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Inactivation of biofilm cells of foodborne pathogen by aerosolized sanitizers

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ABSTRACT

The objective of this study was to determine the effect of aerosolized sanitizers on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* biofilms. Biofilms were formed on a stainless steel and polyvinyl chloride (PVC) coupon by using a mixture of three strains each of three foodborne pathogens. Six day old biofilms on stainless steel and PVC coupons were treated with aerosolized sodium hypochlorite (SHC; 100 ppm) and peracetic acid (100, 200, and 400 ppm) in a model cabinet for 5, 10, 30, and 50 min. Treatment with 100 ppm PAA was more effective than the same concentration of SHC with increasing treatment time. Exposure to 100 ppm SHC and PAA for 50 min significantly ($p<0.05$) reduced biofilm cells of three foodborne pathogens (0.50 to 3.63 log CFU/coupon and 2.83 to more than 5.78 log CFU/coupon, respectively) compared to the control treatment. Exposure to 200 and 400 ppm PAA was more effective in reducing biofilm cells. Biofilm cells were reduced to below the detection limit (1.48 log CFU/coupon) between 10 and 30 min of exposure. The results of this study suggest that aerosolized sanitizers have a potential as a biofilm control method in the food industry.

1. Introduction

Biofilm can be defined as a community of microbes attached to each other and embedded in an organic polymer matrix, adhering to a surface (Carpentier and Cerf, 1993; Costerton, 1995). It is a natural tendency of bacteria to attach to wet surfaces, to multiply and to embed themselves in a slimy matrix composed of extracellular polymeric substances that they produce, forming a biofilm (Simões et al., 2010). Biofilm exists in a variety of environments, including food processing industry (Zottola and Sasahara, 1994). Several factors in the food processing environment, such as flowing water, attachment surfaces, and sufficient nutrients, pH, water activity (a_w), and temperature are suitable for biofilm formation (Brooks and Flint, 2008; Gibson et al., 1999). Also biofilm formation of microorganisms was affected by environmental parameters, including pH, a_w, and temperature (Giaouris et al., 2005). In addition, gene expression patterns of bacteria are related to the production of biofilms. Differential gene expression compared with that of planktonic bacteria is required to form biofilms (Becker et al., 2001; Beloin and Ghigo, 2005). Several foodborne pathogens including *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* have been reported to produce biofilms (Chae and Schraft, 2000; Wirtanen et al., 2000), and the presence of biofilms can lead to contamination of food product (Hall-Stoodley et al., 2004).

It is difficult to completely remove biofilms formed on food processing surfaces (Baumann et al., 2009). Regular disinfection is one strategy to avoid biofilm formation. However, it is hard to disinfect food processing surfaces frequently (Meyer, 2003). Also, it is hard to disinfect inaccessible surfaces such as storage tank, pump exteriors, walls, and ceilings (Chmielewski and Frank, 2003; Kumar and Anand, 1998). It may result in biofilms formation. Also pipe lines and bends in pipes are common sources related with biofilm formation (Wong, 1998).

Various cleaning methods such as electrolyzed water (Ayebah et al., 2005), ultrasound (Baumann et al., 2009), irradiation (Byun et al., 2007), and bacteriophage (Sharma et al., 2005) have been evaluated to remove biofilms on food processing surfaces. Also washing with various sanitizers including peracetic acid (Fatemi and Frank, 1999), chlorine (Joseph et al., 2001), hydrogen peroxide (DeQueiroz and Day, 2007), and ozone (Robbins et al., 2005) have been tested to control microbial biofilms. However, these methods could not be applied to clean inaccessible environmental surfaces.

Aerosolization is the dispersion of a liquid material as a fine mist in air (Oh et al., 2005b). This technique has been applied for room disinfection. Although spray sanitizing may be effective in disinfection of environmental surfaces (Gibson et al., 1999), fine aerosol mists have better penetration property than trigger spray in assessment of surface bioburden (Hiom et al., 2003). Gaseous sanitizers also could be

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applied in disinfection of inaccessible environmental surfaces (Vaid et al., 2010). However, sophisticated equipment is needed to generate gaseous sanitizer, and the number of applicable sanitizers is limited (Oh et al., 2005a). In comparison, aerosolization technique have broad spectrum of applicable sanitizers.

