CENTER for PRODUCE SAFETY

CPS 2020 RFP
FINAL PROJECT REPORT

Project Title
Validation of sanitizer disinfection of wash water in dump tank operation of apple packing lines

Project Period
January 1, 2021 – December 31, 2022 (extended to February 28, 2023)

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Objectives

1. *Assess the efficacies of selected sanitizers to eliminate Listeria monocytogenes in wash water and cross-contamination in a simulated dump tank system.*
2. *Verify the selected sanitizer disinfections in representative commercial apple packing lines.*

Funding for this project was provided partly through the CPS Campaign for Research.

FINAL REPORT

Abstract

The water in dump tanks used for apple packing is commonly reused and recirculated, which leads to high levels of organic matter and a point of possible cross-contamination. This study aimed to compare and validate critical operating parameters for commercially used sanitizers against *L. monocytogenes* in dump tank water, and to further verify their efficacy on multiple apple packing lines. Laboratory studies showed that the efficacy of chlorine against *Listeria* in simulated dump tank water (SDTW) and on apples is affected by its concentration and the level of organic matter present. At 25 ppm free available chlorine (FC) concentration, its effectiveness decreases significantly with an increase in organic load in SDTW. However, at higher concentrations of 50-100 ppm FC, the impact of organic matter on the efficacy of chlorine is reduced, resulting in reductions of approximately 5 $\log_{10}$ CFU/mL in SDTW and approximately 1 $\log_{10}$ CFU/apple in apples. The efficacy of PAA at 40–80 ppm was not affected by organic matter in SDTW during up to 5 min of contact and resulted in reductions of ~6 $\log_{10}$ CFU/mL in SDTW and 1.3–1.8 $\log_{10}$ CFU/apple on apples after 5-min exposure at 1000 and 4000 ppm COD. The study also found that while the application of sanitizers in SDTW reduced the transfer of *L. monocytogenes* from contaminated water to clean apples or from contaminated apples to uninoculated apples or water, it was not able to eliminate cross-contamination. The effectiveness of sanitizer application in commercial dump tanks against *Listeria* were further validated in four commercial packing facilities using *Enterococcus faecium* NRRL B-2354 as a surrogate for *Listeria*. In-plant testing found the effectiveness of chlorine or PAA against the *Listeria* surrogate in commercial dump tanks varied between different facilities. Application of chlorine at 50 ppm FC and 60 ppm PAA in commercial dump tanks resulted in reductions of 1.4–1.6 and 1.6–1.8 $\log_{10}$ CFU/apple of the *Listeria* surrogate on inoculated fruits, respectively. However, the application of chlorine and PAA reduced cross-contamination but could not completely prevent it, and there was bacterial transfer of 2.3–2.6 and 2.3–2.5 $\log_{10}$ CFU/apple to uninoculated fruits, respectively. This study provides practical information for the industry on the effectiveness of chlorine and PAA against *L. monocytogenes* in dump tank water systems. The study also emphasizes the importance of monitoring water quality and maintaining effective levels of sanitizers to ensure the microbial safety of apples during postharvest processing.

Background

Apples are a crucial global commodity, particularly in Washington (WA). Recent *Listeria monocytogenes* outbreaks in various fruits, including cantaloupes (CDC, 2012), caramel apples (FDA, 2014), and stone fruits (Chen et al., 2016) and an increasing number of recalls of fresh apple related to *L. monocytogenes* highlight the importance of controlling this pathogen in apples. During processing in commercial apple packing lines, fresh apples are sorted, washed, and packed for further distribution and marketing. Pathogenic microorganisms, including *L. monocytogenes*, can be introduced to apples at any stage, but the packing environment is generally considered a significant source.

The dump tank and flume systems, which transport apples from harvest bins to the packing line, are the first points of contact for fresh apples and have an average contact time of 2.0 minutes, ranging from 0.5 to 5.0 minutes, depending on the dimensions and practices of a particular packing facility. Water in the dump system is commonly recirculated and reused. As a result, water in dump tanks is high in organic matter (Zhou, Luo, Turner, Wang, & Schneider,

2014) and can become a point of cross-contamination for foodborne pathogens, posing a safety risk to consumers. Once contaminated, *L. monocytogenes* is difficult to eradicate from fresh apples in subsequent antimicrobial interventions (Shen et al., 2019) and can persist on apple surfaces for a long time (Sheng, Edwards, Tsai, Hanrahan, & Zhu, 2017). Proper management of postharvest water sanitation has been widely emphasized given prominent outbreaks involving *L. monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* linked to various produce including mangoes (Beatty, LaPorte, Phan, Van Duyne, & Braden, 2004; Sivapalasingam et al., 2003), cantaloupe (FDA, 2011, 2013), leafy greens (FDA, 2019), and others (Laughlin et al., 2019).

Apple packers rely on wash water disinfectants to enhance product safety. Chlorine, a strong oxidant, is the most used sanitizer in the dump tank and flume system due to its low cost and minimal impact on nutrition and quality of produce. However, its effectiveness varies dramatically depending on process water conditions, such as pH and organic load (Beuchat, Nail, Adler, & Clavero, 1998; Francis et al., 2012). Peroxyacetic acid (PAA) is another commonly used sanitizer in the fresh produce industry, approved by FDA for use on fresh produce at a maximum concentration of 80 ppm without a further rinse (FDA, 2020). Its antimicrobial efficacies are minimally influenced by the organic load at previously tested conditions (Davidson, Kaminski-Davidson, & Ryser, 2017; Gonzalez, Luo, Ruiz-Cruz, & Mcevoy, 2004; Mathew, Muyyarikkandy, Bedell, & Amalaradjou, 2018). However, the level of organic matter in the dump tank and flume section is highly variable depending on the source of fruit, time of processing, and water management practices. The level of chemical oxygen demand (COD), an indicator of level of organic matter in water, can be as high as near 4000 ppm. There is limited scientific data documenting the effectiveness of current water disinfection preventive controls in reducing human pathogens, particularly for *L. monocytogenes*, under commercial practices. Moreover, there is a lack of information on whether the current commonly used chemical sanitizers, chlorine and PAA, in dump tank water will achieve the desired microbial reduction under commercial practices.

The overall objective of this study was to comparatively assess and validate critical operating parameters for commercially used sanitizers against foodborne pathogens in dump tank water, thus cross-contamination, and to further verify this efficacy on multiple apple packing lines.

Research Methods and Results

Objective 1. Assess efficacies of selected sanitizers to eliminate *L. monocytogenes* in wash water and cross-contamination in a simulated dump tank system

Methods

1. Apple cultivar selection

Granny Smith apples were selected for the studies due to their involvement in recent apple *L. monocytogenes* outbreak and recalls.

2. Dump tank water simulation

Simulated dump tank water (SDTW) was created using a mixture of apple exudates, decayed apple tissues, and soil samples from an apple orchard in Yakima, WA, following our published method (Sheng, Shen, Su, et al., 2020). The levels of organic matter in the SDTW were determined by measuring chemical oxygen demand (COD) using the standard method (APHA, 1992). In brief, samples were digested in HR+ COD digestion vials (HACH, Loveland, CO) at 150°C for 2 h using a digital reactor block (DRB200, HACH) and then measured with a colorimeter (DR900, HACH). COD levels in apple process can range from 20 to 3900 ppm (Mundi et al.,

2017). To represent typical and worst-case scenarios of organic matter levels encountered in an apple packing facility, COD levels in the treatment solutions were adjusted to 1000 ppm and 4000 ppm, respectively, using tap water before the experiment.

3. Bacterial strains

Three strains of *L. monocytogenes*, including NRRL B-57618, NRRL B-33053, and NRRL B-33466, were obtained from USDA-ARS culture collections and were stored in trypticase soy broth (BD, Sparks, MD) with 0.6% yeast extract (Fisher Scientific, Fair Lawn, NJ) (TSBYE) and 20% glycerol at -80°C.

4. Inoculum preparation

Before inoculation, each strain was synchronized twice in growth phase by culturing in TSBYE broth at 37°C for 24 h, then pelletized by centrifugation. The resulting pellets were then re-suspended in 0.1% peptone water to achieve the target population density. To prepare a 3-strain *L. monocytogenes* inoculum cocktail, equal volumes of each respective strain suspension were mixed.

5. Inoculation of water and apples

Inoculation of water: Water containing different levels of organic matter (1000 ppm or 4000 ppm COD) was inoculated with the above prepared 3-strain *L. monocytogenes* cocktail to achieve a final inoculation level of 1×10^9 CFU/ml of *L. monocytogenes*.

Inoculation of apples: Non-waxed Granny Smith apples at commercial maturity, without cuts or bruises, were obtained from industry cooperators. Each apple was individually and separately inoculated with 1×10^6 CFU/apple of 3-strain cocktail of *L. monocytogenes* per our established methods (Shen et al., 2019; Sheng et al., 2017; Sheng, Shen, & Zhu, 2020). The inoculated apples underwent sanitizer treatment 48 h after inoculation.

6. Antimicrobial sanitizer disinfection of inoculated apples

Chlorine was prepared using Accu-Tab (Pace International, Wapato, WA) in tap water (0 ppm COD) or SDTW with 1000 ppm COD to achieve initial free available chlorine (FC) of 25, 50, and 100 ppm. The pH of each chlorine solution was adjusted to 6.8. The FC concentrations were confirmed using the Taylor K-2006 test kit (Taylor Water Technologies LLC, Sparks, MD) immediately after preparation. Chlorine solutions with initial FCs of 50 and 100 ppm in combination with 250 ppm Nature's Shield 440-BF (NS440; Pace International) was prepared per the manufacturer's instruction. PAA was prepared with Bioside HS (EnviroTech, Modesto, CA) in tap water or SDTW at an initial level of 10, 20, 40, 60, and 80 ppm. The peracetic acid (PAA) concentration was confirmed using the PAA titration kit immediately after preparation (AquaPhoenix Scientific, Hanover, PA). The stability of chlorine and PAA in SDTW was monitored for up to 30 min. For each intervention treatment, 10 inoculated Granny Smith apples were submerged in 3 L of respective sanitizer solution for 2- or 5-min. Sanitizer solutions prepared in tap water were used as reference values to assess the efficacy of each sanitizer at stable concentrations. A tap water wash was used as a negative control. Immediately after treatment, each apple was individually transferred to a sterile stomacher bag (Fisher Scientific) for survival analysis. Each experiment was repeated independently three times.

