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International Journal of Food Microbiology 91 (2004) 13–18
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*

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Received 15 August 2002; received in revised form 12 April 2003; accepted 19 May 2003

Abstract

The effects of chlorine and pH on the bactericidal activity of electrolyzed (EO) water were examined against *Escherichia coli* O157:H7 and *Listeria monocytogenes*. The residual chlorine concentration of EO water ranged from 0.1 to 5.0 mg/l, and the pH effect was examined at pH 3.0, 5.0, and 7.0. The bactericidal activity of EO water increased with residual chlorine concentration for both pathogens, and complete inactivation was achieved at residual chlorine levels equal to or higher than 1.0 mg/l. The results showed that both pathogens are very sensitive to chlorine, and residual chlorine level of EO water should be maintained at 1.0 mg/l or higher for practical applications. For each residual chlorine level, bactericidal activity of EO water increased with decreasing pH for both pathogens. However, with sufficient residual chlorine (greater than 2 mg/l), EO water can be applied in a pH range between 2.6 (original pH of EO water) and 7.0 while still achieving complete inactivation of *E. coli* O157:H7 and *L. monocytogenes*.

Keywords: Electrolyzed water; Chlorine; pH; Oxidation–reduction potential; *Escherichia coli* O157:H7; *Listeria monocytogenes*

1. Introduction

Escherichia coli O157:H7 and *Listeria monocytogenes* are food-borne pathogens of major public concern in the Unites States, and food-borne outbreaks of their infections have occurred in a variety of foods including poultry, meat, egg, milk, fruits, and vegetables (Beuchat, 1995; Rocourt and Cossart, 1997; Doyle et al., 1997). Therefore, developing effective methods for reducing or eliminating these pathogens is important for the hazard analysis and critical control points (HACCP) systems for the food industry.

Electrolyzed (EO) water, which is generated from anodic electrolysis of a dilute salt (NaCl) solution in an electrolytic cell, has a strong bactericidal activity against most pathogenic bacteria (Kim et al., 2000a,b; Venkitanarayanan et al., 1999). Electrochemically produced active chlorine species (dissolved Cl_2 gas, HOCl, OCl^-) and the corresponding high oxidation–reduction potential (ORP) have been associated with the strong bactericidal activity of EO water (Kim et al., 2000a). The pH of EO water will also affect its bactericidal activity, because it can change the relative

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doi:10.1016/S0168-1605(03)00334-9

fractions of chlorine species in the solution (Len et al., 2000).

Complete inactivation of *E. coli* O157:H7 and *L. monocytogenes* was observed when about 10 $\log_{10}$ CFU/ml of the pathogens were treated with acidic EO water (pH 2.6) at an ORP level of 1160 mV and a residual chlorine concentration of 56 mg/l for 30 s (Kim et al., 2000a,b). However, none of the previous studies provided information on the sensitivity of the two pathogens to EO water as a function of residual chlorine concentration and pH.

The objective of this study was to examine the effects of chlorine concentration and pH on the bactericidal effect of EO water against *E. coli* O157:H7 and *L. monocytogenes*.

2. Materials and methods

2.1. Bacterial cultures

E. coli O157:H7 F500 (human feces isolate) and *L. monocytogenes* ScottA (human isolate) were maintained in 10 ml of tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA) at 37 °C by daily transfer. For experiments, each bacterial strain was cultured separately in 100 ml of TSB in 250-ml Erlenmeyer flasks at 37 °C overnight without shaking. Following incubation, 100 ml of each culture was harvested by centrifugation (4000 × *g* for 10 min), washed twice with sterile deionized water, and resuspended in sterile deionized water to obtain a final cell concentration of 10 $\log_{10}$ CFU/ml. Bacterial concentrations were estimated by measuring the absorbance of bacterial suspension at 600 nm using a spectrophotometer (Beckman DU520, Beckman Instruments, Fullerton, CA, USA) and confirmed by plating 0.1-ml portions of appropriately diluted culture on tryptic soy agar (TSA) (Difco Laboratories) plates and incubating the plates at 37 °C for 48 h.

2.2. Preparation of EO water

EO water was generated from the anodic electrolysis of 0.1% NaCl solution in a commercial EO water generator (model ROX 20 TA, Hoshizaki Electric, Toyoake, Japan) at a setting of 14 A. The pH and ORP of EO water were measured using a dual scale pH/ORP meter (Accumet model 15, Fisher Scientific, Pittsburgh, PA, USA). The residual chlorine concentration was determined by iodometric method using a chlorine test kit (Hach, Ames, IA, USA). The pH, ORP, and residual chlorine concentration of EO water used for the study were 2.57, 1082 mV, and 50 mg/l, respectively.

