Journal of Food Protection, Vol. 67, No. 7, 2004, Pages 1377–1383

Effects of Water Source, Dilution, Storage, and Bacterial and Fecal Loads on the Efficacy of Electrolyzed Oxidizing Water for the Control of *Escherichia coli* O157:H7

S. M. L. STEVENSON,[1,2] S. R. COOK,[1,2] S. J. BACH,[1] AND T. A. MCALLISTER[1]*

[1]*Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada T1J 4B1; and* [2]*Department of Microbiology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1*

MS 03-373: Received 21 August 2003/Accepted 25 January 2004

ABSTRACT

To evaluate the potential of using electrolyzed oxidizing (EO) water for controlling *Escherichia coli* O157:H7 in water for livestock, the effects of water source, electrolyte concentration, dilution, storage conditions, and bacterial or fecal load on the oxidative reduction potential (ORP) and bactericidal activity of EO water were investigated. Anode and combined (7:3 anode:cathode, vol/vol) EO waters reduced the pH and increased the ORP of deionized water, whereas cathode EO water increased pH and lowered ORP. Minimum concentrations (vol/vol) of anode and combined EO waters required to kill 10^4 CFU/ml planktonic suspensions of *E. coli* O157:H7 strain H4420 were 0.5 and 2.0%, respectively. Cathode EO water did not inhibit H4420 at concentrations up to 16% (vol/vol). Higher concentrations of anode or combined EO water were required to elevate the ORP of irrigation or chlorinated tap water compared with that of deionized water. Addition of feces to EO water products (0.5% anode or 2.0% combined, vol/vol) significantly reduced ($P < 0.001$) their ORP values to <700 mV in all water types. A relationship between ORP and bactericidal activity of EO water was observed. The dilute EO waters retained the capacity to eliminate a 10^4 CFU/ml inoculation of *E. coli* O157:H7 H4420 for at least 70 h regardless of exposure to UV light or storage temperature (4 versus 24°C). At 95 h and beyond, UV exposure reduced ORP, significantly more so ($P < 0.05$) in open than in closed containers. Bactericidal activity of EO products (anode or combined) was lost in samples in which ORP value had fallen to ≤848 mV. When stored in the dark, the diluted EO waters retained an ORP of >848 mV and bactericidal efficacy for at least 125 h; with refrigeration (4°C), these conditions were retained for at least 180 h. Results suggest that EO water may be an effective means by which to control *E. coli* O157:H7 in livestock water with low organic matter content.

Escherichia coli O157:H7 represents a threat to the long-term sustainability of the cattle industry *(41).* This pathogen can exist as an asymptomatic transient resident within the gut of cattle *(7, 19).* Numerous outbreaks and human fatalities as a result of *E. coli* O157:H7 infections have been documented over the past few decades *(2).* Of the 65 patients admitted to the hospital during a recent outbreak in Walkerton, Ontario, Canada, 27 developed hemolytic uremic syndrome and 6 died as a direct result of the infection *(9).* This outbreak is an important case study because the source of contamination was identified as the local water supply rather than the consumption of food contaminated with *E. coli* O157 *(9).*

Cattle, especially those raised in intensive feedlot operations, have been implicated as the source of water contamination and subsequent outbreaks of *E. coli* O157:H7 infection in humans *(13, 31).* A number of environmental niches within the feedlot environment have been identified in which *E. coli* O157:H7 can remain viable for extended periods of time *(7, 30, 32, 33, 45).* For example, this pathogen can survive in water troughs for up to 6 months *(33, 45)*; thus, contaminated water may be at least partially responsible for transmission of *E. coli* O157:H7 among feedlot cattle.

Numerous studies have been conducted on the control of *E. coli* O157:H7 in the environment and how to restrict transmission of this pathogen *(7, 18, 46).* Thermal inactivation, UV irradiation, and chlorine and ozone treatments can reduce or eliminate *E. coli* O157:H7 in water *(46).* Although useful in municipal water treatment systems, many of these technologies are expensive and require considerable operational expertise, making their implementation in commercial feedlots impractical.

A number of studies have recently been conducted to evaluate the bactericidal activity of electrolyzed oxidizing (EO) water *(6, 15, 17, 28).* This technology was developed in Russia *(39)* and was based on passing a 0.1% (wt/vol) sodium chloride solution through an electrolyzing chamber containing anode and cathode electrodes separated by a diaphragm. The process of electrolysis produces two distinct solutions, anode (pH <2.5) and cathode (pH >11.0) EO waters. EO water has shown a strong bactericidal effect on pathogenic bacteria such as *E. coli* O157:H7 *(8, 12, 43)* and has potential as a water treatment strategy for commercial feedlots. Before such a practice can be recommended, however, it is important to determine how factors

* Author for correspondence. Tel: 403-327-4561; Fax: 403-382-3156; E-mail: mcallister@agr.gc.ca

such as concentration, storage, temperature, and water source influence the bactericidal activity of EO water.

The objective of this study was to determine the antimicrobial stability of EO water produced from three water sources under various storage conditions. The minimum dose of anode and combined anode-cathode EO waters required to kill *E. coli* O157:H7 was also determined.

MATERIALS AND METHODS

Production of EO waters. EO waters (anode EO and cathode EO) were produced using a lab model membrane-cell electrolysis system (Biostel North America, Inc., Innisfail, Alberta) and aqueous solutions of electrolyte salts (Biostel). Products from the anode stream and the cathode stream were studied individually (i.e., as anode EO water or as cathode EO water) or as a manually produced 7:3 (vol/vol) combination of anode and cathode EO waters, as recommended by the manufacturer. These preparations were denoted A_x, C_x, and AC_x, respectively, where x is the concentration (%, wt/vol) of electrolyte solution used to generate the EO products.

