Effectiveness of slightly acidic electrolyzed water on bacteria reduction: in vitro and spray evaluation

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ABSTRACT

Bacterial inactivation is a crucial aspect of sanitation and hygiene. The effectiveness of slightly acidic electrolyzed water (SAEW) for reduction or removal of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* was evaluated. The bactericidal activity of SAEW and sodium hypochlorite (NaOCl) against *E. coli* and *P. aeruginosa* were compared through in vitro experiments. The effectiveness of SAEW spray was tested against *S. epidermidis*. Results showed that SAEW had a more powerful bactericidal activity than NaOCl at the same available chlorine concentrations. For *E. coli*, SAEW decreased the bacterial counts from 8.4 $\log_{10}$ CFU/mL to less than 3.9 $\log_{10}$ CFU/mL; NaOCl with the same available chlorine of 0.5 mg/L, caused a decrease from 8.4 $\log_{10}$ CFU/mL to 7.1 $\log_{10}$ CFU/mL. For *P. aeruginosa*, SAEW caused bacterial counts to decrease from 8.5 $\log_{10}$ CFU/mL to less than 4.1 $\log_{10}$ CFU/mL against 8.5 $\log_{10}$ CFU/mL to 6.2 $\log_{10}$ CFU/mL for NaOCl with the same available chlorine of 0.5 mg/L. Spray experiments showed that 10 mg/L of SAEW spray decreased the bacterial counts of *S. epidermidis* from 3.7 $\log_{10}$ CFU/m^3 to 2.8 $\log_{10}$ CFU/m^3, with 20 mg/L causing a reduction from 3.8 $\log_{10}$ CFU/m^3 to 0 CFU/m^3. The overall findings of this study indicate that SAEW may be a promising disinfectant agent either as a solution or spray.

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INTRODUCTION

Sanitization and hygiene play an important role in food preservation and consumption because some microorganisms such as bacteria can be harmful to the health of people, animals or plants. Bacteria are highly adaptable and have low nutrient requirements, making them available nearly everywhere with enough water. They are therefore well known as a source of contamination in tap water and raw or slightly cooked food. This contamination can cause dangerous infections in people with immunodeficiency or open wounds. Numerous sanitizers, such as sodium hypochlorite, chlorine dioxide, hydrogen peroxide, organic acids and ozone, have been extensively used for their effectiveness in reducing or removing pathogenic bacteria in agriculture and the food industry, markets, hospitals, care homes, layer housing environments, households, etc. (*Koide et al., 2011*).

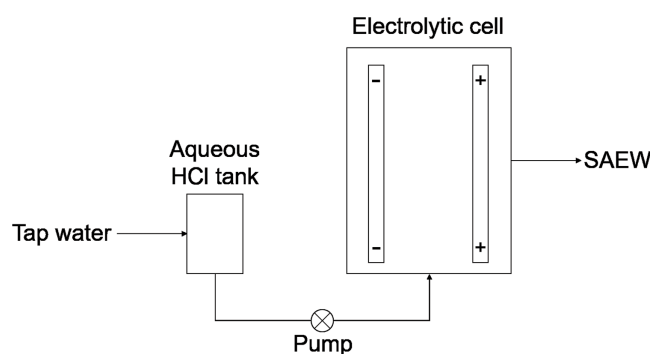
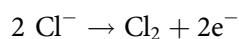



Figure 1 Schematic representation of SAEW production.

However, the majority of chemical disinfectants in use are difficult to handle, potentially toxic, corrosive, volatile and often not very effective (*Forghani & Oh, 2013*; *Koseki et al., 2004*).

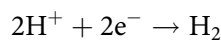
Thus, the use of slightly acidic electrolyzed water (SAEW) has been introduced as an alternative and novel method to decontaminate surface and airborne pathogenic microorganisms. In Japan, SAEW has been authorized as: a food additive by the Japanese Ministry of Health, Labor and Welfare (June 2002); a specific pesticide by the Japanese Ministry of the Environment and the Ministry of Agriculture, Forestry and Fisheries (March 2014); and a cultivation material of organic JAS agricultural products by the Japanese Ministry of Agriculture, Forestry and Fisheries (March 2014). In Japan, the current maximum allowed concentration of free chlorine in SAEW is 80 mg/L. SAEW has been applied in Japan in food line production, hospitals, nursing homes, kindergartens, restaurants and other places where high levels of personal hygiene management are required. It is used as a substitute for sanitizers and deodorizers containing hazardous chemical materials and, thus, prevent accidental ingestion and handling of hazardous chemicals. With climate change and the subsequent risk of increasing airborne infectious diseases, there is a growing opportunity for the SAEW market.

