Bactericidal activity of strong acidic hypochlorous water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* in biofilms attached to stainless steel

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Abstract This study aims to investigate the bactericidal activity of strong acidic hypochlorous water (SAHW) against *Escherichia coli* O157:H7 and *L. monocytogenes* in bacterial biofilms. The bactericidal activity of SAHW against both bacteria in colony biofilm increased with the elevation of the available chlorine concentration (ACC) and extension of the treatment time. The survived cell counts of *E. coli* O157:H7 and *L. monocytogenes* in the biofilms were significantly ($p < 0.05$) decreased compare to tap water at more than 30 mg/L of ACC in SAHW and 15 s of treatment time. *E. coli* O157:H7 and *L. monocytogenes* in the biofilms reduced to less than the detection limit by treatment of 50 mg/L of ACC in SAHW for 300 and 600 s, respectively. SAHW may be a potential disinfecting agent for removing bacterial biofilms from food processing equipment and other facilities.

Keywords Bactericidal activity · Strong acidic hypochlorous water · *Escherichia coli* O157:H7 · *Listeria monocytogenes* · Biofilm

Introduction

E. coli O157:H7 and *L. monocytogenes* are the foremost foodborne pathogens that frequently cause fatal human infections [1, 2]. These microorganisms contaminate food through a variety of environmental sources and food

processing facilities, such as floor drains, storage tanks, and conveyer belts [3, 4]. Some strains have been known to persistently reside in food processing environments for extended periods of time via forming biofilms. Bacteria generally exist in one of the two types of population: planktonic, freely existing in bulk solution, and sessile, a unit attached to a surface or within the confines of a biofilm. Biofilms can be defined as a sessile bacterial community of cells that live attached to each other, to surfaces of foods, and to processing facilities [5]. Bacteria growing in biofilms are a major concern for food industries because of their higher resistance to a wide range of sanitizers, disinfectants, and antimicrobial agents as compared with planktonic cells [6, 7]. The surviving cells in biofilms can spread into food products or onto equipment surfaces, which can result in cross-contamination or post-contamination concerns [7–9]. In addition, biofilms formed in food processing facilities reduce heat transfer, increase energy loss and fluid frictional resistance, and accelerate corrosion [10]. Thus, more effective disinfection methods have become an urgent requirement.

Using electrolyzed oxidizing water as a sanitizer is a novel technology developed in Japan. It has been employed in agriculture, livestock management, medical sterilization, food sanitation, and areas that rely on antimicrobial methodologies [11–13]. Strong acidic hypochlorous water (SAHW) is generated by electrolysis of a sodium chloride solution (0.2% NaCl) in a chamber where anode and cathode electrodes are separated by a septum (diaphragm). SAHW has a pH value of approximately 2.6, an oxidation–reduction potential (ORP) of 1150 mV, and a chlorine concentration ranging between 40 and 90 ppm [11]. SAHW has strong bactericidal activity and can be applied to the food industry for sanitary measures. Moreover, SAHW has been reported to exhibit strong bactericidal activities against many pathogens, including *E. coli*

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O157:H7, *Salmonella enteritidis*, *L. monocytogenes*, *Campylobacter jejuni*, *Enterobacter aerogenes*, and *Staphylococcus aureus* [12–15]. In addition, studies have demonstrated the potential use of SAHW as an effective antimicrobial agent for vegetables [16], poultry [17, 18], sea foods [15], and food contact surfaces such as cutting boards [14], glasses, ceramic tiles, and vitreous china [19]. However, there is little research [20] about the removal of bacterial biofilms by electrolyzed water. Therefore, this study aims to investigate the bactericidal activity of SAHW against biofilms of *E. coli* O157:H7 and *L. monocytogenes* attached to surface of stainless steel.