This study was conducted to evaluate the effectiveness of aerosolized sodium hypochlorite (SHC) and peracetic acid against biofilm of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* formed on stainless steel and PVC. Different concentrations of sanitizer and exposure times were evaluated to guide appropriate application of aerosolization technique.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

Three isolates each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19586, ATCC 43174, DT 104), and *L. monocytogenes* (ATCC 7644, ATCC 19114, ATCC 19115) were obtained from the School of Food Science bacterial culture collection, Washington State University (Pullman, WA, USA). Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 10 ml of tryptic soy broth (TSB; Difco, Sparks, MD, USA) at 37 °C for 24 h, harvested by centrifugation at 4000×*g* for 20 min at 4 °C, and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in sterile phosphate-buffered saline (PBS; pH 7.4), corresponding to approximately 10^7–10^8 CFU/ml.

2.2. Preparation of stainless steel and PVC coupons

Stainless steel (no. 4 grade) and PVC were fabricated into coupons (5 cm×2 cm). Stainless steel and PVC coupons were washed in detergent solution, immersed in 15% phosphoric acid solution for 20 min, and rinsed with distilled water. After washing, coupons were dried in laminar flow biosafety hood (22±2 °C) for 3 h.

2.3. Biofilm formation

Biofilm was formed by two different procedures. In the procedure A, the method used for biofilm formation was similar to that as described by Kim et al. (2006). Each prepared stainless steel and PVC coupon was immersed in a sterile 50-ml conical centrifuge tube (SPL Lifesciences, Pocheon, Korea) containing 30 ml of each cell suspension of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* (ca. 10^7–10^8 CFU/ml). Conical centrifuge tubes were incubated at 4 °C for 24 h to facilitate attachment of cell. After incubation, coupons were removed from conical centrifuge tubes with a sterile forceps, and washed in 500 ml of distilled water with gentle agitation for 5 s (22±2 °C). Washed coupons were transferred to 50-ml conical centrifuge tube containing 30 ml of TSB, and incubated at 25 °C for 6 days. In the procedure B, each prepared stainless steel and PVC coupon was inoculated with 0.1 ml of each cell suspension of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* (ca. 10^7–10^8 CFU/ml) by depositing droplets with a micropipet at 10 locations. After inoculation, coupons were dried in laminar flow biosafety hood for 3 h (22±2 °C) to facilitate the attachment of bacteria. Coupons were transferred 50-ml conical centrifuge tube containing 5 ml of distilled water, and incubated at 25 °C for 6 days.

2.4. Sanitizer preparation

Sodium hypochlorite solution (100 ppm) was prepared by adding bleach containing≥4% active chlorine (Yuhan Clorox, Seoul, Korea) to distilled water. Peracetic acid (Daesung C&S, Seoul, Korea) was diluted according to the manufacturer's instruction with distilled water to 100, 200, and 400 ppm. The free chlorine concentration was measured with a HI 95771 Chlorine Ultra High Range Meter (Hanna Instruments, Ann Arbor, MI, USA).

2.5. Antibacterial aerosol treatment

Coupons were transferred to a model glass cabinet (80×50×50 cm) for aerosolized sanitizer treatment. Coupons immersed in TSB were washed in 500 ml of distilled water with gentle agitation for 10 s (22± 2 °C) before treatment. Aerosolized sanitizers generated by nebulizer (DRWL-2000, Doore Industrial Co., Gyeonggi, Korea) were put into a cabinet. Size of aerosolized particle was approximately 5.42 to 11.42 μm. Coupons were treated with aerosolized SHC (100 ppm) and PAA (100, 200, and 400 ppm) for 5, 10, 30, and 50 min. All experiments were performed at room temperature (22±2 °C).

2.6. Bacterial enumeration

After treatment, stainless steel and PVC coupons were transferred to sterile 50-ml conical centrifuge tube containing 30 ml of PBS and 3 g of glass beads (425–600 μm; Sigma-Aldrich, St. Louis, MO, USA), and then vortexed for 1 min. Cell suspension was tenfold serially diluted in BPW, and 0.1 ml of undiluted cell suspension or diluents was spread-plated onto each selective medium. Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco), and Oxford agar base (OAB; Difco) with antimicrobic supplement (Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. When low bacterial numbers were anticipated, 250 μl of undiluted cell suspension was plated onto four plates of each respective medium. The plates were incubated at 37 °C for 24–48 h. After incubation, colonies were counted.

2.7. Statistical analysis

All experiments were repeated three times. Data were analyzed by ANOVA using Statistical Analysis System (SAS Institute, Cary, NC, USA) and separation of means by Duncan's multiple range test at a probability level of $p<0.05$.