SAEW is produced by electrolyzing an aqueous solution of NaCl or HCl using a non-membrane electrolytic cell. In this research, we use a system which electrolyzes dilute hydrochloric acid (HCl) solution (3%) in a non-diaphragm electrolytic cell. This is because a HCl based system does not release NaCl, which is responsible for negative effects on infrastructure, such as metal corrosion. A schematic representation of the system is presented in Fig. 1. The aqueous HCl solution is supplied to the electrolytic cell where the following electrolysis reactions take place

$$2 \text{ Cl}^- \rightarrow \text{Cl}_2 + 2\text{e}^-$$

On the anode, the chlorine ion is electrolyzed to chlorine and it undergoes the following reaction with water. As a result, hypochlorous acid is generated, which is the bactericidal chemical

$$H_2O + Cl_2 \rightarrow HOCl + H^+ + Cl^-$$

On the cathode, hydrogen gas is generated.

$$2H^+ + 2e^- \rightarrow H_2$$

Previous studies have shown that SAEW is suitable for the prevention and control of microorganisms due to its bactericidal activity (*Cao et al., 2009*; *Hao et al., 2011*, *2014*; *Issa-Zacharia et al., 2011*, *2010a*; *Kim et al., 2019*; *Zhang et al., 2011*; *Zheng et al., 2014*). SAEW poses a nearly neutral pH value, between pH 5.0 and 6.5, with available chlorine concentration usually around 30 mg/L. At this pH range, the effective form of chlorine compound is mainly hypochlorous acid (HOCl, 95%), a weak acid (pK_a = 7.53, 25 °C), which gives the antimicrobial activity (*Yoshifumi, 2003*). The pH of the solution has important effects on the form of chlorine compounds (ClO$^-$, Cl$_2$ or HOCl). At pH values above 7.5, the hypochlorous acid exists as ClO$^-$ ions concentration, resulting in much lower antimicrobial activity compared to the molecule form (HOCl) (*Dukan, Belkin & Touati, 1999*; *El-Kest & Marth, 1988a*, *1988b*; *Huang et al., 2008*). The advantages of SAEW are its qualities of being non-corrosive, environmentally friendly, stable in storage, inexpensive, ease of water reversion after use, having fewer potential health hazards due to its pH range and that it is highly effective against bacteria. Some disadvantages of the SAEW lay on the fact that users need to purchase and install an equipment to produce SAEW, with the price dependent on the required production capacity. However, there are other available options such as purchasing SAEW packs or portable machines.

There is still a lack of information and understanding on the efficiency of SAEW to inactivate microorganisms. Specifically, there is a lack of research comparing SAEW to other disinfectants such as NaOCl, the most widely used disinfectant in the food industry, at the same free chlorine concentration. Therefore, there is a need to compare the bactericidal activity of SAEW with chlorine-based disinfectants at the same free chlorine concentration. Studies conducted previously compared the antibacterial effectiveness of SAEW at 20–30 mg/L available chlorine concentration with NaOCl at 100–150 mg/L available chlorine concentration. This is because NaOCl is commonly used as a sanitizer in food processing at 50–200 mg/L of available chlorine concentration (*Hao et al., 2011*; *Issa-Zacharia et al., 2011*, *2010a*, *2010b*). Also, there is little, if any, research on the effective dose of SAEW in terms of concentration and duration when sprayed into the air to reduce or eliminate airborne bacteria. Thus, the objectives of this study were: (1) to quantify the bactericidal efficiency of SAEW for inactivation of *Escherichia coli* and *Pseudomonas aeruginosa*, pathogens of major public health concern because they pose high health risk and can also cause death (2) to compare the efficiency of SAEW and sodium hypochlorite (NaOCl) in reducing *E. coli* and *P. aeruginosa* at the same free chlorine concentration; (3) to determine the dose and efficiency of SAEW spray to reduce airborne bacteria by testing it against bioaerosol of *Staphylococcus epidermidis*, which is, usually, a nonpathogenic bacteria, but represents an infection risk for patients with weakened immune systems.

MATERIALS AND METHODS

SAEW preparation

SAEW was generated by electrolysis of a mixture of aqueous dilute solution of HCl (3%) and tap water using an Apia 60 generator (Hokuty Co. Ltd., Kanagawa, Japan). The physicochemical properties of SAEW were measured immediately after production. The pH was measured using the Piccolo pH meter (Hanna Instruments Inc., Woonsocket, RI, USA). The free chlorine concentration was determined using the HI96701 Free Chlorine Photometer. The measurement range for the photometer is 0.00 to 5.00 mg/L and the resolution is 0.01 mg/L from 0.00 to 3.50 mg/L and 0.10 mg/L above 3.50 mg/L. The SAEW was used within 1 h after preparation. SAEW solutions with 0.5 mg/L (in vitro experiments) and 10 mg/L and 20 mg/L (spray experiments) were prepared by diluting SAEW in autoclaved distilled water. The free chlorine (HOCl/OCl$^-$) concentration was verified three times before every experiment.

NaOCl preparation

An analytical grade NaClO solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 2.9 g/L of available chlorine was diluted in autoclaved distilled water to obtain 0.5 mg/L of available chlorine for the experiments. The available chlorine was determined by the HI96701 Free Chlorine Photometer.

