

Roles of Oxidation–Reduction Potential in Electrolyzed Oxidizing and Chemically Modified Water for the Inactivation of Food-Related Pathogens

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ABSTRACT

This study investigates the properties of electrolyzed oxidizing (EO) water for the inactivation of pathogen and to evaluate the chemically modified solutions possessing properties similar to EO water in killing *Escherichia coli* O157:H7. A five-strain cocktail (10^{10} CFU/ml) of *E. coli* O157:H7 was subjected to deionized water (control), EO water with 10 mg/liter residual chlorine (J.A.W-EO water), EO water with 56 mg/liter residual chlorine (ROX-EO water), and chemically modified solutions. Inactivation ($8.88 \log_{10}$ CFU/ml reduction) of *E. coli* O157:H7 occurred within 30 s after application of EO water and chemically modified solutions containing chlorine and 1% bromine. Iron was added to EO or chemically modified solutions to reduce oxidation–reduction potential (ORP) readings and neutralizing buffer was added to neutralize chlorine. J.A.W-EO water with 100 mg/liter iron, acetic acid solution, and chemically modified solutions containing neutralizing buffer or 100 mg/liter iron were ineffective in reducing the bacteria population. ROX-EO water with 100 mg/liter iron was the only solution still effective in inactivation of *E. coli* O157:H7 and having high ORP readings regardless of residual chlorine. These results suggest that it is possible to simulate EO water by chemically modifying deionized water and ORP of the solution may be the primary factor affecting microbial inactivation.

Escherichia coli serotype O157:H7 has caused numerous outbreaks of the life-threatening conditions, hemorrhagic colitis and hemolytic uremic syndrome, particularly in the U.S., where foods such as undercooked ground meat and raw milk have been implicated (1). Nebraska Health and Human Services (4) reported that contaminated lettuce may have been the source of Nebraska's largest *E. coli* O157:H7 outbreak. Blake (6) reported that fecal contamination in a water park was the source of a 1998 *E. coli* O157:H7 outbreak in Georgia. Several additional outbreaks of *E. coli* O157:H7 infection have been reported in other areas of the world including Japan (17, 19), Canada (10), the United Kingdom (25), Mexico (11, 12), China (36), and Argentina (21). Foods of animal origin, principally from dairy cattle, have been associated with *E. coli* O157:H7 infections. However, it is not known if animals are the sole reservoir of *E. coli* O157:H7 or if foods other than beef and milk become contaminated with the organism during processing and handling. Doyle (12) suggested that water was the vehicle of a large community outbreak of *E. coli* O157:H7 infections in the United States. McGowan et al. (23) have also isolated *E. coli* O157:H7 from a water sample taken from a countryside reservoir, providing further evidence that the organism can be waterborne. Spika et al. (30), Carter et al. (10), and Griffin et al. (14) reported that person-to-person spread of the organism has been identified as the primary means of transmission in an outbreak at a

day-care center and implicated as responsible for the second wave of illness in other outbreaks principally at nursing homes. Beuchat (5), Brackett (7), and Nguyen and Carlin (26) indicated that tap water has limited or no effect on killing bacteria that commonly occur on raw and minimally processed produce and may result in cross contamination of food preparation surfaces, serving and eating utensils, and other food items.

Consumers have demonstrated an interest in the quality of water in their homes by choosing to use one or more water-quality improvement technologies. These include aeration, water filtration, reverse osmosis system, water softening, and oxidant application (35). From a health and safety point of view, the most important water treatment process is disinfection. Some disinfection methods include distillation, UV irradiation, ozonization, iodination, and chlorination (35). However, the water treatment processes described above have disadvantages. Distillation takes longer to produce the processed water than other methods. There exists no simple test to determine efficacy of UV treatment. The UV irradiation system also needs an effective cleaning program to ensure that biological or chemical foulants do not block UV transmissions through the water. Ozone is unstable and has a very short life, so it must be generated at the point of use. Efficacy of ozone treatment is also difficult to validate. Treatment of water with iodine can result in an objectionable taste and is relatively costly. A National Academy of Science Report (35) also states that continuous iodine intakes of as little as 0.5 mg/day might

