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Evaluation of Peroxyacetic Acid and Chlorine as Treatments for Surface Water for Post-Harvest Uses in the Produce Industry

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Abstract: Nearly half of foodborne illnesses are linked to produce and nuts, and water used for produce post-harvest activities can contribute to contamination. Surface water serves as an economical source for agricultural activities; however, exposure to the environment increases microbial risks and impacts its physicochemical characteristics. In this study, peroxyacetic acid (PAA) and chlorine (Cl) were evaluated as treatments for simulated surface water to determine their efficacy at achieving 'no detectable generic *Escherichia coli*' in 100 mL. Simulated surface water was prepared to turbidities of 2 and 100 NTU, adjusted to pH 6.5 or 8.4, equilibrated to 32 or 12 °C, inoculated with 5 logs per mL of non-pathogenic (generic) *E. coli*, and treated with Cl 25 ± 2 ppm, PAA 75 ± 5 ppm, or sterile water control (W). Dey-Engley neutralization was followed by enumeration on *E. coli*/Coliform Petrifilm at times (t) 0 to 2880 min (48 h) post-treatment. When not detected, treatments were further evaluated through enrichment in 2X Brain Heart Infusion (BHI) broth. Enrichments were streaked on MacConkey agar (MAC) to confirm *E. coli* absence. All Cl and PAA treated samples were below the test limit of detection (<5 CFU/mL), and *E. coli* was not detected in 5 mL enrichments even at t = 0 (shortly after treatment). These data suggest that Cl and PAA interventions may be effective for treating surface water for post-harvest uses.

Keywords: *Escherichia coli*; chlorine; peroxyacetic acid; produce; post-harvest; surface water

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1. Introduction

The increasing awareness of the health benefits of produce consumption has increased interest in consuming fresh produce commodities. However, produce is also increasingly associated with a foodborne disease burden for both the public health and the food industry sectors, as about 46% of foodborne illnesses with a known food source have been associated with produce and nuts, with leafy greens accounting for most of those illnesses [1]. Foodborne illnesses cost the U.S. government around $17.6 billion annually [2]. Viruses such as norovirus and bacteria, mainly *Salmonella* spp., Shiga toxin-producing *Escherichia coli*, and *Campylobacter* spp., cause the majority of foodborne illnesses in global estimation studies [3,4]. These microbial pathogens have been reported to survive in soil, water, and other environments thereby could potentially contaminate produce upon contact [5,6].

Several produce-associated foodborne disease outbreaks have been traced to the use of contaminated agricultural water. One example is a 2018 outbreak that was caused by *E. coli* O157:H7 in red leaf lettuce, green leaf lettuce, and cauliflower which resulted in 62 illnesses, 25 hospitalizations, and 2 cases of a hemolytic uremic syndrome (HUS) and was traced back to sediment in the agricultural water reservoir [7]. It is important to note that several of the recent produce-related outbreaks had unknown sources of contamination, which

calls for effective microbial contamination prevention and intervention strategies during both preharvest and post-harvest activities.

Surface water, such as pond water and rainwater catchment, is readily available to farmers and is now exploited for various agricultural and industrial activities. In 2015, 74% of total water withdrawals for different uses in the U.S. was from surface water sources, the majority of which was for public supply, irrigation, and industrial uses. Ground water was the source of the remaining withdrawn water for different uses [8]. Since surface water sources are exposed to the environment, they are considered to be unsafe for post-harvest use for fresh produce without treatment, as different studies link their microbial contamination risk to both environmental sources (floods, temperature raises, seasonal differences, etc.) and human activities, such as agricultural activities, cattle rearing, deforestation and more [9–11].

The production of fruits and vegetables is increasing [12], and there have been increased produce contamination patterns. The Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) established science-based minimum standards for safe growing, harvesting, packing and holding of covered fresh produce to be followed by covered produce farmers in order to proactively prevent produce contamination [13]. The FSMA-PSR requires no detectable generic *E. coli* (<1 CFU/100 mL) for water used in the post-harvest handling of produce or on surfaces that will contact produce post-harvest, and the water must be 'safe and of adequate sanitary quality for its intended use' [13]. Most enteric pathogens causing foodborne illnesses have a fecal-oral route and generic *E. coli* is the bacterial indicator used by the FDA to represent the potential for fecal contamination and pathogens in water [13,14].