3. Results

In the procedure A with stainless steel, initial attached cell (2–3 log) increased by 4–5 log after 6 days incubation (data not shown). Table 1 shows survival of biofilm cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* after aerosolized sanitizer treatment. *E. coli* O157:H7 formed biofilm on stainless steel with a cell density of 6.44 log CFU/coupon. Biofilm cells of *E. coli* O157:H7 were significantly ($p<0.05$) reduced by 2.56 log CFU/coupon after 50 min of exposure to 100 ppm SHC compared to distilled water treatment (control treatment). Levels of biofilm cells were reduced to below the detection limit (1.48 log CFU/coupon) after exposure to 100 ppm PAA for 50 min, and 200 and 400 ppm PAA for 10 min. The initial biofilm cells of *S.* Typhimurium were 7.09 log CFU/coupon. Treatment with 100 ppm SHC for 50 min significantly ($p<0.05$) reduced biofilm cells by 0.50 log CFU/coupon compared to the control treatment. Treatment of biofilm cells with 100 ppm PAA brought significant reduction (4.37 log CFU/coupon) in counts compared to the control treatment after 50 min of exposure. Biofilm cells of *S.* Typhimurium could not be detected after exposure to 200 and 400 ppm PAA for 10 min. The cell density of *L. monocytogenes* biofilm was 6.18 log CFU/coupon. The biofilm cells of *L. monocytogenes* were significantly ($p<0.05$) reduced by 2.46 and more than 4.58 log CFU/coupon, respectively, after exposure to 100 ppm SHC and PAA for 50 min compared to the control treatment. Biofilm cells were reduced to below the detection limit (1.48 log CFU/coupon) after 30 min of exposure to 200 and 400 ppm PAA.

Table 1
Survival of biofilm cells of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* formed by procedure A on stainless steel coupon after aerosolized sanitizer treatment[a].

Microorganism	Treatment [b]	Population (log CFU/coupon)			
		5 min	10 min	30 min	50 min
E. coli O157:H7	DW	5.64 ± 0.49A[c]	5.48 ± 0.70A	5.42 ± 0.16A	5.27 ± 0.49A
	SHC (100 ppm)	5.22 ± 0.91A	4.76 ± 0.79A	4.00 ± 0.38C	2.71 ± 1.13B
	PAA (100 ppm)	5.40 ± 0.55A	5.25 ± 0.15A	4.96 ± 0.08B	<1.48C
	PAA (200 ppm)	2.66 ± 1.18B	<1.48B	<1.48D	<1.48C
	PAA (400 ppm)	2.48 ± 1.48B	<1.48B	<1.48D	<1.48C
S. Typhimurium	DW	6.64 ± 0.26A	6.59 ± 0.49A	6.43 ± 0.25A	5.88 ± 0.08A
	SHC (100 ppm)	5.52 ± 0.82A	5.50 ± 0.15C	5.30 ± 0.26B	5.38 ± 0.22B
	PAA (100 ppm)	6.29 ± 0.21A	6.16 ± 0.07B	5.02 ± 0.28B	1.51 ± 0.05C
	PAA (200 ppm)	2.81 ± 1.28B	<1.48D	<1.48C	<1.48D
	PAA (400 ppm)	2.62 ± 1.01B	<1.48D	<1.48C	<1.48D
L. monocytogenes	DW	6.10 ± 0.22A	6.01 ± 0.31A	6.07 ± 0.31A	6.06 ± 0.02A
	SHC (100 ppm)	4.98 ± 0.90A	4.58 ± 0.73B	3.75 ± 0.07B	3.60 ± 0.98B
	PAA (100 ppm)	5.60 ± 0.20A	5.35 ± 0.46AB	3.79 ± 0.07B	<1.48C
	PAA (200 ppm)	3.27 ± 0.79B	1.98 ± 0.50C	<1.48C	<1.48C
	PAA (400 ppm)	2.18 ± 0.70C	1.81 ± 0.58C	<1.48C	<1.48C

[a] Mean ± standard deviation; n = 3 for all treatments.
[b] DW, Distilled water; SHC, Sodium hypochlorite; PAA, Peracetic acid.
[c] Values in the same row that are not followed by the same capital letters are significantly different ($p<0.05$).