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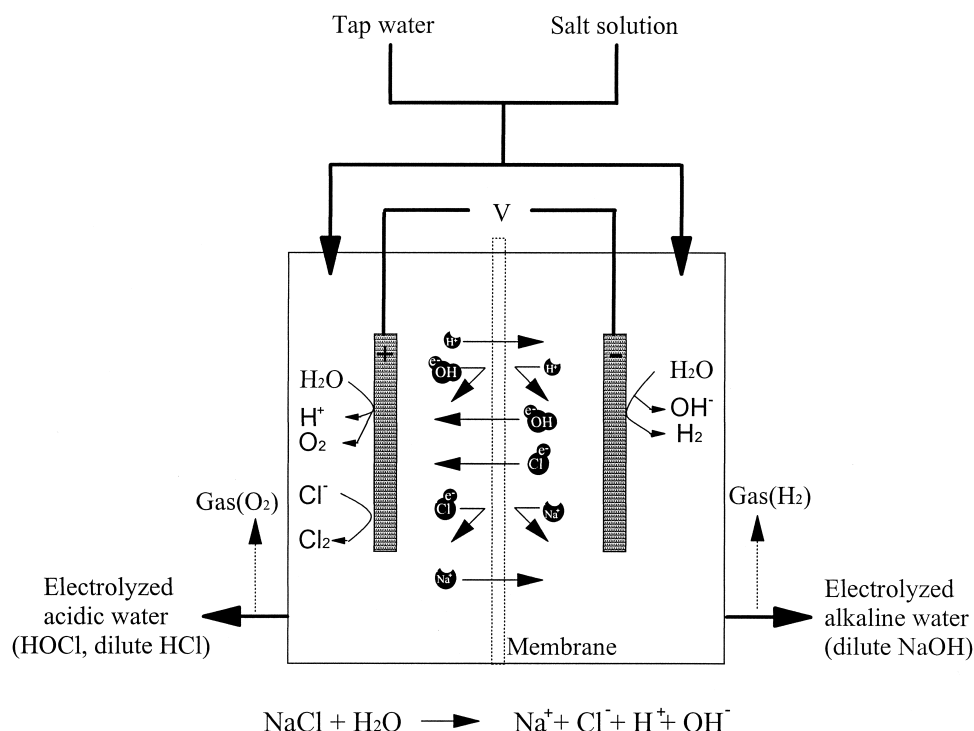


FIGURE 1. Schematic of EO water generator and produced compounds.

have resulted in thyroiditis or increased rates of thyroid carcinomas. Trihalomethanes, by-products formed when chlorine reacts with naturally occurring organic matter such as decayed leaves, have been linked to a higher rate of cancer and have been classified by the U.S. Environmental Protection Agency (27, 33) as possible human carcinogens.

Electrolyzed oxidizing (EO) water has been reported by scientists in Japan to have strong bactericidal effects on most pathogenic bacteria (16, 18, 19). A major advantage of using EO water for inactivation of bacteria is that it is produced using pure water with no added chemicals except sodium chloride (NaCl). Therefore, it has less adverse impact on the environment. The generation of EO water involves reactions in a cell containing inert positively charged and negatively charged electrodes, respectively, separated by a membrane, and through which a very dilute salt water solution passes (Fig. 1). By subjecting the electrodes to direct current voltage, two types of water possessing different characteristics are generated. An electrolyzed basic aqueous solution (pH 11.4 and oxidation–reduction potential [ORP], -795 mV) is produced from the cathode side and has a reducing potential that leads to a reduction of free radicals in biological systems and may be useful in the treatment of organ malfunctions. An electrolyzed strong acid solution is produced from the anode side and has a high ORP (about 1,150 mV), presence of hypochlorous acid (HOCl), and strong bactericidal effect. HOCl is produced during electrolysis of saline-added water, and the amount of HOCl increases in response to the amount of added NaCl (3). However, production of EO water can be modified to reduce the presence of HOCl and still maintains its effectiveness for microbial inactivation to reduce the health concern as chlorinated water.