Unless otherwise specified, deionized water (dH_2O) was used for preparing the electrolyte solution and for diluting the A_x, C_x, and AC_x products. The dH_2O was produced on site at the Lethbridge Research Centre by charcoal filtration of chemically softened municipal water to remove chlorine followed by reverse osmosis and passage through deionization tanks. When water sources other than dH_2O were tested, the water type being studied (e.g., tap water, irrigation water) was used both for preparation of the electrolyte solution and for diluting the EO products.

The Biostel unit was flushed with 7 to 10 liters of sterile dH_2O between production cycles. A minimum of 5 liters of electrolyte solution was processed in any given cycle, which ensured stabilization of electrical current in the system prior to collection of EO products. Anode and cathode EO waters were produced within 2 h of beginning a study and were collected separately into sterile screw-cap 1-liter glass medium bottles. Upon completion of each cycle, the cycle amperage (as indicated on the production unit gauge) and the pH and oxidative reduction potential (ORP) of the A_x, C_x, and AC_x were recorded. Product pH was measured with a pH meter (model 310, Orion Research, Inc., Boston, Mass.), and ORP was determined with a pH/redox meter (Aqua/Lytic MeBgerat, Langen, Germany).

Bacterial strain. The bactericidal effects of EO waters were evaluated using a bovine isolate of *Escherichia coli* O157:H7 (kindly provided by Dr. V. P. J. Gannon, Population and Public Health Branch, Health Canada, Lethbridge, Alberta). The bacterium, *E. coli* O157:H7 strain H4420, was recovered from a 20% glycerol stock culture stored at −80°C by plating onto tryptic soy agar (Difco, BD-Canada, Oakville, Ontario) and incubating overnight at 37°C. A single colony was inoculated into 50 ml of tryptic soy broth (TSB; Difco) and incubated for 18 h at 37°C (250 rpm). The 18-h culture was serially diluted in 50 mM phosphate-buffered saline, pH 7.0 (PBS; Sigma Chemical Co., Oakville, Ontario) and enumerated by plating duplicate 100-μl aliquots of each dilution onto MacConkey agar (Difco) and incubating 18 to 24 h at 37°C. Selected serial dilutions were used for inoculating into EO water products.

Assessment of bactericidal effects. Routine enumerations of 18-h broth cultures of H4420 consistently revealed populations of approximately 3×10^{10} CFU/ml. Therefore, for assessing the bactericidal effects of EO water products in studies 1 through 4, a dilution presumed to deliver an initial bacterial load of 10^4 CFU/ml upon introduction into predispensed EO product was selected for use as inoculum. This initial bacterial load was confirmed in each study by enumeration of the dilution series. Each study was replicated two times.

Where indicated, the viability of H4420 following exposure to EO water preparations was determined by plating duplicate 100-μl aliquots of inoculated EO test sample onto MacConkey agar and incubating for 18 to 24 h at 37°C. This method produced a limit of detection of 10 CFU/ml viable H4420 in inoculated EO products. In the absence of bactericidal effects of EO waters, the initial bacterial load of 10^4 CFU/ml was expected to yield colony counts of approximately 10^3 colonies per plate. As a non-EO control, tubes of predispensed dH_2O were similarly inoculated with H4420 and plated as described.

Reductions in viability of H4420 were expressed as estimated log increments. Uninhibited growth was observed as a lawn (1,000+ colonies). A colony count reduced to 100+ colonies was interpreted as a 1-log reduction ($10^4 \rightarrow 10^3$ CFU/ml); 10 to 99 colonies = 2-log reduction ($10^4 \rightarrow 10^2$ CFU/ml); 1 to 9 colonies = 3-log reduction ($10^4 \rightarrow 10$ CFU/ml). Absence of colonies represented viability reduced to below detectable limits, i.e., $10^4 \rightarrow$ <10 CFU/ml.

Study 1—MICs of EO water treatments and effect of dilution on pH and ORP. Freshly produced $A_{0.1}$, $C_{0.1}$, and $AC_{0.1}$ were diluted with dH_2O to seven concentrations calculated to yield 0, 0.5, 1, 2, 4, 8, and 16% (vol/vol) EO product after addition of inoculum, and the pH and ORP of each dilution were recorded. Triplicate 9-ml volumes of each dilution (and of dH_2O) were dispensed into test tubes, and each tube was inoculated with 1 ml of serially diluted 18-h culture of H4420 containing 10^5 CFU/ml. The tubes were vortexed immediately (5 s) and then allowed to stand for 2 to 5 min. After remixing (vortex, 5 s), duplicate 100-μl aliquots were plated immediately onto MacConkey agar, and H4420 colonies were counted after 18 to 24 h of incubation at 37°C.

On the basis of results obtained in study 1, the concentrations of A_x and AC_x selected for further study were 0.5 and 2.0% (vol/vol), respectively (except when studying effects of dilution). Because of its low bactericidal effect, no further investigation of C_x was conducted.

Study 2—Effect of water source and electrolyte concentration on ORP of EO waters. Waters from three sources (tap water from the City of Lethbridge municipal water supply, irrigation water drawn from the canal system of the St. Mary River Irrigation District, and dH_2O) were used in this study. Each water type was analyzed (Table 1) using APHA methods *(3)* at an accredited commercial laboratory.

The three water types were each used to prepare four concentrations of Biostel electrolytes (0.1, 0.15, 0.2, and 0.25%, wt/vol) for processing in the electrolysis unit. The same three water types were then used to dilute their respective anode ($A_{0.1}$, $A_{0.15}$, $A_{0.2}$, and $A_{0.25}$) and combined ($AC_{0.1}$, $AC_{0.15}$, $AC_{0.2}$, and $AC_{0.25}$) EO water products to concentrations of 0, 0.5, 1, 2, 4, 8, 16, 25, 50, and 100% (vol/vol). Triplicate 10-ml volumes of each dilution were dispensed into test tubes, and their ORP values were determined 10 min after dilution.

