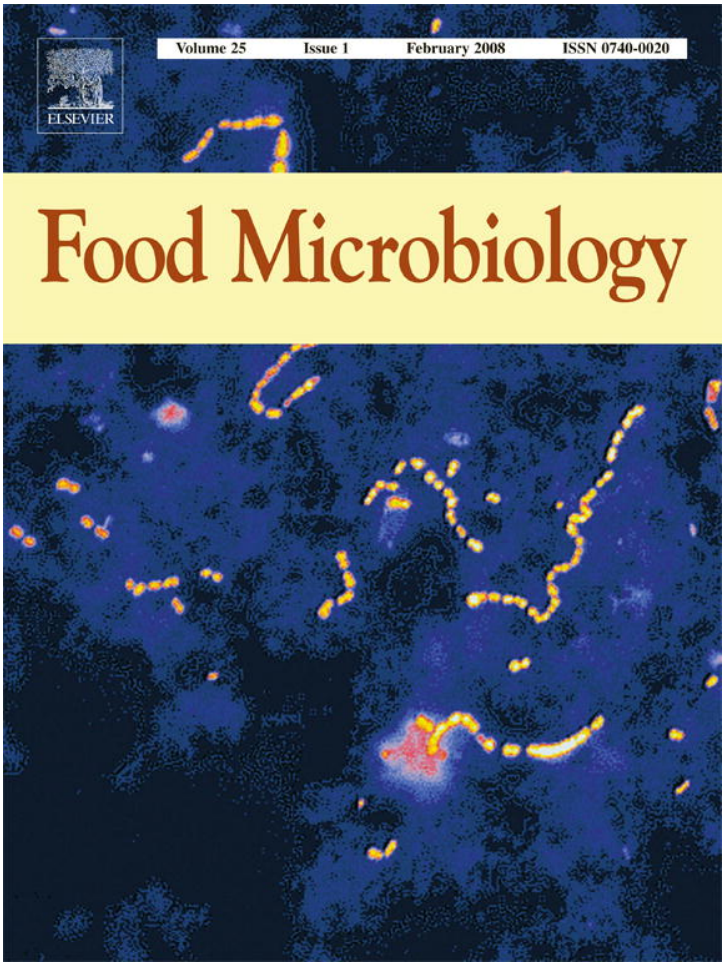

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Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water

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Abstract

Food safety issues and increases in food borne illnesses have promulgated the development of new sanitation methods to eliminate pathogenic organisms on foods and surfaces in food service areas. Electrolyzed oxidizing water (EO water) shows promise as an environmentally friendly broad spectrum microbial decontamination agent. EO water is generated by the passage of a dilute salt solution (~1% NaCl) through an electrochemical cell. This electrolytic process converts chloride ions and water molecules into chlorine oxidants (Cl_2, HOCl/ClO^-). At a near-neutral pH (pH 6.3–6.5), the predominant chemical species is the highly biocidal hypochlorous acid species (HOCl) with the oxidation reduction potential (ORP) of the solution ranging from 800 to 900 mV. The biocidal activity of near-neutral EO water was evaluated at 25 °C using pure cultures of *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis*. Treatment of these organisms, in pure culture, with EO water at concentrations of 20, 50, 100, and 120 ppm total residual chlorine (TRC) and 10 min of contact time resulted in 100% inactivation of all five organisms (reduction of 6.1–6.7 $\log_{10}$ CFU/mL). Spray treatment of surfaces in food service areas with EO water containing 278–310 ppm TRC (pH 6.38) resulted in a 79–100% reduction of microbial growth. Dip (10 min) treatment of spinach at 100 and 120 ppm TRC resulted in a 4.0–5.0 $\log_{10}$ CFU/mL reduction of bacterial counts for all organisms tested. Dipping (10 min) of lettuce at 100 and 120 ppm TRC reduced bacterial counts of *E. coli* by 0.24–0.25 $\log_{10}$ CFU/mL and reduced all other organisms by 2.43–3.81 $\log_{10}$ CFU/mL.