Produce contamination during the post-harvest rinsing process can result from contact with contaminated water and cross contamination from other contaminated produce [15,16] and contaminated food contact surfaces [17]. The use of contaminated water can also lead to pathogen infiltration into produce passively through natural pores or wounds resulting from the exerted hydrostatic pressure on the produce during washing, or actively by the attachment of bacteria from water to produce followed by infiltration [18,19]. Therefore, contamination facilitated by wash water emphasizes the heightened risk that would result from using contaminated surface water in produce washing or for other post-harvest uses.

Chlorine (Cl) and peroxyacetic acid (PAA) are generally the most common and affordable chemical antimicrobial interventions used in the produce industry. To avoid cross-contamination, the antimicrobial concentration and contact time of the water treatment are crucial to reduce risk of cross contamination and potentially prevent future produce outbreaks associated with contaminated water. Previous research has shown that temperature, pH, turbidity, total organic carbon (TOC), and bacterial count affect the survival and growth of bacteria in water, and also affect the treatment efficacy of PAA and chlorine [15,20,21]. Much of the research that focuses on agricultural water as a source of microbial contamination is directed at irrigation water and less on the water used post-harvest. Most post-harvest water use studies don't reflect the use of surface water with its physicochemical characteristics in produce washing or post-harvest [22–24], likely because this use is discouraged due to the higher microbial risk associated with untreated surface water [25]. However, with groundwater sources continuing to deplete and the rising cost municipal water, produce growers are increasingly searching for other sources of water for post-harvest use, including surface water. The FSMA-PSR requires that untreated surface water may not be used as agricultural water during and after harvest, and water in these uses must have no detectable generic *E. coli* in 100 mL in addition to being 'safe and of adequate sanitary quality for its intended use'. For these reasons, it is essential to validate surface water treatments for post-harvest water use [13]. A knowledge gap currently exists regarding chemical efficacy in treating surface water for post-harvest use in the produce industry. This study aims to address this knowledge gap by evaluating the effectiveness of Cl and PAA at reducing *E. coli* populations in simulated surface water. The specific objective is to evaluate the effect of turbidity, temperature, and pH of simulated surface

water on the effectiveness of Cl and PAA at achieving the required 'no detectable generic *E. coli*' in 100 mL requirement as described by the FSMA-PSR for agricultural water used post-harvest by covered farms [13].

2. Materials and Methods

2.1. Bacterial Strains

Generic *E. coli* strains isolated from feces and purchased from the American Type Culture Collection (ATCC) were used in this study, and included ATCC 8739, ATCC 13706, and ATCC 23631. These strains were recommended for use in water and antimicrobial chemical efficacy studies [26–28]. Frozen stock cultures were streaked for isolation on Nutrient Agar plates (N.A.; Difco™, Sparks, MD, USA), incubated at 37 °C for 24 ± 2 h and a single *E. coli* colony was used for inoculum preparation (described below). Prior to initiating the study, all pure cultures were plated on MacConkey agar (MAC; Thermo Scientific™, Remel™, Lenexa, KS, USA) and *E. coli*/Coliform Petrifilm™ (EC Petrifilm, 3M™, Saint Paul, MN, USA) to document appearance and ensure proper colony counting during the inoculation study. Pure *E. coli* cultures grew pink on MacConkey agar (MAC), and blue to purplish colonies with gas bubbles grew on EC Petrifilm.

2.2. Inoculum Preparation

The working inoculum cocktail of the three strains was prepared by transferring one isolated colony of each ATCC strain into 10 mL Brain Heart Infusion broth (BHI broth; Thermo Scientific™ Oxoid, Hants, UK) tubes separately. The three tubes (one tube per strain) were incubated at 37 °C for 24 ± 2 h to achieve a concentration of 10^{8-9} CFU/mL. The separate cultured BHI broth tubes were transferred to 15-mL conical tubes and centrifuged at 4300× *g*, for 15 min at 4 °C. The supernatant was discarded, and the pellets were then resuspended separately in 10 mL Phosphate Buffered Dilution Water (PBDW; EMD Millipore Corporation, Billerica, MA, USA). The remaining equal volumes (≈27 mL total) of the three strains were then mixed in a 50-mL centrifuge tube to form a 3-strain cocktail. The inoculum was diluted 1:10 in PBDW to generate a final target concentration of ~$1.0 \times 10^{7-8}$ CFU/mL. The cocktail concentration was enumerated at the beginning and end of the trial to ensure that the *E. coli* populations had not changed during the inoculation process. To ensure that strains were used in equal proportion in the working inoculum, the concentration of each strain was separately enumerated, and the working cocktail was also enumerated by diluting in PBDW and plating in duplicate on EC Petrifilm. The EC Petrifilm plates were then incubated at 37 °C for 48 ± 4 h. Colonies (blue and purple with the gas bubble) were counted on EC Petrifilm.