Culture preparation

Liquid-dried cultures of *E. coli* (NBRC 3972), *P. aeruginosa* (NBRC 13275) and *S. epidermidis* (NBRC 100911) were obtained from the Biological Resource Center (NBRC) of the National Institute of Technology and Evaluation (NITE), Japan. Microorganisms were revived according to L-dried culture reactivation procedures provided by the manufacturer: the L-dried culture ampoule was carefully and aseptically snap opened, a few drops of 0.9% NaCl solution was added using a sterile pasteur pipette, and then gently agitated for 2 min approximately. The suspension was placed in agar plates and left undisturbed at 35 °C for 24 h (bacterial stock). The culture medium placed in petri dishes consists of a mixture of 1 g of magnesium sulfate heptahydrate (MgSO$_4$ · 7H$_2$O, Nacalai Tesque, Inc., Kyoto, Japan), 10 g of hypolypepton (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan), 2 g of yeast extract B2 (Oriental Yeast, Co., Ltd., Tokyo, Japan) and 15 g of agar powder (Wako Pure Chemical Industries Ltd., Osaka, Japan), in one L of distilled water. Bacterial culture of *E. coli, P. aeruginosa* and *S. epidermidis* were prepared to conduct experiments by transferring several colonies from the bacterial stock to five mL of 0.9% NaCl solution using a sterile inoculation loop and shaken using a vortex mixer (Vortex-Genie 2; Scientific Industries Inc., Bohemia, NY, USA).

One milliliter of each bacteria suspension (approximately 8.0 log$_{10}$ CFU/mL) was added to nine mL of sterilized water, SAEW 0.5 mg/L, or NaOCl 0.5 mg/L. The mixture was shaken using a vortex mixer and left undisturbed for 1 min. Following treatment with SAEW, inactivation experiments were conducted by transferring one mL of each treated sample to a sterile tube containing nine mL of neutralizing buffer solution (0.5% sodium thiosulfate solution, Na$_2$S$_2$O$_3$). The tube was shaken using the vortex mixer. After 5 min of

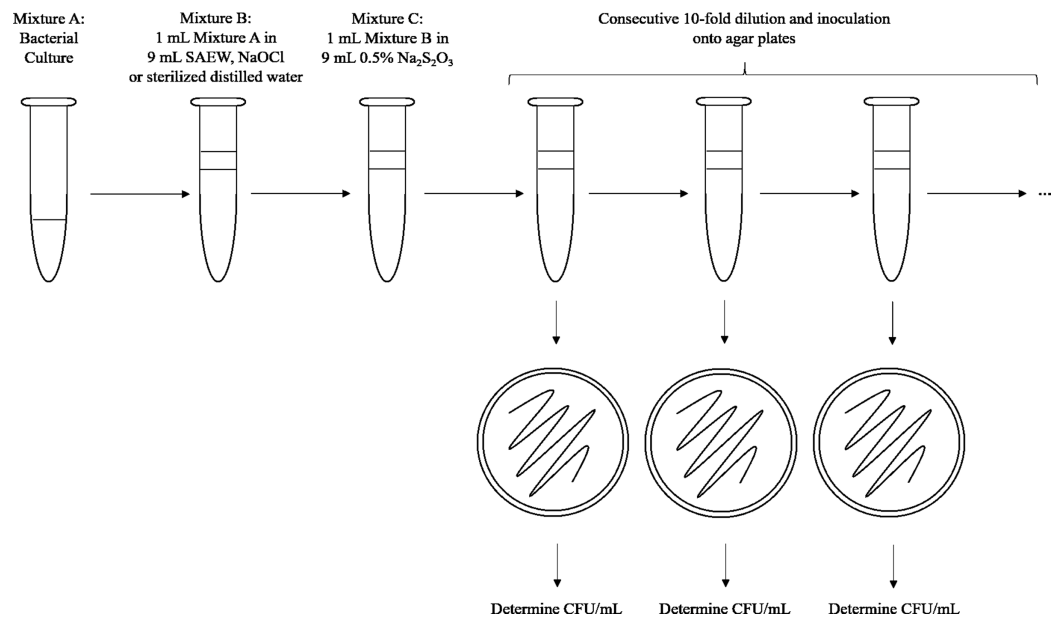



Figure 2 Experimental sequence for in vitro tests.

neutralization, the viable count of bacteria cells in each sample was determined by plating 0.1 mL of bacterial mixture either directly or after serial dilution (1:10) in sterile 0.9% NaCl solution. The plates were incubated at 35 °C for 24 h. After this period, the bacterial colony was counted and the bactericidal activity of SAEW and NaOCl evaluated. Microbial counts were expressed as $\log_{10}$ CFU/mL sample, with the reduction in bacterial population also calculated and presented as $\log_{10}$ CFU/mL to compare the inactivation effects of both SAEW and NaOCl. The effect of the neutralizing solution (0.5% sodium thiosulfate solution) alone was also tested on bacterial cultures to ensure that the observed bacterial reductions were solely attributed to treatment solutions and not due to sodium thiosulfate solution. Sterilized distilled water was used as control for this experiment. Each experimental case was replicated 6 times to assure repeatability. A schematic representation of the in vitro experiment is presented in Fig. 2.

