

TITLE: EVALUATION OF EXPERIMENTAL ENZYMATIC CLEANING AGENTS IN THE REMOVAL OF BACTERIAL BIOFILM UNDER DIFFERENT CONDITIONS

AUTHORS: BRESCIANI, T.M.¹; GARCIA, M.R.P.¹; MAZON, T.²; AOYAMA, H.¹; LEITE, D.S.¹; MIRANDA, M.A.³

INSTITUTIONS: 1. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS, SP (CIDADE UNIVERSITÁRIA ZEFERINO VAZ, S/N; CEP 13083-970, CAMPINAS – SP, BRAZIL); 2. CENTRO DE TECNOLOGIA DA INFORMAÇÃO “RENATO ARCHER”, CAMPINAS, SP (RODOVIA DOM PEDRO I (SP-65), KM 143,6; CEP 13069-901, CAMPINAS – SP, BRAZIL); 3. INSTITUTO FEDERAL DE SÃO PAULO – CAMPUS CAMPINAS, CAMPINAS, SP (AV. COMENDADOR ALADINO SELMI, S/N; CEP 13069-901, CAMPINAS – SP, BRAZIL)

ABSTRACT

The bacteria's ability to form biofilm is an important element of its pathogenicity, and its occurrence is a real challenge for today's health care system. It is of extreme importance for health care institutions the efficient development of cleansing agents that can remove biofilm. The use of enzymes and biotechnology components in solutions, resistant to changes in temperature and pH, must be carefully developed to ensure effective performance. Hence, this work's goal was to evaluate the efficiency of experimental cleaning agents containing protease and carbohydrate class enzymes in the removal of *Escherichia coli* biofilm under different temperature, pH and exposure time conditions. We assayed bacterial biofilm destabilization from the growth of *Escherichia coli* ATCC 35218 in BHI medium using serine proteases (E1 and E2) and carbohydratase (E3). Biofilm removal was evaluated using an experimental cleaning agent plus enzymes in different concentrations (0.5% - 5.0%), temperatures (25° C and 45° C), pH (6.0 and 7.0) and exposure (0.5h and 2.0h). SDS (0.25%) and NaCl (0.9%) were used as positive and negative controls, respectively. Residual biomass (Violet Crystal) and cell viability (Tetrazolium salt) were measured by optical density spectrophotometer. Statistical significance was determined for each set of data using ANOVA test and Tukey test of parametric and two-tailed ($\alpha = 0.05$). The results for the studies of the isolated enzymes revealed 85% of biomass removal and cell death for proteases E1 and E2, regardless of the other analyzed conditions. However, carbohydratase E3 showed 50% biomass removal and 65% cell death at pH 6.0, for any concentration or exposure time. E3 showed no significant effects of biomass removal and cell death at pH 7.0. The enzymes E1, E2 and E3, acting synergistically, promoted the removal of 86.19% of biomass and 94.87% of cell death in the concentration of 5% of E1 and E2 and 0.5% of E3, at pH 6.0, 45° C and 2h. The enzymes E1 and E2, acting synergistically, promoted the removal of 86.5% of biomass, regardless of the time, and 96.3% of cell death in 2h and pH 7.0. In this way, the addition of enzymes to cleaning agents promotes a beneficial effect, in which their efficiency depends on conditions such as: enzymes kind, pH and time of exposure.

Keywords: *Escherichia coli*, enzymes, protease, carbohydratase, biofilm removal

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