Study 3—Effect of fecal contamination on ORP of EO waters. Fresh fecal material (total wet weight 3 kg) from six yearling steers housed at the Lethbridge Research Centre was collected from the pen floors. The steers were fed a corn silage-based diet and were cared for in accordance with the guidelines set out by the Canadian Council on Animal Care *(1)*. The fecal samples were

TABLE 1. *Analyses of water from three sources used for preparation of electrolyte solution and for dilution of EO water product in study 2*

Characteristic	Values for each water source[a]		
	Deionized	Tap	Irrigation
Fecal coliform bacteria (CFU/100 ml)	<1[b]	<1[b]	24
Total *E. coli* (CFU/100 ml)	<1[b]	<1[b]	16
pH	6.68	8.14	8.24
Calcium (ppm)	<0.05[b]	41.8	34.8
Magnesium (ppm)	<0.05[b]	12.8	13.2
Sodium (ppm)	5.0	12.0	9.1
Ammonia N (ppm)	<0.05[b]	<0.05[b]	0.4
Total organic carbon (ppm)	0.31	2.10	8.9

[a] Deionized water is produced on site at the Lethbridge Research Centre from chemically softened municipal water. Tap water is the treated (chlorinated) municipal water supply for the City of Lethbridge. Irrigation water is surface water drawn from the St. Mary River Irrigation District canal system.

[b] Below detectable limit.

transported in sealed plastic bags to the laboratory (0.5 km), where they were combined into a single homogenate, and aliquots (1 ± 0.1 g) were dispensed into 360 sterile test tubes.

EO water products $A_{0.2}$, $A_{0.25}$, $AC_{0.2}$, and $AC_{0.25}$ were prepared and diluted as described for study 2 using tap, distilled, and irrigation water, and 10-ml volumes were added to triplicate tubes of fecal homogenate. The tubes were vortexed for 10 s, and the ORP of each fecal suspension was determined.

Study 4—Effect of storage variables on ORP and bactericidal efficacy of EO waters. The effects of exposure to UV light, storage temperature (4 versus 24°C), and storage in open versus closed vessels were investigated using 0.5% $A_{0.3}$ and 2.0% $AC_{0.3}$. The diluted EO products and dH_2O as a control were each dispensed in 10-ml volumes into 180 test tubes (six storage conditions × 10 sampling times × triplicate samples). Half of the tubes were capped with standard friction fit test tube caps (closed storage) and half were left open. The capped and uncapped tubes were further divided into three groups. One group was placed in a 24°C cabinet equipped with a 91-cm 30-W blacklight bulb (Model G30T8, GE Lighting North America, Oakville, Ontario) that provided 60 $\mu W/cm^2$ of UV exposure. The second group was boxed (for darkness) and kept at room temperature (24°C), and the third group was placed in a refrigerated (4°C) cabinet, also in the dark.

After 2, 5, 10, 25, 50, 70, 95, 125, 150, and 180 h of storage, triplicate tubes of each sample type were retrieved from storage, and the ORP was measured and recorded. Each tube was inoculated with 0.1 ml of serially diluted 18-h culture of H4420 containing 10^6 CFU/ml (for an initial bacterial load of 10^4 CFU/ml) and processed as described for study 1.

Study 5—Effect of bacterial load on the bactericidal efficacy of EO waters. An 18-h culture of H4420 in TSB was serially diluted in PBS, and 0.1 ml of dilutions 10^{-1} to 10^{-7} were used to inoculate triplicate predispensed 10-ml volumes of 0.5% $A_{0.2}$, 2.0% $AC_{0.2}$, and dH_2O (as a 0% EO water control). Following inoculation, the samples were processed as described for study 1.

Statistical analysis. Comparison of treatments was performed utilizing variance components for compound symmetry using SAS Proc Mixed *(34)*. Means were separated using LSMEANS.

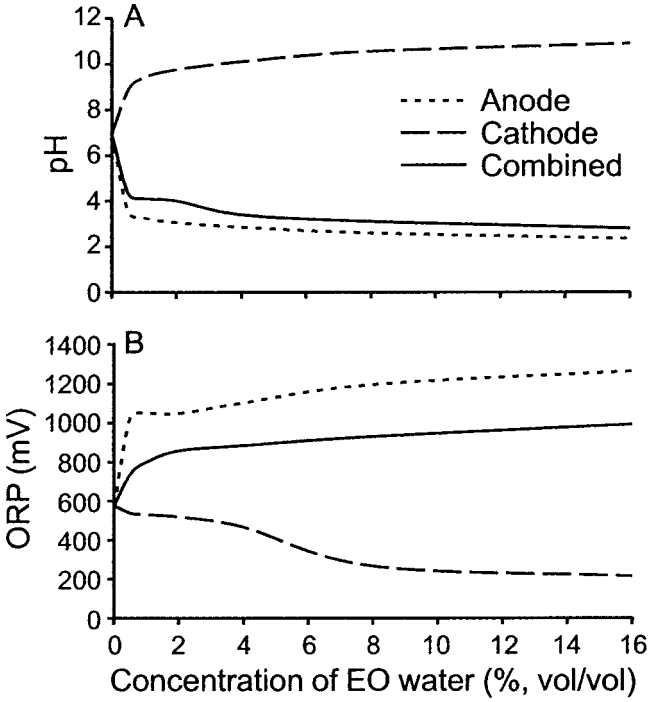

FIGURE 1. *Effect of dilution on the pH (A) and oxidative reduction potential (ORP) (B) of the electrolyzed oxidizing (EO) products of membrane-cell electrolysis of a 0.1% (wt/vol) solution of electrolytes in reverse osmosis/deionized water (dH_2O). Dilutions of the anode and cathode EO waters and of the 7:3 (vol/vol) combination of the two (combined) were prepared with dH_2O and tested for pH and ORP within 2 h of production. Mean values are shown (*n = 6*).*

RESULTS

Study 1—MICs of EO water treatments and effect of dilution on pH and ORP. At production (across studies), undiluted A_x had a pH of 2.02 to 2.24 and an ORP of 1,020 to 1,410 mV. The undiluted C_x had a pH of 11.11 to 12.57 and an ORP of −800 to −936 mV, and AC_x had a pH of 2.60 to 2.83 and an ORP of 1,176 to 1,329 mV. In solution, $A_{0.1}$ and $AC_{0.1}$ markedly reduced the pH of dH_2O and increased its ORP at concentrations as low as 0.5% (vol/vol), whereas cathode EO water ($C_{0.1}$) increased pH and reduced ORP (Fig. 1).