Materials and methods

Preparation of stainless steel chips

Stainless steel (SS) chips were prepared using the method of Dewanti and Wong [21] with some modifications. SS type 304 with a #4 finish, commonly used in food processing equipment and contact surfaces, was cut into 1 × 1 cm chips. The SS chips were washed in a hot detergent solution (1% Micro; International Products Co., Trenton, NJ, USA) for 1 h, rinsed with distilled water, immersed in 70% alcohol for 30 min, rinsed again with distilled water, and air-dried at room temperature. The prepared SS chips were placed in the culture tubes with 25 mL of TSB and sterilized by autoclaving at 121 °C for 15 min.

Preparation of SAHW

SAHW was prepared by electrolyzing 0.2% NaCl solution using mini SUPER WATER JED-007 (UP Co., Yokohama, Japan) according to the manufacturer's instructions. SAHW with different chlorine concentrations were prepared by diluting SAHW with distilled water. These SAHW samples were prepared just before test. The available chlorine concentration (ACC) of SAHW was measured by a pocket colorimeter (HACH Co., Colorado, USA). The ORP and pH of SAHW were measured using a pH/ISE meter (Istek Co., Seoul, Korea) equipped with an ORP sensor and a pH sensor.

Bacterial culture preparation

E. coli O157:H7 ATCC 25922 and *L. monocytogenes* ATCC 15313 were used in this study. Each strain was individually enriched in tryptic soy broth (TSB, Difco, Franklin Lakes, NJ, USA) at 35 °C for 18–24 h. Enriched cultures were pooled into a sterile centrifuge tube and cells were harvested by centrifugation at 5 °C for 15 min (3000×g). Pelleted cells were washed twice with phosphate-buffered saline (PBS, pH 7.2) and re-suspended in

fresh PBS (pH 7.2) to obtain a final cell concentration of 1.0×10^5 CFU/mL.

Biofilm development on an SS chip

Each strain was cultured in 50 mL of TSB at 37 °C for 24 h. Two milliliters of each culture (1.0×10^5 CFU/mL) was inoculated into a tube of 25 mL fresh TSB, where one SS chip was submerged. The tubes were incubated at 37 °C for 8 days to develop a biofilm on the SS chip. The SS chip was rinsed with 25 mL of PBS (pH 7.2) twice to remove the unattached cells every 2 days and then continuously incubated in 25 mL of fresh TSB.

Treatment of SAHW against bacterial biofilms on an SS chip

The SS chip incubated at 37 °C for 8 days was removed from the tube containing PBS using a sterile nipper and submerged in 100 mL of fresh PBS (pH 7.2) for 2 min with agitation to remove the unattached cells. The SS chip was then submerged in each tube containing 50 mL of SAHW with different ACC and hand-shaken for 15, 30, 60, 180, and 300 s. After the reaction, the SS chip was submerged immediately into 100 mL of fresh PBS (pH 7.2) for 2 min to stop the reaction. Positive control SS chip was treated by 50 mL of tap water instead of SAHW. Negative control SS chip was not treated by SAHW or tap water.

Bactericidal activity of SAHW against *E. coli* O157:H7 and *L. monocytogenes* in the biofilm on an SS chip

The SS chip treated with SAHW or tap water was placed in 25 mL of fresh PBS (pH 7.2) containing 2% Tween 80 (Sigma–Aldrich, St. Louis, MO, USA) and sonicated in an ultrasonic washer 4020P (500 W, 60 Hz, KODO Co., Seoul, Korea) for 3 min to detach the bacterial cells from the chip. The bactericidal activity of SAHW against *E. coli* O157:H7 and *L. monocytogenes* in the biofilm on the SS chip was evaluated by survival viable cell count (log CFU/cm²). The viable cell count was measured by the pour plate method with plate counts agar (PCA, Difco, Franklin Lakes, NJ, USA) at 37 °C for 48 h. All experiments were performed in triplicate.