Spray experiment

Spraying SAEW is potentially an effective way to decontaminate pathogenic microorganisms in the air. Experiments were conducted in a clean booth (transparent plastic clean room) 0.9 m × 0.9 m × 0.9 m in dimension, average volume of 0.729 m^3, equipped with a HEPA filter on the top to allow only clean air enter to the room as shown in Fig. 3. An air sampler (MBS-1000 manufactured by Midori Anzen Co. Ltd., Shibuya-Ku, Japan) and a 90 mm diameter plastic plate agar culture medium were placed inside the cabin and set to collect 20 L of air after 20 min. A small fan (Eluteng, 120 mm) was placed and set at 1,500 rpm to assure uniform distribution of bacteria and SAEW during experiments. An electric air pump (SABLE, 200 L/min) was also installed inside the clean booth. *S. epidermidis* suspension (200 mL, 10^5 CFU/mL) was prepared in a 0.9% NaCl solution and placed in a sprayer (ultrasonic humidifier, KX 80UP-VT, 90 mL/h). SAEW

(A) Front view   (B) Top view

HEPA filter

Height: 0.9 m

Width: 0.9 m

Length: 0.9 m

SAEW sprayer

HEPA filter

Bacteria sprayer

Fan

Air pump

Air Sampler

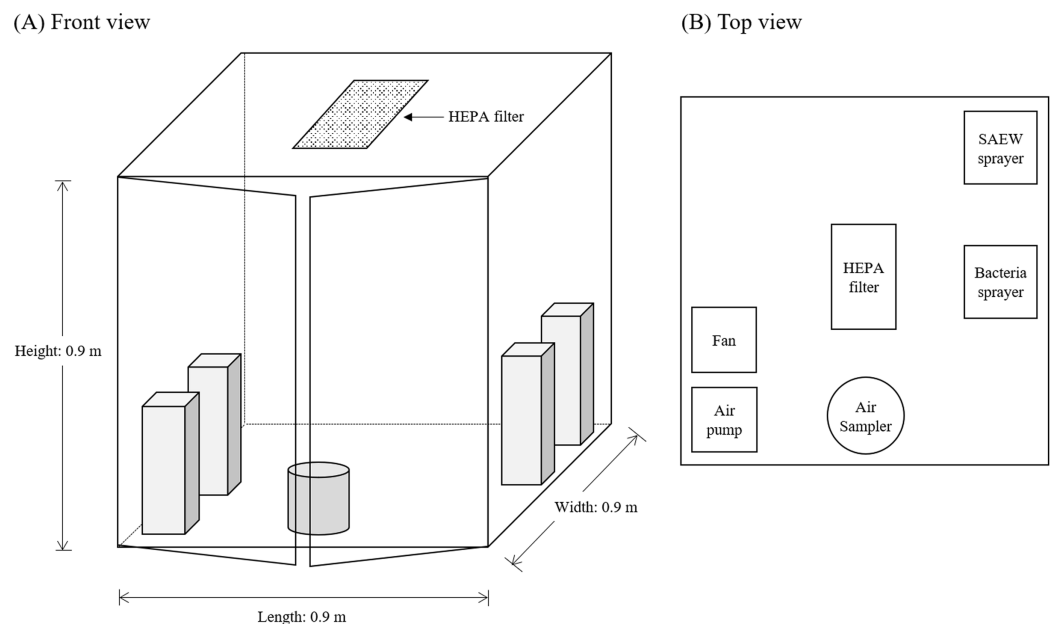



Figure 3 Design of spray experiments: (A) front view, (B) top view.

solution of 10 and 20 mg/L was prepared and poured in a sprayer (ultrasonic humidifier, Ottostyle Uruoi+, 300 mL/h).

After setting the system inside the clean booth and making sure that each unit could be turned on and off from outside the booth, except the air pump that was set to collect air samples after 20 min, the door was closed. The electric air pump was turned on to pump out the air inside the booth and allow clean air to enter the clean room for about 7 min. Once the air inside was clean, bacteria suspension was sprayed for 3 min at a rate of 90 mL/h (4.5 mL of bacterial suspension release). SAEW solution was sprayed following bacterial spraying in a rate of 300 mL/h for 10, 20, 30 and 60 s and was left undisturbed for 5 min before collecting air samples with the air sampler. Experiments were conducted at 25 °C and three replicate trials were done for each case.

RESULTS AND DISCUSSION

In vitro results

The pH of stock solutions and the available chlorine concentration of sterilized distilled water, SAEW dilutions and NaOCl dilutions used for all experiments are presented in Table 1. SAEW had a pH value ranging between 5.5 and 5.8 and a free chlorine concentration of around 29 mg/L whereas NaOCl had a pH around 8.7 and an available chlorine concentration of 2,900 mg/L. Concentrated solutions of SAEW and NaOCl were used to prepare diluted solutions for experiments.

