CPS CENTER for PRODUCE SAFETY

CPS 2010 RFP
FINAL PROJECT REPORT

Project Title
Impact of organic load on sanitizer efficacy and recovery of *E. coli* O157:H7 during commercial lettuce processing

Project Period
January 1, 2011 through December 31, 2011; NCE to February 29, 2012

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Objectives
(1) Quantify the impact of organic load on the efficacy of a chlorine-based sanitizer against *E. coli* O157:H7 in both a model bench-top system (2) and a pilot-scale processing line for leafy greens.

FINAL REPORT

Abstract

Although chlorine is routinely used in wash water by leafy green processors to reduce microbial populations, efficacy of this sanitizer is reduced as organic load increases in the water. The aims of this study were to determine the ability of a commercial chlorine-based sanitizer (XY-12, Ecolab, Inc., St. Paul, MN), alone and with an acidifier, to reduce *Escherichia coli* O157:H7 populations on shredded iceberg lettuce during simulated commercial fluming and drying, and to assess the relationship between various physicochemical parameters and organic load of the wash water on sanitizer efficacy. A 4L bench-top model was tested as a cost-effective method to determine the impact of organic load on chlorine efficacy against *E. coli* O157:H7 in simulated leafy green processing water. In a pilot-scale leafy green processing line, we determined the impact of organic load on chlorine and acidified chlorine efficacy against *E. coli* O157:H7 in processing water and on iceberg lettuce. Physicochemical parameters including oxidation/reduction potential, maximum filterable volume, chemical oxygen demand, and total solids of the wash water from both systems were also correlated to sanitizer efficacy. Results showed that the chlorine-based sanitizer + citric acid were significantly more effective ($P < 0.05$) than the sanitizer alone at reducing *E. coli* O157:H7 populations on iceberg lettuce, in wash water, and on equipment surfaces. In addition, the physicochemical parameters of the wash water that were examined may provide leafy green processors with various alternatives to assess sanitizer efficacy in the presence of increasing organic load. However, intervention steps in addition to sanitizing wash water also need to be considered since *E. coli* O157:H7 was recovered from all processed samples.

Background

Wash water quality has been a major focus of the produce industry for many years with these concerns heightened in response to three nationwide outbreaks in 2006 traced to fresh-cut lettuce and baby spinach. Despite the widespread use of chemical sanitizers in produce wash water, efficacy of these sanitizers remains problematic due to the presence of organic material in the water that decreases antibacterial activity.

The two aims of this study were to 1) determine the ability of sodium hypochlorite, alone and with an acidifier to reduce *Escherichia coli* O157:H7 populations on shredded iceberg lettuce during simulated commercial fluming and drying, and 2) assess the relationship between various physicochemical parameters and organic load of the wash water on sanitizer efficacy.

Research Methods and Results

Objective 1

Methods. A 4-L glass carboy with a spigot (Figure 16) was used to assess the chlorine-based sanitizer XY-12 (Ecolab, Inc., St. Paul, MN) at free chlorine concentrations of 30, 50 and 100 ppm in triplicate against a 4-strain avirulent, GFP-labeled *E. coli* O157:H7 cocktail in wash water containing 0, 1, 5 or 10% (w/v) blended iceberg lettuce solids, with sanitizer-free water serving as the control ($n = 48$). After inoculating the water at 6 log CFU/ml, 50-ml water samples were collected through the opened spigot at 10-sec intervals over 90 sec and neutralized with concentrated Difco™ Neutralizing Buffer. Water was assessed for temperature, pH, Chemical Oxygen Demand (COD), Oxidation/Reduction Potential (ORP), total solids, turbidity at 663 nm,

and maximum filterable volume (MFV) using a 0.45 µm membrane. The neutralized samples were appropriately diluted and surface-plated on TSAYE + ampicillin with or without membrane filtration to enumerate *E. coli* O157:H7.

Results. *E. coli* O157:H7 reductions of 5.88, 0.25 and 0.07 log CFU/ml were seen at organic loads of 1, 5 and 10%, respectively, after 90 sec of exposure to 100 ppm of free chlorine (Figure 1). Increasing organic load correlated with COD, total solids, MFV and turbidity ($P < 0.05$) (Table 1).

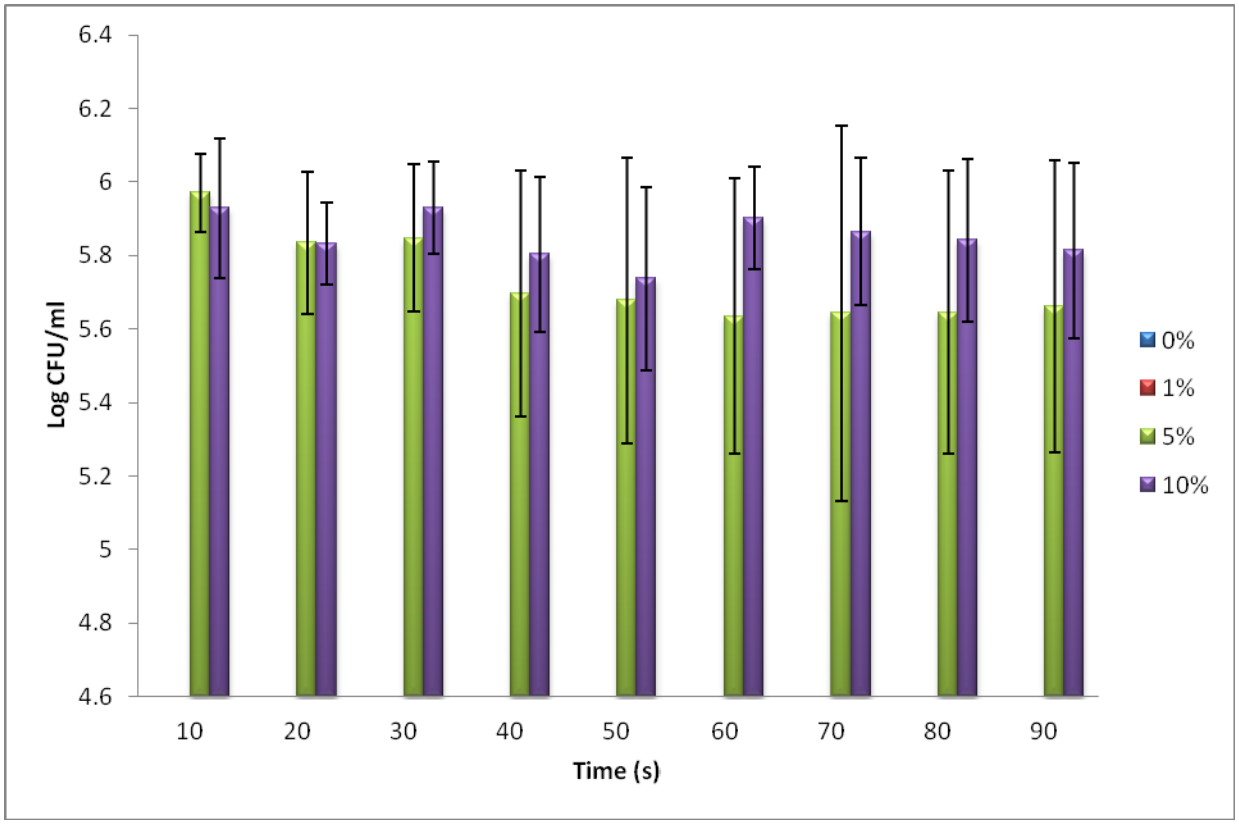

Figure 1. Populations of E. coli *O157:H7 recovered in simulated wash water containing 100 ppm free chlorine and an organic load of 0, 1, 5, or 10%.*

Table 1. Physicochemical parameters of simulated wash water containing 100 ppm free chlorine and an organic load of 0, 1, 5, or 10%.