Bacterial morphology

The morphological changes of *E. coli* O157:H7 and *L. monocytogenes* were assessed by scanning electron microscopy (SEM) using a scanning electron microscope (S-4700, HITACHI Co., Tokyo, Japan). The SS chips were rinsed, re-suspended in PBS, and fixed with 1% glutaraldehyde (Sigma–Aldrich) in 0.2 M cacodylate buffer

(Sigma–Aldrich) overnight at 4 °C. The samples were then rinsed with PBS, dehydrated through a series of graded ethanol washes (50, 70, 90, and 100% for 5 min each), and sputter-coated with gold (JEOL JFC 1100). An image analysis system (SigmaScan Pro 5; SPSS Science, Chicago, IL) was used to quantify the adhered bacterial cells cultured with or without treatment of SAHW.

Statistical analysis

All experiments were performed in triplicate and data were recorded as mean values $\pm$ SD. Data were analyzed using a one-way analysis of variance and Duncan multiple range test with SPSS (version 19, IBM, Seoul, Korea) statistical software. A level of significance of $p < 0.05$ was used for all comparisons.

Results and discussion

Properties of SHAW

The properties of SAHW are shown in Table 1. The ACC, pH, and ORP of SAHW were 54 mg/L, 2.33, and 1115 mV, respectively. When SAHW was diluted by distilled water to

Table 1 Properties of strong acidic hypochlorous water (SAHW)

Available chlorine concentration (mg/L)	pH	Oxidation–reduction potential (mV)
50 $\pm$ 1	2.37 $\pm$ 0.2	1113 $\pm$ 7
40 $\pm$ 1	2.46 $\pm$ 0.3	1108 $\pm$ 5
30 $\pm$ 1	2.56 $\pm$ 0.2	1096 $\pm$ 6
20 $\pm$ 1	2.73 $\pm$ 0.1	1070 $\pm$ 10
10 $\pm$ 1	3.01 $\pm$ 0.1	1015 $\pm$ 8

Mean of triplicate experiments $\pm$ SD

obtain ACC concentrations from 10 to 50 mg/L used in this study, the range of ORP and pH of diluted SAHW were 1015–1113 mV and 2.37–3.01, respectively. *E. coli* O157:H7 and *L. monocytogenes* suspensions with initial cell number of 8.8 log CFU/mL were not detected after treatment with 5 mg/L of ACC in SAHW for 30 s (data not shown).

Bactericidal activity of SAHW against *E. coli* O157:H7 and *L. monocytogenes* in biofilms

The bactericidal activity of SAHW against *E. coli* O157:H7 in the biofilms on SS chips is shown in Table 2. The initial viable cell count of *E. coli* O157:H7 in the biofilms on SS chips was 6.70 log CFU/cm². The bactericidal activity of SAHW against *E. coli* O157:H7 in the biofilms increased with the elevation of ACC and extension of treatment time. The survived cell counts of *E. coli* O157:H7 in the biofilms significantly ($p < 0.05$) decreased as compared with tap water at more than 60 s of treatment time regardless of ACC in SAHW. The survived cell counts of *E. coli* O157:H7 in the biofilms significantly ($p < 0.05$) decreased compare to tap water at more than 30 mg/L of ACC in SAHW regardless of treatment time. Moreover, the survived cell counts reduced to less than the detection limit by treatment of SAHW with 50 mg/L of ACC and 300 s of treatment.

The bactericidal activity of SAHW against *L. monocytogenes* in the biofilms on SS chips is shown in Table 3. The initial viable cell count of *E. coli* O157:H7 in the biofilms attached to SS chips was 5.80 log CFU/cm². The bactericidal activity of SAHW against *L. monocytogenes* in the biofilms increased with the elevation of ACC and the extension of treatment time. The survived cell counts of *L. monocytogenes* in the biofilms reduced to less than the detection limit by the treatment of SAHW with 50 mg/L of ACC for 600 s. The bactericidal activity of SAHW against