7. Antimicrobial sanitizer disinfection of water

The inoculated SDTW with 1000 ppm or 4000 ppm COD was subjected to chlorine (25, 50 and 100 ppm FC) or PAA (20, 40, 60 and 80ppm) for different durations (0, 0.5, 2, and 5 min). In addition, the strengthening effects of the GRAS sanitizer, octanoic acid (caprylic acid, Sigma-Aldrich, St. Louis, MO) at 800 ppm, in the efficacy of 20 ppm PAA were further tested in SDTW

with 0 or 1000 ppm COD. The experiment was conducted with 4 replicates per sanitizer treatment for the selected COD level and treatment duration, and all studies were repeated at least three times independently.

8. Evaluation of cross-contamination

Water-to-apple contamination: Uninoculated, unwaxed apples were immersed in the SDTW (1000 ppm COD) spiked with ~10^6 CFU/ml of the 3-strain *L. monocytogenes* cocktail and treated with the selected sanitizers for 2 min. SDTW without any sanitizer treatment served as a control. After washing, 12 apples per sanitizer treatment were sampled for survival analysis. In addition, the number of *L. monocytogenes* survivors in the sanitizer treated SDTW was determined by analyzing four water samples per treatment. All studies were repeated three times independently.

Apple-to-water and apple-to-apple contamination: Both inoculated and uninoculated apples (48h post-inoculation/post-wash) were introduced to SDTW (1000 ppm COD) treated with the selected sanitizers for a maximum of 5 min. At each time point (0, 2, and 5 minutes), process water was collected from the treatment tank (four samples per sanitizer and contact time combination) for survival analysis. In addition, 12 apples (10 uninoculated and 2 inoculated) were sampled for survival analysis at each time point and sanitizer treatment. Apples that were not treated with sanitizer were used as a control. The experiments were conducted independently three times.

9. Analysis of microbial survival

To determine the population of *L. monocytogenes* on apples, each apple was placed in a stomacher bag with 10 mL of neutralizing buffer and hand-rubbed for 80 sec. The detached bacterial suspension was serially diluted and plated onto duplicate TSAYE (TSBYE with 1.5% agar) plates overlaid with Modified Oxford agar (MOX: BD) and incubated at 37°C for 48 h. For uninoculated apples, 1 mL of bacterial suspension was also enriched in 9 mL of buffered *Listeria* enrichment broth (BLEB; BD) at 30°C for 48 h, and the presumable *L. monocytogenes* colonies were streaked onto CHROMagar™ Listeria (DRG International Inc., Springfield, NJ) plates for confirmation. The residual *L. monocytogenes* populations in spent sanitizer solutions were evaluated by both direct plating and membrane filtration methods according to our published method (Sheng, Shen, Ulloa, et al., 2020).

10. Statistical analysis:

The data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, utilizing IBM SPSS software (Chicago, IL). Statistical significance was set at a P-value of less than or equal to 0.05. The data were reported as mean ± standard error of the mean (SEM).

Results

1. Effectiveness of chlorine and PAA solutions against *L. monocytogenes* in SDTW at different organic loads

Initially, SDTW was prepared using decayed apples, apple exudates, and soil from an apple orchard, all of which can contribute to high levels of chemical oxygen demand (COD) in commercial dump tank water. The COD level of the prepared SDTW showed a strong correlation with other water quality parameters such as turbidity, biological oxygen demand (BOD), conductivity, and total suspended solids.

The efficacy of chlorine against *L. monocytogenes* in SDTW with or without organic matter were concentration-dependent and decreased with increasing organic matter levels (**Fig. 1**). After 5 minutes of contact, 25, 50, and 100 ppm chlorine in tap water led to reductions of 2.1, 5.7, and

6.5 $\log_{10}$ CFU/ml, respectively, while in 1000 ppm COD, the reductions were 0.8, 4.9, and 5.8 $\log_{10}$ CFU/ml, and in 4000 ppm COD, the reductions were 0.5, 4.1, and 5.1 $\log_{10}$ CFU/ml (Fig. 1). Significantly higher survival rates of *L. monocytogenes* were observed in SDTW with 4000 ppm COD compared to tap water (0 ppm COD) ($P < 0.05$), regardless of chlorine concentration (Fig. 1).

The efficacy of PAA against *L. monocytogenes* was unaffected by organic matter at a contact time of up to 5 minutes, regardless of COD levels in SDTW (as shown in **Fig. 2**). Higher concentrations of PAA led to rapid inactivation of *L. monocytogenes*, with 60 and 80 ppm PAA resulting in reductions of 5.8-6.0 $\log_{10}$ CFU/ml in SDTW with or without organic matter after 2 minutes of contact (**Fig. 2C, D**). In SDTW with 1000 ppm COD, 40 ppm PAA exhibited comparable efficacy to 100 ppm chlorine, with a 5-minute contact resulting in reductions of 5.8-5.9 $\log_{10}$ CFU/ml of *L. monocytogenes* regardless of COD levels (**Fig. 2B**). Exposure to 20 ppm PAA for 5 minutes led to reductions of 3.1-3.2 $\log_{10}$ CFU/ml of *L. monocytogenes* in SDTW (**Fig. 2A**).

The addition of 800 ppm octanoic acid (OA) in 20 ppm PAA solution had a synergistic effect against *L. monocytogenes* in both tap water and SDTW with 1000 ppm COD (**Fig. 3**). However, the extent of the increase may not justify the use of OA in PAA intervention due to the additional cost incurred.

2. The efficacies of chlorine and PAA against *L. monocytogenes* on apples in SDTW

The effectiveness of chlorine against *L. monocytogenes* was significantly affected by the presence of organic matter when the initial free chlorine (FC) concentrations were between 25-100 ppm, especially at an initial FC of 25 ppm (**Fig. 4**), which was consistent with the decrease in FC in SDTW (**Table 1**). A 2-minute exposure of 25 ppm FC chlorine led to 0.9 and 0.3 $\log_{10}$ CFU/apple of *L. monocytogenes* in water without and with 1000 ppm COD, respectively, which was correlated to the rapid depletion of FC in SDTW (Table 1). However, chlorine at initial FCs of 50-100 ppm was less affected by organic matter, reducing *L. monocytogenes* by approximately 1.0-1.1 and 0.9 $\log_{10}$ CFU/apple on apples in tap water and SDTW with 1000 ppm COD, respectively. Increasing the contact time from 2 to 5 min did not improve the antimicrobial efficacy of chlorine in water with or without organic matter (Fig. 4). Nature's Shield 440, an organic citrus acid buffer, is often used with chlorine during commercial apple packing. However, adding 250 ppm Nature's Shield 440 did not enhance the effectiveness of chlorine in SDTW (**Fig. 5**).

The efficacy of PAA against *L. monocytogenes* on apples in SDTW was not influenced by organic matter at concentrations of 20-80 ppm and contact times up to 5 min ($P > 0.05$) (**Fig. 6**). This result is consistent with the stable concentrations of PAA during a 5-min contact period, as shown in **Table 2**. In clean water or SDTW with 1000 ppm COD, a 2-min exposure to PAA at concentrations of 20, 40, 60, and 80 ppm resulted in reductions of *L. monocytogenes* on apples by 1.2, 1.3, 1.4-1.5, and 1.7 $\log_{10}$ CFU/apple, respectively.

However, the efficacy of PAA at a concentration of 10 ppm was significantly reduced in SDTW with 1000 ppm COD compared to that in clean water (Fig. 6), which is consistent with dramatic decrease in PAA concentration in the solution (Table 2). Increasing contact time from 2 min to 5 min only slightly increased the efficacy of PAA (Fig. 6).

3. Effectiveness of sanitizer solutions in controlling *L. monocytogenes* cross-contamination in SDTW

The use of chlorine at concentrations up to 100 ppm and PAA at concentrations up to 80 ppm did not completely eliminate the cross-contamination of *L. monocytogenes* in SDTW during 2 or 5-min exposure, but reduced the transfer of bacteria from contaminated SDTW to apples and decreased the level of *L. monocytogenes* level in the wash solution (**Table 3**). When no sanitizer was used, a 2-min exposure to contaminated SDTW resulted in a high attachment of *L. monocytogenes* to apples (6.3 $\log_{10}$ CFU/apple). Chlorine at 25 ppm FC was the least effective

sanitizer, but still led to a reduction of 2.2 $\log_{10}$ CFU/apple on apple surfaces and a reduction of 1.4 $\log_{10}$ CFU/ml in SDTW compared to SDTW with no sanitizers. Increasing the concentration of chlorine and PAA resulted in a lower recovery of *L. monocytogenes* on apple surfaces and in spent washed solutions.

Similarly, during 2 or 5-min exposure in SDTW, *L. monocytogenes* can be transferred from the inoculated apples to uninoculated apples and SDTW by 3.6-3.7 $\log_{10}$ CFU/apple and 3.5 $\log_{10}$ CFU/ml, respectively (**Tables 4 and 5**). The use of sanitizer, regardless of type and initial concentration, reduced but did not eliminate the transfer of *L. monocytogenes* to uninoculated apples and SDTW, regardless of contact time. The cross-contamination of *L. monocytogenes* decreased with the increased sanitizer concentration.

The data from the study indicate that controlling cross-contamination from wash water to produce surfaces is more challenging than controlling foodborne pathogens in the process water itself. Therefore, it is highly recommended for the apple industry to treat the SDTW before packing to reduce the risk of contamination. It should be noted that a high level of *L. monocytogenes* was used in the study to demonstrate log reduction.

Objective 2. Verify the selected sanitizer disinfections in representative commercial apple packing lines

Methods

1. Apple cultivar selection

Granny Smith apples were used for the pilot dump tank testing. For commercial tank testing, Gala apples were selected in addition to Granny Smith apples.

2. Bacterial strain

Enterococcus faecium NRRL B-2354 carrying rifampicin resistance was used in Objective 2; this strain was obtained from Dr. Trevor Suslow's lab at University of California, Davis, and stored at -80°C (Shen et al., 2020).

3. Apple inoculation

The inoculation was conducted as described in the Objective 1 studies (Su, Shen, Chiu, Green, & Zhu, 2022).

4. Effectiveness of sanitizers in reducing bacteria cross-contamination in the pilot-scale dump tank system.

Dump tank water: The study collected water from the dump tank of the chosen apple packing facility towards the end of its usage life and used it for sanitizer intervention in the pilot dump tank (**Fig. 7**).