2.3. Water Preparation

Simulated agricultural surface water was prepared in the lab targeting turbidity of 100 NTU and 2 NTU according to the FDA/Environmental Protection Agency (EPA) protocol for the development and registration of treatments for preharvest agricultural water [29], with modifications. Briefly, 300 mg and 2 mg of PTI Arizona test dust (T.D.; PTI Powder Technology Inc., Arden Hills, MN, USA) were each added in 2 L of DI water to make 100 NTU and 2 NTU finished turbid water, respectively. The water turbidity was determined and verified using the Hatch 2100Q Laboratory Turbidimeter (Hatch Company, Loveland, CO, USA). Next, 3.2 g of sea salt (Sigma Aldrich, Saint Louis, MO, USA) was added [target 1350–1650 mg/L total dissolved solids (TDS)] in both 2 L turbid water batches and vigorously shaken to dissolve. Contrary to the EPA protocol, the two water batches were then autoclaved at 121 °C for 15 min for sterility, due to issues with T.D. microbial contamination. Autoclaving was verified to not change the water turbidity by measuring the water turbidity before and after autoclaving. After autoclaving, 20 mg and 4 mg of Humic Acid (H.A.; Sigma Aldrich, Co., Saint Louis, MO, USA) (target of 10 mg/L and 2 mg/L TOC in the final turbid water) was added in two sterile 1 L screw cap bottles; 500 mL from the 100 NTU and 2 NTU sterile water batches were added to the

bottles, respectively for mixing, vigorously shaken for at least two min to fully hydrate and dissolve the H.A. and added back to their respective bulk (2 L) solutions. Humic acid sterility was validated on BHI agar prior to use since it was added post water autoclaving.

Each of the 2 L water batches was then separated into two equal volume batches and then standardized to achieve a final pH of 6.5 in one batch and 8.4 in the other batch using 1N HCl and/or 5N NaOH (Thermo Scientific™, Fairlawn, NJ, USA) as needed. Each of the four newly made batches (100 NTU, 6.5 pH; 100 NTU, 8.4 pH; 2 NTU, 6.5 pH; 2 NTU, 8.4 pH) was then split into two equal volumes (400 mL), resulting in a total of eight water samples. One of each set of turbidity-pH combination was equilibrated overnight (8–10 h) at 32 °C and the other at 12 °C. After overnight temperature equilibration, each final water sample was plated on BHI agar and EC Petrifilm during the preliminary work, and only on EC Petrifilm during the inoculation study, to ensure sterility before inoculation. Figure 1 shows a summarized flowchart of the inoculated water sample preparation.

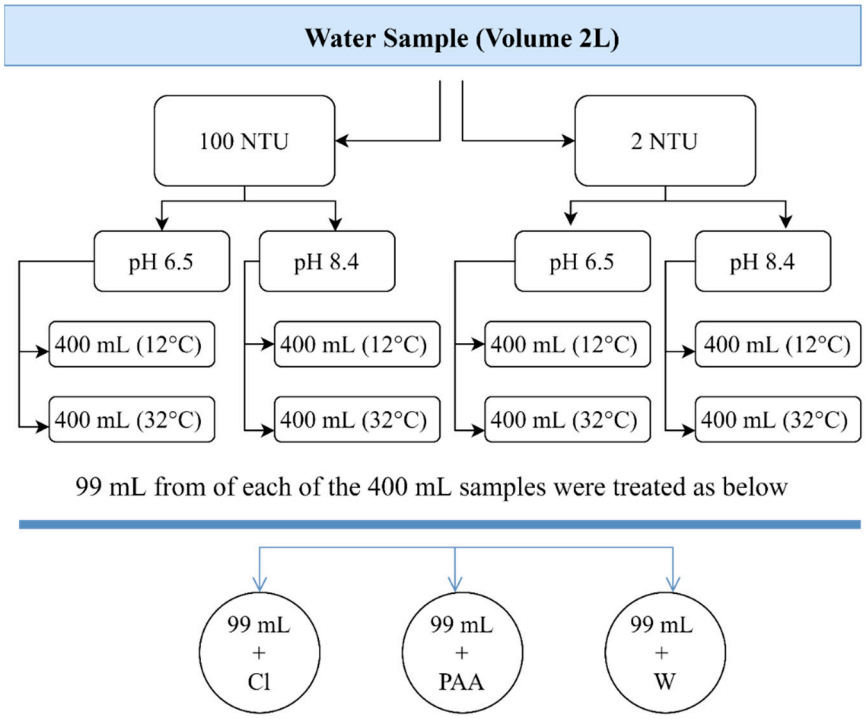

Figure 1. Flow chart of the inoculated water sample preparation and treatment at two pH and temperature levels. Six (99 mL) bottles made from one pH level were treated at the same time.