Table 2 Bactericidal activity of SAHW against *E. coli* O157:H7 in the biofilms on SS chips

Treatment time (s)	Survived viable cell count (log CFU/cm ²)					
	Tap water	ACC of SAHW (mg/L)				
		10	20	30	40	50
0	^a 6.70 $\pm$ 0.29 ^a	^a 6.70 $\pm$ 0.29 ^a	^a 6.70 $\pm$ 0.29 ^a	^a 6.70 $\pm$ 0.29 ^a	^a 6.70 $\pm$ 0.29 ^a	^a 6.70 $\pm$ 0.29 ^a
15	^a 6.36 $\pm$ 0.42 ^a	^a 6.04 $\pm$ 0.52 ^a	^a 5.80 $\pm$ 0.52 ^a	^b 4.95 $\pm$ 1.10 ^b	^a 5.42 $\pm$ 1.06 ^b	^b 3.66 $\pm$ 0.23 ^c
30	^a 6.34 $\pm$ 0.52 ^a	^{ab} 5.03 $\pm$ 0.40 ^{ab}	^{ab} 5.45 $\pm$ 1.34 ^a	^{bc} 3.73 $\pm$ 0.46 ^b	^{ab} 4.10 $\pm$ 0.57 ^b	^b 3.75 $\pm$ 0.66 ^b
60	^a 6.14 $\pm$ 0.28 ^a	^b 4.71 $\pm$ 0.65 ^b	^b 3.61 $\pm$ 0.39 ^b	^c 3.54 $\pm$ 0.12 ^b	^b 3.43 $\pm$ 0.37 ^b	^b 3.30 $\pm$ 0.15 ^b
180	^a 5.87 $\pm$ 0.13 ^a	^b 3.57 $\pm$ 0.36 ^b	^b 3.44 $\pm$ 0.67 ^b	^c 3.48 $\pm$ 0.12 ^b	^b 3.08 $\pm$ 0.77 ^b	^c 1.93 $\pm$ 0.81 ^c
300	^a 5.82 $\pm$ 0.13 ^a	^b 3.63 $\pm$ 0.22 ^b	^b 3.04 $\pm$ 0.90 ^b	^d 1.47 $\pm$ 0.81 ^c	^c 2.03 $\pm$ 0.91 ^c	^d <1.00 $\pm$ 0.00 ^d

Cell numbers are given in mean of triplicate experiments $\pm$ SD. Different letters in the column before the cell counts showed significant difference in treatment time ($p < 0.05$). Different letters in the row after the cell counts showed significant difference in the available chlorine concentration (ACC) ($p < 0.05$)

Table 3 Bactericidal activity of SAHW against *L. monocytogenes* in the biofilms on steels chips

Treatment time (s)	Survived viable cell count (log CFU/cm ²)					
	Tap water	ACC of SAHW (ppm)				
		10	20	30	40	50
0	^a 5.80 ± 0.21 ^a	^a 5.80 ± 0.21 ^a	^a 5.80 ± 0.21 ^a	^a 5.80 ± 0.21 ^a	^a 5.80 ± 0.21 ^a	^a 5.80 ± 0.21 ^a
15	^a 5.30 ± 0.51 ^a	^a 4.41 ± 0.29 ^b	^a 4.46 ± 0.79 ^b	^{ab} 3.61 ± 0.35 ^c	^{ab} 3.69 ± 0.18 ^c	^b 3.28 ± 0.28 ^c
30	^a 5.34 ± 0.54 ^a	^a 3.67 ± 0.22 ^b	^{ab} 3.55 ± 0.61 ^b	^{ab} 3.60 ± 0.20 ^b	^{ab} 3.66 ± 0.17 ^b	^b 3.44 ± 0.08 ^b
60	^a 4.81 ± 0.23 ^a	^{ab} 3.81 ± 0.72 ^{ab}	^{ab} 3.79 ± 0.93 ^{ab}	^{ab} 3.42 ± 0.24 ^b	^b 3.32 ± 0.13 ^b	^b 3.02 ± 0.45 ^b
180	^a 4.57 ± 0.30 ^a	^{ab} 3.57 ± 0.25 ^{ab}	^{ab} 3.90 ± 0.41 ^{ab}	^b 3.36 ± 0.21 ^b	^b 3.56 ± 0.22 ^b	^b 3.09 ± 0.86 ^b
300	^b 4.34 ± 0.17 ^a	^{ab} 3.39 ± 0.24 ^{ab}	^b 3.14 ± 0.39 ^{ab}	^b 3.24 ± 0.46 ^{ab}	^b 3.24 ± 0.62 ^{ab}	^b 3.09 ± 0.34 ^b
600	^b 4.44 ± 0.50 ^a	^{ab} 3.30 ± 0.19 ^b	^c 1.62 ± 1.07 ^c	^c 1.92 ± 0.82 ^c	^c 1.43 ± 0.74 ^c	^c <1.00 ± 0.00 ^c

