Differential efficacy of selected antimicrobials on the mechanical stability of mixed biofilms formed by *Bacillus cereus* and *Pseudomonas fluorescens*

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Biofilms are assemblages of microbes growing on a surface and held together by the presence of a matrix composed of diverse extracellular polymeric substances. In some settings, such as in water treatment facilities, biofilm formation is desirable. But in many situations biofilms can have a negative impact on human activities. For example, biofilms cause steel corrosion and biofilms have been implicated in several chronic human infections such those occurring in the lungs of patients with cystic fibrosis. Bacteria in biofilms have intrinsic mechanisms that protect them from even the most aggressive environmental stress conditions, namely the exposure to chemical antimicrobials. Thus, there is a need to develop alternative approaches to control biofilms in both industrial and biomedical settings. Mechanical stress is a parameter often involved simultaneously in the disinfection and removal of biofilms, as the application of sole chemical agents tends to leave the biofilm intact. Under well-designed conditions the synergy of chemical and mechanical treatments may provide efficient solutions for biofilm control.

In this study, mixed biofilms formed by *Bacillus cereus* and *Pseudomonas fluorescens* were developed in a novel bioreactor rotating system under a constant Reynolds number of agitation ($N`Re_A$) of 2400 using ASI 316 stainless steel as adhesion surface. *B. cereus* was isolated from a disinfectant solution and identified by 16S rRNA sequence analysis and was used the *P. fluorescens* type strain (ATCC 13525). The biofilms were allowed to grow for seven days. Afterwards, the biofilm mechanical stability was assessed by sequential exposure to increasing $N`Re_A$, respectively, 4000, 8100, 12100 and 16100. In order to assess the role of chemical agents on mechanical stability, the biofilms were exposed to selected chemical agents followed by mechanical treatments. A cationic surfactant, cetyltrimethyl ammonium bromide (CTAB), and an aldehyde-based biocide, glutaraldehyde (GTA), were tested and their effects on biofilm mechanical stability were evaluated. Control experiments, *i.e.* without biofilm exposure to the chemicals, were conducted in parallel. It was found that increasing $N`Re_A$ increased biofilm removal, but total removal was not achieved for all the conditions tested. For the experiment without chemical addition (only mechanical treatment), the biofilm removed was about 83 % of the total biofilm mass. The removal, of biofilms previously exposed to CTAB and GTA, was 64 % and 92 %, respectively. This result shows that GTA increased removal when comparing with the control experiment, while CTAB increased biofilm mechanical stability and consequently decreased significantly biomass removal. The high percentage of removal occurred with the implementation of a $N`Re_A$ of 8100 for the control experiment, 8100 and 12100 (similar values) for biofilms pre-treated with CTAB and 4000 and 8100 for biofilms pre-treated with GTA. For all the conditions tested, biofilm removal was dependent on the $N`Re_A$ applied ($P < 0.05$). At the end of the experiment the population viability of the remaining biofilm was assessed showing that about 80 % of the population from CTAB and GTA pre-treated biofilms was in a viable state.

This study demonstrates that GTA increased biomass removal due to mechanical stress, while CTAB increased biofilm mechanical stability. However, even with the synergistic chemical and mechanical treatments, for every scenario studied, total biofilm eradication was not achieved. Also, the distinct nature of the chemical antimicrobials tested, suggests that multiple interactive forces contribute to the mechanical stability of mixed *B. cereus* and *P. fluorescens* biofilms.

Keywords: antimicrobial agents; biofilm control; mechanical stability; mixed biofilms