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Free chlorine loss during spray of membrane-less acidic electrolyzed water (MLAEW) and its antimicrobial effect on airborne bacteria from poultry house

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Abstract. *Spray-application of membrane-less acidic electrolyzed water (MLAEW) is a novel technique for disinfection in livestock houses. This study investigated the loss of free chlorine (FC, the major germicidal component in MLAEW) over distance during spray, as affected by air temperature and initial FC concentration. The antimicrobial effect of MLAEW on airborne bacteria from an aviary laying-hen house was examined. MLAEW was prepared with two FC concentrations (app. 15 and 60 mg L^{-1}), and was sprayed at three air temperatures (18, 25, 32°C). The original MLAEW solution and MLAEW aerosols collected at 0, 25, and 50 cm from the spray nozzle were analyzed for FC concentrations. Bacteria were immersed into these MLAEW samples and numerated for viable count after 0.5-, 2-, and 5-min treatments. MLAEW aerosols collected at 0 cm lost 11.7 – 13.2% FC as compared to the original MLAEW solution. This initial loss was affected neither by the initial FC concentration (P = 0.13) nor by air temperature (P = 0.57). The rate of FC loss during travelling was 0.79 – 0.87 % per centimeter of aerosol travel distance (% cm^{-1}) at 18°C, 1.08 – 1.15 % cm^{-1} at 25°C, and 1.35 – 1.49 % cm^{-1} at 32°C. This travelling loss was affected by air temperature (P = 0.02), but not by initial FC concentration (P = 0.38). Bacteria were completely inactivated in 0.5 min when treated with MLAEW samples with FC > 16.8 mg L^{-1}, in 2 min when FC > 13.8 mg L^{-1}, and in 5 min when FC > 7.2 mg L^{-1}. Airborne bacteria from aviary hen house can be effectively inactivated by MLAEW with adequate FC concentration and contact time. During spray, antimicrobial efficacy of MLAEW aerosols decreased over distance due to FC loss which exacerbates at higher air temperature.*

Keywords. Electrolyzed water, inactivation, airborne microorganisms, livestock, spraying

Introduction

Livestock and poultry production facilities are associated with much higher concentrations of airborne microorganisms compared to ambient environment (Miao et al., 2010; Nimmermark et al., 2009; Zhao et al., 2011a). The airborne microorganisms and their harmful components may not only jeopardize health status of animal and caretaker within barn (Kirychuk et al., 2006; Singh and Schwartz, 2005; Wilson, 2004; Zhao et al., 2011c), but also pose risk of spreading disease between barns if pathogenic species are emitted outside (Gloster and Alexandersen, 2004; Zhao et al., 2013). In response to or in anticipation of state or federal legislation on animal welfare, some egg producers are building or planning to build aviary cage-free hen-housing systems. While the aviary systems (featuring littered floor in addition to perches and nestboxes) well accommodate hen natural behaviors (e.g. dustbathing, foraging, perching etc.), they tend to have higher levels of airborne microorganisms than cage housing system (Groot Koerkamp et al., 1998). Consequently, practical means to improve the indoor air quality in such alternative housing systems are highly desirable.

Membrane-less acidic electrolyzed water (MLAEW) is an antimicrobial agent that is produced by electrolyzing a dilute solution of sodium chloride (NaCl) or hydrochloric acid (HCl), generating the major germicidal component – free chlorine (including ClO^-, HClO and Cl_2). Compared to the traditional membrane acidic electrolyzed water (pH < 3.0, oxidation reduction potential 'ORP' > 1000 mV), the MLAEW has similar antimicrobial ability, but is less corrosive and is easier and cheaper to produce due to its near neutral pH value (6 – 7) and lower ORP. In the past decade, MLAEW has been increasingly gaining interests as a disinfectant in agriculture, dentistry, medicine and food industry (Huang et al., 2008; Sakurai et al., 2003). Recently, MLAEW spray was applied in swine and poultry houses to inactivate airborne microorganisms. Chuang et al. (2011) reported that the level of total airborne bacteria was reduced by 70% by spraying MLAEW in a cage hen house. Wu et al. (2010) found a reduction of 98% in total bacteria and of 68% in fungi after spraying MLAEW in a swine house. In addition, our recent research revealed significant microbial inactivation from MLAEW application in a pilot-scale aviary hen environment.

