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

Contact

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

Authors

Paul Dawson and Claudia Ionita

Project Period

January 1, 2020 – July 31, 2021

Acknowledgements

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

Summary

The overall goal of this project is to develop tools for environmental sampling and sanitation frequency in packinghouses. Biofilms formed by background microbiota collected in stone fruit packinghouses and *Listeria monocytogenes* are being grown 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. The model can be translated into a user-friendly Excel add-in. By entering specific environmental parameters, the add-in could be used to predict microbial behavior in the packinghouse and anticipate optimal sampling time and sanitation intervals.

Methods

L. monocytogenes Scott A and *L. monocytogenes* Petite Scott A (ATCC 49594) were used for biofilm formation on stainless steel coupons. For mixed biofilm experiments, we included 2 strains isolated from stone fruit packinghouses, identified as *Bacillus* and *Pseudomonas*. Microorganisms were first cold adapted for biofilm growth at 4°C. Two types of biofilms were grown as monoculture or mixed biofilms: (i) colony biofilms on top of sterile polycarbonate filters, and (ii) liquid conditions. A set of coupons were coated with a mineral oil–fruit coating prior to inoculation. Biofilms were grown at 4°C, then coupons were removed at selected time intervals for plate counts. A set of samples were exposed to chlorine at 4°C. Results were modeled with the Gompertz equation (DMFit) to compare biofilm growth parameters.

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.

Results to Date

L. monocytogenes biofilms can grow at low temperatures in conditions simulating the packinghouse with nutrients and moisture. Monoculture and mixed culture biofilms were developed for 16 days (**Figure 1** and **Figure 2**). The mixed culture biofilms showed overall longer lag phase (116.67 and 103.91 hours) and maximal growth rate (0.05 and 0.09) than monoculture biofilms (**Table 1**). There were no differences in biofilm growth regarding the presence of the mineral oil–based coating. When biofilms were treated with 100 and 200 ppm chlorine, the sanitizer was not very effective at low temperatures (**Figure 3**). The presence of mineral oil–based coating further reduced chlorine efficacy. Biofilm slouching occurred around 340 hours of growth, based on the decline in the plate counts. This observation and other specific biofilm parameters will be verified with confocal microscopy.

Benefits to the Industry

A science-based 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. The Excel add-in will indicate the growth rate of the hypothetical biofilms in conditions specific to the packinghouse and allow improved sanitation schedule, to avoid product contamination and to ultimately reduce the presence of *L. monocytogenes*. The add-in will be targeted to reflect specific conditions in the packinghouse. For example, by entering environmental parameters it could predict possible biofilm growth in the cold storage of the packinghouse. Our results seem to suggest that more targeted sanitation practices and EMPs can be used in the stone fruit packinghouse.

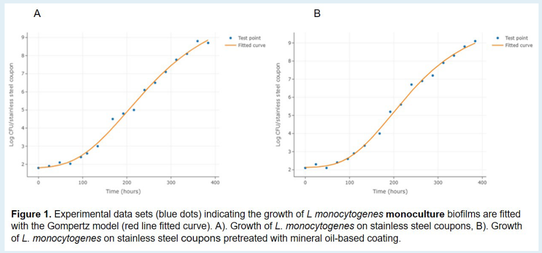

Figure 1. Experimental data sets (blue dots) indicating the growth of *L. monocytogenes* **monoculture** biofilms are fitted with the Gompertz model (red line fitted curve). A). Growth of *L. monocytogenes* on stainless steel coupons, B). Growth of *L. monocytogenes* on stainless steel coupons pretreated with mineral oil-based coating.

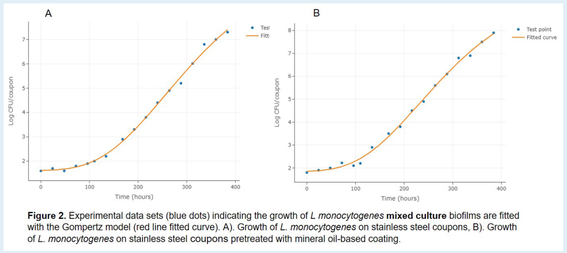

Figure 2. Experimental data sets (blue dots) indicating the growth of *L. monocytogenes* **mixed culture** biofilms are fitted with the Gompertz model (red line fitted curve). A). Growth of *L. monocytogenes* on stainless steel coupons, B). Growth of *L. monocytogenes* on stainless steel coupons pretreated with mineral oil-based coating.

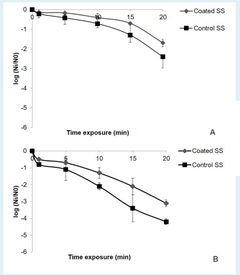

Figure 3. Log reduction of *L monocytogenes* mixed biofilms on stainless steel and PVC coupons in the presence of 100 ppm chlorine (A), and 200 ppm chlorine (B). Data points are means of 3 independent experiments and the vertical bars represent standard deviations.

Table 1. Biofilm model values of maximal growth rate, lag time and model fitness.

Parameters	Monoculture stainless steel	Monoculture stainless steel coated with fruit wax	Mixed biofilm stainless steel	Mixed biofilm stainless steel coated with fruit wax
y0 (logCFU)	1.78	2.01	1.62	1.85
yEnd (logCFU)	9.03	9.16	10.07	8.29
Lag time (hours)	83.92	84.78	116.67	103.91
μmax/h	0.02	0.02	0.05	0.09
Model R2	0.993	0.992	0.996	0.996
Model RMSE	0.49	0.42	0.28	0.31