2.3. Testing bactericidal activity of EO water

For the study of chlorine effect, EO water (generated at 14 A) was appropriately diluted in deionized water to obtain final residual chlorine concentrations of 0.1 to 5.0 mg/l. One milliliter (equivalent to 10 $\log_{10}$ CFU/ml) of each bacterial strain was separately added to 99 ml of the diluted EO water at different residual chlorine concentrations or deionized water (no residual chlorine) in a sterile beaker at ambient temperature (23 ± 2 °C) for 30 s with agitation at 150 rpm. For the study of pH effect, three different buffer solutions (100 mM tartaric acid for pH 3.0 and 5.0, 100 mM monobasic sodium phosphate for pH 7.0) were prepared by adjusting pH with 5 N NaOH. EO water (generated at 14 A) was appropriately diluted in each buffer to obtain final residual chlorine concentrations of 0.1 to 5.0 mg/l, and the ORP and pH values of the diluted EO water were measured before inoculation. One milliliter (equivalent to 10 $\log_{10}$ CFU/ml) of each bacterial strain was separately added to 99 ml of the buffered EO water at different residual chlorine concentrations or deionized water (control) in a sterile beaker at ambient temperature (23 ± 2 °C) for 30 s with agitation at 150 rpm.

After treatments, 1 ml of each sample was serially diluted (1:10) in 9 ml of sterile neutralizing buffer (Difco Laboratories) and the populations of *L. monocytogenes* or *E. coli* O157:H7 were determined by plating 0.1 ml of each dilution in duplicate on TSA plates. Bacterial population was measured as the number of colonies on the TSA plates after incubation at 37 °C for 48 h. For enrichment, 1 ml of each sample solution after treatment was transferred to a 150-ml Erlenmeyer flask containing 20 ml of sterile TSB and incubated at 37 °C for 48 h. Following enrichment, the culture was streaked on TSA plates, and the plates were incubated at 37 °C for 48 h before counting. The whole experiment was replicated twice.

3. Results and discussion

3.1. Effect of chlorine on bactericidal activity of EO water

The effect of chlorine on the inactivation of *E. coli* O157:H7 and *L. monocytogenes* was evaluated using diluted EO water with residual chlorine concentrations ranging from 0.1 to 5.0 mg/l. Using EO water with residual chlorine concentrations of 0.1 and 0.2 mg/l, the populations of *E. coli* O157:H7 were reduced by about 5.0 and 7.0 $\log_{10}$ CFU/ml, respectively (Table 1). However, only 3.5 and 5.8 $\log_{10}$ CFU/ml of *L. monocytogenes* were inactivated by EO water with 0.1 and 0.2 mg/l residual chlorine, respectively (Table 2). Complete inactivation of both pathogens was achieved at residual chlorine levels equal to or greater than 1.0 mg/l. These results indicate that the bactericidal activity of EO water increases with increasing residual chlorine concentration, and *E. coli* O157:H7 is more sensitive to chlorine than *L. monocytogenes*. This high susceptibility of *E. coli* O157:H7 to residual chlorine has also been demonstrated in chlorinated water. More than 7.0 $\log_{10}$ CFU/ml reduction of *E. coli* O157:H7 was reported when the cells were exposed to water containing 0.25 mg/l residual chlorine for 1 min (Zhao et al., 2001).

Table 1
Bactericidal activity of diluted EO water against *E. coli* O157:H7 F500 as a function of residual chlorine[a]

Residual chlorine (mg/l)	Surviving population ($\log_{10}$ CFU/ml)	Water properties	
		pH	ORP (mV)
0 (control)	7.94 ± 0.11	5.82 ± 0.07	350 ± 26
0.1	2.90 ± 0.18	5.32 ± 0.06	757 ± 10
0.2	0.33 ± 0.60	4.97 ± 0.01	787 ± 20
0.5	< 1.0[b]	4.56 ± 0.03	863 ± 7
1.0	ND[c]	4.21 ± 0	915 ± 7
2.0	ND	3.92 ± 0	960 ± 3
5.0	ND	3.51 ± 0.01	1019 ± 4

EO water generated at 14 A was appropriately diluted in deionized water with stirring to obtain final residual chlorine concentrations of 0.1 to 5.0 mg/l. The initial population of *E. coli* O157:H7 F500 was 7.94 $\log_{10}$ CFU/ml.

[a] Values are the means of two replicated measurements ± standard deviation.

[b] Positive by an enrichment procedure and no detectable survivors by a direct plating procedure.