Though the effectiveness of MLAEW spray on microbial inactivation in the livestock houses has been confirmed, some aspects of this novel technique are not well explored in details. Firstly, loss of free chlorine (FC) in the MLAEW aerosols during spray has not been well studied. Free chlorine in MLAEW aerosols may be lost due to Cl_2 volatilization, as a result, the germicidal effect of MLAEW aerosols is attenuated. The magnitude of FC loss is affected by factors such as air temperature and initial aerosol diameter and is exacerbated over distance from spray origin. Secondly, although much research has been dedicated to characterize the inactivation of food-associated microorganisms by MLAEW with different FC levels, the required FC concentration of MLAEW for inactivating airborne microorganisms in an aviary poultry house is unknown. In order to achieve precise management and optimize antimicrobial effect of MLAEW application in aviary house, the above mentioned aspects need to be addressed.

The objective of this study was to investigate the FC loss from the MLAEW aerosols over distances from spray origin at different air temperatures (18, 25, or 32°C) and the effect of FC concentration on such loss (15 vs. 60 mg L^{-1}). The changes in pH and ORP of the aerosols were also examined. Airborne bacteria were sampled from a commercial aviary house and challenged with the MLAEW at different FC concentrations collected under above conditions.

Materials and Methods

MLAEW production

A cylindrical plastic electrolyzing container (Height × Diameter = 32 × 19 cm) was used to produce MLAEW in this study (Figure 1). This container consisted of a water tank and a lid installed with one anode and two cathode metal plates (cast iron). The three electrode plates were identical in size (L × W = 15 × 12.5 cm) and were fixed in parallel with the anode plate in middle. The gap between two adjacent plates was 1 cm. A faucet was installed near the bottom of the container to obtain MLAEW without opening the lid.

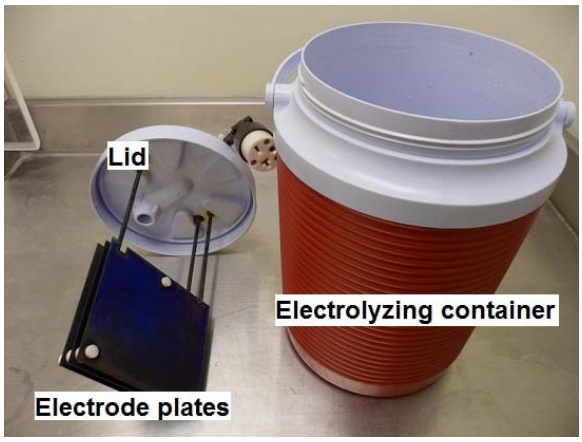

Figure 1. A Picture of the electrolyzing container and components.

The MLAEW was produced by electrolyzing 5-L 0.1% NaCl solution (5 g NaCl in 5 L tap water) at 8 VDC. Based on our previous experiments, the FC production rate was 4.9 mg L^{-1} min^{-1} at this NaCl concentration and voltage. In this experiment, MLAEW with low (app. 15 mg L^{-1}) and high (app. 60 mg L^{-1}) FC concentrations was produced by electrolyzing the 0.1% NaCl solution for 3 and 12 min, respectively. Since spraying liquid with pH > 7 may potentially increase ammonia (NH_3, an alkaline gas) emissions in animal houses (Ogink et al., 2012), the pH of the MLAEW was adjusted to 6 – 7 by adding HCl after electrolyzing.

MLAEW spray and collection

MLAEW was sprayed using a spray gun (PILOT Mini, 0.5 mm nozzle, Walther Pilot NA, Chesterfield, MI) connected to an air compressor (Model # 204100, Campbell Hausfeld, Harrison, OH) at 1.4 bar. The initial aerosol size distribution was delineated using Particle Image Velocimetry (PIV) technology. The PIV system takes two images at a 5×10^{-7} s interval using a high resolution CCD camera (PCO 1600, PCO-TECH Inc., Romulus, MI). Knowing the size of one pixel represents, the PIV technology determines the size of each aerosol by counting the number of pixels an aerosol covers in the image. The size distribution profile (volumetric frequency of aerosols at different sizes) was developed by combining the size information of all aerosols in a small area (1×1 cm) near the nozzle. The velocity of each aerosol can be also determined by dividing its relative locomotion in the two consecutive images by the imaging interval.