Keywords: Electrolyzed oxidizing water; *Escherichia coli*; *Salmonella typhimurium*; *Staphylococcus aureus*; *Listeria monocytogenes*; *Enterococcus faecalis*; *E. coli*

1. Introduction

Problems related to food safety are an important public health issue. Many plant and animal pathogens pose serious concerns within the agricultural community due to their high degree of contagion and resulting high mortality rates (Cupp et al., 2004). Exposure to food borne pathogens results in approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths in US each year (Mead et al., 1999) and food borne illnesses associated with produce have increased 5.3% from 1973 to 1997 (Sivapalasingam et al., 2004). In addition to illness and loss of human life, the US economic environmental loss due to microbial pathogens that cause human diseases is estimated at 6.5 billion US dollars per year (Pimentel et al., 2001). The recent contamination of spinach with *Escherichia coli* resulted in three deaths, numerous hospitalizations, and economic losses to the fresh greens industry estimated at 150 million dollars (Hileman, 2006). In addition to *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis* are common food borne pathogens that can cause illness and death (Mead et al., 1999).

Food safety issues and increases in food borne illnesses have promulgated the development of new sanitation

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methods to eliminate pathogenic organisms on foods. Electrolyzed oxidizing water (EO water) shows promise as an environmentally friendly broad spectrum microbial decontamination agent. EO water is generated by the passage of a dilute salt solution (~1% NaCl) through an electrochemical cell where the anode and cathode are separated by a diaphragm. This electrolytic process facilitates the conversion of chloride ions and water molecules into chlorine oxidants (Cl_2, HOCl/ClO^-), within the anode chamber. At an acidic to neutral pH, the predominant chemical species is the highly biocidal hypochlorous acid species (HOCl) with the oxidation reduction potential (ORP) of the solution ranging from 800 to 1000 mV.

Research conducted by others using acidic EO water indicates that these solutions show promise as a microbial decontamination agent for use in hospitals, food preparation, water purification, and for control of foliar diseases of plants (Park et al., 2004; Buck et al., 2002, 2003; Al-Haq et al., 2002; Venkitanarayanan et al., 1999a, b). The use of acidic EO water has limited potential for long-term applications due to its strong acidity (pH <3). At this pH, dissolved Cl_2 gas can be rapidly lost due to volatilization, decreasing the biocidal effectiveness of the solution over time (Len et al., 2000) and creating a human health and safety issue. The high acidity of the solution may adversely affect equipment and surfaces by causing corrosion and may be phytotoxic to plants.

Near-neutral solutions of EO water (pH 6.5; ORP = 800–900 mV) contain primarily hypochlorous acid (HOCl) (≈95%), the hypochlorite ion (ClO^-) (≈5%), and trace amounts of Cl_2. The high ORP of the solution disrupts the outer membrane, thus facilitating the transfer of HOCl across the cell membrane, resulting in further oxidation of intracellular reactions and respiratory pathways such as the glutathione disulfide-glutathione (GSSG/2GSH) cellular redox couple (Liao et al., 2007). The application of EO water at a near-neutral pH minimizes human health and safety issues from Cl_2 off-gassing, reduces corrosion of surfaces, and limits phytotoxic side effects while maximizing the application of the hypochlorous acid species. The objectives of this study were to evaluate the effectiveness of using near-neutral EO water for inactivation of bacteria, its potential for use in reducing bacterial counts during post-harvest rinsing applications, and as a microbial decontaminant of surfaces in food service areas.