The overall objective of this study was to investigate the properties of EO water for the inactivation of *E. coli* O157:H7. There were two specific objectives of this study. The first objective was to produce chemically modified water from deionized water with properties (pH, residual chlorine, and ORP) similar to EO water without using electrolysis. The second objective was to evaluate the influence of water properties (pH, residual chlorine, and ORP) on *E. coli* O157:H7.

MATERIALS AND METHODS

EO water produced from two different generators was used for this study. The first EO water was produced from J.A.W-020 (N.I.A. Chemical, Nippon Intek Co., Japan) at setting 9 and the other one was produced from ROX-20TA (Hoshizaki Electric Inc., Japan) at a setting of 14 amperage. After reaching stable EO water properties (about 1 min after turning on the machine), EO water was collected from the anode side of the EO water generators for the experiment. Deionized water was chemically modified by changing pH, ORP, and residual chlorine levels to obtain properties similar to EO water produced from the above two different EO water generators (J.A.W-020 and ROX-20TA). The pH of deionized water was modified by different acid solutions (acetic acid, ascorbic acid, citric acid, hydrochloric acid, and oxalic acid; certified for biological work, Fisher Scientific Co., Fair Lawn, N.J.) at various concentrations. ORP was modified by addition of bromine water (1% and 6% vol/vol, certified for biological work, Fisher Scientific Co.) to deionized water. Residual chlorine concentration was modified by addition of chlorine water (1% or 6% vol/vol, certified for biological work, Fisher Scientific Co.) or 5.25% sodium hypochlorite (Clorox, Oakland, Calif.) to deionized water.

Preparation of treatment solutions. Different chemically modified water was produced by adjusting one of the three prop-

TABLE 1. Properties of deionized water containing various chemicals

Solution	Concentration (%)	pH	ORP (mV)	Residual chlorine (mg/liter)
Deionized water		5.6	453	0
Acetic acid	2.0	2.7	571	0
Hydrochloric acid	0.0025 N	2.6	530	0
Ascorbic acid	0.5	2.7	167	0
Citric acid	0.4	2.6	543	0
Oxalic acid	0.02	2.7	469	0
Bromine water	Saturated	2.0	1,039	0
	1.0	3.5	981	0
	6.0	3.4	988	0
Chlorine water	Saturated	1.8	1,182	2,017
	1.0	3.9	998	13
	6.0	2.9	1,160	60
Clorox ^a		11.2	693	43,833
EO water (J.A.W-020) ^b		2.5	1,123	10
EO water (ROX-20TA) ^c		2.6	1,160	56

^a A 5.25% sodium hypochlorite (NaOCl) solution.

^b Electrolyzed oxidizing water produced from J.A.W-020 generator.

^c Electrolyzed oxidizing water produced from ROX-20TA generator.

erties (pH, ORP, and residual chlorine) at a time using different acids, bromine water, chlorine water, or dilute sodium hypochlorite solution (Table 1). Based on the results from the chemically modified water (Table 1), chemically modified water with properties matching EO water was prepared (Table 2) using the combination of acid and chlorine water. In each case, pH and ORP were monitored immediately after preparation using a pH meter (Accumet model 15, Fisher Scientific Co.) with a pH and ORP electrode. The residual chlorine of EO and chemically modified water was determined with a chlorine test kit (Hach Co., Ames, Iowa).