Figure 7. Image of the pilot dump tank system

Sanitizer treatment: Chlorine was tested at concentration of 25, 50, and 100 ppm FC, while PAA was tested at concentrations of 40, 60, 80 ppm. The effectiveness of the selected sanitizer treatments in preventing bacterial transfer from apple to apple, apple to process water, and water to apples were evaluated using the pilot dump tank (Fig. 7) in ~102 L of commercial dump tank water.

Apple-to-apple (water) cross-contamination: Ten inoculated apples were introduced along with 40 uninoculated apples and exposed to each sanitizer treatment for either 2 or 5 minutes. At both time points, five inoculated apples and 20 uninoculated apples were sampled. Process water was further collected from the treatment tank (three samples per sanitizer and contact time combination) at each time point for survival analysis.

5. In-plant testing in four commercial apple packing facilities in Washington State

Commercial packing facilities selected in the study: Four commercial apple packing facilities (Facility A-D) were selected for in-plant testing. Each facility had a dump tank water system followed by a pre-sorting roller table where the apples that passed through the dump tank were collected. The validation was conducted in each facility at the end of the dump tank water's usage life, just before disposal, representing the worst-case scenario with the highest level of organic matter or COD level.

Sanitizer treatment in commercial dump tank: Each packing facility was tested with both chlorine at 50 ppm FC and PAA at 60 ppm in separate visits. The FC levels and PAA concentrations in processing water were measured using the FAS-DPD chlorine test kit (Taylor Water Technologies LLC) and PAA titration kit (Aquaphoenix Scientific), respectively.

Apple processing in the packinghouse: For each sanitizer test at each packing facility, both Granny Smith and Gala apples were tested. In each test, a half bin of apples of the selected variety, consisting of 200 inoculated apples and 800 uninoculated apples, was introduced into the commercial dump tank for dwell times of 2 and 5 minutes. The treated inoculated and uninoculated apples were then collected at the pre-sorting table (**Fig. 8**). Granny Smith and Gala apples were tested separately in the dump tank in all facilities except for one facility where Granny Smith and Gala apples were placed in the same bin and then run through the dump tank together. At each time point, ~100 inoculated apples and ~400 uninoculated apples were collected at the pre-sorting tables. Apples collected at each sampling point were individually placed into Whirl-Pak bags, immediately rinsed with 10 ml of neutralizer solution to stop the sanitizer action, and then placed into a cooler.

Figure 8. Apple sampling at pre-sorting table

Water sampling during in-plant test: At each facility, water samples were collected from the dump tank at three different locations for microbial analysis and measurement of physicochemical parameters, with two samples taken per location, for a selected sanitizer treatment and contact time. The water collected was neutralized with a sodium thiosulfate solution to immediately stop the sanitizer action after treatment. Water samples before sanitizer intervention were also taken at each facility.

Water physicochemical parameters: COD was assayed as described in Objective 1. Biological oxygen demand (BOD) was measured following the Hach method 8043 (Hach, 2017). Turbidity was measured with the DR-900 colorimeter, conductivity was measured with the Orion Star A212

Conductivity meter (Thermo Scientific), pH was measured with the Orion 8302BNUMD Ross Ultra pH/ATC Triode (Thermo Scientific), and ORP was measured with the Orion 9678BNWP electrode (Thermo Scientific); all connected to the Orion Versa Star Pro electrochemistry meter. The Hardness and alkalinity of water samples were measured with Total Hardness test kit (Hach) and Total Alkalinity DRT kit (Lamotte, Chestertown, MD), respectively. Total suspended solid (TSS) of water samples was measured following the Hach Method 8158 (Hach, 2015) using Grade 691 Glass Microfibre filters (VWR International LLC, Radnor, PA) and a 47 mm Sterifil Aseptic filter system (Sigma-Aldrich). The water temperature was measured with a Model.9847N digital thermometer (Taylor Water Technologies LLC).

Survival of *E. faecium* analysis: The apple and water samples were chilled and transported to the microbiology laboratories for quantitative and qualitative (if below LOD) analysis of *E. faecium* NRRL B-2354 on water/apple samples, following our established methods (Shen et al., 2020). Microbiological analyses were performed on all samples within 20 hours of collection.

To enumerate *E. faecium* NRRL B-2354 on inoculated apple surfaces, the appropriate dilution of the detached microbial suspension was plated onto TSAYE plates containing 40 µg/ml of rifampicin (TSAYE+Rif). For uninoculated apples, the detached bacterial suspension of four apples was pooled, and 1.0 ml of the pooled suspension was plated onto three TSAYE+Rif plates for quantitative enumeration or enriched in enterococcosel broth for 24h for qualitative detection of cross-contamination. Enrichment-positive enterococci were streaked onto enterococcosel broth supplemented with 1.5% agar (EA) and TSAYE+Rif plates, and further confirmed with PCR targeting the vanB gene (Dutkamalen, Evers, & Courvalin, 1995; Jayaratne & Rutherford, 1999).

6. Statistical analysis

The data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, utilizing IBM SPSS software (Chicago, IL). Statistical significance was set at a P-value of less than or equal to 0.05. The data were reported as mean ± standard error of the mean (SEM).

Results

1. Effectiveness of chlorine and PAA in controlling *E. faecium* NRRL B-2354 cross-contamination in the pilot dump tank system using commercial dump tank water

The use of chlorine in dump tank water reduced *E. faecium* counts on the inoculated Granny Smith apples as well as its transfer from inoculated apples to uninoculated apples and dump tank water (**Fig. 9**). Chlorine at 25, 50, and 100 ppm FC for 2 min contact reduced *E. faecium* on inoculated apples by 0.93, 1.2, and 1.4 $\log_{10}$ CFU/apple, respectively (**Fig. 9A**). During 2 or 5-min exposure in the pilot dump tank, *E. faecium* was found to transfer from the inoculated apples to uninoculated apples by 1.1-2.0 $\log_{10}$ CFU/apple (**Fig. 9B**), with no significant difference observed between different chlorine concentrations. Low counts of *E. faecium* were detected in spent dump tank water treated with 25 ppm FC and 50 ppm FC. *E. faecium* in dump tank water treated with 100 ppm FC was below the limit of detection (**Fig. 9C**).

Similarly, the use of PAA in dump tank water reduced *E. faecium* counts on the inoculated Granny Smith apples as well as its transfer from inoculated apples to uninoculated apples and dump tank water (**Fig. 10**). PAA at 40, 60, and 80 ppm for 2-min contact reduced *E. faecium* on inoculated apples by 1.4, 1.7, and 2.2 $\log_{10}$ CFU/apple, respectively (**Fig. 10A**). During 2 or 5-min exposure in the pilot dump tank, *E. faecium* was found to transfer from the inoculated apples to uninoculated apples by 1.3-1.9 $\log_{10}$ CFU/apple (**Fig. 10B**). Low counts of *E. faecium* were detected in spent dump tank water treated with up to 80 ppm PAA (**Fig. 10C**).

Based on the pilot tank results, chlorine at 50 ppm FC and 60 ppm PAA were selected for in-plant testing in the commercial packing facilities.

2. Effectiveness of chlorine and PAA in controlling *E. faecium* NRRL B-2354 cross-contamination in the commercial dump tank

Physicochemical parameters of the commercial dump tank water of four packing facilities are listed in **Table 6**. The COD of dump tank water varied across the facilities, ranging from 265 ppm to 2580 ppm. The log reduction of *E. faecium* on Granny Smith and Gala apples in chlorinated dump tank water varied among facilities (**Fig. 11A and C**), with no significant difference between the 2-min and 5-min contact times. On average, the reduction in *E. faecium* on Granny Smith and Gala apples was similar, with a mean reduction of approximately 1.4 $\log_{10}$ CFU/apple on Granny Smith apples and 1.4-1.6 $\log_{10}$ CFU/apple on Gala apples (**Fig. 11B and D**). The transfer of *E. faecium* from inoculated to uninoculated apples varied across facilities, regardless of the apple variety (**Fig. 12A and C**). On average, there were 2.3-2.6 $\log_{10}$ CFU/apple transferred to Granny Smith apples during 2 or 5-min contact, and ~2.6 $\log_{10}$ CFU/apple transferred to Gala apples during same period (**Fig. 12B and D**). **Table 7** presents the result of *E. faecium* detection in the dump tank water. Despite the typical low levels of *E. faecium* in chlorinated water with up to 0.74 $\log_{10}$ CFU/250ml, all four facilities tested positive for *E. faecium* (Table 7).

Table 8 lists the physicochemical parameters of commercial dump tank water from four packing facilities used for PAA testing. The COD of dump tank water of the four participating facilities varied, ranging from 993 ppm to 2497 ppm. The log reduction of *E. faecium* on Granny Smith and Gala apples in PAA-treated dump tank water varied among facilities (**Fig. 13A and C**), with no significant difference in the reduction between the 2 or 5-min contact times. On average, the reduction in *E. faecium* on Granny Smith and Gala apples was similar, with a mean reduction of approximately 1.6-1.7 $\log_{10}$ CFU/apple on both Granny Smith and Gala apples (**Fig. 13B and D**). The transfer of *E. faecium* from inoculated to uninoculated apples varied across facilities, regardless of the apple variety (**Fig. 14A and C**). On average, ~2.3 $\log_{10}$ CFU/apple transferred to Granny Smith apples during 2 or 5-min contact, and ~2.5 $\log_{10}$ CFU/apple transferred to Gala apples during the same period (**Fig. 14B and D**). Table 9 shows *E. faecium* detection result in the dump tank water. Similar to the chlorine intervention, low levels of *E. faecium* were detected in 60 ppm PAA-treated dump tank water, ranging from 0.21 $\log_{10}$ CFU/250ml to 0.51 $\log_{10}$ CFU/250ml, and the water samples from all four facilities tested positive for *E. faecium* (**Table 9**).

Outcomes and Accomplishments

Through extensive laboratory testing and in-plant testing in commercial facilities, this study demonstrated that *L. monocytogenes* can easily spread from contaminated apples to wash water and uninoculated apples, as well as from contaminated process water to apples. The laboratory studies revealed that sanitizer treatment in process water can reduce *L. monocytogenes* in wash water and on apple surfaces, and limit bacterial transfer but cannot eliminate cross-contamination. The findings from lab testing were further validated in four representative commercial packing facilities using *E. faecium* NRRL B-2354 as a surrogate of *L. monocytogenes*.

