofilms are less susceptible to the action of BZK, sign of a possible protective effect of one species to another. Since few reports specifically address interactions within multi-species biofilms, these results highlight the importance of understanding the multiple behaviours of biofilms as a community and a complex web of interactions.

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