ENVIROTECH

CASE STUDY

The Effectiveness of Water, Sodium Hypochlorite Bleach, and Peroxyacetic Acid (PAA) in Eradicating a Wild Field Strain of E. coli O157:H7 from the Surface of Cucumbers

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Background

The contamination of produce by pathogenic organisms such as E. coli O157:H7 bacteria can have a devastating impact upon the industry. Two years ago the baby spinach industry all but collapsed when product contaminated with E. coli O157:H7 bacteria sickened consumers. Last year wreaked similar devastation when tomatoes were allegedly contaminated with harmful Salmonella bacteria that reached the consumer. Vigilant companies are adopting a chemical intervention step of treating their produce with FDA approved sanitizers as part of their HACCP programs.

A major processor of fresh cucumbers sought to improve the safety of their product and wanted to know whether treatment of their produce with a solution of peroxyacetic acid would be an effective means of accomplishing this task. In particular, the company was most concerned about the microorganism E. coli O157:H7 because of its extreme virulence. See the data table below:

TABLE I:

Estimated infectious dose of bacteria species

Bacteria Species	Estimated infectious dose *(bacteria cell number)*	Disease
E. coli O157:H7	10 to 100	Hemorrhagic colitis
E. coli	1,000,000 to 100,000,000	Traveler's diarrhea
Salmonella	100 to 1,000,000,000	Salmonellosis

Principal source: *Foodborne Pathogens: Risks and Consequences, Report No. 122, CAST-Council for Agricultural Science and Technology, September 1994.*

A very simple experiment was devised: The surfaces of cucumbers supplied by the processing plant were inoculated with a field culture of E. coli O157:H7 by spraying a suspension of the culture onto the outside surfaces of the cucumbers and allowing to dry. The cucumber processor also supplied the field culture, as these are considered hardier and more difficult to eradicate than those available from the laboratory institutions such as the ATTC. After inoculating the cucumber surfaces, the produce was then rinsed with either

(1) plain city water
(2) a solution of sodium hypochlorite bleach, or
(3) a solution of peroxyacetic acid.

The amount of viable E. coli O157:H7 bacteria rinsed from the surface of the cucumbers was measured by plating the respective rinse solutions. Recognizing that it is not the amount of bacteria in the rinse solutions that pose a menace to the public, rather it is the amount of bacteria still resident on the cucumber surface that is critical; the surfaces were swabbed for bacteria after the respective rinses in order to determine the effectiveness of eradication.

The cucumbers were exposed to each rinsing program for a low and a high concentration of chemical treatment. A one-minute contact time was used to simulate the exposure time of cucumbers being sprayed on a conveyor belt.

Materials and Methods

a) Low Concentration Study

A wild field strain of E. coli O157:H7 bacteria was cultured in nutrient broth (Sigma, St. Louis, MS) by incubation for two days at 35 oC. The bacteria were separated from the nutrient broth by centrifugation, and resuspended in 150 ml of city water contained in a spray bottle. A determination of the amount of E. coli O157:H7 bacteria in suspension was performed through common plating techniques, (described later) but was found to be Too-Numerous-To-Count (TNTC).

The cucumbers used were supplied fresh from the processor. Table II provides the average dimensions.

TABLE II:

Average weight /g	272.2
Average length /cm	16.8
Average circumference/ cm	17.9
Calculated average surface area /cm2	352

Twenty-five cucumbers were evenly spaced apart on an aluminum foil sheet. Holding the spray bottle about 6 inches from the cucumbers, the outer surfaces were doused with a fine spray of the E. coli O157:H7 suspension. Care was taken to ensure that the cucumbers were covered evenly. The cucumbers were left to dry overnight, whereupon a second and third E. coli O157:H7 inoculation was applied the following morning.

When the cucumbers were dry, 15 of the 25 were randomly sorted into three groups of five.

