Optimization of slightly acidic electrolyzed water spray for airborne culturable bacteria reduction in animal housing

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Abstract: Slightly acidic electrolyzed water (SAEW) spray has been considered as a novel approach for airborne bacteria reduction in animal housing. This study aimed to optimize the operating parameters of SAEW spray based on the size distribution of sprayed aerosols, the available chlorine travelling loss in sprayed aerosols, and the reduction efficiency of airborne culturable bacteria (CB). The optimized operating parameters were the nozzle orifice diameter and the spray pressure. The size distribution characteristics of sprayed SAEW aerosols under different operating parameters were quantified. The pH and available chlorine concentrations of the original SAEW solution and the SAEW aerosols collected at 0 m, 0.25 m, 0.50 m, 0.75 m, and 1.00 m from the spray nozzle head were analyzed. A bioaerosol nebulizing generator was used to simulate animal housing environment in an environmentally-controlled chamber. Six SAEW spray trials under different operating parameters were conducted at a spray dosage of 80 mL/m^2 in the chamber. Airborne CB concentrations before and after spray were measured to investigate the reduction efficiency of airborne CB. Dv(50), the volume-based diameter below which 50% of the particle being present, increased with the nozzle orifice diameter and decreased with the spray pressure. The travelling loss of available chlorine in the sprayed SAEW aerosols was greatly dependent on Dv(50). SAEW spray with medium size sprayed aerosols (Dv(50) = 86.62 μm, 67.94 μm, and 54.53 μm) showed significantly higher airborne CB reduction efficiencies than large (Dv(50)=121.80 μm and 96.00 μm) or small size aerosols (Dv(50) = 42.57 μm). The spray operating parameters that provide medium size sprayed aerosols (Dv(50) ~ 60-90 μm) are recommended for SAEW spray in animal housing.

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1 Introduction

In recent years, the indoor environment in animal housing has received increasing attention because of the much higher concentrations of airborne microorganisms compared with the ambient environment. Airborne microorganisms at high concentrations along with their toxic components may not only jeopardize the health of the animals and workers[1-3], but cause a risk of disease outbreak by transmitting through the air[4-6]. Reducing airborne bacteria concentration is a critical approach to improve the indoor air environment in animal housing.

To address the challenge, slightly acidic electrolyzed water (SAEW) spray has been recognized as an effective and environment-friendly approach to improve the air quality in animal housing by reducing the airborne bacteria and particulate matter concentrations[7-10]. SAEW is produced by electrolyzing a dilute solution of

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sodium chloride (NaCl) or hydrochloric acid (HCl) [11,12]. Its high bactericidal effect is due to the available chlorine, including ClO^-, HClO, and Cl_2[13,14]. The bactericidal effectiveness of SAEW spray utilized in animal housing is relevant to the available chlorine concentration (ACC) and spray dosage[7,9]. However, some aspects of this novel technique have not been scientifically explored in detail, such as the available chlorine travelling loss in SAEW aerosols and the bactericidal effectiveness under different spray operating parameters. Different size distributions of sprayed aerosols are not quantified when operating SAEW spray under different nozzle orifice diameters and spray pressures. Different initial sprayed aerosol diameter may be related to the bactericidal effectiveness of SAEW aerosols. In order to enhance the antimicrobial effect of SAEW spray and figure out the optimal spray operating parameters in animal housing, the above-unknown aspects need to be addressed.

This study aimed to optimize the operating parameters (nozzle orifice diameter and spray pressure) of SAEW spray based on the size distribution of sprayed aerosols, the available chlorine loss in sprayed aerosols, and the efficiency of airborne culturable bacteria (CB) reduction in a simulated animal housing environment.

2 Materials and methods

2.1 Production of SAEW

SAEW with an ACC of 30 mg/L and pH of 6.0-6.1 was produced using an SAEW generator (Purester MP-600T, Morinaga Engineering Co., Ltd., Tokyo, Japan). HCl solution (9%) was diluted with tap water before electrolysis in an electrolysis cell to produce SAEW.

