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Effect of Slightly Acidic Electrolyzed Water for Inactivating *Escherichia coli* O157:H7 and *Staphylococcus aureus* Analyzed by Transmission Electron Microscopy

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ABSTRACT

The use of different available chlorine concentrations (ACCs) of slightly acidic electrolyzed water (SAEW; 0.5 to 30 mg/liter), different treatment times, and different temperatures for inactivating *Escherichia coli* O157:H7 and *Staphylococcus aureus* was evaluated. The morphology of both pathogens also was analyzed with transmission electron microscopy. A 3-min treatment with SAEW (pH 6.0 to 6.5) at ACCs of 2 mg/liter for *E. coli* O157:H7 and 8 mg/liter for *S. aureus* resulted in 100% inactivation of two cultures (7.92- to 8.75-log reduction) at 25°C. The bactericidal activity of SAEW was independent of the treatment time and temperature at a higher ACC ($P > 0.05$). *E. coli* O157:H7 was much more sensitive than *S. aureus* to SAEW. The morphological damage to *E. coli* O157:H7 cells by SAEW was significantly greater than that to *S. aureus* cells. At an ACC as high as 30 mg/liter, *E. coli* O157:H7 cells were damaged, but *S. aureus* cells retained their structure and no cell wall damage or shrinkage was observed. SAEW with a near neutral pH may be a promising disinfectant for inactivation of foodborne pathogens.

Foodborne illnesses are prevalent all over the world and have continued to be of major public and governmental concern in recent years. *Escherichia coli* O157:H7 and *Staphylococcus aureus* are common foodborne pathogens that can cause human illness and death *(20)*. Several outbreaks of food poisoning have been associated with *E. coli* O157:H7 and *S. aureus* in foods such as dairy products, poultry, eggs, meat, fruits, and vegetables *(8)*. Therefore, an effective method for reducing or eliminating these pathogens is crucial for food safety and human health.

Acidic electrolyzed water (AEW), also known as electrolyzed oxidizing water, has been regarded as a potential disinfectant with environmentally friendly broad spectrum antimicrobial effects. AEW has low pH (<2.7) and high oxidation reduction potential (ORP; >1,000 mV) and contains available chlorine concentrations (ACCs) that depend on the AEW generator setting. AEW is commonly generated through electrolysis of dilute NaCl solution in a cell with anode and cathode electrodes, which are separated by a membrane, and is obtained from the anode side. AEW exhibits strong bactericidal activity against many pathogens such as *E. coli* O157:H7, *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Listeria monocytogenes (10, 12, 13, 24, 26)* and has been used to reduce pathogens on fruits, vegetables, eggs, poultry carcasses, pork, and seafood and in food processing facilities *(3, 11, 14, 17, 18, 22)*. However, AEW has limited potential for long-term applications because of its strong acidity (pH of <2.7), which causes the corrosion of equipment *(7)*. At this low pH, dissolved Cl_2 gas can be rapidly lost due to volatilization, decreasing the bactericidal activity of AEW with time *(5)* and adversely affecting human health and the environment.

Slightly acidic electrolyzed water (SAEW), with a pH value of 5.0 to 6.5 and containing a high concentration of hypochlorous acid (HOCl; approximately 95%), is generated by electrolysis of dilute hydrochloric acid (HCl) and/or NaCl solution in a nonmembrane electrolytic cell *(9)*. The bactericidal activity of hypochlorous acid is 80 times greater than that of hypochlorite ion (ClO^-) for inactivating *E. coli* at the same chlorine concentration and treatment time *(2)*. Therefore, SAEW may improve the bactericidal activity through maximizing the use of hypochlorous acid, thus reducing corrosion of surfaces and minimizing human health and safety issues from off-gassing of Cl_2 *(7)*. A 2-min in vitro treatment using SAEW (ACC > 4 mg/liter) resulted in an 8.2-log reduction of *Salmonella* Enteritidis, and SAEW at an ACC of 15 mg/liter reduced the population of *Salmonella* Enteritidis on shell eggs by 6.5 log CFU/g after a 3-min treatment *(4)*. SAEW (pH 6.1 and ACC of 20 mg/liter) used with fresh cut cabbage reduced total aerobic bacteria by 1.5 log CFU/g and yeasts and molds by 1.3 log CFU/g *(16)*. The disinfectant efficacy of SAEW was equivalent to or higher than that of NaOCl solution (pH 9.6 and ACC of about 150 mg/liter). Although many studies have been conducted on the enumeration of bacterial