2.4. Water Inoculation

One mL was removed from each of the prepared 400 mL turbid water samples and used for sterility testing. Then, each 399 mL sample, defined by turbidity and pH of test water samples (e.g., 100 NTU-8.4-32 °C and 100 NTU-8.4-12 °C) was inoculated with 1 mL of the working inoculum to achieve a target concentration of ca. 5 log CFU/mL. The concentration of each water sample was enumerated before antimicrobial treatment to document initial *E. coli* concentration. Briefly, dilutions were made in PBDW and plated on EC Petrifilm, which were then incubated at 37 °C for 48 ± 4 h. Typical *E. coli* colonies were counted on EC Petrifilm. Following enumeration, each inoculated sample was then separated into three equal (99 mL) bottles and placed back at their respective temperature for approximately 30 min for temperature equilibration.

2.5. Antimicrobial Treatment Preparation

The two antimicrobial chemicals used were SaniDate15 (PAA; BioSafe Systems, LLC, East Hartford, CT, USA) at 75 ± 5 ppm of PAA and Ultra Clorox germicidal bleach (Cl); Clorox Professional Products Company, Oakland, CA, USA) at 25 ± 3 ppm of available

free chlorine as a final concentration in the treated water samples. The concentrations were chosen based on the EPA labels for each product, which can be easily found using the Produce Safety Alliance (PSA) Labeled Sanitizers for Produce spreadsheet [30,31], and summarizes approved sanitizers and their respective concentrations for produce. Stock solutions of PAA and Cl were prepared and were validated to achieve final concentration (as mentioned above), in the final 100 mL water samples as part of a preliminary study. The treatments were prepared in sterile, aluminum foil-covered flasks to protect against light degradation. The free chlorine concentration was confirmed using a Hanna free and total chlorine high range portable photometer (HI96734; Hanna Instruments, Woonsocket, RI, USA). The PAA treatment concentration was prepared from SaniDate15 following manufacturer recommendations, and its concentration was confirmed using the peroxyacetic acid test kit (BioSafe Systems, LLC, East Hartford, CT, USA) according to manufacturer instructions. The concentrations of both working solutions were measured at the beginning and the end of the inoculation study to ensure that no change in concentration occurred during the course of use. Sterile DI water was used as a control (W).

2.6. Antimicrobial Application and Microbial Analysis

Sample bottles were treated by adding a 1 mL aliquot of the appropriate treatment solution (Cl, PAA) or water (W) into each 99 mL bottle of simulated agricultural water. The bottles were then swirled for 10 s to evenly distribute the sanitizer and the time 0-min sample was then immediately collected (hereafter referred to as t = 0). Each sample bottle was sampled at 0 (t = 0), 5, 10, 60, 1440, and 2880 min post-treatment and enumerated for *E. coli*. Briefly, 1 mL of the treated sample was neutralized in 9 mL of Dey/Engley Neutralizing Buffer (D/E; Difco™, Sparks, MD, USA), and 5 mL were neutralized in 45 mL of D/E in Whirl-Pak® bags (Nasco, Madison, WI, USA). At each sampling point, the 1 mL aliquot was collected first (immediately), and the 5 mL aliquot was collected second. Therefore, at t = 0, the mL aliquot represents a sample collected immediately after 10 s of contact time (mixing), while the 5 mL aliquot was collected second (<1 min after the the 10 s contact time). Subsequent dilutions were made from D/E neutralized tubes using 9 mL PBDW and enumerated on EC Petrifim. The EC Petrifilm were then incubated at 37 °C for 48 ± 4 h, and the typical colonies were enumerated.

The EPA protocol calls for the use of a non-selective agar (e.g., BHI agar) for enumeration [29]. However, preliminary work demonstrated issues with contamination and questionable counts. To overcome this challenge, EC Petrifilm were validated as an effective, more selective alternative as part of a preliminary study. To the Whirl-Pak® bags containing 5 mL of sample neutralized in 45 mL of D/E, 50 mL of 2X BHI was added, resulting in 1X BHI upon dilution with the sample and D/E, and incubated at 37 °C for 24 ± 2 h for enrichment and recovery of generic *E. coli*. Because the FSMA-PSR requires 'no detectable generic *E. coli*' in 100 mL agricultural water used with produce post-harvest, the enrichment step was important for detecting generic *E. coli* that might be present, but below the limit of detection for EC Petrifilm. After incubation, the enriched BHI samples were then streaked on MAC and incubated at 37 °C for 18–24 h to determine the presence/absence of *E. coli* in treated samples at a detection limit of 20 CFU/100 mL of original sample. Any MAC plates with pink colonies were interpreted as positive for *E. coli*. Immediately following the 10 min sampling point, sample bottles were returned to their respective temperatures and stored in between treatments, and only briefly removed for each subsequent sampling point.