A digital chlorine test kit (RC-3F; Kasahara Chemical Instruments Corporation, Saitama, Japan) and a dual-scale pH meter (HM-30 R; DKKTOA Corporation, Tokyo, Japan) were used to determine the ACC and pH, respectively. The pH meter was calibrated using commercial standard buffers with pH of 4.01 and 6.86 supplied by the manufacturer.

2.2 SAEW spray system

The spray system (Nantong Wujing High-pressure Pump Co., Ltd., China) consists of a strainer, a high-pressure pump with a pressure regulator, a pressure monitor, a flow rate monitor, and an array of pipe with slip-lok tees coupling with nozzles. Full cone nozzles with different orifice diameters of 0.1 mm, 0.2 mm, 0.3 mm, 0.4 mm and 0.5 mm were used in this experiment, under different spray pressures of 0.138 MPa, 0.207 MPa and 0.276 MPa (20 psi, 30 psi and 40 psi). The flow rates were recorded and given in Table 1.

Table 1 Flow rates with different nozzle orifice diameters and spray pressures (mL/min)

Spray pressure /MPa	Nozzle orifice diameter/mm				
	0.1	0.2	0.3	0.4	0.5
0.138	14.3±0.9	32.1±0.4	34.6±1.0	36.3±1.2	36.2±0.9
0.207	25.7±0.3	64.2±0.8	65.9±1.5	90.4±5.0	81.7±3.3
0.276	33.5±0.5	80.8±0.5	85.0±1.5	125.5±3.3	113.6±0.3

2.3 Aerosol size distribution and available chlorine travelling loss measurement

The characteristics of the size distribution of sprayed SAEW aerosols was determined using a Spraytec system (Malvern Instruments LTD, Worcestershire, UK), which can measure aerosol size distribution data using laser diffraction technique. The spray nozzle was set 0.25 m above the laser line. The Spraytec system can cover a size range from 0.1-2000 μm sprayed aerosols and access the size distribution data including Dv(90), Dv(50), Dv(10), and the Span. For a volume-based size distribution, Dv(90), Dv(50), and Dv(10) are the diameter below which 90%, 50% and 10% of the particles being present in volume, respectively. They are the most common data for demonstrating laser diffraction results based on a volume distribution. Span measures the width of the distribution, and is the most common data used to express distribution width. Consequently, the span decreases as the distribution becomes narrower. The span is calculated using Equation (1).

$$Span = \frac{Dv(90) - Dv(10)}{Dv(50)} \tag{1}$$

Available chlorine travelling loss was measured in a clean room without any mechanical ventilation. Sprayed SAEW aerosols were simultaneously collected by dry-clean glass Petri dishes at a distance of 0 m, 0.25 m, 0.50 m, 0.75 m and 1.00 m from the spray nozzle head. The ACC and pH of collected aerosols were measured by a digital chlorine test kit (RC-3F; Kasahara Chemical Instruments Corporation, Saitama, Japan) and a dual-scale pH meter (HM-30 R; DKKTOA Corporation, Tokyo, Japan), respectively.

2.4 Experimental set-up for airborne CB reduction

A 1.2 m×1.2 m×1.2 m isolator made of acrylic glass with negative pressure high-efficiency particulate arresting (HEPA) filters was used as the experimental space. Spray nozzles were installed at the top center of the isolator for SAEW spray.

Figure 1 shows the overview of the experimental design to investigate the reduction efficiency of airborne CB by SAEW spray. Prior to spray, aerosols containing bacteria were generated in the isolator, followed by airborne CB sampling. After the before-spray sampling, SAEW spray (80 mL/m^2) was performed under different combinations of nozzle orifice diameter and spray pressure. As shown in Table 2, six combinations of nozzle orifice diameter and spray pressure (Trail 1-6) were selected to spray SAEW due to the varied Dv(50) achieved in the experiment. These spray trials were randomly operated with 7-8 replications each. After spray, airborne CB sampling was performed again. Details on the methods for aerosolization and airborne CB sampling are given in Sections 2.5 and 2.6, respectively.

