# Applying the Ozone Water Spraying for Inactivating *E. Coli* bioaerosols

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**Abstract.** This work applied ozone water spraying to explore the feasibility of inactivating bioaerosol. The inactivating efficiency using ozone water (OW) spraying individually against airborne strains *Escherichia coli* (*E. coli)* bioaerosols was evaluated in the testing chamber. The setting air exchanged rate (ACH) in this work were 0.5 and 1.0 $h^{-1}$. The OW operating concentration of 0.5 and 1.0 ppm were sprayed into the testing chamber for inactivating bioaerosols, respectively. Higher OW spraying concentration showed better inactivating results. The inactivation constant of *E.Coli* against WO 0.5 and 1.0 ppm spraying under ACH 1.0 $h^{-1}$ was 0.236 and 0.435 ($min^{-1}$). Moreover, the bioaerosol removal efficiency would increase with ACH. The inactivation decay coefficient of WO 1.0 ppm spraying against *E.Coli* was 0.312 and 0.435 ($min^{-1}$) under ACH 1.0 and 0.5 $h^{-1}$. The results demonstrated that the WO spraying technology was effective in inactivating bioaerosols.

## 1. Introduction

Nowadays, biological pollution in indoor air has a significant impact on human health in the world[1].Exposure to biological contamination of bacteria and related biotoxins in indoor environments may lead to sick building syndrome (SBS), allergic rhinitis, respiratory tract irritation, and asthma[2-3]. Over the past few decades, numerous strategies and technologies for removing indoor bioaerosols have been developed according to the health needs. Thus, various bioaerosol-cleaning techniques, including electret[4], ozone[5], ultraviolet germicidal[6], photocatalyst[7], negative air ions[8], plasma [9], and membrane-less electrolyzed water spraying[10] are applied to remove indoor bioaerosols.

In this study, the ozone water spraying has been applied in indoor bioaerosols inactivating. In recent years, ozone water has been used for disinfection of food and agricultural products. The advantage of ozone water is that its high oxidizing ability can effectively inactivate microorganisms. Several studies indicated that bacteria were inactivating by using ozone water under various conditions[11-12]. Restaino et al. (1995) studied the antimicrobial ability by using the ozonated water against gram-positive and gram-negative bacteria [14]. The ozonated water could effectively inactivate *Salmonella typhimurium* and *Escherichia coli* cells. However, this technology is mainly used for food and agricultural sterilization applications, and is rarely used for bioaerosols inactivating in indoor environments. In the past, the ozone in gas phase was directly used to inactivate bioaerosls in indoor

environments. But this method will cause excessive ozone concentration in indoors. Using ozone water spray technology, ozone is coated in the water film, which can solve the problem of excessive ozone concentration. The goal of this work is to investigate the inactivating ability of ozone water spraying against bioaerosols under various operating factors, ozone water concentration and ventilation rate in simulated-environment chamber.

## 2. Experimental methods

**Generation of ozone water spraying.** The ozone water is generated by laboratory's hand-made equipment in this work. Ozone aqueous solution is diluted with high concentration ozone water and deionlized water (Milli-Q, Millipore, Billerica, MA, USA). The ozone water concentration was setting on 0.5 and1.0 ppm. The ozone water was pumped and sprayed in the testing chamber with the working pressure of 70 kg/cm$^2$. The ozone water spraying was generated by passing through 4 μm orifice diameter nozzle.

**Bacterial suspension preparation.** *Escherichia coli* K12S (BCRC 14894) was used as challenging bioaerosols in the study. 100 μL aliquot of *Escherichia coli* bacterial pure culture was transferred and grown overnight in 10 mL tryptic soya broth (BactoTM TSB, BD, Dickinson and Company, NJ, USA) at 37 ± 1$^o$C to prepare suspensions (equivalent to 10 colony forming unit (CFU)/mL). The suspensions were poured into a tube and centrifuged at 7,000 rpm in 10 minutes. Resulting pellet was resuspended in 10mL sterile deionlized water (Milli-Q, Millipore, Billerica, MA, USA) and centrifuged again at the same conditions. The final pellet of the bacteria cells (equivalent to 107 CFU/ml) from the second centrifugation was suspended in 10 mL pre-sterilized water for subsequent chamber experiments.

**Experimental system.** Figure.1 depicts the bioaerosol inactivating experimental system. The system was including bioaerosol nebulizer, charge neutralizer, makeup air equipment, ozone water spraying device and bioaerosol sampler. The challenging bacterial strains were aerosolized by a three-jet Collison nebulizer (BGI Inc., Waltham, MA). The bacterial bioaerosols were dried by the diffusion dryer. The dried bioaerosols then passed through Kr-85 neutralizer (model 3077, TSI Inc.) to neutralize themselves become Boltzmann charge equilibrium. After passing through the Kr-85 neutralizer, the bioaerosols would flow into the stainless-steel simulated-environmental chamber($80 \times 80 \times 80$ cm$^3$). This system applied makeup air system for simulating the real indoor environments. These systems included two fans and one pump for controlling and maintaining the he stable airflow and total air exchange rate (ACH) in the testing chamber. Two total ACH parameters, 0.5 and 1.0 h$^{-1}$ were chosen for the testing. The relative humidity (RH) inside the testing chamber was 30%, which was controlling the ratio of dry gas stream flow rate to humidified gas stream flow rate. The humidified gas stream was generated by a water vapor saturator.