Cell numbers are given in mean of three replicate experiments ± SD. Different letters in the column before the cell counts showed significant difference in treatment time ($p < 0.05$). Different letters in the row after the cell counts showed significant difference in ACC ($p < 0.05$)

L. monocytogenes in the biofilms on SS chips was similar to that of *E. coli* O157:H7, but the bactericidal activity of SAHW against *E. coli* O157:H7 in the biofilms was a little better than that against *L. monocytogenes*. This may be due to the differences in the cell wall structures between gram-negative (*E. coli* O157:H7), and gram-positive bacteria (*L. monocytogenes*) or chemical properties (exopolysaccharide) of their biofilms [15].

Morphological changes of *E. coli* O157:H7 and *L. monocytogenes* in biofilms

The morphological change of *E. coli* O157:H7 in the biofilms observed by SEM after treating with SAHW are shown in Fig. 1. Figure 1(A) shows that the *E. coli* O157:H7 cells were tightly attached to the SS chips by forming a complex three-dimensional structure. These cells are attached to each other by excreted extracellular polymeric substances [5, 10]. However, only a few cells remained on the SS chips without an extracellular matrix after treating with SAHW (ACC, 50 mg/L) for 5 min [Fig. 1(B)]. Similar change was observed in the *L.*

monocytogenes in the biofilms (Fig. 2). While the *L. monocytogenes* cells were tightly attached to the SS chips [Fig. 2(A)], few cells were remained on the SS chips after treating with SAHW (ACC, 50 mg/L) for 10 min [Fig. 2(B)].

There are few reports about the effects of chlorine concentration, pH, and ORP values of SAHW against pathogenic bacteria. Kim et al. [22] have developed chemically modified water from deionized water with the same properties (i.e., pH, chlorine, and ORP) as SAHW without electrolysis and reported that ORP was the primary factor responsible for the bactericidal activity. However, Koseki et al. [23] reported that ORP is not the main factor of bactericidal activity because the higher ORP of ozonated water did not show higher disinfectant effect than the lower ORP of SAHW. They further defined that the free chlorine of SAHW, mainly hypochlorous acid (HOCl), produces hydroxyl radical (·OH) that acts on bacteria. The bactericidal activity improves with higher concentration of ·OH produced by higher HOCl concentration in SAHW. Len et al. [24] reported that the relative concentrations of aqueous molecular chlorine, HOCl, hypochlorite ion

Fig. 1 Scanning electron micrographs of *E. coli* O157:H7 in biofilms on SS chips. (A) Scanning electron micrographs of *E. coli* O157:H7 in the biofilm before treatment with SAHW; (B) scanning electron micrographs of *E. coli* O157:H7 in the biofilm after treatment with SAHW (ACC, 50 mg/L) for 5 min

Fig. 2 Scanning electron micrographs of *L. monocytogenes* in biofilms on SS chips. (A) Scanning electron micrographs of *L. monocytogenes* in the biofilm before treatment with SAHW; (B) scanning electron micrographs of *L. monocytogenes* in the biofilm after treatment with SAHW (ACC, 50 mg/L) for 10 min

(OCl^-), and chlorine gas (Cl_2) were also the factors responsible for the bactericidal activity. SAHW with the maximum concentration of HOCl had the maximum bactericidal activity at pH 4.