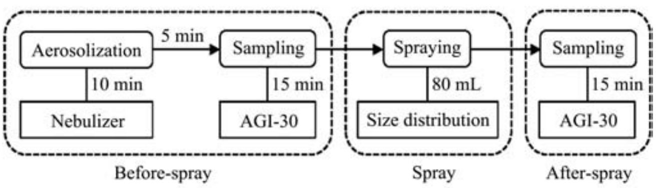

Figure 1 Overview of the experimental set-up

Table 2 Characteristics of the spray trails

Trials	Spray pressure /MPa	Nozzle orifice diameter/mm	Dv(50) /μm	Spray volume/mL	Replications
Trial 1	0.138	0.5	121.80±3.97	79.6±1.2	8
Trial 2	0.138	0.4	96.00±4.42	81.0±1.5	8
Trial 3	0.138	0.2	86.62±2.91	82.3±1.7	8
Trial 4	0.207	0.5	67.94±1.83	80.1±0.9	8
Trial 5	0.207	0.3	54.53±1.72	80.8±1.2	7
Trial 6	0.276	0.2	42.57±2.00	81.7±1.3	8

2.5 Aerosolization

Frozen beads containing *Escherichia coli* O157:H7 (*E. coli*) were rolled on a sterilized tryptic soy agar that was then incubated at 37ºC for 24 h. The colonies of *E. coli* were scraped off and transferred into a 40 mL sterilized liquid nutrient broth (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China). The *E. coli* concentration of the suspension was approximately 9 $\log_{10}$ CFU (colony forming units)/mL. A 6 Jet Collision Nebulizer (Collision MRE 6 Jet, BGI Collision Nebulizer, BGI Inc., MA, USA) connecting to an air compressor was used to spray the liquid suspension as small droplets containing the microorganism-attached particles. The nebulizer with the 40 mL suspension was placed at the center of the chamber at the height of 40 cm. It was operated for aerosolization for 10 min at an air pressure of 0.276 MPa (40 psi) in this study. The mass median diameter (MMD), geometric standard deviation (GSD) and count median diameter (CMD) were given by the manufacturer, which was 2.5 μm, 1.8 μm, and 0.89 μm, respectively.

2.6 Airborne CB sampling and analysis

The airborne CB sampling was conducted by an all-glass impinger (AGI-30, Ace Glass, Inc., NJ, USA) located at the center of the chamber at the height of 0.40 m. The impinger was connected to a vacuum pump (Rocker R410, Rocker Scientific Co., Ltd, Taiwan) with clear Tygon PVC tubing. Airborne CB was collected for 15 min with 15 mL collecting fluid (sterilized 0.9% physiological saline solution) in the impinger at a stable operating flow rate of 12.5 L/min. After sampling, the collecting fluid with airborne CB (original sample) was serially diluted (1:10) in sterilized 0.9% physiological saline solution. Then 0.5 mL of the original and the diluted samples were plated on Petri dishes with sterilized tryptic soy agar for duplication. The Petri dishes were incubated at 37ºC for 24 h. After incubation, colonies in the Petri dishes with 30-300 colonies were enumerated. The volume of the original sample was measured by a pipette. The airborne CB concentration was calculated using Equation (2).

$$C_{bacteria} = \frac{N_1 V_1}{V_2 Q t} \times 10^a \tag{2}$$

where, $C_{bacteria}$ is the airborne CB concentration, CFU/m^3; N_1 is the average number of colonies in Petri dishes with 30-300 colonies where 10^{-a} liquid samples are cultured, CFU; V_1 is the total volume of the original sample, mL; a is the dilution factor of the original sample; V_2 is the volume of 10^{-a} original sample plated on trypticase soy agar (0.5 mL); Q is the airflow rate through the impinger (12.5 L/min = 0.0125 m^3/min); t is the sampling duration, min.

To assess the different effectiveness on airborne CB reduction among the six spray trails, the reduction efficiency was calculated using Equation (3).

$$R_{reducion} = \frac{C_{before} - C_{after}}{C_{before}} \times 100\% \qquad (3)$$

where, $R_{removal}$ is the airborne CB reduction efficiency by SAEW spray, %; C_{before} is the airborne CB concentration measured before spray, CFU/m^3; C_{inlet} is the airborne CB concentration measured after spray, CFU/m^3.