In the procedure B with stainless steel, bacterial populations increased by approximately 1–1.5 log CFU/coupon from initial inoculation levels of foodborne pathogens (data not shown). Biofilm cells formed by the procedure B were showed a similar reduction patterns to those formed by the procedure A (Table 2). Exposure to 200 and 400 ppm PAA was more effective than 100 ppm SHC in reducing biofilm cells with increasing the treatment time.

The results for inactivation of biofilm formed on PVC by aerosolized sanitizers were represented in Tables 3–4. Reduction patterns of biofilm cells on PVC were similar to those of biofilm cells on stainless steel. With increasing treatment time, PAA treatment was more effective than treatment with same concentration of SHC. Also similar to biofilms on stainless steel, biofilms formed on PVC by the procedure B were more resistant to aerosolized sanitizers than these formed by the procedure A.

4. Discussion

Biofilms are more resistant to antimicrobial agents compared to planktonic cells, and this makes their elimination from food processing environment a big challenge (Simões et al., 2009). Resistance to antimicrobial agents may be in part by virtue of complex structure or the polysaccharides (Meyer, 2003; Sharma and Anand, 2002). Thus mature biofilms rather than planctonic cells or early-stage biofilms must be considered when disinfection protocols have to be optimized (Ibusquiza et al., 2011). Several disinfection methods have been evaluated to remove biofilms on food processing surfaces. Ayebah et al. (2005) used electrolyzed water (EO) to inactivate *L. monocytogenes* biofilms on stainless steel surfaces. Treatment with acidic EO water for 30 to 120 s reduced the bacteria population by 4.3 to 5.2 log CFU/coupon. Ultrasound treatment (20 kHz, 120 W) at a distance of 2.54 cm from biofilm chip reduced levels of *L. monocytogenes* biofilms by 3.8 log CFU/ml after 60 s (Baumann et al., 2009). Recently, Chorianopoulos et al. (2011) reported nanostructured TiO_2 thin films on stainless steel and glass is alternative means of disinfecting contaminated surface. The biofilm of *L. monocytogenes* on glass decreased by 3 log CFU/cm^2 when TiO_2 was activated by ultraviolet A light for 90 min. However, effective methods to control biofilms on inaccessible area are needed in the food industry.

In the present study, aerosolized sanitizers showed significant antimicrobial effect against biofilm cells of foodborne pathogens on stainless steel and PVC. Oh et al. (2005a) reported aerosolized peroxyacetic acid exhibited a 3–4 log reduction in population of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on lettuce leaves.

Table 2
Survival of biofilm cells of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* formed by procedure B on stainless steel coupon after aerosolized sanitizer treatment[a].

Microorganism	Treatment[b]	Population (log CFU/coupon)			
		5 min	10 min	30 min	50 min
E. coli O157:H7	DW	7.70 ± 0.52A[c]	7.71 ± 0.42A	7.75 ± 0.35A	7.72 ± 0.40A
	SHC (100 ppm)	7.54 ± 0.37A	7.58 ± 0.39A	7.40 ± 0.73A	5.76 ± 0.49B
	PAA (100 ppm)	7.62 ± 1.16A	7.27 ± 0.03A	5.58 ± 0.43B	4.20 ± 0.12C
	PAA (200 ppm)	6.75 ± 0.29A	5.73 ± 0.24B	<1.48C	<1.48D
	PAA (400 ppm)	6.59 ± 0.25A	5.33 ± 0.03B	<1.48 C	<1.48D
S. Typhimurium	DW	8.06 ± 0.14A	8.05 ± 0.10A	7.86 ± 0.44A	7.80 ± 0.11A
	SHC (100 ppm)	7.83 ± 0.03A	7.52 ± 0.12B	7.37 ± 0.56A	6.07 ± 0.71B
	PAA (100 ppm)	7.23 ± 0.85AB	7.14 ± 0.08C	5.09 ± 0.05B	2.02 ± 0.78C
	PAA (200 ppm)	6.85 ± 0.38B	5.98 ± 0.04D	<1.48C	<1.48D
	PAA (400 ppm)	6.71 ± 0.35B	5.90 ± 0.10D	<1.48C	<1.48D
L. monocytogenes	DW	7.04 ± 0.07A	7.11 ± 0.73A	7.02 ± 0.94A	6.68 ± 0.80A
	SHC (100 ppm)	6.91 ± 0.39AB	6.37 ± 0.67A	5.79 ± 0.52AB	4.30 ± 0.71B
	PAA (100 ppm)	5.91 ± 1.09B	5.30 ± 0.61B	5.05 ± 1.16B	3.64 ± 0.10B
	PAA (200 ppm)	6.00 ± 0.42AB	5.17 ± 0.17B	<1.48C	<1.48C
	PAA (400 ppm)	3.84 ± 0.07C	1.98 ± 0.50C	<1.48C	<1.48C

[a] Mean ± standard deviation; n = 3 for all treatments.
[b] DW, Distilled water; SHC, Sodium hypochlorite; PAA, Peracetic acid.
[c] Values in the same row that are not followed by the same capital letters are significantly different ($p<0.05$).