Park et al. [25] investigated the effects of chlorine and pH on the bactericidal activity of SAHW against *E. coli* O157:H7 and *L. monocytogenes* and demonstrated that SAHW is very effective for inactivating *E. coli* O157:H7 and *L. monocytogenes* in a wide pH range (between 2.6 and 7.0) if sufficient free chlorine (greater than 2 mg/L) is present. The bactericidal activity and ORP of SHAW increased with decreasing pH at certain chlorine concentrations. Liao et al. [26] reported that the ORP of SAHW could damage the outer and inner membranes of *E. coli* O157:H7 based on fluorescent and spectroscopic measurements. The redox state of the glutathione disulfide-glutathione couple (GSSG/2GSH) can serve as an important indicator of the redox environment. There are many redox couples in a cell that work together to maintain the redox environment. The inactivation mechanism hypothesized that ORP could damage the redox state of GSSG/2GSH and then penetrate the outer and inner membranes of bacterial cell, thus releasing intracellular components and finally causing the necrosis of *E. coli* O157:H7. Thus, the bactericidal activity of SAHW derives from the combined action of the pH, ORP, and free chlorine.

Chlorine has been widely used in chemical disinfection and is known to not only kill microbes but also remove the exopolysaccharides from the bacterial surface [27]. Therefore, chlorine has been investigated in many studies [28–31] for its bactericidal effect against bacterial biofilms. Several factors have been suggested to account for biofilm tolerance, for example, slow growth and presence of an exopolysaccharide matrix that can slow the diffusion of antibiotics, as well as the presence of unknown resistance mechanisms [32]. Nevertheless, this study demonstrated that SAHW is effective to kill *E. coli* O157:H7 and *L. monocytogenes* in the biofilms on SS chips. These results are similar to the results from previous studies [28–31]. Furthermore, Ayebah and Hung [33] reported that SAHW

did not have any adverse effects on SS, showing no metal corrosion after the use of SAHW on the food processing equipment surfaces. From above results, it can be concluded that SAHW may be a potential disinfecting agent for removing bacterial biofilms from food processing equipment and other facilities.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. *Int. J. Med. Microbiol.* 295: 405–418 (2005)
- Swaminathan B, Peter GS. The epidemiology of human listeriosis. *Microbiol. Infect.* 9: 1236–1243 (2007)
- Murphy HM, Payne SJ, Gagnon GA. Sequential UV- and chlorine-based disinfection to mitigate *Escherichia coli* in drinking water biofilms. *Water Res.* 42: 2083–2092 (2008)
- Carpentier B, Chassaing D. Interactions in biofilms between *Listeria monocytogenes* and resident microorganisms from food industry premises. *Int. J. Food Microbiol.* 97: 111–122 (2004)
- Chmielewski RAN, Frank JF. Biofilm formation and control in food processing facilities. *Comprehensive Reviews Food Sci. Food Safe.* 2: 22–32 (2003)
- Sharma M, Anand SK. Biofilms evaluation as an essential component of HACCP for food/dairy processing industry—a case. *Food Cont.* 13: 469–477 (2002)
- Kumar CG, Anand SK. Significance of microbial biofilms in food industry. *Int. J. Food Microbiol.* 42: 9–27 (1998)
- Petrak T, Kalodera Z, Novakovic P. Bacteriological comparison of parallel and counter flow water chilling of poultry meat. *Meat Sci.* 53: 269–271 (1999)
- Chye FY, Abdullah A, Ayob MK. Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiol.* 21: 535–541 (2004)
- Garrett TR, Bhakoo M, Zhang ZB. Bacterial adhesion and biofilms on surfaces. *Natural Sci.* 18: 1049–1056 (2008)
- Hotta K, Suzuki T. Electrolyzed water: Formation principle, physicochemical property and function. *Bioscience & Industry.* 57: 22–26 (1999)
- Hotta K. The use of acidic electrolyzed water for sanitary or hygienic measure in food and medical field. *Food Processing & Ingredients.* 36: 10–12 (2001)