The findings provide valuable information on the effectiveness of chlorine and PAA against *L. monocytogenes* in dump tank water systems and emphasize the importance of monitoring water quality and maintaining effective levels of sanitizers to ensure the microbial safety of apples during postharvest processing. Furthermore, the study offers data-driven guidelines for apple packers on water change intervals in the dump tank system based on the correlation between physicochemical parameters of the water and antimicrobial efficacy of the selected sanitizers.

These findings offer important reference points for the apple industry to validate or verify process controls in compliance with industry expectations and federal preventive control requirements. Also, the study establishes baseline parameters for an alternative intervention method for the apple industry to improve antimicrobial efficacy against foodborne pathogens.

Summary of Findings and Recommendations

The study revealed that *L. monocytogenes* can be easily spread from contaminated apples to wash water and uninoculated apples, and from contaminated process water to apples. The use of PAA and chlorine in process water can reduce *L. monocytogenes* in wash water and on apple surfaces and limit bacterial transfer but they cannot eliminate cross-contamination. Chlorine efficacy was highly dependent on levels of organic matter. In addition, the effectiveness of chlorine or PAA against the *Listeria* surrogate in commercial dump tanks varied among different facilities. While application of chlorine at 50 ppm FC and 60 ppm PAA in commercial dump tanks resulted in reductions of 1.4-1.6 log and 1.6-1.7 log of the *Listeria* surrogate on inoculated fruits, respectively, there was still a 2.3-2.6 log and 2.3-2.5 log bacterial transfer to uninoculated fruits, respectively. Moreover, all dump tank water treated with 50 ppm FC or 60 ppm PAA were positive for the *Listeria* surrogate.

Based on the findings, the apple industry is recommended to treat dump tank water with sanitizers and regularly monitor sanitizer concentrations and COD levels during packing to reduce the risk of *L. monocytogenes* contamination. However, the unique settings of each packing line should be taken into consideration before adopting the results of the study. It is important to implement multiple interventions to reduce the risk of *L. monocytogenes* contamination and ensure the safety of fresh apples.

APPENDICES

Publications and Presentations

Wang, R., X. Shen, Y. Su, F. Critzer, and M. J. Zhu. 2023. Chlorine and peroxyacetic acid inactivation of *Listeria monocytogenes* in simulated apple dump tank water. *Food Control*, 144: 109314.

Su, Y., X. Shen, T. Chiu, T. Green, and M. J. Zhu. 2022. Efficacy of chlorine and peroxyacetic acid to control *Listeria monocytogenes* on apples in process water. *Food Microbiology*, 106: 104033.

Wang, R., X. Shen, F. Critzer, and M. J. Zhu. Efficacies of chlorine and peroxyacetic acid against *Listeria monocytogenes* in simulated apple dump tank water. Presented at the 2022 International Association for Food Protection Annual Meeting, Pittsburgh, PA, July 31-August 3, 2022.

Su, Y., X. Shen, T. Chiu, T. Green, and M. J. Zhu. Efficacy of sanitizer treatments in simulated dump tank water against *Listeria monocytogenes* on apples. Presented at the 2022 International Association for Food Protection Annual Meeting, Pittsburgh, PA, July 31-August 3, 2022.

Budget Summary

This project was awarded $349,269 in grant funds. All funds will be utilized by the end of the project period in the execution of the planned and modified objectives associated with this project.

Tables 1–9 and Figures 1–6, 9–14 (see below)

Tables and Figures

Table 1. Change of free chlorine concentrations in chlorinated SDTW of 3L system

	SDTW (1000 ppm COD)					Tap water
Initial FC	0.5 min	2 min	5 min	10 min	30 min	30 min
25 ppm	18.2 ± 0.6	15.5 ± 0.5	10.2 ± 0.6	8.2 ± 0.6	6.5 ± 0.9	25 ± 0.0
50 ppm	44.7 ± 0.4	41.1 ± 0.6	38.9 ± 0.6	36.9 ± 0.9	30.4 ± 0.4	50 ± 0.0
100 ppm	100.0 ± 0.0	94.7 ± 0.4	90.7 ± 0.4	87.8 ± 0.2	81.3 ± 0.4	100 ± 0.0

Data are presented as Mean ± SEM, n = 3. COD: chemical oxygen demand; FC: free chlorine; SDTW: simulated dump tank water with 1000 ppm COD. Limit of detection for chlorine solution was 0.2 ppm. FC of chlorine solutions prepared with tap water (0 ppm COD) were used as controls.

Table 2. Alteration of PAA concentrations in the PAA-treated SDTW in 3L system

Initial PAA Concentration	SDTW (1000 ppm COD)					Tap water
	0.5 min	2 min	5 min	10 min	30 min	30 min
10 ppm	10.0 ± 0.0	6.3 ± 0.3	2.7 ± 0.7	1.0 ± 0.0	< LOD	10.0 ± 0.0
20 ppm	20.0 ± 0.0	20.0 ± 0.0	16.3 ± 1.3	12.0 ± 1.2	10.0 ± 0.0	20.0 ± 0.0
40 ppm	40.0 ± 0.0	40.0 ± 0.0	38.3 ± 1.7	32.0 ± 1.0	30.7 ± 0.7	40.0 ± 0.0
60 ppm	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0	55.0 ± 0.0	51.7 ± 1.7	60.0 ± 0.0
80 ppm	80.0 ± 0.0	80.0 ± 0.0	80.0 ± 0.0	78.3 ± 1.7	76.7 ± 1.7	80.0 ± 0.0

Data are presented as Mean ± SEM, n = 3. COD: chemical oxygen demand; PAA: peroxyacetic acid; LOD: limit of detection, which is 0.5 ppm of PAA; SDTW: simulated dump tank water with 1000 ppm COD. PAA solution prepared with tap water (0 ppm COD) were used as controls.

Table 3. Transferring of *L. monocytogenes* from sanitizer treated SDTW to apples

Treatment	Recovery on Apples (log_{10} CFU/apple)	Survivors in Wash Solution (log_{10} CFU/ml)
SDTW	6.3 ± 0.0^{a}	6.9 ± 0.1^{a}
25 ppm chlorine	4.1 ± 0.0^{b}	5.5 ± 0.0^{b}
50 ppm chlorine	3.3 ± 0.1^{c}	2.5 ± 0.0^{c}
100 ppm chlorine	2.9 ± 0.1^{de}	1.8 ± 0.1^{d}
20 ppm PAA	3.2 ± 0.1^{c}	2.7 ± 0.1^{c}
40 ppm PAA	3.0 ± 0.1^{cd}	1.9 ± 0.1^{d}
60 ppm PAA	2.7 ± 0.0^{de}	1.5 ± 0.1^{de}
80 ppm PAA	2.6 ± 0.1^{e}	1.4 ± 0.0^{e}
PAA + OA	2.4 ± 0.0^{f}	1.4 ± 0.0^{e}

The initial inoculation level is 6.8 ± 0.0 log_{10} CFU/ml. SDTW: simulated dump tank water with 1000 ppm chemical oxygen demand. Contact time: 2 min. PAA: peroxyacetic acid; PAA+OA: 80 ppm PAA + 800 ppm octanoic acid. Data were reported as Mean ± SEM, averaged from 3 independent studies (12 apples and 4 wash solution samples per treatment per independent study). $^{a\text{-}f}$ mean within a column without the same letter differ significantly ($P < 0.05$).

Table 4. Transfer of *L. monocytogenes* from inoculated apples to uninoculated apples and washing solutions during 2-min wash in SDTW

Treatments (Initial Concentration)	Inoculated apple[1]		Uninoculated apple[2]		Washing solution		
	Survival (log_{10} CFU/apple)	Reduction (log_{10} CFU/apple)	Plating (log_{10} CFU/apple)	Enrichment[3] (+/total)	Plating (log_{10} CFU/ml)	MF (log_{10} CFU/100ml)	Enrichment (+/total)
SDTW	5.98 ± 0.04^{a}	0.17 ± 0.01^{a}	3.62 ± 0.03^{a}	36/36	3.48 ± 0.04^{a}	/	12/12
25 ppm FC	5.92 ± 0.03^{a}	0.25 ± 0.04^{a}	2.46 ± 0.09^{b}	36/36	1.42 ± 0.05^{b}	/	12/12
50 ppm FC	5.49 ± 0.03^{b}	0.75 ± 0.02^{b}	1.65 ± 0.14^{c}	30/36	1.01 ± 0.09^{c}	/	12/12
100 ppm FC	5.28 ± 0.06^{c}	1.01 ± 0.06^{c}	0.56 ± 0.14^{e}	12/36	0.40 ± 0.08^{d}	/	9/12
20 ppm PAA	4.97 ± 0.07^{d}	1.22 ± 0.02^{c}	1.61 ± 0.16^{c}	28/36	1.35 ± 0.05^{b}	/	12/12
40 ppm PAA	4.94 ± 0.02^{d}	1.35 ± 0.03^{d}	1.44 ± 0.15^{c}	27/36	< LOD	1.07 ± 0.12^{a}	11/12
60 ppm PAA	4.82 ± 0.03^{d}	1.48 ± 0.02^{e}	1.00 ± 0.16^{d}	20/36	< LOD	0.89 ± 0.13^{a}	10/12
80 ppm PAA	4.59 ± 0.06^{e}	1.70 ± 0.05^{f}	0.86 ± 0.15^{de}	18/36	< LOD	0.73 ± 0.14^{a}	9/12

[1]Initial *L. monocytogenes* level of apples before cross-contamination was 6.26 ± 0.05 log_{10} CFU/apple. [2]Uninoculated apples were introduced at 12 uninoculated apples to 3 inoculated apples. Studies were repeated independently three times. [3]Enrichment results as positive/total samples tested from three independent experiments. FC: free chlorine; LOD: limit of detection; PAA: peroxyacetic acid; SDTW: simulated dump tank water with 1000 ppm chemical oxygen demand. LOD for apples was 10 CFU/apple. LOD for SDTW solution was 1 CFU/ml for direct plating and 1 CFU/100ml for the membrane filtration method. Means ± SEM, n = 9 for inoculated apples, n = 36 for uninoculated apples, and n = 12 for SDTW samples. [a-f]mean values within a column without a common letter differ significantly ($P < 0.05$).