Bacterial bioaerosols were collected by BioStage single-stage viable cascade impactor (SKC Inc., USA) with tryptic soya agar (BactoTM TSA, BD, Dickinson and Company, NJ, USA). In each bioaeosols sampling, the bioaerosol impactor was operated at flow rate of 28.3 L/min and sampling time of 30 seconds. For each sample, three replicates were performed. To calculate the inactivating efficiency of bioaerosols and the initial testing bioaerosol concentrations, the time-bioaerosol concentration curve in the testing chamber was built by continuously delivering bioaerosols and collecting the samples at 30 minute intervals. The first-order decay constants of bioaerosols concentrations without and with using the ozone water spray in the testing chamber were defined as natural decay constant ($k_n$) and inactivation constant ($k_a$).

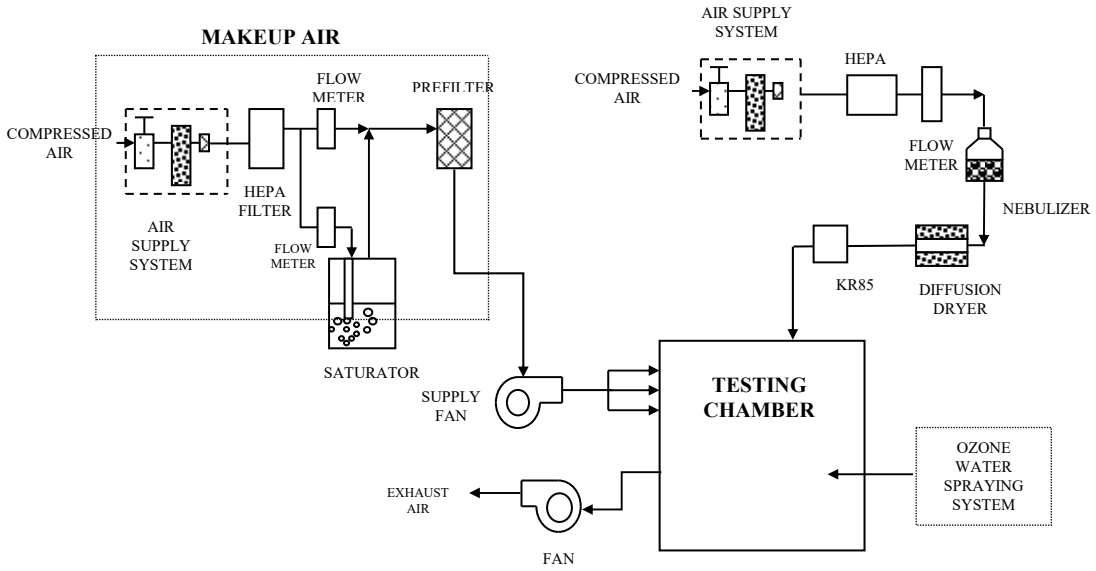


Figure1. Schematic diagram of experimental setup

## 3. Results and discussions

**The concentration curve of *E. Coli* bioaerosols in test chamber.** Figure 2 shows the linear relationship between *E. Coli* bioaerosol concentration and delivery time. After 50 minutes of delivery, the *E. coli* bioaerosol concentration can reach about $3 \times 10^4$ CFU/m$^3$. This concentration is enough for the bioaerosol removal or inactivating in the testing system. Therefore, the initial *E coli* bioaerosol concentrations of $3 \times 10^4$ CFU/m$^3$ were setting for each experiments of natural decay, air exchange, and onoze water spraying inactivating.

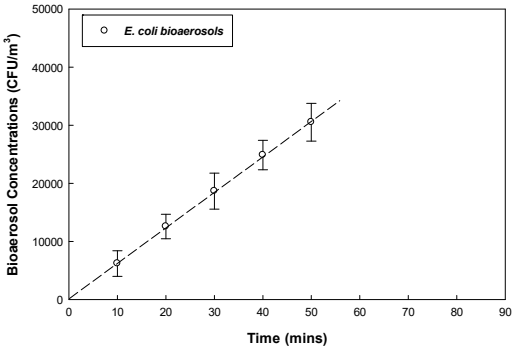


Figure 2. Concentration curve of *E. Coli* bioaerosols delivery in the test chamber