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populations before and after treatment (e.g., sodium chloride and chlorine-containing compounds), limited information is available on the morphological changes to the bacterial cells. Visual information is useful for providing insight into the microstructure of the cell *(8)*. To elucidate the bactericidal mechanism of electrolysis, a relatively resistant bacterium, *Pseudomonas aeruginosa,* was analyzed by electron microscopy *(21)*. The effect of pH and ORP values of AEW on bacterial cell morphology was examined using transmission electron microscopy *(15, 19)*.

The objective of this study was to evaluate the bactericidal activity of SAEW for inactivation of *E. coli* O157:H7 and *S. aureus* at different ACCs, treatment times, and temperatures and to examine the effect of SAEW on the cell morphology of the two pathogens using transmission electron microscopy.

MATERIALS AND METHODS

Bacterial cultures. Freeze-dried pure cultures of *E. coli* O157:H7 (human feces isolate) and *S. aureus* ATCC 6538 (raw milk isolate) were obtained from the China Veterinary Culture Collection (CVCC, Beijing, China). Cultures were hydrated according to manufacturer's directions and propagated in tryptic soy broth supplemented with 0.6% (wt/vol) yeast extract (TSBYE; CVCC) at 37°C for 24 h. Cells of each culture were collected by centrifugation at 3,000 × *g* for 10 min at 4°C (TDL-60B, Anke, Shanghai, China). The supernatant was decanted, and the pellets were resuspended in 10 ml of sterile 0.1% peptone water, centrifuged again at 3,000 × *g* for 10 min at 4°C, and then diluted in the same peptone water. Counts of viable bacteria were obtained by plating 0.1 ml of a 10-fold serial dilution of broth cultures onto sterile tryptic soy agar supplemented with 0.6% (wt/vol) yeast extract (TSAYE; CVCC) and incubating the plates at 37°C for 24 h. The population of *E. coli* O157:H7 or *S. aureus* in each culture was approximately 8.0 log CFU/ml.

Preparation of slightly acidic electrolyzed water. Slightly acidic electrolyzed water was produced using an SAEW generator (Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China) that basically consisted of a nonmembrane electrolytic cell with anode and cathode electrodes. SAEW with a pH of 6.2 to 6.3, ORP of 849.3 to 857.0 mV, and ACC of 10 to 30 mg/liter was produced by electrolysis of a mixture of 17 mM NaCl and 1.2 mM hydrochloric acid aqueous solution in the SAEW generator at 20 V for 3 and 5 min, respectively. The resulting SAEW was diluted in sterile deionized water to obtain solutions with ACCs of 0.5 to 8 mg/liter. Sterile deionized water was used as the control. All chemicals used were of analytical grade.

The pH, ORP, and ACC of the SAEW were measured immediately before each bactericidal experiment. The pH and ORP values were measured using a dual scale pH/ORP meter (HM-30R, DKK-TOA Corporation, Tokyo, Japan) with a pH electrode (GST-5741C) or an ORP electrode (PST-5721C). The ACC was determined by a colorimetric method using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan).

Bactericidal experiments of SAEW. Nine-milliliter aliquots of SAEW of various ACCs (0.5, 1, 1.5, 2, 4, 5, 6, 8 and 10 mg/liter) and of sterile deionized water (control) were placed into separate sterile screw-cap tubes. One milliliter of *E. coli* O157:H7 or *S. aureus* (approximately 8.0 log CFU/ml) was individually added to the prepared tubes. The tubes were mixed and continuously shaken by hand at an ambient temperature of 25 ± 2°C for 3 min.

To investigate the effects of treatment time and temperature on the bactericidal activity of SAEW, the experiments were performed by adding 1 ml of *E. coli* O157:H7 or *S. aureus* into sterile screw-cap tubes that contained 9 ml of SAEW with ACCs of 1.5, 6, or 8 mg/liter (treatment) or 9 ml of sterile deionized water (control). Tubes were kept at 4°C (in an ice water bath) and 20°C or in a preheated water bath (HHS1-N1, Beijing Changan Scientific Instrument Plant, Beijing, China) at 50°C for 0, 0.5, 1, 3, 5, 8, or 10 min.

