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Effects of Active Chlorine, Oxidation-reduction Potential, and pH on the Bactericidal Activity of Chlorinated Water

Hoon Park*

Division of Applied Biological Sciences, Sunmoon University

Abstract

The effects of active chlorine and oxidation-reduction potential (ORP) on the inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* were evaluated using chlorinated water. The bactericidal activity of chlorinated water increased with active chlorine concentration, and *E. coli* 0157:H7 was found to be more sensitive to chlorinated water than *L. monocytogenes*. Complete inactivation was observed at active chlorine levels equal to or higher than 5 mg/L as Cl_2. The pH effect on the bactericidal activity of chlorinated water was also examined at pH 3, 5 and 7. The bactericidal activity increased with the decrease of pH for both pathogens, and complete inactivation was observed regardless of pH at active chlorine levels equal to or higher than 15 mg/L as Cl_2. High oxidation strength of chlorinated water at high active chlorine level and low pH could be responsible for its strong bactericidal activity. The results indicate that chlorinated water can be applied in a wide pH range between 3 and 7 for inhibiting both pathogens.

Key words: chlorine, pH, oxidation-reduction potential, *Escherichia coli* O157:H7, *Listeria monocytogenes*

Introduction

Chlorinated water has been utilized in various fields, such as food sanitation, water treatment, medical sterilization, and areas that rely on antimicrobial methodologies (Brackett 1987; White 1999). Especially, in food industries, chlorinated water has been frequently used in many countries to wash poultry and vegetables at various stages of handling and processing since it has a strong antimicrobial activity against most pathogenic bacteria. Besides chlorine, inorganic and organic chloramines and chlorine dioxide also has been used as sanitizer, however, their antimicrobial activity varies (Kim et al., 2000).

Various species of chlorine (HOCl, OCl^-, Cl_2) are present in a solution and the relative distribution of HOCl and OCl^- depends on the pH of a solution, with the former being the more active oxidizing agent (White, 1999). Therefore, variability in oxidation or antimicrobial efficiency can be dependent on the pH of a solution. Previous studies suggested that chlorine species inactivate bacterial cells by 1) inactivation of enzymes involved in respiration, 2) oxidation of sulfhydryl compounds on cell surface, 3) inhibition of ATP generation, and 4) retardation of active transport (Albrich et al., 1986; Barrette et al., 1989; Hurst et al., 1991; Leyer & Johnson, 1997).

Foodborne outbreaks of *Escherichia coli* O157:H7 and *Listeria monocytogenes* infections have been frequently reported in a variety of foods including meats, poultry egg, milk, fruits, and vegetables (Rocourt & Cossart, 1997; Doyle et al., 1997; Singh et al., 2002). To date, little information was provided about the antimicrobial activity of chlorinated water as a function of active chlorine, oxidation-reduction potential (ORP), and pH against foodborne pathogens such as *E. coli* O157:H7 and *L. monocytogenes*. The pH of chlorinated water will change ORP and the relative fractions of chlorine species that have different bactericidal activities.

The objective of this study was to examine the effects of active chlorine, ORP, and pH on the bactericidal effectiveness of chlorinated water against *E. coli* O157:H7 and *L. monocytogenes*.

Materials and Methods

Bacterial cultures. Two strains of foodborne pathogenic bacteria tested in this study were *E. coli* O157:H7 F500 (human feces isolate) and *L. monocytogenes* ScottA. The strains were maintained in 10 ml of tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA) at 37°C by daily transfer. Each strain was separately cultured in 100 ml of TSB

Corresponding author: Hoon Park, Division of Applied Biological Sciences, Sunmoon University, Asan, Chung Nam, 336-708, Republic of Korea
Tel: 041-530-2262; Fax: 041-530-2917
E-mail: hpark@sunmoon.ac.kr

in 250-mL Erlenmeyer flasks at 37°C overnight without shaking, harvested by centrifugation (4,000 ×g for 10 min), washed twice with sterile deionized water, and resuspended in sterile deionized water. Bacterial concentrations were estimated by measuring the A_{600} of bacterial suspension and confirmed by plating 0.1-mL portions of appropriately culture on tryptic soy agar (TSA) (Difco Laboratories) plates and incubating the plates at 37°C for 48 hr.

Preparation of chlorinated water. Chlorinated water was prepared to have various concentrations by diluting concentrated chlorine water (Fisher Scientific Co., Fair Lawn, NJ, USA) with deionized water or buffered water. The pH and ORP of chlorinated water were measured using a dual scale pH/ORP. The active chlorine concentration was determined by iodometric method using a chlorine test kit.