2. Methods and materials

2.1. Preparation of near-neutral electrolyzed oxidizing water

Near-neutral EO water was generated using an EcaFlo® system (model C101; Integrated Environmental Technologies, Little River, SC) equipped with a C-100 electrolytic cell operating at 20 V, 50 A, and 79.5 L/h. The C-100 cells are constructed of titanium, ceramic, and plastic. The anode and cathode of the C-100 cells are separated by a ceramic diaphragm, which allows for the separate production of oxidizing anolyte solutions (EO water) and reducing catholyte solutions. The EO water was generated using a continuous supply of room temperature dilute salt water (~1%NaCl in tap water). The patented EcaFlo® system generates near-neutral (pH 6.5–6.7) EO water while maintaining a high oxidation/reduction potential (ORP = 800–900 mV). The pH and ORP were measured using an Oakton 110 series meter equipped with pH and ORP electrodes (model #s WD-35615-24 and 35805-15, Oakton Instruments, Vernon Hills, IL). At this pH range (6.5–6.7), chemical speciation calculations indicate that the predominant chlorine species (96%) is hypochlorous acid (HOCl) (Faust and Aly, 1998). The concentration of total residual chlorine (TRC) species was determined using iodometric titration (Method 8209; detection limit = 0.1 mg/L, HACH Co., Ames, IA).

2.2. Preparation of inoculums

Freeze-dried (Kwik-Stik™) cultures of *E. coli* (designated as O157:H7, ATCC 35150), *S. typhimurium* (ATCC 13311), *S. aureus* (ATCC 6538), *L. monocytogenes* (ATCC 7644), and *E. faecalis* (ATCC 29212) were obtained from MicroBioLogics® (St. Cloud, MN). Individual cultures were hydrated according to manufacturer's directions and grown in BD™ BBL™ trypticase™ soy broth at 35 °C for 24 h. The viable cell count of each culture was verified using serial dilution and heterotrophic plate count/spread plate methodology using BBL™ standard methods agar and incubated at 25 °C for 24 h. The inoculum's strength in each culture was ~10^8 CFU/mL.

2.3. Procedure for EO treatment of pure cultures

A volume of 100 μL of each stock culture (~10^8 CFU/mL) was combined with 9.9 mL of sterile water or 9.9 mL of EO water, (20, 50, 100, or 120 ppm TRC; pH 6.5–6.7; ORP = 800–900 mV), for a final cell count of 10^6 CFU/mL per treatment. The treatments were vortexed and incubated at 25 °C for 10 min. Following 10 min treatments, experiments were stopped by diluting with sterile phosphate buffer solution (1/100). Following this dilution, 100 μL of each treatment was plated on to BBL™ standard methods agar and incubated at 35 °C for 48 h. The colonies were enumerated using heterotrophic plate count methodology. Each treatment was performed in triplicate. A broth-enrichment experiment was conducted to detect the presence of low numbers of survivors that would not be detected by direct plating. A volume of 100 μL of each treatment solution was combined with 14.9 mL of BD™ BBL™ trypticase™ soy broth and incubated at 35 °C for 48 h. Following enrichment, each broth solution was vortexed and 100 μL of each broth treatment was plated on to BBL™ standard methods agar and incubated at 35 °C for 48 h. The colonies were enumerated using

heterotrophic plate count methodology. Each treatment was performed in triplicate.

2.4. Preparation and inoculation of lettuce and spinach leaves

Pre-packaged iceberg lettuce and spinach were purchased from a local grocery store and maintained at 4 °C prior to testing. Lettuce and spinach leaves were surface-sterilized by immersion in 70% methanol for 30 s, rinsed in sterile distilled water, and allowed to air-dry (Buck et al., 2002). Sterilized leaves were inoculated by pipetting 100 μL of $\sim 10^7$ CFU/mL bacterial suspension on to the surface of each leaf, which resulted in a bacterial count of $\sim 10^6$ CFU applied to a leaf surface area of 4 cm^2. The inoculated leaves were placed in a laminar flow safety hood (25 °C; 90% humidity) for 4 h to allow for bacterial attachment to the leaf surfaces. Previous studies indicate that bacteria attach to leaf surfaces within 1 h of inoculation (Yang et al., 2003). Each bacterium was tested separately and each treatment was performed in triplicate.