Table 3
Survival of biofilm cells of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* formed by procedure A on PVC coupon after aerosolized sanitizer treatment[a].

Microorganism	Treatment [b]	Population (log CFU/coupon)			
		5 min	10 min	30 min	50 min
E. coli O157:H7	DW	6.18 ± 0.96A[c]	5.96 ± 0.41A	5.87 ± 0.49A	5.88 ± 0.38A
	SHC (100 ppm)	5.19 ± 1.05AB	4.69 ± 0.14C	4.06 ± 0.74B	3.54 ± 0.22B
	PAA (100 ppm)	5.66 ± 0.52AB	5.45 ± 0.19B	3.43 ± 0.22B	1.57 ± 0.17C
	PAA (200 ppm)	4.29 ± 1.27B	<1.48D	<1.48C	<1.48D
	PAA (400 ppm)	1.49 ± 0.10C	<1.48D	<1.48C	<1.48D
S. Typhimurium	DW	6.74 ± 0.36A	6.79 ± 0.45A	6.75 ± 0.58A	6.39 ± 0.59A
	SHC (100 ppm)	6.20 ± 0.10B	4.79 ± 0.60C	4.06 ± 0.24B	2.76 ± 0.91B
	PAA (100 ppm)	5.73 ± 0.65BC	5.73 ± 0.15B	4.53 ± 0.73B	<1.48C
	PAA (200 ppm)	5.26 ± 0.24C	<1.48D	<1.48C	<1.48C
	PAA (400 ppm)	1.77 ± 0.29D	<1.48D	<1.48C	<1.48C
L. monocytogenes	DW	6.44 ± 0.04A	6.40 ± 0.01A	5.75 ± 0.45A	5.29 ± 0.03A
	SHC (100 ppm)	4.68 ± 1.09BC	4.58 ± 0.29C	3.28 ± 0.44C	2.24 ± 0.68B
	PAA (100 ppm)	5.82 ± 0.44AB	5.34 ± 0.15B	4.58 ± 0.24B	<1.48C
	PAA (200 ppm)	3.76 ± 0.92CD	<1.48D	<1.48D	<1.48C
	PAA (400 ppm)	2.72 ± 1.30D	<1.48D	<1.48D	<1.48C

[a] Mean ± standard deviation; n = 3 for all treatments.
[b] DW, Distilled water; SHC, Sodium hypochlorite; PAA, Peracetic acid.
[c] Values in the same row that are not followed by the same capital letters are significantly different ($p<0.05$).

Gibson et al. (1999) reported high pressure (17.2, 34.5, 51.7, and 68.9 bar) spraying method with a sanitizer is effective at reducing biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, aerosol generated by high pressure spraying method should be applied in a short distance to bacterial biofilms (125–250 mm). On the other hand, aerosolization method can deliver sanitizers to inaccessible areas and have higher penetrating activity. Oh et al. (2005b) found aerosolized peroxyacetic acid and hydrogen peroxide diffused effectively in huge semi-trailer. Levels of *Bacillus cereus*, *Listeria innocua*, *S. aureus*, and *S.* Typhimurium were reduced by an average of 3.09, 7.69, 6.93, and 8.18 log units per plate, respectively. Also they reported aerosolized sanitizers represented antimicrobial effect regardless of height and orientation. These properties may facilitate the use of aerosolization method as a sanitation procedure of food processing plants. It may effectively reduce biofilm cells of foodborne pathogens in inaccessible food processing surfaces.