The pattern of effects of EO products on H4420 viability was reflective of pH and ORP observations (i.e., C_x differed from A_x and AC_x). Growth of H4420 on MacConkey agar following exposure to dH_2O (i.e., 0% $A_{0.1}$ and $AC_{0.1}$) was consistent with uninhibited populations enumerated in the culture used as inoculum (3×10^8 CFU/ml). Viability of H4420 was reduced to below detectable limits (<10 CFU/ml) by exposure to $AC_{0.1}$ at concentrations of ≥2% (vol/vol). At 0.5 or 1.0%, no inhibition of H4420 by $AC_{0.1}$ relative to exposure to dH_2O was observed. All of the $A_{0.1}$ concentrations tested (0.5 to 16%, vol/vol) reduced H4420 viability to <10 CFU/ml (no growth observed). In contrast, only the highest concentrations of $C_{0.1}$ (8 and 16%) affected viability of H4420, and

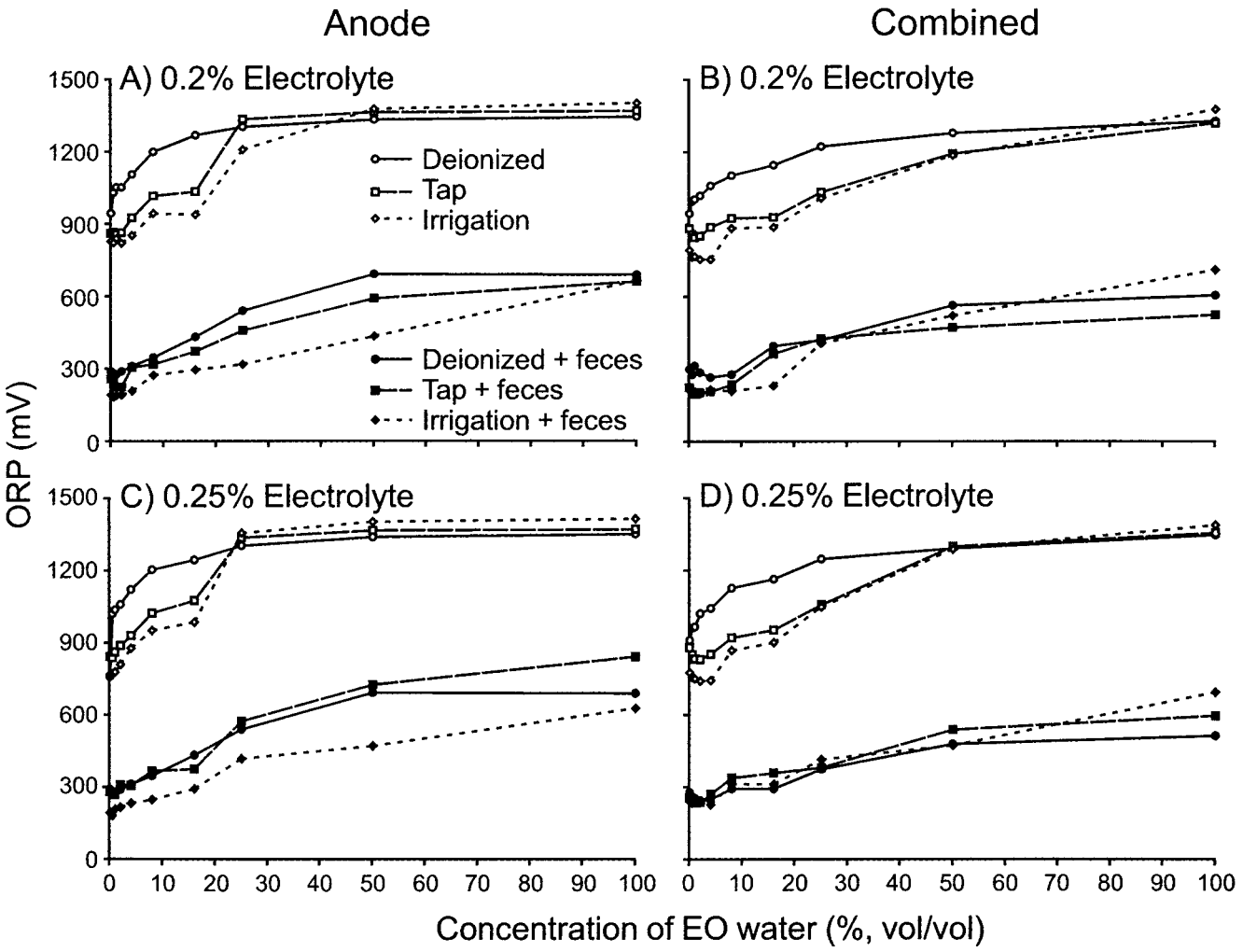

FIGURE 2. *Effect of the concentration of electrolyte solutions (0.2 or 0.25%, wt/vol) used to generate EO water products and of the concentration (vol/vol) of the anode (A_x) and combined (AC_x) EO waters so generated on their oxidative reduction potential (ORP) in the absence (open symbols) or presence (closed symbols) of fecal contamination. Combined EO water (AC_x) is a 7:3 (vol/vol) mixture of undiluted anode and cathode EO waters. Electrolyte solutions and dilutions of A_x and AC_x were prepared using deionized, tap, or irrigation water. Mean values are shown* (n = 6).

they exerted only a single log reduction (i.e., <1,000 colonies per plate were observed).