Table 5. Transfer of *L. monocytogenes* from inoculated apples to uninoculated apples and washing solutions during 5-min wash in SDTW

Treatments (Initial Concentrtion)	Inoculated apple[1]		Uninoculated apple[2]		Washing solution		
	Survival (log_{10} CFU/apple)	**Reduction** (log_{10} CFU/apple)	**Plating** (log_{10} CFU/apple)	**Enrichment[3]** (+/total)	**Plating** (log_{10} CFU/ml)	**MF** (log_{10} CFU/100ml)	**Enrichment** (+/total)
SDTW	6.01 ± 0.04^{a}	0.24 ± 0.05^{e}	3.71 ± 0.02^{a}	/	3.53 ± 0.01	/	/
100 ppm FC	5.13 ± 0.06^{b}	1.13 ± 0.06^{d}	0.56 ± 0.12^{de}	18/36	< LOD	< LOD	2/12
20 ppm PAA	5.02 ± 0.04^{bc}	1.21 ± 0.01^{d}	1.44 ± 0.12^{b}	31/36	1.03 ± 0.12	/	12/12
40 ppm PAA	4.93 ± 0.06^{c}	1.36 ± 0.02^{c}	1.11 ± 0.16^{bc}	24/36	< LOD	0.76 ± 0.07^{a}	11/12
60 ppm PAA	4.80 ± 0.05^{d}	1.48 ± 0.01^{b}	0.84 ± 0.15^{cd}	21/36	< LOD	0.66 ± 0.07^{a}	11/12
80 ppm PAA	4.56 ± 0.07^{e}	1.73 ± 0.03^{a}	0.39 ± 0.12^{e}	13/36	< LOD	0.26 ± 0.08^{b}	8/12

[1]Initial *L. monocytogenes* level of inoculated apples is 6.20-6.30 log_{10} CFU/apple. [2]Uninoculated apples were introduced at 12 uninoculated apples to 3 inoculated apples. Studies were repeated independently three times. [3]Enrichment results as positive/total samples tested from three independent experiments. FC: free chlorine; LOD: limit of detection; PAA: peroxyacetic acid; SDTW: simulated dump tank water with 1000 ppm chemical oxygen demand. LOD of apples was 10 CFU/apple. LOD of SDTW solutions was 1 CFU/ml for direct plating and 1 CFU/100ml for the membrane filtration method. Mean ± SEM, n = 9 for inoculated apples, n = 36 for uninoculated apples, and n = 12 for SDTW samples. [a-e]mean values within a column without a common letter differ significantly ($P < 0.05$).

Table 6. Physicochemical parameters of commercial dump tank water during chlorine testing

Facility	Turbidity (FAU)	Conductivity (μs/cm)	ORP (mV)	Hardness (ppm)	TSS (mg/L)	Temperature (°C)
A	46 ± 2	383.1 ± 2.9	1111.9 ± 6.5	223 ± 8	64.3 ± 7.5	/
B	113 ± 6	549.9 ± 1.0	641.4 ± 0.9	183 ± 28	142.5 ± 18.0	14.4 ± 0.2
C	29 ± 1	746.6 ± 4.1	867.2 ± 10.1	1667 ± 42	42.5 ± 6.9	13.2 ± 0.0
D	92 ± 5	1031.5 ± 11.2	511.1 ± 1.5	403 ± 6	152.5 ± 9.9	18.7 ± 0.0

All measurements were taken during processing. Data were expressed as mean ± standard error mean, n = 3. ORP, oxygen reduction potential; TSS, total suspended solids.

Table 7. Transfer of *E. faecium* from inoculated apples to chlorinated dump tank water

Apple variety	Time	Facilities	Direct plating (log_{10} CFU/ml)	Membrane filtration (Log_{10} CFU/250 ml)	Enrichment (+/total)
GSA	2 min	A	<LOD	/	/
		B	/	/	6/6
		C	<LOD	0.45 ± 0.11	5/6
		D	<LOD	0.26 ± 0.09	4/6
	5 min	A	<LOD	/	/
		B	/	/	6/6
		C	<LOD	0.00 ± 0.00	1/6
		D	<LOD	0.00 ± 0.00	2/6
Gala	2 min	A	<LOD	0.39 ± 0.20	4/6
		B	/	/	6/6
		C	<LOD	0.38 ± 0.16	4/6
		D	<LOD	0.31 ± 0.11	4/6
	5 min	A	<LOD	0.66 ± 0.31	5/6
		B	/	/	6/6
		C	<LOD	0.73 ± 0.17	5/6
		D	<LOD	0.83 ± 0.06	6/6

Chlorine was applied at 50 ppm free chlorine, pH 6.8. Data are reported as Mean ± SEM. Chemical oxygen demand level in the dump tank water varied from 265 ppm to 2580 ppm, and the volume of water in the dump tank ranged from 11,000 – 15,000 liters. Each apple variety was represented by 200 inoculated apples and 800 uninoculated apples per variety. Enumeration data of water sample from facility B data was not included due to the loss of rifamycin activity under hot weather conditions.

Table 8. Physicochemical parameters of commercial dump tank water during peroxyacetic acid testing

Facility	Turbidity (FAU)	Conductivity (µs/cm)	ORP (mV)	Hardness (ppm)	TSS (mg/L)	Temperature (°C)
A	47 ± 1	454.9 ± 2.2	486.7 ± 2.6	245 ± 10	40.9 ± 12.0	/
B	119 ± 5	982.4 ± 4.3	497.7 ± 0.4	450 ± 0	520.0 ± 13.8	13.1 ± 0.2
C	49 ± 4	581.5 ± 4.8	569.0 ± 4.7	1733 ± 42	41.7 ± 2.5	10.0 ± 0.0
D	89 ± 2	378.6 ± 1.6	586.1 ± 9.0	247 ± 7	136.7 ± 4.8	18.7 ± 0.0

All measurements were taken during processing. Data were expressed as mean ± standard error mean, n = 3. ORP, oxygen reduction potential; TSS, total suspended solids.

Table 9. Transfer of *E. faecium* from inoculated apples to peroxyacetic acid treated dump tank water

Apple variety	Time	Facilities	Direct plating (log_{10} CFU/ml)	Membrane filtration (Log_{10} CFU/250 ml	Enrichment (+/total)
GSA	2 min	A	<LOD	/	/
		B	/	/	6/6
		C	<LOD	0.70 ± 0.22	6/6
		D	<LOD	0.33 ± 0.12	4/6
	5 min	A	<LOD	/	/
		B	/	/	6/6
		C	<LOD	0.21 ± 0.21	2/6
		D	<LOD	0.22 ± 0.11	3/6
Gala	2 min	A	<LOD	0.62 ± 0.12	6/6
		B	/	/	6/6
		C	<LOD	0.08 ± 0.08	2/6
		D	<LOD	0.18 ± 0.08	3/6
	5 min	A	<LOD	0.68 ± 0.10	6/6
		B	/	/	6/6
		C	<LOD	0.31 ± 0.20	3/6
		D	<LOD	0.26 ± 0.09	4/6

Peroxyacetic acid was applied at 60 ppm. Membrane filtration data are reported as Mean ± SEM. Chemical oxygen demand level in the dump tank water varied from 993 ppm to 2497 ppm, and the volume of water in the dump tank ranged from 11,000 – 15,000 liters. Each apple variety was represented by 200 inoculated apples and 800 uninoculated apples per variety. Enumeration data of water sample from facility B data was not included due to the loss of rifamycin activity under hot weather conditions.

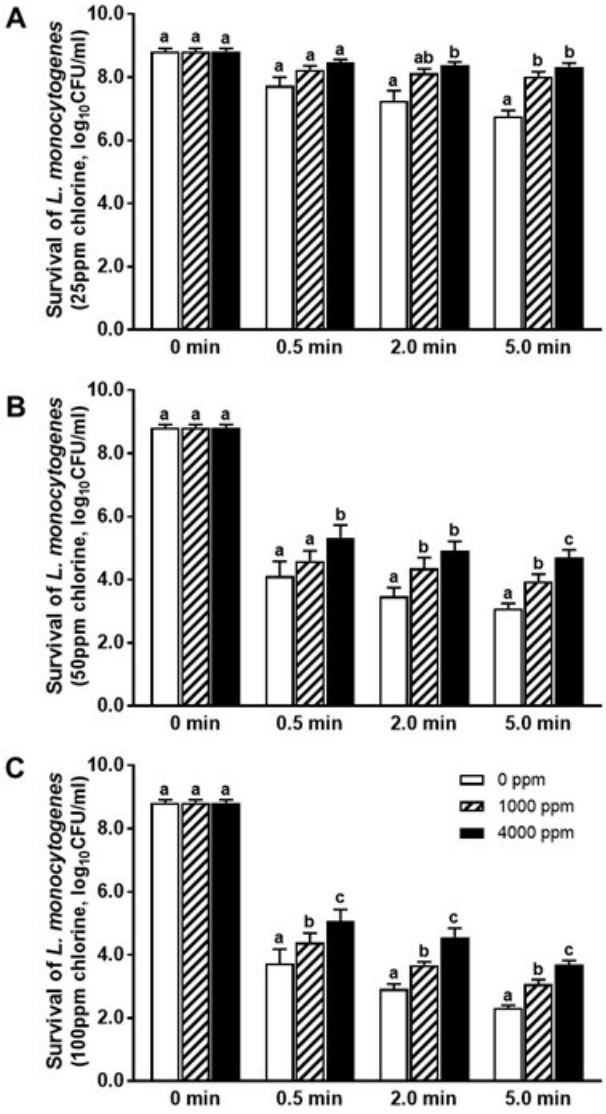


Figure 1. Survival of *L. monocytogenes* in chlorinated simulated dump tank water (SDTW) with different organic loads. (A) 25 ppm, (B) 50 ppm, (C) 100 ppm. 0 ppm: tap water with 0 ppm chemical oxygen demand (COD); 1000 ppm: SDTW with 1000 ppm COD; 4000 ppm: SDTW with 4000 ppm COD. Mean ± SEM, averaged from 6-8 independent studies with 3 replicates per independent study. Histogram bars with the same letter do not differ significantly at a *P*-value of 0.05.