2.5. Inactivation of bacteria rinsed from inoculated leaves

Inoculated leaves were placed individually in sterile plastic beakers containing 50 mL sterile deionized water (control) or EO water (4, 20, 50, 100, or 120 ppm TRC, pH 6.3–6.5; ORP = 800–900 mV). The leaves were agitated for 10 min at 25 °C. Immediately following treatment, the leaves were individually rinsed in 50 mL of sterile deionized water for 2 min at 25 °C. These rinse water solutions were individually plated (100 μL) onto BBL™ standard methods agar and incubated at 35 °C for 48 h. The colonies were enumerated using heterotrophic plate count methodology. Each treatment was performed in triplicate. The percent reduction of bacteria washed from the leaf surfaces and inactivated by EO, as measured by a 10 min EO dip and a sterile water rinse was calculated relative to the control using the following formula:

$$\%\ \text{reduction} = 100 \times \frac{\text{Control}_{\text{CFU/mL}} - \text{Treatment}_{\text{CFU/mL}}}{\text{Control}_{\text{CFU/mL}}}.$$

2.6. Residue experiment

Lettuce and spinach leaves were immersed in tap water, EO water (4, 20, 50, 100, or 120 ppm TRC; pH 6.3–6.5; ORP = 800–900 mV), or 0.5% bleach and then air-dried for 4 h. The leaves were rinsed in 25 mL deionized water for 10 min. The rinse water was analyzed for TRC as a measure of the residue left on the leaves following treatment.

2.7. EO treatment of surfaces

Surfaces in food service and other common areas in two schools were wiped with a sterile cotton swab. Any organisms on the swabs were transferred onto plates of BBL™ standard methods agar using the streak plate method (swab 1). These surfaces were sprayed with EO water (TRC = 278–310 ppm; ORP = 872–885 mV; pH 6.38) and allowed to air-dry (15–30 min). The surfaces were then re-swabbed in a different region of the treatment area and plated (swab 2). The treatment area was 900 cm^2 and it was assumed that bacteria were present on the surfaces in a homogeneous distribution. The plates were incubated at 35 °C for 48 h. Percent bacterial growth on the plates was determined by placing a grid of known surface area over the plate and counting the grids that were covered with growth. The percent reduction of bacteria on the surfaces was calculated using the following formula:

$$\%\ \text{reduction} = 100 \times \frac{\text{Swab 1}_{\%\ \text{growth}} - \text{Swab 2}_{\%\ \text{growth}}}{\text{Swab 1}_{\%\ \text{growth}}}.$$

2.8. Statistical analysis

Statistical calculations were performed using SigmaStat 3.5 (SysStat Software, Inc., Richmond, CA). The calculations included mean, standard deviation, and Kruskal–Wallis one-way analysis of variance on ranks to determine statistically significant differences.

3. Results

3.1. Inactivation of bacteria in pure culture using near-neutral EO water

The biocidal activity of near-neutral EO water (ORP = 800–900 mV; pH 6.3–6.5) was evaluated at 25 °C using pure cultures of *E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes*, and *E. faecalis*. At time zero, the control and treatment groups for all five organisms contained bacterial counts of approximately 6 $\log_{10}$ CFU/mL. Treatment of organisms with 4 ppm TRC and 10 min of contact time did not effectively eliminate bacterial growth. Ten minutes of contact time resulted in 100% inactivation (reduction of 6.1–6.7 $\log_{10}$ CFU/mL) of all five organisms at concentrations of 20, 50, 100, and 120 ppm TRC, as evidenced by no detectable survivors via direct plate and negative broth enrichments (Fig. 1).

