



Abstract

exist in the food-processing environment and are a concern due to increased resistance mechanisms against antibiotics and disinfectants. Bi-culture biofilms were developed on five common food surface materials for up to 14 days. Materials evaluated included Buna-n rubber, nylon, ultra high molecular weight polyethylene (uhmw), and stainless steel type 304 with #3 and #2b finishes were used and submerged in sterile tryptic soy broth (TSB). All surfaces were sterilized by autoclaving prior to inoculation into sterile Tryptic Soy Broth and subsequent biofilm development. The TSB was refreshed every 24 hours to ensure constant nutrient supply during biofilm development. Samples were removed after 1, 3, 7, and 14 days of development and treated with Sandia National Laboratories Decon Foam-200 (DF-200). DF-200 is a unique combination of quaternary ammonium salts and hydrogen peroxide originally developed for use in chemical and biological agents of warfare. The test coupons were exposed to DF-200 for 1, 5, or 10 minutes. Residual bacterial populations were enumerated on selective media for *Listeria monocytogenes* and *Pseudomonas putida*. Scanning Electron Microscopy (SEM) was used to view the cells adhered to each of the surfaces after the 14 days of biofilm development. A GLM model in SAS was used for statistical analysis comparing biofilms in the Least Squares of Means. Statistical analysis was used to analyze differences between surfaces, treatments, and time in age. In general, the hydrophobic surfaces (buna-n, nylon, polyethylene) had the greatest adhesion ($P \leq 0.05$). Overall resistance to the sanitizer did not increase with biofilm age as expected for either organism ($P > 0.05$). Overall the DF-200 was effective resulting in complete decontamination of both *Listeria monocytogenes* and *Pseudomonas putida* on all test surfaces.

Introduction

biofilms are problematic in the food processing environment due to the potential for post-process production contamination, especially with ready-to-eat foods. Various food surfaces contaminated with biofilms have demonstrated increased resistance mechanisms to antimicrobial treatments by regulatory agencies. The objective of this research was to develop bi-culture *Listeria monocytogenes* and *Pseudomonas putida* biofilms onto five common food contact surfaces and evaluate the effectiveness of Sandia DF-200 for decontamination and removal of the biofilms. This was accomplished using traditional enumeration and characterization with Scanning Electron Microscopy (SEM).

Materials and Methods

Bacterial Cultures: five strain mixtures of *L. monocytogenes* (101M, 109, and 108M, donated by Dr. Beauchat, Univ. of Georgia, and ATCC serovar 4c, ATCC serovar 3) and *Pseudomonas putida* (poultry skin isolate, donated by Dr. Joe Frank, Univ. of Georgia). **Methodology:** 1 cm² pieces made of nylon, uhmw polyethylene, buna-n nitrile rubber, and stainless steel type 304 with #3 and #2b finishes were used and submerged in sterile tryptic soy broth (TSB). Samples were inoculated with a 5:1 ratio of *L. monocytogenes* to *P. putida*. Biofilms were developed for up to 14 days statically at 35°C. After 24 hour, 72 hour, 7 day, or 14 day biofilm formation, test coupons were aseptically removed from the TSB and rinsed thoroughly with phosphate buffered saline (PBS) to remove unattached cells. Coupons were then treated with 10 mL DF-200 for 1 minute, 5 minutes, or 10 minutes. Samples were then neutralized and enumerated. **SEM:** The surfaces were fixed by a series of various washings and critical point drying. The surfaces were mounted and coated with a palladium/gold mixture. The samples were viewed using the S-3500N Scanning Electron Microscope equipped with the S-6542 Absorbed Electron Detector (Hitachi Science Systems, Ltd. 1040 Ichige, Hitachinaka, Ibaraki Pref 312-0033, Japan).

Discussion

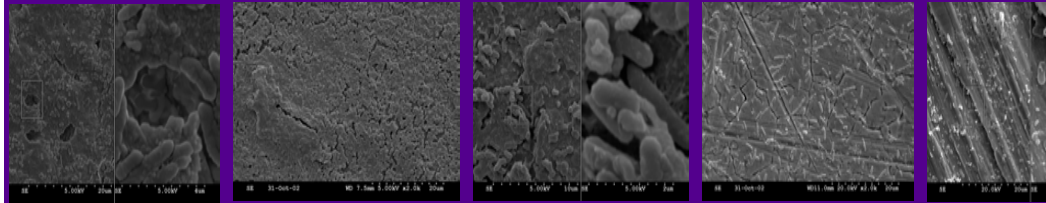
The results indicate that Sandia DF-200 was highly effective with contact times as low as 1 minute for complete decontamination of both *L. monocytogenes* and *P. putida*. SEM shows the highly contaminated surfaces prior to treatment with DF-200. The SEM of the treated surfaces demonstrate that the DF-200 removes the cells from the various surfaces. In general, the hydrophobic surfaces (buna-n, nylon, polyethylene) had the greatest adhesion ($P \leq 0.05$). Overall resistance to the sanitizer did not increase with biofilm age as expected for either organism ($P > 0.05$). Overall the DF-200 was extremely effective resulting in complete decontamination of both *L. monocytogenes* and *P. putida* on all test surfaces.

Acknowledgements

I would like to acknowledge Cecelia Williams, Mark Tucker, and Bruce Kelley (Sandia National Laboratories), John Boyer (Dept. of Statistics), Kent Hampton (Dept. of Entomology, KSU), and Daniel Boyle (Dept. of Biology, KSU) for their help in conducting this research.

Results

Buna-n Nylon Polyethylene SS #3 SS #2b
Control Samples: Prior to treatment with DF-200. Average adhesion = 6-7 log CFU/cm²



Treated Samples: After 1 min treatment with DF-200. Average reduction = 6-7 log CFU/cm²

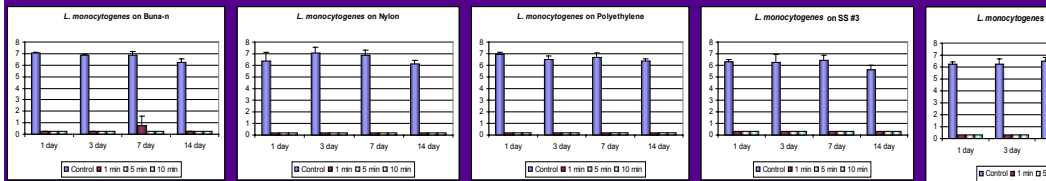
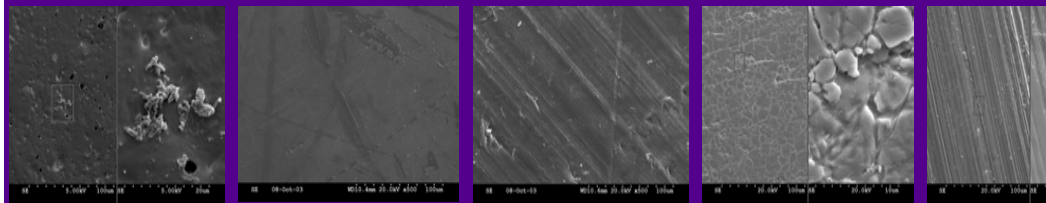


Chart data shown for recovered *L. monocytogenes*, Log CFU/cm². Results indicate hydrophobic surfaces had increased adhesion, $P \leq 0.05$. Resistance to DF-200 did not occur over time, $P > 0.05$.