Preparation of inocula. Five strains of *E. coli* O157:H7 (CA1, raw ground beef isolate; E0019, calf feces isolate; E09, beef isolate; 932, human feces isolate; F500, human feces isolate) were used in the study. Cultures were transferred three times to tryptic soy broth (pH 7.3, Difco, Detroit, Mich.) by loop inoculation at successive 24-h intervals and incubated at 37°C. Twenty-four-hour cultures of *E. coli* O157:H7 were then centrifuged for 10 min at maximum speed (1,800 × g, 24°C) in a clinical centrifuge (model IEC 809, International Equipment Company, Mass.). The pellets were resuspended in 5 ml of 0.1% (wt/vol) peptone (Difco) water, centrifuged at 1,800 × g for 10 min and then resuspended in 5 ml of 0.1% peptone water. Equal volume (2 ml) of five cultures was mixed to give an inoculum containing approximately 10¹⁰ CFU/ml of *E. coli* O157:H7.

Procedures for inoculation. One milliliter of cell inoculum (*E. coli* O157:H7, 10¹⁰ CFU/ml) was added to 100 ml of EO water or chemically modified solutions at 24°C. After the 30-s treatment, 1 ml of the solution was serially diluted in sterile 0.1% peptone water and 0.1 ml was deposited on tryptic soy agar (Difco). Inocula were uniformly distributed on the agars using sterile bent glass rods. The plates were incubated at 37°C for 24 h. Each sample was also inoculated into tryptic soy broth for enrichment

TABLE 2. Formulation of chemically modified water with similar properties as EO water

Solution		pH	ORP (mV)	Residual chlorine (mg/liter)
EO water ^a	J.A.W-020	2.5	1,123	10
2% AA ^b + 1% Cl ^c		2.6	1,123	13
0.0025 N HCl ^d + 1% Cl		2.7	1,100	13
EO water ^e	ROX-20TA	2.6	1,160	56
2% AA + 6% Cl		2.5	1,180	60
0.0025 N HCl + 6% Cl		2.5	1,170	60
2% AA + 1% Br ^f		2.6	993	0

^a Electrolyzed oxidizing water produced from J.A.W-020 generator (Nippon Intek Co.).

^b Acetic acid solution.

^c Chlorine water.

^d Hydrochloric acid.

^e Electrolyzed oxidizing water produced from ROX-20TA generator (Hoshizaki Electric Inc.).

^f Bromine water.

to detect the presence of the low number of survivors that would not be detected by direct plating. Three independent replicate trials were conducted for each treatment solution.

Statistical analysis. Data were analyzed using the general linear model procedures (31). Comparisons of means were performed using Duncan's multiple range test. A number 1.00 was assigned to the log₁₀ CFU/ml value for statistical analysis purpose (15) when either no bacteria were detected on tryptic soy agar plates, or the presence of bacteria was detected only by an enrichment procedure.

RESULTS AND DISCUSSION

Effects of various chemicals on the properties (pH, ORP, and residual chlorine) of deionized water are presented in Table 1. All acid solutions were successfully used to reduce the pH of deionized water to 2.6 to 2.7. ORP of the acid solutions ranged from 167 to 571 mV that was much lower than the ORP (1,123 to 1,160 mV) of EO water. No residual chlorine was detected in the acid solutions.

Even though saturated bromine water had a pH of 2.0 and ORP of 1,039 mV, 1% and 6% bromine water used for ORP modification had relatively high pH (3.4 to 3.5) and slightly lower ORP value (981 to 988 mV) compared to those of EO water. Chlorine water and Clorox were used to adjust the residual chlorine of deionized water. Saturated chlorine water had a pH of 1.8, ORP of 1,182 mV, and residual chlorine of 2,017 mg/liter, and Clorox containing 5.25% sodium hypochlorite solution had a pH of 11, ORP of 693 mV, and residual chlorine of 43,833 mg/liter. The 1% and 6% chlorine waters with residual chlorine of 13 and 60 mg/liter, respectively, closely matched the chlorine level of two EO waters. However, both 1% and 6% chlorine water also increased ORP significantly. Because of the alkaline pH of the Clorox solution, Clorox was not used further.