Following treatment, bactericidal experiments were stopped immediately by transferring 1 ml of each treated sample to a sterile tube containing 9 ml of neutralizing buffer solution (0.5% sodium thiosulfate plus 0.03 M phosphate buffer solution, pH 7.2 to 7.4), and the tubes was shaken on a platform shaker at 150 rpm (MIR-S100, Sanyo Electric Biomedical Co., Ltd., Osaka, Japan). After 5 min of neutralization, the viable count of each pathogen was determined by plating 0.1 ml of either undiluted culture or culture that was serially diluted (1:10) in sterile 0.1% peptone water. Cultures were plated on triplicate TSAYE plates, which were incubated at 37°C for 24 h. The detection limit of this method was as low as 1 CFU/ml. An enrichment experiment was conducted to determine the presence of low numbers of survivors that might not be detected by direct plating. One milliliter of the suspension was transferred to a 100-ml flask containing 50 ml of sterile TSBYE and incubated at 37°C for 24 h. The culture solution was streaked on TSAYE plates, and the plates were incubated at 37°C for 48 h before counting *(22)*. Each treatment was replicated in triplicate.

Electron microscopy. *E. coli* O157:H7 and *S. aureus* cells were mixed with SAEW at ACCs of 10 and 30 mg/liter for 3 min, and the available chlorine in the mixtures was neutralized with buffer solution (0.5% sodium thiosulfate plus 0.03 M phosphate buffer solution, pH 7.2 to 7.4). After the treatments, the cells of both cultures were immediately pelleted and fixed by adding 2.5% (vol/vol) glutaraldehyde. The pellets were washed twice with cacodylate buffer (0.05 M, pH 7.4) and postfixed with 1% osmium tetroxide in the same buffer containing 0.05% (wt/vol) ruthenium red. The fixed samples were dehydrated with acetone (30, 50, 70, 80, 90, and 100%), embedded in Epoxy resin, and cut into ultrathin sections. The sections were doubly stained with 4% uranyl acetate and lead citrate and observed with a transmission electron microscopy (JEM-1230, JEOL Ltd., Tokyo, Japan) at 80 kV with a magnification factor of 50,000. Untreated *E. coli* O157:H7 and *S. aureus* cells were observed in the same manner.

Statistical analysis. All experiments had three replications for each treatment and measurement. Mean values for bacterial populations, pH, ORP, and ACC were calculated from the independent triplicate trials. Statistical analysis was performed using the SAS 8.0 software (SAS Institute Inc., Cary, NC). Tukey's studentized range (honestly significantly different) test was used to determine the significant differences among the means at the 5% probability level.

RESULTS AND DISCUSSION

Inactivation of *E. coli* O157:H7 and *S. aureus* by SAEW at various ACCs. The pH and ORP values of SAEW at various ACCs and the surviving populations of *E. coli* O157:H7 and *S. aureus* are shown in Tables 1 and 2, respectively. Sterile deionized water with no chlorine served

TABLE 1. *Inactivation of* E. coli *O157:H7 by slightly acidic electrolyzed water at various available chlorine concentrations*[a]

ACC (mg/liter)	pH	ORP (mV)	Surviving population (log CFU/ml)[b]
0 (control)	6.05 ± 0.07	392.3 ± 9.0	7.92 ± 0.13 A
0.5	6.57 ± 0.01	798.6 ± 5.0	6.27 ± 0.18 B
1	6.55 ± 0.05	801.2 ± 11.0	4.29 ± 0.11 C
1.5	6.54 ± 0.07	805.3 ± 6.0	2.69 ± 0.14 D
2	6.53 ± 0.02	812.5 ± 8.0	ND E
5	6.49 ± 0.03	827.0 ± 5.0	ND E
8	6.42 ± 0.05	847.6 ± 3.0	ND E

[a] Values are means ± standard deviations of triplicate measurements. Treatment time was 3 min.

[b] Means followed by different letters are significantly different as determined by Tukey's studentized range test ($P < 0.05$). ND, no detectable survivors by direct plating procedure.

as the control. The pH (6.31 to 6.57) of SAEW was a little higher than that of the control (pH 6.02 to 6.05), whereas the ORP (798.6 to 849.3 mV) of SAEW was markedly higher than that of the control (392.3 to 395.2 mV). The population of both pathogens in the treated samples was greatly reduced at different available chlorine concentrations compared with the control samples ($P < 0.05$), except for the treatment of *S. aureus* with SAEW at an ACC of 2 mg/liter.

