Research Note: Evaluation of the efficacy of engineered water nanostructures in inactivating airborne bacteria in poultry houses

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ABSTRACT Methods to control microbial contamination in confined livestock facilities are important to the health of both animals and workers. In addition, bacterial contamination is also a food safety issue. The most common disinfection technique employed in livestock facilities is the application of oxidizing agents (e.g., potassium peroxymonosulphate, chlorine, hydrogen peroxide, ozone). However, these techniques are associated with a number of limitations (e.g., toxicity, high cost, corrosiveness). Recently, engineered water nanostructures (**EWNS**) generated using an electrospray system was found effective in inactivating foodborne bacteria. Thus, this study investigated the efficacy of EWNS generated using a laboratory-scale electrospray system in inactivating bacteria found in poultry facilities. The effects of various operating conditions (distance between the injector and grounded electrode of the electrospray system, applied voltage, liquid pH and conductivity, liquid flow rate, and treatment time) on the efficacy were also assessed. In these various experiments, airborne bacterial samples were collected from a pullet room using tryptic soy agar plates and then exposed to EWNS under varying conditions. After treatment, the plates were incubated at 37°C prior to colony counting. Reductions in bacterial concentrations up to 1.26 logs were obtained. The results indicate that the EWNS generated by the electrospray system can be a potential chemical-free alternative to conventional disinfection methods. Future tests will focus on scaling up the system for larger scale trials.

Key words: electrospray system, engineered water nanostructures, microbial inactivation, poultry house

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INTRODUCTION

Microbial contamination in livestock facilities can have a devastating impact on the industry and on public health. Foodborne salmonellosis, for instance, which is commonly associated with consumption of poultry products (Hugas and Beloeil, 2014), was estimated to cause 93.8 million illnesses and 155,000 deaths worldwide each year based on the data collected from 1966−2007 (Majowicz et al., 2010). Thus, effective microbial decontamination is an important consideration in food production.

Current microbial decontamination techniques employed in livestock facilities include disinfection with oxidizing agents (e.g., potassium peroxymonosulphate (active ingredient in Virkon), chlorine, ozone, hydrogen peroxide) (Zhao et al., 2006), fogging with organic acids, spraying with slightly acidic electrolyzed water (Hao et al., 2013), and ultraviolet irradiation (Cossu et al., 2018). However, these techniques are associated with a number of limitations, such as high energy cost, toxicity on animals and humans, and potential damage of materials (Vaze et al., 2018). Recently, engineered water nanostructures (**EWNS**) have been reported for foodborne bacteria inactivation (Pyrgiotakis et al., 2016). EWNS are generated through a combined process of electrospray and ionization using an electrospray system, wherein a high electric field created between a metal capillary holding the water and a counter electrode causes the water droplet at the tip of the capillary to break into fine particles. The process

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generates a large amount of reactive oxygen species (**ROS**) (e.g., hydroxyl and superoxide radicals) embedded in the droplets, which are responsible for the oxidizing and biocidal properties of the EWNS (Vaze et al., 2018). In contrast to electrolyzed water whose droplets are generally in micron range, the sizes of the EWNS droplets are in nanoscale (average diameter ~ 25 nm) (Pyrgiotakis et al., 2014); hence, they can be extremely mobile and remain airborne for hours. In addition, inhaled EWNS were found to be toxicologically benign (Pyrgiotakis et al., 2014), and the process leaves no chemical residues as it mainly uses water. A previous study obtained reductions up to 3.8 logs (99.98%) of foodborne bacteria after treatment with EWNS (Pyrgiotakis et al., 2016). However, although the EWNS have shown potential for microbial inactivation, they have not yet been tested on bacteria found in livestock facilities.

Thus, this study aimed to investigate the efficacy of EWNS generated using a laboratory-scale electrospray system in inactivating airborne bacteria in a poultry house and assess the effects of various operating conditions (distance between the injector and grounded electrode of the electrospray system, applied voltage, liquid pH and conductivity, liquid flow rate, and treatment time) on the efficacy.

