TITLE: SYNERGISTIC PROTEASES AND SURFACTIN ACTION ON THE REMOVAL OF *Escherichia coli* BIOFILM

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ABSTRACT:
Removal of bacterial biofilms from dental-medical-hospital devices is a major challenge for the health-care system. Biofilms contribute to persistent bacterial infections which cause high costs to eradication. Therefore, it is important to develop effective cleaning products, which does not impact the environment and human health, in order to remove the microbial load from those devices. The addition of enzymes and biosurfactants to cleaning agents has a beneficial effect on biofilm removal and meets health and safety requirements. The aim of this study was to assess the efficiency of cleaning agents containing serine proteases and biosurfactant surfactin in different concentrations and time exposure on the removal of *Escherichia coli* ATCC 35218 biofilm. Biofilm was assayed on a 96-well polystyrene plate for 24h at 37ºC, and removal assay was performed using two proteases (E1 and E2) at concentrations of 0.5% and 5.0% and surfactin 0.5%, in 0.5h and 2h. Crystal violet and tetrazolium salt assays were used to detect the residual biomass and cell viability, respectively. The individual proteases test showed 85% removal of total biomass and cell death in 2h of exposure in both concentrations. The two proteases combined removed 86.5% of the biomass, regardless of time, and showed 96.3% of inviable cells in 2h. Surfactin removed 58.5% of total biomass, but 80% of cells remained viable. Surfactin and proteases (5.0%) combined promoted 90% of biomass removal and inviability cells. The results of 0.5h were not statistically relevant. There was no significant difference in biofilm removal in different enzymes concentrations. The best time exposure was during 2h. Hence, E1 and E2 combined increased the efficiency of biomass removal and inviability of bacterial cells of the biofilm.The surfactin addition increased biomass removal, which evidencing a synergistic relation between E1+E2 and E1+E2+surfactin. Thus, the combination of E1, E2, and surfactin showed to be an effective alternative to remove bacterial biofilm and to decrease viable bacterial cells.

Keywords: nosocomial infection, enzymatic detergent, serine proteases

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