On the basis of these observations, the EO preparations selected for further study were the MICs of $A_{0.1}$ and $AC_{0.1}$ (0.5% and 2.0%, vol/vol, respectively).

Study 2—Effect of water source and electrolyte concentration on ORP of EO waters. Responses of ORP to dilution of A_x or AC_x prepared using a given water source (dH_2O, tap, or irrigation) were similar across concentrations of electrolyte solutions. Thus, representative data (from the 0.2 and 0.25% electrolyte concentrations) are presented in Figure 2.

Regardless of water source, increasing the concentration of A_x and AC_x increased the ORP of the diluted EO preparation, although at concentrations ≤25% (vol/vol), the ORP of tap and irrigation EO waters remained lower ($P < 0.001$) than that of EO deionized water. The ORP of tap and irrigation EO products were similar ($P > 0.05$) across the range of concentrations studied for both A_x and AC_x. Elevation of the ORP of deionized water with increasing concentrations of A_x and AC_x (prepared with dH_2O) was more rapid than that observed with tap or irrigation water.

Study 3—Effect of fecal contamination on ORP of EO waters. The presence of fecal material significantly reduced ($P < 0.001$) the ORP of all samples, irrespective of water source or type or concentration of EO product (Fig. 2). Values >700 mV were not observed. In anode EO solutions, the depressive effect of fecal material on ORP was more pronounced with irrigation water than with tap or deionized water.

Study 4—Effect of storage variables on ORP and bactericidal efficacy of EO waters. For the first 70 h of storage, the ORP of 0.5% $A_{0.3}$ and 2.0% $AC_{0.3}$ remained relatively constant (850 to 917 mV) under all conditions studied (Fig. 3). At 95 h and beyond, however, ORP values were lower ($P < 0.05$) in samples exposed to UV light than in samples stored in the dark. With $AC_{0.3}$, the decline in ORP was more pronounced ($P < 0.05$) in open storage vessels than in closed vessels. Among samples stored in the

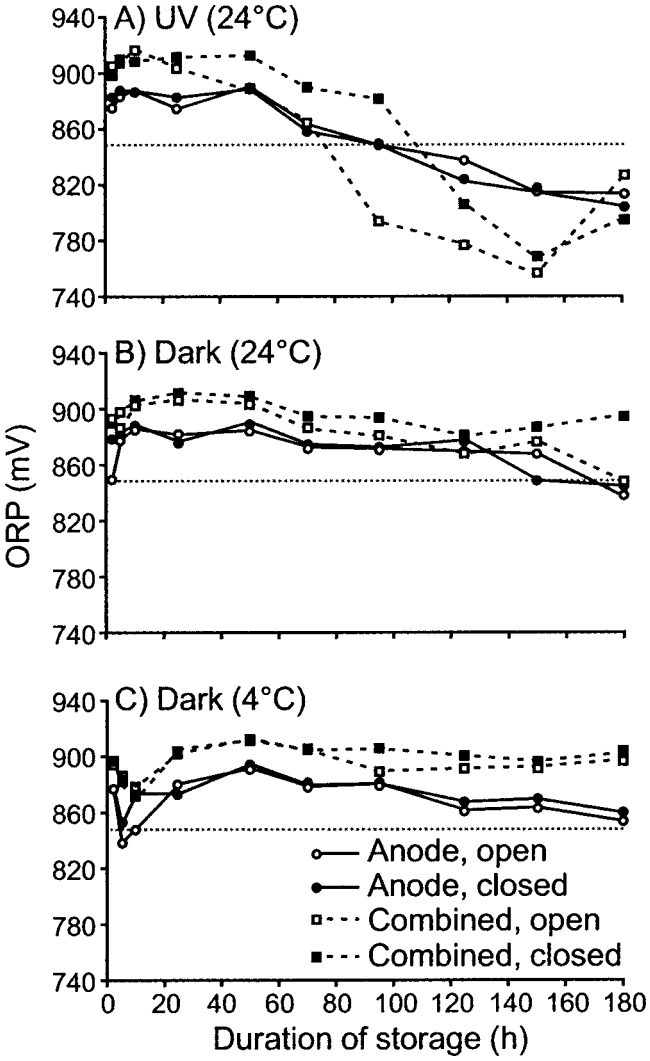

FIGURE 3. *Changes in the oxidative reduction potential (ORP) of 0.5% (vol/vol) $A_{0.3}$ and 2.0% (vol/vol) $AC_{0.3}$ during 180 h of storage in open or closed containers exposed to UV light at room temperature (A), in the dark at room temperature (B), or in the dark with refrigeration (C). Samples with ORP >848 mV (i.e., those above the dotted line) reduced* E. coli *O157:H7 strain H4420 inoculated at 10^4 CFU/ml to below detectable limits (<10 CFU/ml), whereas uninhibited growth of H4420 was observed in those samples with ORP ≤848 mV (at or below the dotted line). Mean values are shown* (n = 6).

dark, ORP values did not differ ($P > 0.05$) between open and closed storage vessels or between $A_{0.3}$ and $AC_{0.3}$, although $AC_{0.3}$ retained numerically higher ORP than did $A_{0.3}$, particularly when stored at 4°C as compared with 24°C.

Full bactericidal efficacy was retained by the $A_{0.3}$ and $AC_{0.3}$ samples in which ORP remained >848 mV, as indicated by a reduction in viability of H4420 from 10^4 CFU/ml initial load to <10 CFU/ml (i.e., no detectable growth) upon exposure to the EO products. Without exception, however, bactericidal effects were lost completely once ORP decreased to ≤848 mV. No difference in H4420 viability (>1,000 colonies/plate) was detectable between inoculated dH_2O controls and inoculated EO products in which ORP was reduced to ≤848 mV.

