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Full Length Article

Minimum Inhibitory Concentration of Slightly Acidic Electrolyzed Water and its Bactericidal Activity on the Surfaces of Broiler Houses

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Abstract

Spraying slightly acidic electrolyzed water (SAEW) has been considered as a novel approach to reduce microbes in poultry houses. The objective of this study is to determine the minimum inhibitory concentrations (MICs) of SAEW for the microbes isolated from broiler houses and to evaluate the effectiveness of SAEW in reducing pathogenic microorganisms on internal surfaces of broiler houses. SAEW with available chlorine concentrations (ACCs) at 10, 20, 30, 40, 50 and 60 mg/L was applied to suspension of Salmonella, *Staphylococcus aureus*, fungi, and total bacteria isolated form the surfaces of broiler houses and also directly to the internal surfaces of broiler houses by wiping or spray. The results showed that SAEW had strong antimicrobial activity against Escherichia coli, Salmonella, *S. aureus* and fungi isolated from broiler barns, and the bactericidal effectiveness increased with the increasing ACC. MIC of SAEW for Salmonella and *S. aureus* isolated form broiler barns is 20 mg/L of ACC, while it is 30 mg/L for *E. coli* and fungi. SAEW with ACC at 50 and 60 mg/L can completely inactivate the total suspended bacteria and fungi isolated from feathers and feed, respectively. SAEW significantly inactivated the total bacteria and fungi on the various surfaces of broiler barns and wiping was more effective than spraying.

Keywords: Slightly acidic electrolyzed water (SAEW); Microbes; Minimum inhibitory concentration (MIC); Disinfection

Introduction

A large amount of chemical disinfectants is used for the disinfection in farms. They are chemical toxic, corrosive and instable, which cause great concerns for livestock husbandry and product safety (Böhm 1998; Gräslund and Bengtsson 2001). Developing an alternative disinfectant that is not a biohazard to animal and human health is crucial for environmental decontamination. Slightly acidic electrolyzed water (SAEW) has been considered as an effective and environment-friendly disinfectant for reducing microbes in farms. It is produced by the electrolysis of dilute sodium chloride or/and hydrochloric acid solution in electrolytic cells without separating membranes (Anonymous 1997). SAEW is an anti-microbial agent 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' > 1,000 mV), the SAEW has a similar anti-microbial ability, but is less corrosive and easier and cheaper to produce due to its near neutral pH value (5.5–6.5) and lower ORP. In the past decade, SAEW has been increasingly gaining interest as a disinfectant in agriculture, dentistry, medicine and food industry (Sakurai *et al*. 2003; Huang *et al*. 2008; Zheng *et al*. 2012, 2016). It has been shown that SAEW can effectively inactivate microorganisms such as *Escherichia coli*, Salmonella, *Staphylococcus aureus*, yeast, mold and virus in the air and on the surfaces (Nan *et al*. 2010; Quan *et al*. 2010; Zhang *et al*. 2011; Hao *et al*. 2013a). SAEW with an available chlorine concentration (ACC) of 20 mg/L has a bactericidal effect against microorganisms in a short period of time (30 s to 60 s) (Okamoto 2006). Yang *et al*. (2011) reported that the level of total airborne bacteria was reduced by 70% by spraying SAEW in a cage hen house. Zhao *et al*. (2014), Hao *et al*. (2013b) and Ji *et al*. (2017) also found SAEW have antimicrobial effect on airborne bacteria from poultry house. Wu (2010) and Hao *et al*. (2013c) found a reduction of 98% in total bacteria and 68% in fungi after spraying SAEW in a swine house. As animal drinking water, SAEW can also be added into drinking water for animals to promote the intestinal health and improve the production performance of animals (Bodas *et al*. 2013; Bügener *et al*. 2014). However, there is little published information regarding the minimum inhibitory concentrations (MICs) of

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SAEW for the microbes isolated from broiler houses and its effectiveness in reducing pathogenic microorganisms on internal surfaces of broiler houses.