3.2. Reduction of bacteria on spinach and lettuce leaves

Variable results were obtained from the 10 min dip treatment of spinach and lettuce leaves using different concentrations of near-neutral EO water and subsequent sterile water rinse. Dipping inoculated spinach leaves in 4 ppm TRC reduced bacterial counts by 0.52–3.77 $\log_{10}$ CFU/mL for all five organisms. Bacterial counts were reduced by 2.14–4.97 $\log_{10}$ CFU/mL for the 20 and 50 ppm TRC treatments. A 4.0–5.0 $\log_{10}$ CFU/mL reduction was observed in the 100 and 120 ppm TRC treatments, for all

organisms tested (Table 1). Treatment of lettuce leaves at 4 ppm TRC resulted in a 0.1 $\log_{10}$ CFU/mL reduction of *E. coli* and 0.1–1.67 $\log_{10}$ CFU/mL reduction for all other test organisms. Lettuce dipped in 20 and 50 ppm TRC reduced *E. coli* counts by 0.13–0.15 $\log_{10}$ CFU/mL and all other organisms were reduced 1.43–2.98 $\log_{10}$ CFU/mL. Dipping of lettuce at 100 and 120 ppm TRC resulted in a 0.24–0.25 $\log_{10}$ CFU/mL reduction of *E. coli* and reduced all other organisms by 2.43–3.81 $\log_{10}$ CFU/mL (Table 2).

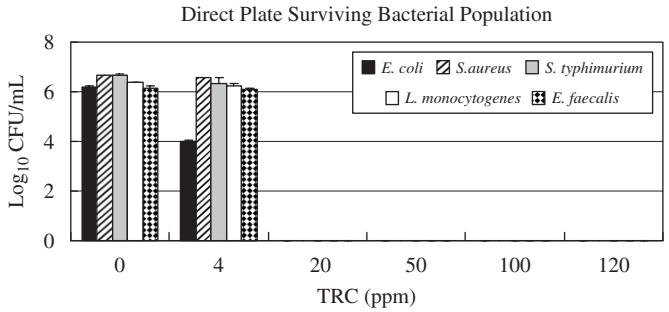

Fig. 1. Inactivation of *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis* ($n = 3$). Zero values at concentrations of 20, 50, 100, and 120 ppm TRC indicate no detectable survivors as evidenced by direct plate count and negative broth enrichment. The initial bacteria populations were reduced by 6.1–6.7 $\log_{10}$ CFU/mL.

3.3. EO residue on spinach and lettuce leaves

Treatment of spinach and lettuce leaves with near-neutral EO water did not leave any significant ($p = 0.416$) residue on the leaves relative to a water rinse. The 0.5% bleach treatment resulted in a significantly higher TRC residue on the spinach (1 ± 1.3 ppm TRC; $p = 0.019$) and

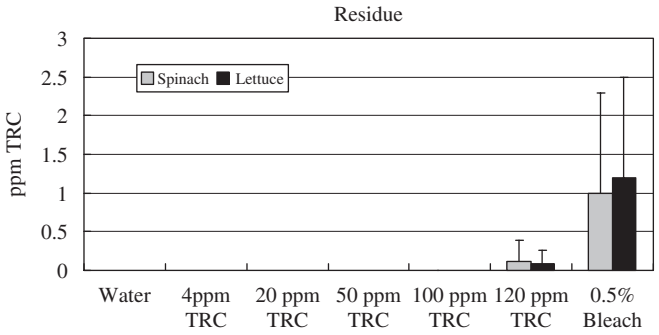

Fig. 2. Analysis of TRC residue on spinach and lettuce leaves rinsed in water, near-neutral EO water, or 0.5% bleach. EO water at concentrations of 4, 20, 50, 100, and 120 ppm TRC did not leave any significant residue on the leaves, relative to the water control ($p = 0.416$). The 0.5% bleach treatment resulted in a significantly higher TRC residue on the spinach and lettuce ($p \leqslant 0.019$) leaves relative to the water and EO rinses. Five replicates per treatment group. Kruskal–Wallis one-way analysis of variance on ranks using SigmaStat 3.5.