2.7 Statistical analysis

Statistical analysis was performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Tukey's studentized range test was used to determine the significant differences among the airborne CB reduction efficiencies of the six spray trails. Differences were considered significant for a 5% probability level.

3 Results and discussion

3.1 Size distribution of the sprayed aerosols

The size distributions of sprayed SAEW aerosols under different nozzle orifice diameters and spray pressures are given in Table 3. The span was quite constant for each combination of nozzle orifice diameter and spray pressure (1.23-1.43). Dv(50), the volume-based diameter below which 50% of the particle being present which is the data reflecting the majority of droplet size diameter, varied depending on different nozzle orifice diameters and spray pressures. For the same spray pressure, Dv(50) increased with the nozzle orifice diameter, indicating a higher nozzle orifice diameter associated with a larger aerosol size spectrum. For the same nozzle orifice diameter, Dv(50) decreased with the spray pressure, indicating a higher spray pressure corresponded with a smaller aerosol size spectrum. The similar trends were reported by other researchers using different measuring techniques[15,16]. The size distribution of sprayed SAEW aerosols has a great impact on the surface area of the aerosols. When spraying SAEW in animal housing, the particle-liquid contact can be enhanced by increasing the surface area of the aerosols. The particle-liquid contact during SAEW spray varies under different nozzle orifice diameters and spray pressures.

Table 3 Size distribution data with different nozzle orifice diameters and spray pressures

Nozzle orifice diameter /mm	Spray pressure/MPa					
	0.138		0.207		0.276	
	Dv(50)/μm	Span	Dv(50)/μm	Span	Dv(50)/μm	Span
0.1	67.62±3.18	1.26±0.01	47.69±2.40	1.29±0.01	37.64±0.76	1.37±0.01
0.2	86.62±2.91	1.32±0.02	48.27±0.55	1.30±0.04	42.57±2.00	1.43±0.05
0.3	92.63±4.42	1.34±0.02	54.53±1.72	1.32±0.02	47.63±0.90	1.31±0.03
0.4	96.00±3.45	1.30±0.05	56.71±0.59	1.33±0.01	49.79±0.53	1.33±0.04
0.5	121.80±3.97	1.23±0.08	60.94±1.83	1.28±0.02	48.88±1.87	1.38±0.04

Note: Dv(50) is the volume-based diameter below which 50% of the particle being present. Span measures the width of the distribution. The narrower the distribution, the smaller the span becomes.

3.2 Available chlorine travelling loss

Results showed that pH of the sprayed SAEW aerosols collected at each distance from the spray head was quite constant (6.1-6.3) for each combination of nozzle orifice diameter and spray pressure. Figure 1 shows the ACC of sprayed SAEW aerosols collected at different distances from the nozzle head when spraying SAEW under different nozzle orifice diameter and spray pressure. The ACC of sprayed SAEW aerosols collected at a distance of 0 m from the nozzle head was lower than that of the original SAEW (24-27 mg/L vs. 30 mg/L), indicating a 10%-20% initial available chlorine loss. Similar to results shown above, Zhao et al.[17] reported an 11.7%-13.2% initial available chlorine loss when spraying SAEW with a Dv(50) of 80 μm. However, a direct comparison of the initial available chlorine loss between this study and the study conducted by Zhao et al. is lacking due to different spray systems (hydraulic vs. air) used. Regardless of the spray technique, the initial loss of available chlorine was within expectations because it was a result of sudden aerosolization near the nozzle in a short period of time.