[c] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

Table 2
Bactericidal activity of diluted EO water against *L. monocytogenes* ScottA as a function of residual chlorine[a]

Residual chlorine (mg/l)	Surviving population ($\log_{10}$ CFU/ml)	Water properties	
		pH	ORP (mV)
0 (control)	7.94 ± 0.05	5.81 ± 0.04	352 ± 5
0.1	4.48 ± 0.09	5.32 ± 0.02	733 ± 28
0.2	2.18 ± 0.32	4.99 ± 0.14	767 ± 33
0.5	< 1.0[b]	4.61 ± 0.05	841 ± 16
1.0	ND[c]	4.26 ± 0.04	895 ± 11
2.0	ND	3.97 ± 0.03	941 ± 11
5.0	ND	3.53 ± 0.03	1006 ± 12

EO water generated at 14 A was appropriately diluted in deionized water with stirring to obtain final residual chlorine concentrations of 0.1 to 5.0 mg/l. The initial population of *L. monocytogenes* ScottA was 8.0 $\log_{10}$ CFU/ml.

[a] Values are the means of two replicated measurements ± standard deviation.

[b] Positive by an enrichment procedure and no detectable survivors by a direct plating procedure.

[c] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

The ORP of a solution is an indicator of its oxidizing or reducing strength, with higher positive ORP values indicating a greater oxidizing strength. The ORP values of diluted EO water containing only 0.1 and 0.2 mg/l of residual chlorine were significantly higher than the ORP of deionized water (Tables 1 and 2). The ORP value of EO water also increased with increasing chlorine concentration and reached around 1000 mV at 5 mg/l residual chlorine. This indicates that chlorine in the EO water is a strong oxidizing agent and may be responsible for its bactericidal activity. The strong oxidation strength of EO water might cause the oxidation of sulfhydryl compounds on cell surfaces (Leyer and Johnson, 1997) and other key metabolic systems (Albrich et al., 1986; Barrette et al., 1989; Hurst et al., 1991), resulting in the inhibition of bacterial cell growth.

The pH values of diluted EO water at residual chlorine levels of 0.5 and 1.0 mg/l, at which most bacteria were inhibited, were about 4.6 and 4.2, respectively (Tables 1 and 2). This suggests that a very low pH (2.6) observed for the original EO water may not be necessary for bacterial inhibition during EO water applications.

3.2. Influence of pH on bactericidal activity of EO water

The effect of pH on the inactivation of *E. coli* O157:H7 and *L. monocytogenes* was evaluated at three pH levels (3.0, 5.0, and 7.0) with residual chlorine concentrations ranged from 0.1 to 5.0 mg/l. At residual chlorine concentrations of 0.1, 0.2, and 0.5 mg/l, the surviving populations of *E. coli* O157:H7 decreased with decreasing pH of EO water (Table 3). A similar pattern of reduction in populations of *L. monocytogenes* was also observed (Table 4). These results indicated that decreasing pH values increased the sensitivity of both pathogens to EO water at a given residual chlorine concentration.

At a residual chlorine concentration of 1.0 mg/l, bacterial colonies were observed only at pH 7.0 for both pathogens after EO water treatment. At a residual chlorine concentration of 2.0 mg/l or above, complete microbial inactivation was observed regardless of pH. These results indicate that the effect of chlorine on bacterial inhibition is more significant than the effect of pH. No significant reduction of both pathogens was observed as indicated by the similar bacterial population of the control (treated with buffer solutions) and the initial population before treatment (Tables 3 and 4).

Fig. 1 shows the ORP values of pH adjusted EO water used in the study at different residual

Table 3
Bactericidal activity of diluted EO water against *E. coli* O157:H7 F500 as a function of pH

Residual chlorine (mg/l)	Surviving population[a] ($\log_{10}$ CFU/ml)		
	pH 3.0	pH 5.0	pH 7.0
0 (control)	7.79 ± 0.04	7.89 ± 0.18	7.75 ± 0.08
0.1	4.25 ± 0.11	4.66 ± 0.19	7.60 ± 0.11
0.2	2.64 ± 0.30	3.02 ± 0.18	5.81 ± 0.15
0.5	1.15 ± 0.23	1.19 ± 0.22	1.38 ± 0.48
1.0	ND[b]	ND	< 1.0[c]
2.0	ND	ND	ND
5.0	ND	ND	ND

EO water generated at 14 A was appropriately diluted in deionized water with stirring to obtain final residual chlorine concentrations of 0.1 to 5.0 mg/l. The initial population of *E. coli* O157:H7 F500 was 7.89 $\log_{10}$ CFU/ml.

[a] Values are the means of two replicated measurements ± standard deviation.

[b] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

[c] Positive by an enrichment procedure and no detectable survivors by a direct plating procedure.

