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Effectiveness of Electrolyzed Water as a Sanitizer for Treating Different Surfaces

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ABSTRACT

The effectiveness of electrolyzed (EO) water at killing *Enterobacter aerogenes* and *Staphylococcus aureus* in pure culture was evaluated. One milliliter (approximately 10^9 CFU/ml) of each bacterium was subjected to 9 ml of EO water or control water (EO water containing 10% neutralizing buffer) at room temperature for 30 s. Inactivation (reduction of >9 $\log_{10}$ CFU/ml) of both pathogens occurred within 30 s after exposure to EO water containing approximately 25 or 50 mg of residual chlorine per liter. The effectiveness of EO water in reducing *E. aerogenes* and *S. aureus* on different surfaces (glass, stainless steel, glazed ceramic tile, unglazed ceramic tile, and vitreous china) was also evaluated. After immersion of the tested surfaces in EO water for 5 min without agitation, populations of *E. aerogenes* and *S. aureus* were reduced by 2.2 to 2.4 $\log_{10}$ CFU/cm^2 and by 1.7 to 1.9 $\log_{10}$ CFU/cm^2, respectively, whereas washing with control water resulted in a reduction of only 0.1 to 0.3 $\log_{10}$ CFU/cm^2. The washing of tested surfaces in EO water with agitation (50 rpm) reduced populations of viable cells on the tested surfaces to <1 CFU/cm^2. For the control water treatment with agitation, the surviving numbers of both strains on the tested surfaces were approximately 3 $\log_{10}$ CFU/cm^2. No viable cells of either strain were observed in the EO water after treatment, regardless of agitation. However, large populations of both pathogens were recovered from control wash solution after treatment.

Bacterial contamination on food-processing surfaces, including stainless steel, glass, cast iron, polypropylene, and Formica, that results in food spoilage or transmission of disease has been extensively reported *(2, 5, 10, 23).* Many commercial disinfecting cleaning agents, such as potassium persulphate, isopropanol, hydrogen peroxide, sodium dichloroisocyanurate, ethanol and phenol derivatives *(1),* quaternary ammonium compound, and chlorine *(20),* have been shown to be effective against foodborne pathogens in suspension tests. However, microorganisms attached to the surfaces are less susceptible to chemical sanitizers than are their free-living counterparts because sanitizers have a limited ability to penetrate the protective layer of microbial polymers *(9, 15).* Bacterial contamination could also occur on non-food-contact surfaces such as ceramic tiles, vitreous china, stainless steel, and glassware (found in bathrooms and in laundry, microbiological testing laboratory, swimming pool, and medical facilities) if these surfaces are not completely sanitized. These contaminated surfaces can serve as reservoirs of pathogens and transfer diseases via hand-to-surface contact *(7).* Hospital-acquired infections have also frequently occurred through the transmission of pathogens such as *Staphylococcus aureus* and *Enterobacter aerogenes* from poorly sanitized surfaces *(6, 7).* Therefore, the development of an effective sanitizer is necessary to reduce or eliminate bacterial populations on different surfaces.

Electrolyzed (EO) water, which is generated by the electrolysis of a dilute salt (NaCl) solution, has a strong bactericidal effect on *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella* Enteritidis, *Campylobacter jejuni,* and *Bacillus cereus* because it contains hypochlorous acid (10 to 90 mg/liter) and has a high oxidation reduction potential (ORP) (ca. 1,100 mV) *(12, 13, 21).* Previous studies have also revealed that EO water is highly effective in reducing or eliminating foodborne pathogens on kitchen cutting boards and on various food products, such as poultry and vegetables *(18, 19, 22).* In addition, EO water is very effective in eliminating *L. monocytogenes* biofilms on stainless steel *(14).*

The objective of this project was to evaluate the effectiveness of EO water as a sanitizer for treating different surfaces according to the specifications outlined in Environmental Protection Agency Standard Operating Procedure DIS/TSS-10 *(8)* in order to determine the efficacy of a sanitizer on different hard surfaces. Specifically, this project was undertaken to evaluate the efficacy of EO water in reducing *S. aureus* (as recommended by the Environmental Protection Agency Standard Operating Procedure) and *E. aerogenes* ATCC 13048 (often associated with coliform spoilage as a result of fecal contamination) on glass, stainless steel, glazed ceramic tile, unglazed ceramic tile, and vitreous china.

MATERIALS AND METHODS

Bacterial cultures. *S. aureus* ATCC 6538 and *E. aerogenes* ATCC 13048 (American Type Culture Collection, Rockville, Md.)

