Verification and validation of environmental monitoring programs for biofilm control in the packinghouse

Contact

Paul Dawson
Clemson University
Department of Food, Nutrition and Packaging Sciences
pdawson@clemson.edu

Authors

Paul Dawson, Claudia Ionita (Co-PI)

Project funding dates

January 1, 2020 – December 31, 2020

Acknowledgements

Center for Produce Safety, USDA Specialty Crop Block Grant Program – Farm Bill, and California Stone Fruit Association

Summary

The aim of this project is to develop informational tools for environmental sampling and sanitation frequency in packinghouses. The team will grow biofilms formed by background microbiota collected in stone fruit packinghouses and by *Listeria monocytogenes* (Lm) under conditions simulating industry settings. The main findings regarding biofilm growth rate and transfer will be then validated in pilot plant studies, in which background microbiota will be inoculated and allowed to develop as biofilms on selected surfaces. Data from biofilm growth and transfer experiments will be used to build a mathematical model of biofilm development, and ultimately designed as a user-friendly Excel add-in. The add-in could be developed into a practical tool to predict microbial behavior in the packinghouse and anticipate optimal sampling time and sanitation intervals.

Benefits to the Industry

A science-based packinghouse sampling plan can be developed from these results such as sufficient number and location of samples, frequency and time intervals between sampling, and time of the shift that samples should be taken. In addition, understanding biofilm growth rates in conditions specific to the packinghouse will allow improved sanitation schedules, to avoid product contamination to ultimately reduce the presence of *L. monocytogenes* in packinghouses. Our results seem to suggest that more targeted sanitation practices and EMPs can be used in the packinghouse. Specifically, the fruit contact surfaces in the wash-wax area should be cleaned and exposed to sanitizers for longer contact time, whereas the dry, sorting area could be sanitized with shorter exposure time.

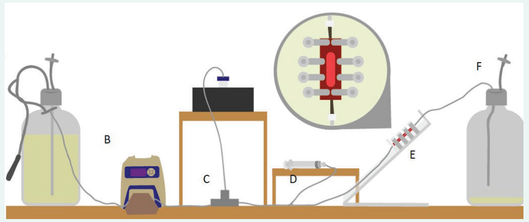

Figure 1. Diagram of flow-through biofilm apparatus. Sterile media (A) is pumped through sterile silicone tubing (B-peristaltic pump) to a flow-breaker/air trap (C) and then flow cell which contains the tested surface (E). Microorganisms are inoculated through a separate port (D) with a sterile syringe. Biofilm waste and bulk phase are collected in the waste container (F).

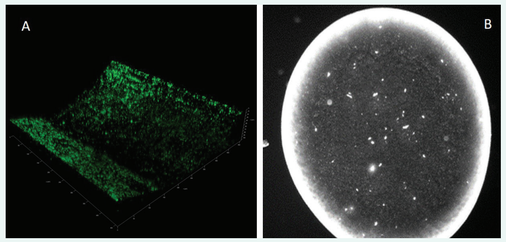

Figure 2. (A) Confocal image of flow cell mixed-species biofilm (on TPU), and (B) Far-field fluorescent image of colony-grown mixed-species biofilm (on TPU).

Objectives

1. Determine physiological and structural parameters (growth rate, microbial composition, morphology, and erosion or detachment) of microbial biofilms of resident microbiota and *Listeria monocytogenes* on food-contact surfaces in zone 1 in a simulated packinghouse environment.
2. Determine factors (microbial, physical, environmental) important in *L. monocytogenes* biofilm persistence and colonization of the packinghouse.
3. Validate the biofilm development parameters in a pilot plant study; build a mathematical model of biofilm growth and detachment and develop an Excel add-in to predict optimal sampling time and sanitation schedule.

Methods

Biofilms of mixed species (*L. monocytogenes* (Lm), *Pseudomonas* and *Burkholderia*) were grown on fabricated surfaces commonly found in the packinghouse, using a flow-through biofilm apparatus (**Figure 1**). In previous work, we tested two surfaces: Hyrel® (fruit holders) and Hycar® (belts and table coatings). In this project we tested 3×1 inch coupons of thermoplastic urethane (TPU) and polyvinyl chloride (PVC), materials found as conveyer belts or rollers and other fruit contact surfaces. Two types of surface modifications were used: (i) new material, ~0.5-micron surface roughness, and (ii) rough material, ~2-micron surface roughness. Coupons were also used for biofilms grown as colony biofilms, to simulate a different contamination and biofilm growth scenario in the packinghouse. Biofilms were treated with 0, 20, 100, or 200 ppm of active chlorine to determine Lm inactivation.