Organic Load	COD (mg O2/L)	Total Solids (g)	Max. Filterable Vol. (ml)	Turb. (@ 663 nm)
0	3 (C)	0.0065 (D)	50 (A)	0 (C)
1%	649 (C)	0.0158 (C)	20 (B)	0.034 (C)
5%	3330 (B)	0.0302 (B)	10 (C)	0.124 (B)
10%	5880 (A)	0.0454 (A)	6 (C)	0.281 (A)

Objective 2

Methods. Flume tank water containing 0, 2.5, 5, or 10% (w/v) blended iceberg lettuce and a commercial chlorine-based produce sanitizer (XY-12, Ecolab, St. Paul, MN) at a concentration of 50 ppm free chlorine (pH 8.1) was adjusted to pH 6.5 using citric acid (CA) (Sigma-Aldrich, St. Louis, MO). Sanitizer efficacy against a 4-strain avirulent, GFP-labeled *E. coli* O157:H7 cocktail was then assessed in triplicate trials by processing dip-inoculated (5.4 kg) followed by uninoculated (3 x 5.4 kg, each) heads of iceberg lettuce at 10 min intervals in a pilot-scale lettuce processing line that included a Urschel TransSlicer shredder, step conveyor, 3.3-m flume tank equipped with a holding gate, shaker table and centrifugal dryer (22.7 kg capacity) with sanitizer-free water (pH 7.3) serving as the control (n = 36). Shredded lettuce (25 g) and water samples (50 ml) were collected and neutralized every 30 sec from the 3.3-m flume tank along with additional water samples at 2-min intervals between batches and nine equipment samples (100 cm^2) after processing (Figure 2). Wash water was also assessed for various physicochemical parameters, including temperature, pH, chemical oxygen demand (COD), oxidation/reduction potential (ORP), total solids, turbidity at 663 nm, and maximum filterable volume (MFV) using a 0.45 µm membrane. All samples were appropriately neutralized in Difco™ Neutralizing Buffer, diluted and surface-plated on TSAYE + ampicillin with or without membrane filtration to enumerate *E. coli* O157:H7.

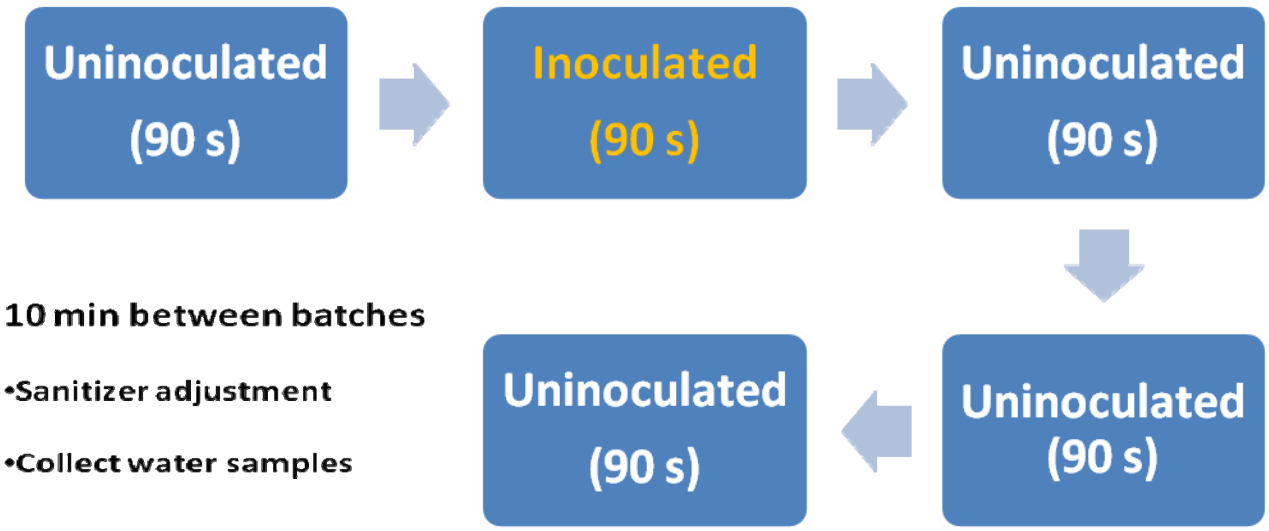

Figure 2. Design for iceberg lettuce processing in a pilot-scale leafy green processing line.

Results. Populations of *E. coli* O157:H7 recovered from inoculated iceberg lettuce after processing with wash water containing 10% organic load plus either 50 ppm free chlorine, 50 ppm free chlorine + citric acid, or water without sanitizer were not significantly different (P > 0.05). However, in the third batch of uninoculated lettuce processed after the inoculated batch, significantly fewer (P < 0.05) *E. coli* O157:H7 were recovered from lettuce using citric acid-

supplemented chlorine compared to water without sanitizer with populations of 1.27 and 3.05 log CFU/g, respectively (Figure 3).

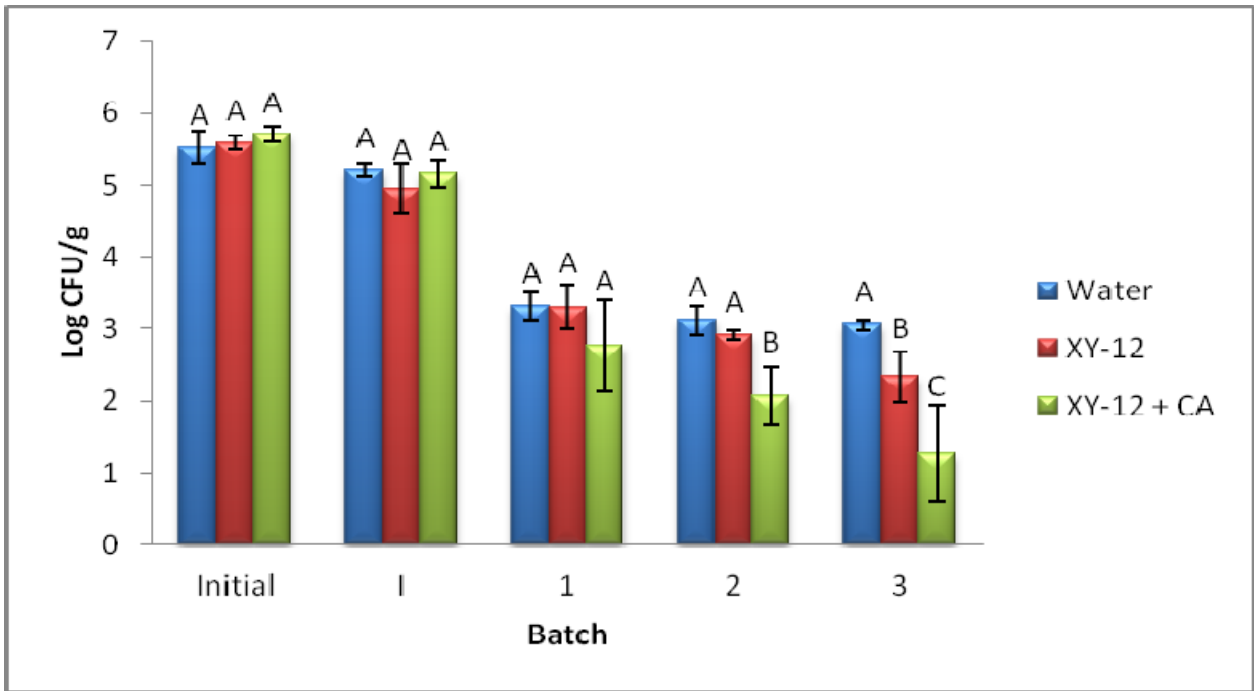

Figure 3. Populations of E. coli *O157:H7 recovered from iceberg lettuce after centrifugation of each batch of lettuce during processing with wash water containing an organic load of 10% with 50 ppm free chlorine, 50 ppm free chlorine + citric acid, or water without sanitizer.*

Significantly higher (P < 0.05) numbers of *E. coli* O157:H7 were recovered from the inoculated batch of lettuce and from the first and third uninoculated batches processed in wash water containing 50 ppm free chlorine + citric acid and organic loads of 5 and 10%. Final populations of *E. coli* O157:H7 recovered from the third batch of uninoculated lettuce were 1.27 log CFU/g with a 10% organic load compared to 0.73 log CFU/g without an organic load (Figure 4).