MATERIALS AND METHODS

Electrospray System

In this study, the electrospray system consisted of a 30-G needle (Metal Hub, Fisher Scientific, Ottawa, Ontario, Canada) with an inner diameter of 0.159 mm and an outer diameter of 0.311 mm, coupled to a 2.5 mL syringe (1000 series Gas Tight, Hamilton, Reno, NV). The size of the needle used was chosen based on the diameters of the droplets produced in a preliminary trial; needles with smaller diameter produced smaller size droplets. The liquid flow rate through the needle was controlled using a syringe pump (NE-1000, New Era Pump Systems Inc., Farmingdale, NY). As shown in Figure 1, the pump was mounted on top of a plexiglass chamber (35 cm × 35 cm × 35 cm). The needle was connected to a high voltage power supply (APM-30KIPNX, Kasuga Denkie Inc., Japan). The counter electrode (5.9 cm in diameter and 0.1-cm thick, made of alumina) was grounded and positioned underneath the needle. The counter electrode was supported by an adjustable polyvinyl chloride (**PVC**) platform so that its distance from the tip of the needle could be varied.

Airborne Bacteria Collection and EWNS Treatment

Airborne bacterial samples were collected by exposing (passive sampling) tryptic soy agar (**TSA**) plates (DF0369176, BD Difco, Fisher Scientific, Ontario, Canada) to the air inside a pullet house of the Poultry Research and Teaching Unit of the University of Saskatchewan for five minutes. After sample collection, the agar plates were placed in a sterile Ziploc bags and brought to the laboratory. In each agar plate, a small portion of the agar (approximately 1.5 cm × 1.5 cm)

