RESEARCH IN PROCESS

Preventive sanitation measures for *Listeria monocytogenes* biofilms in critical postharvest sites

SUMMARY

Surfaces in the packinghouse can have intrinsic properties, making them difficult to sanitize. The main goals of this project are to identify these surfaces, characterize the surface roughness and hydrophobicity, use these surfaces for biofilm formation in the laboratory, and design methodologies for biofilm inactivation. In a previous CPS-funded project we assembled a biofilm apparatus with flow-through cells, where *Listeria monocytogenes* (*Lm*) biofilms are grown under laminar flow conditions. In this project to date, wood coupons were mounted on the flow cells and biofilms were grown on the coupons for 48 hours and then treated with 200 ppm chlorine. The wood coupons also were characterized for their surface properties. Results indicated that surfaces with increased roughness/waviness require longer contact time with the sanitizer for microbial inactivation.

OBJECTIVES

1. Construct testing beds from fabricated surfaces in the laboratory to replicate real-life conditions found in the packinghouse, by identifying areas, materials, specific environmental conditions, and microflora in the packinghouse that can harbor *Lm*. These test beds will be validated with conditions found in the packinghouse.
2. Determine cleaning and cleaning-and-sanitizing procedures for biofilms (*Lm* single culture and in co-culture with the resident microflora) grown in conditions simulating the packinghouse. Compare and validate efficacy of the tested sanitizers against *Lm* biofilms with the EPA requirements for hard surface (porous and non-porous) sanitizers.

METHODS

Construct test beds with fabricated surfaces to replicate real-life conditions found in the packinghouse:

- Materials (wood coupons) were obtained from a peach packinghouse.
- Coupons were characterized for surface roughness, sterilized with 75% ethanol and used to grow *L. monocytogenes* biofilms on their surface; some samples were treated with mineral oil to simulate a soiling event.
- *L. monocytogenes* Scott A was transformed with plasmid pNF8 to constitutively express green fluorescent protein (GFP) – the plasmid encodes for erythromycin resistance.
- Coupons were mounted on the biofilm apparatus (flow-through enclosures with laminar flow) and biofilms were grown for 48 hours.
- Samples were treated with 200 ppm free chlorine for 5 minutes and then 20 minutes.
- Control samples were treated only with phosphate buffer.
- Chlorine was inactivated at the end of the treatment, and the surviving population was determined by plating on tryptic soy agar supplemented with erythromycin.

RESULTS TO DATE

- Biofilm growth was conducted in a flow-through apparatus (constructed in our previous CPS-funded project), consisting of a media reservoir connected through tubing and connectors to a pump and then to a flow-break/air bubble trap device (**Figure 1**). Bacterial inoculum is introduced through a different port, with a sterile syringe that can be clamped on/off. Microorganisms are allowed to attach for 1 hour in the flow cells and then media is pumped through to the waste reservoir. Test surfaces are sandwiched between a machined silicone gasket and a sterile glass slide in the flow cells.
- Wood coupons were characterized for surface roughness (**Figure 2**).
- *L. monocytogenes* can form biofilms on less-than-optimal surfaces such as wood.
- Surfaces with increased roughness promote bacterial attachment.
- Surfaces with crevasses and pits should be exposed to sanitizer (chlorine) for longer time for microbial inactivation.
- Soiling of wood surfaces reduces sanitizer (chlorine) efficacy (**Figure 3**).

BENEFITS TO THE INDUSTRY

- Identification of critical microenvironments for a seek-and-destroy approach, where pathogens are eliminated before posing risks for product contamination.
- Outcomes of this project will be communicated with equipment manufacturers for better equipment design.
- Characterization of the conditions favorable for *L. monocytogenes* contamination of stone fruit in the packinghouse and design of measures to reduce pathogen survival and avoid cross-contamination.