Treatment of *E. coli* O157:H7 by SAEW with an ACC of 0.5, 1, and 1.5 mg/liter at 25°C for 3 min reduced the populations by 1.65, 3.63, and 5.23 log CFU/ml, respectively (Table 1). At an ACC of 2 mg/liter, 100% inactivation of *E. coli* O157:H7 by SAEW was obtained, but the population of *S. aureus* was only reduced by about 0.30 log CFU/ml (Table 2). When the ACC of SAEW was increased to 4 and 6 mg/liter, the reductions of *S. aureus* were 1.67 and 2.03 log CFU/ml, respectively. *S. aureus* was completely inactivated by SAEW with an ACC ≥ 8 mg/liter. Results indicate that the bactericidal activity of SAEW increases with increasing ACC, and *E. coli* O157:H7 is very susceptible to available chlorine whereas *S. aureus* was much more chlorine tolerant. The populations of both pathogens in the control samples were not reduced.

TABLE 2. *Inactivation of* S. aureus *by slightly acidic electrolyzed water at various available chlorine concentrations*[a]

ACC (mg/liter)	pH	ORP (mV)	Surviving population (log CFU/ml)[b]
0 (control)	6.02 ± 0.05	395.2 ± 6.0	8.75 ± 0.21 A
2	6.55 ± 0.03	810.5 ± 7.0	8.45 ± 0.11 A
4	6.54 ± 0.04	832.1 ± 4.0	7.08 ± 0.19 B
6	6.48 ± 0.01	842.7 ± 8.0	6.72 ± 0.14 B
8	6.42 ± 0.05	844.6 ± 5.0	ND C
10	6.31 ± 0.02	849.3 ± 9.0	ND C

[a] Values are means ± standard deviations of triplicate measurements. Treatment time was 3 min.

[b] Means followed by different letters are significantly different as determined by Tukey's studentized range test ($P < 0.05$). ND, no detectable survivors by direct plating procedure.

SAEW produced by electrolysis of dilute hydrochloric acid and NaCl solution in a nonmembrane electrolytic cell had powerful bactericidal activity against *E. coli* O157:H7 and *S. aureus*. In SAEW or neutral electrolyzed water with a near neutral pH (6.0 to 6.5), the most effective form of chlorine is hypochlorous acid (approximately 95%), which is an active bactericidal agent *(9)*. When five pure cultures of pathogenic organism were treated with neutral electrolyzed water (pH 6.5 to 6.7) with total residual chlorine concentrations of 20, 50, 100, and 120 mg/liter for 10 min, 100% inactivation (reduction of 6.1 to 6.7 log CFU/ml) was obtained for all the pathogens *(7)*. *Salmonella* Enteritidis cultures were completely inactivated (reduction of 8.2 log CFU/ml) by SAEW with a pH of 6.3 to 6.5 and ACCs of more than 4 mg/liter at 4, 20, and 45°C for 2 min *(4)*. SAEW had bactericidal activity similar to that of AEW (pH 2.6 to 2.7, ORP > 1,000 mV) and sodium hypochlorite solution (pH 12.8, ORP of 460 mV) with the same ACC and contact time. The population of viable *Vibrio vulnificus* cells was reduced by 2.2 log CFU/ml after treatment with sodium hypochlorite solution with an ACC of 35 mg/liter for 60 s, but no cells survived (a reduction of 5.7 log CFU/ml) treatment with weakly acidic electrolyzed water (pH 5.9) for 30 s *(25)*. Similar results were obtained for *Vibrio parahaemolyticus*. The result indicates that weakly acidic electrolyzed water kills these microorganisms more quickly than a chemical product such as sodium hypochlorite, even at equivalent ACCs. SAEW (pH 6.1, ACC of 20 mg/liter) as a disinfectant for fresh cut cabbage had the same bactericidal ability as did a sodium hypochlorite solution with a pH of 9.6 and an ACC of 150 mg/liter *(16)*. Approximately 1.64- and 1.72-log reductions of *E. coli* O157:H7 on beef were obtained after treatment with AEW (pH 2.3 to2.7, ACC of 50 mg/liter) and SAEW (pH 6.2, ACC of 5 mg/liter), respectively *(6)*. No significant differences in the bactericidal activity of these AEW and SAEW treatments were found for inactivating *E. coli* O157:H7 on beef.