Study 5—Effect of bacterial load on the bactericidal efficacy of EO waters. Enumeration of the serially diluted H4420 culture confirmed that using the 10^{-1} to 10^{-7} dilutions as inoculum resulted in initial bacterial loads of 10^7 to 10^1 CFU/ml, respectively, in the dH_2O, 0.5% $A_{0.2}$, or 2.0% $AC_{0.2}$. Colonies developed on all of the control (dH_2O) plates in numbers consistent with uninhibited growth. Exposure to $A_{0.2}$ reduced the viability of H4420 to <10 CFU/ml (no growth observed) at all initial bacterial loads ≤10^4 CFU/ml, but at initial loads of 10^5 CFU/ml (or higher) no bactericidal effect was observed (>1,000 colonies per plate). The $AC_{0.2}$ also eradicated H4420 (<10 CFU/ml) at initial loads of ≤10^4 CFU/ml and reduced the viability of the 10^5 CFU/ml H4420 challenge by approximately 2 log (<100 colonies per plate). Other than with 8 and 16% cathode EO water in study 1, this was the only instance in which H4420 was not either completely uninhibited (>10^3 colonies per plate, indistinguishable from dH_2O controls) or eradicated to below detectable levels (i.e, <10 CFU/ml, no growth observed).

DISCUSSION

Numerous studies have examined the ability of EO water to kill food-related pathogens *(14, 15, 42).* Anode EO water is currently being used in a number of dental and medical applications *(10, 11, 24–27, 35–38).* In the majority of these situations either concentrated anode EO water or a 50:50 mixture of anode and cathode water is employed *(16, 43, 44).* Use of similar solutions in commercial beef production facilities is impractical because of the large volumes of water consumed (25 to 65 liters per animal per day) and the detrimental effects that concentrated solutions may have on the intestinal microbial populations that are essential for feed digestion *(22).* The present study demonstrated that anode and a mixture of anode-cathode EO water at concentrations as low as 0.5 and 2%, respectively, could effectively kill *E. coli* O157:H7 in laboratory culture.

The bactericidal activity of EO water has been attributed to free chlorine in the form of hypochlorous acid (HOCl) *(14, 15, 20).* Hypochlorous acid penetrates the bacterial cell wall and inhibits key metabolic systems, including the action of membrane transport proteins and ATP synthesis *(20, 28).* Several researchers have examined the stability of bactericidal activity in concentrated EO water *(16, 20, 21).* In the present study, exposure to UV light dramatically reduced ORP and the bactericidal activity of both dilute anode and a mixture of anode-cathode EO water, more so with open than with closed storage vessels. UV light enhances photodecomposition of chlorine *(4)* and thus probably reduces the effective concentration of the bactericidal agent, HOCl. Len et al. *(20)* reported that atmospheric exposure of EO water reduced its effectiveness as a result of the loss of chlorine by evaporation. To optimize the effectiveness of EO water in commercial livestock production facilities, the EO water must be protected from long periods of exposure to the atmosphere or UV light.

The acidic nature of anode water undoubtedly contributes to its antimicrobial properties. Acid treatment has been and is currently being employed as a pathogen control

mechanism in slaughterhouses *(40)*. In a related study (data not shown), the pH of undiluted anode, cathode, and combined EO water treatments remained stable for at least 2 months when stored sealed in the dark. Nonetheless, in the present study, the bactericidal activity of 0.5% anode EO water was no longer evident 95 h after production. Furthermore, at the MICs determined in study 1, combined EO water was as effective as anode EO water at killing *E. coli* O157:H7, even though the pH of the AC_x was substantially higher.

The ORP was correlated closely with the bactericidal activity of EO water. Kim et al. *(15)* also suggested that ORP is a greater determinant than pH for bactericidal activity in EO water. When chlorine is added to water, HOCl is formed in addition to free hydrogen and the hypochlorite ion (ClO^-). Because HOCl has no charge, it can penetrate microbial cell walls more easily. It is hypothesized that high (>1,100 mV) ORP is related to the concentration of HOCl *(23)*. ORP values of >650 mV are recommended for the disinfection of drinking water *(23)*. Data from the present study suggest that ORP values ≥850 mV are necessary to kill *E. coli* O157:H7 strain H4420.

Combined (2.0%, vol/vol) and anode (0.5%, vol/vol) EO waters exerted little or no effect on *E. coli* O157:H7 when cell density exceeded 10^4 CFU/ml. Although *E. coli* O157:H7 contamination would not commonly reach this level in livestock water, defecation by an animal shedding *E. coli* O157:H7 into the water source could result in similar bacterial loads. Combined EO water (2.0%, vol/vol) exerted a moderate bactericidal effect (2-log reduction) against an *E. coli* O157:H7 challenge of 10^5 CFU/ml, whereas anode EO water did not. In our laboratory, bactericidal activity of anode water was lost 180 h after production whereas the combined EO water remained effective for at least 180 h at room temperature. The apparent greater stability of the combined EO water may account for its greater bactericidal activity as compared with anode EO water.

In previous studies using EO water treatments for the control of foodborne and waterborne pathogens, deionized water has been used for the electrolyzing process *(6, 14, 15, 28, 43, 44)*. The use of deionized water in livestock production facilities is impractical, and application of electrolyzed water in agricultural systems would require the use of either surface or ground water. In the present study, increased concentrations of anode and combined EO water were required to achieve ORP levels similar to those needed to maintain bactericidal activity. If irrigation water is used in electrolysis, the concentration of combined EO water may have to be at least doubled to achieve the same ORP level as that obtained with deionized water. The concentration of EO water would also likely have to be adjusted with seasonal changes in the quality, e.g., organic matter content, of irrigation water.