Results of bacteria inactivation using 0.5 mg/L of SAEW and 0.5 mg/L of NaOCl are presented in Table 2 for *E. coli* and *P. aeruginosa*. Results obtained in this research showed that bacterial cultures were completely inactivated when using 0.5 mg/L of SAEW and
Table 1 Physicochemical properties (pH and free chlorine concentration) of tested solutions.

	Unit	Sterilized distilled water	SAEW (stock)	NaOCl (stock)
pH of stock solution	–	6.9 ± 0.5	5.6 ± 0.1	8.7 ± 0.04
Free chlorine concentration of concentrated solutions	mg/L	NA	28.9 ± 1.1	2,900
Free chlorine concentration of diluted solutions	mg/L	NA	In vitro 0.5 Spray 10.0 ± 0.5 20.2 ± 0.3	In vitro 0.5

Note:
NA: not applicable.

Table 2 Results of bacterial reduction or inactivation using SAEW and NaOCl. The experiments were repeated 5–6 times.

	Sterilized distilled water	SAEW 0.5 mg/L	NaOCl 0.5 mg/L
Escherichia coli	0.06 ± 0.03	>4.49 ± 0.003	1.28 ± 0.91
Pseudomonas aeruginosa	0.15 ± 0.11	>4.38 ± 0.005	2.31 ± 0.67

Note:
Unit: $\log_{10}$ CFU/mL.

partly reduced when applying 0.5 mg/L of NaOCl. SAEW with an available chlorine concentration of 0.5 mg/L with 1 min of treatment time showed a bacterial reduction capacity of more than 4.5 $\log_{10}$ CFU/mL for *E. coli* and a bacterial reduction capacity of more than 4.4 $\log_{10}$ CFU/mL for *P. aeruginosa*. On the other hand, NaOCl with an available chlorine concentration of 0.5 mg/L and also treated for 1 min showed a bacterial reduction capacity of 1.3 $\log_{10}$ CFU/mL for *E. coli* and a bacterial reduction capacity of 2.3 $\log_{10}$ CFU/mL for *P. aeruginosa*. The available chlorine in SAEW, even at low dosage, is the most important component in reducing bacteria count. Bacterial populations of 8.4 $\log_{10}$ CFU/mL for *E. coli*, and 8.5 $\log_{10}$ CFU/mL for *P. aeruginosa* were found in control experiments using sterilized distilled water. Results of in vitro experiments showed that SAEW is effective at low dosage which makes it suitable to use not only at industrial levels or factories, but also at home and public places such as restaurants where hygiene must be assured. SAEW can also be considered as an alternative solution to conventional sanitizers such as NaOCl which is usually applied in higher dosage (*Issa-Zacharia et al., 2010b*; *Koseki et al., 2004*).

The difference in bactericidal activity between SAEW and NaOCl lays on the presence of hypochlorous acid (HOCl), a very effective disinfectant, rather than the total available chlorine concentration. This is true even at concentrations of less than 0.1 mg/L (*Ludovici, Phillips & Jeter, 1975*). SAEW consists mainly of HOCl, whereas the ion OCl$^-$ is the major chlorine specie present at the pH in commercial NaOCl dilutions (50–200 mg/L of available chlorine concentrations). It is believed that HOCl's bactericidal activity is due to its ability to diffuse through the microbial cell, causing damage to the plasma membrane and DNA, and also inhibiting enzyme activity essential for growth (*Fukuzaki, 2006*). Conversely, the poor germicidal activity of the OCl$^-$ specie is because

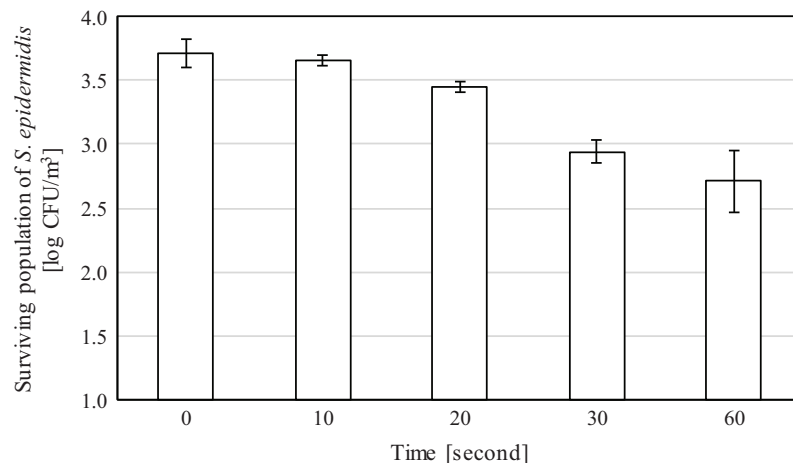



Figure 4 Bacteria population after spraying 10 mg/L of SAEW. The bacteria population decreases after spraying 10 mg/L of SAEW for 0–60 s.

penetration of this ion is prevented by the hydrophobic layer of microbe's plasma membrane (*Fukuzaki, 2006*).