Inactivation of *E. coli* O157:H7 and *S. aureus* by SAEW at various treatment times and temperatures. The surviving populations of *E. coli* O157:H7 and *S. aureus* treated by SAEW with pH 6.51 to 6.54, ORPs of 812.6 to 842.7 mV, and ACCs of 1.5 or 6 mg/liter for 0, 0.5, 1, 3, 5, 8, and 10 min at 25 ± 2°C are presented in Table 3. The surviving populations of both pathogens were decreased with increasing treatment time. At 0 min, the populations of *E. coli* O157:H7 and *S. aureus* in the control and treated samples were 7.87 and 8.45 log CFU/ml, respectively. Treatment of SAEW for 0.5, 1, and 3 min resulted reductions of 2.04, 3.28, and 5.13 log CFU/ml, respectively, for *E. coli* O157:H7 and 0.44, 1.39, and 2.02 log CFU/ml, respectively, for *S. aureus*. Complete (100%) inactivation of *E. coli* O157:H7 (about 7.87-log reduction) and *S. aureus* (about 8.45-log reduction) in the treated samples was achieved with treatment times equal to or greater than 5 and 10 min, respectively.

TABLE 3. *Effect of treatment time on the bactericidal activity of slightly acidic electrolyzed water for inactivating* E. coli *O157:H7 and* S. aureus[a]

	Surviving population (log CFU/ml)			
	E. coli O157:H7		*S. aureus*	
Treatment time (min)	Control	Treated	Control	Treated
0	7.87 ± 0.29	7.87 ± 0.29	8.45 ± 0.19	8.45 ± 0.19
0.5	7.88 ± 0.15	5.84 ± 0.10	8.42 ± 0.27	7.98 ± 0.13
1	7.92 ± 0.22	4.64 ± 0.15	8.43 ± 0.36	7.04 ± 0.12
3	7.91 ± 0.13	2.78 ± 0.07	8.54 ± 0.15	6.52 ± 0.09
5	7.87 ± 0.11	ND[b]	8.49 ± 0.22	5.86 ± 0.16
8	7.86 ± 0.26	ND	8.52 ± 0.33	4.09 ± 0.21
10	7.87 ± 0.52	ND	8.47 ± 0.08	ND

[a] Values are means ± standard deviations of triplicate measurements. Available chorine concentrations were 1.5 mg/liter for *E. coli* O157:H7 and 6 mg/liter for *S. aureus* at 25 ± 2°C.
[b] ND, no detectable survivors by direct plating procedure.

Table 4 illustrates the bactericidal activity of SAEW against *E. coli* O157:H7 and *S. aureus* at various ACCs (1.5, 6, and 8 mg/liter) and treatment temperatures (4, 25, and 50°C) for 3 min. The populations of both pathogens in the treated samples were significantly reduced compared with the control by SAEW at the various treatment temperatures ($P < 0.05$). A reduction of 4.92 log CFU/ml for *E. coli* O157:H7 was obtained after treatment with SAEW with an ACC of 1.5 mg/liter at 25°C. This reduction is significantly greater than that found for samples treated at 4°C (4.72-log reduction) and 50°C (3.83-log reduction) ($P < 0.05$). When the ACC was 8 mg/liter, treatment temperature had no markedly effect on the bactericidal activity of SAEW ($P > 0.05$) against *E. coli* O157:H7. Similar results were observed for *S. aureus* treated by SAEW at ACCs of 6 and 8 mg/liter. However, there was no reduction in the population of either pathogen in the control samples at the various temperatures.

The bactericidal activity of SAEW against *E. coli* O157:H7 and *S. aureus* was affected by treatment time and temperature at lower ACCs, whereas this activity was independent of treatment time and temperature at higher ACCs. When the ACC was 8 mg/liter, 100% inactivation of both pathogens was observed at 4, 25, and 45°C, which suggests that the ACC in the SAEW may be the most important factor for killing pathogens. Similar results were reported for *V. vulnificus* and *V. parahaemolyticus* treated with weakly acidic electrolyzed water (pH 5.9), whose bactericidal activity was primarily affected by ACC rather than treatment time *(25)*.