Table 1
Bactericidal activity of EO water during washing of inoculated spinach leaves as measured by a 10 min EO dip and a sterile water rinse

TRC (mg/L)	Surviving population[a] ($\log_{10}$ CFU/mL) in rinse water				
	E. coli	*S. aureus*	*S. typhimurium*	*L. monocytogenes*	*E. faecalis*
0 (control)	4.07 ± 0.06[a]	4.62 ± 0.25	5.00 ± 0.19	4.97 ± 0.30	4.33 ± 0.35
4	3.63 ± 0.2 (48–81)[b]	4.10 ± 0.17 (66–75)	1.59 ± 3.46 (0–100)	1.20 ± 2.08 (91–100)	1.43 ± 2.48 (0–100)
20	2.82 ± 0.38 (91–97)	2.58 ± 2.24 (60–100)	2.86 ± 0.81 (94–99)	2.03 ± 0.45 (98–99)	1.47 ± 0.58 (98–99)
50	1.45 ± 1.29 (96–99)	1.15 ± 1.00 (99–100)	2.72 ± 0.18 (98–99)	ND[c] (100)	ND (100)
100	ND (100)	ND (100)	1.07 ± 1.10 (99–100)	ND (100)	ND (100)
120	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)

[a]Surviving population ($\log_{10}$ CFU/mL) values are the means of three measurements ± standard deviation.
[b]Values in parentheses represent the range of percent (%) reductions of bacterial populations washed from spinach surfaces as measured by a 10 min EO dip and a sterile water rinse. The percent reductions are calculated relative to the survival of bacteria in the control rinse (0 mg/L TRC).
[c]Indicates no detectable survivors via direct plating procedure.

Table 2
Bactericidal activity of EO water during washing of inoculated lettuce leaves as measured by a 10 min EO dip and a sterile water rinse

TRC (mg/L)	Surviving population[a] ($\log_{10}$ CFU/mL) in rinse water				
	E. coli	*S. aureus*	*S. typhimurium*	*L. monocytogenes*	*E. faecalis*
0 (control)	4.25 ± 0.02[a]	4.65 ± 0.16	5.25 ± 0.10	4.52 ± 0.64	4.49 ± 0.20
4	4.19 ± 0.09 (0–30)[b]	4.62 ± 0.41 (33–40)	4.42 ± 0.10 (85–86)	4.01 ± 0.30 (16–74)	2.83 ± 2.46 (80–100)
20	4.11 ± 0.09 (11–44)	3.22 ± 0.75 (61–99)	3.84 ± 0.75 (84–99)	1.53 ± 1.39 (91–100)	1.69 ± 1.81 (90–99)
50	4.10 ± 0.02 (27–31)	1.86 ± 1.83 (90–100)	2.35 ± 2.04 (94–98)	1.99 ± 1.73 (80–100)	1.89 ± 1.67 (86–99)
100	4.00 ± 0.007 (34–54)	1.96 ± 1.83 (97–99)	1.91 ± 1.83 (96–100)	2.09 ± 1.85 (47–100)	1.11 ± 1.16 (97–99)
120	4.00 ± 0.10 (32–57)	2.25 ± 1.96 (99–100)	2.25 ± 1.96 (98–99)	0.87 ± 1.5 (93–100)	0.68 ± 1.18 (90–99)

[a]Surviving population ($\log_{10}$ CFU/mL) values are the means of three measurements ± standard deviation.
[b]Values in parentheses represent the range of percent (%) reductions of bacterial populations washed from lettuce surfaces as measured by a 10 min EO dip and a sterile water rinse. The percent reductions are calculated relative to the survival of bacteria in the control rinse (0 mg/L TRC).