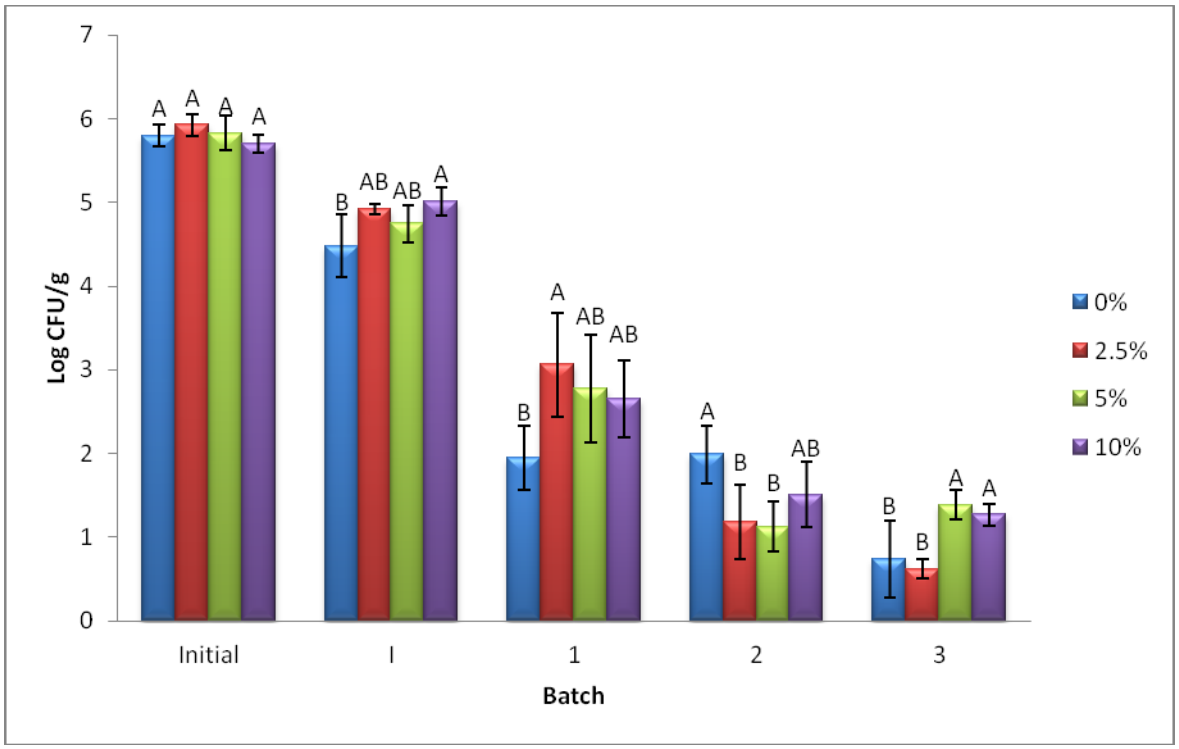

Figure 4. Populations of E. coli *O157:H7 on iceberg lettuce after centrifuging each batch of lettuce during processing with wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10%.*

Significantly fewer ($P < 0.05$) *E. coli* O157:H7 were recovered from wash water containing a 10% organic load 30 sec after processing each batch of uninoculated iceberg lettuce when the wash water contained 50 ppm free chlorine + citric acid. Populations of *E. coli* O157:H7 recovered while processing the third batch of uninoculated lettuce in flume water containing chlorine + citric acid were 2.4 log CFU/ml less than populations recovered from sanitizer-free water (Figure 5).

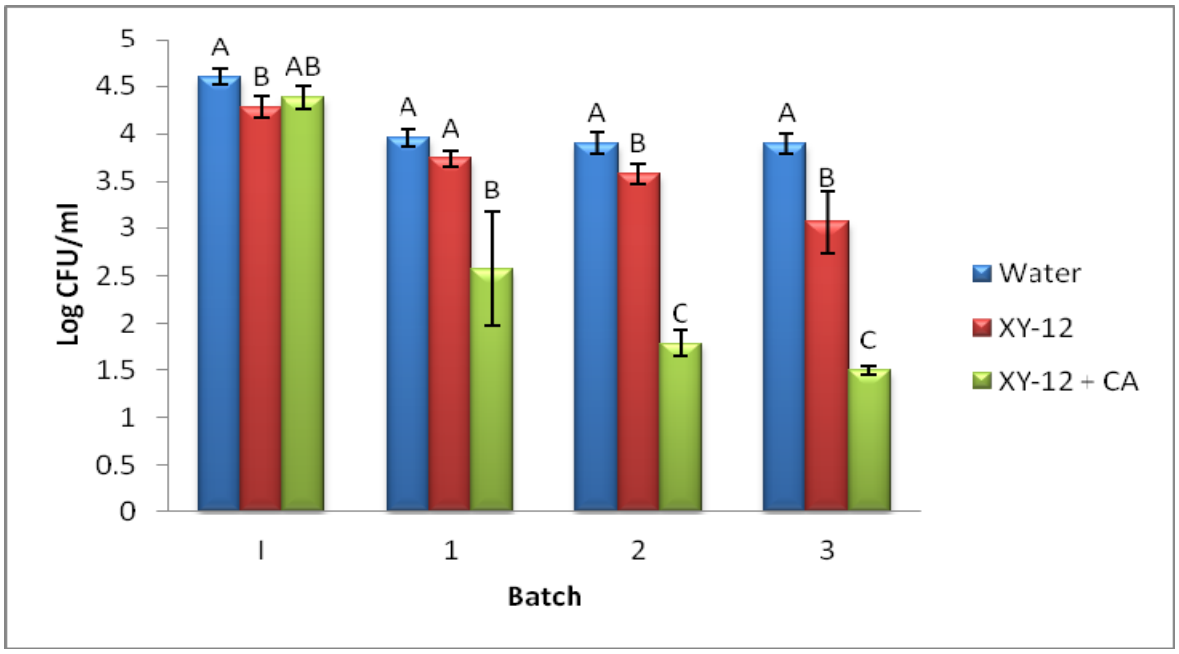

Figure 5. Populations of E. coli *O157:H7 recovered from wash water 30 sec after processing each batch of iceberg lettuce with wash water containing an organic load of 10% with 50 ppm free chlorine, 50 ppm free chlorine + citric acid, or water without sanitizer.*

Significantly higher ($P < 0.05$) populations of *E. coli* O157:H7 were recovered during the processing of all batches of lettuce in wash water containing 2.5, 5, and 10% organic load with 50 ppm free chlorine + citric acid. Populations of *E. coli* O157:H7 recovered while processing the third batch of uninoculated lettuce at 0% organic load were 2.65 log CFU/ml lower than populations recovered while processing with an organic load of 10% (Figure 6).