Morphological analysis of *E. coli* O157:H7 and *S. aureus* treated with SAEW. The effects of SAEW with ACCs of 10 and 30 mg/liter on the morphology of *E. coli* O157:H7 and *S. aureus* are shown in Figure 1. The cellular morphology was analyzed both before and after treatment with SAEW using transmission electron microscopy. The cells of both pathogens had intact cellular membranes before treatment (Fig. 1a and 1d). *E. coli* O157:H7 was much less tolerant to SAEW than was *S. aureus* at both ACCs. After treatment with SAEW at an ACC of 10 mg/liter for 3 min, the outer membrane of *E. coli* O157:H7 cell was destroyed (arrow in Fig. 1b). With increasing ACC (30 mg/liter) of SAEW, the morphological structure of *E. coli* O157:H7 cells was markedly damaged, destroyed, or deformed (arrow in Fig. 1c). A distinct separation of the cytoplasmic cellular

TABLE 4. *Effect of treatment temperature on the bactericidal activity of slightly acidic electrolyzed water for inactivating* E. coli *O157:H7 and* S. aureus[a]

		Surviving population (log CFU/ml)			
		E. coli O157:H7		*S. aureus*	
Temp (°C)	Treatment group	1.5 mg/liter	8 mg/liter	6 mg/liter	8 mg/liter
4	Control	7.74 ± 0.25 A	7.72 ± 0.33	7.87 ± 0.17 A	7.86 ± 0.26
	Treated	3.02 ± 0.12 B	ND[b]	4.91 ± 0.13 B	ND
25	Control	7.73 ± 0.17 A	7.72 ± 0.24	7.89 ± 0.09 A	7.85 ± 0.12
	Treated	2.81 ± 0.19 C	ND	4.61 ± 0.23 C	ND
50	Control	7.75 ± 0.29 A	7.72 ± 0.31	7.87 ± 0.18 A	7.86 ± 0.35
	Treated	3.92 ± 0.16 B	ND	4.95 ± 0.15 B	ND

[a] Values are means ± standard deviations of triplicate measurements. Treatment time was 3 min. Within a column, means followed by different letters are significantly different as determined by Tukey's studentized range test ($P < 0.05$).
[b] ND, no detectable survivors by direct plating procedure.