The objective of this study is to 1) determine the MICs of SAEW for the Salmonella, *S. aureus,* fungi, and total bacteria isolated from broiler houses and 2) to evaluate the effectiveness of SAEW in reducing the total bacteria and fungi on internal surfaces of broiler houses.

Materials and Methods

SAEW preparation

SAEW at available chlorine concentration (ACC) of 10, 20, 30, 40, 50 and 60 mg/L was generated by electrolyzing 9% (w/v) HCl solution using an SAEW generator (Beijing Zhouji Ziyuan Huanbao Technology Co. Ltd., Beijing, China). HCl solution (9%) was diluted with tap water before electrolysis in an electrolysis cell to produce SAEW. The pH and oxidation-reduction potential (ORP) values of SAEW were measured using a dual scale pH/ORP meter (Shanghai Kangyi Technology Co. Ltd., Shanghai, China) bearing pH and ORP electrodes. The ACC of SAEW was determined by a colorimetric method with a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corp., Saitama, Japan). Sterilized distilled water (ACC 0 mg/L) was used as the control. The properties of SAEW are shown in Table 1.

Collection and preparation of bacterial cultures

The types of microorganisms and its collection media used in this study are shown in Table 2. Samples were collected by wiping the surfaces of floor, walls, glass, trough, sink and iron wires and equipment using sterilized cotton swabs soaked in sterilized saline, respectively. Each sampling area was 2 cm^2 which was wiped 20 times repeatedly. The cotton swab head was removed and dropped into 10 mL sterilized saline. The supernatant was collected after intensive mixing. The collected suspensions were spread on the plates with sterilized medium described in Table 2, respectively. All the plates were cultured at 37°C for 48–72 h, except that the fungi plates were cultured at 28°C. A single colony of each of the typically grown strains was then picked and streaked to the new plates. This selection steps were repeated until pure colonies of each strain were confirmed. The pure colonies were stored at 4°C before use.

Minimum inhibitory concentration determination of SAEW

Each isolated bacterial strain was suspended in sterile physiological saline and inoculated to a plate count agar (PCA) with gradient method. The plates were cultured at 37°C for 48–72 h, except that the fungi plates were cultured at 28°C. Then the number of colonies was counted. The concentration of microorganisms for each bacterial suspension was calculated using Eq. 1.

$$P = 50N \cdot 10^x / L \qquad (1)$$

Where, P is the total number of microorganisms for each bacterial suspension, CFU/mL; N is the average number of colonies per plate, CFU; x is the dilution ratio; L is the inoculation volume, mL.

A 0.1 mL aliquot of each bacterial suspension with the determined concentration was mixed with 0.9 mL SAEW at ACC of 10, 20, 30, 40, 50 and 60 mg/L, respectively. Another 0.1 mL liquid was mixed with sterile distilled water as the control. After mixing for 5 min, 1 mL mixture was added to 9 mL 0.1% sodium thiosulfate solution for another 5 min mixing. Then 0.1 mL of each mixture was inoculated on PCA. The number of microbes in each bacterial suspension after sterilization was also calculated using Equ.1. The sterilization rate was calculated using Eq.2.

$$\text{Sterilization rate (\%)} = 100\,(Pc-P_T)/Pc \qquad (2)$$

Where, Pc is the total number of microbial colonies before disinfection, CFU/mL; P_T is the total number of microbial colonies after disinfection, CFU/mL.

Manure, feed, dust, 1g for each, and 10 chicken feathers were collected. These 4 samples were placed into a tube with 100 mL of sterile saline to make a bacterial suspension. A 1 mL aliquot of the suspension was used to determine the sterilization rate for total bacteria and fungi, respectively.

Disinfection of the surfaces

After routine cleaning of the broiler barn, SAEW with ACC at 0 (the control), 10, 20, 30, 40, 50 and 60 mg/L were used for wiping or spraying on the internal surfaces of the broiler house, respectively, including ground, walls, troughs, metal wires. Microbial samples were collected 5 min before and after disinfection, respectively, using the same method of wiping the surfaces. The sterilization rate was calculated using Equ.2.