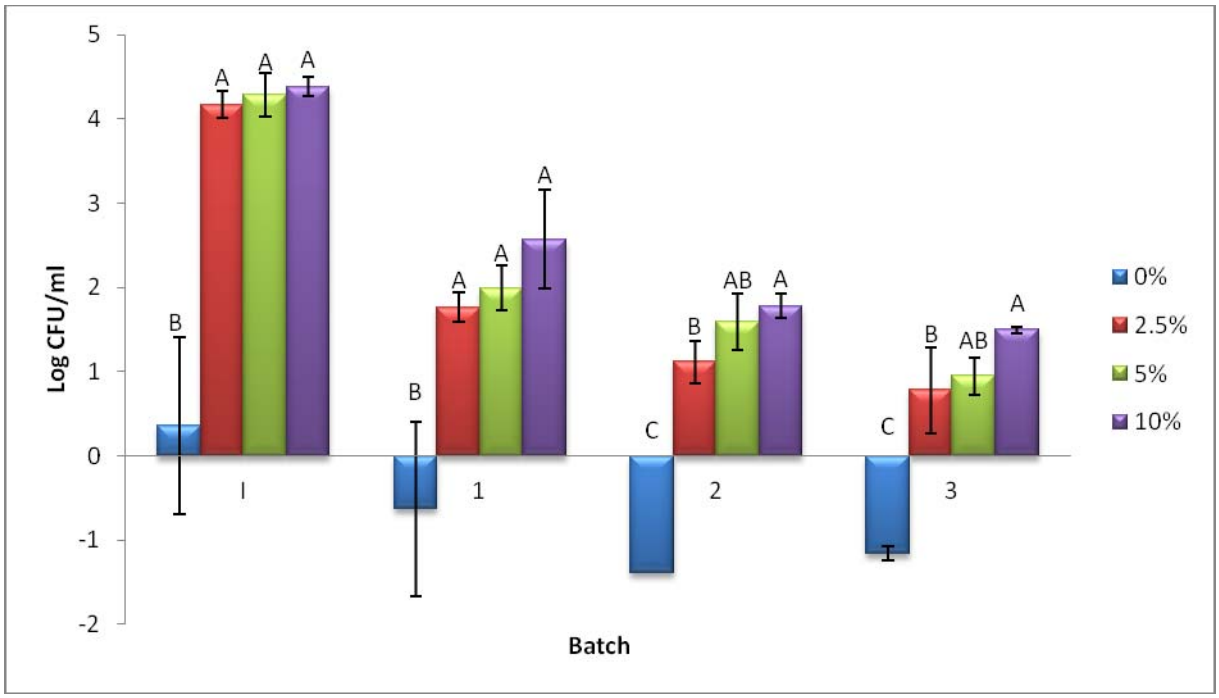

Figure 6. Populations of E. coli *O157:H7 recovered from wash water after the first 30 sec of processing of each batch of iceberg lettuce with wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10%.*

Significantly less ($P < 0.05$) *E. coli* O157:H7 was recovered from centrifugation water after processing each batch of uninoculated iceberg lettuce when the wash water contained a 10% organic load and 50 ppm free chlorine + citric acid. A 2.12 log CFU/ml difference between sanitizer-free wash water and wash water containing 50 ppm free chlorine + citric acid was seen in centrifugation water from the third batch of uninoculated lettuce (Figure 6).

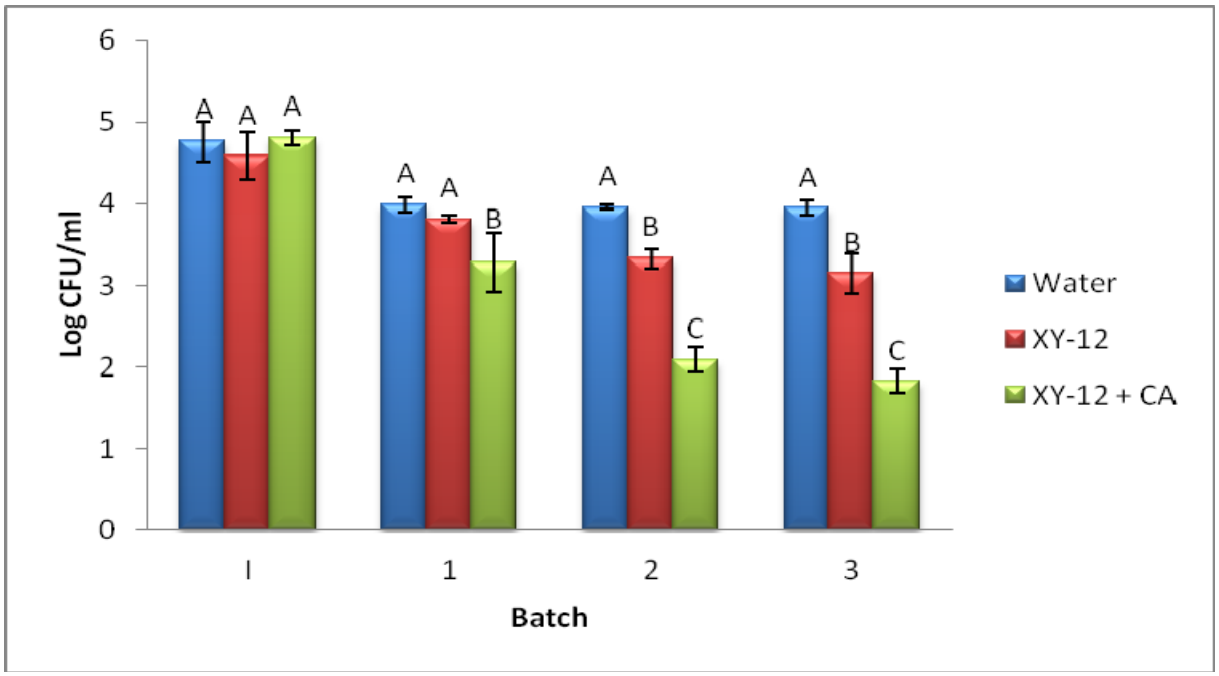

Figure 7. Populations of E. coli *O157:H7 recovered from centrifugation water after processing of each batch of iceberg lettuce with wash water containing an organic load of 10% with 50 ppm free chlorine, 50 ppm free chlorine + citric acid, or water without sanitizer.*

Significantly higher ($P < 0.05$) populations of *E. coli* O157:H7 were recovered from centrifugation water after the processing all batches of lettuce in wash water containing 2.5, 5, and 10% organic load with 50 ppm free chlorine + citric acid. Centrifugation water populations from the third batch of uninoculated lettuce at 0% organic load were 2.65 log CFU/ml lower than populations recovered using an organic load of 10% (Figure 8).

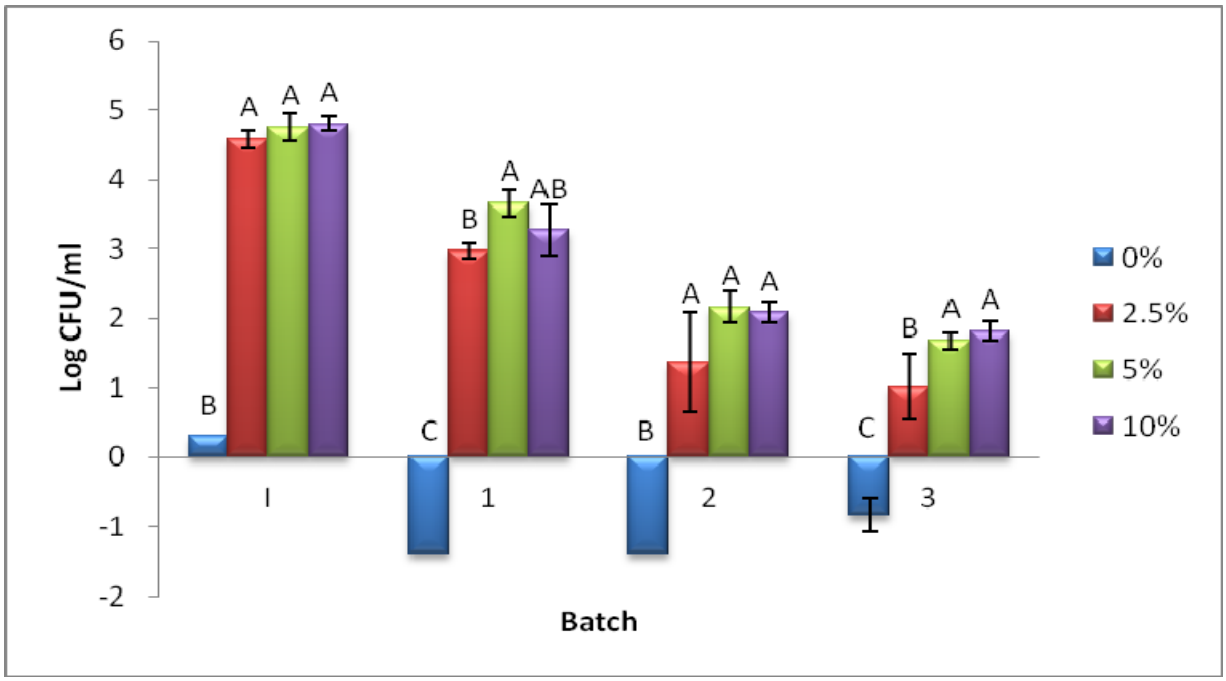

Figure 8. Populations of E. coli *O157:H7 recovered from centrifugation water after processing of each batch of iceberg lettuce with wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10%.*

Significantly fewer ($P < 0.05$) *E. coli* O157:H7 were recovered from all 9 equipment surface samples (100 cm^2) when wash water contained 10% organic load and 50 ppm free chlorine + citric acid than when water alone was used (Figure 9).