Sample treatment. In order to determine the effect of active chlorine on antimicrobial activity, chlorinated water was properly diluted to obtain final active chlorine concentrations of 0.1-10 mg/L as Cl_2. One milliliter (equivalent to 10^{10} CFU/ml) of each bacterial strain was separately added to 99 ml of chlorinated water or deionized water (no active chlorine) in a sterile beaker and incubated at ambient temperature (23±2°C) for 30 sec with stirring.

For the experiment of pH effects on antimicrobial activity, three different pH levels of stock buffer solutions were prepared; the pH 3 buffer was 100 mM in tartaric acid, the pH 5 buffer was 100 mM in tartaric acid, and the pH 7 buffer was 100 mM in monobasic sodium phosphate. Chlorinated water was appropriately diluted in each buffer to obtain final active chlorine concentrations of 0.2 to 15 mg/L as Cl_2, and the ORP and pH values of the chlorinated water were measured before inoculation. One milliliter (equivalent to 10^{10} CFU/mL) of each bacterial strain was separately added to 99 ml of the buffered chlorinated water or deionized water (control) in a sterile beaker and incubated at ambient temperature (23±2°C) for 30 sec with stirring. Following treatments, 1 mL of each sample was serially diluted (1:10) in 9 mL of sterile neutralizing buffer (Difco Laboratories) and the populations of *E. coli* O157:H7 or *L. monocytogenes* were determined by plating 0.1 mL of each dilution in duplicate on TSA plates. Bacterial viability was measured as the number of colonies appearing on the duplicate TSA plates after incubation at 37°C for 48 hr. 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 hr. Following enrichment, the culture was streaked on TSA plates, and the plates were incubated at 37°C for 48 hr. Two independent replicates were conducted.

Results and Discussion

Effects of active chlorine and ORP on bactericidal activity. The effect of active chlorine on bactericidal activity was evaluated using chlorinated water, which contained active chlorine levels ranging from 0.1 to 10 mg/L as Cl_2. The effect of active chlorine on the antimicrobial activity of *E. coli* O157:H7 and *L. monocytogenes* are shown in Table 1 and 2, respectively. At the active chlorine concentrations of 0.5 and 1.0 mg/L, the populations of *E. coli* 0157:H7 were reduced by about 4.9 and 6.1 $\log_{10}$ CFU/mL, respectively (Table 1). At the same active chlorine levels, about 2.0 and 4.8 $\log_{10}$ CFU/mL of *L. monocytogenes* were inactivated, respectively (Table 2).

Table 1. Bactericidal activity of chlorinated water against *E. coli* O157:H7 as a function of active chlorine

Active chlorine (mg/L)	Surviving population ($\log_{10}$ CFU/mL)	Water properties	
		pH	ORP (mV)
0	7.78 ± 0.10	6.20 ± 0.03	405 ± 16
0.1	6.05 ± 0.07	5.65 ± 0.07	601 ± 9
0.2	5.39 ± 0.15	5.26 ± 0.06	665 ± 7
0.5	2.95 ± 0.18	4.84 ± 0.06	722 ± 12
1	1.74 ± 0.23	4.50 ± 0.02	802 ± 15
2	< 1.0[a]	4.15 ± 0.03	860 ± 11
5	ND[b]	3.72 ± 0.05	916 ± 8
10	ND	3.39 ± 0.02	955 ± 10

Values are the means of two replicated measurements ± standard deviation.
The initial population of *E. coli* O157:H7 was 7.86 $\log_{10}$ CFU/mL.
[a]Positive by an enrichment procedure and detectable survivors by a direct plating procedure.
[b]Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

Table 2. Bactericidal activity of chlorinated water against *L. monocytogenes* as a function of active chlorine

Active chlorine (mg/L)	Surviving population ($\log_{10}$ CFU/mL)	Water properties	
		pH	ORP (mV)
0	7.79 ± 0.06	6.26 ± 0.12	412 ± 14
0.1	7.63 ± 0.10	5.59 ± 0.02	610 ± 17
0.2	7.02 ± 0.05	5.26 ± 0.06	657 ± 3
0.5	5.88 ± 0.14	4.84 ± 0.03	717 ± 16
1	3.15 ± 0.23	4.47 ± 0.05	805 ± 10
2	1.14 ± 0.31	4.12 ± 0.02	860 ± 8
5	ND[a]	3.67 ± 0.01	925 ± 11
10	ND	3.36 ± 0	962 ± 9

Values are the means of two replicated measurements ± standard deviation.
The initial population of *Listeria monocytogenes* was 7.92 $\log_{10}$ CFU/mL.
[a]Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

These results indicate that the bactericidal activity of chlorinated water increases with active chlorine concentration, and *E. coli* 0157:H7 is more sensitive to chlorinated water than *L. monocytogenes*. Complete inactivation of both pathogens was observed at active chlorine levels equal to or greater than 5 mg/L as Cl_2.