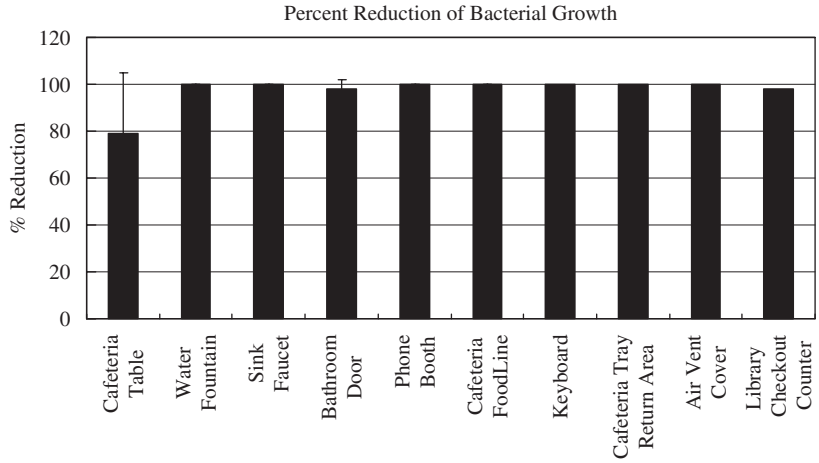

Fig. 3. Percent reduction of bacterial growth on surfaces in 2 schools. $N = 3$ for cafeteria table, water fountain, sink faucet, bathroom door, phone booth, and cafeteria food line. $N = 1$ for keyboard, cafeteria tray return, air vent cover, and library checkout counter.

lettuce (1.2 ± 1.2 ppm TRC; $p < 0.001$) leaves relative to the water and EO rinses (Fig. 2).

3.4. Treatment of surfaces in food service areas using near-neutral EO water

A variety of surfaces in food service and other common contact areas of two schools were sprayed with near-neutral EO water to evaluate its ability to reduce bacterial populations on surfaces. Treatment of surfaces with EO water containing 278–310 ppm TRC (pH 6.38) resulted in 79–100% reduction of microbial growth (Fig. 3).

4. Discussion

The effectiveness of near-neutral EO water to reduce or eliminate microorganisms in pure culture, on structural surfaces in food service areas, and on food surfaces has been assessed using five organisms. These organisms (*E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes*, and *E. faecalis*) are common food borne pathogens reported to be present on meats, poultry, fish, vegetables, and food processing equipment (Mead et al., 1999). In the present work, treatment of these organisms with near-neutral EO water (20, 50, 100, and 120 ppm TRC; pH 6.3–6.5; ORP 800–900 mV; 10 min contact time) resulted in 100% inactivation of all five organisms in pure culture (reduction of 6.1–6.7 $\log_{10}$ CFU/mL). Other studies have also observed complete inactivation of *E. coli*, *Salmonella enteritidis*, and *L. monocytogenes* in pure culture using acidic EO water (pH 2.5; 82 ppm free chlorine) (Venkitanarayanan et al., 1999a) and slightly alkaline EO water (pH 8.0; 63 ppm free chlorine) (Deza et al., 2003). Strongly acidic EO solutions have biocidal properties, but cause corrosion of surfaces and rapidly lose chlorine (Cl_2) through the evolution of chlorine gas, thus reducing the biocidal effectiveness of the solutions. Alkaline EO solutions contain primarily hypochlorite ions (OCl^-, 80–95%), which have less biocidal effectiveness and leave a chlorine residue on surfaces. Dip treatments of spinach and lettuce in near-neutral EO solutions (4, 20, 50, 100, and 120 ppm TRC) which contain primarily HOCl did not leave a chlorine residue on the leaves. In contrast, treatment with 0.5% bleach (hypochlorite, ClO^-) left a significantly higher ($p < 0.001$) chlorine residue on the leaves.