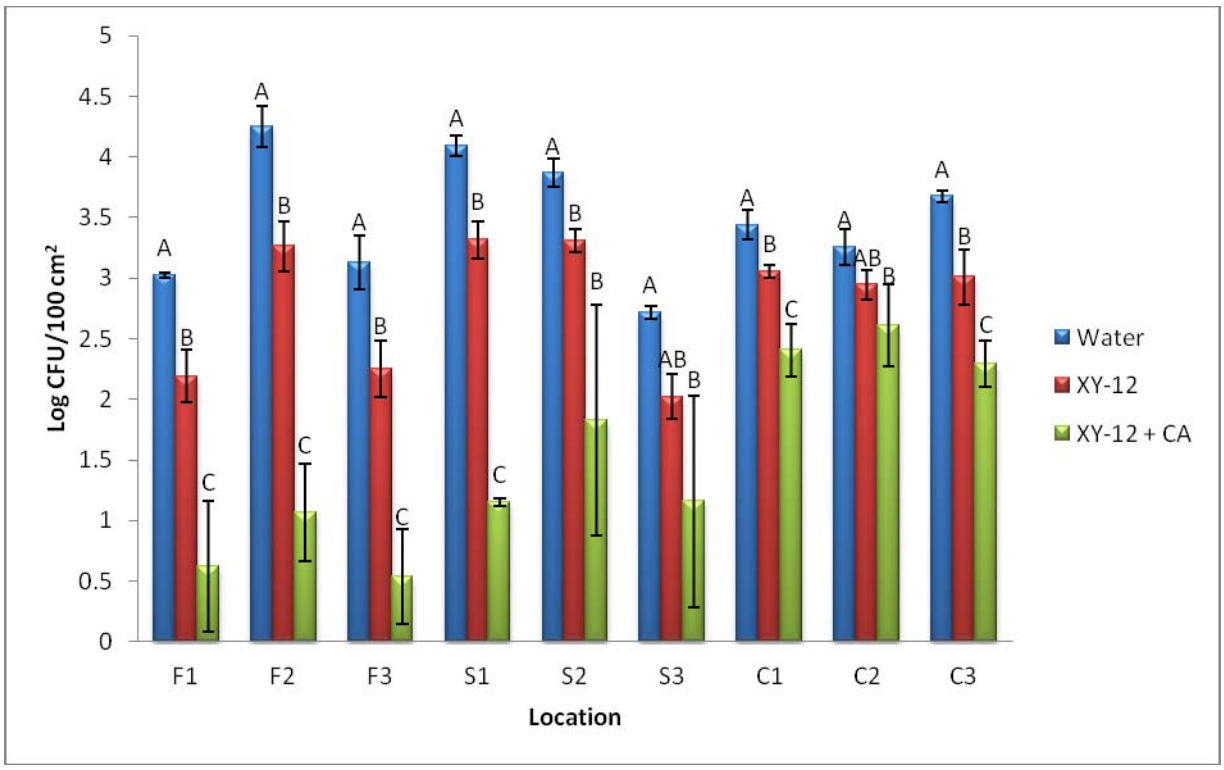

Figure 9. Populations of E. coli *O157:H7 recovered from equipment surfaces (3 each from flume tank (F1-F3), shaker table (S1-S3), and centrifugal dryer (C1-C3), Figures 17 and 18) after processing all batches of iceberg lettuce with wash water containing an organic load of 10% with 50 ppm free chlorine, 50 ppm free chlorine + citric acid, or water without sanitizer.*

Significantly higher ($P < 0.05$) populations were recovered from 7 of the 9 equipment surface samples (100 cm^2) when the wash water contained 50 ppm free chlorine + citric acid at both 5 or 10% organic loads (Figure 10).

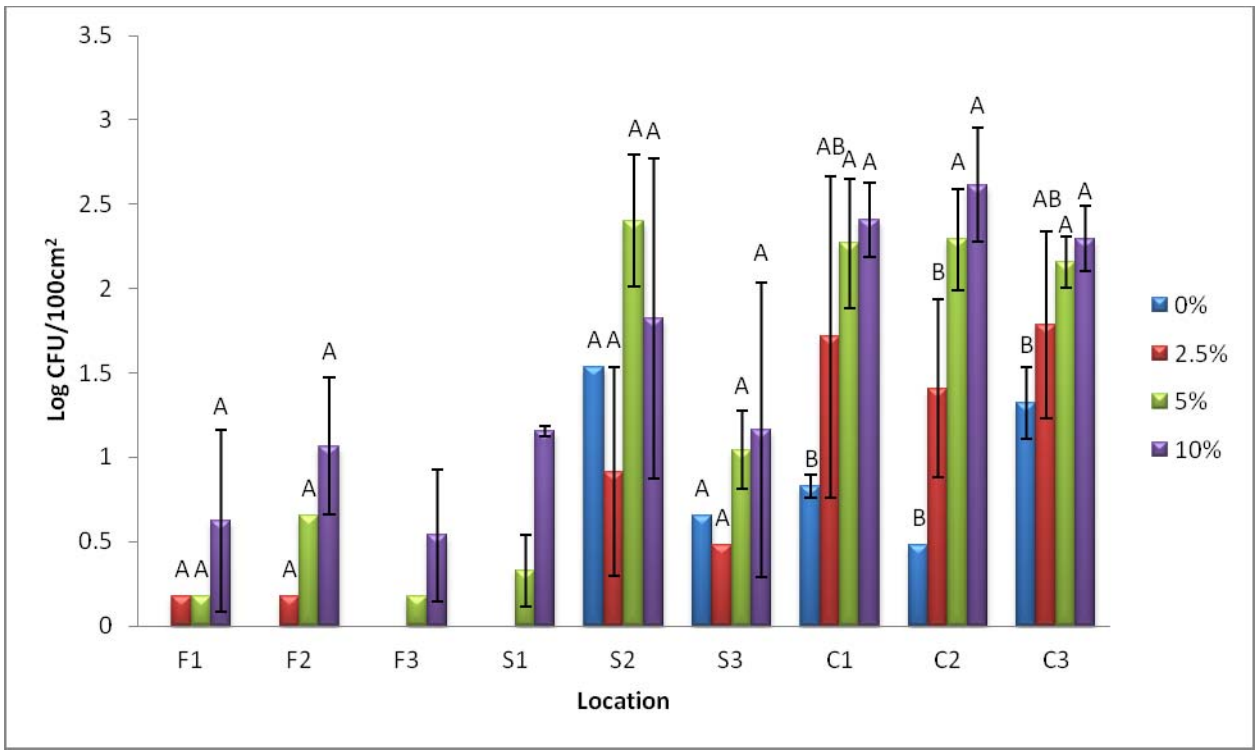

Figure 10. Populations of E. coli *O157:H7 recovered from equipment surfaces (3 each from flume tank (F1-F3), shaker table (S1-S3), and centrifugal dryer(C1-C3), Figures 17 and 18) after processing all batches of iceberg lettuce with wash water with 50 ppm free chlorine + citric acid containing an organic load of 0, 2.5, 5, or 10%.*

In order to determine how the different physicochemical parameters of the wash water correlated to sanitizer efficacy, we chose to compare the efficacy of 50 ppm free chlorine + citric acid to log reductions for *E. coli* O157:H7 seen after 30 sec of processing the first of three uninoculated batches following the inoculated batch. At this time point, there was significantly less ($P < 0.05$) reduction of *E. coli* O157:H7 in wash water containing 2.5, 5, and 10% organic loads compared to water with a 0% organic load. The vertical green vertical line at 2.5% organic load (see Figures 11 – 15) established a distinct test point for each physicochemical parameter that could be used by industrial processors as a limit to better ensure sanitizer efficacy against *E. coli* O157:H7. Both oxidation/reduction potential (Figure 11) and maximum filterable volume (Figure 12) of the wash water positively correlated with the log reduction of *E. coli* O157:H7. In contrast, turbidity (Figure 12), total solids (Figure 14), and chemical oxygen demand (Figure 15) negatively correlated with *E. coli* O157:H7 log reductions.