**The natural decay for *E. Coli* bioaerosols.** Figure 3 shows the *E. Coli* bioaerosols' decay behavior under the different ACH in the testing chamber. The natural decay constants ($k_n$) of *E. coli* bioaerosols under ACH of 0, 0.5 and 1.0h$^{-1}$ were 0.012, 0.098 and 0.185 (min$^{-1}$), respectively. This result indicated increasing fresh air volume in the testing chamber performs in significantly bioaerosols removal. When ACH is 0 hr$^{-1}$, the natural decay constant ($k_n$ = 0.012) of *E. coli* bioaerosol is quite low. It demonstrated the effects of gravity deposition and wall loss on bioaerosols were not obviously in the chamber. The data also indicates over 90% bioaerosols would be removal for 30 minutes after fresh air delivering into testing chamber.

**The inactivation efficiency of ozone water spraying against bioaerosols.** Figure 4 shows inactivating behavior of *E. coli* bioaerosol with 1.0 ppm OW spraying under ACH of 0.5 and 1.0 hr$^{-1}$. The decay constant ($k_a$) of *E. coli* bioaerosols with 1.0 ppm OW spraying under ACH of 0.5 and 1.0 hr$^{-1}$ were 0.291 and 0.435 (min$^{-1}$), respectively. Using 1.0 ppm OW spraying in the testing chamber under

ACH of 0.5 and 1.0 $hr^{-1}$, *E.coli* bioaerosol concentrations decreased from $3 \times 10^4$ to 0 $CFU/m^3$ in 30 and 20minutes. Comparison of $k_n$ under the same operating condition without OW spraying intervention ($k_n$ = 0.098 and 0.185 at ACH of 0.5 and 1.0 $h^{-1}$), the OW spraying shows the significant inactivating ability against *E. coli* bioaerosol. According to the experimental data, it is also finding that OW spraying performed the better bioaerosol inactivating ability than bioaerosol removal efficiency of the ACH.

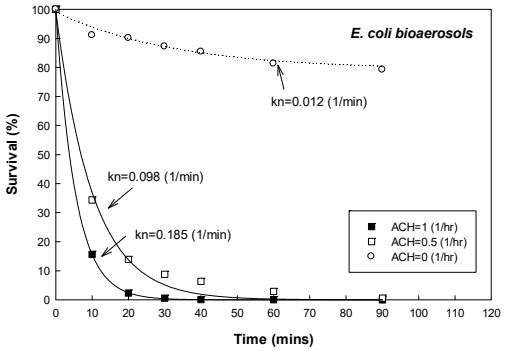


Figure 3. Natural ventilation decay of *E. Coli* bioaerosol in the test chamber

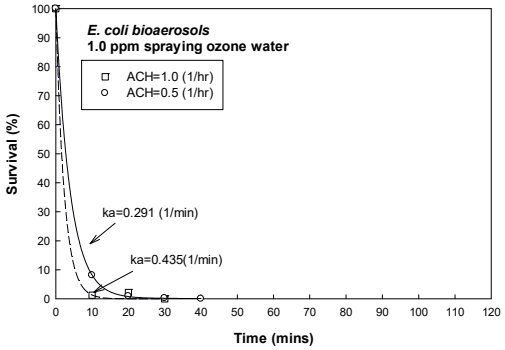


Figure 4. Inactivation efficiency of *E. coli* bioaerosols using ozone water spraying in different ACH

**Effect of ozone water concentration on inactivating efficiency.** Figure 5 indicates inactivating behavior of *E.coli* bioaerosol against 0.5 and 1.0 ppm ozone water spraying under the ACH of 1.0 $h^{-1}$. The inactivating constant $k_a$ with using 0.5 and 1.0 ppm OW spray were around 0.236 and 0.435 ($min^{-1}$). The data showed that higher OW concentration performed better inactivating ability against bioaerosols.

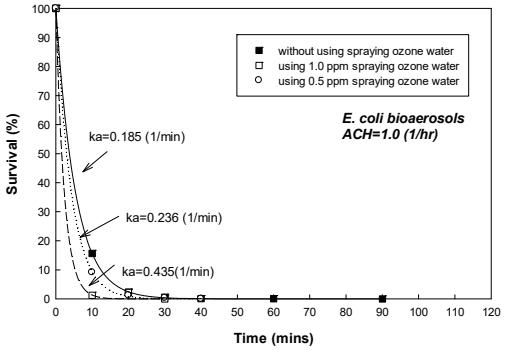


Figure 5. Inactivation efficiency of *E. coli* bioaerosols using ozone water spraying in different ozone water concentration

## 4. Summary

Results demonstrate that the ozone water effectively in *E.coli* bioaerosols during the testing simulated-environment chamber. The inactivating decay constant of *E. coli* bioaerosols against ozone water would increase with ozone water concentration and ACH. The ozone water inactivating capability was the major effect on bioaerosol inactivation under testing experiment.

**Acknowledgements**

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