2.7. Statistical Analysis

All experimental procedures were replicated three times. Statistical analyses were conducted with the Statistical Analysis Software (SAS version 9.4; Cary, NC, USA). All data were analyzed as a repeated measures model using the PROC MIXED linear mixed model at significance level of 0.05. The best covariance structure was determined and used in the model. The Least Squares Means (LSMEANS) were calculated and used to identify statistical significance between individual treatments using the Tukey-Kramer

adjustment for multiple comparisons. The main effects of time, temperature, turbidity, pH and treatment, as well as all two-way interactions, were evaluated for statistical significance. Three- and four-way interactions were not included in the model to avoid potential issues with model nonconvergence. Main effects and interactions not statistically significant were removed from the model using backwards elimination.

3. Results

All Cl and PAA samples were below the detection limit (5 CFU/mL) for EC Petrifilm and *E. coli* was not detected on MAC at all time points for all Cl and PAA treated samples. This indicates that both 25 ppm of available free chlorine and 75 ppm PAA were able to achieve approximately a 5-log reduction of *E. coli* following a 10 s contact time. The main effects of time ($p = 0.0004$), temperature ($p < 0.0001$), pH ($p = 0.0010$), and treatment ($p < 0.0001$) were statistically significant. The main effect of turbidity was not statistically significant ($p > 0.05$). However, the following two-way interactions were significant: temperature × time ($p = 0.0002$), treatment × time ($p < 0.0001$), treatment × pH ($p < 0.0001$), and treatment × temperature ($p < 0.0001$). Data will be discussed according to these significant interactions.

When comparing *E. coli* populations at each temperature across time when combining all treatments (Cl, PAA, and W), a significant difference was not observed until the 1440- and 2880-min sampling points. Because *E. coli* was not detected from the PAA and Cl samples at any sampling point, the variability shown in Table 1 is the result of variability in control samples. Therefore, for the control samples held at 32 °C (with no treatment), *E. coli* populations increased at 1440- and 2880- min sampling points in comparison to the control samples held at 12 °C ($p \leq 0.05$). No significant differences in *E. coli* concentrations were observed in samples stored at 12 °C at any sampling time points.

Table 1. Average *E. coli* populations in all studied simulated agricultural surface water analyzed by storage temperature and time. The temperature × time interaction was significant ($p = 0.0002$) and does not include pH, turbidity, or treatment effects.

***E. coli* Survival (Log CFU/mL) (L.S. Means ± S.E.)**						
Temperature (°C)	**Time (min)**					
	0	5	10	60	1440	2880
12	1.8 ± 0.024^{Aa}	1.8 ± 0.026^{Aa}	1.7 ± 0.026^{Aa}	1.7 ± 0.031^{Aa}	1.7 ± 0.030^{Aa}	1.7 ± 0.031^{Aa}
32	1.8 ± 0.024^{Aa}	1.7 ± 0.026^{Aa}	1.7 ± 0.026^{Aa}	1.7 ± 0.031^{Aa}	2.0 ± 0.030^{Bb}	2.0 ± 0.031^{Bb}

Note: ^{AB} Values with different uppercase superscripts in the same row indicate significant differences between sampling points for a temperature. ^{ab} Values with different lowercase superscripts in the same column indicate significant differences between temperature at a sampling point. Interaction between temperature and time was significant ($p = 0.0002$).

Beginning at sampling point 0, *E. coli* was not detected in the Cl and PAA samples on both the EC Petrifilm and MAC. When comparing the treatments at each sampling point, the W treatment was significantly different than both the C and PAA treatments ($p < 0.0001$, whereas the Cl and PAA treatments were not statistically different ($p = 1.0000$). However, the W control samples did change over time, with a significant ($p < 0.0001$) increase of 0.3 to 0.4 log CFU/mL from time points 0, 5, 10, and 60 min in comparison to 1440 min and 2880 min (Table 2).