Results to Date

Previously isolated strains from stone fruit packinghouses were identified as strong biofilm-forming microbes: a *Pseudomonas* sp. and a *Burkholderia* sp. Microscopy experiments of Lm mixed-species biofilms determined that flow-through biofilms developed after 48 hours, and mature colony-based biofilms developed over 72 hours of growth (**Figure 2**). Biofilms had increased biomass on fabricated, rough surfaces compared with smooth controls.

Chlorine treatment was least effective on flow-through biofilms, where Lm in mixed-species biofilms required more than 10 min exposure to 200 ppm chlorine for 5-log reductions (**Tables 1–2** for TPU and **Tables 3–4** for PVC). After 25 min exposure to 200 ppm chlorine, complete inactivation of Lm in mixed-species biofilms on smooth surfaces was achieved; however, Lm in biofilms on rough surfaces was detected through enrichment. In colony-grown biofilms, no surviving Lm was detected after 5 minute treatment with 200 ppm chlorine.

	Chlorine (ppm)			
Time (min)	**0**	**20**	**100**	**200**
0	8.04 ± 0.02	7.86 ± 0.11	6.98 ± 0.53	5.65 ± 0.31
5	8.42 ± 0.17	7.43 ± 0.91	7.01 ± 0.11	4.93 ± 0.87
10	7.92 ± 0.54	6.98 ± 0.72	6.43 ± 0.33	2.74 ± 0.72
20	8.54 ± 0.39	7.01 ± 0.54	5.06 ± 0.71	2.03 ± 0.89
25	8.31 ± 0.41	6.96 ± 0.32	4.09 ± 0.27	Not detected

Table 1. Chlorine disinfection of smooth thermoplastic urethane (TPU) coupons of biofilms grown in flow cells. Average of 2 independent experiments is represented as log CFU/ml for Listeria monocytogenes in recovery buffer (± standard deviation).

	Chlorine (ppm)			
Time (min)	**0**	**20**	**100**	**200**
0	8.94 ± 0.32	8.01 ± 0.70	7.32 ± 0.22	7.49 ± 0.44
5	9.02 ± 0.11	8.20 ± 0.49	6.86 ± 0.29	5.16 ± 0.29
10	8.95 ± 0.13	7.03 ± 0.12	6.02 ± 0.15	4.38 ± 0.21
20	9.04 ± 0.41	6.56 ± 0.27	5.15 ± 0.67	2.15 ± 0.65
25	8.77 ± 0.65	6.09 ± 0.31	3.23 ± 0.51	Positive after enrichment

Table 2. Chlorine disinfection of 2-micron roughness TPU coupons of biofilms grown in flow cells. Average of 2 independent experiments is represented as log CFU/ml for Lm in recovery buffer (± SD).

	Chlorine (ppm)			
Time (min)	**0**	**20**	**100**	**200**
0	7.61 ± 0.15	7.02 ± 0.31	6.59 ± 0.13	6.44 ± 0.27
5	7.95 ± 0.10	6.33 ± 0.51	6.02 ± 0.17	5.64 ± 0.31
10	8.09 ± 0.25	5.10 ± 0.52	5.08 ± 0.74	3.67 ± 0.61
20	8.11 ± 0.62	4.36 ± 0.97	4.25 ± 0.87	2.35 ± 0.55
25	8.06 ± 0.14	2.55 ± 0.86	4.19 ± 0.39	Not detected

Table 3. Chlorine disinfection of smooth PVC coupons of biofilms grown in flow cells. Average of 2 independent experiments is represented as log CFU/ml for LM in recovery buffer (± SD).

	Chlorine (ppm)			
Time (min)	**0**	**20**	**100**	**200**
0	8.29 ± 0.14	7.33 ± 0.5	7.12 ± 0.1	6.59 ± 0.42
5	8.62 ± 0.25	6.81 ± 0.31	6.23 ± 0.24	5.21 ± 0.11
10	8.87 ± 0.20	4.93 ± 0.22	4.02 ± 0.24	3.98 ± 0.48
20	8.15 ± 0.29	3.86 ± 0.32	3.15 ± 0.26	2.45 ± 0.25
25	8.68 ± 0.75	2.79 ± 0.40	2.18 ± 0.35	Positive after enrichment

Table 4. Chlorine disinfection of 2-micron roughness PVC coupons of biofilms grown in flow cells. Average of 2 independent experiments is represented as log CFU/ml for Lm in recovery buffer (± SD).