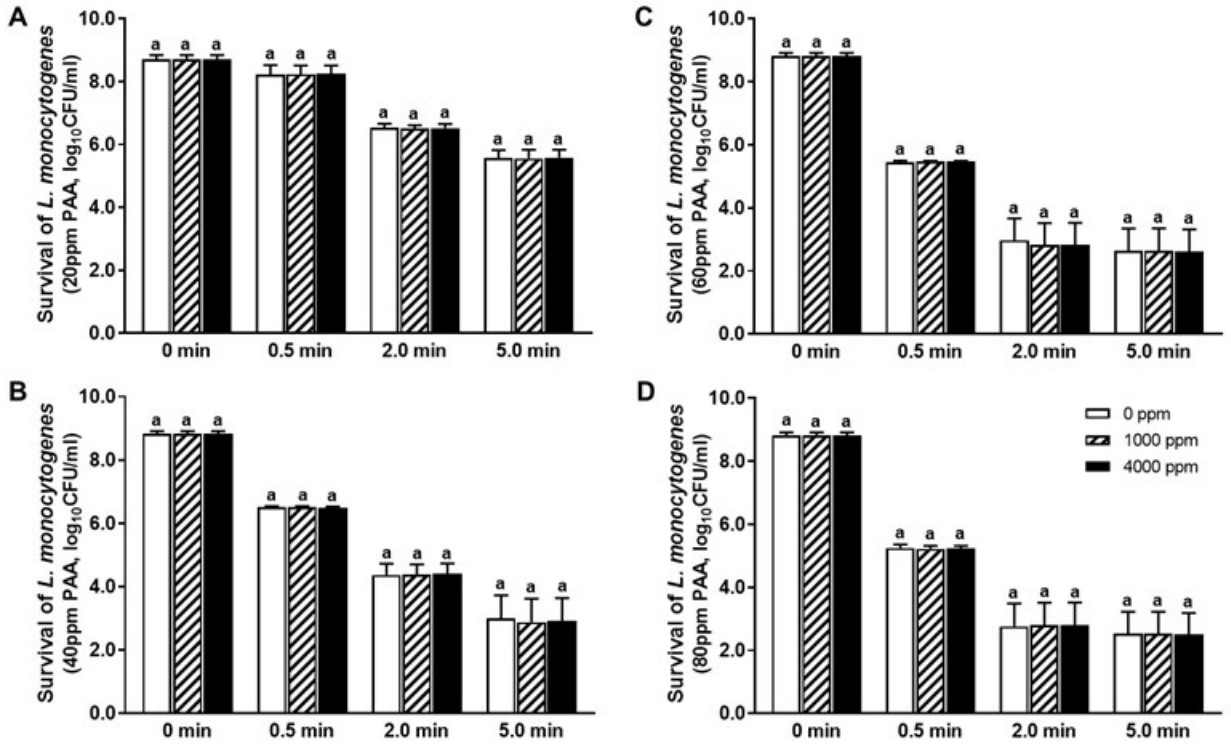


Figure 2. Survival of *L. monocytogenes* in peroxyacetic acid (PAA)-treated simulated dump tank water (SDTW) with different organic loads. (A) 20 ppm, (B) 40 ppm, (C) 60 ppm, and (D) 80 ppm. 0 ppm: tap water with 0 ppm chemical oxygen demand (COD); 1000 ppm: SDTW with 1000 ppm COD; 4000 ppm: SDTW with 4000 ppm COD. Mean ± SEM, averaged from 6-8 independent studies with 3 replicates per independent study. Histogram bars with the same letter do not differ significantly at a *P*-value of 0.05.

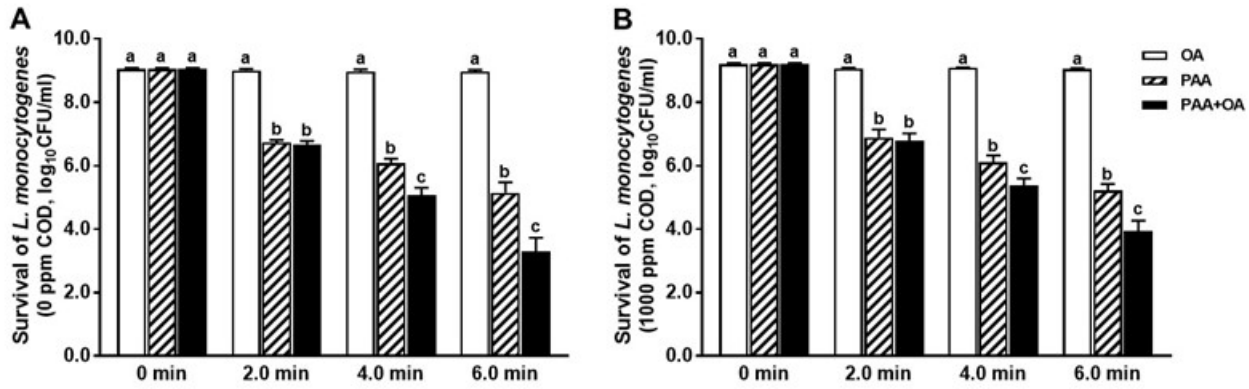
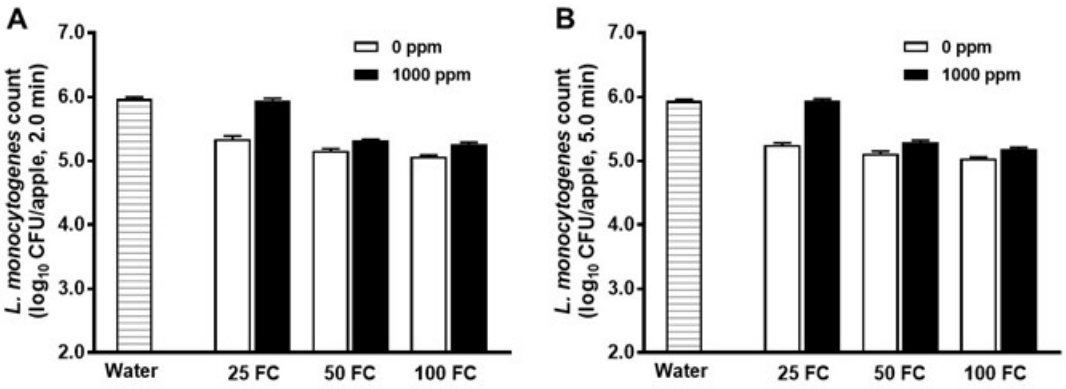


Figure 3. Survival of *L. monocytogenes* in peroxyacetic acid with or without octanoic acid treated simulated dump tank water. (A) Tap water (0 ppm COD), (B) SDTW with 1000 ppm chemical oxygen demand (COD). OA: octanoic acid applied at 800 ppm; PAA: peroxyacetic acid at 20 ppm; PAA+OA: 20 ppm PAA + 800 ppm OA. Mean ± SEM. Experiment was repeated 3 times independently with 3 replicates per time point of each independent study. Histogram bars within each sampling point without common letter differ significantly at a *P*-value of 0.05.



C

COD (ppm)	2.0 min contact			5.0 min contact		
	25 FC	50 FC	100 FC	25 FC	50 FC	100 FC
0	0.88 ± 0.02^{aA}	1.04 ± 0.02^{aB}	1.14 ± 0.02^{aC}	0.97 ± 0.02^{aA}	1.11 ± 0.03^{aB}	1.18 ± 0.03^{aB}
1000	0.25 ± 0.02^{bA}	0.87 ± 0.04^{bB}	0.94 ± 0.03^{bB}	0.28 ± 0.03^{bA}	0.94 ± 0.04^{bB}	1.03 ± 0.03^{bB}

Figure 4. Efficacies of chlorine against *Listeria monocytogenes* on apples in simulated dump tank water with 0 or 1000 ppm chemical oxygen demand (COD). A-B. Representative bar graph of *L. monocytogenes* survival on apples post 2- or 5-min treatment, respectively. C. $\log_{10}$ CFU/apple reduction of *L. monocytogenes* on apples. FC: free chlorine. Data are presented as Mean ± SEM, averaged from three independent studies; there are 10 apples per treatment within each independent experiment. $^{a-b}$ mean values within columns or $^{A-C}$ mean values across rows with different letters denote significant differences ($P \leq 0.05$).

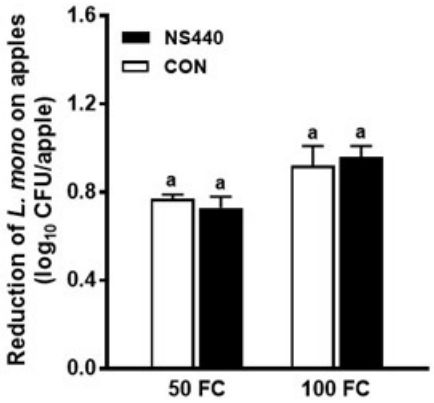
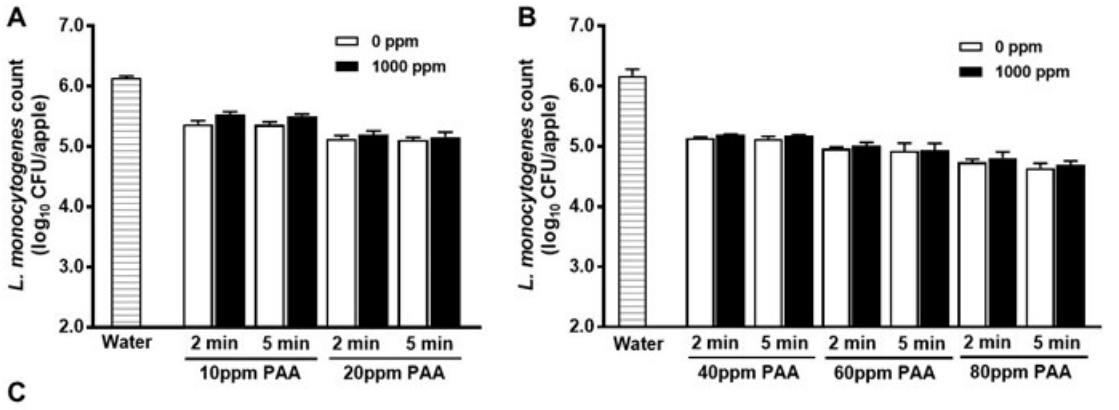