13. Shimizu Y, Hurusawa DJ. Antiviral, antibacterial, and antifungal actions of electrolyzed oxidizing water through electrolysis. *Dental J.* 37: 1055–1062 (1992)
14. Venkitanarayanan K, Ezeike GO, Hung YC, Doyle MP. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolyzed oxidizing water. *J. Food Prot.* 62: 857–860 (1999).
15. Kim C, Hung YC, Brackett RE. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. *Int. J. Food Microbiol.* 61: 199–207 (2000)
16. Koseki S, Itoh K. 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 (2001)
17. Fabrizio KA, Cutter CN. Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella Typhimurium* and *Listeria monocytogenes*. *J. Food Prot.* 66: 1379–1384 (2003)
18. Park CM, Hung YC, Brackett RE. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. *Int. J. Food Microbiol.* 72: 77–83 (2002).
19. Park H, Hung YC, Kim C. Effectiveness of electrolyzed water as a sanitizer for treating different surfaces. *J. Food Prot.* 65: 1276–1280 (2002)
20. Kim C, Hung YC, Brackett RE. Inactivation of *Listeria monocytogenes* biofilms by electrolyzed oxidizing water. *J. Food Proc. Pres.* 25: 91–100 (2001)
21. Dewanti R, Wong ACL. Influence of culture conditions on biofilm formation by *Escherichia coli* O157:H7. *Food Microbiol.* 26: 147–164 (1995)
22. Kim C, Hung YC, Brackett RE. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of food-borne pathogens. *Inter. J. Food Microbiol.* 61: 199–207 (2000).
23. Koseki S, Yoshida K, Isobe S, Itoh K. Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. *J. Food Prot.* 67: 1247–1251 (2004)
24. Len SV, Hung YC, Erickson M, Kim C. Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. *J. Food Prot.* 63: 1534–1537 (2000)
25. Park H, Hung YC, Chung DH. Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Inter. J. Food Microbiol.* 91: 13–18 (2004)
26. Liao LB, Chen WM, Xiao XM. The generation and inactivation mechanism of oxidation–reduction potential of electrolyzed oxidizing water. *J. Food Eng.* 78: 1326–1332 (2007)
27. Alasri A, Roques C, Cabassud C. Effects of different biocides on a mixed biofilm produced on a Tygon tube and on ultrafiltration membranes. *Spectra* 2000. 168: 21–24 (1992)
28. Richard R. Efficacy of sanitizers on *Listeria monocytogenes* biofilms. *Food Aust.* 51: 624–625 (1999)
29. Lewis K. Riddle of biofilm resistance. *Antimicro. Agents Chemo.* 45: 999–1007 (2001)
30. Byun MW, Kim JH, Kim DH. Effects of irradiation and sodium hypochlorite on the micro-organisms attached to a commercial food container. *Food Microbiol.* 24: 544–548 (2007)
31. Ueda S, Kuwabara Y. Susceptibility of biofilm *Escherichia coli*, *Salmonella enteritidis* and *Staphylococcus aureus* to detergents and sanitizers. *Biocon. Sci.* 12: 149–153 (2007)
32. Spoering AM, Lewis K. Biofilm and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J. Bacteriol.* 183: 6746–6751 (2001)
33. Ayebah B, Hung YC. Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *J. Food Proc. Eng.* 28: 247–264 (2005)