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TABLE 1. *Inactivation of* E. aerogenes *and* S. aureus *by EO water*[a]

Treatment	Water properties			Surviving population (log_{10} CFU/ml)	
	pH	ORP (mV)	Residual chlorine (mg/liter)	*E. aerogenes*	*S. aureus*
Control[b]	2.57 ± 0.03	526 ± 8	0	7.98 ± 0.04	8.03 ± 0.03
EO water (50 mg/liter)[c]	2.53 ± 0.01	1,178 ± 4	53.1 ± 1.9	ND[d]	ND
EO water (25 mg/liter)[e]	2.79 ± 0.02	1,163 ± 4	26.9 ± 1.1	ND	ND
EO water (10 mg/liter)[f]	3.18 ± 0.02	1,116 ± 6	11.3 ± 0.8	ND	3.92 ± 0.11

[a] Values are the means of four readings from two replicated measurements ± standard deviations.
[b] The control solution was obtained by neutralizing residual chlorine in the EO water (generated at a setting of 14 A) with neutralizing buffer.
[c] EO water was generated at a setting of 14 A.
[d] ND, negative by an enrichment procedure; no detectable survivors by a direct plating procedure.
[e] Twofold-diluted EO water containing approximately 25 mg of residual chlorine per liter.
[f] Fivefold-diluted EO water containing approximately 10 mg of residual chlorine per liter.

were maintained in 10 ml of tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) at 37°C by daily transfer. For the treatment of pure culture, a 24-h culture of each bacterium was harvested by centrifugation (4,000 × *g* for 10 min), washed twice with 0.1 M phosphate-buffered saline (PBS, pH 7.2), and resuspended in PBS to obtain final populations of about 9 log_{10} CFU/ml. For the washing of different surfaces, 5 ml of a 24-h culture of each bacterium (about 10^9 CFU/ml) in TSB was transferred into a sterile test tube, and 250 μl of Bacto Horse Serum (Difco) was added to the bacterial suspension. Bacterial concentrations were estimated by measuring the absorbance of the bacterial suspension at 600 nm with a spectrophotometer (Beckman DU520, Beckman Instruments Inc., Fullerton, Calif.) and confirmed by plating 0.1-ml portions of appropriately diluted culture on tryptic soy agar (TSA; Difco) plates. TSA plates were then incubated at 37°C for 48 h before counting.

Preparation of treatment solutions. EO water was generated with a Hoshizaki EO water generator (ROX 20 TA, Hoshizaki Electric Inc., Toyoake, Aichi, Japan) at a setting of 14 A. Freshly prepared EO water with a residual chlorine concentration of about 50 mg/liter was used for the study. Treatment solutions with 10 and 25 mg of residual chlorine per liter were prepared by dilution with sterilized deionized water. The control solution was prepared by completely removing residual chlorine from EO water (generated at a setting of 14 A) by adding 50 ml of neutralizing buffer (Difco) to 450 ml of EO water and mixing for 60 min. The pH and ORP of EO water were measured immediately before treatment with a dual-scale pH meter (Accumet model 15, Fisher Scientific Co., Fair Lawn, N.J.) with pH and ORP electrodes. The residual chlorine was determined by an iodometric method with a chlorine test kit (Hach Co., Ames, Iowa).

Treatment of pure culture. A volume of 9 ml of EO water (containing approximately 10, 25, or 50 mg of residual chlorine per liter) or control water was transferred to sterile screw-cap tubes. One milliliter (equivalent to 9 log_{10} CFU/ml) of each bacterium was added to each tube containing 9 ml of a treatment solution at room temperature (23 ± 2°C) for 30 s. Following treatments, 1 ml of each sample was serially diluted (1:10) in 9 ml of neutralizing buffer, and populations of *E. aerogenes* and *S. aureus* were determined by plating 0.1 ml of each dilution in duplicate on TSA plates. Bacterial viability was measured as the number of colonies on TSA plates after incubation at 37°C for 48 h. For enrichment, a volume of 1 ml of each sample after treatment was also transferred to separate 150-ml Erlenmeyer flasks containing 20 ml of sterile TSB and incubated at 37°C for 48 h. Two independent replicate trials were conducted for the treatment.