No effect of temperature was observed for Cl ($p = 0.3468$) and PAA ($p = 0.3468$) treatments. However, the impact of temperature was significant for W control samples ($p < 0.0001$), with an increase of 0.3 logs CFU/mL in samples stored at 32 °C compared to samples at 12 °C. When comparing each treatment at 12 °C, the W treatment was significantly different ($p < 0.0001$) than both the Cl and PAA treatments, whereas the Cl and PAA treatments were statistically the same ($p = 1.000$). The same was observed at 32 °C, where W was significantly different than Cl and PAA ($p < 0.0001$), but the Cl and PAA were statistically the same ($p = 1.0000$).

Table 2. *E. coli* populations in simulated agricultural surface water analyzed by antimicrobial treatment and time. The treatment × time interaction was significant ($p = 0.0001$) and did not include pH, turbidity, or temperature effects. Therefore, data associated with pH, turbidity, or temperature are displayed according to treatment and time.

L.S. Means ± S.E. *E. coli* Survival (Log CFU/mL)						
Treatments	**Time (min)**					
	0	5	10	60	1440	2880
Cl	0.0 ± 0.024 ^{Aa}	0.0 ± 0.026 ^{Aa}	0.0 ± 0.026 ^{Aa}	0.0 ± 0.031 ^{Aa}	0.0 ± 0.030 ^{Aa}	0.0 ± 0.031 ^{Aa}
PAA	0.0 ± 0.024 ^{Aa}	0.0 ± 0.026 ^{Aa}	0.0 ± 0.026 ^{Aa}	0.0 ± 0.031 ^{Aa}	0.0 ± 0.030 ^{Aa}	0.0 ± 0.031 ^{Aa}
W	5.3 ± 0.024 ^{Ab}	5.3 ± 0.026 ^{Ab}	5.2 ± 0.026 ^{Ab}	5.2 ± 0.031 ^{Ab}	5.6 ± 0.030 ^{Bb}	5.6 ± 0.031 ^{Bb}

Note: *E. coli* not detected is indicated as 0.0 log CFU/mL. ^{AB} Values with different uppercase superscripts in the same row indicate significant differences between sampling points for a treatment. ^{ab} Values with different lowercase superscripts in the same column indicate significant differences between treatments at a sampling point. Interaction between treatment and time was significant ($p < 0.0001$).

Although the pH x treatment interaction was significant ($p < 0.0001$), there was no effect of pH on the Cl treatment ($p = 1.0000$) and PAA treatment ($p = 1.0000$). However, the W treatment was significantly different ($p < 0.0001$) at each pH, with a mere increase of 0.1 log CFU/mL detected in the 6.5 pH (5.3 log CFU/mL) compared to the 8.4 pH (5.4 log CFU/mL). When comparing each treatment at pH 6.5 and 8.4, the Cl and PAA treatments were statistically similar ($p = 1.0000$), while the W treatment was significantly different ($p < 0.0001$) than both the Cl and PAA treatments.

4. Discussion

This study provides initial insights for farmers and the produce industry regarding the efficacy of two chemical interventions that may be efficacious for treating surface water sources for post-harvest use in the produce industry. The objective of this study was to evaluate the efficacy of 75 ppm PAA and 25 ppm of free chlorine at reducing *E. coli* populations in simulated surface water with varying turbidity, pH, and temperatures to satisfy the FSMA-PSR requirement of 'no detectable generic *E. coli*' in 100 mL agricultural water used post-harvest. It should be noted that the FSMA-PSR specifies 'no detectable generic *E. coli*' in a 100 mL sample; however, the EPA protocol followed in this study did not use a 100 mL sample for testing at each time point and only a 5 mL sample was enriched for presence/absence testing. While the data presented herein suggest 'no detectable *E. coli*' following treatment, they are limited to the 5 mL of water sampled at each time point. Regardless of turbidity, pH, and temperature, both PAA and Cl were able to achieve 'no detectable generic *E. coli*' in 5 mL of simulated surface water, beginning at the t = 0 sampling point, which was collected immediately following a 10 s mixing period and followed by neutralization in D/E. *E. coli* populations in the water control sample did not decline. These data generally suggest that pH (6.5 & 8.4), temperature (12 °C & 32 °C), holding time (0 to 2880 min), and turbidity (2 & 100 NTU) had no significant impact on the efficacy of PAA (75 ppm) and Cl (25 ppm) at reducing generic *E. coli* in simulated surface water.