MLAEW with either low or high FC concentration was sprayed at 18, 25 and 32°C air temperature in a climate-controlled room. The sprayed MLAEW aerosols were collected using glass petri-dishes at three distances, i.e. 0, 25, and 50 cm, from the nozzle of the spray gun. Characteristics of MLAEW at farther distances were of less interest because in practice MLAEW would be sprayed to the source (litter) of airborne microorganisms from a short distance to minimize the FC loss, thus achieve the optimal antimicrobial effect. The four MLAEW samples (one original and three collected at different distances) were transferred to individual dark tubes before further analysis.

Analysis of MLAEW

Immediately after MLAEW samples were obtained, FC, pH, and ORP of the samples were analyzed. Free chlorine was quantified using a colorimeter (Martini MI-413 Free & Total Chlorine, Milwaukee Instruments, Rocky Mount, NC, USA). This colorimeter can measure (N,N-diethyl-p-phenylenediamine, or 'DPD' method) free or total chlorine up to 10 mg L^{-1}. Whenever the FC concentration in an MLAEW sample exceeded this limit, its diluted sample (using deionized water) was analyzed and the FC concentration in the original sample was calculated using the dilution factor. Values of pH and ORP were measured using respective meters (pH 3300i, WTW, Weilheim, Germany).

Collection of airborne bacteria

On the same day of MLAEW spray application, airborne bacteria were collected from a commercial aviary house in central Iowa. The aviary house measured 150.8 × 21.4 m and had a capacity of 50,000 laying hens. The hens were introduced to this house at 16 weeks of age. They were kept in the aviary colonies until 22 week old when they were given litter floor access for dustbathing and foraging about 8 – 10 h per day. In total, eight times of air samplings (on eight different days) were conducted in December 2012 when hens were at 33 – 36 weeks of age.

The airborne bacteria were collected at 30 cm above litter floor for 25 min using an all glass impinger (AGI-30, Ace Glass Incorporated, Vineland, NJ) with 20 mL of physiological saline. Twenty milliliter of physiological saline was used as collection medium. The AGI-30 was designed for collecting total airborne microorganisms without distinguishing their size at a nominal air flow rate of 12.5 L min^{-1}. After sampling, the liquid microbial sample was transferred to a 50 mL vortex tube and kept at 4°C before further use. The thermal environment in the aviary house was monitored using a temperature and relative humidity (RH) sensor (HOBO® Pro Series, ONSET, Bourne, MA) on the sampling days, and the average temperature and RH were 23.1 ± 1.2°C and 68 ± 7%, respectively, during air sampling. The ventilation was at minimal level, app. 0.7 m^3 h^{-1} hen^{-1}, during air sampling.

Inactivation of airborne bacteria by MLAEW

To investigate the antimicrobial effect of MLAEW, MLAEW samples (original and those collected at different distances) of three spray events were used to treat the bacteria collected from the aviary hen house. A volume of 1.8 mL of MLAEW sample (treatment) and sterile deionized water (control) was separately prepared in sterilized tubes at room temperature. An aliquot (0.2 mL) of the liquid bacterial sample was individually added to the prepared tubes and mixed by vortexing for 5 s. After 0.5, 2 and 5 min, 0.2 mL of each treated sample was transferred to a sterile tube containing 1.8 mL of neutralizing buffer solution (0.5 sodium thiosulphate + 0.03 M phosphate buffer solution, pH = 7.1) and mixed by vortexing. The samples were neutralized for 5 min, then viable count of total bacteria and Gram-negative bacteria in each sample were determined by plating 0.2 mL portions directly or after serially diluted (1:10) in physiological saline on trypticase soy agar (TSA, for total bacteria, Catalog No. R455002, Fisher Scientific, Hanover Park, IL) and Macconkey No. 3 (for Gram-negative bacteria, Catalog No. OXCM0115B, Fisher Scientific, Hanover Park, IL) plates. The plates were aerobically incubated at 37°C for 24 h (total bacteria) or 48 h (Gram-negative bacteria).

An enrichment experiment was further conducted to determine the presence of low survivals that might not be detected using direct plating. For total bacteria, 0.5 mL of the suspension was transferred to a sterile tube containing 50 mL of trypticase soy broth (TSB, Catalog No. R455052, Fisher Scientific, Hanover Park, IL), and incubated at 37°C for 48 h. Following enrichment, 0.5 mL culture suspension was spread on TSA plate, and the plate was incubated at 37°C for 48 h before counting. The same procedure was applied to Gram-negative bacteria

enrichment and culturing, except Macconkey broth (7185, Neogen Corp., Lansing, MI) and Macconkey No. 3 agar were used.