To produce a chemically modified solution with properties closely matching with EO water, combinations of

TABLE 3. Populations of *E. coli* O157:H7 after a 30-s treatment in deionized water and chemically modified solutions

Treatment	Surviving population (log ₁₀ CFU/ml) ^a
Deionized water	9.82 A
2% acetic acid	9.68 A
1% bromine water	ND B ^b
1% chlorine water	<1.0 B ^c

^a Values in a column sharing a common letter are not significantly different ($P \leq 0.05$); initial population was 9.88 log₁₀ CFU/ml.

^b Not detected on enrichment.

^c Not detectable on direct plate count but positive on enrichment.

acid, chlorine water, and bromine water were used (Table 2). Chemically modified solutions containing 2% acetic acid and 1% chlorine water (pH 2.6; ORP, 1,123 mV; and residual chlorine, 13 mg/liter) yielded similar properties to J.A.W-EO water (pH 2.5; ORP, 1,123 mV; and residual chlorine, 10 mg/liter). Another way to generate chemically modified water with similar properties to J.A.W-EO water was using 0.0025 N hydrochloric acid solution containing 1% chlorine water (pH 2.7; ORP, 1,100 mV; and residual chlorine, 13 mg/liter). It was also possible to duplicate the properties of ROX-EO water by chemically modifying deionized water with 2% acetic acid plus 6% chlorine water (pH 2.5; ORP, 1,180 mV; and residual chlorine, 60 mg/liter) or with 0.0025 N hydrochloric acid solution containing 6% chlorine water (pH 2.5; ORP, 1,170 mV; and residual chlorine, 60 mg/liter). The properties of 1% bromine water plus 2% acetic acid (pH 2.6; ORP, 993 mV; and residual chlorine, 0 mg/liter) were close to pH and ORP properties of J.A.W-EO water but without residual chlorine.

Three chemically modified solutions—2% acetic acid (for pH modification), 1% bromine water (for ORP modification), and 1% chlorine water (for chlorine modification)—were first selected for the microbial challenge study. Populations of *E. coli* O157:H7 after a 30-s treatment are shown in Table 3. The 2% acetic acid solution (pH 2.7; ORP, 571 mV; and residual chlorine, 0 mg/liter) was not effective in reducing population of *E. coli* O157:H7. However, inactivation of *E. coli* O157:H7 occurred within 30 s after application of 1% bromine water (pH 3.5; ORP, 981 mV; and residual chlorine, 0 mg/liter) and 1% chlorine water (pH 3.9; ORP, 998 mV; and residual chlorine, 13 mg/liter). Results in Table 3 indicate that ORP, residual chlorine or the combination of both contributed to the microbial inactivation. Marriott (22) reported that HOCl existing in chlorinated water is responsible for the lethal reactions associated with the bacterial cell membrane, DNA denaturation, or disruption of protein synthesis. Albrich and Hurst (2) noted that the microbicidal action of HOCl arises from interruption of energy-linked cellular respiration due to bacterial cells exposed to HOCl undergo irreversible oxidation of cytochrome *b*, carotene, and adenine nucleotides. Several researchers also reported that HOCl will cause profound alteration of microbial cell membrane metabolite transport capabilities (8, 34) and denaturation of enzymes on the cell surface and lowering of the cytoplasmic pH due to proton

TABLE 4. Populations of *E. coli* O157:H7 after a 30-s treatment in deionized, chemically modified, and EO water

Treatment	Surviving population (log ₁₀ CFU/ml) ^a
Deionized water	9.82 A
EO water ^b	<1.0 B ^c
2% AA ^d + 1% Cl ^e	ND B ^f
0.0025 N HCl ^g + 1% Cl	<1.0 B
EO water ^h	ND B
2% AA + 6% Cl	ND B
0.0025 N HCl + 6% Cl	ND B
2% AA + 1% Br ⁱ	ND B

^a Values in a column sharing a common letter are not significantly different ($P \leq 0.05$); initial population was 9.88 log₁₀ CFU/ml.

^b Electrolyzed oxidizing water produced from J.A.W-020 generator (Nippon Intek Co.).

^c Not detectable on direct plate count but positive on enrichment.

^d Acetic acid.

^e Chlorine water.