Each group of five cucumbers was placed in a one-gallon Ziploc bag for contact with one of the following rinse solutions:

(1) 500 ml of Modesto city water *(control)*
(2) 500 ml of Modesto city water containing 37.5 ppm *(as Cl2)* of sodium hypochlorite bleach *(this is the same molar concentration of 40 ppm PAA).*
(3) 500 ml of Modesto city water containing 40 ppm of peroxyacetic acid *(PAA)*.
The cucumbers were then gently tumbled in the rinse solution for exactly one minute, after which they were removed.

Each set of 5 wet cucumbers were then assigned to another microbiology technician who was unaware which rinsing solution had been used on which set of cucumbers Using gloved hands to hold the cucumber, the technician swabbed half of the surface of the still-wet cucumber with a Q-tip-like swab known as a Quickswab. No area of the surface cucumber was contacted more than once with the swab, which was rotated slightly between swabbing strokes for uniform distribution of bacteria onto the absorbent pad. This was followed by vortexing the swab into the 1 ml of nutrient broth that accompanied the Quickswab in order to dislodge the bacteria from the swab and into the aqueous phase. Another swab was then used to swab the other half of the cucumber in identical fashion. Thus, two fresh Quickswabs were used for each cucumber. The nutrient broth containing viable bacteria swabbed from the cucumbers were subsequently serially diluted (100,102 and 104) using Butterfield's buffer, and plated onto 3M petrifilms for fecal coliforms.

b) High Concentration Study

The above test was repeated at using double the concentration of PAA and NaOCl. This testing was performed on a separate day, with a fresh culture of E. coli O157:H7 and a fresh set of cumbers from the processor. The only other change being that following the cucumber rinses, the rinse solutions were immediately processed by a microbiology technician unaware of their identities. Each rinse solution was plated onto 3M Petrifilms for fecal coliforms at 100, 102 and 104 dilutions in Butterfield's buffer.

RESULTS AND DISCUSSION

Microbiological Quality of the Rinsed Cucumbers

LOW CONCENTRATION STUDY

It is obvious that it is not the amount of bacteria in the rinse solutions that pose a threat to the public; rather it is the amount of bacteria still resident on the cucumber surface after the rinsing step that is the important public health issue. Therefore, after the respective rinses, the surfaces of the cucumbers were swabbed in order to determine the relative effectiveness of each rinsing/sanitizing program.

Table III reports the data in terms of log10 reduction in surface-associated E. coli O157:H7 bacteria compared to the amount removed by the city water control rinse. The figures in parentheses express the results in terms of the % reduction in surfaceassociated bacteria over and above what was achieved with just the city water control rinse.

TABLE III:

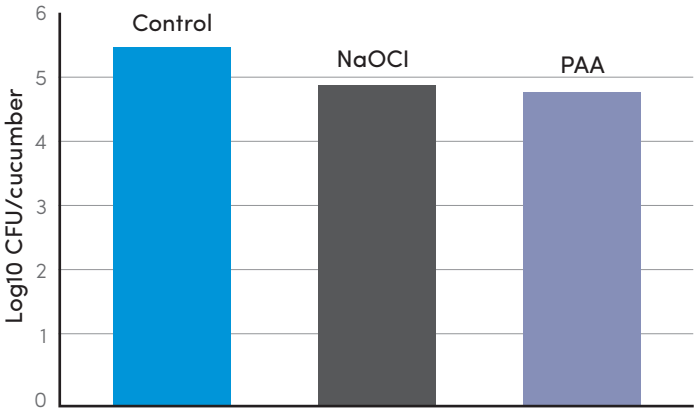
Log[10] CFU/cucumber of E. coli O157:H7 Bacteria Remaining on the Surface Subsequent to the Respective Rinses

	Mean Log[10] CFU/cucumber *(One minute)*	Mean Log[10] Reduction CFU/cucumber compared to City Water Control *(%)*
City water *(control)*	5.62	-
37.5 ppm *(as Cl_2)* NaOCl	4.9	0.72 *(80.9%)*
40 ppm PAA	4.86	0.76 *(82.6%)*