Statistical analysis

Each treatment (air temperature × FC concentration) was repeated six times. The FC loss in MLAEW aerosols was categorized into initial loss and travelling loss rate. The initial loss was calculated using Eq. 1; while the travelling loss rate (% per cm of aerosol travel distance, or % cm^{-1}) was the slope in the linear regression of FC concentrations in MLAEW aerosols vs. the correspondent distance (0, 25, or 50 cm from the nozzle) where the aerosols were collected. Differences in FC concentration and antimicrobial effect among treatments were compared using General Linear Model (GLM) of Statistical Analysis System (SAS 9.2, SAS Institute Inc., Cary, NC) at the significance level of 0.05. Bonferroni procedure was used to adjust multiple comparisons. Minimum FC concentration required for complete bacterial inactivation was expressed as a function of air temperature, distance between nozzle and target, and MLAEW-bacteria contact time.

$$L_i = C_i / C_0 \qquad (1)$$

L_i: initial loss, %

C_i: free chlorine concentration in original MLAEW solution for spray, mg L^{-1}

C_0: free chlorine concentration in MLAEW aerosols collected at 0 cm from nozzle, mg L^{-1}

Results

Figure 2 shows the size distribution of the aerosols in a 1×1 cm area near the spray gun with 1.4 bar compressed air. The frequency curve peaked at 34 μm, indicating the most aerosols produced were at this size. The cutoff diameter by volume (D_{v50}, aerosol diameter corresponding to 50% cumulative volumetric frequency) was 80 μm. The average velocity of the aerosols was 60.5 m s^{-1}.

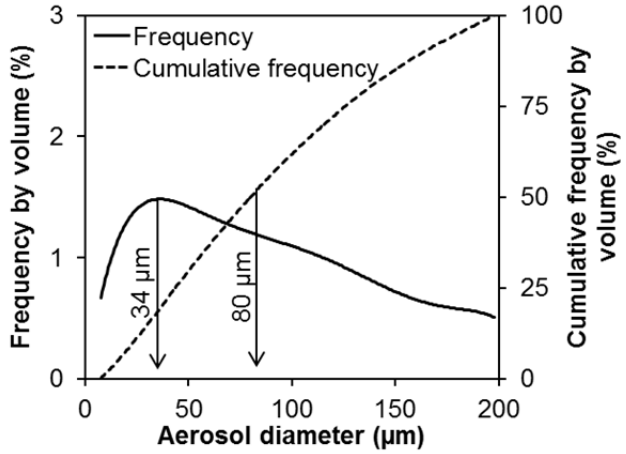

Figure 2. Aerosol size distribution (nebulized using spray gun with 0.5 mm nozzle at 1.4 bar)

The FC concentration was 15.0 – 16.2 mg L^{-1} in the original MLAEW electrolyzed for 3 min, and 56.0 – 59.3 mg L^{-1} when electrolyzed for 12 min (Table 1). The FC concentration in the MLAEW aerosols collected at 0 cm from the nozzle was slightly lower than the original MLAEW solution, reflecting an 11.7 – 13.2% initial loss. There was no difference in initial loss between low and high concentration groups (P = 0.13) or among the three air temperatures (P = 0.57). The FC concentrations of MLAEW aerosols decreased significantly over distance (P = 0.01), with a

traveling loss rate of 0.79 – 1.49% cm^{-1}. Statistical analysis showed that travelling loss exacerbated as air temperature increased (P = 0.02). However, no effect of initial FC concentration on traveling loss was found (P = 0.38).