Attachment of cells to the test surfaces. Test surfaces used in this study were 25.8-cm^2 portions of glass (Fisherbrand slides, catalog no. 12-567, Fisher Scientific), stainless steel (type 304, no. 4 finish, Washington Specialty Metal, Athens, Ga.), glazed ceramic tile (Moonstone surface cap angle matte, US Ceramic Tile Co., East Sparta, Ohio), unglazed ceramic tile (Red Flat Quarry, US Ceramic Tile Co.), and vitreous china (Roma, Artisan Collection, Lewisville, Tex.). All test surfaces except glass were precut to a size of 5 by 5 cm for this study. All surfaces were scrubbed for 1 min with a cleansing solution (Micro, International Products Co., Burlington, Vt.) and then rinsed twice with deionized water. All surfaces were then sterilized by autoclaving at 121°C for 15 min before use. A 0.1-ml volume of bacterial culture (10^8 CFU/ml) in 5% Bacto Horse Serum was applied and spread evenly on a marked area of 6.45 cm^2 on each of the tested surfaces with a sterile disposable inoculating loop. After inoculation, the surfaces were air dried under a biosafety hood for 30 min at 30°C.

Washing treatment. Duplicate sets of four samples of each test material were immersed in 500 ml of EO or control water in a Sterilite container (34 by 20 by 10 cm; Sterilite, Townsend, Mass.) at room temperature (23 ± 2°C) for 5 min. Treatments with agitation (50 rpm) were conducted on a platform shaker (Model No. G33, New Brunswick Scientific, Edison, N.J.). At the end of the treatment, all samples were immediately removed from treatment solutions, and two samples were randomly selected (from among four samples) for use in determining the number of viable cells on tested surfaces. Each of two selected samples was immersed in 100 ml of neutralizing buffer, and cells were recovered by vigorous agitation with the same platform shaker at 200 rpm for 2 min. The numbers of viable cells in the neutralizing buffer and in the treatment solution after treatment were then determined through serial dilutions in 9 ml of sterile neutralizing buffer followed by direct plating of 0.1 ml of each dilution in duplicate on TSA plates and incubation at 37°C for 24 h before counting. The initial bacterial populations on the tested surfaces before treatment were also determined by the recovery of cells in neutralizing buffer by the same procedure used for the tested surface after treatment. No loss in viability during drying was observed throughout the study. For enrichment, 5 ml each of neutralizing buffer and treatment solution after use were also transferred to separate 250-ml Erlenmeyer flasks containing 100 ml of sterile TSB and incubated at 37°C for 48 h. Following enrichment,

TABLE 2. *Populations of* E. aerogenes *on treated surfaces and in wash solution after treatments without agitation*

Non-food-contact surface	Population on surface (log_{10} CFU/cm^2)[a]			Population in wash solution after treatment (log_{10} CFU/ml)[b]	
	Initial	After control wash[c]	After EO water wash[d]	Control solution	EO water
Glass	6.11 ± 0.11	5.92 ± 0.05	3.88 ± 0.13	2.49 ± 0.06	ND[e]
Stainless steel	6.19 ± 0.07	5.98 ± 0.05	3.76 ± 0.14	2.54 ± 0.05	ND
Glazed ceramic tile	6.15 ± 0.10	6.01 ± 0.06	3.93 ± 0.20	2.62 ± 0.03	ND
Unglazed ceramic tile	6.17 ± 0.10	5.97 ± 0.06	3.96 ± 0.15	2.54 ± 0.06	ND
Vitreous china	6.18 ± 0.09	5.87 ± 0.11	3.93 ± 0.12	2.55 ± 0.04	ND

[a] Values are the means of 12 readings from three replicated measurements ± standard deviations.
[b] Values are the means of six readings from three replicated measurements ± standard deviations.
[c] The control solution was obtained by neutralizing residual chlorine in the EO water (generated at a setting of 14 A) with neutralizing buffer.
[d] EO water was generated at a setting of 14 A.
[e] ND, negative by an enrichment procedure; no detectable survivors by a direct plating procedure.

the culture was streaked on TSA plates, and the plates were incubated at 37°C for 48 h before counting *(8).* Three independent replicate trials were conducted for the treatment.