Figure 1 is a graphical representation of the data shown in Table III

FIGURE 1:

Log[10] CFU/cucumber of E. coli O157:H7 in Test Solutions



Study 1: 37.5ppm NaOCl / 40ppm PAA

The data in Table III and Figure 1 indicate that at these low concentrations of NaOCl and PAA, a one-minute rinse effects only a moderate reduction in surface-associated E. coli O157:H7 bacteria compared to the amount present following just a rinse with city water. It can also be seen that at equivalent molar concentrations of PAA and NaOCl the performance of both active ingredients in eradicating surface-associated E. coli O157:H7 bacteria is of no statistical difference. Faced with the need to achieve a better degree of microbiological control, the options were to increase the contact time, increase the temperature or increase the concentration of the active ingredients. Since the plant may not have the option of increasing the contact time, and since increasing the temperature of the rinse water is impractical, it was decided to double the concentration of active ingredients to 80 ppm PAA and 75 ppm NaOCl. Note that 80 ppm PAA represent the maximum dose allowed by the FDA under 21 CFR 173.315.

RESULTS AND DISCUSSION

Microbiological Quality of the Rinsed Cucumbers

HIGH CONCENTRATION STUDY

Table IV reports the data in terms of log10 reduction in surface-associated E. coli O157:H7 bacteria compared to the amount removed by the city water control rinse. The figures in parentheses express the results in terms of the % reduction in surface-associated bacteria over and above what was achieved with just the city water control rinse.

TABLE IV:

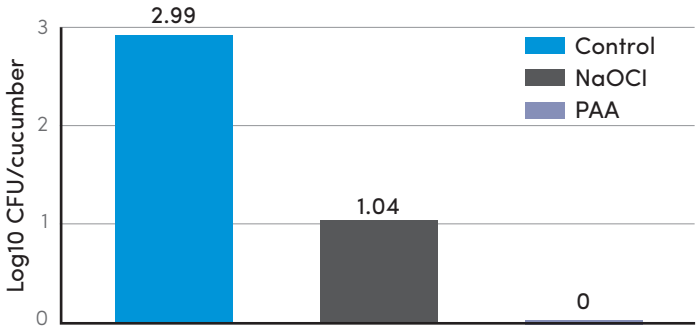
Log[10] CFU/cucumber of E. coli O157:H7 Bacteria Remaining on the Surface Subsequent to the Respective Rinses

	Mean Log[10] CFU/cucumber *(One minute)*	Mean Log[10] Reduction CFU/cucumber compared to City Water Control *(%)*
City water *(control)*	2.99	-
75 ppm *(as Cl_2)* NaOCl	1.04	1.95 *(98.8%)*
40 ppm PAA	0.0	>2.99 *(100%)*

Figure 2 is a graphical representation of the data shown in Table IV.

FIGURE 2:

Log[10] CFU/cucumber of E. coli O157:H7 in Test Solutions



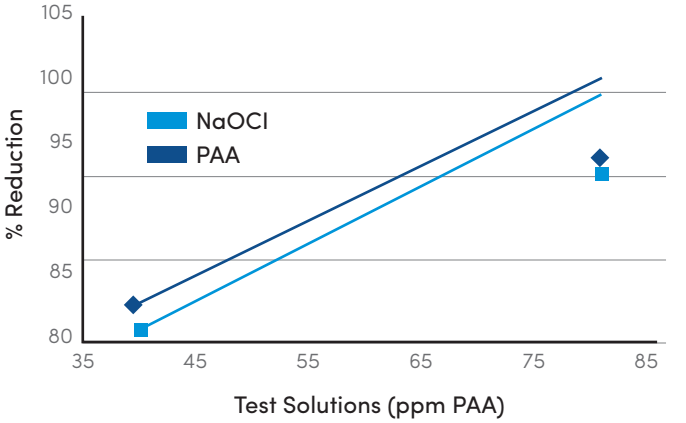
Study 2: 37.5ppm NaOCl / 40ppm PAA

As expected, at the higher concentrations used both NaOCl and PAA confer much better microbiological eradication properties than at the lower concentrations of active ingredient. On the other hand, PAA is now seen to exert significantly higher performance than NaOCl and totally eradicated all surface-associated E. coli O157:H7 bacteria affording an almost 3 log10 reduction of the microorganism per cucumber. The relatively poor performance of the NaOCl bleach solution might well be explained by adversely high pH conditions that develop when it is introduced to water (the pH of Modesto city water used was 7.67). This situation would be even more exacerbated if the source water was of naturally high pH, or if the rinse water was recycled and bleach was being continually introduced to make up for chemical demand losses. Under either scenario, the implementation of a bleach program would be improved by pH adjustment with a food grade acid such as phosphoric acid. Obviously this increases the complexity of the treatment program.

Figure 3 graphically illustrates how improved eradication of surface-associated E. coli O157:H7 varies with the concentration of active ingredients.

FIGURE 3:

% Reduction of E.coli O157:H7 As a Function of Concentration



The difference of performance between the NaOCl bleach solution and the PAA solution has important implications in commercial processing environments. Certainly, the PAA rinse outperforms NaOCl bleach at short one-minute contact times when the products are employed at high molar equivalent concentrations. These shorter time scenarios represent those typically encountered when the cucumbers are sprayed on a conveyor belt during commercial processing. If the plant is ill-equipped to lengthen the contact time in a dump or soak tank, then PAA represents a better antimicrobial safeguard to the food supply than does NaOCl bleach.

How Much Surface-Associated E. coli O157:H7 is Removed by Simple Rinsing in City Water?

Up to now the discussion has centered on the performance enhancements of NaOCl and PAA solutions in comparison to the rinse with city water alone. But the question remains:

How well does a simple city water rinse remove the surface-associated E. coli O157:H7?

This number can be calculated from the results of swabbing inoculated but unrinsed cucumbers and comparing the numbers to inoculated cucumbers rinsed in city water. **Table V** reports the data.

TABLE V:

	Average log^{10} CFU E. coli O157:H7/ cucumber
Unrinsed cucumbers	3.38
One –minute city water rinse	2.69

From **Table V**, it can be seen that washing cucumbers with 500 ml of city water for one-minute yields a plate count of log10 2.69 CFU surface-associated E. coli O157:H7 bacteria per cucumber compared to an initial amount present of log^{10} 3.38.

$$\text{\% removal by water wash} = \frac{\text{No. of E. coli O157:H7 still resident on the cucumber}}{\text{Initial No. of E. coli O157:H7 per cucumber}} \times 100$$

$$= \frac{2.69}{3.38} \times 100$$

$$= 79.6\%$$

Water alone appears to be an inefficient way of removing surface-associated E. coli O157:H7 bacteria from the cucumber, since only 79.6% can be washed into the aqueous phase after a one-minute rinse. Combined with the data in Table II, which reports the mean log10 CFU bacteria per cucumber resident on the surface after the respective rinses, it is possible to calculate the relative E. coli O157:H7 bacteria removal/eradication efficiency of each rinse program after one minute. Table IV summarizes the results.

TABLE VI:

Relative E. coli O157:H7 Removal/Eradication Effectiveness of the Respective Rinses after One Minute *(High Concentration Data)*

	City Water	75 ppm *(as Cl2)* NaOCl	80 ppm PAA
% Removal/ eradication from cucumber surface	79.6	98.8	100

Although the high concentration (75 ppm) of NaOCl appears to be a fairly effective weapon in the eradication of surface-associated E. coli O157:H7 bacteria, from Figure 2 and Table III it can be calculated there are still 10 CFU of bacteria still resident on the cucumber. Table I indicates that this would represent an infectious dose were the cucumber to have been consumed.