Table 1. Free chlorine (FC) in original MLAEW solution and in MLAEW aerosols nebulized at two initial concentrations (low: 15.0 – 16.2 mg L^{-1}, high: 56.0 – 59.3 mg L^{-1}) and three air temperatures (18, 25, and 32 °C), and initial and travelling FC losses. MLAEW aerosols were collected at three distances (0, 25 and 50 cm) from the spray nozzle. (n = 6)

Initial FC Conc.	Air Temp.	FC conc. (±SD, mg L^{-1})				Initial loss[1] (±SD, %)	Traveling loss rate[2] (±SD, % cm^{-1})
		Original	Aerosol Travel Distance (cm)				
			0	25	50		
Low	18°C	$15.0^{a,A}$ ± 1.2	$13.2^{a,B}$ ± 0.7	$10.3^{a,C}$ ± 1.0	$7.5^{a,D}$ ± 1.7	11.8^{a} ± 1.0	0.87^{a} ± 0.12
	25°C	$16.2^{a,A}$ ± 1.0	$14.3^{a,B}$ ± 1.0	$9.0^{a,C}$ ± 1.2	$6.1^{ab,D}$ ± 2.0	11.7^{a} ± 0.8	1.15^{b} ± 0.10
	32°C	$15.5^{a,A}$ ± 1.2	$13.5^{a,B}$ ± 0.5	$7.4^{b,C}$ ± 0.5	$3.0^{b,D}$ ± 2.4	12.4^{a} ± 1.5	1.49^{c} ± 0.14
High	18°C	$56.0^{b,A}$ ± 3.4	$48.6^{b,B}$ ± 2.7	$37.6^{c,C}$ ± 2.9	$29.5^{c,D}$ ± 6.4	$13.2^{a,}$ ± 2.2	0.79^{a} ± 0.12
	25°C	$57.2^{b,A}$ ± 4.6	$50.1^{b,A}$ ± 4.7	$34.5^{c,B}$ ± 4.9	$23.0^{cd,C}$ ± 6.4	$12.4^{a,}$ ± 0.9	1.08^{b} ± 0.10
	32°C	$59.3^{b,A}$ ± 6.9	$51.8^{b,A}$ ± 7.6	$32.7^{c,B}$ ± 5.6	$17.8^{d,C}$ ± 5.9	12.8^{a} ± 1.6	1.35^{c} ± 0.09

[1] FC loss in MLAEW aerosols collected at 0 cm as compared to original MLAEW solution
[2] FC loss rate during MLAEW aerosols' traveling, expressed as % loss of the FC concentration at 0 cm per cm of distance traveled.
^{a,b,c,d} Means in the column with different superscript letters are significantly different (P < 0.05).
^{A,B,C,D} Means in the row (under "FC concentration" category) with different superscript letters are significantly different (P < 0.05).

Table 2 shows the pH and ORP of the MLAEW solutions and aerosols. Since the initial FC concentration did not affect either pH (P = 0.44) or ORP (P = 0.67), their values were pooled. The pH and ORP slightly increased after spraying. GLM analysis showed that travel distance (but not air temperature) has significant impact on pH (P = 0.03) and ORP (P = 0.04) of MLAEW.

Table 2. Oxidizing reduction potential (ORP) and pH value of original MLAEW solution and of MLAEW aerosols nebulized at three air temperatures. MLAEW aerosols were collected at three distances from nozzle. (±SD, n = 12)

	Air Temp.	Original Solution	Aerosol Travel Distance (cm)		
			0	25	50
pH (±SD)	18°C	6.7^{A} ± 0.2	7.0^{B} ± 0.1	7.1^{B} ± 0.1	7.2^{C} ± 0.1
	25°C	6.9^{A} ± 0.2	7.1^{B} ± 0.1	7.2^{B} ± 0.1	7.2^{B} ± 0.2
	32°C	6.7^{A} ± 0.1	7.0^{B} ± 0.1	7.2^{B} ± 0.1	7.2^{B} ± 0.2
ORP (±SD, mV)	18°C	803^{A} ± 22	851^{B} ± 28	847^{B} ± 27	836^{AB} ± 26
	25°C	784^{A} ± 53	813^{A} ± 48	799^{A} ± 58	787^{A} ± 57
	32°C	812^{A} ± 18	845^{B} ± 35	831^{AB} ± 39	815^{AB} ± 38

^{A,B,C,D} Means in the row with different superscript letters are significantly different (P < 0.05).