^f Not detected on enrichment.

^g Hydrochloric acid.

^h Electrolyzed oxidizing water produced from ROX-20TA generator (Hoshizaki Electric Inc.).

ⁱ Bromine water.

permeation through membrane in response to the increased pH gradient (2, 13, 20).

Populations of *E. coli* O157:H7 that survived a 30-s treatment in EO waters and chemically modified solutions with similar properties as EO water (Table 2) are shown in Table 4. Inactivation of *E. coli* O157:H7 occurred within 30 s after application of EO water and chemically modified water solutions containing 1% chlorine water plus 2% acetic acid, 1% chlorine water plus 0.0025 N hydrochloric acid, 6% chlorine water plus 2% acetic acid, 6% chlorine water plus 0.0025 N hydrochloric acid, and 1% bromine water plus 2% acetic acid. In other words, chemically modified waters that simulate properties of EO water were very effective in killing *E. coli* O157:H7. Hayashibara et al. (16) and Iwasawa et al. (18, 19) also reported that EO water has strong killing effects on various bacteria (*E. coli*, *Pseudomonas*, *Enterobacter*, *Shigella*, *Staphylococcus*, and *Vibrio*).

Because of the difficulty of chemically modifying water to achieve desired residual chlorine and ORP, alternative methods of neutralizing chlorine or reducing ORP were attempted. The effect of iron (Iron Powder, Fisher Scientific Co.) addition on the ORP, pH, and residual chlorine content of chemically modified and EO water was evaluated. The properties of J.A.W-EO water and ROX-EO water were investigated with the addition of 100 mg/liter iron. The properties of chemically modified water containing 2% acetic acid plus 1% or 6% chlorine water and 100 mg/liter iron were also investigated. Residual chlorine equal to that of the EO water was achieved by addition of neutralizing buffer (10% vol/vol of 5.2 g/liter neutralizing buffer, Difco). The pH, ORP, and residual chlorine concentration in these solutions were determined immediately after the ORP of

TABLE 5. pH, ORP, and residual chlorine of chemically modified and EO water containing iron and neutralizing buffer

Solution	pH	ORP (mV)	Residual chlorine (mg/liter)
EO water ^a + 100 mg/liter iron	2.6	322	10
2% AA ^b + 1% Cl ^c + 100 mg/liter iron	2.7	303	13
EO water ^d + 100 mg/liter iron	2.7	1,122	56
2% AA + 6% Cl + 100 mg/liter iron	2.4	256	60
EO water ^a + neutralizing buffer ^e	2.4	391	0
EO water ^d + neutralizing buffer	2.5	540	0

^a Electrolyzed oxidizing water produced from J.A.W-020 generator (Nippon Intek Co.).

^b Acetic acid solution.

^c Chlorine water.

^d Electrolyzed oxidizing water produced from ROX-20TA generator (Hoshizaki Electric Inc.).

^e Ten milliliters of 5.2 g/liter neutralizing buffer solution.

the treatment solution stabilized. Deionized water was used as a control in this study. Adding 100 mg/liter iron to the J.A.W-EO water reduced the ORP to 322 mV without affecting the pH and residual chlorine (Table 5). For ROX-EO water, the ORP reading was still high (1,122 mV) even 2 h after the addition of 100 mg/liter iron. The ORP of chemically modified water with the addition of 100 mg/liter iron decreased to 200–300 mV probably due to chemical reactions of chlorine compound and iron.

Compared to iron addition on EO water and other chemically modified water, it took longer for the ORP reading of EO water to stabilize, which may suggest that HOCl existing in the EO water is more stable than those in chemically modified water. Results in Table 5 also suggest that iron might reduce the oxidation potential of the solution acting as a reducing agent. Swaddle (32) reported that chloride can form complexes with Fe²⁺, facilitating its departure by reducing its effective charge, by forming FeCl⁺ (aq) or even FeCl₂ (aq). Silver et al. (29) agreed with Swaddle (32) that iron chloride was serving as a reducing agent. The residual chlorine of the EO water was reduced to 0 mg/liter within 10 min of the addition of neutralizing buffer (Table 5). The neutralizing buffer also reduced the ORP of J.A.W-EO water and ROX-EO water to 391 and 540 mV, respectively.