Microbiological Quality of the Rinse Waters

The results of measuring the viability of E. coli O157:H7 bacteria washed into the aqueous phase have important ramifications in commercial processing operations where wash and rinse water is recycled and reused. The more effective a chemical program is in eradicating aqueous phase bacteria, the less likely that rinsed cucumbers will be recontaminated by redeposition of viable bacteria present in the recycled water. Table VII records the log10 CFU/ml of the E. coli O157:H7 bacteria in the 500 ml of water used to rinse 5 cucumbers whose surfaces had been inoculated with E. coli O157:H7. The data presented is for the high concentration study for a one-minute contact time.

TABLE VII:

Log^{10} CFU/ml E. coli O157:H7 Bacteria in the Respective Rinse Waters

	Log^{10} CFU/ml *(One minute)*	**Log^{10} CFU Reduction** *(%)*
City water *(control)*	2.78	-
75 ppm *(as Cl_2)* NaOCl	0	>2.78 *(100%)*
40 ppm PAA	0	>2.78 *(100%)*

It can be seen with either chemical intervention treatment program, no viable bacteria are present in the aqueous phases. E. Coli O157:H7 bacteria washed from the surface of the cucumbers are totally eradicated by either NaOCl bleach or PAA.

CONCLUSIONS

- At low concentrations of NaOCl (37.5 ppm) and PAA (40 ppm), a one-minute rinse effects only a moderate reduction in surface-associated E. coli O157:H7 bacteria compared to the amount present following just a rinse with city water.
- At these low molar equivalent concentrations, the performance of PAA and NaOCl in eradicating surface-associated E. coli O157:H7 bacteria are of no statistical difference.
- At higher concentrations both NaOCl (75 ppm) and PAA (80 ppm) confer much better microbiological eradication properties than at the lower concentrations of active ingredient. On the other hand, PAA is now seen to exert significantly higher performance than NaOCl and totally eradicated all surface-associated E. coli O157:H7 bacteria effecting an almost 3 log10 reduction of the microorganism per cucumber.
- The relatively poor performance of the NaOCl bleach solution (at the higher concentration) might well be explained by adversely high pH conditions that develop when it is introduced to water (the pH of Modesto city water used was 7.67). This situation would be even more exacerbated if the source water was of naturally high pH, or if the rinse water was recycled and bleach was being continually introduced to make up for chemical demand losses. Under either scenario, the implementation of a bleach program would be improved by pH adjustment with a food grade acid such as phosphoric acid. Obviously this increases the complexity of the treatment program.
- The PAA rinse outperforms NaOCl bleach at short one-minute contact times when the products are employed at high molar equivalent concentration. These shorter contact time scenarios represent those typically encountered when the cucumbers are sprayed on a conveyor belt during commercial processing. If the plant is ill-equipped to lengthen the contact time in a dump or soak tank, then PAA represents a better antimicrobial safeguard to the food supply than does NaOCl bleach.
- Water alone appears to be an inefficient way of removing surface-associated E. coli O157:H7 bacteria from the cucumber, since only 79.6% can be washed into the aqueous phase after a one-minute rinse. Furthermore since viable bacteria remain in the water, in commercial processing operations where wash and rinse water is recycled and reused, the greater is the chance that the cucumbers will be recontaminated by redeposition of bacteria onto the produce.
- When the rinse water contains either PAA (80 ppm) or NaOCl (75 ppm as Cl2), E. coli O157:H7 bacteria washed from the surface of the cucumber is 100% eradicated for both chemical intervention programs, effectively eliminating the possibility of recontamination by redeposition.
- Although the high concentration (75 ppm) of NaOCl appears to be a fairly effective weapon in the eradication of surface-associated E. coli O157:H7 bacteria, there would have still been sufficient present are still to cause infection were the cucumber to have been consumed.