The lack of influence of pH 6.5 is strongly supported by other publications placing it in the optimal pH ranges of efficiency for both PAA and chlorine sanitizers [32,33]. A pH of 8.4 was also used because it is slightly basic, which can reportedly affect the efficacy of chlorine sanitizers by converting the most efficient form of chlorine (HOCl) into the less effective form (OCl^-) [32,34]. However, in this study a simulated surface water of pH 8.4 had no effect on the overall efficacy of either the Cl or PAA. A study [35] also reported similar log reductions of *E. coli* at a similar pH, with a ~6 log reduction of *E. coli* in hard water of about 500 ppm hardness, at pH of 8.5 using 12 ppm of hypochlorite in 15 s. The difference in time required between the cited report [35] and the present study may be attributed to the higher dosage of chlorine (25 ppm) that was used in the present study. Also, another study [34] demonstrated that it is only at pH > 8.5 that OCl^- concentration begins to exceed that of HOCl, which would then shift the equilibrium to the less effective

form and, therefore, reduce the efficacy. Conversely, PAA has a broader pH efficiency, which includes the pH of 8.4 tested in this study [33].

Simulated surface water turbidity was neither a significant main effect ($p > 0.05$), nor contributed to any significant two-way interactions, which suggests that turbidity values of 100 NTU and 2 NTU do not impact the efficacy of Cl (25 ppm) and PAA (75 ppm) when used as antimicrobial interventions for reducing generic *E. coli* when all other variables are held constant. Total organic content associated with turbidity has been found to be the more likely cause of chlorine demand than turbidity [36] when water was filtered losing 99% of its turbidity but keeping 90% of its initial unfiltered chlorine demand. The TOC used in this study was very low (10 mg/L and 2 mg/L respective to turbidity), hence it didn't affect the efficiency of Cl or PAA. Also, a previous study [37] observed no significant effect of TOC (500 and 750 mg/L) on the efficacy of PAA at 75 ppm. However, this also contradicts a number of studies that reported that organic matter content and turbidity significantly impact antimicrobial efficacy of both PAA and Cl [21,36,38–40], with Cl generally more impacted than PAA [40]. Most of the above studies that reported a significant impact of turbidity and organic content on the effectiveness of both Cl and PAA were either evaluating: (1) a very high organic content (e.g.,: chemical oxygen demand (COD): 500–1500 mg O_2/L) or (2) a very low concentration of antimicrobial treatment (e.g., 4.5–6 mg/L PAA). A concentration of 50 ppm PAA or greater is reportedly not affected by organic content [41]. The concentrations used in this study (25 ppm of free available chlorine and 75 ppm of PAA) followed the EPA label of sanitizers use in produce handling [30,31]. The PAA and Cl concentrations were most likely reduced by organic matter, but if the starting concentration is high enough to quench the organic content demand, leaving enough residual disinfectant concentration, then overall treatment efficacy is not significantly impacted by turbidity and organic matter [38,39,42].

A study [43] demonstrated that washing produce, such as lettuce, will over time significantly increase the turbidity, as well as the total organic content of the wash water, which will then reduce the available Cl concentration as well as Cl efficacy. Hence, even though the total turbidity and organic content (TOC) of the water used in the present study (100 NTU and 2 NTU, and 10 mg/L and 2 mg/L respectively) didn't affect the efficacy of both Cl and PAA for the purpose of surface water disinfection, if the treated surface water is to be used in produce washing, then the residual concentrations of both treatments should be studied using different sources of water and with varied organic content.

E. coli reductions achieved by both Cl and PAA were statistically significant at both 12 °C and 32 °C when compared to the water control samples ($p < 0.0001$), and a difference in efficacy was not observed for Cl or PAA when comparing each individual treatment at 12 °C and 32 °C ($p = 0.3468$) (Table 1). This suggests that both 75 ppm PAA and 25 ppm Cl would be individually effective at a range of temperatures, indicating they are both effective for treating surface water sources during different seasons (i.e., water temperatures). The efficacy of antimicrobial interventions generally increases as the temperature increases; however, the present study was effective at achieving no detectable *E. coli* in 5 mL of simulated surface water at the 12 °C temperature, indicating efficacy also at a cooler temperature. When comparing Cl efficacies at 4 °C and 50 °C, a study [44] found comparable antimicrobial efficacy of chlorine at both temperatures. Granted, contrary to our study, a previous study [44] looked at the microbial disinfection on carrots instead of water, and the antimicrobial effect on produce is known to be smaller than in the wash water. On the contrary, using smaller concentrations (2 mg/L), one of the studies [45] observed greater water disinfection when the temperature increased from 12 °C to 25 °C in secondary effluent water. The difference in other physicochemical characteristics, such as pH and organic content, as well as the higher concentrations of antimicrobial interventions used in the present study, might explain why temperature did not significantly impact treatment efficacy.