Results

Microbicidal effect on microorganism isolated from broiler barns

The microbicidal activity of SAEW on pure microbes isolated from broiler barns is shown in Table 3. SAEW with an ACC of ≥ 30 mg/L could inhibit the growth of *Escherichia coli* and fungi isolated from broiler barns. For *Salmonella* and *S. aureus*, SAEW with an ACC ≥ 20 mg/L could inhibit their growth. After a treatment with SAEW at ACC 20 mg/L, *E. coli* and fungi could still grow a small number of colonies., but could be completely inhibited

Table 1: Main characteristics of the slightly acidic electrolyzed water

ACC mg/L	pH	ORP, mV
0	5.67 ± 0.13	1012.6 ± 12.4
10	6.01 ± 0.11	1008.3 ± 21.5
20	5.89 ± 0.08	1003.7 ± 29.4
30	5.83 ± 0.09	1007.1 ± 47.7
40	5.88 ± 0.11	999.3 ± 40.1
50	6.07 ± 0.14	998.4 ± 29.0
60	6.07 ± 0.07	1002.5 ± 13.9

Table 2: Microbial groups isolated form the broiler house and the selective media

Microbe	Media
Total bacteria and fungi	Nutrient Agar (NA)
E. coli	Mc Conkey (MCA)
Salmonella	SS agar
Staphylococcus aureus	BCP Medium
Fungi	Sabaurauds agar (SDA)

Table 3: Efficiency of slightly acidic electrolyzed water for inactivation of microbes isolated form chicken barns

Microbes	Colonies (log_{10}CFU/mL)	SAEW concentration (mg/L)						
		0	10	20	30	40	50	60
E. coli	5.12±0.11	+++	+++	+--	---	---	---	---
Salmonella	4.79±0.17	+++	+++	---	---	---	---	---
S. aureus	5.77±0.21	+++	+++	---	---	---	---	---
fungi	6.23±0.10	+++	+++	+--	---	---	---	---

The processing time for all treatments is 5 min. + means there was still microbial growth after plating. - no microorganisms growth after plating and after re-plating. +++, -- --, ++- and +-- represent the results of three consecutive operations

by SAEW with an ACC at 30 mg/L. It shows that SAEW with an ACC of 30 mg/L can completely inhibit the growth of *E. coli*, Salmonella, *S. aureus* and fungi isolated from broiler houses, which is consistent with the results of previous studies (Cao *et al*. 2009; Guentzel *et al.* 2010; Nan *et al*. 2010).

The microbicidal efficiency of SAEW treatment on total bacteria and fungi isolated from faces, feathers and feed was shown in Table 4, respectively. It shows an increasing trend along with ACC. The treatment with SAEW at 20 mg/L ACC for 5 min inactivated 50.51% microorganisms in the fecal suspension. The sterilization rate increased to 83.64 and 99%, when using SAEW with an ACC of 30 and 40 mg/L, respectively. The sterilizing effect of SAEW for microorganisms in feed also increases with increasing ACC. SAEW with an ACC of 10 mg/L inactivated 55.36% microorganisms in the feed suspension. With the ACC at 30 and 40 mg/L, the SAEW sterilization rate could reach to about 95 and 99%, respectively. SAEW with an ACC of 60 mg/L could completely sterilize all the microorganisms in the feed suspension. Great antimicrobial effect on microbes by SAEW on broiler chicken feathers could also be proved. The sterilization rate reached 94.96% at the ACC of 20 mg/L and the microorganisms in the feather suspension was completely eliminated at the ACC of 20 mg/L.

