

TITLE: INITIAL MICROBIAL LOAD, TEMPERATURE AND TIME OF CONTACT AFFECT THE BIOFILM FORMATION OF *ALICYCLOBACILLUS ACIDOTERRESTRIS* IN RUBBER SURFACE

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ABSTRACT:

Alicyclobacillus spp. is a genus of spore-forming bacteria, which can be found in the food industry in the form of biofilms. Biofilms can be a focus of food contamination, leading to deterioration of products. The objective of this study was to evaluate the effect of the initial microbial load (low - 2 log CFU/ml or high - 5 log CFU/ml), temperature (28 °C and 45 °C), and contact time (4, 8, 24, 48, and 72 h) on the biofilm formation of *A. acidoterrestris* CBMAI 0244^T on rubber surface using processed reconstituted orange juice as culture medium. In the low initial load of *A. acidoterrestris*, the highest biofilm formation occurred after 48 h of contact at 28 °C (3.72 log CFU/cm²) and 45 °C (4.73 log CFU/cm²). In addition, a reduction of *A. acidoterrestris* sessile cell counts was observed after 72 h of contact at both temperatures on the rubber surface, probably due to the release of biofilm fragments. Moreover, the presence of spores in the biofilm was not observed (count below the detection limit: <3 log CFU/cm²). In high initial load of *A. acidoterrestris*, the biofilm formation was observed on the rubber surface after a few hours, within 8 h and 4 h for 28 °C and 45 °C, respectively. The highest biofilm formation was observed at 28 °C and 45 °C after 72 h of contact, with counts of 4.56 log CFU/cm² and 4.79 log CFU/cm², respectively. In addition, at high initial *A. acidoterrestris* populations, the spore formation was detected in biofilms from rubber surface since 48 h of contact (counts around 3.5 log CFU/cm²). Therefore, the high initial load of *A. acidoterrestris* led to biofilm formation on rubber surface more rapidly, suggesting that hygiene procedures must be performed frequently. Biofilm formation was observed from 48 h of contact at a low initial load of *A. acidoterrestris*, while at high initial microbial load, the biofilms were formed from 4 h of contact. In addition, at 28 °C it was necessary a longer contact time (from 48 h) for the formation of denser biofilms. On the other hand, at 45 °C, a short period of time was sufficient for a dense biofilm formation of *A. acidoterrestris*.

Keywords: *Alicyclobacillus acidoterrestris*, concentrated orange juice, rubber, spores