Differences in the survival percentages of bacteria on spinach and lettuce leaves may be attributable to contact time, structural characteristics of the vegetable surfaces, and protective mechanisms of adhesive microbial biofilms. Ten-minute dip treatments using near-neutral EO water at concentrations of 100 and 120 ppm TRC reduced bacterial populations of *E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes*, and *E. faecalis* by 4.0–5.0 $\log_{10}$ CFU/mL on the surfaces of spinach leaves. Concentrations of 4, 20, and 50 ppm TRC reduced populations of these organisms by 0.52–4.97 $\log_{10}$ CFU/mL on spinach leaves. Treatment of iceberg lettuce leaves resulted in a 0.1–0.25 $\log_{10}$ CFU/mL reduction of *E. coli* and a 0.1–3.81 $\log_{10}$ CFU/mL reduction of *S. typhimurium*, *S. aureus*, *L. monocytogenes*, and *E. faecalis*. The surfaces of iceberg lettuce leaves contain folds and crevices which can trap bacteria and shield them from inactivation by EO water. Previous studies using chlorinated water indicate that *E. coli* (7.9–8.1 $\log_{10}$ CFU/cm^2) attached to the edges of lettuce and trapped within lettuce tissues survived a 5 min treatment in chlorine water (200 ppm) (Takeuchi and Frank, 2000).

Increases in contact time for dip treatments and more vigorous agitation may increase the effectiveness of the near-neutral EO water. Sharma and colleagues reported increased reductions of *E. coli* on alfalfa sprouts as a function of increased contact time using acidic EO water at a concentration of 76 ppm TRC (Sharma and Demirci, 2003). Populations of *E. coli*, *S. enteritidis*, and *L. monocytogenes* on smooth tomato surfaces were reduced by 4.4–5.01 $\log_{10}$ CFU/cm^2 after a 60 s treatment with slightly alkaline EO water (pH 8; 89 ppm TRC) (Deza et al., 2003). Yang et al. (2003) reported a 2.0 $\log_{10}$ CFU/g

reduction in *S. typhimurium*, *E. coli*, and *L. monocytogenes* on the surfaces of romaine lettuce after a 5 min dip in neutral EO water (pH 7; 300 ppm TRC). Low reductions of bacteria despite the use of EO water may also be attributable to the presence of biofilms on the surfaces of the vegetables. Microbial secretion of adhesive biofilms facilitates the attachment of bacteria to surfaces and may protect organisms from the oxidative effects of electrolyzed water treatments (Yang et al., 2003).

Electrolyzed oxidizing water is an effective sanitizer of surfaces and equipment in food-processing operations. Acidic EO water (pH 2.5; 87–200 ppm TRC) effectively eliminated bacteria populations on ceramic platform surfaces in fish markets (Huang et al., 2006) and resulted in a 5–6 $\log_{10}$ CFU/mL reduction in populations of *E. coli* and *L. monocytogenes*, on plastic cutting boards (Venkitanarayanan et al., 1999b). Slightly alkaline EO water (pH 8; 63 ppm TRC) eliminated populations of *E. coli*, *L. monocytogenes*, *Pseudomonas aeruginosa*, and *S. aureus* on stainless and glass surfaces (Deza et al., 2005). In this study, near-neutral EO water (278–310 ppm TRC; pH 6.38) effectively reduced or eliminated (79–100%) bacterial populations on a variety of surfaces in food service and other common contact areas in two schools. The use of near-neutral EO water to clean surfaces, minimizes corrosion and reduces the negative risk and exposure that is associated with the use and disposal of traditional decontamination agents.

In conclusion, this study demonstrates the effectiveness of using near-neutral electrolyzed water as a microbial decontamination agent for structural surfaces that contain mixed populations of heterotrophic bacteria and as a rinse treatment to reduce bacterial populations on spinach and lettuce. Future studies should include different washing/dipping/contact time regimes designed to mimic commercial processing operations in addition to other types of fruits, vegetables, porous/non-porous surfaces, and microbial pathogens.

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