Results from this study show that aerosolized 100 ppm SHC and PAA showed a similar degree of antimicrobial effect against biofilm cells formed on stainless steel and PVC. However, with increasing treatment time, 100 ppm PAA was more effective than 100 ppm SHC. Aerosolized PAA (200 and 400 ppm) were more effective to remove biofilms of three foodborne pathogens. Oxidizing agents including chlorine and PAA are frequently used to kill or remove biofilms (Meyer, 2003). Chlorine is known to remove exopolysaccharide as well as to kill microorganisms (Ronner and Wong, 1993). PAA widely used as peracid sanitizer, is a more potent biocide than hydrogen peroxide (Chmielewski and Frank, 2003). Several researches have been conducted to evaluate antimicrobial effect of chlorine and PAA against biofilm cells. Generally, PAA is considered to be more effective than chlorine. It maintains activity in the presence of organic loads (McDonnell and Russell, 1999). Norwood and Gilmour (2000) found that 200 ppm free chlorine treatment did not significantly reduce biofilm cells of *L. monocytogenes*. PAA sanitizer was more effective than same concentration of chlorine sanitizer for inactivation of *L. monocytogenes* and *Pseudomonas* biofilm cells grown in milk (Fatemi and Frank, 1999). Harkonen et al. (1999) reported that peroxide based sanitizers were more effective than hypochlorite for inactivation of bacterial biofilms.

Method of biofilm formation similar to the procedure A in this study has been used in other studies (Fatemi and Frank, 1999; Kim et al., 2008). In the present study, different method (procedure B) was used to form biofilms. In the procedure B, biofilms were formed by drying the bacterial suspension on stainless steel and PVC coupons. Biofilms formed by the procedure B were more resistant to

Table 4
Survival of biofilm cells of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* formed by procedure B on PVC coupon after aerosolized sanitizer treatment[a].

Microorganism	Treatment [b]	Population (log CFU/coupon)			
		5 min	10 min	30 min	50 min
E. coli O157:H7	DW	7.46 ± 0.26A[c]	7.49 ± 0.27A	7.39 ± 0.25A	7.01 ± 0.71A
	SHC (100 ppm)	7.27 ± 0.58AB	7.17 ± 0.07AB	6.96 ± 0.08B	5.62 ± 1.16B
	PAA (100 ppm)	7.37 ± 0.23A	6.85 ± 0.12B	5.32 ± 0.19C	4.18 ± 0.15C
	PAA (200 ppm)	6.76 ± 0.09BC	6.23 ± 0.15 C	<1.48D	<1.48D
	PAA (400 ppm)	6.40 ± 0.09C	5.18 ± 0.45D	<1.48D	<1.48D
S. Typhimurium	DW	8.03 ± 0.23A	7.89 ± 0.11A	7.83 ± 0.07A	7.83 ± 0.36A
	SHC (100 ppm)	8.01 ± 0.29A	7.61 ± 0.37A	7.14 ± 0.11B	6.40 ± 1.06B
	PAA (100 ppm)	7.56 ± 0.30AB	7.42 ± 0.73AB	3.91 ± 0.13C	3.50 ± 0.15C
	PAA (200 ppm)	6.99 ± 0.58B	6.56 ± 0.61B	<1.48D	<1.48D
	PAA (400 ppm)	7.08 ± 0.08B	5.43 ± 0.64C	<1.48D	<1.48D
L. monocytogenes	DW	7.29 ± 0.70A	7.08 ± 0.64A	6.58 ± 0.20A	6.68 ± 0.95A
	SHC (100 ppm)	7.32 ± 0.51A	7.06 ± 0.75A	5.19 ± 0.24B	4.16 ± 0.32B
	PAA (100 ppm)	6.93 ± 0.20AB	6.44 ± 0.05AB	4.37 ± 0.11C	1.85 ± 0.68C
	PAA (200 ppm)	6.28 ± 0.70B	5.59 ± 0.94B	<1.48D	<1.48D
	PAA (400 ppm)	4.75 ± 0.05C	4.19 ± 0.06C	<1.48D	<1.48D

[a] Mean ± standard deviation; n = 3 for all treatments.
[b] DW, Distilled water; SHC, Sodium hypochlorite; PAA, Peracetic acid.
[c] Values in the same row that are not followed by the same capital letters are significantly different ($p<0.05$).

aerosolized sanitizers than these formed by the procedure A. Further study is needed to verify the high resistance of biofilm formed by the procedure B.

In conclusion, control and removal of biofilms are important issue for food processing industry. This study showed that aerosolized sanitizers were able to inactivate foodborne pathogens within biofilms effectively. It may provide cleaning procedure for food industry to control biofilms in food processing facilities.

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