

## ***P. fluorescens* biofilm control using bacteriophage $\Phi$ S1**

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*Pseudomonas fluorescens* biofilms contribute to the spoilage of dairy industry products due to the proteolytic activity of some *Pseudomonas fluorescens* strains. The eradication of these biofilms is difficult using the traditional chemical biocides due to the low removal action of these agents. Additionally chemical control leaves inactivated cells attached to the surface that tends to provide an ideal environment for further bacterial adhesion and growth. Bacteriophages can be seen as good alternative biofilm control agents due to their high specificity, efficacy against biocide resistant bacteria and because they are innocuous to the environment. The use of the bacteriophage phi-S1 as control agent of *P. fluorescens* biofilms at early stages of formation and in the mature stage was investigated in this work. Phage infection of attached cells was studied using a parallel plate flow cell mounted on an inverted microscope and with an automatic image analysis system that enables *in situ* and real time enumeration of cells. After phage infection the recolonization of the surfaces was also assessed with this system. The results obtained showed that phage infection caused a removal of about  $93.5 \pm 1.51\%$  of the adhered cells. Additionally *P. fluorescens* recolonization of the surfaces was no longer possible due to the adsorption of phages (assessed by EDS) that remained on the glass surfaces after being washed during one hour with buffer. Five days old biofilms and planktonic cells in different growth phases were infected with phage (with initial MOI of 0.5). The infection was monitored by measuring the amount of cell lysis, PFU release and decrease in biomass. The results obtained revealed that although the rate of cell lysis and PFU release was lower in biofilms than those in exponential planktonic cultures ( $p < 0.05$ , ANOVA), the decrease in the biomass after 200 minutes was approximately the same of infection and about  $85.27 \pm 1.41\%$ . This work demonstrated that phages are important tools to be considered in industrial control of biofilms because they are able to cause significant biomass reduction of early stages and mature biofilms and prevent cell recolonization of the surfaces.