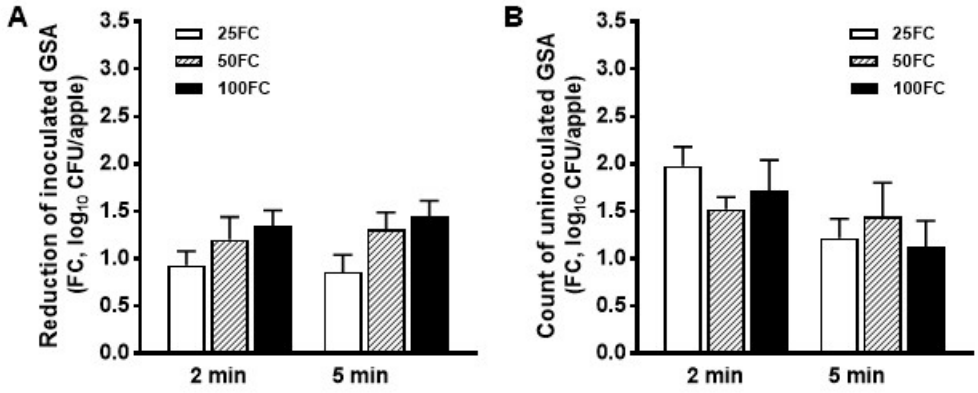
Figure 5. Efficacies of chlorine with or without 250 ppm Nature's Shield 440 against *Listeria monocytogenes* on apples in simulated water with 1000 ppm chemical oxygen demand (COD). NS440: chlorine with 250 ppm Nature's Shield 440; CON: chlorine; FC: free chlorine; *L. mono*: *L. monocytogenes*. Data are presented as $\log_{10}$ CFU/apple reduction averaged from three independent studies; there are 10 apples per treatment within each independent experiment. [a] Histogram bars at respective treatment with common letter did not differ significantly ($P \leq 0.05$).



C

Time/COD	Water	10 ppm PAA	20 ppm PAA	40 ppm PAA	60 ppm PAA	80 ppm PAA
2 min						
0 ppm	0.27 ± 0.04	1.00 ± 0.01^{aA}	1.21 ± 0.01^{aB}	1.30 ± 0.01^{aC}	1.49 ± 0.01^{aD}	1.73 ± 0.03^{aE}
1000 ppm	/	0.83 ± 0.02^{bA}	1.18 ± 0.01^{aB}	1.27 ± 0.01^{aC}	1.42 ± 0.03^{aD}	1.66 ± 0.02^{aE}
5 min						
0 ppm	0.22 ± 0.05	1.07 ± 0.06^{aA}	1.26 ± 0.02^{aB}	1.36 ± 0.01^{aC}	1.51 ± 0.02^{aD}	1.81 ± 0.01^{aE}
1000 ppm	/	0.93 ± 0.06^{bA}	1.23 ± 0.02^{aB}	1.34 ± 0.02^{aC}	1.52 ± 0.04^{aD}	1.82 ± 0.06^{aE}

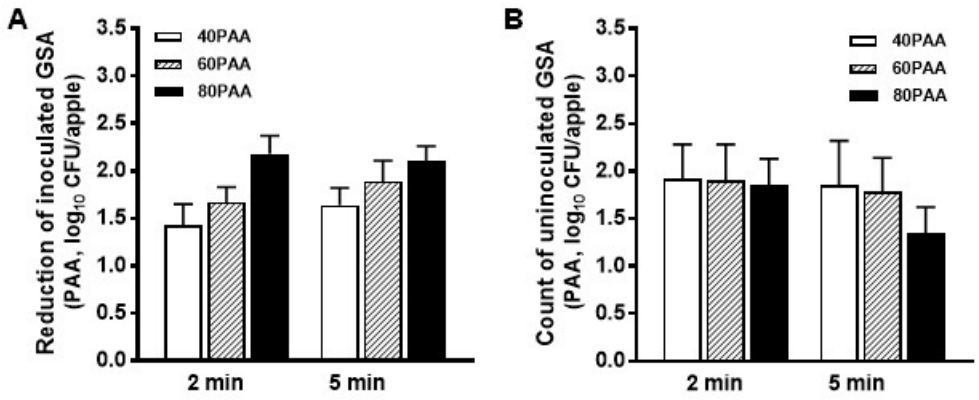
Figure 6. Efficacies of peroxyacetic acid against *Listeria monocytogenes* on apples in simulated dump tank water with 0 or 1000 ppm chemical oxygen demand (COD) A-B. Representative bar graph of *L. monocytogenes* survival on apples after 2-min or 5-min intervention. C. $\log_{10}$ CFU/apple reduction of *L. monocytogenes* on apples. PAA: peroxyacetic acid. Mean ± SEM, averaged from three independent studies; there are 10 apples per treatment within each independent experiment. $^{a-b}$ mean values within columns or $^{A-E}$ mean values across rows with different letters denote significant differences ($P \leq 0.05$).



C Residual *E. faecium* in spent dump tank water (log_{10} CFU/250 ml)

Contact time	25FC	50FC	100FC
2 min	0.12 ± 0.12	< LOD	< LOD
5 min	0.10 ± 0.06	0.15 ± 0.10	< LOD

Figure 9. Effectiveness of chlorine in controlling *E. faecium* NRRL B-2354 cross-contamination from apple to apple and apple to water in the pilot dump tank system using dump tank water from commercial facilities. A. Reduction of *E. faecium* on the inoculated apples after 2- or 5-min treatment, respectively. B. Bacteria transferred to the uninoculated apples after 2- or 5-min treatment. C. Bacteria transferred to dump tank water after 2- or 5-min treatment, enumerated by membrane filtration method. FC: free chlorine. 25FC: 25 ppm free chlorine; 50FC: 50 ppm free chlorine; 100FC: 100 ppm free chlorine; GSA: Granny Smith apples; LOD: limit of detection. Data are presented as Mean ± SEM, averaged from three independent studies, with 20 apples per treatment within each independent experiment. This study utilized dump tank water from a different facility for each independent study. The level of chemical oxygen demand ranged from 260 to 1497 ppm.



C Residual *E. faecium* in spent dump tank water (log_{10} CFU/250 ml)

Contact time	40PAA	60PAA	80PAA
2 min	1.04 ± 0.09	0.46 ± 0.21	0.30 ± 0.14
5 min	0.60 ± 0.27	0.27 ± 0.14	0.13 ± 0.09

Figure 10. Effectiveness of peroxyacetic acid (PAA) in controlling *E. faecium* NRRL B-2354 cross-contamination from apple to apple in the pilot dump tank system using dump tank water from commercial facilities. A. Reduction of *E. faecium* on the inoculated apples after 2- or 5-min treatment, respectively. B. Bacteria transferred to the uninoculated apples after 2- or 5-min treatment. C. Bacteria transferred to dump tank water after 2- or 5-min treatment, enumerated by membrane filtration method. 40PAA: 40 ppm PAA; 60PAA: 60 ppm PAA; 80PAA: 80 ppm PAA; GSA: Granny Smith apples. Data are presented as Mean ± SEM, averaged from three independent studies, with 20 apples per treatment within each independent experiment. This study utilized dump tank water from a different facility for each independent study. The level of chemical oxygen demand ranged from 975 to 5353 ppm.

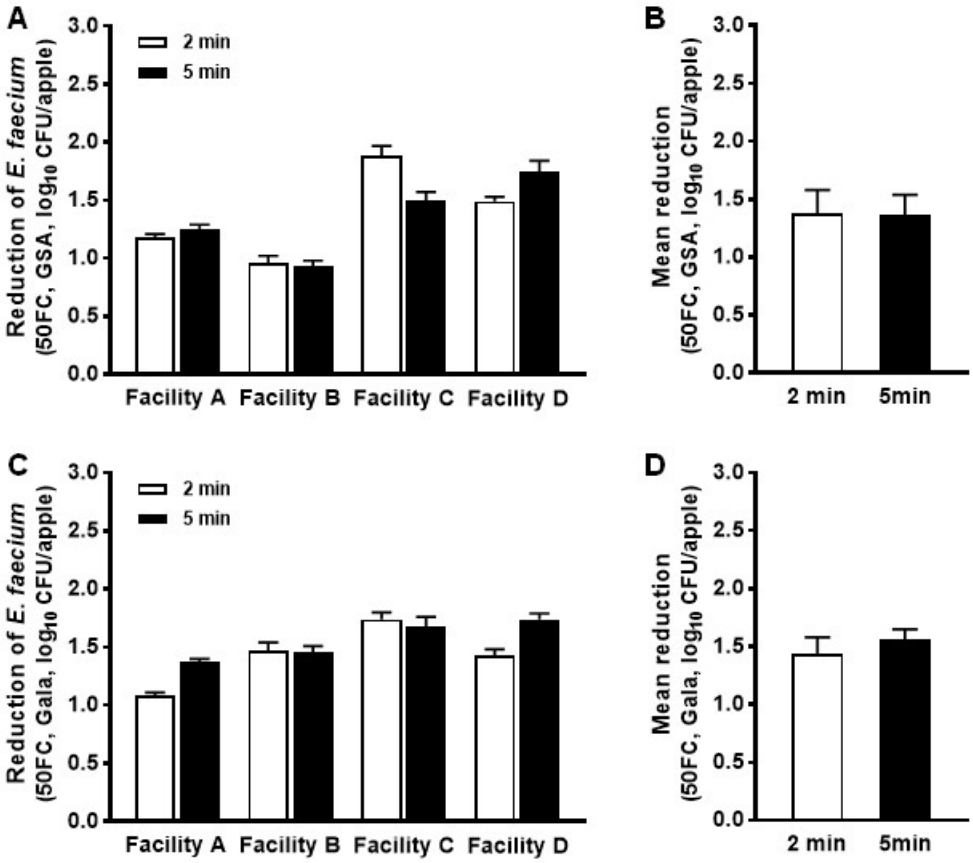


Figure 11. Effectiveness of chlorine in controlling *E. faecium* NRRL B-2354 on the inoculated apples in the commercial dump tank systems. Chlorine was applied at 50 ppm free chlorine, pH 6.8. A-B. Reduction of *E. faecium* on the inoculated Granny Smith apples (GSA) after 2- or 5-min contact, with panel A representing results for individual facilities and panel B showing the mean reduction. C-D. Reduction of *E. faecium* on the inoculated Gala apples after 2- or 5-min contact, with panel C representing results for individual facilities and panel D showing the mean reduction. The data are presented as Mean ± SEM, with 90-110 apples per variety per facility. The chemical oxygen demand level of dump tank water ranged from 265 ppm to 2580 ppm.

