

**TITLE:**INFLUENCE OF SANITATION PROCEDURES IN THE ELIMINATION OF *Bacillus cereus* AND *Bacillus licheniformis* BIOFILMS ISOLATED FROM INDUSTRIAL BOVINE GELATINE

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**ABSTRACT:**

Gelatine processing has several steps that allow the survival of sporulated microorganisms, such as the *Bacillus* genus. The main strategies used in the control of biofilms are sanitation procedures, which consists of the combined use of detergents and disinfectants. The objective of this work was to evaluate the influence of sanitation procedures in the elimination of *B. cereus* and *B. licheniformis* biofilms. These microorganisms were isolated from the environment of a bovine gelatine processing industry. Biofilms were formed after immersion of stainless steel coupons in semi-finished bovine gelatine (10 g of bovine gelatine in 90 mL of sterile distilled water at 40 °C) inoculated with approximately 10<sup>2</sup> CFU/mL of *B. cereus* or *B. licheniformis*. After 48 hours of contact at 35 °C, the coupons were submitted to different sanitation procedures: 1- (alkaline cleaning: pre-rinse, 2% alkaline detergent and final rinse); 2- (disinfection: pre-rinse, 0.2% peracetic acid); 3- (alkaline cleaning + disinfection: pre-rinse, 0.2% alkaline detergent, rinse, and 0.2% peracetic acid). The alkaline detergent and disinfectant were applied at room temperature for 20 min each. For *B. licheniformis*, the biofilm count was 7.87 log CFU/cm<sup>2</sup>. In this case, procedures 2 and 3 were able to eliminate biofilm, leaving counts below the detection limit (<3 log CFU/cm<sup>2</sup>). Procedure 1 reduced only 2.63 log, and the biofilm count after application of the alkaline detergent was 5.24 log CFU/cm<sup>2</sup>. For *B. cereus*, the biofilm count was 6.82 log CFU/cm<sup>2</sup>. In this case, only procedure 3 was able to reduce the biofilm to counts below the detection limit (<3 log CFU/cm<sup>2</sup>). Procedure 1 reduced *B. cereus* biofilm counts to 4.30 log CFU/cm<sup>2</sup> (reduction of 2.52 log). Direct application of peracetic acid to biofilm (procedure 2) reduced counts to 4.88 log CFU/cm<sup>2</sup> (reduction of 1.94 log). Although the two microorganisms belong to the same genus, *B. cereus* was more resistant to sanitation procedures and was only eliminated when the procedure 3 was applied. In addition, only the application of the alkaline detergent did not eliminate any of the biofilms. Therefore, the results demonstrated the importance of combining the methods (cleaning + disinfection) for an efficient reduction of the studied biofilms.

**Keywords:***Bacillus*, bovine gelatine, disinfectant, stainless steel.