Table 4: The number of colonies in suspension of dust, faces, feathers and feed before and after SAEW treatment

Sources	ACC (mg/L)	Colonies (CFU/L)		Sterilization rate ± SE (%)
		Before	After	
Faces	10	1.98×10^7	1.56×10^7	21.21 ± 9.89
	20		9.80×10^6	50.51 ± 23.00
	30		3.24×10^6	83.64 ± 31.97
	40		2.08×10^4	99.89 ± 11.03
	50		<1	99.99
	60		<1	99.99
Feed	10	2.24×10^3	1.00×10^3	55.36 ± 29.09
	20		8.71×10^2	61.12 ± 18.88
	30		115	94.87 ± 6.68
	40		6.88	99.99 ± 22.04
	50		<1	99.99
	60		Not detectable	100.00
Feather	10	6.83×10^5	4.73×10^5	30.75 ± 12.12
	20		3.44×10^4	94.96 ± 4.99
	30		8.81×10^2	99.87 ± 26.72
	40		<1	99.99
	50		Not detectable	100.00
	60		Not detectable	100.00

The processing time for all treatments is 5 min

Table 5: Sterilization effect of SAEW for surface disinfection in broiler barns

Surface	ACC (mg/L)	Wipe		Spray	
		Inactivation Rate (%)	Standard Error (±)	Inactivation Rate (%)	Standard Error (±)
Floor	30	89.87a	9.27	76.44b	20.05
	40	95.77a	13.02	85.13b	14.02
	50	98.00a	21.45	90.18b	6.67
	60	100.00a	11.53	97.03a	10.31
Wall	30	95.23a	8.98	93.00a	6.96
	40	94.12a	10.17	94.42a	6.11
	50	100.00a	8.66	94.92a	8.33
	60	100.00a	20.05	99.00a	9.95
Glass	30	98.76a	13.02	86.88b	7.92
	40	99.77a	11.45	90.69b	13.58
	50	100.00a	11.53	97.00a	15.22
	60	100.00a	8.98	100.00a	8.87
Feed trough	30	92.23a	10.17	77.00b	9.65
	40	94.12a	8.66	79.12b	8.19
	50	98.55a	12.80	90.92b	15.52
	60	100.00a	13.02	96.07a	11.71
Sink	30	91.99a	21.45	69.67b	8.40
	40	95.77a	11.53	75.58b	13.11
	50	98.00a	8.98	90.16b	10.45
	60	100.00a	10.17	92.70b	11.43
Iron wire	30	90.00a	8.66	73.03b	8.98
	40	94.12a	18.05	85.49b	10.04
	50	97.92a	6.26	94.34a	8.76
	60	99.00a	8.13	98.10a	9.05

All samples were collected 5 min after disinfection. In the same line, values with different small letters mean significant difference at $P < 0.05$

Disinfection efficiency on the surfaces

Table 5 shows the sterilization effects of SAEW on the internal surfaces of the broiler house. As shown in Table 5, the sterilization effect on the internal surfaces of the broiler barn increased with the increasing ACC. However, the sterilization effect differed from the disinfection method. The disinfection effect of each method varied with the different conditions of the surface. Wiping using SAEW at an ACC of 30 mg/L inactivated 93.01% microbes on average on the surfaces of various facilities. The order of effectiveness from the highest to the lowest was: glass (98.76%) > wall (95.23%) > feed trough (92.23%) > water trough (91.99%) > metal wire (90.00%) > ground (89.87%). the average inactivation rate was only 78.99% when spraying SAEW with ACC of 30 mg/L. the average sterilization rate could reached to 92.92% for SAEW with ACC of 50 mg/L. The order of sterilization rate from the highest to the lowest was: glass (97.00) > wall (94.92%) > iron wire (94.34%) > feed trough (90.92%) > ground (90.18%) > sink. It indicated that facilities surface material and disinfection methods have impacts on the bactericidal effect of SAEW.