Table 3 lists the survival of total bacteria treated with deionized water (control), original MLAEW solution (with low initial FC concentration) and its aerosols that were nebulized at 18, 25 and 32°C and were collected at 0, 25 and 50 cm from the nozzle. Total bacteria count remained similar before and during the 5-min treatment with deionized water (average FC concentration = 0.3 mg L^{-1}). All original MLAEW solutions (average FC concentration = 15.4 mg L^{-1}) reduced the total bacteria count below the detection limit (2.7 log CFU mL^{-1}) of direct plating culture in 0.5-

min treatment, and completely inactivated the bacteria after 2-min treatment. The MLAEW aerosols collected at 0 cm from the nozzle (average FC concentration = 13.6 mg L^{-1}) completely inactivated the bacteria in 5-min treatment. The MLAEW aerosols collected at 25 cm from the nozzle reduced bacteria count by 0.6 – 1.9 log CFU mL^{-1} in 0.5-min treatment, by > 1.7 log CFU mL^{-1} in 2-min treatment, and by > 2.5 log CFU mL^{-1} in 5-min treatment, respectively. The MLAEW aerosols collected at 50 cm from the nozzle reduced bacteria count by 0.3 – 1.2 log CFU mL^{-1} in 0.5-min treatment, by 1.3 – 1.9 log CFU mL^{-1} in 2-min treatment, and by > 2.0 log CFU mL^{-1} in 5-min treatment.

Table 3. Inactivation of airborne total bacteria from aviary hen house by deionized water (control), original MLAEW solution (low free chlorine 'FC' concentration) and its aerosols nebulized at three air temperatures and collected at three distances from nozzle. (n=3)

Treatment agent	Air Temp.	Agent FC Conc. (±SD, mg L^{-1})	Bacteria concentration (±SD, log CFU mL^{-1})			
			Before treatment	Treatment duration (min)		
				0.5	2	5
Deionized water	-	0.3 ± 0.0	5.3 ± 0.1	5.2 ± 0.2	5.3 ± 0.1	5.3 ± 0.2
Original MLAEW	18°C	15.2 ± 1.0	5.3 ± 0.1	< 2.7[1]	ND[2]	ND
MLAEW aerosol (0 cm)		13.1 ± 0.7		< 2.7	< 2.7	ND
MLAEW aerosol (25 cm)		10.2 ± 1.0		3.4 ± 0.1	< 2.7	ND
MLAEW aerosol (50 cm)		7.3 ± 0.7		4.1 ± 0.3	3.4 ± 0.1	< 2.7
Original MLAEW	25°C	15.9 ± 0.9	5.2 ± 0.2	< 2.7	ND	ND
MLAEW aerosol (0 cm)		14.0 ± 0.5		< 2.7	< 2.7	ND
MLAEW aerosol (25 cm)		8.7 ± 1.2		4.6 ± 0.1	3.5 ± 0.0	2.7[3]
MLAEW aerosol (50 cm)		5.8 ± 1.0		4.4 ± 0.3	3.8 ± 0.4	3.2 ± 0.1
Original MLAEW	32°C	15.0 ± 0.9	5.4 ± 0.1	< 2.7	ND	ND
MLAEW aerosol (0 cm)		13.8 ± 0.7		< 2.7	< 2.7	ND
MLAEW aerosol (25 cm)		7.4 ± 0.7		4.8 ± 0.2	3.3 ± 0.2	2.7
MLAEW aerosol (50 cm)		3.0 ± 1.6		5.1 ± 0.3	4.1 ± 0.1	3.1 ± 0.3

[1] All three samples was negative by direct plating culture, but at least one of the samples was positive by enrichment.
[2] All three samples was negative by both enrichment and direct plating culture.
[3] Only one sample of the three samples was positive by direct plating culture.

The original MLAEW solutions with high FC concentrations and their aerosol samples killed all bacteria in 0.5-min treatment, except for the aerosol samples collected at 50 cm and 32°C air temperature which required 2-min treatment for complete inactivation (data not tabulated). No Gram-negative bacteria were detected in the air samples from the aviary house either by direct plating culture or by enrichment; therefore its inactivation using MLAEW was not examined.

Figure 3 shows the bacterial inactivation using MLAEW at different FC concentrations. It can be seen that MLAEW with lower FC concentrations required more contact time to increase inactivate of bacteria. Specifically, the MLAEW with FC < 10 mg L^{-1} inactivated bacteria by 62.9% (14.9 – 99.6%) in 0.5-min treatment, by 96.4% (90.7 – 99.6%) in 2-min treatment, and by 99.6% (98.3 – 100%) in 5-min treatment. More than 99% of bacteria were killed when treated by MLAEW with FC > 10 mg L^{-1} within 0.5 min. The complete bacteria inactivation (negative by enrichment) required FC concentration > 16.8 mg L^{-1} in 0.5-min treatment, > 13.9 mg L^{-1} in 2-min treatment and > 7.2 mg L^{-1} in 5-min treatment. Combining the FC loss during spraying over distance (Table 1), the following model (Eq. 2) was developed to estimate the minimum FC level

in the MLAEW for complete bacterial inactivation in aviary houses using air spray technique (aerosol cutoff diameter = 80 μm). Values in the brackets are the standard errors of coefficients.

