

BIOFILMS

REMOVAL AND DISINFECTION OF *LISTERIA MONOCYTOGENES* AND POULTRY SOIL-CONTAINING BIOFILMS USING CHEMICAL CLEANING AND SANITIZING AGENTS UNDER STATIC CONDITIONS

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Cleaning and sanitizing the food processing environment often involves the application of chemical agents in the form of foam or gel (viscous liquid or thin film) to avoid the use of high pressure sprays and hand scrubbing that can facilitate the spread of pathogenic bacteria. In addition, these chemical agents are often applied without application of heat to ambient or cold surfaces. The objective of this research was to evaluate the effectiveness of cleaning and sanitizing chemicals applied under static conditions without application of heat for the removal of *Listeria monocytogenes* biofilms coated with soil of poultry origin. Chemicals evaluated were alkali and neutral cleaning compounds, sodium hypochlorite, acidified sodium chlorite, peroxyacetic acid, peroxyacetic acid/octanoic acid mixture, and quaternary ammonium compound sanitizing agents. Biofilms were prepared by growing *L. monocytogenes* on stainless steel for 24 h at 25°C. The resulting biofilms were then coated with chicken serum albumin and rendered chicken fat. Chemical treatments were at 4°C or 25°C for 1 to 30 min. At 25°C, the alkali cleaning agent removed 99% of fat and 93% of protein after 30 min exposure. The neutral cleaning agent was equally effective at removing fat, but removed only 77% of protein. The alkali cleaning agent also effectively removed *L. monocytogenes* biofilm coated with protein, decreasing cell numbers on the surface by over 7 log₁₀ after 10 min exposure. Acidified sodium chlorite and peracetic acid/octanoic acid mixture were the most effective sanitizers at killing *L. monocytogenes* biofilm coated with fat and protein, both achieving > 5 log₁₀ reduction after 1 min exposure at 25°C. A combination of 10 min cleaning with alkali and 30 min sanitizing with acidified sodium chlorite achieved > 7 log₁₀ reduction of *L. monocytogenes* to nearly undetectable levels (> 0.2 cfu/50 cm²) at 25°C. The combination of alkali cleaning (10 min) and use of either acidified sodium chlorite or peracetic acid/octanoic acid (10 min) were effective at inactivating the *L. monocytogenes* biofilm at 4°C, achieving > 6.0 and 5.3 log₁₀ reductions, respectively. This research has demonstrated that processing plant environmental surfaces can be effectively cleaned and sanitized using static application of chemicals on surfaces and ambient and cold temperatures.

HEAT INACTIVATION OF *LISTERIA MONOCYTOGENES*-CONTAINING BIOFILMS

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Recent outbreaks of *Listeria monocytogenes* have been associated with the consumption of contaminated processed meat products and refrigerated products and have raised concern that recontamination is occurring during or after processing. Possible sources of recontamination in food processing plants could be due to ineffective cleaning and redeposition of soil especially in stagnant areas such as joints and dead ends. The objective of this research was to develop a predictive model to determine the importance of time and temperature for predicting survival of *Listeria monocytogenes*, *Pseudomonas*, and *Listeria*-*Pseudomonas* mixed culture biofilms formed on stainless steel and buna-N rubber coupon surfaces.

Coupons were added to 10% TSB inoculated with 0.1% *Pseudomonas spp M21*, *L. monocytogenes*, or 1:4 *Pseudomonas spp M21*-*L. monocytogenes* mixed culture and incubated for 4 h at 25°C. After attachment, coupon surfaces were rinsed with phosphate buffer and transferred to 10% TSB and incubated for 48 h at 25°C. Duplicate coupons were tested for each heating time (1, 3, 5, or 15 min) and temperature (70, 72, 75, or 77°C). Heat treated samples were enumerated using the fraction negative enumeration method. Positive controls were vortexed with glass beads and enumerated using PCA and *Listeria* selective agar. The experiment was repeated six times.

Time was the predominant predictive factor for biofilm survival on stainless steel while both temperature and time contributed equally to predicting the survival of biofilm on buna-N rubber. Overall, *Pseudomonas* was more heat resistant than *Listeria* on stainless steel, probably due to its higher initial load. On rubber, *Listeria* in the mixed culture biofilm had the greatest probability of survival. *Pseudomonas* in biofilms on stainless steel has a 16% probability of survival after heat treatment of 77°C for 15 min and 0.04% on buna-N rubber. For *Listeria* in

biofilms, the probability of survival is 7% on stainless steel and 0.094% on buna-N while in mixed culture biofilms, the probability of survival of *Listeria* was 0.3% on stainless steel and 0.4% on buna-N rubber.