For each combination of nozzle orifice diameter and spray pressure, the ACC of sprayed SAEW aerosols considerably decreased over the distance from the nozzle head. The ACC of sprayed SAEW aerosols showed a decreasing tread with the spray pressure but an increasing trend with the nozzle orifice diameter. For the same spray pressure, a higher nozzle orifice diameter corresponded with a smaller travelling loss of available chlorine. For example, at a spray pressure of 0.207 MPa, the travelling loss from 0 m to 0.75 m was 63%, 55% and 51% for 0.1 mm, 0.3 mm and 0.5 mm nozzle orifice diameter, respectively. For the same nozzle orifice diameter, higher spray pressures resulted in higher travelling losses. For example, for 0.3 mm nozzle orifice diameter, the travelling loss from 0 m to 0.25 m was 32%, 38% and 44% at a spray pressure of 0.138 MPa, 0.207 MPa and 0.276 MPa, respectively. These results emphasized that the travelling loss of available chlorine was greatly impacted by the initial sprayed aerosol diameter, which was related to the nozzle orifice diameter and the spray pressure. Small size sprayed aerosols promote the gas-liquid contact, but consequently increase the travelling loss of available chlorine presumably caused by the evaporation of SAEW aerosols. The smaller are the SAEW aerosols, the higher evaporation rates are to be expected. The Cl_2 off-gas will increase due to the increasing chlorine vapour pressure, resulting in an increase of the travelling loss of available chlorine.

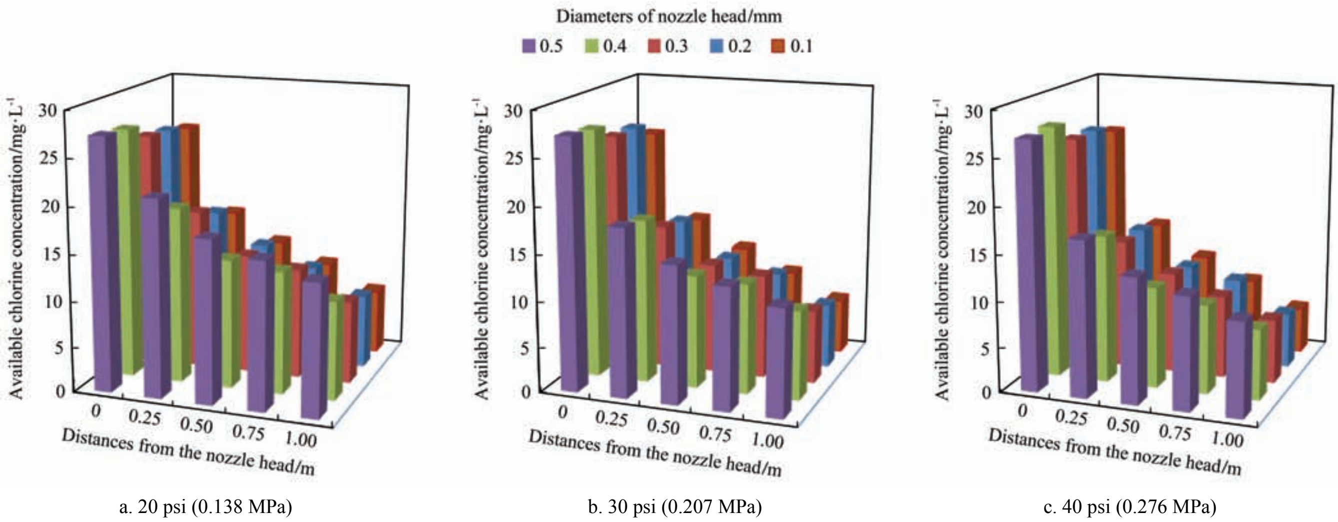

Figure 2 Available chlorine concentrations at different distances from the nozzle head when spraying slightly acidic electrolyzed water under different nozzle orifice diameters and spray pressures