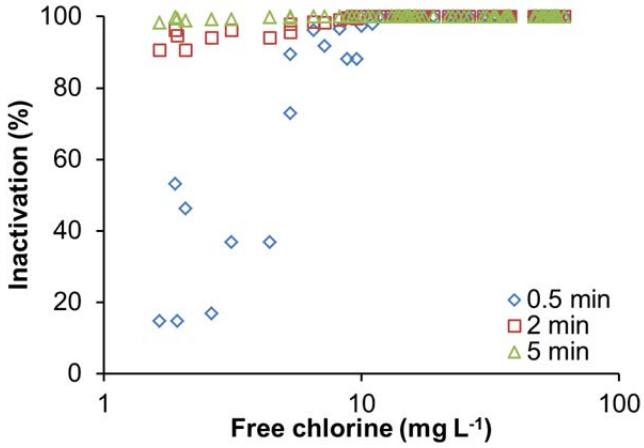

Figure 3. Inactivation of bacteria collected from an aviary hen house treated with MLAEW at different free chlorine concentrations and treatment time of 0.5, 2 or 5 min.

$$FC_{min} = 6.88(\pm 1.14)e^{0.026(\pm 0.002)D + 0.033(\pm 0.004)Temp - 0.193(\pm 0.014)Time} \quad \text{(Adjusted } R^2 = 0.89\text{)} \qquad (2)$$

FC_{min}: minimum FC concentration for complete bacterial inactivation, mg L^{-1}

D: distance between spray nozzle and target, cm

Temp: air temperature at which MLAEW is sprayed, °C

Time: contact time that is needed for complete bacterial inactivation, min

Discussion

Spray of MLAEW is increasingly used to inactivate the airborne microorganisms in animal and other agricultural/food production situations (Kim et al., 2005; Northcutt et al., 2007). However, loss of the antimicrobial components during spray and their influencing factors has not been well understood; as a result, precise management of MLAEW application is compromised. The current study investigated changes in MLAEW characteristics over spray distance under different air temperatures and initial FC concentrations, and inactivation of airborne bacteria from a commercial aviary hen house using the MLAEW. Our results show significant FC loss during the air spray. This loss must be considered in practical application since it is the sprayed aerosols, but not the original MLAEW solution, that eventually contact with microorganisms and play the antimicrobial role. Similar to the finding in the current study, Wu (2010) reported a 39.2 – 59.3% FC loss in MLAEW aerosols (diameter = 5 – 8 μm) collected at 1 m distance from the nozzle. However, direct comparison of FC loss from this current study and from Wu's study is difficult because of the different spray techniques (hydraulic vs. air) and parameters (e.g. initial aerosol size and air temperature, etc.) used in the two studies.

The FC loss during spray was further categorized into initial and traveling losses. It was found that initial losses were similar (11.7 – 13.2%) regardless of air temperature at which spray was done. This was within expectation because the initial loss is a result of sudden aerosolization near the nozzle in a short period, thus is mainly determined by spray technique and initial aerosol diameter and original, but not air temperature. In contrast, the traveling loss rate was positively related to the air temperature ($P < 0.05$), presumably because the aerosols evaporated faster at higher temperatures, which in turn increased the relative exposure surfaces of aerosols and release and decomposition of the germicidal components (Koide et al., 2009).

This positive relation would also be attributed to the fact that the aerosols were heated up faster at higher air temperature so that Cl_2 off-gas increased due to the higher chlorine vapor pressure. Specifically, we found 0.79 – 0.87 % FC loss per cm of MLAEW aerosol travel distance at 18°C versus 1.35 – 1.49 % cm^{-1} loss at 32°C. This result suggests that higher initial FC concentrations are needed when MLAEW is sprayed at a higher air temperature in order to obtain final aerosols with similar antimicrobial effect. No significant effect of initial FC concentration on FC loss was noticed.