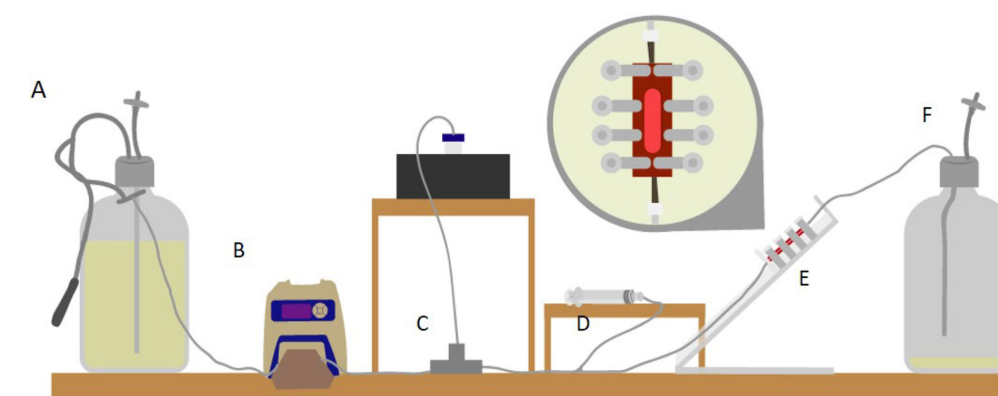
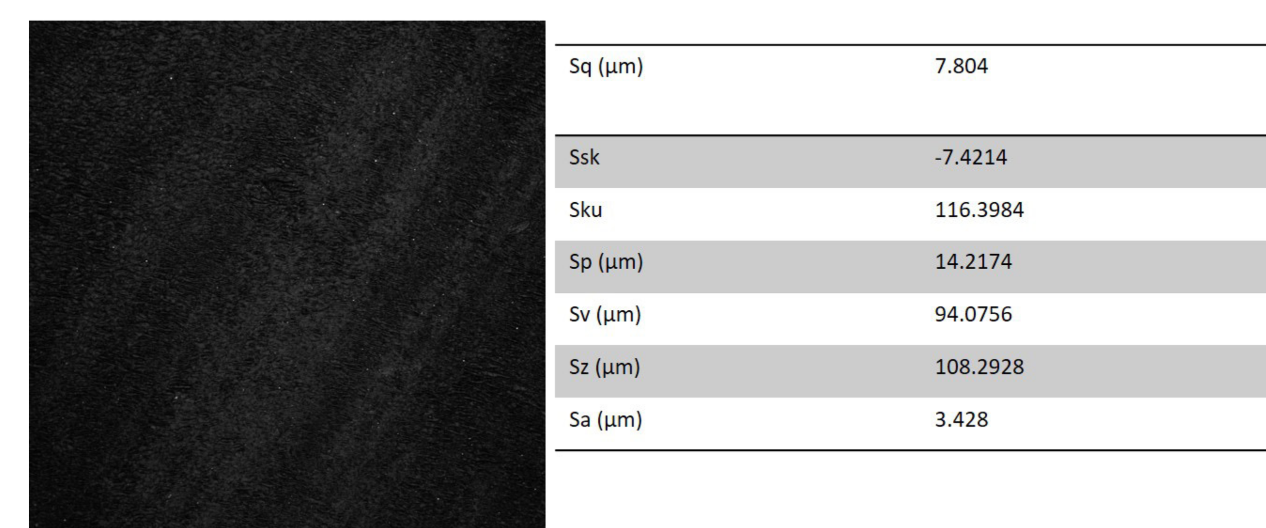


Figure 1. Diagram of the biofilm apparatus assembled with flow-through enclosures. (A) Media reservoir, (B) pump, (C) flow break/air bubble trap, (D) port with sterile syringe for microbial inoculation, (E) flow cell, and (F) waste reservoir. Insert shows a flow cell.



Parameter	Value
Sq (µm)	7.804
Ssk	-7.4214
Sku	116.3984
Sp (µm)	14.2174
Sv (µm)	94.0756
Sz (µm)	108.2928
Sa (µm)	3.428

Figure 2. Confocal microscopy and surface roughness measurements of wood samples collected from a local packinghouse. Images and roughness measurements were obtained with an Olympus LEXT OLS4100 digital microscope, magnification 10X. The following parameters were measured and represent the average of 5 samples (measurements taken for each sample in 5 different spots): Sq, root mean square height; Ssk, skewness; Sku, kurtosis; Sp, maximum peak height; Sv, maximum pit height; Sz, maximum height; and Sa, arithmetical mean height.

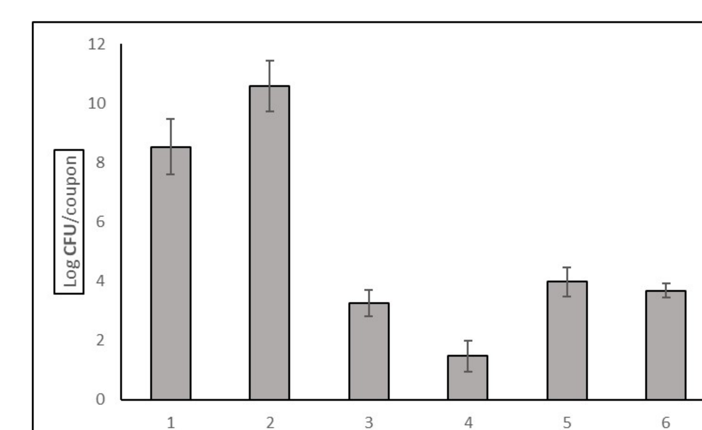


Figure 3. *L. monocytogenes* Scott A biofilm formation on wood coupons and inactivation by 200 ppm chlorine. (1) Wood coupon controls; (2) wood coupons soaked briefly in mineral oil control, before inoculation; (3) Wood treated with chlorine for 5 min, (4) wood treated with chlorine for 20 min, (5) wood coupon with mineral oil treated with chlorine for 5 min, and (6) wood coupon with mineral oil treated with chlorine for 20 min.

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