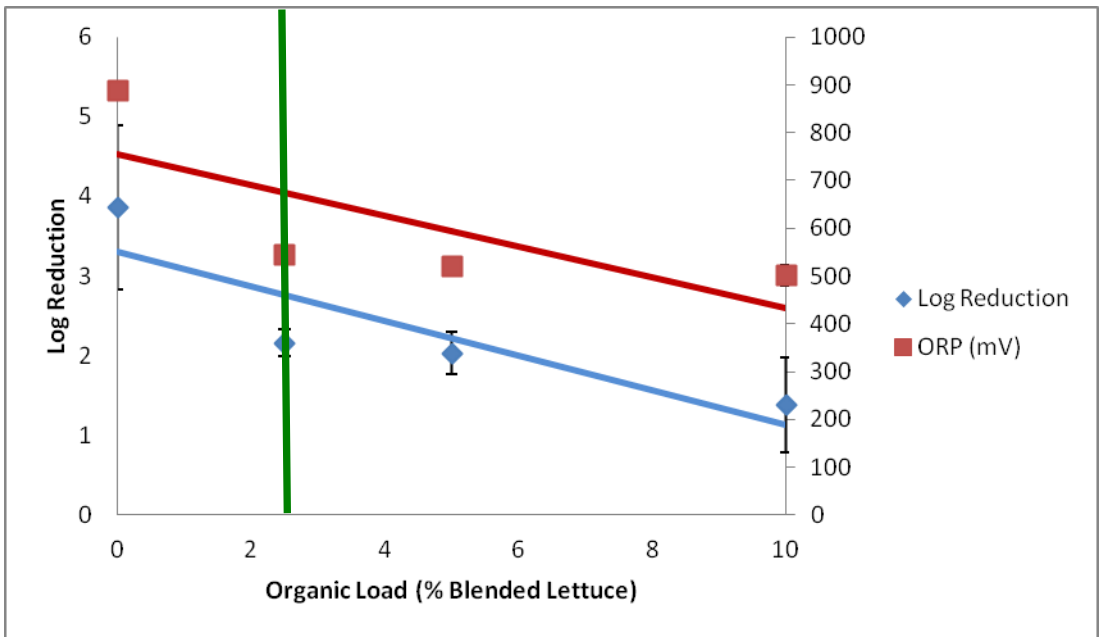

Figure 11. Oxidation/reduction potential (ORP) of wash water correlated to log reduction of E. coli *O157:H7 in wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10% after 30 sec of processing the first of three batches of uninoculated lettuce following the inoculated batch of lettuce.*

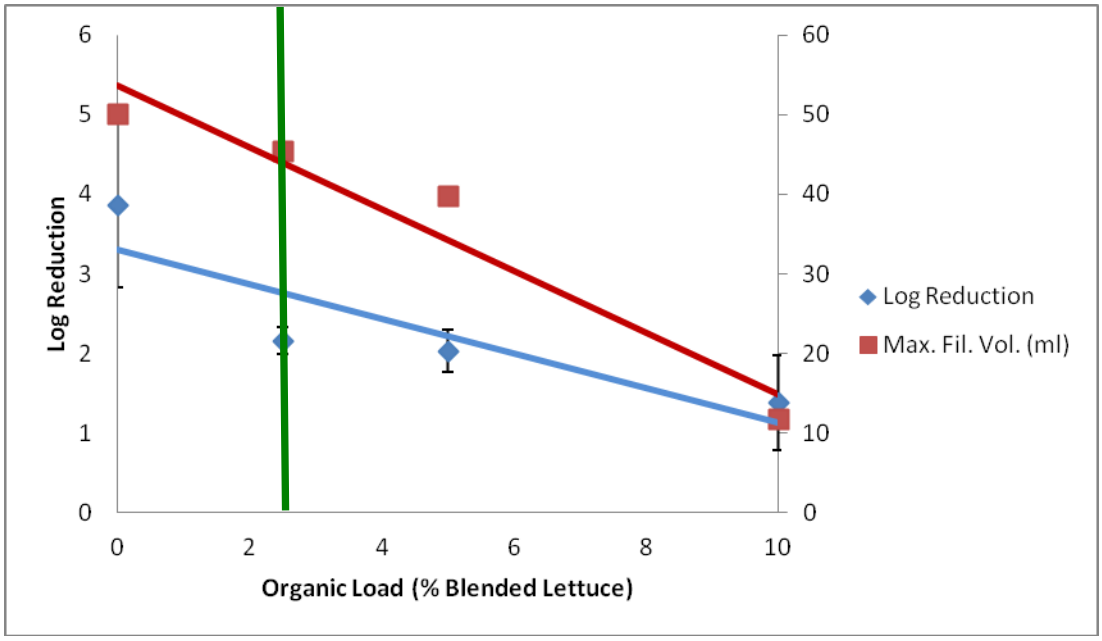

Figure 12. Maximum filterable volume (MFV) of wash water correlated to log reduction of E. coli *O157:H7 in wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10% after 30 sec of processing the first of three batches of uninoculated lettuce following the inoculated batch of lettuce.*

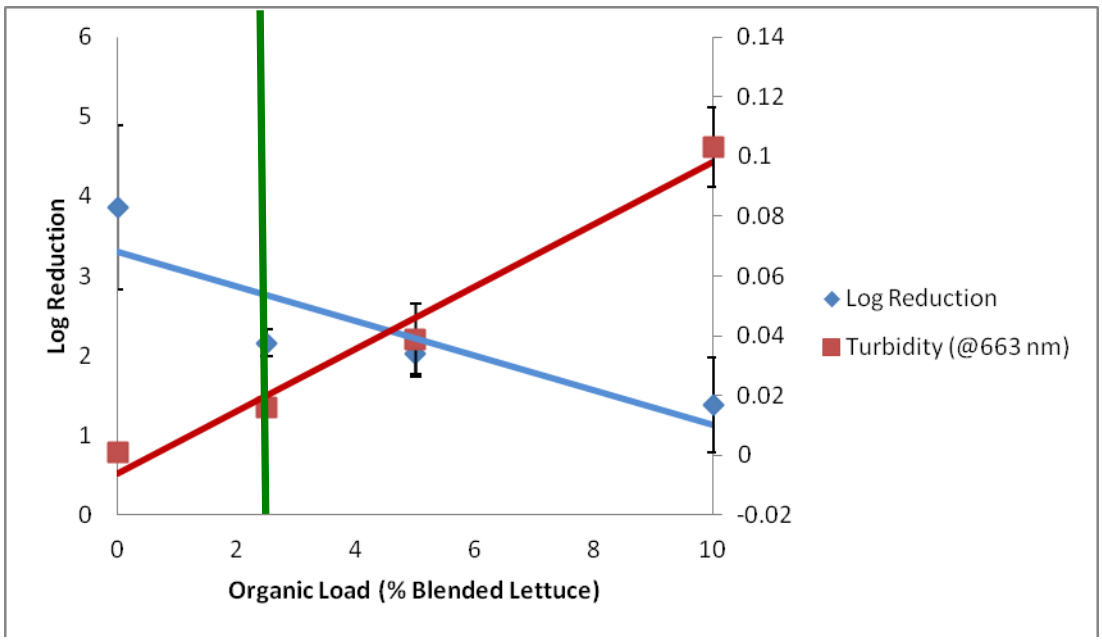

Figure 13. Turbidity (at 663 nm) of wash water correlated to log reduction of E. coli *O157:H7 in wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10% after 30 sec of processing the first of three batches of uninoculated lettuce following the inoculated batch of lettuce.*