Nan et al. (2010), *Kim et al. (2019)* and *Kiura et al. (2002)* analyzed the bacterial cellular morphology before and after treatment with solutions containing free chlorine by transmission electron microscopy. *Nan et al. (2010)* and *Kim et al. (2019)* found that *E. coli* cells were remarkably damaged, destroyed or deformed after exposure to SAEW with free chlorine concentrations between 10 and 60 mg/L. *Kiura et al. (2002)* observed damage of the outer membrane of *P. aeruginosa* and inactivation of cytoplasmic enzyme after exposure to free chlorine concentration solutions, which, according to this research team, are clear indicators of the bactericidal activity of free chlorine solutions. The bactericidal effect of SAEW lies in the synergy between high oxidation-reduction potential (ORP, equal to or greater than 900 mV) and HOCl. First, a series of ORP reactions take place which damage the outer and inner membrane and inactivate the defense mechanism of bacteria. Subsequently, HOCl can penetrate the bacterial cell and oxidize it, resulting in cell inactivation, inhibition of ATP generation or death (*Issa-Zacharia et al., 2011*).

Spray experiments

In the spray experiments, a great reduction in the population of the bacteria *S. epidermidis* was observed after spraying 10 and 20 mg/L of SAEW. This indicates that SAEW can be probably used as an alternative to purifying the air in food processing factories, agricultural facilities, hospitals, care homes, fruit and vegetable markets, restaurants, etc. even at lower concentrations and for short periods of time. Results presented in Figs. 4 and 5 showed a gradual decrease in bacterial population as the SAEW spraying time was increased from 10 to 60 s. The average initial bacterial concentration for experiments using 10 mg/L was 3.72 $\log_{10}$ CFU/m^3, whereas for experiments using 20 mg/L, it was 3.76 $\log_{10}$ CFU/m^3. Reduction in terms of $\log_{10}$ CFU/m^3 and percentages are presented in Table 3. From this table, a decrease of 0.06, 0.26, 0.77 and 1.00 $\log_{10}$ CFU/m^3 or 12%, 44%, 82% and 89% was observed in bacteria population after spraying 10 mg/L of SAEW for

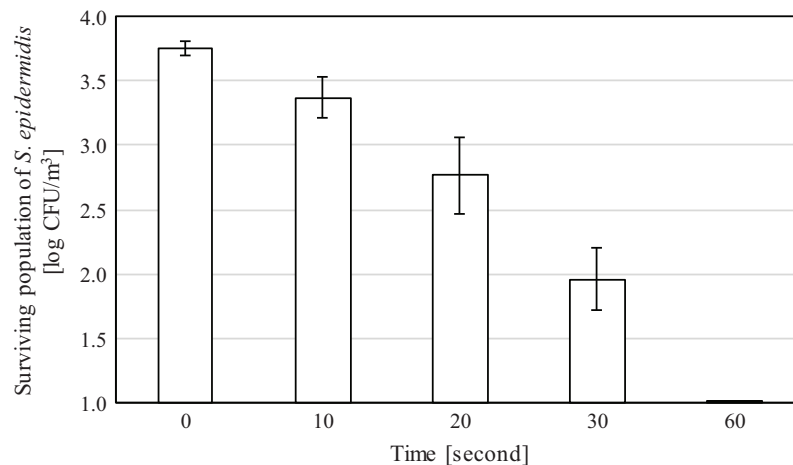



Figure 5 Bacteria population after spraying 20 mg/L of SAEW. The bacteria population decreases after spraying 20 mg/L of SAEW for 0–60 s.

Table 3 Results of spray experiments in both log scale and percentage. The experiments were repeated 3 times.

Time	SAEW 10 mg/L		SAEW 20 mg/L	
	Reduction $\log_{10}$ CFU/m³	Reduction percentage	Reduction $\log_{10}$ CFU/m³	Reduction percentage
10 s	0.06 ± 0.06	11.6 ± 13.4	0.39 ± 0.21	55.7 ± 19.9
20 s	0.26 ± 0.12	43.6 ± 16.3	0.99 ± 0.28	88.3 ± 7.8
30 s	0.77 ± 0.20	81.8 ± 9.0	1.80 ± 0.25	98.3 ± 0.8
60 s	1.00 ± 0.27	88.9 ± 5.5	3.76 ± 0.06	100 ± 0.0

10, 20, 30 and 60 s respectively at a 300 mL/h rate. Also, a decrease in 0.39, 0.99, 1.80 and 3.76 $\log_{10}$ CFU/m³ or 56%, 88%, 98% and 100% was observed in bacteria population after spraying 20 mg/L of SAEW for 10, 20, 30 and 60 s respectively at a 300 mL/h rate. This indicates that SAEW can be sprayed at lower concentrations and that continuous supply is probably not required. From the overall results, it appears that 20 mg/L of SAEW for 60 s is enough to almost completely eliminate the bacteria in the air.