The present study resulted in no detectable *E. coli* in 5 mL of simulated surface water at all sampling points, including t = 0 (less than 1 min), after treatment of simulated surface

water by both Cl and PAA (inoculated with ~5 log CFU/mL) (Table 2). The Cl and PAA were mixed into each water sample for 10 s prior to collecting the t = 0 sampling point and enumerating generic *E. coli* populations on EC Petrifilm. Because all MAC plates were also negative for *E. coli*, the data presented herein suggest that *E. coli* is rapidly killed by both Cl and PAA in 5 mL of simulated surface water. It is important to mention that the 1 mL aliquot for D/E neutralization and plating on EC Petrifilm was removed first at each sampling point and the 5 mL was subsequently transferred to a bag containing 45 mL D/E. Therefore, it was likely within 30–60 s (for enrichment) after each sampling point that the 5 mL aliquot was neutralized. Regardless, *E. coli* was rapidly killed within the first minute of exposure. This correlates to a study [35], that observed a reduction of 6 logs CFU/mL of *E. coli* from hard water (500 ppm) in 15 s using 12 ppm sodium hypochlorite. Two previous studies, one [46] observed greater than a 4 log reduction of *E. coli* in 15 min using 6 ppm of PAA, and another [47] observed over a 3 log reduction in coliforms following a less than 4 min contact time using 15 ppm PAA. This rapid kill reported in the literature and the present study is likely associated with the high starting concentration for both PAA and Cl. A study [46] observed a reduction in the contact time required for increased PAA efficiency as concentration increased, with only 15 min needed by 6 mg/L concentration to achieve a >4 log reduction of *E. coli* compared to the 25 min required by 3 mg/L to achieve ~1 log reduction of *E. coli*.

The rapid biocidal activity (~5 log reductions after a 10 s contact time) of both the PAA and Cl treatments is only associated with the ATCC reference strains used in this study. Studies report that the efficacy of chemical interventions may be different depending on the pathogen or microorganisms, including either a phenotypic or genotypic resistance [48,49]. Similarly, studies [46,50] reported that environmental *E. coli* strains demonstrated increased resistance when compared to their reference strains. When microorganisms are exposed to harsh environments they develop new mechanisms of defense, which may include genetic alterations, such as mutations, gene transfer, expression of silent genes; or phenotypic alterations, such as biofilm formation, and others that aren't as well understood [51,52].

5. Conclusions

This study found that PAA and Cl rapidly killed *E. coli* in simulated surface water. The lack of *E. coli* recovery from 5 mL samples enriched and streaked to MAC suggest that *E. coli* were eliminated at the t = 0 (less than 1 min) sampling point to less than 1 CFU in 5 mL (−0.7 logs) or <20 CFU in 100 mL (1.3 logs). Therefore, this study concludes that both PAA and Cl were able to achieve reductions in *E. coli* of ~6 log (5 logs/mL–(−0.7 logs/mL) = 5.7 logs/mL) within the brief mixing period. Efficacy of PAA and C was not impacted by time, turbidity, temperature, and pH, as *E. coli* was not detected in all treated samples at the t = 0 sampling point.

Based on the results described herein, this study demonstrates that PAA (75 ppm) and Cl (25 ppm) are effective antimicrobial interventions for treating surface waters of a variety of physicochemical characteristics. According to the utilized FDA/EPA protocol (with modifications), because these treatments were able to reduce approx. 5 log CFU/mL of generic *E. coli* in water representing a variety of physicochemical characteristics (temperature, pH and turbidity), these treatments are effective to meet EPA expectations (achieving >3-log reduction) for use as surface water interventions [29]. Furthermore, because PAA and Cl reduced the concentration of *E. coli* to the point of not being detected from a 5 mL sample (approximately 6-log reduction), these data suggest that PAA and Cl may be effective for treating surface water that will be used for post-harvest uses in the produce industry, as required by the FSMA-PSR.

However, it cannot be overemphasized enough that this study is somewhat limited in scope because only two temperatures, two pH levels, two turbidity levels, and two treatments were evaluated against three strains of generic *E. coli* inoculated into simulated (laboratory prepared) surface water. Furthermore, the qualitative criterion of "safe and of adequate sanitary quality for its intended use" was not evaluated. Therefore, these

data cannot be extrapolated to all surface water conditions and/or microorganisms. As an example, the organic matter in the simulated surface water, which can greatly influence antimicrobial intervention efficacy, was low in the present study. Hence, further research must be completed with the Cl and PAA treatments in a variety of surface waters that represent different organic matter levels/types to determine if these treatments at the same concentration are effective when used for naturally occurring surface water sources. It is also important to evaluate these chemical interventions at their respective concentrations against wild-type microflora in a variety of surface water sources to ensure efficacy.

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