RESULTS AND DISCUSSION

Effectiveness of EO water in killing *E. aerogenes* and *S. aureus* pure culture. Properties (pH, ORP, and residual chlorine) of EO water and the treatment solutions used in the bacterium inactivation studies are presented in Table 1. No residual chlorine was detected in the control solution. The initial populations of *E. aerogenes* and *S. aureus* used in this study were 8.0 and 8.04 log_{10} CFU/ml, respectively. Inactivation (reduction of >9 log_{10} CFU/ml) of *E. aerogenes* and *S. aureus* occurred within 30 s after exposure to EO water containing approximately 25 or 50 mg of residual chlorine per liter. *S. aureus* was more resistant than *E. aerogenes* to the diluted EO water containing approximately 10 mg of residual chlorine per liter. After exposure to EO water containing approximately 10 mg of residual chlorine per liter for 30 s, the population of *E. aerogenes* decreased to an undetectable level, whereas the surviving population of *S. aureus* was 3.9 log_{10} CFU/ml. These results confirm earlier findings *(13)* that EO water with 10 mg of residual chlorine per liter is very effective, reducing the populations of *E. coli* O157:H7, *L. monocytogenes,* and *B. cereus* vegetative cells to undetectable levels after a 60-s treatment. Zhao et al. *(24)* found that most *E. coli* O157:H7 strains are very sensitive to chlorine and that a reduction of >7 log_{10} CFU/ml can be achieved with 0.25 mg of free chlorine per liter. In the meantime, no difference in bacterial counts between the control-treated population and the initial population was observed throughout the study.

Sanitizing effectiveness of EO water in treating different test surfaces. EO water generated at 14 A had an initial pH of 2.55, an ORP of 1,181 mV, and a residual chlorine level of 52.8 mg/liter. The control solution had a pH of 2.54, an ORP of 379 mV, and no residual chlorine. The initial populations of *E. aerogenes* and *S. aureus* were approximately 6.1 log_{10} CFU/cm^2 regardless of the type of surface in question (Tables 2 and 3). The survival characteristics of *E. aerogenes* and *S. aureus* after exposure to EO water or control solution without agitation are presented in

TABLE 3. *Populations of* S. aureus *on treated surfaces and in wash solution after treatments without agitation*

Non-food-contact surfaces	Population on surface (log_{10} CFU/cm^2)[a]			Population in wash solution after treatment (log_{10} CFU/ml)[b]	
	Initial	After control wash[c]	After EO water wash[d]	Control solution	EO water
Glass	6.10 ± 0.10	5.87 ± 0.14	4.37 ± 0.05	2.49 ± 0.05	ND[e]
Stainless steel	6.14 ± 0.07	5.94 ± 0.08	4.32 ± 0.08	2.60 ± 0.06	ND
Glazed ceramic tile	6.05 ± 0.08	5.96 ± 0.10	4.34 ± 0.07	2.65 ± 0.05	ND
Unglazed ceramic tile	6.11 ± 0.08	5.97 ± 0.07	4.30 ± 0.05	2.56 ± 0.10	ND
Vitreous china	6.12 ± 0.09	6.02 ± 0.08	4.25 ± 0.06	2.51 ± 0.03	ND

[a] Values are the means of 12 readings from three replicated measurements ± standard deviations.
[b] Values are the means of six readings from three replicated measurements ± standard deviations.
[c] The control solution was obtained by neutralizing residual chlorine in the EO water (generated at a setting of 14 A) with neutralizing buffer.
[d] EO water was generated at a setting of 14 A.
[e] ND, negative by an enrichment procedure; no detectable survivors by a direct plating procedure.

TABLE 4. *Populations of* E. aerogenes *on treated surfaces and in wash solution after treatments with agitation at 50 rpm*

Non-food-contact surfaces	Population on surface (log_{10} CFU/cm^2)[a]			Population in wash solution after treatment (log_{10} CFU/ml)[b]	
	Initial	After control wash[c]	After EO water wash[d]	Control solution	EO water
Glass	6.09 ± 0.09	3.32 ± 0.07	ND[e]	4.69 ± 0.08	ND
Stainless steel	6.12 ± 0.08	3.34 ± 0.08	ND	4.63 ± 0.12	ND
Glazed ceramic tile	6.14 ± 0.05	3.22 ± 0.14	ND	4.73 ± 0.07	ND
Unglazed ceramic tile	6.14 ± 0.06	3.17 ± 0.12	ND	4.75 ± 0.08	ND
Vitreous china	6.13 ± 0.09	3.17 ± 0.11	ND	4.67 ± 0.05	ND

[a] Values are the means of 12 readings from three replicated measurements ± standard deviations.
[b] Values are the means of six readings from three replicated measurements ± standard deviations.
[c] The control solution was obtained by neutralizing residual chlorine in the EO water (generated at a setting of 14 A) with neutralizing buffer.
[d] EO water was generated at a setting of 14 A.
[e] ND, negative by an enrichment procedure; no detectable survivors by a direct plating procedure.