Ammonia is a major air pollutant in poultry houses that can have adverse effects on the occupants and the ecosystem. In practice, the pH value of the MLAEW aerosols should ideally remain acidic (< 7) to suppress, or at least not stimulate, NH_3 volatilization from litter and manure. Sprayed at pH of 6.7 – 6.9, it was found the pH of MLAEW aerosols increased over 7 after spraying, likely due to release of acidic components, e.g. HCl and Cl_2, from the aerosols. This suggests that the field MLAEW application may require a pH value lower than 6.7. At the same time, it should be noted that the elevation of pH (0.3 – 0.5) during spray is quite small. Slight increase in ORP was noticed for MLAEW aerosols collected at 0 cm compared to the original MLAEW solution, following a gradually decrease while aerosols were travelling. Although ORP was suggested to be the primary factor responsible for the antimicrobial effect (Kim et al., 2000), a more recent research revealed that higher ORP did not show better germicidal effect; instead, FC played the primary role in microbial inactivation (Koseki and Itoh, 2001). In fact, it was shown that MLAEW with low ORP (238 mV) had better bactericidal activity than high ORP disinfectant at the same FC concentration (Cao et al., 2009).

Several studies have been carried out to examine bactericidal effect of MLAEW on poultry-related bacteria. While all the studies reported significant bacteria reduction by MLAEW solutions, discrepancies exist in their bactericidal effect. Cao et al. (2009) observed MLAEW (FC = 6 mg L^{-1}, pH = 6.35, ORP = 238 mV) completely inactivated *Salmonella enteritidis* within 2 min; whereas Venkitanarayanan et al. (1999) found that 10 min was needed to eliminate all *S. enteritidis* with MLAEW (FC = 43 – 48.5 mg L^{-1}, pH 2.63, ORP = 1160 mV). Similarly, for *Listeria monocytogenes*, one study reported a complete inactivation within 10 min (Venkitanarayanan et al., 1999); while another study detected quite a few bacteria after 15-min treatment with MLAEW (Fabrizio and Cutter, 2003). The discrepancies were assumed to arise from differences in experimental conditions (e.g. room temperature) and the characteristics of MLAEW used in these studies. In the current study, all total bacteria collected from the aviary hen house were killed after 0.5-min treatment with MLAEW > 16.8 mg L^{-1}, after 2-min treatment with MLAEW > 13.9 mg L^{-1}, and after 5-min treatment with MLAEW > 7.2 mg L^{-1} (pH = 6.7 – 7.2, ORP = 784 – 851 mV). Our results demonstrated that bactericidal effect of MLAEW depends on the FC level, namely, the lower the FC concentration of MLAEW, the longer the contact time required for effective inactivation.

A model (Eq. 2) was developed to predict minimal FC requirement for complete inactivation of airborne bacteria in aviary hen house as a function of air temperature, distance between nozzle and target, and contact time. It should be noted that this model was developed by assuming a perfect contact between bacteria and MLAEW (i.e., bacteria were fully immersed into MLAEW). In practical situation, the perfect contact may be not readily achieved as the MLAEW aerosols cannot capture and encompass every single bacterium targeted. Therefore, this model must be interpreted as the minimum FC requirement for practice; and future work is needed to refine and validate the model to delineate field situations.

No Gram-negative bacteria were recovered from air in the aviary house; therefore, the inactivation test could not be performed. The reasons for the negative air sample could be that the house air was free of Gram-negative bacteria, or their concentration was below the detection limit of the AGI-30 (Zhao et al., 2011b). Moreover, the number of air sampling in this

experiment was relative small, which could be another reason for the negative results. Previous studies have reported the Gram-negative only account for a small portion in the total bacteria in livestock houses (Bródka et al., 2012; Zucker et al., 2000), therefore, are more difficult to recover.

Conclusions

This study demonstrated that MLAEW is effective to inactivating airborne bacteria collected from an aviary house; however, FC loss and decreased antimicrobial effect of MLAEW aerosols occurred during spray. The decrease of the antimicrobial component and effect exacerbated over aerosol travel distance and at higher air temperatures. The MLAEW with lower FC concentration need longer contact time to inactivate the bacteria. The FC concentrations need to be at least 16.8, 13.9 and 7.2 mg L^{-1} for a complete bacterial inactivation in 0.5-, 2- and 5-min treatments under the current experimental conditions. Further verification of the lab-scale results and modification of the resultant empirical model under field conditions are needed.

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