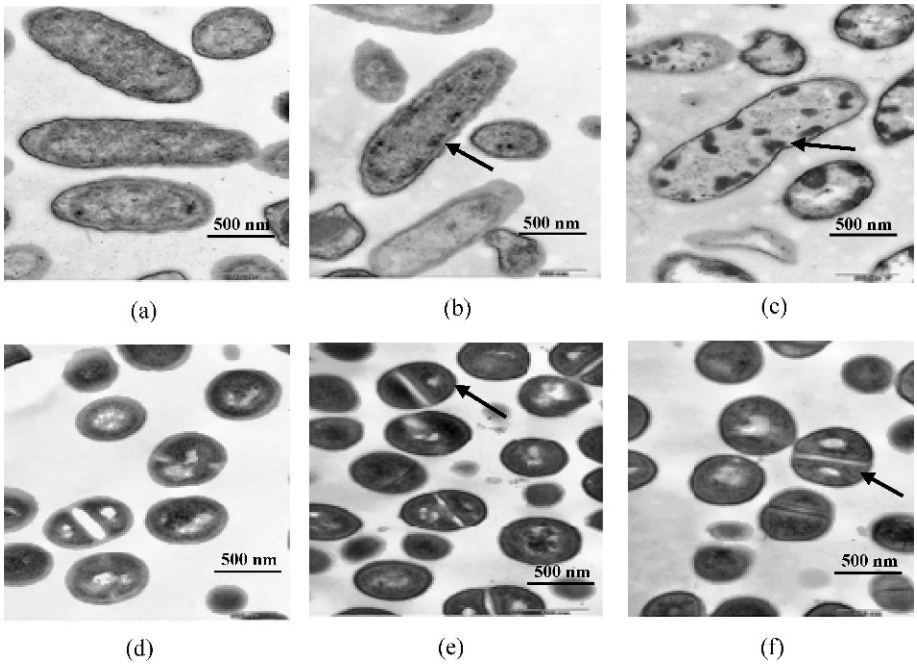

FIGURE 1. *Transmission electron micrographs of* E. coli *O157:H7 and* S. aureus *cultures treated with slightly acidic electrolyzed water at available chlorine concentrations of 10 and 30 mg/liter for 3 min: (a) 0 mg/liter (control,* E. coli *O157:H7), (b) 10 mg/liter (treated,* E. coli *O157:H7), (c) 30 mg/liter (treated,* E. coli *O157:H7), (d) 0 mg/liter (control,* S. aureus*), (e) 10 mg/liter (treated,* S. aureus*), (f) 30 mg/liter (treated,* S. aureus*). Magnification is ×50,000; scale bar is 500 nm. Arrows indicate the outer cell membrane of* E. coli *O157:H7 slightly damaged (b); the cell membrane of* E. coli *O157:H7 severely damaged and irregular aggregation of cytoplasmic small granules (c), and the cell membrane of* S. aureus *not evidently damaged (e and f).*

material from the cell membrane was found in *E. coli* O157:H7 cells treated with SAEW with an ACC of 30 mg/liter. However, the cellular structure of *S. aureus* cells was not evidently damaged by SAEW with an ACC of 10 or 30 mg/liter (arrows in Fig. 1e and 1f). This result corroborates those of the inactivation experiments, suggesting that *S. aureus* cells are more tolerant than *E. coli* O157:H7 cells to the effects of chlorine (Tables 1 and 2).

Park et al. *(24)* also found that *E. coli* O157:H7 was very sensitive to chlorine. The morphological damage to *E. coli* O157:H7 cells was significantly greater than that to *S. aureus* cells when NaCl concentrations were increased from 5 to 10%. *E. coli* O157:H7 cells experienced more morphological damage, destruction, or alteration with increasing NaCl concentration, whereas *S. aureus* cells maintained cellular structure, and no severe cell wall or plasma membrane damage and/or shrinkage was observed *(8)*. The outer and inner membranes of *E. coli* O157:H7 cells were damaged by electrolyzed oxidizing water (pH 2.5, ORP of 1,150 mV, and ACC of 60 mg/liter), leading to the inactivation *(17)*.

The morphology of *P. aeruginosa* cells treated with electrolyzed strong acid water was analyzed using transmission electron microscopy *(15)*. Electrolyzed strong acid water with low free chlorine concentrations caused in a smaller number of breaks and blebs on the cell than did treatment with solutions with high free chlorine concentrations, but even the low concentrations were sufficient to kill the bacteria. In the present study, a similar result was found for *S. aureus*. Although the structure of *S. aureus* cells was not significantly altered by SAEW at an ACC of 10 and 30 mg/liter, complete inactivation of *S. aureus* was found by bacteriological analysis.

The presence of hypochlorous acid, high ORPs, and low pH values are the main factors associated with the bactericidal activity of electrolyzed water. Previous work suggested that hypochlorous acid inactivates bacterial cells by oxidation of cell surface sulfhydryl compounds, inactivation of enzymes, and inhibition of ATP generation *(19, 23)*. However, the mechanism of inactivation of microbial cells by electrolyzed water is still not clear.

SAEW with a near neutral pH is less corrosive to processing equipment and less irritating to hands than is AEW, which is strongly acidic (pH < 2.7, ORP of 1,000 mV or more, and ACC of 40 to 90 mg/liter *(1)*. SAEW also is less phytotoxic to plants and less prone to safety issues from Cl_2 off-gassing *(7)*. Cui et al. *(5)* reported that the factor (especially available chlorine) responsible for the bactericidal effect of SAEW was more stable with time than the corresponding factor in AEW under various storage conditions. The ACC of AEW diminished after 6 days of storage under open-light or open-dark conditions and lost its bactericidal activity, but the bactericidal activity of SAEW was not markedly affected by storage conditions, possibly because dissolved chlorine does not decrease as much with time in SAEW as in AEW.

In conclusion, SAEW with a near neutral pH value (6.0 to 6.5) is effective for inactivating *E. coli* O157:H7 and *S. aureus* at low ACCs. However, the mechanism of this bactericidal effect is not clear. Further studies are required to understand the mechanism by which SAEW inactivates microorganisms.

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