The ORP of chlorinated water was proportional to the concentration of active chlorine. The ORP of chlorinated water was significantly higher than that of deionized water even at very low chlorine levels, due to the presence of strong oxidative active chlorine species. The data suggested that over 5 mg/L of active chlorine concentrations and 900 mV of ORP were strong enough to kill both pathogens.

Influence of pH on bactericidal activity. The effect of pH on the bactericidal activity of chlorinated water was evaluated against both pathogens under active chlorine levels ranging from 0.2 to 15 mg/L as Cl_2. At the same active chlorine concentration, decreasing pH values increased the sensitivity of both pathogens. The active chlorine concentration of 2 mg/L was effective to completely kill both pathogens at pH 3, whereas more than 5 mg/L is required at pH 5 (Table 3 & 4). The strong bactericidal activity observed at pH 3 could be due to the relative high amount of HOCl (Oldham & Mayland, 1994; Len et al., 2000; Park et al., 2004). At 10 mg/L (as Cl_2) chlorine level, bacterial colonies were observed only at pH 7 for both pathogens. However, at more than 15 mg/L (as Cl_2) chlorine level, complete microbial inactivation was observed regardless of pH. No significant reduction of both pathogens was observed upon exposure to buffer treatments (control) at each pH. The values of surviving bacterial populations in

Table 3. Bactericidal activity of chlorinated water against *E. coli* O157:H7 at different levels of active chlorine and pH

Active chlorine (mg/L)	Surviving population (log_{10} CFU/mL)		
	pH 3	pH 5	pH 7
0	7.74 ± 0.16	7.76 ± 0.18	7.85 ± 0.13
0.2	4.03 ± 0.12	6.23 ± 0.09	7.76 ± 0.20
0.5	2.08 ± 0.35	3.14 ± 0.11	7.78 ± 0.16
1	< 1.0[a]	1.64 ± 0.15	6.51 ± 0.27
2	ND[b]	ND	3.97 ± 0.32
5	ND	ND	1.52 ± 0.23
10	ND	ND	ND
15	ND	ND	ND

Values are the means of two replicated measurements ± standard deviation.
The initial population of *E. coli* O157:H7 was 7.85 log_{10} CFU/mL.
[a]Positive by an enrichment procedure and detectable survivors by a direct plating procedure.
[b]Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

Table 4. Bactericidal activity of chlorinated water against *L. monocytogenes* at different levels of active chlorine and pH

Active chlorine (mg/L)	Surviving population (log_{10} CFU/mL)		
	pH 3	pH 5	pH 7
0	7.82 ± 0.04	7.75 ± 0.10	7.79 ± 0.14
0.2	6.01 ± 0.09	7.79 ± 0.13	7.81 ± 0.03
0.5	4.23 ± 0.14	6.24 ± 0.21	7.77 ± 0.12
1	1.92 ± 0.20	4.92 ± 0.14	7.48 ± 0.10
2	ND[b]	3.27 ± 0.15	6.23 ± 0.09
5	ND	< 1.0[a]	3.24 ± 0.06
10	ND	ND	1.77 ± 0.06
15	ND	ND	ND

Values are the means of two replicated measurements ± standard deviation.
The initial population of *Listeria monocytogenes* was 7.90 log_{10} CFU/mL.
[a] Positive by an enrichment procedure and detectable survivors by a direct plating procedure.
[b] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

Table 3 and 4 showed that *E. coli* O157:H7 is more sensitive to chlorinated water than *L. monocytogenes* at any pH levels.

Table 5 shows the ORP values of buffered chlorine water at each active chlorine level. At all chorine levels, the ORP values increased with the decrease of pH, suggesting that the stronger bactericidal activity observed at lower pH could be due to the corresponding higher ORP. The lower ORP at higher pH is probably due to the large fraction of OCl^- ions, which are less oxidative than HOCl. Previously, Park et al. (2004) reported the effects of chlorine and pH on efficacy of electrolyzed oxidizing (EO) water for inactivating *E. coli* O157:H7 and *L. monocytogenes* in a wide pH range (between 2.6 and 7.0). The observed strong bactericidal activity of EO water was primarily due to the presence of chlorine species. Consistently, for each chlorine content, bactericidal activity and ORP of chlorinated water increased with decreasing pH. However, in this study, chlorinated water was found to be

Table 5. ORP values of bufferred chlorine water at different levels of active chlorine and pH

