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# Evaluation of the conditions for bacterial biofilm removal from E. coli by proteases present in enzymatic detergents

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**Abstract**

The development of effective cleaning products to remove microbial loads has been of extreme importance for the Sterilization Centers of Dental-Medical-Hospital Materials. The addition of enzymes to these products has promoted a beneficial effect, which efficiency depended on the biofilm and the enzymes evaluated. Thus, the objective of this work was to analyze the efficiency of such cleaning agents that contain enzymes of the protease class in the removal of bacterial biofilm of E. coli under different conditions of time, temperature and pH.

***Key words:***
*Proteases, bacterial biofilm, E. coli.*

## Introduction

One of the major health care challenge in hospital infections is to get an efficient way to remove dirtiness and bacterial biofilms from medical devices avoiding high costs for the health care system[1]. Corrosive chemicals and high temperatures can not be used for this purpose due to the delicacy of some medical devices, constituting a great difficulty of cleaning in materials centers and sterilization. In this way, softer solutions, such as enzymatic detergents, must be carefully developed to ensure effective performance. The objective of this work was to evaluate the efficiency of these cleaning agents containing enzymes of the protease class in the removal of the bacterial biofilm of *E. coli* under different conditions of time, temperature and pH.

## Results and Discussion

*Escherichia coli* ATCC 35218 biofilm was developed in BHI medium for 24h at 37°C in 96-well plates for further analysis of biofilm removal[2]. Two enzymes of the class of the serine proteases (E1 and E2) at concentrations of 0.5% and 5.0%, diluted in base detergent were evaluated. The following conditions were used: pH values of 6.0 and 7.0, time exposure of 0.5h and 2.0h, and temperatures of 30ºC and 45ºC. 0.25% SDS and 0.9% NaCl were used as positive and negative controls, respectively. The residual biomass and the cell viability were determined using crystal violet and tetrazolium salt, respectively. At the different pH values evaluated, E1 and E2 were able to remove the bacterial biofilm according to the results presented in Chart 1.

**Chart 1.** Summary of biofilm removal capacity for the proposed enzymes.

| Enzyme diluted in standard $H_2O$ | Biofilm removal capability | Total biomass | Bacterial viability |
|---|---|---|---|
| E1 | Yes | ++ | +++ |
| E2 | | ++ | +++ |

Legend: +++ Strong (> 80% in the evaluated methods);
++ Moderate (> 50% in the evaluated methods).

Removal of biofilm at pH 6.0 was higher than at pH 7.0 (not shown), however all the results obtained were performed at pH 7.0, according to the characteristics of the product. It was observed that E1 and E2 removed 65% and 55% of biomass, respectively, in 0.5h, regardless the enzyme dilution. However, at 2.0h of exposure time, 90% of biofilm removal was observed. In a joint action, independently of the enzyme dilution, E1 and E2 promoted 85% and 95% of cell death, respectively. Individually, the proteases did not show any increase in the cell death. Regardless the enzyme dilution and the exposure time, the increase of temperature from 30ºC to 45ºC did not promote significant changes in the biomass removal. On the other hand, the cell death was strongly affected by the association of exposure time and temperature.

## Conclusions

Our results suggest the importance of a detailed study of the influence of pH, temperature and exposure time to improve the bacterial biofilm removal and cell death, two essential factors for an effective cleaning of medical devices.

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