3.3 Airborne culturable bacteria reduction

The concentration and reduction efficiency of airborne CB for each trial are given in Table 4. The reduction efficiency of airborne CB reflecting a combined effect of SAEW spray and physical deposition of airborne CB in the test chamber varied from different trails. Statistical analysis showed that the reduction efficiencies had no significant difference among Trails 3, 4 and 5 (p=0.99, 0.85, and 0.63). Trails 3, 4 and 5 had significant higher reduction efficiencies than Trails 1, 2 and 6 (p<0.05). The reduction efficiencies significantly differed among Trails 1, 2 and 6 (p<0.05). These results indicated that the size distribution of sprayed SAEW aerosols had a great effect on airborne CB efficiency during SEAW spray. SAEW spray with medium size sprayed aerosols (Dv(50)=86.62 μm, 67.94 μm and 54.53 μm) showed significantly higher airborne CB reduction efficiencies than SAEW spray with large (Dv(50)=121.80 μm and 96.00 μm) or small size sprayed aerosols (Dv(50)=42.57 μm). SAEW was reported to be a highly effective bactericide when bacteria are directly exposed to SAEW aerosols[7,9,10]. Compared to large size sprayed aerosols, SAEW spray with medium size sprayed aerosols promotes the gas-liquid contact and increases the airborne CB reduction efficiency. Compared to small size sprayed aerosols, medium size sprayed aerosols have lower travelling loss of the available chlorine from evaporation of the SAEW aerosols.

For a SAEW spray system with full cone nozzles, optimizing the combination of nozzle orifice diameter and spray pressure is critical to enhance the reduction efficiency of airborne CB by SAEW spray. The spray operating parameters that provide medium size sprayed aerosols (Dv(50)~60-90 μm) are recommended for SAEW spray in animal housing. SAEW spray can be optimized by selecting the desired combination of nozzle orifice diameter and spray pressure. However, challenge exists as high spray pressure often associates with high energy consumption and safety risk, while nozzle with small orifice diameter has high occurrence of clogging issues, all of which should be taken into consideration for selecting appropriate nozzle orifice diameter and spray pressure. In the current study, the airborne CB reduction efficiency was evaluated in a simulated animal housing environment. Bio-aerosols generated by the nebulizer have a 2.5 μm MMD, which is lower than the MMD of bio-aerosols in commercial animal buildings[18-21]. Further studies using common MMD of bio-aerosols in commercial animal buildings are recommended. Results shown in this study provide valuable instructions on optimizing SAEW spray for airborne CB reduction in animal housing by selecting an appropriate combination of nozzle orifice diameter and spray pressure.

Table 4 Concentration and reduction efficiency of airborne culturable bacteria by slightly acidic electrolyzed water spray

Trials	Airborne culturable bacteria concentration/$\times10^5$ CFU·m^{-3}						Reduction efficiency/ %
	Before spray			After spray			
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	
Trail 1	1.92	1.05	1.42±0.31	1.17	0.58	0.80±0.23	44.0±6.9 d
Trail 2	2.92	1.25	1.92±0.63	1.45	0.43	0.77±0.38	61.2±6.7 c
Trail 3	2.40	1.04	1.53±0.46	0.46	0.10	0.22±0.13	85.7±6.4 a
Trail 4	3.12	1.23	2.16±0.64	0.45	0.16	0.27±0.09	86.8±4.5 a
Trail 5	2.70	1.46	2.11±0.45	0.62	0.26	0.36±0.12	82.3±5.8 a
Trail 6	2.94	1.23	1.97±0.62	0.96	0.32	0.57±0.24	71.8±4.6 b

Note: Values followed by different letters in the column are significantly different (p<0.05).

4 Conclusions

The size distributions of sprayed SAEW aerosols under different nozzle orifice diameters and spraying pressures were determined. The effects of sprayed SAEW aerosols with different size distributions on available chlorine travelling loss and airborne CB reduction were assessed. Results provide findings as below:

1) The volume-based diameter of sprayed SAEW aerosols increased with the nozzle orifice diameter (0.138 MPa, 0.207 MPa, and 0.276 MPa) and decreased with the spray pressure (0.5 mm, 0.4 mm, 0.3 mm, 0.2 mm, and 0.1 mm).

2) The travelling loss of available chlorine was greatly dependent on the volume-based diameter of sprayed SAEW aerosols. Medium size sprayed SAEW aerosols (Dv(50)=86.62 μm, 67.94 μm, and 54.53 μm) showed significantly higher airborne CB reduction efficiencies than large and small size aerosols (Dv(50)=121.80 μm, 96.00 μm, and 42.57 μm).

3) The spray operating parameters that provide medium size sprayed aerosols (Dv(50)~60-90 μm) are recommended for SAEW spray in animal housing.

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