Introduction of feces (1 g/10 ml) immediately reduced the ORP of solutions of anode EO or combined EO waters. The decline of ORP to <700 mV even in undiluted EO water suggests that no bactericidal activity would remain in water troughs with fecal contamination levels approaching 10% (wt/vol). The effectiveness of chlorine as a bactericidal agent is reduced in the presence of organic matter because of the formation of monochloramines *(23)*. This finding is consistent with the role of chlorine in the bactericidal activity of EO water. Others have proposed that contamination of drinking water contributes to the transfer of *E. coli* O157:H7 among cattle in feedlots *(5, 29)*. This contamination is often due to animals defecating in the water bowl. Hygienic practices such as daily cleaning of water bowls would still have to be practiced even if EO water were included in drinking water.

Relatively low concentrations of electrolyzed water (2 to 5%) have the potential to reduce the water-mediated transmission of pathogens in the livestock environment. The efficacy of this approach will be substantially improved when high-quality water with a low concentration of mineral and organic matter is used in the electrolysis process. Accumulation of organic matter in water troughs is likely to eliminate the bactericidal activity of EO water. Consequently, use of EO water is not a substitute for regular hygienic practices such as the cleaning of water bowls. The relative simplicity of the electrolysis process and the ease with which it can be installed into livestock production systems may make it an effective method of reducing the prevalence of bacteria in livestock water.

ACKNOWLEDGMENTS

This study was conducted with technical and financial support from Biostel (North America) Ltd. The authors thank Fred Van Herk of the Lethbridge Research Centre for his assistance with statistical analysis.

REFERENCES

1. Canadian Council on Animal Care. 1993. Guidelines to the care and use of experimental animals, 2nd ed., vol. 1. Canadian Council on Animal Care, Ottawa, Ontario.
2. Chalmers, R. M., H. Aird, and F. J. Bolton. 2000. Waterborne *Escherichia coli* O157. *Symp. Ser. Soc. Appl. Microbiol.* 29:124S–132S.
3. Clesceri, L. S., A. E. Greenberg, and A. D. Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
4. Cosson, H., and W. R. Ernst. 1994. Photodecomposition of chlorine dioxide and sodium-chlorate in aqueous solutions by irradiation with ultraviolet light. *Ind. Eng. Chem. Res.* 33:1468–1475.
5. Elder, N. G., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallager, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 in feces, hides and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:1519–1525.
6. Fabrizio, K. A., R. R. Sharma, A. Demirci, and C. N. Cutter. 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. *Poult. Sci.* 81:1598–1605.
7. Hancock, D., T. Besser, J. Lejeune, M. Davis, and D. Rice. 2001. The control of VTEC in the animal reservoir. *Int. J. Food Microbiol.* 66:71–78.
8. Hayashibara, T., A. Kadowaki, N. Yuda, M. Yamanaka, K. Akagawa, K. Tagusa, and K. Matsui. 1994. A study on the disinfection. Microbicidal effects of electrolyzed oxidizing water. *Jpn. J. Med. Technol.* 43:555–561.
9. Health Canada. 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May–June. Population and Public Health Branch. Available at: http://www.hcsc.gc.ca/hpb/lcdc/publicat/ccdr/00vol26/dr2620eb.html. Accessed 15 February 2002.
10. Horiba, N., K. Hiratsuka, T. Onoe, T. Yoshida, K. Suzuki, T. Mat-