A few studies reported on the application of SAEW spray for the reduction of airborne bacteria in animal housing. *Weichao et al. (2016)* reported on the optimization of different operating parameters (nozzle orifice diameter and spray pressure) when using SAEW spray with 30 mg/L of available chlorine concentration to improve the indoor air environment in animal housing. *Hao et al. (2014)* studied the distribution and subsequent reduction of airborne bacteria and fungi after 30 min of SAEW spraying in tunnel ventilated layer breeding houses. They found that both bacteria and fungi population sharply decreased after 30 min of SAEW with 250 mg/L of available chlorine concentration exposure. *Zheng et al. (2013)* reported on the use of SAEW spray with 150–250 mg/L of available chlorine concentration in laying-hen houses for 30 min and found that the efficiency of SAEW on the inactivation of pure cultures increased with increasing available

chlorine concentration. These research teams held experiments using SAEW with available chlorine concentrations from 30 to 250 mg/L and for long periods of time. Therefore, our contribution lay on the fact that SAEW spray with much lower concentration (10 and 20 mg/L of available chlorine concentration) and reduced exposure time (intermittent or not continuous application) is effective and can be applied to public places where clean air is necessary, while guaranteeing people's health and safety needs.

CONCLUSIONS

The results obtained in this study demonstrated that SAEW exhibits higher bactericidal activity compared to NaClO when tested at 0.5 mg/L and was effective in reducing and/or eliminating *E. coli* and *P. aeruginosa*.

Escherichia coli counts decreased more than 4.5 $\log_{10}$ CFU/mL when SAEW was used and 1.3 $\log_{10}$ CFU/mL when using NaOCl, both with the same available chlorine of 0.5 mg/L. *P. aeruginosa* counts decreased more than 4.4 $\log_{10}$ CFU/mL when SAEW was used and 2.3 $\log_{10}$ CFU/mL when using NaOCl, both with the same available chlorine of 0.5 mg/L. This indicates that at the same chlorine concentration, SAEW is more effective in reducing or eliminating bacterial count.

Spray experiments showed that bacterial counts gradually decreased as SAEW spray was applied for 10, 20, 30 and 60 s. After spraying 10 mg/L of SAEW for 60 s, *S. epidermidis* counts decreased from 3.7 $\log_{10}$ CFU/m^3 to 2.8 $\log_{10}$ CFU/m^3. After spraying 20 mg/L of SAEW for 60 s, bacterial count decreased from 3.8 $\log_{10}$ CFU/m^3 to 0 CFU/m^3. The latter result indicates that 1 min of 20 mg/L of SAEW is enough to eliminate 3.8 $\log_{10}$ CFU/m^3 of *S. epidermidis*.

Considering that SAEW is environmentally friendly and poses low potential for damage to human health, it could be used as an effective disinfectant, in both solution and spray form, in the food industry, industry in general, or at home.

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Competing Interests

Kenji Nakamura is employed by Toyotomi Co. Ltd., Research Department.

Author Contributions

- Angelica Naka conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Masaya Yakubo conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Kenji Nakamura conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Midori Kurahashi conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, proposed solutions to problems during experiments, and approved the final draft.

Data Availability

The raw measurements are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8593#supplemental-information.

REFERENCES

Cao W, Zhu ZW, Shi ZX, Wang CY, Li BM. 2009. Efficiency of slightly acidic electrolyzed water for inactivation of *Salmonella enteritidis* and its contaminated shell eggs. *International Journal of Food Microbiology* **130(2)**:88–93 DOI 10.1016/j.ijfoodmicro.2008.12.021.

Dukan S, Belkin S, Touati D. 1999. Reactive oxygen species are partially involved in the bacteriocidal action of hypochlorous acid. *Archives of Biochemistry and Biophysics* **367(2)**:311–316 DOI 10.1006/abbi.1999.1265.

El-Kest SE, Marth EH. 1988a. Inactivation of Listeria monocytogenes by chlorine. *Journal of Food Protection* **51(7)**:520–524 DOI 10.4315/0362-028X-51.7.520.

El-Kest SE, Marth EH. 1988b. Temperature, pH, and strain of pathogen as factors affecting inactivation of Listeria monocytogenes by chlorine. *Journal of Food Protection* **51(8)**:622–625 DOI 10.4315/0362-028X-51.8.622.

Forghani F, Oh D-H. 2013. Hurdle enhancement of slightly acidic electrolyzed water antimicrobial efficacy on Chinese cabbage, lettuce, sesame leaf and spinach using ultrasonication and water wash. *Food Microbiology* **36(1)**:40–45 DOI 10.1016/j.fm.2013.04.002.

Fukuzaki S. 2006. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. *Biocontrol Science* **11(4)**:147–157 DOI 10.4265/bio.11.147.