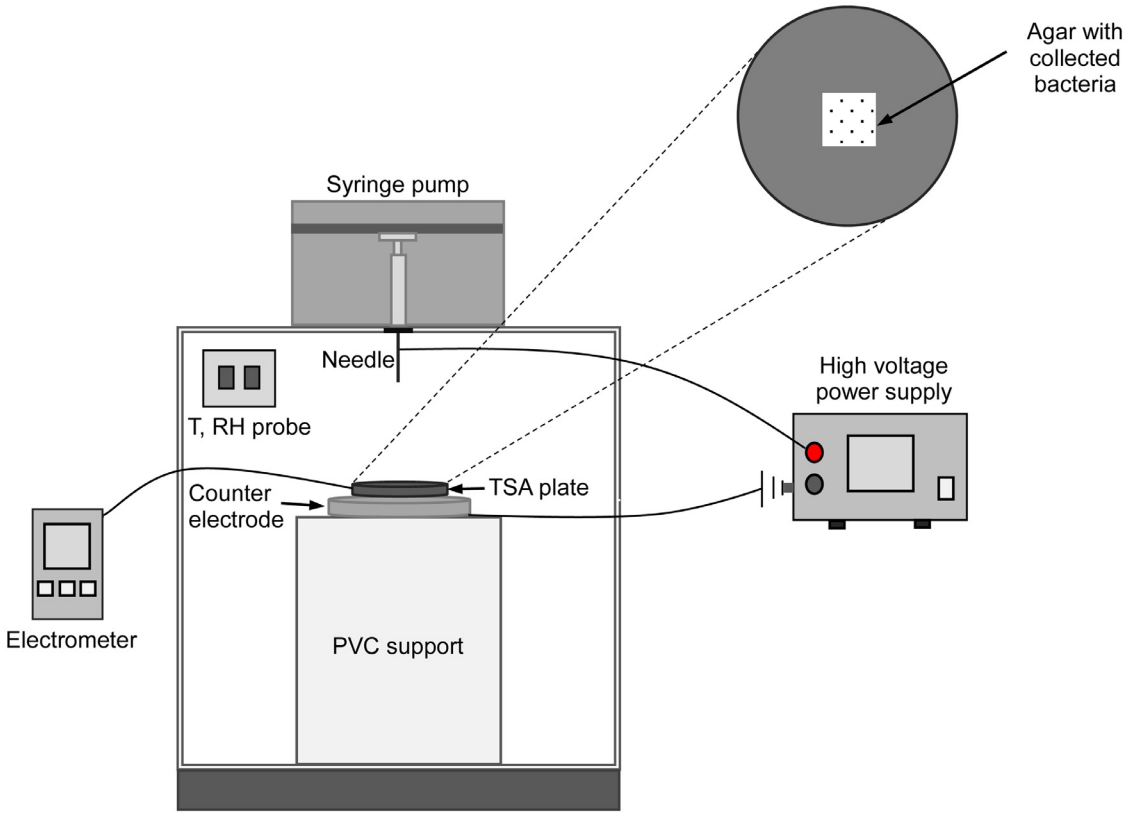

Figure 1. Schematic diagram of the experimental setup.

Table 1. Operating conditions evaluated and efficacy of the EWNS under each operating condition.

Parameter	Levels tested[a]	Mean log reduction ± S.D. (log)	Mean percent reduction ± S.D. (%)
Voltage (kV)	+6.6	0.68 ± 0.08	78.8 ± 3.8
	−6.6	0.72 ± 0.15	79.6 ± 7.8
	−7.6	1.12 ± 0.25	91.5 ± 4.2
Distance between needle tip and counter electrode (cm)	2	1.26 ± 0.25	93.8 ± 3.9
	3	0.68 ± 0.16	77.9 ± 8.1
	4	0.62 ± 0.38	70.9 ± 17.9
Liquid flow rate (μL/min)	1	0.44 ± 0.09	62.7 ± 8.2
	2	0.72 ± 0.15	79.6 ± 7.8
	4	0.44 ± 0.12	62.2 ± 10.8
Conductivity (mS/cm)	0.06	0.69 ± 0.07	79.2 ± 5.4
	0.20	0.72 ± 0.15	79.6 ± 7.8
	14.72	0.94 ± 0.20	87.9 ± 5.4
pH	**7.0**	0.72 ± 0.15	77.9 ± 8.1
	8.5	1.13 ± 0.24	91.7 ± 3.6
	10.0	0.91 ± 0.16	86.9 ± 4.9
Treatment time (min)	15	0.46 ± 0.06	65.4 ± 10.1
	25	0.68 ± 0.16	77.9 ± 8.1
	35	0.84 ± 0.08	85.5 ± 2.6

[a]Bold numbers were the baseline operating parameters (parameters that were maintained, while the specific parameter to be evaluated was varied).

was cut and retained in the plate, while the other portion was discarded. The remaining agar in the plate was placed directly under the needle of the electrospray system for EWNS treatment at various operating conditions listed in Table 1. Milli-Q water (ultrapure water; MilliporeSigma, Ontario, Canada), reverse osmosis (**RO**) water, and saline water (0.1 g of NaCl in 75 mL RO water) were used to obtain liquids with conductivities of 0.06, 0.20, and 14.72 mS/cm, respectively. A liquid with a pH of 7.0 was obtained by using RO water, while those with pH of 8.5 and 10.0 were obtained by adding appropriate amount of NaOH to RO water. The effects of the operating parameters were evaluated by keeping the baseline operating conditions (the bold numbers in Table 1) constant, while the specific parameter to be evaluated was varied. For instance, in evaluating the influence of liquid flow rate, the flow rate was varied from 1 to 4 μL/min, while the pH, conductivity, distance, and applied voltage were maintained at 7, 0.20 mS/cm, 3 cm, and -6.6 kV, respectively. For each condition, 6 agar plates were used: 3served as treatment (exposed to the EWNS) and three served as control (not exposed to the EWNS or any water droplet). Further, each operating condition was performed in triplicate. After treatment, the treated agar plates were placed in an incubator (650D, Fisher Scientific, Canada) at 37°C for 8 h prior to colony counting. The mean chamber temperature and relative humidity during the entire trials were approximately 20°C and 25%, respectively.