sumoto, and H. Nakamura. 1999. Bactericidal effect of electrolyzed neutral water on bacteria isolated from infected root canals. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 87:83–87.
11. Inoue, Y., S. Endo, K. Kondo, H. Ito, H. Omori, and K. Saito. 1997. Trial of electrolyzed strong acid aqueous solution lavage in the treatment of peritonitis and intraperitoneal abscess. *Artif. Organs* 21:28–31.
12. Iwasawa, A., H. Nakamura, K. Nakamura, and T. Muria. 1993. Bactericidal effect of aqua oxidation water. *Clin. Pharmacol. Ther.* 3: 1555.
13. Jackson, S. G., R. B. Goodbrand, R. P. Johnson, V. G. Odorico, D. Alves, K. Rahn, J. B. Wilson, M. K. Welch, and R. Khakhria. 1998. *Escherichia coli* O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm. *Epidemiol. Infect.* 120:17–20.
14. Kim, C., Y. C. Hung, and R. E. Brackett. 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. *Int. J. Food Microbiol.* 61:199–207.
15. Kim, C., Y. C. Hung, and R. E. Brackett. 2000. Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. *J. Food Prot.* 63:19–24.
16. Kiura, H., K. Sano, S. Morimatsu, T. Nakano, C. Morita, M. Yamaguchi, T. Maeda, and Y. Katsuoka. 2002. Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. *J. Microbiol. Methods* 49:285–293.
17. Koseki, S., and K. Itoh. 2001. Prediction of microbial growth in fresh-cut vegetables treated with acidic electrolyzed water during storage under various temperature conditions. *J. Food Prot.* 64: 1935–1942.
18. LeJeune, J. T., T. E. Besser, N. L. Merrill, D. H. Rice, and D. D. Hancock. 2001. Livestock drinking water microbiology and the factors influencing the quality of drinking water offered to cattle. *J. Dairy Sci.* 84:1856–1862.
19. LeJeune, J. T., T. E. Besser, D. H. Rice, and D. D. Hancock. 2001. Methods for the isolation of water-borne *Escherichia coli* O157. *Lett. Appl. Microbiol.* 32:316–320.
20. Len, S. V., Y. C. Hung, D. Chung, J. L. Anderson, M. C. Erickson, and K. Morita. 2002. Effects of storage conditions and pH on chlorine loss in electrolyzed oxidizing (EO) water. *J. Agric. Food Chem.* 50:209–212.
21. Len, S. V., Y. C. Hung, M. Erickson, and C. Kim. 2000. Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. *J. Food Prot.* 63:1534–1537.
22. McAllister, T. A., H. D. Bae, G. A. Jones, and K.-J. Cheng. 1994. Microbial attachment and feed digestion in the rumen. *J. Anim. Sci.* 72:3004–3018.
23. McPherson, L. L. 1993. Understanding ORP's role in the disinfection process. *Water Eng. Manage.* 140(11):29–31.
24. Middleton, A. M., M. V. Chadwick, J. L. Sanderson, and H. Gaya. 2000. Comparison of a solution of super-oxidized water (Sterilox) with glutaraldehyde for the disinfection of bronchoscopes, contaminated in vitro with *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare* in sputum. *J. Hosp. Infect.* 45:278–282.
25. Miyamoto, M., K. Inoue, Y. Gu, M. Hoki, S. Haji, and H. Ohyanagi. 1999. Effectiveness of acidic oxidative potential water in preventing bacterial infection in islet transplantation. *Cell Transplant.* 8:405–411.
26. Morita, M., Y. Tanji, K. Mizoguchi, T. Akitsu, N. Kijima, and H. Unno. 2002. Characterization of a virulent bacteriophage specific for *Escherichia coli* O157:H7 and analysis of its cellular receptor and two tail fiber genes. *FEMS Microbiol. Lett.* 211:77–83.
27. Nakae, H., and H. Inaba. 2000. Effectiveness of electrolyzed oxidized water irrigation in a burn-wound infection model. *J. Trauma* 49:511–514.
28. Park, H., Y. C. Hung, and R. E. Brackett. 2002. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. *Int. J. Food Microbiol.* 72:77–83.
29. Pell, A. N. 1997. Manure and microbes: public and animal health problem? *J. Dairy Sci.* 80:2673–2681.
30. Rahn, K., S. Renwick, R. Johnson, J. Wilson, R. C. Clarke, D. Alves, S. McEwen, H. Lior, and J. Spika. 1997. Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. *Epidemiol. Infect.* 119:251–259.
31. Rasmussen, M. A., and T. A. Casey. 2001. Environmental and food safety aspects of *Escherichia coli* O157:H7 infections in cattle. *Crit. Rev. Microbiol.* 27:57–73.
32. Rice, E., C. Johnson, D. Wild, and D. Reasoner. 1992. Survival of *Escherichia coli* O157:H7 in drinking water associated with a waterborne disease of hemorrhagic colitis. *Lett. Appl. Microbiol.* 15: 38–40.
33. Rice, E. W., and C. H. Johnson. 2000. Short communication: survival of *Escherichia coli* O157:H7 in dairy cattle drinking water. *J. Dairy Sci.* 83:2021–2023.
34. SAS. 1995. User's guide version 6. Statistical Analysis Systems Institute, Inc., Cary, N.C.
35. Sasai-Takedatsu, M., T. Kojima, A. Yamamoto, K. Hattori, S. Yoshijima, S. Taniuchi, S. Namura, H. Akamatsu, T. Horio, and Y. Kobayashi. 1997. Reduction of *Staphylococcus aureus* in atopic skin lesions with acid electrolytic water—a new therapeutic strategy for atopic dermatitis. *Allergy* 52:1012–1016.
36. Selkon, J. B., J. R. Babb, and R. Morris. 1999. Evaluation of the antimicrobial activity of a new super-oxidized water, Sterilox, for the disinfection of endoscopes. *J. Hosp. Infect.* 41:59–70.
37. Shetty, N., S. Srinivasan, J. Holton, and G. L. Ridgway. 1999. Evaluation of microbicidal activity of a new disinfectant: Sterilox 2500 against *Clostridium difficile* spores, *Helicobacter pylori*, vancomycin resistant *Enterococcus* species, *Candida albicans* and several *Mycobacterium* species. *J. Hosp. Infect.* 41:101–105.
38. Shimada, K., K. Ito, and S. Murai. 2000. A comparison of the bactericidal effects and cytotoxic activity of three types of oxidizing water, prepared by electrolysis, as chemical dental plaque control agents. *Int. J. Antimicrob. Agents* 15:49–53.
39. Shtannikov, E. V., and M. Morozovla. 1977. Use of electrolyzed solutions of table salt for water decontamination. *Gig. Sanit.* 8:15–19.
40. Uyttendaele, M., E. Jozwik, A. Tutenel, L. De Zutter, J. Uradzinski, D. Pierard, and J. Debevere. 2001. Effect of acid resistance of *Escherichia coli* O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue and effect of modified atmosphere packaging on survival of *Escherichia coli* O157:H7 on red meat. *J. Food Prot.* 64:1661–1666.
41. Van Donkersgoed, J., J. Berg, A. Potter, D. Hancock, T. Besser, D. Rice, J. LeJeune, and S. Klashinsky. 2001. Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *Can. Vet. J.* 42:714–720.
42. Venczel, L. V., M. Arrowood, M. Hurd, and M. D. Sobsey. 1997. Inactivation of *Cryptosporidium parvum* oocysts and *Clostridium perfringens* spores by a mixed-oxidant disinfectant and by free chlorine. *Appl. Environ. Microbiol.* 63:1598–1601.
43. Venkitanarayanan, K. S., G. O. Ezeike, Y. C. Hung, and M. P. Doyle. 1999. Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 65:4276–4279.
44. Venkitanarayanan, K. S., G. O. Ezeike, Y. C. Hung, and M. P. Doyle. 1999. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolyzed oxidizing water. *J. Food Prot.* 62:857–860.
45. Wang, G., and M. P. Doyle. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *J. Food Prot.* 61:662–667.
46. Zhao, T., M. P. Doyle, P. Zhao, P. Blake, and F. M. Wu. 2001. Chlorine inactivation of *Escherichia coli* O157:H7 in water. *J. Food Prot.* 64:1607–1609.