Table 4
Bactericidal activity of diluted EO water against *L. monocytogenes* ScottA as a function of pH

Residual chlorine (mg/l)	Surviving population[a] ($\log_{10}$ CFU/ml)		
	pH 3.0	pH 5.0	pH 7.0
0 (control)	7.73 ± 0.10	7.66 ± 0.20	7.74 ± 0.12
0.1	3.90 ± 0.16	4.70 ± 0.22	7.68 ± 0.19
0.2	2.56 ± 0.18	4.04 ± 0.12	7.72 ± 0.08
0.5	1.46 ± 0.50	1.75 ± 0.66	3.08 ± 0.44
1.0	ND[b]	ND	< 1.0[c]
2.0	ND	ND	ND
5.0	ND	ND	ND

EO water generated at 14 A was appropriately diluted in deionized water with stirring to obtain final residual chlorine concentrations of 0.1 to 5.0 mg/l. The initial population of *L. monocytogenes* ScottA was 7.78 $\log_{10}$ CFU/ml.

[a] Values are the means of two replicated measurements ± standard deviation.

[b] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

[c] Positive by an enrichment procedure and no detectable survivors by a direct plating procedure.

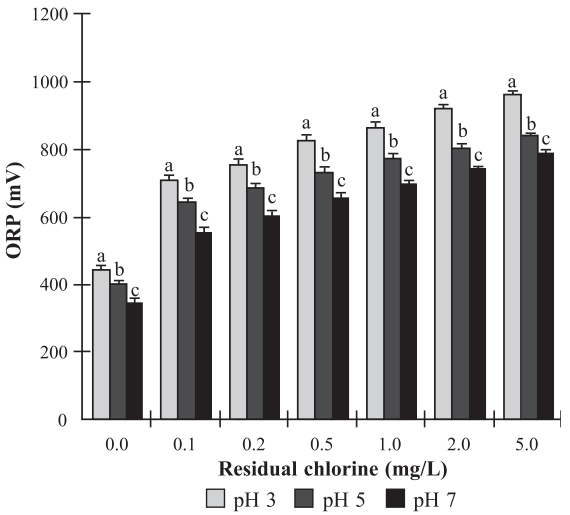

Fig. 1. ORP values of buffered EO water used for the inactivation of *E. coli* O157:H7 and *L. monocytogenes* at different levels of residual chlorine and pH. Data points are the means of eight replicated measurements. Error bars represent standard deviations.

chlorine levels. At each residual chorine level, the ORP values increased significantly ($\alpha = 0.05$) with decreasing pH. This suggests that the observed stronger bactericidal activity at lower pH could be due to the corresponding higher ORP. The reason for high ORP at low pH may be explained as follows. At acidic pH (<pH 5.0), where HOCl constitutes more than 97% of the total chlorine (White, 1999), the ORP increased with decreasing pH due to the Nernst Law (Oldham and Mayland, 1994). When pH exceeds 5.0, the fraction of OCl^-, which has less oxidative and bactericidal effect than HOCl, dramatically increases with pH, and hence the ORP is lower at higher pH. Another reason may be that the buffer itself has higher ORP at lower pH as demonstrated at zero residual chlorine level in Fig. 1.

Comparing the results presented in Tables 1 and 3 on the inactivation of *E. coli* O157:H7 at the residual chlorine concentration of 0.2 mg/l, the diluted EO water without pH adjustment (Table 1), whose pH is about 5.0, had stronger bactericidal activity than the corresponded pH 5.0-buffered EO water (Table 3). This phenomenon could be explained by the difference in ORP values between the diluted and the buffered EO water. The ORP values of the diluted EO water were about 100 mV higher than those of the pH 5.0-buffered EO water at the same residual chlorine concentration (Table 5). This difference may be due to the slight reaction of free chlorine with buffer solution. The high ORP values may contribute to the stronger bactericidal activity of the diluted EO water (Tables 1 and 3). This observation also supports the study of Kim et al. (2000a) that the ORP of EO water is a primary factor, which contributes to EO water's the bactericidal activity. Similar differences between the diluted and the buffered EO water on the bactericidal activity of *L. monocytogenes* (Tables 2 and 4) can also be explained based on the difference in ORP values.

Our results demonstrated that EO water is very effective for inhibiting *E. coli* O157:H7 and *L. monocytogenes* in a wide pH range (between 2.6 and 7.0), if sufficient residual chlorine is present. The residual chlorine concentration of EO water will only need to be maintained at a very low level for practical applications, because EO water containing only 1.0 to 2.0 mg/l of residual chlorine showed very strong bactericidal activity against *E. coli* O157:H7 and *L. monocytogenes*.

Table 5
Comparison of ORP values between pH-unadjusted dilute EO water and pH 5.0-buffered EO water

Residual chlorine (mg/l)	Diluted EO water		pH 5.0-buffered EO water
	pH[a]	ORP (mV)[b]	ORP (mV)[c]
0.1	5.32 ± 0.06	757 ± 10	648 ± 10
0.2	4.97 ± 0.01	787 ± 20	657 ± 23
0.5	4.56 ± 0.03	863 ± 7	741 ± 4

[a] Values are from Table 1.
[b] Values are from Table 1.
[c] Values are the means of four replicated measurements ± standard deviation.

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