Data and Statistical Analyses

The efficacy of the EWNS in inactivating the collected poultry barn bacteria under each operating condition was assessed using a modified log reduction equation described in Equation 1 and percent reduction equation described in Equation 2.

$$log\ reduction = Abs\left[Log_{10}\left(\frac{\frac{CFU\ in\ A_{eff}}{A_{eff}}}{\frac{CFU\ in\ A_{control}}{A_{control}}}\right)\right], \quad (1)$$

$$\%\ reduction = \left[1 - \left(\frac{\frac{CFU\ in\ A_{eff}}{A_{eff}}}{\frac{CFU\ in\ A_{control}}{A_{control}}}\right)\right] \times\ 100, \quad (2)$$

Where $A_{control}$ is the area of the control agar plate (approximately 225 mm^2), A_{eff} is the effective area, which is the area in the treated agar covered with the nanodroplets, CFU in A_{eff} is the number of colony forming units (**CFU**) in the effective area, and CFU in $A_{control}$ is the number of CFU in the control area. Depending on the operating condition, the effective area varied, which ranged from 5.1 mm^2 to 96.9 mm^2; hence, modified log and percent reduction equations were used to consider this variation.

Analysis of variance (**ANOVA**) and Student's t test (SPSS v.26; IBM Corp., NY) were used to determine significant differences between the various operating conditions.

RESULTS AND DISCUSSION

The EWNS generated by the electrospray system developed in this study reduced the poultry barn bacteria by up to 1.26 logs or 94% (Table 1), which is higher than the 59% obtained by Hao et al. (2013) using slightly acidic electrolyzed water. Although the negative charging (−6.6 kV) resulted in relatively higher mean log reduction (0.72 log) than the positive charging (+6.6 kV), which resulted in 0.68 log, the difference was not significant ($P > 0.05$). However, the −7.6 kV applied voltage resulted in significantly higher log reduction ($P < 0.05$) than the −6.6 kV, indicating that increasing the applied voltage could result in higher inactivation, which could be attributed to enhanced ROS production at higher voltage levels.

The results show that increasing the distance between the needle tip and the counter electrode from 2 cm to 4 cm decreased the log reduction from 1.26 to 0.62; however, there was no significant deference ($P > 0.05$) between 3 cm (0.68 log) and 4 cm (0.62 log). A shorter distance between needle tip and counter electrode results

in higher current and more electrical charges due to higher electric field (Si et al., 2021), thus producing more ROS.

The 2 μL/min liquid flow rate resulted in significantly higher log reduction (0.72 log) than the other 2 flow rates (1 and 4 μL/min) ($P < 0.05$). Si et al. (2021) found a higher *Escherichia coli* reduction on stainless steel coupons at 1 μL/min than at 2 and 4 μL/min. The difference on the results could be due to the type of surfaces or the bacteria present.

There was no significant difference ($P > 0.05$) on the log reductions obtained from the three liquids (i.e., RO, Milli-Q, and saline water) with different conductivities at the range tested. The results also show that higher pH resulted in higher log reduction; however, no significant difference was observed between pH 8.5 and pH 10. Khan et al. (2018) found that higher liquid pH could result in higher ROS production. Moreover, longer exposure or treatment time resulted in higher bacterial inactivation as the bacteria experience more protein and DNA damage at longer exposure to EWNS (Vaze et al., 2018).

The results indicate that EWNS can deactivate common poultry barn bacteria. Moreover, the results could provide insights for larger scale evaluation of the efficacy of EWNS in decontaminating livestock facilities.

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DISCLOSURES

The authors declare that there is no conflict of interest.

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