Populations of *E. coli* O157:H7 that survived a 30-s treatment in chemically modified and EO water with added iron and neutralizing buffer are presented in Table 6. Chemically modified J.A.W-EO water containing 100 mg/liter iron was not effective in killing *E. coli* O157:H7. Table 6 also shows that populations of *E. coli* O157:H7 were not affected by chlorine solution containing 2% acetic acid plus 100 mg/liter iron. However, ROX-EO water with the addition of 100 mg/liter iron was still effective in killing bacteria. The EO waters with 10 ml of neutralizing buffer (5.2 g/liter) added had a pH of 2.4 to 2.5, ORP of 391 to 540 mV, and no residual chlorine. These solutions were not effective in reducing populations of *E. coli* O157:H7. The

TABLE 6. Populations of *E. coli* O157:H7 after a 30-s treatment in deionized water and chemically modified water with iron and neutralizing buffer

Treatment	Surviving population (log ₁₀ CFU/ml) ^a
Deionized water	9.76 A
EO water ^b + 100 mg/liter iron	9.71 A
2% AA ^c + 1% Cl ^d + 100 mg/liter iron	9.65 A
EO water ^e + 100 mg/liter iron	ND B ^f
2% AA + 6% Cl + 100 mg/liter iron	9.75 A
EO water ^b + neutralizing buffer ^g	9.41 A
EO water ^e + neutralizing buffer	9.40 A

^a Values in a column sharing a common letter are not significantly different ($P \leq 0.05$); initial population was 9.88 log₁₀ CFU/ml.

^b Electrolyzed oxidizing water produced from J.A.W-020 generator (Nippon Intek Co.).

^c Acetic acid solution.

^d Chlorine water.

^e Electrolyzed oxidizing water produced from ROX-20TA generator (Hoshizaki Electric Inc.).

^f Not detected on enrichment.

^g Ten milliliters of 5.2 g/liter neutralizing buffer solution.

only modified solution (Table 6) that was effective in inactivation of *E. coli* O157:H7 was ROX-EO water with 100 mg/liter iron. This solution is also the only one that still had high ORP readings regardless of residual chlorine concentration.

The possible reason that the ROX-EO water with 100 mg/liter iron added was still effective by inactivated *E. coli* O157:H7 may be due to insufficient time for the added iron to reduce oxidation potential in the solution. After 2 h application of the iron, the ORP value in the ROX-EO water was still high (ORP, 1,122 mV). However, the ORP reading of the ROX-EO water containing 100 mg/liter iron eventually dropped to 300–400 mV after 10 h (data not shown).

Results in this study suggest that ORP of the treatment solutions may be the primary factor affecting microbial inactivation. This observation appears consistent with the reports concerning water disinfection applications by McPherson (24) and Carlson (9) where the ORP value of the solution has been demonstrated to be more meaningful than the concentration of residual (free) or total chlorine. McPherson (24) also reported that ORP became a world standard in 1968 when a German Federal Health Office Laboratory first proved that the killing rate of *E. coli* bacteria was dependent on ORP and not on residual chlorine. Carlson (9) and Robbs et al. (28) also noted that the killing of bacteria was not based on a defined chlorine reaction and that higher ORPs were required for complete kill of *E. coli*. Hence, chlorine measurement alone cannot guarantee disinfection, but ORP provides a single measurement of the total oxidation capability, regardless of the pH and the concentration of chlorine.

In summary, ORP should be considered as the primary indication of disinfection (or oxidation) capability. The properties of EO water could be simulated by chemically

modifying deionized water with acetic acid and chlorine water. EO water and chemically modified deionized water possessing similar properties of EO water were effective in inactivation of *E. coli* O157:H7 within 30 s treatment time.

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