Hao J, Liu H, Liu R, Dalai W, Zhao R, Chen T, Li L. 2011. Efficacy of slightly acidic electrolyzed water (SAEW) for reducing microbial contamination on fresh-cut cilantro. *Journal of Food Safety* **31(1)**:28–34 DOI 10.1111/j.1745-4565.2010.00261.x.

Hao X, Cao W, Li B, Zhang Q, Wang C, Ge L. 2014. Slightly acidic electrolyzed water for reducing airborne microorganisms in a layer breeding house. *Journal of the Air & Waste Management Association* **64(4)**:494–500 DOI 10.1080/10962247.2013.870940.

Huang Y-R, Hung Y-C, Hsu S-Y, Huang Y-W, Hwang D-F. 2008. Application of electrolyzed water in the food industry. *Food Control* **19(4)**:329–345 DOI 10.1016/j.foodcont.2007.08.012.

Issa-Zacharia A, Kamitani Y, Miwa N, Muhimbula H, Iwasaki K. 2011. Application of slightly acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. *Food Control* **22(3–4)**:601–607 DOI 10.1016/j.foodcont.2010.10.011.

Issa-Zacharia A, Kamitani Y, Morita K, Iwasaki K. 2010a. Sanitization potency of slightly acidic electrolyzed water against pure cultures of *Escherichia coli* and *Staphylococcus aureus*, in comparison with that of other food sanitizers. *Food Control* **21(5)**:740–745 DOI 10.1016/j.foodcont.2009.11.002.

Issa-Zacharia A, Kamitani Y, Tiisekwa A, Morita K, Iwasaki K. 2010b. In vitro inactivation of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. using slightly acidic electrolyzed water. *Journal of Bioscience and Bioengineering* **110(3)**:308–313 DOI 10.1016/j.jbiosc.2010.03.012.

Kim H-J, Tango CN, Chelliah R, Oh D-H. 2019. Sanitization efficacy of slightly acidic electrolyzed water against pure cultures of *Escherichia coli*, *Salmonella enterica*, *Typhimurium*, *Staphylococcus aureus* and *Bacillus cereus spores*, in comparison with different water hardness. *Scientific Reports* **9(1)**:4348 DOI 10.1038/s41598-019-40846-6.

Kiura H, Sano K, Morimatsu S, Nakano T, Morita C, Yamaguchi M, Maeda T, Katsuoka Y. 2002. Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. *Journal of Microbiological Methods* **49(3)**:285–293 DOI 10.1016/S0167-7012(01)00385-2.

Koide S, Shitanda D, Note M, Cao W. 2011. Effects of mildly heated, slightly acidic electrolyzed water on the disinfection and physicochemical properties of sliced carrot. *Food Control* **22(3–4)**:452–456 DOI 10.1016/j.foodcont.2010.09.025.

Koseki S, Yoshida K, Isobe S, Itoh K. 2004. Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. *Journal of Food Protection* **67(6)**:1247–1251 DOI 10.4315/0362-028X-67.6.1247.

Ludovici PP, Phillips RA, Jeter WS. 1975. Comparative inactivation of bacteria and viruses in tertiary-treated wastewater by chlorination. In: Johnson JD, ed. *Disinfection Water and Wastewater*. Ann Arbor: Ann Arbor Science Publishers, 359–390.

Nan S, Li Y, Li B, Wang C, Cui X, Cao W. 2010. Effect of slightly acidic electrolyzed water for inactivating *Escherichia coli* O157: H7 and *Staphylococcus aureus* analyzed by transmission electron microscopy. *Journal of Food Protection* **73(12)**:2211–2216 DOI 10.4315/0362-028X-73.12.2211.

Weichao Z, Li N, Xue H, Baoming L, Jiafa Z. 2016. Optimization of slightly acidic electrolyzed water spray for airborne culturable bacteria reduction in animal housing. *International Journal of Agricultural and Biological Engineering* **9**:185–191.

Yoshifumi H. 2003. Improvement of the electrolysis equipment and application of slightly acidic electrolyzed water for dairy farming. *Journal of the Japanese Society of Agricultural Machinery* **65**:27–29.

Zhang C, Lu Z, Li Y, Shang Y, Zhang G, Cao W. 2011. Reduction of *Escherichia coli* O157: H7 and *Salmonella enteritidis* on mung bean seeds and sprouts by slightly acidic electrolyzed water. *Food Control* **22(5)**:792–796 DOI 10.1016/j.foodcont.2010.11.018.

Zheng W, Kang R, Wang H, Li B, Xu C, Wang S. 2013. Airborne bacterial reduction by spraying slightly acidic electrolyzed water in a laying-hen house. *Journal of the Air & Waste Management Association* **63(10)**:1205–1211 DOI 10.1080/10962247.2013.812815.

Zheng W, Zhao Y, Xin H, Gates RS, Li B, Zhang Y, Soupir ML. 2014. Airborne particulate matter and culturable bacteria reduction from spraying slightly acidic electrolyzed water in an experimental aviary laying-hen housing chamber. *Transactions of the ASABE* **57**:229–236.