Discussion

SAEW with germicidal activity is generated by electrolyzing a dilute salt solution or dilute hydrochloric acid solution el. Its available chlorine exists in the form of hypochlorous acid (HClO) and hypochlorite (ClO^-). Due to the slightly acidic conditions (5.5–6.5), the available chlorine is mainly in the form of HClO and the sterilization effect of HClO is about 80 times that of ClO^- (Anonymous 1997). It has been reported that SAEW can effectively sterilize microorganisms such as *Escherichia coli*, Salmonella, *S. aureus*, yeasts, molds and viruses that exist on the surfaces and in the air (Nan *et al*. 2010; Quan *et al*. 2010; Zhang *et al*. 2011; Hao *et al*. 2013a).

The results of this study confirmed that SAEW has a great germicidal effect for disinfection in broiler barns. A treatment for 5 min with SAEW at ACC of 20 mg/L could completely inhibit the growth of *Salmonella* and *S. aureus* isolated from broiler barns. At an ACC of 30 mg/L, SAEW could completely sterilize *E. coli* and fungi. It shows that a minimum of 20 mg/L ACC is required for sterilization of *Salmonella* and *S. aureus* with a 5 min treatment time for a separated strain of microbes suspended in saline, while the ACC is 30 mg/L for *E. coli* and fungi isolated from broiler barns. This result is slightly different from previous reports in which SAEW with ACC at 10 mg/L for 5 min could completely suppress the growth of *E. coli*, Salmonella and *S. aureus* (Cao *et al*. 2009; Nan *et al*. 2010) and SAEW with ACC at 25 mg/L could inhibit fungal growth after 10 min treatment (Guentzel *et al*. 2010). The reason for the difference may be due to individual differences of the microorganisms from different regions or the difference of the organic content in the germ suspension extracted from the sample. Because the ACC in SAEW decreases with the increase of organic matter in the solution, this will weaken its germicidal ability (Park *et al*. 2009). A slight difference in the minimum inhibitory concentration of SAEW required for inactivation was also observed for the same microbial strain from different sources in broiler barns. For example, SAEW with an ACC of 30 mg/L inactivated 83.64% of the microorganisms in the feces suspension, 94.87% in the suspension of the feed and 99.87% in the suspension of feathers. When the ACC reached 50 mg/L, SAEW killed all the microbes in the suspension of feathers. At ACC 60 mg/L, SAEW killed all microorganisms in the feed suspension. These observations indicate that there is a difference in the tolerance of microorganisms from different sources to SAEW. To reach a same inactivation rate, a higher ACC is required to treat the germs in feces suspension, which may be related to the different microbial species in different samples.

The inactivation rate of SAEW is affected by the method application of wiping and spraying when used for sanitizing the surfaces in broiler barns. The results showed that using SAEW with ACC at 30 mg/L, the wipe method could inactivate 93.01% of microbes on average for various surfaces in broiler barns, while the spray method could only inactivate 78.99% of the germs. The materials in facilities also has an impact on the sterilization rate. The reason for the difference in sterilization rates between the two methods was that the spray disinfection operation itself leads to the loss of available chlorine in SAEW (Hsu *et al*. 2004), thereby reduces the effectiveness of disinfection and sterilization on the surfaces in the facility.

Conclusion

SAEW has strong germicidal activity against *Escherichia coli*, Salmonella, *S. aureus* and fungi isolated from broiler houses. The germicidal effect of SAEW increases with increasing ACC. After a treatment for 5 min, SAEW with ACC at 20 mg/L could completely inhibit the growth of *Salmonella* and *S. aureus* isolated from broiler house. With ACC at 30 mg/L, SAEW completely inhibited the growth of *E. coli* and fungi. For mixed microbes of different sources from broiler barns, SAEW with an ACC of 50 mg/L could kill all the microorganisms in the suspension of feathers. SAEW with an ACC at 60 mg/L could sterilize all microorganisms in the feed suspension. To completely kill microorganism in feces, a higher ACC is required. SAEW could significantly reduce the microbial content on the surface of facilities in broiler houses, but its sterilization effect was affected by the application method. Wiping using SAEW with the ACC at 30 mg/L could sterilize 93.01% of germs, while spraying SAEW could averagely sterilize only 78.99% of the germs, vindicating that the wiping method is more effective than the spraying method in surface disinfection.

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