Active chlorine(mg/L)	ORP (mV)		
	pH 3	pH 5	pH 7
0	432 ± 21	411 ± 14	397 ± 7
0.2	715 ± 8	643 ± 13	566 ± 4
0.5	774 ± 12	709 ± 6	621 ± 15
1	824 ± 23	758 ± 10	662 ± 17
2	869 ± 10	802 ± 7	701 ± 16
5	921 ± 5	857 ± 18	753 ± 11
10	967 ± 8	903 ± 12	812 ± 14
15	1006 ± 3	950 ± 5	869± 9

Data points are the means of two replicated measurements.

required more active chlorine to completely inactivate *E. coli* O157:H7 and *L. monocytogenes* at the equivalent pH levels. This can possibly be explained by lower ORP values of chlorinated water than those of EO water at the same active chlorine levels. The higher ORP of EO water may be due to the presence of other oxidants except chlorine species.

This study demonstrates that chlorinated water is very effective for inhibiting the *E. coli* O157:H7 and *L. monocytogenes* in a wide pH range between 3 and 7. The results suggest that in actual applications, the active chlorine level of chlorinated water can be significantly reduced from a normal value (50-200 mg/L active chlorine as Cl_2), since the two pathogens are very sensitive to active chlorine. This study also suggests that drinking water containing active chlorine appears likely to be effective on reducing the populations of chlorine-sensitive pathogens such as *E. coli* 0157:H7, but its antimicrobial activity may be dependent on its pH and active chlorine level. The typical pH range of drinking water is between 6.5 and 8.5 (White 1999). According to WHO drinking water standards, 2-3 mg/L of chlorine should be added to water in order to gain a satisfactory disinfection, and the maximum amount of chlorine one can use is 5 mg/L (WHO 2004).

Conclusions

The bactericidal activity of chlorinated water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* was proportional to the active chlorine concentration and ORP values. At the same active chlorine concentration, the bactericidal activity against both pathogens increased with the decrease of pH value, due to the increase of ORP value. The chlorinated water containing equal to or higher than 15 mg/L of active chlorine was effective in completely killing both pathogens, regardless of pH, suggesting that chlorinated water could be applied for inhibiting foodborne pathogens in a wide pH ranges.

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References

Albrich JM, Gilbaugh III JH, Callahan KB and Hurst JK. 1986. Effects of the putative neutrophile-generated toxin, hypochlorous acid, on membrane permeability and transport systems of *Escherichia coli*. J. Clin. Invest. **78**: 177-184

Brackett RE. 1987. Antimicrobial effect of chlorine on *Listeria monocytogenes*. J. Food Prot. **50**: 999-1003

Barrette WC, Hannum DM, Wheeler WD and Hurst JK. 1989. General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. Biochemistry **28**: 9172-9178

Doyle MP, Zhao T, Meng J and Zhao S. 1997. *Escherichia coli* O157:H7. In: Doyle MP, Beuchat LR, Montville MJ, (Eds.), Food Microbiology, Fundamentals and Frontiers. ASM Press, Washington DC, USA. pp 171-191

Hurst JK, Barrette WC, Michel BR and Rosen H. 1991. Hypochlorous acid and myeloperoxidase-catalyzed oxidation of iron-sulfur clusters in bacterial respiratory dehydrogenase. Eur. J. Biochem. **202**: 1275-1282

Kim C, Hung YC and Brackett RE. 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. Int. J. Food Microbiol. **61**: 199-207

Len SV, Hung YC, Erickson M and Kim C. 2000. Ultraviolet spectrophotometric characterization and bacterial properties of electrolyzed oxidizing water as influenced by amperage and pH. J. Food Prot. **63**: 1534-1537

Leyer GJ and Johnson EA. 1997. Acid adaptation sensitizes *Salmonella typhimurium* to hypochlorous acid. Appl. Environ. Microbiol. **63**: 461-467

Oldham HB and Mayland JC. 1994. Fundamental of electrochemical science. Academic Press Inc., San Diego, USA. pp120-122

Park H, Hung YC and Chung D. 2004. Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* 0157:H7 and *Listeria monocytogenes*. Int. J. Food Microbiol. **91**: 13-18

Rocourt J and Cossart P. 1997. *Listeria monocytogenes*. In: Doyle MP, Beuchat LR, Montville MJ, (Eds.), Food Microbiology, Fundamentals and Frontiers. ASM Press, Washington DC, USA. pp337-352

Singh N, Singh RK, Bhunia AK and Stroshine RL. 2002. Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* 0157:H7 on lettuce. Food Microbiol. **19**: 183-193

White GC. 1999. Handbook of chlorination and alternative disinfectants. (4th Eds.), John Wiley & Sons, New York, USA. pp103-287

WHO. 2004. Guidelines for drinking water quality. (3rd ed), World Health Organization, Geneva, Switzerland.

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