Tables 2 and 3. After the immersion of test surfaces in EO water for 5 min without agitation, the populations of *E. aerogenes* and *S. aureus* were reduced by 2.2 to 2.5 log_{10} CFU/cm^2 and by 1.7 to 1.9 log_{10} CFU/cm^2, respectively. Washing of inoculated surfaces with the control solution had only a minimal effect (a reduction of about 0.1 to 0.3 log_{10} CFU/cm^2). The populations of *E. aerogenes* and *S. aureus* in wash solutions immediately after treatment are also shown in Tables 2 and 3. No viable cells of either bacterium were detected in the EO water after treatment. However, >2 log_{10} CFU of viable cells per ml were recovered from the control wash solution after treatment.

Washing of inoculated surfaces in EO water with agitation at 50 rpm decreased the populations of both bacteria on different test surfaces to undetectable levels, whereas the control treatment resulted in a reduction of approximately 3 log_{10} CFU/cm^2 for both bacteria (Tables 4 and 5). The observed reduction after the control treatment could be due to the removal of cells from inoculated surfaces by agitation. No viable cells of either bacterium were observed in EO water after treatment (Tables 4 and 5). However, average counts of 4.6 to 4.9 log_{10} CFU/ml were recovered from the control solution after treatment, suggesting that significant amounts of attached cells were removed from the inoculated surfaces during agitation (compared with approximately 2.5 log_{10} CFU/ml for the treatment without agitation).

To date, the antimicrobial mechanisms of EO water have not been fully understood, but the presence of chlorine *(16)* and high ORP values *(12)* have been reported to contribute to the antimicrobial capacity of EO water. Previous studies have also suggested that hypochlorous acid (an undissociated form of chlorine) penetrates microbial cell membranes and subsequently exerts its antimicrobial action through the oxidation of key metabolic systems *(3, 4, 11).* In the current study, EO water treatment was less effective without agitation than with agitation, perhaps because of the limited ability of chlorine in EO water to penetrate attached microbial cell layers. Without agitation, the TSB medium could possibly react with EO water and form combined chlorine and hence reduce the local active chlorine concentration at or near the tested surfaces *(17).* Complete inactivation of both bacteria on tested surfaces after EO water treatment with agitation was observed, perhaps be-

TABLE 5. *Populations of* S. aureus *on treated surfaces and in wash solution after treatments with agitation at 50 rpm*

Non-food-contact surfaces	Population on surface (log_{10} CFU/cm^2)[a]			Population in wash solution after treatment (log_{10} CFU/ml)[b]	
	Initial	After control wash[c]	After EO water wash[d]	Control solution	EO water
Glass	6.13 ± 0.07	3.13 ± 0.09	ND[e]	4.79 ± 0.09	ND
Stainless steel	6.16 ± 0.06	3.20 ± 0.11	ND	4.71 ± 0.05	ND
Glazed ceramic tile	6.14 ± 0.06	3.17 ± 0.09	ND	4.86 ± 0.04	ND
Unglazed ceramic tile	6.13 ± 0.08	3.19 ± 0.14	ND	4.69 ± 0.05	ND
Vitreous china	6.10 ± 0.09	3.18 ± 0.11	ND	4.72 ± 0.03	ND

[a] Values are the means of 12 readings from three replicated measurements ± standard deviations.
[b] Values are the means of six readings from three replicated measurements ± standard deviations.
[c] The control solution was obtained by neutralizing residual chlorine in the EO water (generated at a setting of 14 A) with neutralizing buffer.
[d] EO water was generated at a setting of 14 A.
[e] ND, negative by an enrichment procedure; no detectable survivors by a direct plating procedure.

cause (i) the cells removed from the surfaces during agitation were immediately inactivated in EO water, (ii) agitation facilitates the penetration of EO water into the remaining cells on the test surfaces, or (iii) the well-mixed EO water resulting from agitation allows chlorine to react with cells more efficiently.

In conclusion, this study revealed that EO water treatment could be used as an effective method for reducing microbial contamination on different surfaces. The similar levels of effectiveness of EO water in reducing microbial contamination on many diverse surfaces suggested that EO water might well be as effective on a number of other surfaces as well. The observed complete inactivation of *S. aureus* and *E. aerogenes* in EO water after treatment indicates that EO water can also prevent cross-contamination from treatment solutions. Since EO water is produced on-site and on demand for direct use, it can also reduce health hazards for workers by eliminating the need to handle concentrated chemicals.

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