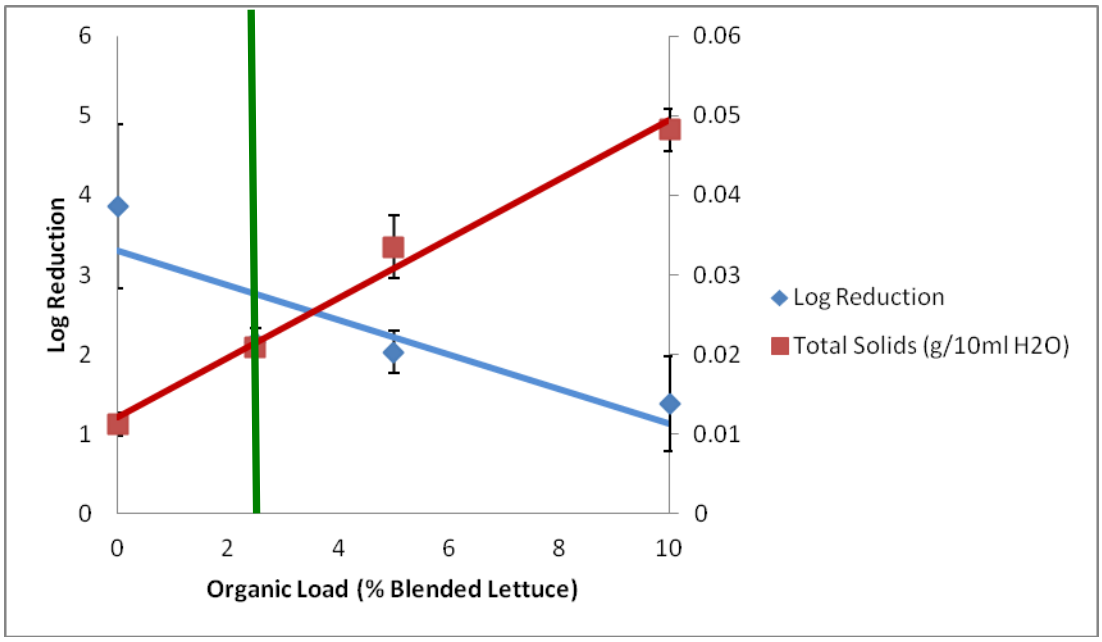

Figure 14. Total solids (g/10 ml water) of wash water correlated to log reduction of E. coli *O157:H7 in wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10% after 30 sec of processing the first of three batches of uninoculated lettuce following the inoculated batch of lettuce.*

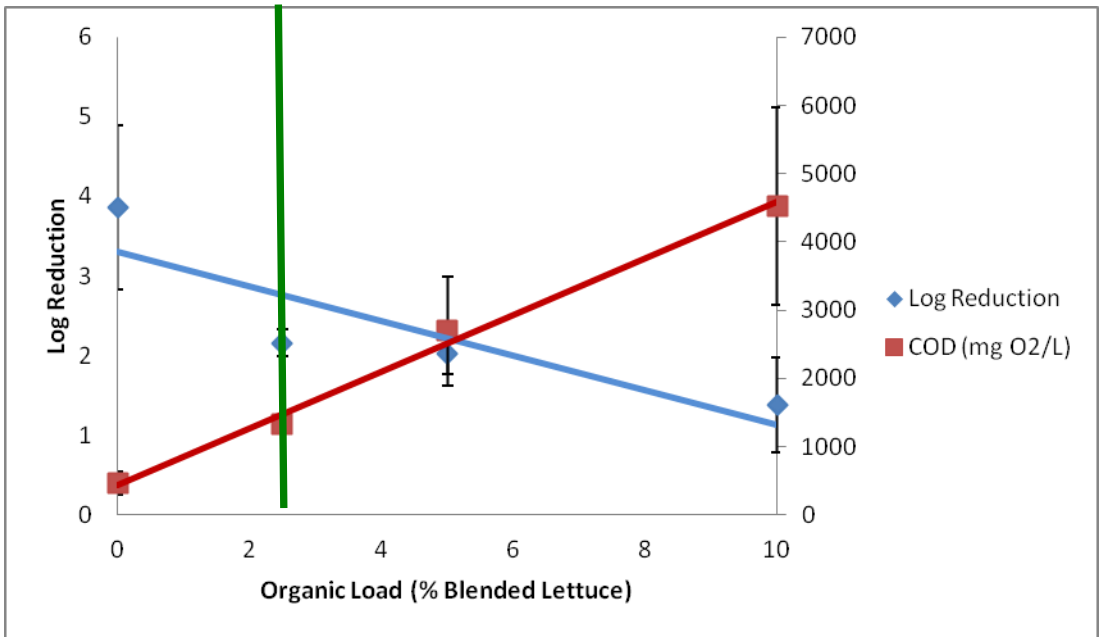

Figure 15. Chemical Oxygen Demand (mg O2/L) of wash water correlated to log reduction of E. coli *O157:H7 in wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10% after 30 sec of processing the first of three batches of uninoculated lettuce following the inoculated batch of lettuce.*

Outcomes and Accomplishments

The two goals of this study were to 1) determine the ability of sodium hypochlorite, alone and with an acidifier to reduce *Escherichia coli* O157:H7 populations on shredded iceberg lettuce during simulated commercial processing, and 2) assess the relationship between various physicochemical parameters and organic load of the wash water on sanitizer efficacy. Both goals were met in this study by work completed in a laboratory and in a pilot-scale leafy green processing line. Work completed in this study determined that sodium hypochlorite was effective at reducing *E. coli* O157:H7 populations in wash water, on iceberg lettuce, and on equipment surfaces. Efficacy of chlorine was significantly enhanced by the addition of citric acid. It was determined that various physicochemical parameters tested correlated to organic load and sanitizer efficacy.

Summary of Findings and Recommendations

When comparing the populations of *E.* coli O157:H7 recovered, the results from Objective 1 (bench top model) did not correlate those from Objective 2 (the pilot-scale processing line) However, a similar trend was noticed between the two models in that an increasing organic load did correlate to a decrease in sanitizer efficacy (Table 1, Figures 10 – 14). From objective 2, we determined that supplementing chlorine with citric acid to reduce the pH of the wash water to 6.5 significantly ($P < 0.05$) increased the efficacy of the wash water against *E. coli* O157:H7, even at a 10% organic load. However, a significant decrease ($P < 0.05$) in sanitizer efficacy was seen, even at 2.5% organic load compared to no organic load in the sanitizing wash water. This leads us to recommend that lettuce processors, if they are relying upon chlorine as the sanitizer in their wash water, use citric acid to reduce the pH of the wash water to 6.5 and monitor ORP, maximum filterable volume, turbidity, total solids, and COD to determine if their wash water will be effective against *E. coli* O157:H7.

Elliot Ryser, Michigan State University
Impact of organic load on sanitizer efficacy and recovery of E. coli O157:H7 during commercial lettuce processing

APPENDICES

Publications and Presentations (required)

Presentations

1. Davidson, G.R., C.N. Kaminski, L. Ren, and E.T. Ryser. 2012. Impact of organic load on *Escherichia coli* O157:H7 persistence during pilot-scale processing of iceberg lettuce with acidified sodium hypochlorite. Abst. Ann. Mtg. Int. Assoc. Food Prot. Providence, RI. July 22- 25.

2. Davidson, G.R., Y. Xu, and E.T. Ryser. 2011. Persistence of *Escherichia coli* O157:H7 during pilot-scale processing of iceberg lettuce using flume water containing sanitizers and an organic load. Abst. Ann. Mtg. Int. Assoc. Food Prot. Milwaukee, WI. July 31- August 4. *Gordon Davidson was awarded 1st place in the Developing Scientist competition for this technical presentation.*

3. Davidson, G.R., H. Wang, and E.T. Ryser. 2011. Impact of organic load on sanitizer efficacy against *Escherichia coli* O157:H7 in simulated leafy green processing water. Abst. Ann. Mtg. Int. Assoc. Food Prot. Milwaukee, WI. July 31- August 4.

Publications

1. Davidson, G.R., C.N. Kaminski, and E.T. Ryser. 2011. Persistence of *Escherichia coli* O157:H7 during pilot-scale processing of iceberg lettuce using flume water containing sanitizers and an organic load. *J. Food Prot*. (In preparation).

2. Davidson, G.R., C.N. Kaminski, and E.T. Ryser. 2011. Impact of organic load on sanitizer efficacy against *Escherichia coli* O157:H7 in simulated leafy green processing water. *J. Food Prot*. (In preparation).

Budget Summary (required)

Grant Award Amount	Total Advance Amount	Remaining Advance Balance	Total Grant Funds Invoiced	Remaining Grant Balance	Program Income Earned	Match / In-Kind Fund Commitment	Match/In-Kind Funds spent
$70,104	$ 0	$ 0	$70.104	$ 0	$ 0	$ 0	$ 0

Tables and Figures (optional)

Figure 16. Carboy used in Objective 1.