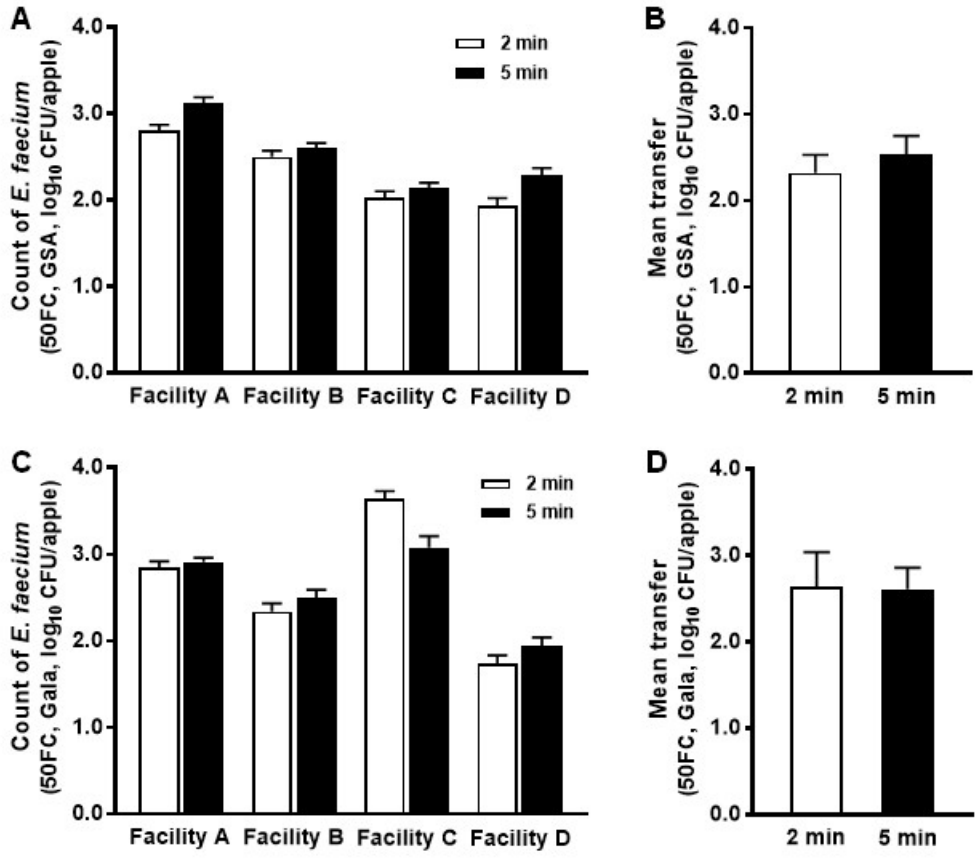


Figure 12. Effectiveness of chlorine in controlling *E. faecium* NRRL B-2354 cross-contamination from apple to apple in the commercial dump tank practice. Chlorine was applied at 50 ppm free chlorine, pH 6.8. A-B. The count of *E. faecium* transferred to uninoculated Granny Smith apples (GSA) after 2- or 5-min contact, with panel A presenting results for individual facilities and panel B showing the mean reduction. C-D. The counts of *E. faecium* transferred to the inoculated Gala apples after 2- or 5-min treatment, with panel C presenting results for individual facilities and panel D showing the mean reduction. Data are presented as Mean ± SEM. There are 360-400 apples per variety per facility. Chemical oxygen demand level ranged from 265 ppm to 2580 ppm.

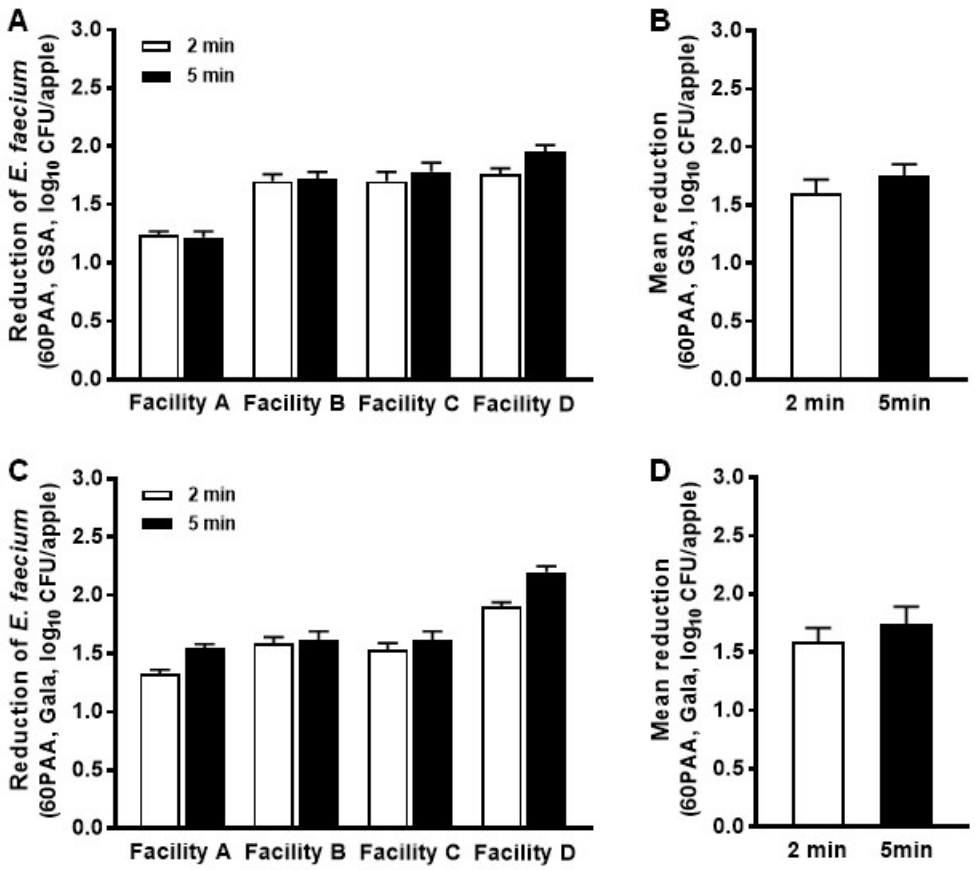


Figure 13. Effectiveness of peroxyacetic acid (PAA) in controlling *E. faecium* NRRL B-2354 on the inoculated apples in the commercial dump tank systems. PAA was applied at 50 ppm free chlorine, pH 6.8. A-B. Reduction of *E. faecium* on the inoculated Granny Smith apples (GSA) after 2- or 5-min contact, with panel A representing results for individual facilities and panel B showing the mean reduction. C-D. Reduction of *E. faecium* on the inoculated Gala apples after 2- or 5-min contact, with panel C representing results for individual facilities and panel D showing the mean reduction. The data are presented as Mean ± SEM, with 90-110 apples per variety per facility. The chemical oxygen demand level of dump tank water ranged from 993 ppm to 2497 ppm.

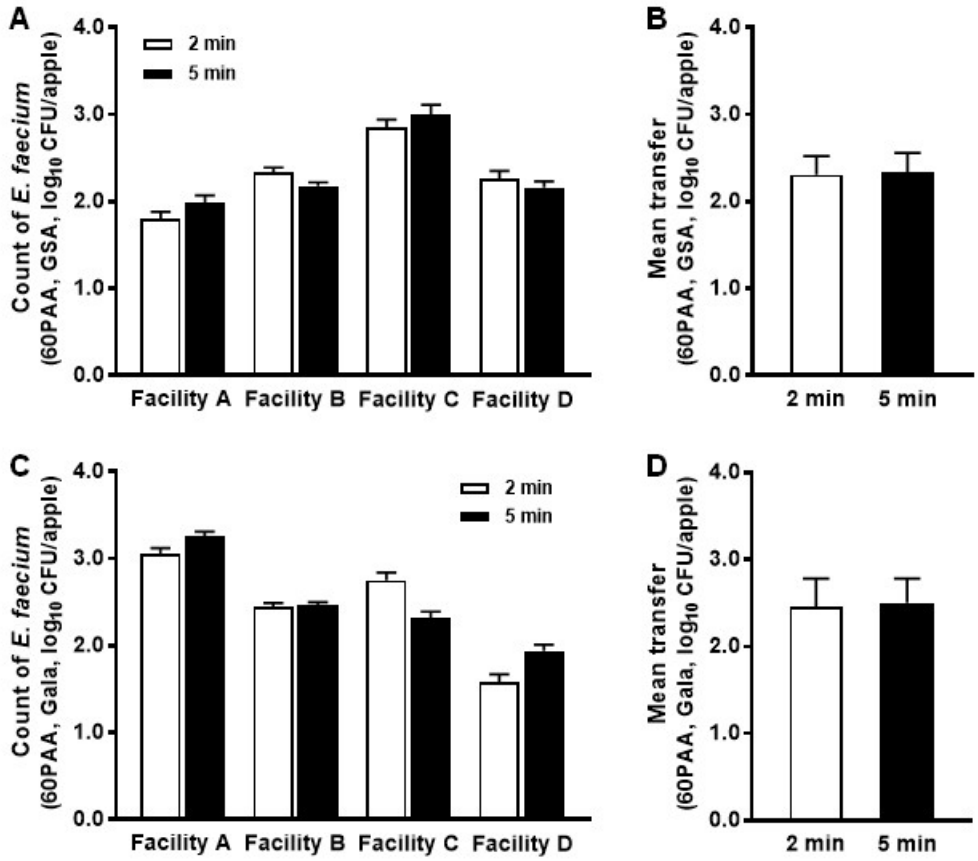


Figure 14. Effectiveness of peroxyacetic acid (PAA) in controlling *E. faecium* NRRL B-2354 cross-contamination from apple to apple in the commercial dump tank practice. PAA was applied at 50 ppm free chlorine, pH 6.8. A-B. The count of *E. faecium* transferred to uninoculated Granny Smith apples (GSA) after 2- or 5-min contact, with panel A presenting results for individual facilities and panel B showing the mean reduction. C-D. The counts of *E. faecium* transferred to the inoculated Gala apples after 2- or 5-min treatment, with panel C presenting results for individual facilities and panel D showing the mean reduction. Data are presented as Mean ± SEM. There are 360-440 apples per variety per facility. The chemical oxygen demand level of dump tank water ranged from 993 ppm to 2497 ppm.

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