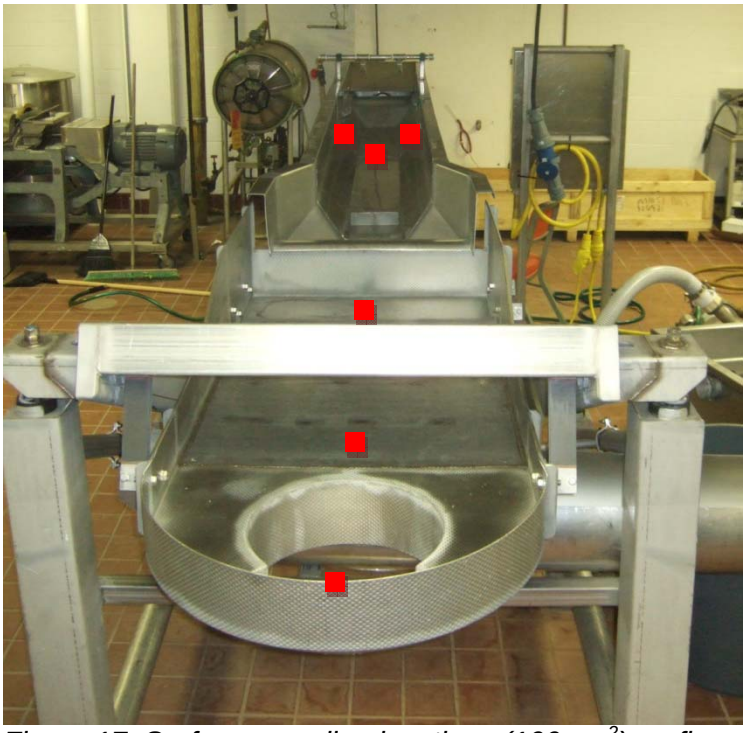

Figure 17. Surface sampling locations (100 cm^2) on flume tank and shaker table.

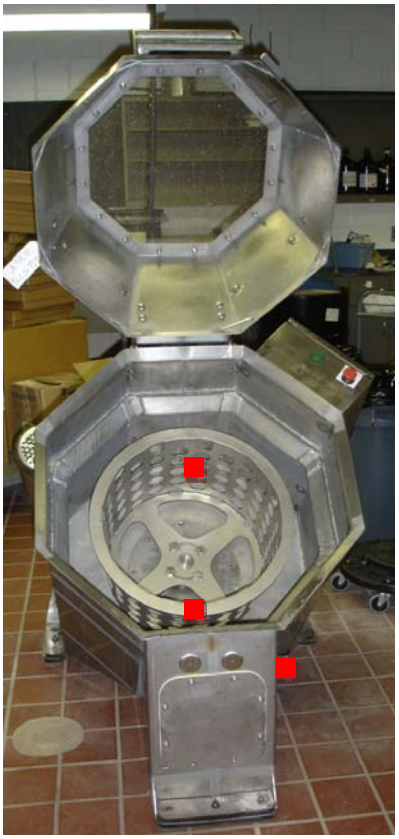

Figure 18. Surface sampling locations (100 cm2) in centrifugal dryer.

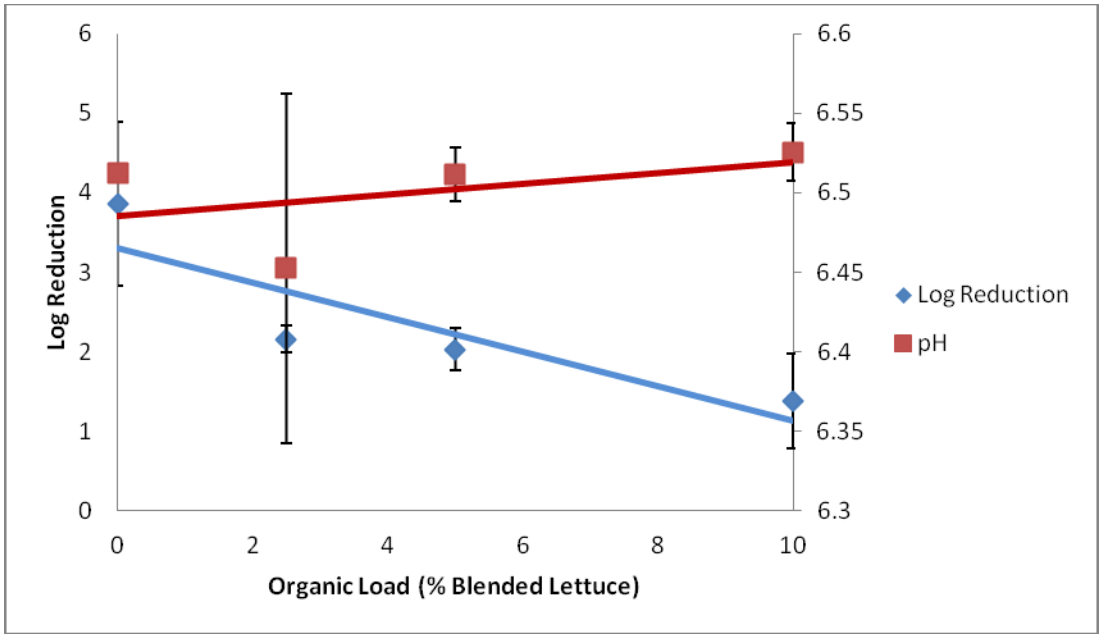

Figure 19. pH of wash water correlated to log reduction of E. coli *O157:H7 in wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10% after 30 sec of processing the first of three batches of uninoculated lettuce following the inoculated batch of lettuce.*

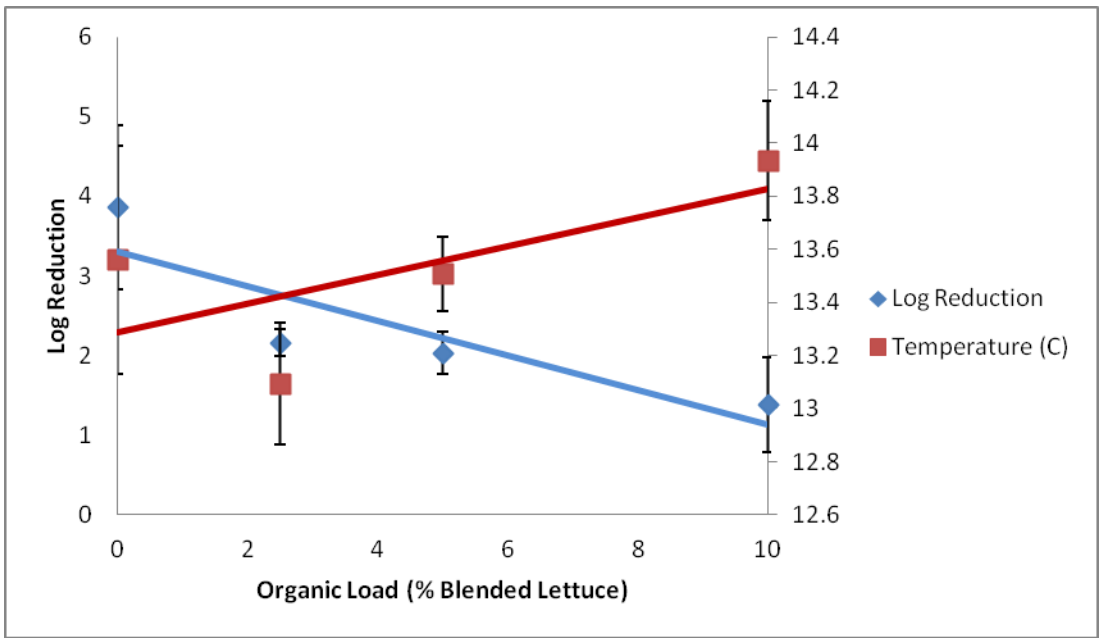

Figure 20. Temperature of wash water correlated to log reduction of E. coli *O157:H7 in wash water containing 50 ppm free chlorine + citric acid and an organic load of 0, 2.5, 5, or 10% after 30 sec of processing the first of three batches of uninoculated lettuce following the inoculated batch of lettuce.*